Autism Studies & Related Medical Conditions

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1- Autism and Mitochondrial Dysfunction (19 citations):

Blasi, F., E. Bacchelli, et al. (2006). "SLC25A12 and CMYA3 gene variants are not associated with autism in the IMGSAC multiplex family sample." Eur J Hum Genet **14**(1): 123-6.

Autism is a severe neurodevelopmental disorder with a complex genetic predisposition. Linkage findings from several genome scans suggest the presence of an autism susceptibility locus on chromosome 2q24-q33, making this region the focus of candidate gene and association studies. Recently, significant association with autism has been reported for single-nucleotide polymorphisms (SNPs) in the SLC25A12 and CMYA3 genes on chromosome 2q. We attempted to replicate these findings in the collection of families from the International Molecular Genetic Study of Autism Consortium (IMGSAC), using the transmission disequilibrium test and case-control comparison. Our study failed to reveal any significant association for the SNPs tested at either locus, suggesting that these variants are unlikely to play a major role in genetic susceptibility to autism in our sample.

Clark-Taylor, T. and B. E. Clark-Taylor (2004). "Is autism a disorder of fatty acid metabolism? Possible dysfunction of mitochondrial beta-oxidation by long chain acyl-CoA dehydrogenase." Med Hypotheses **62**(6): 970-5.

Long chain acyl-CoA dehydrogenase (LCAD) has recently been shown to be the mitochondrial enzyme responsible for the beta-oxidation of branched chain and unsaturated fatty acids [Biochim. Biophys. Acta 1393 (1998) 35; Biochim. Biophys. Acta 1485 (2000) 121]. Whilst disorders of short, medium and very long chain acyl dehydrogenases are known, there is no known disorder of LCAD deficiency in humans. Experimental LCAD deficiency in mice shows an acyl-carnitine profile with prominent elevations of unsaturated fatty acid metabolites C14:1 and C14:2 [Hum. Mol. Genet. 10 (2001) 2069]. A child with autism whose acyl-carnitine profile also shows these abnormalities is presented, and it is hypothesized that the child may have LCAD deficiency. Additional metabolic abnormalities seen in this patient include alterations of TCA energy production, ammonia detoxification, reduced synthesis of omega-3 DHA, and abnormal cholesterol metabolism. These metabolic changes are also seen as secondary abnormalities in dysfunction of fatty acid beta-oxidation, and have also been reported in autism. It is hypothesized that LCAD deficiency may be a cause of autism. Similarities between metabolic disturbances in autism, and those of disorders of fatty acid beta-oxidation are discussed.

Filipek, P. A., J. Juranek, et al. (2004). "Relative carnitine deficiency in autism." J Autism Dev Disord **34**(6): 615-23.

A random retrospective chart review was conducted to document serum carnitine levels on 100 children with autism. Concurrently drawn serum pyruvate, lactate, ammonia, and alanine levels were also available in many of these children.

Values of free and total carnitine ($p < 0.001$), and pyruvate ($p = 0.006$) were significantly reduced while ammonia and alanine levels were considerably elevated ($p < 0.001$) in our autistic subjects. The relative carnitine deficiency in these patients, accompanied by slight elevations in lactate and significant elevations in alanine and ammonia levels, is suggestive of mild mitochondrial dysfunction. It is hypothesized that a mitochondrial defect may be the origin of the carnitine deficiency in these autistic children.

Filipek, P. A., J. Juranek, et al. (2003). "Mitochondrial dysfunction in autistic patients with 15q inverted duplication." Ann Neurol **53**(6): 801-4.

Two autistic children with a chromosome 15q11-q13 inverted duplication are presented. Both had uneventful perinatal courses, normal electroencephalogram and magnetic resonance imaging scans, moderate motor delay, lethargy, severe hypotonia, and modest lactic acidosis. Both had muscle mitochondrial enzyme assays that showed a pronounced mitochondrial hyperproliferation and a partial respiratory chain block most parsimoniously placed at the level of complex III, suggesting candidate gene loci for autism within the critical region may affect pathways influencing mitochondrial function.

Fillano, J. J., M. J. Goldenthal, et al. (2002). "Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome." J Child Neurol **17**(6): 435-9.

A group of 12 children clinically presenting with hypotonia, intractable epilepsy, autism, and developmental delay, who did not fall into previously described categories of mitochondrial encephalomyopathy, were evaluated for mitochondrial respiratory enzyme activity levels, mitochondrial DNA, and mitochondrial structural abnormalities. Reduced levels in specific respiratory activities were found solely in enzymes with subunits encoded by mitochondrial DNA in seven of eight biopsied skeletal muscle specimens evaluated. Five cases exhibited increased levels of large-scale mitochondrial DNA deletions, whereas pathogenic point mutations previously described in association with mitochondrial encephalomyopathies were not found. Mitochondrial structural abnormalities were present in three of four patients examined. Our findings suggest that mitochondrial dysfunction, including extensive abnormalities in specific enzyme activities, mitochondrial structure, and mitochondrial DNA integrity, may be present in children with a clinical constellation including hypotonia, epileptic seizures, autism, and developmental delay. The acronym HEADD is presented here to facilitate pursuit of mitochondrial defects in patients with this clinical constellation after other causes have been excluded.

Gargus, J. J. and F. Imtiaz (2008). "Mitochondrial energy-deficient endophenotype in autism." American Journal of Biochemistry and Biotechnology **4**(2): 198-207.

While evidence points to a multigenic etiology of most autism, the pathophysiology of the disorder has yet to be defined and the underlying genes

and biochemical pathways they subserve remain unknown. Autism is considered to be influenced by a combination of various genetic, environmental and immunological factors; more recently, evidence has suggested that increased vulnerability to oxidative stress may be involved in the etiology of this multifactorial disorder. Furthermore, recent studies have pointed to a subset of autism associated with the biochemical endophenotype of mitochondrial energy deficiency, identified as a subtle impairment in fat and carbohydrate oxidation. This phenotype is similar, but more subtle than those seen in classic mitochondrial defects. In some cases the beginnings of the genetic underpinnings of these mitochondrial defects are emerging, such as mild mitochondrial dysfunction and secondary carnitine deficiency observed in the subset of autistic patients with an inverted duplication of chromosome 15q11-q13. In addition, rare cases of familial autism associated with sudden infant death syndrome (SIDS) or associated with abnormalities in cellular calcium homeostasis, such as malignant hyperthermia or cardiac arrhythmia, are beginning to emerge. Such special cases suggest that the pathophysiology of autism may comprise pathways that are directly or indirectly involved in mitochondrial energy production and to further probe this connection three new avenues seem worthy of exploration: 1) metabolomic clinical studies provoking controlled aerobic exercise stress to expand the biochemical phenotype, 2) high-throughput expression arrays to directly survey activity of the genes underlying these biochemical pathways and 3) model systems, either based upon neuronal stem cells or model genetic organisms, to discover novel genetic and environmental inputs into these pathways.

Graf, W. D., J. Marin-Garcia, et al. (2000). "Autism associated with the mitochondrial DNA G8363A transfer RNA(Lys) mutation." J Child Neurol **15**(6): 357-61.

We report a family with a heterogeneous group of neurologic disorders associated with the mitochondrial DNA G8363A transfer ribonucleic acid (RNA)Lys mutation. The phenotype of one child in the family was consistent with autism. During his second year of life, he lost previously acquired language skills and developed marked hyperactivity with toe-walking, abnormal reciprocal social interaction, stereotyped mannerisms, restricted interests, self-injurious behavior, and seizures. Brain magnetic resonance imaging (MRI) and repeated serum lactate studies were normal. His older sister developed signs of Leigh syndrome with progressive ataxia, myoclonus, seizures, and cognitive regression. Her laboratory studies revealed increased MRI T2-weighted signal in the putamen and posterior medulla, elevated lactate in serum and cerebrospinal fluid, and absence of cytochrome c oxidase staining in muscle histochemistry. Molecular analysis in her revealed the G8363A mutation of the mitochondrial transfer RNA(Lys) gene in blood (82% mutant mitochondrial DNA) and muscle (86%). The proportions of mutant mitochondrial DNA from her brother with autism were lower (blood 60%, muscle 61%). It is likely that the origin of his autism phenotype is the pathogenic G8363A mitochondrial DNA mutation. This

observation suggests that certain mitochondrial point mutations could be the basis for autism in some individuals.

Lerman-Sagie, T., E. Leshinsky-Silver, et al. (2004). "Should autistic children be evaluated for mitochondrial disorders?" J Child Neurol **19**(5): 379-81.

Autism is etiologically heterogeneous; medical conditions are implicated in only a minority of cases, whereas metabolic disorders are even less common. Recently, there have been articles describing the association of autism with mitochondrial abnormalities. We critically review the current literature and conclude that mitochondrial disorders are probably a rare and insignificant cause of pure autism; however, evidence is accumulating that both autosomal recessive and maternally inherited mitochondrial disorders can present with autistic features. Most patients will present with multisystem abnormalities associated with autistic behavior. Finding biochemical or structural mitochondrial abnormalities in an autistic child does not necessarily imply a primary mitochondrial disorder but can also be secondary to technical inaccuracies or another genetic disorder. Clinicians should be careful in diagnosing a mitochondrial disorder in an autistic child because it has important implications for accurate genetic counseling, prognosis, and therapy.

Lombard, J. (1998). "Autism: a mitochondrial disorder?" Med Hypotheses **50**(6): 497-500.

Autism is a developmental disorder characterized by disturbance in language, perception and socialization. A variety of biochemical, anatomical and neuroradiographical studies imply a disturbance of brain energy metabolism in autistic patients. The underlying etiology of a disturbed bioenergetic metabolism in autism is unknown. A likely etiological possibility may involve mitochondrial dysfunction with concomitant defects in neuronal oxidative phosphorylation within the central nervous system. This hypothesis is supported by a frequent association of lactic acidosis and carnitine deficiency in autistic patients. Mitochondria are vulnerable to a wide array of endogenous and exogenous factors which appear to be linked by excessive nitric oxide production. Strategies to augment mitochondrial function, either by decreasing production of endogenous toxic metabolites, reducing nitric oxide production, or stimulating mitochondrial enzyme activity may be beneficial in the treatment of autism.

Oliveira, G., A. Ataide, et al. (2007). "Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions." Dev Med Child Neurol **49**(10): 726-33.

The objective of this study was to estimate the prevalence of autistic spectrum disorder (ASD) and identify its clinical characterization, and medical conditions in a paediatric population in Portugal. A school survey was conducted in elementary schools, targeting 332,808 school-aged children in the mainland and 10,910 in the Azores islands. Referred children were directly assessed using the Diagnostic

and Statistical Manual of Mental Disorders (4th edn), the Autism Diagnostic Interview-Revised, and the Childhood Autism Rating Scale. Clinical history and a laboratory investigation was performed. In parallel, a systematic multi-source search of children known to have autism was carried out in a restricted region. The global prevalence of ASD per 10,000 was 9.2 in mainland, and 15.6 in the Azores, with intriguing regional differences. A diversity of associated medical conditions was documented in 20%, with an unexpectedly high rate of mitochondrial respiratory chain disorders.

Oliveira, G., L. Diogo, et al. (2005). "Mitochondrial dysfunction in autism spectrum disorders: a population-based study." Dev Med Child Neurol **47**(3): 185-9.

A minority of cases of autism has been associated with several different organic conditions, including bioenergetic metabolism deficiency. In a population-based study, we screened associated medical conditions in a group of 120 children with autism (current age range 11y 5mo to 14y 4mo, mean age 12y 11mo [SD 9.6mo], male:female ratio 2.9:1). Children were diagnosed using Diagnostic and Statistical Manual of Mental Disorders criteria, the Autism Diagnostic Interview--Revised, and the Childhood Autism Rating Scale; 76% were diagnosed with typical autism and 24% with atypical autism. Cognitive functional level was assessed with the Griffiths scale and the Wechsler Intelligence Scale for Children and was in the normal range in 17%. Epilepsy was present in 19 patients. Plasma lactate levels were measured in 69 patients, and in 14 we found hyperlactacidemia. Five of 11 patients studied were classified with definite mitochondrial respiratory chain disorder, suggesting that this might be one of the most common disorders associated with autism (5 of 69; 7.2%) and warranting further investigation.

Poling, J. S., R. E. Frye, et al. (2006). "Developmental regression and mitochondrial dysfunction in a child with autism." J Child Neurol **21**(2): 170-2.

Autistic spectrum disorders can be associated with mitochondrial dysfunction. We present a singleton case of developmental regression and oxidative phosphorylation disorder in a 19-month-old girl. Subtle abnormalities in the serum creatine kinase level, aspartate aminotransferase, and serum bicarbonate led us to perform a muscle biopsy, which showed type I myofiber atrophy, increased lipid content, and reduced cytochrome c oxidase activity. There were marked reductions in enzymatic activities for complex I and III. Complex IV (cytochrome c oxidase) activity was near the 5% confidence level. To determine the frequency of routine laboratory abnormalities in similar patients, we performed a retrospective study including 159 patients with autism (Diagnostic and Statistical Manual of Mental Disorders-IV and Childhood Autism Rating Scale) not previously diagnosed with metabolic disorders and 94 age-matched controls with other neurologic disorders. Aspartate aminotransferase was elevated in 38% of patients with autism compared with 15% of controls (P <.0001). The serum creatine kinase level also was abnormally elevated in 22 (47%) of 47 patients

with autism. These data suggest that further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent.

Pons, R., A. L. Andreu, et al. (2004). "Mitochondrial DNA abnormalities and autistic spectrum disorders." J Pediatr **144**(1): 81-5.

OBJECTIVES: To further characterize mtDNA defects associated with autistic features, especially the A3243G mtDNA mutation and mtDNA depletion.Study design Five patients with autistic spectrum disorders and family histories of mitochondrial DNA diseases were studied. We performed mtDNA analysis in all patients and magnetic resonance spectroscopy in three. RESULTS: Three patients manifested isolated autistic spectrum features and two had additional neurologic symptoms. Two patients harbored the A3243G mutation. In two others, the A3243G mutation was not found in accessible tissues but was present in tissues from their mothers. The fifth patient had 72% mtDNA depletion in skeletal muscle. CONCLUSIONS: Autistic spectrum disorders with or without additional neurologic features can be early presentations of the A3243G mtDNA mutation and can be a prominent clinical manifestation of mtDNA depletion. Mitochondrial dysfunction should be considered in patients who have autistic features and associated neurologic findings or who have evidence of maternal inheritance.

Ramoz, N., J. G. Reichert, et al. (2004). "Linkage and association of the mitochondrial aspartate/glutamate carrier SLC25A12 gene with autism." Am J Psychiatry **161**(4): 662-9.

OBJECTIVE: Autism/autistic disorder (MIM number 209850) is a complex, largely genetic psychiatric disorder. The authors recently mapped a susceptibility locus for autism to chromosome region 2q24-q33 (MIM number 606053). In the present study, genes across the 2q24-q33 interval were analyzed to identify an autism susceptibility gene in this region. METHOD: Mutation screening of positional candidate genes was performed in two stages. The first stage involved identifying, in unrelated subjects showing linkage to 2q24-q33, genetic variants in exons and flanking sequence within candidate genes and comparing the frequency of the variants between autistic and unrelated nonautistic subjects. Two single nucleotide polymorphisms (SNPs) that showed evidence for divergent distribution between autistic and nonautistic subjects were identified, both within SLC25A12, a gene encoding the mitochondrial aspartate/glutamate carrier (AGC1). In the second stage, the two SNPs in SLC25A12 were further genotyped in 411 autistic families, and linkage and association tests were carried out in the 197 informative families. RESULTS: Linkage and association were observed between autistic disorder and the two SNPs, rs2056202 and rs2292813, found in SLC25A12. Using either a single affected subject per family or all affected subjects, evidence for excess transmission was found by the Transmission Disequilibrium Test for rs2056202, rs2292813, and a two-locus G*G haplotype.

Similar results were observed using TRANSMIT for the analyses. Evidence for linkage was supported by linkage analysis with the two SNPs, with a maximal multipoint nonparametric linkage score of 1.57 and a maximal multipoint heterogeneity lod score of 2.11. Genotype relative risk could be estimated to be between 2.4 and 4.8 for persons homozygous at these loci. CONCLUSIONS: A strong association of autism with SNPs within the SLC25A12 gene was demonstrated. Further studies are needed to confirm this association and to decipher any potential etiological role of AGC1 in autism.

Rossignol, D. A. and J. J. Bradstreet (2008). "Evidence of mitochondrial dysfunction in autism and implications for treatment." American Journal of Biochemistry and Biotechnology **4**(2): 208-217.

Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. Exposure to environ-mental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions. Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.

Segurado, R., J. Conroy, et al. (2005). "Confirmation of association between autism and the mitochondrial aspartate/glutamate carrier SLC25A12 gene on chromosome 2q31." Am J Psychiatry **162**(11): 2182-4.

OBJECTIVE: Autism is a neurodevelopmental disorder with childhood onset and a known major genetic component. A recent study identified a highly significant association between autism and a two-single-nucleotide-polymorphism haplotype in the SLC25A12 gene, with a homozygote genotype relative risk between 2.4 and 4.8. The authors' goal was to investigate this association with autism in Irish affected child-parent trios because replication in an independent sample is essential in the validation of such potentially important findings. METHOD:

Markers rs2056202 and rs2292813 were genotyped in a total of 158 trios (442 individuals). The Transmission Disequilibrium Test was used to examine these markers for association with autism. RESULTS: In agreement with the recent study, the authors found significant association between autism and the C alleles of both rs2056202 and rs2292813 as well as the two-marker haplotype. CONCLUSIONS: These findings provide replication of the association between autism and SLC25A12.

Silverman, J. M., J. D. Buxbaum, et al. (2007). "Autism-related routines and rituals associated with a mitochondrial aspartate/glutamate carrier SLC25A12 polymorphism." Am J Med Genet B Neuropsychiatr Genet.

Evidence for a genetic association between autism and two single nucleotide polymorphisms (SNPs), rs2056202 and rs2292813, in the mitochondrial aspartate/glutamate carrier (SLC25A12) gene led us to ask whether any of the four previously identified familial traits in autism spectrum disorders (ASD) varied by these SNPs. In 355 ASD cases from 170 sibships we examined levels of the four traits in these SNPs using ANCOVA models. The primary models selected unrelated affected cases and used age and sex as covariates. An ancillary set of models used all affected siblings and included "sibship" as a random effects independent variable. We found significantly lower levels of routines and rituals associated with the presence of the less frequent A allele in rs2056206. No other significant differences were observed. The rs2056202 polymorphism may be associated with levels of routines and rituals in autism and related disorders. (c) 2007 Wiley-Liss, Inc.

Trushina, E. and C. T. McMurray (2007). "Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases." Neuroscience **145**(4): 1233-48.

In recent years, it has become increasingly clear that mitochondrial dysfunction and oxidative damage are major contributors to neuronal loss. Free radicals, typically generated from mitochondrial respiration, cause oxidative damage of nucleic acids, lipids, carbohydrates and proteins. Despite enormous amount of effort, however, the mechanism by which oxidative damage causes neuronal death is not well understood. Emerging data from a number of neurodegenerative diseases suggest that there may be common features of toxicity that are related to oxidative damage. In this review, while focusing on Huntington's disease (HD), we discuss similarities among HD, Friedreich ataxia and xeroderma pigmentosum, which provide insight into shared mechanisms of neuronal death.

Tsao, C. Y. and J. R. Mendell (2007). "Autistic disorder in 2 children with mitochondrial disorders." J Child Neurol **22**(9): 1121-3.

Autistic disorder is a heterogeneous disorder. The majority of the cases are idiopathic, and only a small number of the autistic children have associated secondary diagnosis. This article reports 2 children with mitochondrial disorders

associated with autistic disorder fulfilling the diagnostic criteria of the American Psychiatric Association Manual of Psychiatric Diseases, 4th edition, and briefly reviews the literature on autistic disorder associated with mitochondrial disorders.

2- Autism and Gastrointestinal Inflammation (101 citations)

Afzal, M. A. and P. D. Minor (2002). "Vaccines, Crohn's disease and autism." Mol Psychiatry **7 Suppl 2**: S49-50.

Afzal, M. A., P. D. Minor, et al. (2001). "Measles virus persistence in specimens of inflammatory bowel disease and autism cases." Dig Dis Sci **46**(3): 658-60.

Afzal, N., S. Murch, et al. (2003). "Constipation with acquired megarectum in children with autism." Pediatrics **112**(4): 939-42.

> OBJECTIVE: Recent evidence suggests that autistic children may have significant gastrointestinal symptoms. Although constipation occurs in 2% to 5% of healthy children, its clinical diagnosis is often difficult in children with behavioral disorders. We thus aimed to assess the prevalence of fecal loading in autistic children with gastrointestinal symptoms and to identify possible predictors of constipation. METHODS: We studied abdominal radiographs of 103 autistic children (87 boys) who were referred for gastroenterological assessment, in comparison with 29 control radiographs from children who were referred to the emergency department, most with abdominal pain. Radiographs were scored independently, in blinded manner, by 4 pediatric gastroenterologists and a radiologist. The severity of constipation was determined using a validated index. Details of stool habit, abdominal pain, dietary history, and laxative use were obtained from case notes. RESULTS: The incidence of constipation in the control subjects with abdominal pain was higher than reported for normal children. Despite this, moderate or severe constipation was more frequent in the autistic group than in the control subjects (36% vs 10%). Analysis of rectosigmoid loading showed more striking differences (54.4% of autistic children had moderate/severe loading or acquired megarectum compared with 24.1% of control subjects). Multivariate regression analysis showed consumption of milk to be the strongest predictor of constipation in the autistic group, whereas stool frequency, gluten consumption, soiling, and abdominal pain were not predictive of constipation. CONCLUSIONS: Constipation is a frequent finding in children with gastrointestinal symptoms and autism, particularly in the rectosigmoid colon, often with acquired megarectum. The absence of any correlation between the clinical history and the degree of fecal impaction in autistic children confirms the importance of an abdominal radiograph in the assessment of their degree of constipation.

Ashwood, P., A. Anthony, et al. (2003). "Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology." J Clin Immunol **23**(6): 504-17.

> Inflammatory intestinal pathology has been reported in children with regressive autism (affected children). Detailed analysis of intestinal biopsies in these children indicates a novel lymphocytic enterocolitis with autoimmune features;

however, links with cognitive function remain unclear. To characterize further, the nature and extent of this disease we examined the mucosal infiltrate using flow cytometry. Duodenal, ileal, and colonic biopsies were obtained from 52 affected children, 25 histologically normal, and 54 histologically inflamed, developmentally normal controls. Epithelial and lamina propria lymphocyte populations were isolated and examined by multicolor flow cytometry. Adjacent biopsies were assessed by semiquantitative histopathology. At all sites, CD3(+) and CD3(+)CD8(+) IEL as well as CD3(+) LPL were significantly increased in affected children compared with developmentally normal noninflamed control groups ($p<0.01$) reaching levels similar to inflamed controls. In addition, two populations--CD3(+)CD4(+) IEL and LP CD19(+) B cells--were significantly increased in affected children compared with both noninflamed and inflamed control groups including IBD, at all sites examined ($p<0.01$). Histologically there was a prominent mucosal eosinophil infiltrate in affected children that was significantly lower in those on a gluten- and casein-free diet, although lymphocyte populations were not influenced by diet.The data provide further evidence of a pan-enteric mucosal immunopathology in children with regressive autism that is apparently distinct from other inflammatory bowel diseases.

Ashwood, P., A. Anthony, et al. (2004). "Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10." J Clin Immunol **24**(6): 664-73.

A lymphocytic enterocolitis has been reported in a cohort of children with autistic spectrum disorder (ASD) and gastrointestinal (GI) symptoms. This study tested the hypothesis that dysregulated intestinal mucosal immunity with enhanced pro-inflammatory cytokine production is present in these ASD children. Comparison was made with developmentally normal children with, and without, mucosal inflammation. Duodenal and colonic biopsies were obtained from 21 ASD children, and 65 developmentally normal paediatric controls, of which 38 had signs of histological inflammation. Detection of CD3+ lymphocyte staining for spontaneous intracellular TNFalpha, IL-2, IL-4, IFNgamma, and IL-10, was performed by multicolor flow cytometry. Duodenal and colonic mucosal CD3+ lymphocyte counts were elevated in ASD children compared with noninflamed controls ($p<0.03$). In the duodenum, the proportion of lamina propria (LP) and epithelial CD3(+)TNFalpha+ cells in ASD children was significantly greater compared with noninflamed controls ($p<0.002$) but not coeliac disease controls. In addition, LP and epithelial CD3(+)IL-2+ and CD3(+)IFNgamma+, and epithelial CD3(+)IL-4+ cells were more numerous in ASD children than in noninflamed controls ($p<0.04$). In contrast, CD3(+)IL-10+ cells were fewer in ASD children than in noninflamed controls ($p<0.05$). In the colon, LP CD3(+)TNFalpha+ and CD3(+)IFNgamma+ were more frequent in ASD children than in noninflamed controls ($p<0.01$). In contrast with Crohn's disease and non-Crohn's colitis, LP and epithelial CD3(+)IL-10+ cells were fewer in ASD children

than in nondisease controls (p<0.01). There was a significantly greater proportion of CD3(+)TNFalpha+ cells in colonic mucosa in those ASD children who had no dietary exclusion compared with those on a gluten and/or casein free diet (p<0.05). There is a consistent profile of CD3+ lymphocyte cytokines in the small and large intestinal mucosa of these ASD children, involving increased pro-inflammatory and decreased regulatory activities. The data provide further evidence of a diffuse mucosal immunopathology in some ASD children and the potential for benefit of dietary and immunomodulatory therapies.

Ashwood, P. and A. J. Wakefield (2006). "Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms." J Neuroimmunol **173**(1-2): 126-34.

Gastrointestinal pathology, characterized by lymphoid nodular hyperplasia and entero-colitis, has been demonstrated in a cohort of children with autistic spectrum disorder (ASD). Systemic and intestinal mucosal immune dysregulation was assessed in ASD children with gastrointestinal (GI) symptoms (n = 18), and typically developing controls (n = 27), including non-inflamed controls (NIC) and inflamed GI control children with Crohn's disease (CD), by analysis of intracellular cytokines in CD3+ lymphocytes. In both peripheral blood and mucosa, CD3+ TNFalpha+ and CD3+ IFNgamma+ were increased in ASD children compared with NIC (p < 0.004) and reached levels similar to CD. In contrast, peripheral and mucosal CD3+ IL-10+ were markedly lower in ASD children with GI symptoms compared with both NIC and CD controls (p < 0.02). In addition, mucosal CD3+ IL-4+ cells were increased (p < 0.007) in ASD compared with NIC. There is a unique pattern of peripheral blood and mucosal CD3+ lymphocytes intracellular cytokines, which is consistent with significant immune dysregulation, in this ASD cohort.

Balzola, F., V. Barbon, et al. (2005). "Panenteric IBD-like disease in a patient with regressive autism shown for the first time by the wireless capsule enteroscopy: another piece in the jigsaw of this gut-brain syndrome?" Am J Gastroenterol **100**(4): 979-81.

Balzola, F., C. Daniela, et al. (2005). "Autistic enterocolitis: confirmation of a new inflammatory bowel disease in an Italian cohort of patients." Gastroenterology **128**(Suppl. 2): A303.

Barcia, G., A. Posar, et al. (2008). "Autism and coeliac disease." J Autism Dev Disord **38**(2): 407-8.

Black, C., J. A. Kaye, et al. (2002). "Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database." BMJ **325**(7361): 419-21.

OBJECTIVES: To assess whether children with autism are more likely to have a history of gastrointestinal disorders than children without autism. DESIGN:

Nested case-control study. SETTING: UK General Practice Research Database. SUBJECTS: Children born after 1 January 1988 and registered with the General Practice Research Database within 6 months of birth. OUTCOME MEASURES: Chronic inflammation of the gastrointestinal tract, coeliac disease, food intolerance, and recurrent gastrointestinal symptoms recorded by the general practitioner. RESULTS: 9 of 96 (9%) children with a diagnosis of autism (cases) and 41 of 449 (9%) children without autism (matched controls) had a history of gastrointestinal disorders before the index date (the date of first recorded diagnosis of autism in the cases and the same date for controls). The estimated odds ratio for a history of gastrointestinal disorders among children with autism compared with children without autism was 1.0 (95% confidence interval 0.5 to 2.2). CONCLUSIONS: No evidence was found that children with autism were more likely than children without autism to have had defined gastrointestinal disorders at any time before their diagnosis of autism.

Black, D., H. Prempeh, et al. (1998). "Autism, inflammatory bowel disease, and MMR vaccine." Lancet **351**(9106): 905-6; author reply 908-9.

Bolte, E. R. (1998). "Autism and Clostridium tetani." Med Hypotheses **51**(2): 133-44.
Autism is a severe developmental disability believed to have multiple etiologies. This paper outlines the possibility of a subacute, chronic tetanus infection of the intestinal tract as the underlying cause for symptoms of autism observed in some individuals. A significant percentage of individuals with autism have a history of extensive antibiotic use. Oral antibiotics significantly disrupt protective intestinal microbiota, creating a favorable environment for colonization by opportunistic pathogens. Clostridium tetani is an ubiquitous anaerobic bacillus that produces a potent neurotoxin. Intestinal colonization by C. tetani, and subsequent neurotoxin release, have been demonstrated in laboratory animals which were fed vegetative cells. The vagus nerve is capable of transporting tetanus neurotoxin (TeNT) and provides a route of ascent from the intestinal tract to the CNS. This route bypasses TeNT's normal preferential binding sites in the spinal cord, and therefore the symptoms of a typical tetanus infection are not evident. Once in the brain, TeNT disrupts the release of neurotransmitters by the proteolytic cleavage of synaptobrevin, a synaptic vesicle membrane protein. This inhibition of neurotransmitter release would explain a wide variety of behavioral deficits apparent in autism. Lab animals injected in the brain with TeNT have exhibited many of these behaviors. Some children with autism have also shown a significant reduction in stereotyped behaviors when treated with antimicrobials effective against intestinal clostridia. When viewed as sequelae to a subacute, chronic tetanus infection, many of the puzzling abnormalities of autism have a logical basis. A review of atypical tetanus cases, and strategies to test the validity of this paper's hypothesis, are included.

Buie, T. M. (2005). "Gastroesophageal reflux in children with autism: how do children present and can one test these children?" J Pediatr Gastroenterol Nutr **41**(4): 505.

Background: Gastroesophageal Reflux (GER) is primarily diagnosed by symptom description. Children with autism have core difficulty communicating and atypical social relatedness. For this reason, identification of GER in autistic children may be difficult. The prevalence of GER in autism remains unknown, but several reports identify esophagitis as a finding in autistic children undergoing endoscopy. Aims: To evaluate autistic children with GI complaints and aggression or self-injurious behavior in order to determine if these behaviors may be symptoms of GER. Methods: Six consecutive autistic children (ages 8–19 years) undergoing endoscopy and scheduled for BRAVO (wireless) pH probe were evaluated for histology and pH meter results. Findings: GER was identified in 5 of 5 patients tested by BRAVO pH testing. Esophagitis was seen in 3 of 6 patients biopsied. See tables below. Conclusions: 1. Gastroesophageal reflux can be tested in children with autism using wireless BRAVO pH probe technology. 2. Aggresive or self-injurious behavior may be a manifestation of pain from GER and should prompt consideration of further investigation. 3. Further study of non-classic GI symptoms needs to be considered in children with autism.

Cade, R., M. Privette, et al. (2000). "Autism and schizophrenia: intestinal disorders." Nutritional Neuroscience **3**: 57-72.

DeFelice, M. L., E. D. Ruchelli, et al. (2003). "Intestinal cytokines in children with pervasive developmental disorders." Am J Gastroenterol **98**(8): 1777-82.

OBJECTIVES: A relationship between autism and gastrointestinal (GI) immune dysregulation has been postulated based on incidence of GI complaints as well as macroscopically observed lymphonodular hyperplasia and microscopically determined enterocolitis in pediatric patients with autism. To evaluate GI immunity, we quantitatively assessed levels of proinflammatory cytokines, interleukin (IL)-6, IL-8, and IL-1beta, produced by intestinal biopsies of children with pervasive developmental disorders. METHODS: Fifteen patients, six with pervasive developmental disorders and nine age-matched controls, presenting for diagnostic colonoscopy were enrolled. Endoscopic biopsies were organ cultured, supernatants were harvested, and IL-6, IL-8, and IL-1beta levels were quantified by ELISA. Tissue histology was evaluated by blinded pathologists. RESULTS: Concentrations of IL-6 from intestinal organ culture supernatants of patients with pervasive developmental disorders (median 318.5 pg/ml, interquartile range 282.0-393.0 pg/ml) when compared with controls (median 436.9 pg/ml, interquartile range 312.6-602.5 pg/ml) were not significantly different ($p = 0.0987$). Concentrations of IL-8 (median 84,000 pg/ml, interquartile range 16,000-143,000 pg/ml) when compared with controls (median 177,000 pg/ml, interquartile range 114,000-244,000 pg/ml) were not significantly different ($p = 0.0707$). Concentrations of IL-1beta (median 0.0 pg/ml, interquartile range 0.0-94.7 pg/ml) when compared with controls (median 0.0

pg/ml, interquartile range 0.0-60.2 pg/ml) were not significantly different (p = 0.8826). Tissue histology was nonpathological for all patients. CONCLUSIONS: We have demonstrated no significant difference in production of IL-6, IL-8, and IL-1beta between patients with pervasive developmental disorders and age-matched controls. In general, intestinal levels of IL-6 and IL-8 were lower in patients with pervasive developmental disorders than in age-matched controls. These data fail to support an association between autism and GI inflammation.

D'Eufemia, P., M. Celli, et al. (1996). "Abnormal intestinal permeability in children with autism." Acta Paediatr **85**(9): 1076-9.

We determined the occurrence of gut mucosal damage using the intestinal permeability test in 21 autistic children who had no clinical and laboratory findings consistent with known intestinal disorders. An altered intestinal permeability was found in 9 of the 21 (43%) autistic patients, but in none of the 40 controls. Compared to the controls, these nine patients showed a similar mean mannitol recovery, but a significantly higher mean lactulose recovery (1.64% +/- 1.43 vs 0.38% +/- 0.14; P < 0.001). We speculate that an altered intestinal permeability could represent a possible mechanism for the increased passage through the gut mucosa of peptides derived from foods with subsequent behavioural abnormalities.

Erickson, C. A., K. A. Stigler, et al. (2005). "Gastrointestinal factors in autistic disorder: a critical review." J Autism Dev Disord **35**(6): 713-27.

Interest in the gastrointestinal (GI) factors of autistic disorder (autism) has developed from descriptions of symptoms such as constipation and diarrhea in autistic children and advanced towards more detailed studies of GI histopathology and treatment modalities. This review attempts to critically and comprehensively analyze the literature as it applies to all aspects of GI factors in autism, including discussion of symptoms, pathology, nutrition, and treatment. While much literature is available on this topic, a dearth of rigorous study was found to validate GI factors specific to children with autism.

Finegold, S. M., D. Molitoris, et al. (2002). "Gastrointestinal microflora studies in late-onset autism." Clin Infect Dis **35**(Suppl 1): S6-S16.

Some cases of late-onset (regressive) autism may involve abnormal flora because oral vancomycin, which is poorly absorbed, may lead to significant improvement in these children. Fecal flora of children with regressive autism was compared with that of control children, and clostridial counts were higher. The number of clostridial species found in the stools of children with autism was greater than in the stools of control children. Children with autism had 9 species of Clostridium not found in controls, whereas controls yielded only 3 species not found in children with autism. In all, there were 25 different clostridial species found. In gastric and duodenal specimens, the most striking finding was total absence of non-spore-forming anaerobes and microaerophilic bacteria from

control children and significant numbers of such bacteria from children with autism. These studies demonstrate significant alterations in the upper and lower intestinal flora of children with late-onset autism and may provide insights into the nature of this disorder.

Fombonne, E. (1998). "Inflammatory bowel disease and autism." Lancet **351**(9107): 955.

Fombonne, E. and E. H. Cook (2003). "MMR and autistic enterocolitis: consistent epidemiological failure to find an association." Mol Psychiatry **8**(2): 133-4.

Furlano, R. I., A. Anthony, et al. (2001). "Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism." J Pediatr **138**(3): 366-72.
OBJECTIVES: We have reported colitis with ileal lymphoid nodular hyperplasia (LNH) in children with regressive autism. The aims of this study were to characterize this lesion and determine whether LNH is specific for autism. METHODS: Ileo-colonoscopy was performed in 21 consecutively evaluated children with autistic spectrum disorders and bowel symptoms. Blinded comparison was made with 8 children with histologically normal ileum and colon, 10 developmentally normal children with ileal LNH, 15 with Crohn's disease, and 14 with ulcerative colitis. Immunohistochemistry was performed for cell lineage and functional markers, and histochemistry was performed for glycosaminoglycans and basement membrane thickness. RESULTS: Histology demonstrated lymphocytic colitis in the autistic children, less severe than classical inflammatory bowel disease. However, basement membrane thickness and mucosal gamma delta cell density were significantly increased above those of all other groups including patients with inflammatory bowel disease. CD8(+) density and intraepithelial lymphocyte numbers were higher than those in the Crohn's disease, LNH, and normal control groups; and CD3 and plasma cell density and crypt proliferation were higher than those in normal and LNH control groups. Epithelial, but not lamina propria, glycosaminoglycans were disrupted. However, the epithelium was HLA-DR(-), suggesting a predominantly T(H)2 response. INTERPRETATION: Immunohistochemistry confirms a distinct lymphocytic colitis in autistic spectrum disorders in which the epithelium appears particularly affected. This is consistent with increasing evidence for gut epithelial dysfunction in autism.

González, L., K. López, et al. (2006). "Endoscopic and histological characteristics of the digestive mucosa in autistic children with gastrointestinal symptoms." Archivos Venezolanos De Puericultura Y Pediatria **69**: 19-25.

Goodwin, M. S., M. A. Cowen, et al. (1971). "Malabsorption and cerebral dysfunction: a multivariate and comparative study of autistic children." J Autism Child Schizophr **1**(1): 48-62.

Horvath, K., J. C. Papadimitriou, et al. (1999). "Gastrointestinal abnormalities in children with autistic disorder." J Pediatr **135**(5): 559-63.

OBJECTIVES: Our aim was to evaluate the structure and function of the upper gastrointestinal tract in a group of patients with autism who had gastrointestinal symptoms. STUDY DESIGN: Thirty-six children (age: 5.7 +/- 2 years, mean +/- SD) with autistic disorder underwent upper gastrointestinal endoscopy with biopsies, intestinal and pancreatic enzyme analyses, and bacterial and fungal cultures. The most frequent gastrointestinal complaints were chronic diarrhea, gaseousness, and abdominal discomfort and distension. RESULTS: Histologic examination in these 36 children revealed grade I or II reflux esophagitis in 25 (69.4%), chronic gastritis in 15, and chronic duodenitis in 24. The number of Paneth's cells in the duodenal crypts was significantly elevated in autistic children compared with non-autistic control subjects. Low intestinal carbohydrate digestive enzyme activity was reported in 21 children (58.3%), although there was no abnormality found in pancreatic function. Seventy-five percent of the autistic children (27/36) had an increased pancreatico-biliary fluid output after intravenous secretin administration. Nineteen of the 21 patients with diarrhea had significantly higher fluid output than those without diarrhea. CONCLUSIONS: Unrecognized gastrointestinal disorders, especially reflux esophagitis and disaccharide malabsorption, may contribute to the behavioral problems of the non-verbal autistic patients. The observed increase in pancreatico-biliary secretion after secretin infusion suggests an upregulation of secretin receptors in the pancreas and liver. Further studies are required to determine the possible association between the brain and gastrointestinal dysfunctions in children with autistic disorder.

Horvath, K. and J. A. Perman (2002). "Autism and gastrointestinal symptoms." Curr Gastroenterol Rep **4**(3): 251-8.

Autism is a collection of behavioral symptoms characterized by dysfunction in social interaction and communication in affected children. It is typically associated with restrictive, repetitive, and stereotypic behavior and manifests within the first 3 years of life. The cause of this disorder is not known. Over the past decade, a significant upswing in research has occurred to examine the biologic basis of autism. Recent clinical studies have revealed a high prevalence of gastrointestinal symptoms, inflammation, and dysfunction in children with autism. Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract. In addition, decreased sulfation capacity of the liver, pathologic intestinal permeability, increased secretory response to intravenous secretin injection, and decreased digestive enzyme activities were reported in many children with autism. Treatment of digestive problems appears to have positive effects on autistic behavior. These new observations represent only a piece of the unsolved autism "puzzle" and should stimulate more research into the brain-gut connection.

Horvath, K. and J. A. Perman (2002). "Autistic disorder and gastrointestinal disease." Curr Opin Pediatr **14**(5): 583-7.

Autistic disorder is a pervasive developmental disorder manifested in the first 3 years of life by dysfunction in social interaction and communication. Many efforts have been made to explore the biologic basis of this disorder, but the etiology remains unknown. Recent publications describing upper gastrointestinal abnormalities and ileocolitis have focused attention on gastrointestinal function and morphology in these children. High prevalence of histologic abnormalities in the esophagus, stomach, small intestine and colon, and dysfunction of liver conjugation capacity and intestinal permeability were reported. Three surveys conducted in the United States described high prevalence of gastrointestinal symptoms in children with autistic disorder. Treatment of the digestive problems may have positive effects on their behavior.

Horvath, K., G. Stefanatos, et al. (1998). "Improved social and language skills after secretin administration in patients with autistic spectrum disorders." J Assoc Acad Minor Phys **9**(1): 9-15.

We report three children with autistic spectrum disorders who underwent upper gastrointestinal endoscopy and intravenous administration of secretin to stimulate pancreaticobiliary secretion. All three had an increased pancreaticobiliary secretory response when compared with nonautistic patients (7.5 to 10 mL/min versus 1 to 2 mL/min). Within 5 weeks of the secretin infusion, a significant amelioration of the children's gastrointestinal symptoms was observed, as was a dramatic improvement in their behavior, manifested by improved eye contact, alertness, and expansion of expressive language. These clinical observations suggest an association between gastrointestinal and brain function in patients with autistic behavior.

Hunter, L. C., A. O'Hare, et al. (2003). "Opioid peptides and dipeptidyl peptidase in autism." Dev Med Child Neurol **45**(2): 121-8.

It has been hypothesized that autism results from an 'opioid peptide excess'. The aims of this study were to (1) confirm the presence of opioid peptides in the urine of children with autism and (2) determine whether dipeptidyl peptidase IV (DPPIV/CD26) is defective in children with autism. Opioid peptides were not detected in either the urine of children with autism (10 children; nine males, one female; age range 2 years 6 months to 10 years 1 month) or their siblings (10 children; seven males, three females; age range 2 years 3 months to 12 years 7 months) using liquid chromatography-ultraviolet-mass spectrometric analysis (LC-UV-MS). Plasma from 11 normally developing adults (25 years 5 months to 55 years 5 months) was also tested. The amount and activity of DPPIV in the plasma were quantified by an ELISA and DPPIV enzyme assay respectively; DPPIV was not found to be defective. The percentage of mononuclear cells expressing DPPIV (as CD26) was determined by flow cytometry. Children with

autism had a significantly lower percentage of cells expressing CD3 and CD26, suggesting that they had lower T-cell numbers than their siblings. In conclusion, this study failed to replicate the findings of others and questions the validity of the opioid peptide excess theory for the cause of autism.

Jass, J. R. (2005). "The intestinal lesion of autistic spectrum disorder." Eur J Gastroenterol Hepatol **17**(8): 821-2.

This editorial briefly reviews the significance of lymphoid nodular hyperplasia in the intestinal tract of children with autistic spectrum disorder. The distinction between physiological and pathological lymphoid hyperplasia of the intestinal tract is of importance in the context of a possible causative link with autism. A primary intestinal lesion may occur as part of the broad spectrum of immunological disorders to which autistic children are prone. This could result in increased intestinal permeability to peptides of dietary origin which may then lead to disruption of neuroregulatory mechanisms required for normal brain development. Alternatively, there could be a primary defect in the translocation and processing of factors derived from the intestinal lumen. These possibilities deserve further investigation and should not be lost in the fog of the controversy regarding the role of measles/mumps/rubella vaccination in the aetiology of autistic spectrum disorder.

Jyonouchi, H., L. Geng, et al. (2005). "Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders." J Pediatr **146**(5): 605-10.

OBJECTIVE: To evaluate an association between cytokine production with common dietary proteins as a marker of non-allergic food hypersensitivity (NFH) and gastrointestinal (GI) symptoms in young children with autism spectrum disorders (ASD). STUDY DESIGN: Peripheral blood mononuclear cells (PBMCs) were obtained from 109 ASD children with or without GI symptoms (GI [+] ASD, N = 75 and GI (-) ASD, N = 34], from children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. We measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMCs stimulated with whole cow's milk protein (CMP), its major components (casein, beta-lactoglobulin, and alpha-lactoalbumin), gliadin, and soy. RESULTS: PBMCs obtained from GI (+) ASD children produced more tumor necrosis factor-alpha (TNF-alpha)/interleukin-12 (IL-12) than those obtained from control subjects with CMP, beta-lactoglobulin, and alpha-lactoalbumin, irrespective of objective GI symptoms. They also produced more TNF-alpha with gliadin, which was more frequently observed in the group with loose stools. PBMCs obtained from GI (-) ASD children produced more TNF-alpha/IL-12 with CMP than those from control subjects, but not with beta-lactoglobulin, alpha-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable. CONCLUSION: A high prevalence of elevated TNF-alpha/IL-12 production by GI (+) ASD PBMCs with CMP and its major

components indicates a role of NFH in GI symptoms observed in children with ASD.

Jyonouchi, H., L. Geng, et al. (2005). "Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention." Neuropsychobiology **51**(2): 77-85.

OBJECTIVE: Our previous study indicated an association between cellular immune reactivity to common dietary proteins (DPs) and excessive proinflammatory cytokine production with endotoxin (lipopolysaccharide, LPS), a major stimulant of innate immunity in the gut mucosa, in a subset of autism spectrum disorder (ASD) children. However, it is unclear whether such abnormal LPS responses are intrinsic in these ASD children or the results of chronic gastrointestinal (GI) inflammation secondary to immune reactivity to DPs. This study further explored possible dysregulated production of proinflammatory and counter-regulatory cytokines with LPS in ASD children and its relationship to GI symptoms and the effects of dietary intervention measures. METHODS: This study includes ASD children (median age 4.8 years) on the unrestricted (n = 100) or elimination (n = 77) diet appropriate with their immune reactivity. Controls include children with non-allergic food hypersensitivity (NFH; median age 2.9 years) on the unrestricted (n = 14) or elimination (n = 16) diet, and typically developing children (median age 4.5 years, n = 13). The innate immune responses were assessed by measuring production of proinflammatory (TNF-alpha, IL-1beta, IL-6, and IL-12) and counter-regulatory (IL-1ra, IL-10, and sTNFRII) cytokines by peripheral blood mononuclear cells (PBMCs) with LPS. The results were also compared to T-cell responses with common DPs and control T-cell mitogens assessed by measuring T-cell cytokine production. RESULTS: ASD and NFH PBMCs produced higher levels of TNF-alpha with LPS than controls regardless of dietary interventions. However, only in PBMCs from ASD children with positive gastrointestinal (GI(+)) symptoms, did we find a positive association between TNF-alpha levels produced with LPS and those with cow's milk protein (CMP) and its major components regardless of dietary interventions. In the unrestricted diet group, GI(+) ASD PBMCs produced higher IL-12 than controls and less IL-10 than GI(-) ASD PBMCs with LPS. GI(+) ASD but not GI(-) ASD or NFH PBMCs produced less counter-regulatory cytokines with LPS in the unrestricted diet group than in the elimination diet group. There was no significant difference among the study groups with regard to cytokine production in responses to T-cell mitogens and other recall antigens. Conclusion: Our results revealed that there are findings limited to GI(+) ASD PBMCs in both the unrestricted and elimination diet groups. Thus our findings indicate intrinsic defects of innate immune responses in GI(+) ASD children but not in NFH or GI(-) ASD children, suggesting a possible link between GI and behavioral symptoms mediated by innate immune abnormalities.

Jyonouchi, H., S. Sun, et al. (2002). "Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder." Neuropsychobiology **46**(2): 76-84.

OBJECTIVES: Children with autism spectrum disorder (ASD) frequently reveal various gastrointestinal (GI) symptoms that may resolve with an elimination diet along with apparent improvement of some of the behavioral symptoms. Evidence suggests that ASD may be accompanied by aberrant (inflammatory) innate immune responses. This may predispose ASD children to sensitization to common dietary proteins (DP), leading to GI inflammation and aggravation of some behavioral symptoms. METHODS: We measured IFN-gamma, IL-5, and TNF-alpha production against representative DPs [gliadin, cow's milk protein (CMP), and soy] by peripheral blood mononuclear cells (PBMCs) from ASD and control children [those with DP intolerance (DPI), ASD siblings, and healthy unrelated children]. We evaluated the results in association with proinflammatory and counter-regulatory cytokine production with endotoxin (LPS), a microbial product of intestinal flora and a surrogate stimulant for innate immune responses. RESULTS: ASD PBMCs produced elevated IFN-gamma and TNF-alpha, but not IL-5 with common DPs at high frequency as observed in DPI PBMCs. ASD PBMCs revealed increased proinflammatory cytokine responses with LPS at high frequency with positive correlation between proinflammatory cytokine production with LPS and IFN-gamma and TNF-alpha production against DPs. Such correlation was less evident in DPI PBMCs. CONCLUSION: Immune reactivity to DPs may be associated with apparent DPI and GI inflammation in ASD children that may be partly associated with aberrant innate immune response against endotoxin, a product of the gut bacteria.

Kawashima, H., T. Mori, et al. (2000). "Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism." Dig Dis Sci **45**(4): 723-9.

It has been reported that measles virus may be present in the intestine of patients with Crohn's disease. Additionally, a new syndrome has been reported in children with autism who exhibited developmental regression and gastrointestinal symptoms (autistic enterocolitis), in some cases soon after MMR vaccine. It is not known whether the virus, if confirmed to be present in these patients, derives from either wild strains or vaccine strains. In order to characterize the strains that may be present, we have carried out the detection of measles genomic RNA in peripheral mononuclear cells (PBMC) in eight patients with Crohn's disease, three patients with ulcerative colitis, and nine children with autistic enterocolitis. As controls, we examined healthy children and patients with SSPE, SLE, HIV-1 (a total of eight cases). RNA was purified from PBMC by Ficoll-paque, followed by reverse transcription using AMV; cDNAs were subjected to nested PCR for detection of specific regions of the hemagglutinin (H) and fusion (F) gene regions. Positive samples were sequenced directly, in nucleotides 8393-8676 (H region) or 5325-5465 (from noncoding F to coding F region). One of eight

patients with Crohn disease, one of three patients with ulcerative colitis, and three of nine children with autism, were positive. Controls were all negative. The sequences obtained from the patients with Crohn's disease shared the characteristics with wild-strain virus. The sequences obtained from the patients with ulcerative colitis and children with autism were consistent with being vaccine strains. The results were concordant with the exposure history of the patients. Persistence of measles virus was confirmed in PBMC in some patients with chronic intestinal inflammation.

Knivsberg, A. M., K. L. Reichelt, et al. (2002). "A randomised, controlled study of dietary intervention in autistic syndromes." Nutr Neurosci **5**(4): 251-61.

Impaired social interaction, communication and imaginative skills characterize autistic syndromes. In these syndromes urinary peptide abnormalities, derived from gluten, gliadin, and casein, are reported. They reflect processes with opioid effect. The aim of this single blind study was to evaluate effect of gluten and casein-free diet for children with autistic syndromes and urinary peptide abnormalities. A randomly selected diet and control group with 10 children in each group participated. Observations and tests were done before and after a period of 1 year. The development for the group of children on diet was significantly better than for the controls.

Knivsberg, A. M., K. L. Reichelt, et al. (2001). "Reports on dietary intervention in autistic disorders." Nutr Neurosci **4**(1): 25-37.

Autism is a developmental disorder for which no cure currently exists. Gluten and/or casein free diet has been implemented to reduce autistic behaviour, in addition to special education, since early in the eighties. Over the last twelve years various studies on this dietary intervention have been published in addition to anecdotal, parental reports. The scientific studies include both groups of participants as well as single cases, and beneficial results are reported in all, but one study. While some studies are based on urinary peptide abnormalities, others are not. The reported results are, however, more or less identical; reduction of autistic behaviour, increased social and communicative skills, and reappearance of autistic traits after the diet has been broken.

Knivsberg, A. M., K. L. Reichelt, et al. (1995). "Autistic symptoms and diet: a follow-up study." Scand J Ed Research **39**: 223-236.

Kuddo, T. and K. B. Nelson (2003). "How common are gastrointestinal disorders in children with autism?" Curr Opin Pediatr **15**(3): 339-43.

We could identify no report that describes the prevalence of gastrointestinal disorders in a representative group of children with a diagnosis of autism compared with appropriate controls. Thus, we found no evidence upon which to base a confident conclusion as to whether gastrointestinal symptoms are more common in children with than without autism. However, the frequency of

gastrointestinal symptoms observed in population-based samples of autistic children indicate that gastrointestinal problems are not nearly as common in children with autism as reports from pediatric gastroenterology clinics suggest.

Kushak, R. I., H. S. Winter, et al. (2005). "Gastrointestinal symptoms and intestinal disaccharidase activities in children with autism." J Pediatr Gastroenterol Nutr **41**(4): 508.

Autistic children frequently suffer from diarrhea, abdominal pain, food intolerance and other gastrointestinal problems (GIP) that may contribute to their behavioral symptoms. Aim: To determine disaccharidase activities in autistic (AI) and nonautistic individuals (NAI) with different GIP. Methods: Specific activities for lactase, sucrase, maltase, and palatinase were studied in duodenal biopsies from 308 AI and 206 NAI selected for endoscopy based on a suspicion of GIP. Disaccharidase activities were analyzed for all patients based upon clinical report or diagnosis of diarrhea, abdominal pain, food sensitivity, failure to thrive (FTT), constipation, GER, or a combination of symptoms. Within each diagnostic category, activities for AI and NAI were determined. Cut off values for lactase, sucrase, maltase, and palatinase deficiency were correspondingly 15, 25, 100, and 5 U/g protein. Disaccharidase activities in intestinal biopsies were determined by Dahlqvist method; protein level was measured by Bradford method. Results: The frequency of GIP among AI and NAI was: diarrhea, 38 vs 18 %; abdominal pain, 36 vs 59 %; food sensitivity, 14 vs 11%; constipation, 4 vs. 0.5%; GER, 3 vs. 11%; FTT, 2 vs. 6%; diarrhea with abdominal pain, 6 vs 5%; diarrhea with food sensitivity, 6 vs 3%; and abdominal pain with food sensitivity, 4 vs 3%. AI with diarrhea (n = 206) demonstrated significantly lower maltase (P , 0.05) activity than NAI with diarrhea. Frequency of lactase deficiency in AI with FTT (n = 5) was significantly higher (80% vs 25%; P , 0.05) than in NAI with FTT and frequency of palatinase deficiency in AI with diarrhea was significantly higher than in NAI (28% vs 11%; P , 0.05) with the same GIP. AI and NAI with other GIP had similar frequency of disaccharidase deficiencies. Conclusion: Clinical indications for endoscopy based on GIP differ in AI and NAI. The clinical relevance of maltase deficiency in behavioral issues of AI with diarrhea needs to be determined. For most AI with GIP, the frequency of disaccharidase deficiency does not appear to differ from NAI.

Levy, S. E., M. C. Souders, et al. (2007). "Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders." Biol Psychiatry **61**(4): 492-7.

BACKGROUND: Gastrointestinal (GI) symptoms and abnormalities in stool consistency are frequently reported by parents of children with autism spectrum disorders (ASD). The purpose of this study was to 1) describe dietary intake of a cohort of children with ASD compared with normative data and 2) determine whether GI symptoms and stool consistency are related to dietary intake. METHODS: Data from diet diaries of children (3-8 years) with ASD (n = 62) were

analyzed by a registered pediatric dietician to compare to RDA standards for total calories, protein, carbohydrate, and fat. Dietary intake was correlated with descriptors of stool consistency using cumulative logistic regression methods. RESULTS: Intake of calories, carbohydrates, and fat were in the average range; protein intake was increased (211% of RDA). Reported frequency of GI abnormalities, including abnormal stool consistency (e.g., bulky or loose), was increased (54%). No statistically significant relationships between stool consistency and dietary intake were observed. CONCLUSIONS: In this sample, there was a high rate of reported gastrointestinal symptoms, despite lack of medical causes. Intake was adequate for calories and carbohydrates and increased for protein. The children did not exhibit excessive carbohydrate intake. There was no association of nutrient intake to changes in stool consistency.

Liu, Z., N. Li, et al. (2005). "Tight junctions, leaky intestines, and pediatric diseases." Acta Paediatr **94**(4): 386-93.

BACKGROUND: Tight junctions (TJs) represent the major barrier within the paracellular pathway between intestinal epithelial cells. Disruption of TJs leads to intestinal hyperpermeability (the so-called "leaky gut") and is implicated in the pathogenesis of several acute and chronic pediatric disease entities that are likely to have their origin during infancy. AIM: This review provides an overview of evidence for the role of TJ breakdown in diseases such as systemic inflammatory response syndrome (SIRS), inflammatory bowel disease, type 1 diabetes, allergies, asthma, and autism. CONCLUSION: A better basic understanding of this structure might lead to prevention or treatment of these diseases using nutritional or other means.

Macdonald, T. T. and P. Domizio (2007). "Autistic enterocolitis: is it a histopathological entity?" Histopathology **51**(4): 552-3.

MacFabe, D. F., D. P. Cain, et al. (2007). "Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders." Behav Brain Res **176**(1): 149-69.

Clinical observations suggest that certain gut and dietary factors may transiently worsen symptoms in autism spectrum disorders (ASD), epilepsy and some inheritable metabolic disorders. Propionic acid (PPA) is a short chain fatty acid and an important intermediate of cellular metabolism. PPA is also a by-product of a subpopulation of human gut enterobacteria and is a common food preservative. We examined the behavioural, electrophysiological, neuropathological, and biochemical effects of treatment with PPA and related compounds in adult rats. Intraventricular infusions of PPA produced reversible repetitive dystonic behaviours, hyperactivity, turning behaviour, retropulsion, caudate spiking, and the progressive development of limbic kindled seizures, suggesting that this compound has central effects. Biochemical analyses of brain homogenates from PPA treated rats showed an increase in oxidative stress

markers (e.g., lipid peroxidation and protein carbonylation) and glutathione S-transferase activity coupled with a decrease in glutathione and glutathione peroxidase activity. Neurohistological examinations of hippocampus and adjacent white matter (external capsule) of PPA treated rats revealed increased reactive astrogliosis (GFAP immunoreactivity) and activated microglia (CD68 immunoreactivity) suggestive of a neuroinflammatory process. This was coupled with a lack of cytotoxicity (cell counts, cleaved caspase 3' immunoreactivity), and an increase in phosphorylated CREB immunoreactivity. We propose that some types of autism may be partial forms of genetically inherited or acquired disorders involving altered PPA metabolism. Thus, intraventricular administration of PPA in rats may provide a means to model some aspects of human ASD in rats.

McGinnis, W. R. (2001). "Mercury and autistic gut disease." Environ Health Perspect **109**(7): A303-4.

Molloy, C. A. and P. Manning-Courtney (2003). "Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders." Autism **7**(2): 165-71.

The purpose of this study was to estimate the prevalence of chronic gastrointestinal symptoms in a general population of children with autism or autistic spectrum disorder (ASD). The study site was a clinic specializing in ASD in a large pediatric medical center serving a 10 county area in the midwestern USA. In a sample of 137 children, age 24-96 months, classified as having autism or ASD by the Autism Diagnostic Observation Schedule-Generic, 24 percent had a history of at least one chronic gastrointestinal symptom. The most common symptom was diarrhea, which occurred in 17 percent. There was no association between chronic gastrointestinal symptoms and a history of developmental regression. The potential phenotypic association between autism and gastrointestinal symptoms is discussed.

O'Brien, S. J., I. G. Jones, et al. (1998). "Autism, inflammatory bowel disease, and MMR vaccine." Lancet **351**(9106): 906-7; author reply 908-9.

Parracho, H. M., M. O. Bingham, et al. (2005). "Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children." J Med Microbiol **54**(Pt 10): 987-91.

Children with autistic spectrum disorders (ASDs) tend to suffer from severe gastrointestinal problems. Such symptoms may be due to a disruption of the indigenous gut flora promoting the overgrowth of potentially pathogenic micro-organisms. The faecal flora of patients with ASDs was studied and compared with those of two control groups (healthy siblings and unrelated healthy children). Faecal bacterial populations were assessed through the use of a culture-independent technique, fluorescence in situ hybridization, using

oligonucleotide probes targeting predominant components of the gut flora. The faecal flora of ASD patients contained a higher incidence of the Clostridium histolyticum group (Clostridium clusters I and II) of bacteria than that of healthy children. However, the non-autistic sibling group had an intermediate level of the C. histolyticum group, which was not significantly different from either of the other subject groups. Members of the C. histolyticum group are recognized toxin-producers and may contribute towards gut dysfunction, with their metabolic products also exerting systemic effects. Strategies to reduce clostridial population levels harboured by ASD patients or to improve their gut microflora profile through dietary modulation may help to alleviate gut disorders common in such patients.

Quigley, E. M. and D. Hurley (2000). "Autism and the gastrointestinal tract." Am J Gastroenterol **95**(9): 2154-6.

Reichelt, K. L. (1991). "[Gluten-free diet in infantile autism]." Tidsskr Nor Laegeforen **111**(10): 1286-7.

Reichelt, K. L., K. Hole, et al. (1981). "Biologically active peptide-containing fractions in schizophrenia and childhood autism." Adv Biochem Psychopharmacol **28**: 627-43.

It is well documented that peptides have a major role in the effective functioning of higher animals at all levels from enzyme stabilization to homeostatic mechanisms governing essential functions such as eating, sexual behavior, and temperature regulation. The effects of exogenously administered peptides on neurotransmitter release, uptake, metabolism and behavioral consequences are also well established. We have attempted to extend these findings by postulating peptidergic neurons as transducers of multisignal inputs, and that development of pathological states may be due to genetically-determined reduced levels of activity of key peptidases, leading to excretion of regulatory peptides into the circulation. We have been able to demonstrate that, in schizophrenia and autism (in well defined clinical cases), the patterns of peptides and associated proteins from urinary samples differ considerably from each other and from normal controls. In addition to this, further purification of the material obtained has led to the discovery of a number of factors capable of modulating the function of major neurotransmitters. Some of these are in the final stages of characterization as peptides, while the remainder are also probably peptides, as purification has been followed by both biological testing and chemical analysis for peptidic material. We have outlined a number of parameters which we consider relevant in any attempt to put psychiatric disorders on a biological foundation. Any new advances in the neurochemical understanding of such disorders must take into consideration the observations of several different disciplines including genetics and psychology. However, at this stage of research it is far too early to speculate on the relevance of the various biological activities to the etiology and symptomatology of schizophrenia and childhood autism.

Reichelt, K. L. and A. M. Knivsberg (2003). "Can the pathophysiology of autism be explained by the nature of the discovered urine peptides?" Nutr Neurosci **6**(1): 19-28.

Opioid peptides derived from food proteins (exorphins) have been found in urine of autistic patients. Based on the work of several groups, we try to show that exorphins and serotonin uptake stimulating factors may explain many of the signs and symptoms seen in autistic disorders. The individual symptoms ought to be explainable by the properties and behavioural effects of the found peptides. The data presented form the basis of an autism model, where we suggest that exorphins and serotonin uptake modulators are key mediators for the development of autism. This may be due to a genetically based peptidase deficiency in at least two or more peptidases and, or of peptidase regulating proteins made manifest by a dietary overload of exorphin precursors such as by increased gut uptake.

Sandler, R. H., S. M. Finegold, et al. (2000). "Short-term benefit from oral vancomycin treatment of regressive-onset autism." J Child Neurol **15**(7): 429-35.

In most cases symptoms of autism begin in early infancy. However, a subset of children appears to develop normally until a clear deterioration is observed. Many parents of children with "regressive"-onset autism have noted antecedent antibiotic exposure followed by chronic diarrhea. We speculated that, in a subgroup of children, disruption of indigenous gut flora might promote colonization by one or more neurotoxin-producing bacteria, contributing, at least in part, to their autistic symptomatology. To help test this hypothesis, 11 children with regressive-onset autism were recruited for an intervention trial using a minimally absorbed oral antibiotic. Entry criteria included antecedent broad-spectrum antimicrobial exposure followed by chronic persistent diarrhea, deterioration of previously acquired skills, and then autistic features. Short-term improvement was noted using multiple pre- and post-therapy evaluations. These included coded, paired videotapes scored by a clinical psychologist blinded to treatment status; these noted improvement in 8 of 10 children studied. Unfortunately, these gains had largely waned at follow-up. Although the protocol used is not suggested as useful therapy, these results indicate that a possible gut flora-brain connection warrants further investigation, as it might lead to greater pathophysiologic insight and meaningful prevention or treatment in a subset of children with autism.

Schneider, C. K., R. D. Melmed, et al. (2006). "Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study." J Autism Dev Disord **36**(8): 1053-64.

Immunoglobulin secretion onto mucosal surfaces is a major component of the mucosal immune system. We hypothesized that chronic gastrointestinal (GI) disturbances associated with autistic disorder (AD) may be due to an underlying deficiency in mucosal immunity, and that orally administered immunoglobulin

would be effective in alleviating chronic GI dysfunction in these individuals. In this pilot study, twelve male subjects diagnosed with AD were evaluated using a GI severity index (GSI) while receiving daily dosing with encapsulated human immunoglobulin. Following eight weeks of treatment, 50% of the subjects met prespecified criteria for response in GI signs and symptoms and showed significant behavioral improvement as assessed by the Autism Behavior Checklist and parent and physician rated Clinical Global Impression of Improvement.

Senior, K. (2002). "Possible autoimmune enteropathy found in autistic children." Lancet **359**(9318): 1674.

Smeeth, L., A. Hall, et al. (2002). "Autism, bowel inflammation, and measles." Lancet **359**(9323): 2112-3.

Song, Y., C. Liu, et al. (2004). "Real-time PCR quantitation of clostridia in feces of autistic children." Appl Environ Microbiol **70**(11): 6459-65.

Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three Clostridium clusters and one Clostridium species, C. bolteae, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle (CT) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of C. bolteae could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for C. bolteae and the following Clostridium groups were statistically significant: mean counts of C. bolteae and clusters I and XI in autistic children were 46-fold (P = 0.01), 9.0-fold (P = 0.014), and 3.5-fold (P = 0.004) greater than those in control children, respectively, but not for cluster XIVab (2.6 x 10(8) CFU/g in autistic children and 4.8 x 10(8) CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.

Taylor, B., E. Miller, et al. (2002). "Measles, mumps, and rubella vaccination and bowel problems or developmental regression in children with autism: population study." BMJ **324**(7334): 393-6.

Objectives: To investigate whether measles, mumps, and rubella (MMR) vaccination is associated with bowel problems and developmental regression in children with autism, looking for evidence of a "new variant" form of autism. Design: Population study with case note review linked to independently recorded vaccine data. Setting: Five health districts in north east London. Participants: 278

children with core autism and 195 with atypical autism, mainly identified from computerised disability registers and born between 1979 and 1998. Main outcome measures: Recorded bowel problems lasting at least three months, age of reported regression of the child's development where it was a feature, and relation of these to MMR vaccination. Results: The proportion of children with developmental regression (25% overall) or bowel symptoms (17%) did not change significantly (P value for trend 0.50 and 0.47, respectively) during the 20 years from 1979, a period which included the introduction of MMR vaccination in October 1988. No significant difference was found in rates of bowel problems or regression in children who received the MMR vaccine before their parents became concerned about their development (where MMR might have caused or triggered the autism with regression or bowel problem), compared with those who received it only after such concern and those who had not received the MMR vaccine. A possible association between non-specific bowel problems and regression in children with autism was seen but this was unrelated to MMR vaccination. Conclusions: These findings provide no support for an MMR associated "new variant" form of autism with developmental regression and bowel problems, and further evidence against involvement of MMR vaccine in the initiation of autism.

Thrower, D. (2002). "Autism, bowel inflammation, and measles." Lancet **359**(9323): 2113.

Torrente, F., A. Anthony, et al. (2004). "Focal-enhanced gastritis in regressive autism with features distinct from Crohn's and Helicobacter pylori gastritis." Am J Gastroenterol **99**(4): 598-605.

BACKGROUND: Immunohistochemistry allowed recent recognition of a distinct focal gastritis in Crohn's disease. Following reports of lymphocytic colitis and small bowel enteropathy in children with regressive autism, we aimed to see whether similar changes were seen in the stomach. We thus studied gastric antral biopsies in 25 affected children, in comparison to 10 with Crohn's disease, 10 with Helicobacter pylori infection, and 10 histologically normal controls. All autistic, Crohn's, and normal patients were H. pylori negative. METHODS: Snap-frozen antral biopsies were stained for CD3, CD4, CD8, gammadelta T cells, HLA-DR, IgG, heparan sulphate proteoglycan, IgM, IgA, and C1q. Cell proliferation was assessed with Ki67. RESULTS: Distinct patterns of gastritis were seen in the disease states: diffuse, predominantly CD4+ infiltration in H. pylori, and focal-enhanced gastritis in Crohn's disease and autism, the latter distinguished by striking dominance of CD8+ cells, together with increased intraepithelial lymphocytes in surface, foveolar and glandular epithelium. Proliferation of foveolar epithelium was similarly increased in autism, Crohn's disease and H. pylori compared to controls. A striking finding, seen only in 20/25 autistic children, was colocalized deposition of IgG and C1q on the subepithelial basement membrane and the surface epithelium. CONCLUSIONS: These findings

demonstrate a focal CD8-dominated gastritis in autistic children, with novel features. The lesion is distinct from the recently recognized focal gastritis of Crohn's disease, which is not CD8-dominated. As in the small intestine, there is epithelial deposition of IgG.

Torrente, F., P. Ashwood, et al. (2002). "Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism." Mol Psychiatry **7**(4): 375-82, 334.

We have reported lymphocytic colitis in children with regressive autism, with epithelial damage prominent. We now compare duodenal biopsies in 25 children with regressive autism to 11 with coeliac disease, five with cerebral palsy and mental retardation and 18 histologically normal controls. Immunohistochemistry was performed for lymphocyte and epithelial lineage and functional markers. We determined the density of intraepithelial and lamina propria lymphocyte populations, and studied mucosal immunoglobulin and complement C1q localisation. Standard histopathology showed increased enterocyte and Paneth cell numbers in the autistic children. Immunohistochemistry demonstrated increased lymphocyte infiltration in both epithelium and lamina propria with upregulated crypt cell proliferation, compared to normal and cerebral palsy controls. Intraepithelial lymphocytes and lamina propria plasma cells were lower than in coeliac disease, but lamina propria T cell populations were higher and crypt proliferation similar. Most strikingly, IgG deposition was seen on the basolateral epithelial surface in 23/25 autistic children, co-localising with complement C1q. This was not seen in the other conditions. These findings demonstrate a novel form of enteropathy in autistic children, in which increases in mucosal lymphocyte density and crypt cell proliferation occur with epithelial IgG deposition. The features are suggestive of an autoimmune lesion.

Uhlmann, V., C. M. Martin, et al. (2002). "Potential viral pathogenic mechanism for new variant inflammatory bowel disease." Mol Pathol **55**(2): 84-90.

AIMS: A new form of inflammatory bowel disease (ileocolonic lymphonodular hyperplasia) has been described in a cohort of children with developmental disorder. This study investigates the presence of persistent measles virus in the intestinal tissue of these patients (new variant inflammatory bowel disease) and a series of controls by molecular analysis. METHODS: Formalin fixed, paraffin wax embedded and fresh frozen biopsies from the terminal ileum were examined from affected children and histological normal controls. The measles virus Fusion (F) and Haemagglutinin (H) genes were detected by TaqMan reverse transcription polymerase chain reaction (RT-PCR) and the Nucleocapsid (N) gene by RT in situ PCR. Localisation of the mRNA signal was performed using a specific follicular dendritic cell antibody. RESULTS: Seventy five of 91 patients with a histologically confirmed diagnosis of ileal lymphonodular hyperplasia and enterocolitis were positive for measles virus in their intestinal tissue compared with five of 70 control patients. Measles virus was identified within the follicular

dendritic cells and some lymphocytes in foci of reactive follicular hyperplasia. The copy number of measles virus ranged from one to 300,00 copies/ng total RNA. CONCLUSIONS: The data confirm an association between the presence of measles virus and gut pathology in children with developmental disorder.

Valicenti-McDermott, M., K. McVicar, et al. (2006). "Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease." J Dev Behav Pediatr **27**(2 Suppl): S128-36.

This is a cross-sectional study that compares lifetime prevalence of gastrointestinal (GI) symptoms in children with autistic spectrum disorders (ASDs) and children with typical development and with other developmental disabilities (DDs) and examines the association of GI symptoms with a family history of autoimmune disease. A structured interview was performed in 50 children with ASD and 2 control groups matched for age, sex, and ethnicity-50 with typical development and 50 with other DDs. Seventy-four percent were boys with a mean age of 7.6 years (SD, +/-3.6). A history of GI symptoms was elicited in 70% of children with ASD compared with 28% of children with typical development ($p < .001$) and 42% of children with DD ($p = .03$). Abnormal stool pattern was more common in children with ASD (18%) than controls (typical development: 4%, $p = .039$; DD: 2%, $p = .021$). Food selectivity was also higher in children with ASD (60%) compared with those with typical development (22%, $p = .001$) and DD (36%, $p = .023$). Family history of autoimmune disease was reported in 38% of the ASD group and 34% of controls and was not associated with a differential rate of GI symptoms. In the multivariate analysis, autism (adjusted odds ratio (OR), 3.8; 95% confidence interval (CI), 1.7-11.2) and food selectivity (adjusted OR, 4.1; 95% CI, 1.8-9.1) were associated with GI symptoms. Children with ASD have a higher rate of GI symptoms than children with either typical development or other DDs. In this study, there was no association between a family history of autoimmune disease and GI symptoms in children with ASD.

Van Heest, R., S. Jones, et al. (2004). "Rectal prolapse in autistic children." J Pediatr Surg **39**(4): 643-4.

Rectal prolapse in children is not uncommon, but surgery is rarely indicated. In mentally challenged adults and children, rectal prolapse occurs more frequently than in the general population and often requires surgical intervention in the second to third decade of life. The authors describe 3 children with autism and mental retardation who presented with rectal prolapse at an earlier age than would be anticipated with mental retardation alone. All 3 children required surgical intervention.

Vojdani, A., A. W. Campbell, et al. (2002). "Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk,

Chlamydia pneumoniae and Streptococcus group A." J Neuroimmunol **129**(1-2): 168-77.

We measured autoantibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of preexisting T-cells and central nervous system antigens to immunocompetent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.

Vojdani, A., T. O'Bryan, et al. (2004). "Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism." Nutr Neurosci **7**(3): 151-61.

The mechanisms behind autoimmune reaction to nervous system antigens in autism are not understood. We assessed the reactivity of sera from 50 autism patients and 50 healthy controls to specific peptides from gliadin and the cerebellum. A significant percentage of autism patients showed elevations in antibodies against gliadin and cerebellar peptides simultaneously. For examining cross-reaction between dietary proteins and cerebellar antigens, antibodies were prepared in rabbits, and binding of rabbit anti-gliadin, anti-cerebellar peptides, anti-MBP, anti-milk, anti-egg, anti-soy and anti-corn to either gliadin- or cerebellar-antigen-coated wells was measured. In comparison to anti-gliadin peptide binding to gliadin peptide at 100%, the reaction of anti-cerebellar peptide to gliadin peptide was 22%, whereas the binding of anti-myelin basic protein (MBP), anti-milk, anti-egg and anti-soy to gliadin was less than 10%. Further examination of rabbit anti-gliadin (EQVPLVQQ) and anti-cerebellar (EDVPLLED) 8 amino acid (AA) peptides with human serum albumin (HSA) and an unrelated peptide showed no binding, but the reaction of these antibodies with both the cerebellar and gliadin peptides was greater than 60%. This cross-reaction was further confirmed by DOT-immunoblot and inhibition studies. We conclude that a subgroup of patients with autism produce antibodies against Purkinje cells and gliadin peptides, which may be responsible for some of the neurological symptoms in autism.

Vojdani, A., J. B. Pangborn, et al. (2003). "Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism." Int J Immunopathol Pharmacol **16**(3): 189-99.

Similar to many complex autoimmune diseases, genetic and environmental factors including diet, infection and xenobiotics play a critical role in the development of autism. In this study, we postulated that infectious agent antigens such as streptokinase, dietary peptides (gliadin and casein) and ethyl mercury (xenobiotic) bind to different lymphocyte receptors and tissue enzyme (DPP IV or CD26). We assessed this hypothesis first by measuring IgG, IgM and IgA antibodies against CD26, CD69, streptokinase (SK), gliadin and casein peptides and against ethyl mercury bound to human serum albumin in patients with autism. A significant percentage of children with autism developed anti-SK, anti-gliadin and casein peptides and anti-ethyl mercury antibodies, concomitant with the appearance of anti-CD26 and anti-CD69 autoantibodies. These antibodies are synthesized as a result of SK, gliadin, casein and ethyl mercury binding to CD26 and CD69, indicating that they are specific. Immune absorption demonstrated that only specific antigens, like CD26, were capable of significantly reducing serum anti-CD26 levels. However, for direct demonstration of SK, gliadin, casein and ethyl mercury to CD26 or CD69, microtiter wells were coated with CD26 or CD69 alone or in combination with SK, gliadin, casein or ethyl mercury and then reacted with enzyme labeled rabbit anti-CD26 or anti-CD69. Adding these molecules to CD26 or CD69 resulted in 28-86% inhibition of CD26 or CD69 binding to anti-CD26 or anti-CD69 antibodies. The highest % binding of these antigens or peptides to CD26 or CD69 was attributed to SK and the lowest to casein peptides. We, therefore, propose that bacterial antigens (SK), dietary peptides (gliadin, casein) and Thimerosal (ethyl mercury) in individuals with pre-disposing HLA molecules, bind to CD26 or CD69 and induce antibodies against these molecules. In conclusion, this study is apparently the first to demonstrate that dietary peptides, bacterial toxins and xenobiotics bind to lymphocyte receptors and/or tissue enzymes, resulting in autoimmune reaction in children with autism.

Wakefield, A., C. Stott, et al. (2006). "Gastrointestinal comorbidity, autistic regression and Measles-containing vaccines: positive re-challenge and biological gradient." Medical Veritas **3**: 796-802.

Background: A temporal association between exposure to measles-containing vaccine (MCV) and autistic-like developmental regression in a sub-set of children with enterocolitis has been reported. Measles virus (MV) was detected in ileal biopsies from these children at higher prevalence than in developmentally normal pediatric controls. This study tested the hypothesis of a dose-response effect of MCV exposure on intestinal pathology, as evidence of a causal association. Methodology/Principle Findings: Children with normal early development and autistic-like developmental regression were divided into two groups: re-exposed children (n=23), who had received more than one dose of a measles-containing

vaccine (MCV), and once-exposed children (n=23), who had received only one dose of MCV. The groups were matched for sex, age, and time-elapsed from first exposure to endoscopy. Com-parisons included: secondary (2o) gastrointestinal (GI) and related physical symptoms and observer-blinded scores of endoscopic and histological disease. Re-exposed children scored significantly higher than once-exposed for 2o physical symptoms including incontinence, presence of severe ileal lymphoid hyperplasia, number of biopsies with epithelial damage and number of children with acute inflammation. Markers of acute inflammation included number of children affected and proportion of biopsies affected. Conclusion/Significance: The data identify a re-challenge effect on symptoms and a biological gradient effect on intestinal pathology, which links MCV exposure to autistic-like developmental regression and enterocolitis.

Wakefield, A. J. (2002). "Enterocolitis, autism and measles virus." Mol Psychiatry **7 Suppl 2**: S44-6.

Wakefield, A. J. (2002). "The gut-brain axis in childhood developmental disorders." J Pediatr Gastroenterol Nutr **34 Suppl 1**: S14-7.

Wakefield, A. J., A. Anthony, et al. (2000). "Enterocolitis in children with developmental disorders." Am J Gastroenterol **95**(9): 2285-95.

OBJECTIVE: Intestinal pathology, i.e., ileocolonic lymphoid nodular hyperplasia (LNH) and mucosal inflammation, has been described in children with developmental disorders. This study describes some of the endoscopic and pathological characteristics in a group of children with developmental disorders (affected children) that are associated with behavioral regression and bowel symptoms, and compares them with pediatric controls. METHODS: Ileocolonoscopy and biopsy were performed on 60 affected children (median age 6 yr, range 3-16; 53 male). Developmental diagnoses were autism (50 patients), Asperger's syndrome (five), disintegrative disorder (two), attention deficit hyperactivity disorder (ADHD) (one), schizophrenia (one), and dyslexia (one). Severity of ileal LNH was graded (0-3) in both affected children and 37 developmentally normal controls (median age 11 yr, range 2-13 yr) who were investigated for possible inflammatory bowel disease (IBD). Tissue sections were reviewed by three pathologists and scored on a standard proforma. Data were compared with ileocolonic biopsies from 22 histologically normal children (controls) and 20 children with ulcerative colitis (UC), scored in an identical manner. Gut pathogens were sought routinely. RESULTS: Ileal LNH was present in 54 of 58 (93%) affected children and in five of 35 (14.3%) controls ($p < 0.001$). Colonic LNH was present in 18 of 60 (30%) affected children and in two of 37 (5.4%) controls ($p < 0.01$). Histologically, reactive follicular hyperplasia was present in 46 of 52 (88.5%) ileal biopsies from affected children and in four of 14 (29%) with UC, but not in non-IBD controls ($p < 0.01$). Active ileitis was present in four of 51 (8%) affected children but not in controls. Chronic colitis

was identified in 53 of 60 (88%) affected children compared with one of 22 (4.5%) controls and in 20 of 20 (100%) with UC. Scores of frequency and severity of inflammation were significantly greater in both affected children and those with UC, compared with controls ($p < 0.001$). CONCLUSIONS: A new variant of inflammatory bowel disease is present in this group of children with developmental disorders.

Wakefield, A. J., P. Ashwood, et al. (2005). "The significance of ileo-colonic lymphoid nodular hyperplasia in children with autistic spectrum disorder." Eur J Gastroenterol Hepatol **17**(8): 827-36.

BACKGROUND: Intestinal mucosal pathology, characterized by ileo-colonic lymphoid nodular hyperplasia (LNH) and mild acute and chronic inflammation of the colorectum, small bowel and stomach, has been reported in children with autistic spectrum disorder (ASD). AIM: To assess ileo-colonic LNH in ASD and control children and to test the hypothesis that there is an association between ileo-colonic LNH and ASD in children. PATIENTS AND METHODS: One hundred and forty-eight consecutive children with ASD (median age 6 years; range 2-16; 127 male) with gastrointestinal symptoms were investigated by ileo-colonoscopy. Macroscopic and histological features were scored and compared with 30 developmentally normal (non-inflammatory bowel disease, non-coeliac disease) controls (median age 7 years; range 1-11; 25 male) showing mild non-specific colitis in 16 cases (13 male) and normal colonic histology in 14 cases (12 male). Seventy-four ASD children and 23 controls also underwent upper gastrointestinal endoscopy. The influence on ileal LNH of dietary restriction, age at colonoscopy, and co-existent LNH elsewhere in the intestine, was examined. RESULTS: The prevalence of LNH was significantly greater in ASD children compared with controls in the ileum (129/144 (90%) vs. 8/27 (30%), $P < 0.0001$) and colon (88/148 (59%) vs. 7/30 (23%), $P = 0.0003$), whether or not controls had co-existent colonic inflammation. The severity of ileal LNH was significantly greater in ASD children compared with controls, with moderate to severe ileal LNH present in 98 of 144 (68%) ASD children versus 4 of 27 (15%) controls ($P < 0.0001$). Severe ileal LNH was associated with co-existent colonic LNH in ASD children ($P = 0.01$). The presence and severity of ileal LNH was not influenced by either diet or age at colonoscopy ($P = 0.2$). Isolated ileal LNH without evidence of pathology elsewhere in the intestine was a rare event, occurring in less than 3% of children overall. On histopathological examination, hyperplastic lymphoid follicles are significantly more prevalent in the ileum of ASD children (84/138; 61%) compared with controls (2/23; 9%, $P = 0.0001$). CONCLUSION: Ileo-colonic LNH is a characteristic pathological finding in children with ASD and gastrointestinal symptoms, and is associated with mucosal inflammation. Differences in age at colonoscopy and diet do not account for these changes. The data support the hypothesis that LNH is a significant pathological finding in ASD children.

Wakefield, A. J., S. H. Murch, et al. (1998). "Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children." Lancet **351**(9103): 637-41.

BACKGROUND: We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder. METHODS: 12 children (mean age 6 years [range 3-10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined. FINDINGS: Onset of behavioural symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children, with measles infection in one child, and otitis media in another. All 12 children had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. Histology showed patchy chronic inflammation in the colon in 11 children and reactive ileal lymphoid hyperplasia in seven, but no granulomas. Behavioural disorders included autism (nine), disintegrative psychosis (one), and possible postviral or vaccinal encephalitis (two). There were no focal neurological abnormalities and MRI and EEG tests were normal. Abnormal laboratory results were significantly raised urinary methylmalonic acid compared with age-matched controls (p=0.003), low haemoglobin in four children, and a low serum IgA in four children. INTERPRETATION: We identified associated gastrointestinal disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers.

Wakefield, A. J., J. M. Puleston, et al. (2002). "Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands." Aliment Pharmacol Ther **16**(4): 663-74.

There is growing awareness that primary gastrointestinal pathology may play an important role in the inception and clinical expression of some childhood developmental disorders, including autism. In addition to frequent gastrointestinal symptoms, children with autism often manifest complex biochemical and immunological abnormalities. The gut-brain axis is central to certain encephalopathies of extra-cranial origin, hepatic encephalopathy being the best characterized. Commonalities in the clinical characteristics of hepatic encephalopathy and a form of autism associated with developmental regression in an apparently previously normal child, accompanied by immune-mediated gastrointestinal pathology, have led to the proposal that there may be analogous mechanisms of toxic encephalopathy in patients with liver failure and some children with autism. Aberrations in opioid biochemistry are common to these

two conditions, and there is evidence that opioid peptides may mediate certain aspects of the respective syndromes. The generation of plausible and testable hypotheses in this area may help to identify new treatment options in encephalopathies of extra-cranial origin. Therapeutic targets for this autistic phenotype may include: modification of diet and entero-colonic microbial milieu in order to reduce toxin substrates, improve nutritional status and modify mucosal immunity; anti-inflammatory/immunomodulatory therapy; and specific treatment of dysmotility, focusing, for example, on the pharmacology of local opioid activity in the gut.

White, J. F. (2003). "Intestinal pathophysiology in autism." Exp Biol Med (Maywood) **228**(6): 639-49.

Autism is a life-long developmental disorder affecting as many as 1 in 500 children. The causes for this profound disorder are largely unknown. Recent research has uncovered pathology in the gastrointestinal tract of autistic children. The pathology, reported to extend from the esophagus to the colon, is described here along with other studies pointing to a connection between diet and the severity of symptoms expressed in autism. The evidence that there is impaired intestinal permeability in autism is reviewed, and various theories are discussed by which a leaky gut could develop. Lastly, some possible ways in which impaired gastrointestinal function might influence brain function are discussed.

Krigsman A, Boris M, Goldblatt A. Frequency of histologic enterocolitis and lymphonodular hyperplasia in autistic children presenting for ileocolonoscopy. IMFAR. May 7th, 2004.

Kuddo T, Nelson KB. How common are gastrointestinal disorders in children with autism. Curr Opin Pediatr 2003: 15(3); 339-343.

Levy S, Souders MC, Wray J, Jawad AF, Gallagher PR, Coplan J, Belchic JK, Gerdes M, Mitchell R, Mulberg AE. Children with autistic spectrum disorders. I: Comparison of placebo and single dose of human synthetic secretin. Arch. Dis. Child. 2003;88;731-736.

Melmed RD, Schneider CK, Fabes RA. Metabolic markers and gastrointestinal symptoms in children with autism and related disorders. J Pediatr Gastroenterol Nutr 2000:31(suppl 2)S31-32.

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3 - Autism and Neuroinflammation (42 citations):

Ahlsen, G., L. Rosengren, et al. (1993). "Glial fibrillary acidic protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders." Biol Psychiatry **33**(10): 734-43.

The cerebrospinal fluid (CSF) of 47 children and adolescents with autism was analyzed for the contents of two astroglial proteins, the glial fibrillary acidic protein (GFA) and S 100. The results were contrasted with those obtained in similarly aged cases with other neuropsychiatric disorders (n = 25) and in normal children (n = 10). S-100 did not discriminate the groups from each other. However, GFA in autism and autistic-like conditions was at a level almost three times that in the normal group. The results could implicate gliosis and unspecific brain damage in autism. An alternative model would be increased synapse turnover regardless of underlying cause.

Bradstreet, J. J., S. Smith, et al. (2007). "Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders." Med Hypotheses **68**(5): 979-87.

Multiple studies now demonstrate that autism is medically characterized, in part, by immune system dysregulation, including evidence of neuroglial activation and gastrointestinal inflammation. This neuroglial process has further been characterized as neuroinflammation. In addition, a subset of autistic children exhibit higher than average levels of androgens. Spironolactone is an aldosterone antagonist and potassium-sparing diuretic with a desirable safety profile. It possesses potent anti-inflammatory and immune modifying properties that might make it an excellent medical intervention for autism spectrum disorders. Furthermore, spironolactone demonstrates substantial anti-androgen properties that might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic autistic children. One case report is briefly reviewed demonstrating objective clinical improvements in an autistic child after spironolactone administration. Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

Braunschweig, D., P. Ashwood, et al. (2007). "Autism: Maternally derived antibodies specific for fetal brain proteins." Neurotoxicology.

Autism is a profound disorder of neurodevelopment with poorly understood biological origins. A potential role for maternal autoantibodies in the etiology of some cases of autism has been proposed in previous studies. To investigate this hypothesis, maternal plasma antibodies against human fetal and adult brain proteins were analyzed by western blot in 61 mothers of children with autistic disorder and 102 controls matched for maternal age and birth year (62 mothers of typically developing children (TD) and 40 mothers of children with non-ASD developmental delays (DD)). We observed reactivity to two protein bands at

approximately 73 and 37kDa in plasma from 7 of 61 (11.5%) mothers of children with autism (AU) against fetal but not adult brain, which was not noted in either control group (TD; 0/62 p=0.0061 and DD; 0/40 p=0.0401). Further, the presence of reactivity to these two bands was associated with parent report of behavioral regression in AU children when compared to the TD (p=0.0019) and DD (0.0089) groups. Individual reactivity to the 37kDa band was observed significantly more often in the AU population compared with TD (p=0.0086) and DD (p=0.002) mothers, yielding a 5.69-fold odds ratio (95% confidence interval 2.09-15.51) associated with this band. The presence of these antibodies in the plasma of some mothers of children with autism, as well as the differential findings between mothers of children with early onset and regressive autism may suggest an association between the transfer of IgG autoantibodies during early neurodevelopment and the risk of developing of autism in some children.

Chez, M. G., T. Dowling, et al. (2007). "Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children." Pediatr Neurol **36**(6): 361-5.

Recent reports implicating elevated cytokines in the central nervous system in a small number of patients studied with autism have reported clinical regression. These studies have not focused on tumor necrosis factor-alpha as a possible marker for inflammatory damage. A series of 10 children with autism had clinical evaluation of their serum and spinal fluid for inflammatory changes and possible metabolic disease as part of their neurological evaluation. Elevation of cerebrospinal fluid levels of tumor necrosis factor-alpha was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in all of the patients studied. The ratio of the cerebrospinal fluid levels to serum levels averaged 53.7:1. This ratio is significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum tumor necrosis factor-alpha levels have been simultaneously measured. This observation may offer a unique insight into central nervous system inflammatory mechanisms that may contribute to the onset of autism and may serve as a potential clinical marker. More controlled study of this potentially important observation may prove valuable.

Connolly, A. M., M. Chez, et al. (2006). "Brain-derived neurotrophic factor and autoantibodies to neural antigens in sera of children with autistic spectrum disorders, Landau-Kleffner syndrome, and epilepsy." Biol Psychiatry **59**(4): 354-63.

BACKGROUND: Brain derived neurotrophic factor (BDNF) elevation in newborn sera predicts intellectual/social developmental abnormalities. Other autoantibodies (AAs) to endothelial cells (ECs) and myelin basic protein (MBP) are also elevated in some children. We tested relationships between BDNF, BDNF AAs, and other AAs in children with these disorders. METHODS: BDNF levels and IgG/IgM autoantibodies to BDNF, ECs, MBP, and histones were measured in children with autism, childhood disintegrative disorder (CDD), pervasive developmental delay-not otherwise specified (PDD-nos), acquired epilepsy,

Landau-Kleffner syndrome (LKS); healthy children (HC), and children with non-neurological illnesses (NNI). RESULTS: Mean BDNF levels were elevated in children with autism and CDD, ($p <$ or $= 0.0002$) compared to HC or NNI. Mean IgG and IgM BDNF AAs were elevated in children with autism, CDD and epilepsy ($p <$ or $= 0.0005$) compared to HC but not to NNI. Mean IgM AA EC titers detected by immunocytochemistry were higher in autism, PDD-NOS, epilepsy, and LKS ($p <$ or $= 0.005$) compared to HC and NNI. While mean ELISA IgG EC AAs were higher in autism and PPD-NOS ($p < 0.005$) compared to HC but not NNI, ELISA IgM EC AAs were higher in children with autism, CDD, PDD-NOS, and epilepsy compared to both HC and NNI ($p < 0.0005$). Mean anti-MBP IgG and IgM titers were higher in all study groups ($p < 0.005$) except for LKS compared to both HC and NNI. CONCLUSION: Children with developmental disorders and epilepsy have higher AAs to several neural antigens compared to controls. The presence of both BDNF AAs and elevated BDNF levels in some children with autism and CDD suggests a previously unrecognized interaction between the immune system and BDNF.

Connolly, A. M., M. G. Chez, et al. (1999). "Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders." J Pediatr **134**(5): 607-13.

OBJECTIVE: Etiologically unexplained disorders of language and social development have often been reported to improve in patients treated with immune-modulating regimens. Here we determined the frequency of autoantibodies to brain among such children. DESIGN: We collected sera from a cohort of children with (1) pure Landau-Kleffner syndrome ($n = 2$), (2) Landau-Kleffner syndrome variant (LKSV, $n = 11$), and (3) autistic spectrum disorder (ASD, $n = 11$). None had received immune-modulating treatment before the serum sample was obtained. Control sera ($n = 71$) were from 29 healthy children, 22 with non-neurologic illnesses (NNIs), and 20 children with other neurologic disorders (ONDs). We identified brain autoantibodies by immunostaining of human temporal cortex and antinuclear autoantibodies using commercially available kits. RESULTS: IgG anti-brain autoantibodies were present in 45% of sera from children with LKSV, 27% with ASD, and 10% with ONDs compared with 2% from healthy children and control children with NNIs. IgM autoantibodies were present in 36% of sera from children with ASD, 9% with LKSV, and 15% with ONDs compared with 0% of control sera. Labeling studies identified one antigenic target to be endothelial cells. Antinuclear antibodies with titers >/=1:80 were more common in children with ASD and control children with ONDs. CONCLUSION: Children with LKSV and ASD have a greater frequency of serum antibodies to brain endothelial cells and to nuclei than children with NNIs or healthy children. The presence of these antibodies raises the possibility that autoimmunity plays a role in the pathogenesis of language and social developmental abnormalities in a subset of children with these disorders.

Cook, E. H., Jr., B. D. Perry, et al. (1993). "Receptor inhibition by immunoglobulins: specific inhibition by autistic children, their relatives, and control subjects." J Autism Dev Disord **23**(1): 67-78.

Forty-two parents of children with autistic disorder, 15 children with autistic disorder, 17 siblings of children with autistic disorder, and 12 unrelated normal adult controls were studied to determine if immunoglobulins isolated from their plasma would inhibit binding of the 5HT1A agonist, [3H]-8-hydroxy-N,N-dipropyl-2-aminotetralin (DPAT) to 5HT1A receptors in human hippocampal membranes. There were no significant differences among the means of percentage inhibition of DPAT binding of parents, children with autistic disorder, siblings, or unrelated controls. In addition, there were no differences in the proportion of subjects with > 15% DPAT inhibition among autistic children, their parents, their siblings, or unrelated controls. Immunoglobulin inhibition was not specific for the 5HT1A receptor binding site, since immunoglobulins inhibited binding to 5HT2, D1, D2, and alpha 2-adrenergic binding sites. The immunoglobulins isolated from normal controls inhibited [3H]-rauwolscine binding at alpha 2-adrenergic sites less than immunoglobulins of children with autistic disorder and their parents and siblings. This study did not support the hypothesis that autoantibodies to 5HT1A or 5HT2 receptors are characteristic of autistic disorder.

Cooper, E. L. (2003). "Neuroimmunology of autism: a multifaceted hypothesis." Int J Immunopathol Pharmacol **16**(3): 289-92.

Cortesi, M., E. Alfei, et al. (2007). "Linking autism, regression and Landau-Kleffner syndrome: integrative role of nerve growth factor." Med Hypotheses **68**(5): 1178-9.

Dalton, P., R. Deacon, et al. (2003). "Maternal neuronal antibodies associated with autism and a language disorder." Ann Neurol **53**(4): 533-7.

Neurodevelopmental disorders could be caused by maternal antibodies or other serum factors. We detected serum antibodies binding to rodent Purkinje cells and other neurons in a mother of three children: the first normal, the second with autism, and the third with a severe specific language disorder. We injected the serum (0.5-1.0 ml/day) into pregnant mice during gestation and found altered exploration and motor coordination and changes in cerebellar magnetic resonance spectroscopy in the mouse offspring, comparing with offspring of mice injected with sera from mothers of healthy children. This evidence supports a role for maternal antibodies in some forms of neurodevelopmental disorder.

Kern, J. K. (2003). "Purkinje cell vulnerability and autism: a possible etiological connection." Brain Dev **25**(6): 377-82.

Autism is a neurological disorder of unknown etiology. The onset of the abnormal growth and development within the brain is also not known. Current thought by experts in autism is that the time of onset is prenatal, occurring prior to 30 weeks gestation. However, autism comprises a heterogeneous population in that

parents report either that their child was abnormal from birth, or that their child was developmentally normal until sometime after birth, at which time the child began to regress or deteriorate. Anecdotal reports suggest that some children with autism have significant illness or clinical events prior to the development of autistic symptoms. Conceivably, these children may become autistic from neuronal cell death or brain damage sometime after birth as result of insult. To support this theory is that marked Purkinje cell loss, the most consistent finding in the autistic disorder, can result from insult. Evidence suggests that the Purkinje cell is selectively vulnerable. This article discusses a theory that the selective vulnerability of the Purkinje cell may play a role in the etiology of autism, and suggests that a future direction in autism research may be to investigate the possibility of neuronal cell loss from insult as a cause of autism. Results of a small pilot survey are also discussed.

Kirkman, N. J., J. E. Libbey, et al. (2008). "How Relevant are GFAP Autoantibodies in Autism and Tourette Syndrome?" J Autism Dev Disord **38**(2): 333-41.

Controversy exists over the role of autoantibodies to central nervous system antigens in autism and Tourette Syndrome. We investigated plasma autoantibody titers to glial fibrillary acidic protein (GFAP) in children with classic onset (33) and regressive onset (26) autism, controls (25, healthy age- and gender-matched) and individuals with Tourette Syndrome (24) by enzyme-linked immunosorbent assays. We found a significant difference in autoantibody titers to GFAP, not accounted for by age, between the Tourette (significantly lower) and regressive autism groups. However, no differences were found between: classic/regressive; classic/controls; classic/Tourette; regressive/controls; or controls/Tourette. Autoantibody responses against GFAP are unlikely to play a pathogenic role in autism or Tourette Syndrome.

Kozlovskaia, G. V., T. P. Kliushnik, et al. (2000). "[Nerve growth factor auto-antibodies in children with various forms of mental dysontogenesis and in schizophrenia high risk group]." Zh Nevrol Psikhiatr Im S S Korsakova **100**(3): 50-2.

The level of autoantibodies (AAb) to nerve growth factor was evaluated in blood serum of 163 children with different forms of mental dysontogenesis of endogenic, residual-organic, psychogenic and deprivative origin. Significant elevation of the level of AAb was found in all forms of psychic dysontogenesis. The most significant elevation of the level of AAb ($p < 0.01$), as compared with the controls (45 children), was characteristic for endogenic forms of dysontogenesis (schizophrenia, early children's autism, schizotypic diathesis). The level of AAb was also found as an indicator of the acuteness of the pathologic state. Besides, its elevation was observed 1-2 weeks prior to the onset of the clinical exacerbation. Elevation of AAb level was also found in psychic dysontogenesis of residual-organic nature (children with perinatal encephalopathy), but it was not so significant as compared with the controls ($p < 0.05\%$). The analysis in the age dynamics of children from this group

revealed, that AAb level may serve as some prognostic index of the severity of psychic dysontogenesis. The level of AAb differs some states in schizotypic diathesis and deprivative dysontogenesis, which are clinically quite similar. The method for the estimation of serum AAb level may be proposed as screening in prophylactic medical examination of children from the first year of life under conditions of pediatric outpatient service for identification of risk-groups by psychic dysontogenesis to perform early special psychoprophylaxis.

Libbey, J. E., H. H. Coon, et al. (2008). "Are There Enhanced MBP Autoantibodies in Autism?" J Autism Dev Disord **38**(2): 324-32.

Autoantibodies to central nervous system antigens, such as myelin basic protein (MBP), may play a role in autism. We measured autoantibody titers to MBP in children with autism, both classic onset and regressive onset forms, controls (healthy age- and gender-matched) and individuals with Tourette syndrome via enzyme-linked immunosorbent assays. We found a significant difference in autoantibody titers to MBP, not accounted for by age or medication, between Tourette and classic autism (both significantly lower) when compared to regressive autism, but not when compared to controls. Autoantibody responses against MBP are unlikely to play a pathogenic role in autism.

MacFabe, D. F., D. P. Cain, et al. (2007). "Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders." Behav Brain Res **176**(1): 149-69.

Clinical observations suggest that certain gut and dietary factors may transiently worsen symptoms in autism spectrum disorders (ASD), epilepsy and some inheritable metabolic disorders. Propionic acid (PPA) is a short chain fatty acid and an important intermediate of cellular metabolism. PPA is also a by-product of a subpopulation of human gut enterobacteria and is a common food preservative. We examined the behavioural, electrophysiological, neuropathological, and biochemical effects of treatment with PPA and related compounds in adult rats. Intraventricular infusions of PPA produced reversible repetitive dystonic behaviours, hyperactivity, turning behaviour, retropulsion, caudate spiking, and the progressive development of limbic kindled seizures, suggesting that this compound has central effects. Biochemical analyses of brain homogenates from PPA treated rats showed an increase in oxidative stress markers (e.g., lipid peroxidation and protein carbonylation) and glutathione S-transferase activity coupled with a decrease in glutathione and glutathione peroxidase activity. Neurohistological examinations of hippocampus and adjacent white matter (external capsule) of PPA treated rats revealed increased reactive astrogliosis (GFAP immunoreactivity) and activated microglia (CD68 immunoreactivity) suggestive of a neuroinflammatory process. This was coupled with a lack of cytotoxicity (cell counts, cleaved caspase 3' immunoreactivity), and an increase in phosphorylated CREB immunoreactivity. We propose that some types of autism may be partial forms of genetically inherited or acquired

disorders involving altered PPA metabolism. Thus, intraventricular administration of PPA in rats may provide a means to model some aspects of human ASD in rats.

MacFabe, D. F., K. Rodríguez-Capote, et al. (2008). "A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and oxidative stress in discrete regions of adult rat brain " American Journal of Biochemistry and Biotechnology **4**(2): 146-166.

Innate neuroinflammatory changes, increased oxidative stress and disorders of glutathione metabolism may be involved in the pathophysiology of autism spectrum disorders (ASD). Propionic acid (PPA) is a dietary and gut bacterial short chain fatty acid which can produce brain and behavioral changes reminiscent of ASD following intraventricular infusion in rats. Adult Long-Evans rats were given intraventricular infusions of either PPA (500ug uL-1, 4µl animal-1) or phosphate buffered saline (PBS) vehicle, twice daily for 7 days. Immediately following the second daily infusion, the locomotor activity of each rat was assessed in an automated open field (Versamax) for 30 min. PPA-treated rats showed significant increases in locomotor activity compared to PBS vehicle controls. Following the last treatment day, specific brain regions were assessed for neuroinflammatory or oxidative stress markers. Immunohistochemical analyses revealed reactive astrogliosis (GFAP), activated microglia (CD68, Iba1) without apoptotic cell loss (Caspase 3 and NeuN) in hippocampus and white matter (external capsule) of PPA treated rats. Biomarkers of protein and lipid peroxidation, total glutathione (GSH) as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) were examined in brain homogenates. Some brain regions of PPA treated animals (neocortex, hippocampus, thalamus, striatum) showed increased lipid and protein oxidation accompanied by decreased total GSH in neocortex. Catalase activity was decreased in most brain regions of PPA treated animals suggestive of reduced antioxidant enzymatic activity. GPx and GR activity was relatively unaffected by PPA treatment while GST was increased perhaps indicating involvement of GSH in the removal of PPA or related catabolites. Impairments in GSH and catalase levels may render CNS cells more susceptible to oxidative stress from a variety of toxic insults. Overall, these findings are consistent with those found in ASD patients and further support intraventricular PPA administration as an animal model of ASD.

Martinez Bermejo, A., I. Pascual Castroviejo, et al. (1989). "[Acquired aphasia syndrome with epilepsy (Landau-Kleffner syndrome) secondary to cerebral arteritis. 4 cases]." Neurologia **4**(8): 296-9.

We report four cases of acquired aphasia with epilepsy syndrome or Landau-Kleffner syndrome in three boys and one girl who were previously in good health. Along with the clinical picture, we describe cerebral arteriographic

findings, which consisted of obstructive arteriopathy of small and medium size vessels in delimited areas. A specific cause of the arteriopathy was not found in any case. We discuss the treatment (corticosteroids and calcium antagonists) and the course of the patients. In front of the possibility of being isolated cases, we discuss the interest of performing cerebral angiographic studies in patients with Landau-Kleffner syndrome in initial stage in order to confirm the consistence of these findings and the adequate treatment.

Mazur-Kolecka, B., I. L. Cohen, et al. (2007). "Altered development of neuronal progenitor cells after stimulation with autistic blood sera." Brain Res **1168**: 11-20.

Changes of brain structure and functions in people with autism may result from altered neuronal development, however, no adequate cellular or animal models are available to study neurogenesis in autism. Neuronal development can be modeled in culture of neuronal progenitor cells (NPCs) stimulated with serum to differentiate into neurons. Because sera from people with autism and age-matched controls contain different levels of numerous biologically active factors, we hypothesized that development of human NPCs induced to differentiate into neurons with sera from children with autism reflects the altered early neuronal development that leads to autism. The control and autistic sera were collected from siblings aged below 6 years that lived in the same environment. The effect of sera on differentiation of NPC neurospheres into neuronal colonies was tested in 72-h-long cultures by morphometry, immunocytochemistry and immunoblotting. We found that sera from children with autism significantly reduced NPCs' proliferation, but stimulated cell migration, development of small neurons with processes, length of processes and synaptogenesis. These results suggest that development of network of processes and synaptogenesis--the specific events in the brain during postnatal ontogenesis--are altered in autism. Further studies in this cell culture model may explain some of the cellular alterations described in autistic patients.

Ni, L., G. Acevedo, et al. (2007). "Toll-like receptor ligands and CD154 stimulate microglia to produce a factor(s) that promotes excess cholinergic differentiation in the developing rat basal forebrain: implications for neurodevelopmental disorders." Pediatr Res **61**(1): 15-20.

Maternal inflammation plays a role in the etiology of certain neurodevelopmental disorders including autism and schizophrenia. Because maternal inflammation can lead to activation of fetal microglia, we have examined effects of inflamed microglia on cultured neural progenitors from rat embryonic septal region and basal forebrain. These cells give rise to cholinergic neurons projecting to cortex and hippocampus. Microglia stimulated with lipopolysaccharide (LPS), peptidoglycan, Poly I:C and CD154 produce conditioned media (CM) that promotes excessive numbers of cholinergic neurons and levels of choline acetyltransferase (ChAT) activity 6-8 times that of untreated cultures. Expression of the neural-specific transcription factor MATH1 increases substantially within 1

h of plating in LPS-CM. Untreated cultures do not attain equivalent levels until 6 h. By contrast, expression of glial-related transcription factors in LPS-CM-treated cultures never attains the elevated levels of untreated cultures. LPS-CM-treated clones derived from individual progenitors labeled with a LacZ-expressing retrovirus showed >2.5-fold increase in the percentage of cholinergic cells compared with untreated clones. Thus, CM from activated microglia prompts excess cholinergic differentiation from undifferentiated progenitors suggesting that microglial inflammation during critical stages can lead to aberrant brain development.

Pardo, C. A., D. L. Vargas, et al. (2005). "Immunity, neuroglia and neuroinflammation in autism." Int Rev Psychiatry **17**(6): 485-95.

Autism is a complex neurodevelopmental disorder of early onset that is highly variable in its clinical presentation. Although the causes of autism in most patients remain unknown, several lines of research support the view that both genetic and environmental factors influence the development of abnormal cortical circuitry that underlies autistic cognitive processes and behaviors. The role of the immune system in the development of autism is controversial. Several studies showing peripheral immune abnormalities support immune hypotheses, however until recently there have been no immune findings in the CNS. We recently demonstrated the presence of neuroglial and innate neuroimmune system activation in brain tissue and cerebrospinal fluid of patients with autism, findings that support the view that neuroimmune abnormalities occur in the brain of autistic patients and may contribute to the diversity of the autistic phenotypes. The role of neuroglial activation and neuroinflammation are still uncertain but could be critical in maintaining, if not also in initiating, some of the CNS abnormalities present in autism. A better understanding of the role of neuroinflammation in the pathogenesis of autism may have important clinical and therapeutic implications.

Pascual-Castroviejo, I., V. Lopez Martin, et al. (1992). "Is cerebral arteritis the cause of the Landau-Kleffner syndrome? Four cases in childhood with angiographic study." Can J Neurol Sci **19**(1): 46-52.

Four children with Landau-Kleffner syndrome were studied over a six year period. They presented with acquired aphasia, epilepsy, and focal or generalized EEG discharges which were exacerbated during sleep. In addition, cerebral angiography demonstrated isolated arteritis of some branches of the carotid arteries in all cases. Computed tomographic and magnetic resonance images were normal. Nicardipine in a dose of 1 to 2 mg/kg/day, added to conventional anticonvulsant drugs provided effective supplementary control of seizures, of paroxysmal EEG discharges, and of language and behavioural disturbances, even several years after the onset of the disorder and in patients whose response to other medications, including steroids, had been poor. Interruption of nicardipine administration was followed by relapse of the language disorder. Repeat

angiography was performed in all four patients and showed recanalization of obstructed vessels in two cases. Focal cerebral vasculitis may be the pathogenesis of the Landau-Kleffner syndrome and calcium channel blockers such as nicardipine may be effective and specific therapy.

Pascual-Castroviejo, I. and S. I. Pascual Pascual (2000). "[Cerebral arteritis and psychic involution in children. A report of one case with a good response to treatment]." Rev Neurol **31**(4): 311-3.

OBJECTIVE: To show the importance of the cerebral arteritis as etiology of the language and the intellectual involution in children. CLINICAL CASE: A boy started to show psychic and language involution since 18 months of life to arrive to an autistic behavior. After showing normal results in all the studies performed in order to investigate the possible etiologies, cerebral arteriography was performed. Cerebral arteritis affecting especially the right opercular artery was disclosed. Oral nicardipine administration was follow-up of a complete recuperation. CONCLUSIONS: Cerebral arteritis is very seldom managed as the cause of intellectual and/or language involution in children as it also occurs with the syndrome of acquired aphasia. However, this pathology has a good response not only to corticoids but also to calcium channel antagonists as it occurred in our patient.

Purcell, A. E., O. H. Jeon, et al. (2001). "Postmortem brain abnormalities of the glutamate neurotransmitter system in autism." Neurology **57**(9): 1618-28.

BACKGROUND: Studies examining the brains of individuals with autism have identified anatomic and pathologic changes in regions such as the cerebellum and hippocampus. Little, if anything, is known, however, about the molecules that are involved in the pathogenesis of this disorder. OBJECTIVE: To identify genes with abnormal expression levels in the cerebella of subjects with autism. METHOD: Brain samples from a total of 10 individuals with autism and 23 matched controls were collected, mainly from the cerebellum. Two cDNA microarray technologies were used to identify genes that were significantly up- or downregulated in autism. The abnormal mRNA or protein levels of several genes identified by microarray analysis were investigated using PCR with reverse transcription and Western blotting. alpha-Amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA)- and NMDA-type glutamate receptor densities were examined with receptor autoradiography in the cerebellum, caudate-putamen, and prefrontal cortex. RESULTS: The mRNA levels of several genes were significantly increased in autism, including excitatory amino acid transporter 1 and glutamate receptor AMPA 1, two members of the glutamate system. Abnormalities in the protein or mRNA levels of several additional molecules in the glutamate system were identified on further analysis, including glutamate receptor binding proteins. AMPA-type glutamate receptor density was decreased in the cerebellum of individuals with autism ($p < 0.05$). CONCLUSIONS: Subjects with autism may have specific abnormalities in the AMPA-type glutamate

receptors and glutamate transporters in the cerebellum. These abnormalities may be directly involved in the pathogenesis of the disorder.

Silva, S. C., C. Correia, et al. (2004). "Autoantibody repertoires to brain tissue in autism nuclear families." J Neuroimmunol **152**(1-2): 176-82.

The hypothesis of an immune dysfunction in autism spectrum disorders has previously been put forward without, however, compelling evidence of a direct relation to its etiology or pathogenesis. To further understand if autoimmunity could play a significant role in autism, we analyzed autoantibody repertoires to brain tissue extract in the plasma of 171 autism children, their parents, and 54 controls, by quantitative immunoblotting. Multiparametric analysis revealed significant differences between patients and controls, and showed that one single reactivity in Section 32 of the blot had the most power to discriminate between these samples. Family correlation coefficients and heritability estimates did not provide any evidence that this reactivity was genetically determined. While the molecular weight of the target protein suggested that it might be an isoform of Myelin Basic Protein (MBP), inhibition assays with human MBP argued against this hypothesis. The study evidences the widespread occurrence of autoreactivities to brain tissue in autism patients, which may represent the immune system's neuroprotective response to a previous brain injury occurred during neurodevelopment. The molecular identification of the target protein in Section 32 will contribute to the understanding of the role of immune responses against brain antigens in autistic patients.

Singer, H. S., C. M. Morris, et al. (2007). "Antibodies against fetal brain in sera of mothers with autistic children." J Neuroimmunol.

Serum antibodies in 100 mothers of children with autistic disorder (MCAD) were compared to 100 age-matched mothers with unaffected children (MUC) using as antigenic substrates human and rodent fetal and adult brain tissues, GFAP, and MBP. MCAD had significantly more individuals with Western immunoblot bands at 36 kDa in human fetal and rodent embryonic brain tissue. The density of bands was greater in fetal brain at 61 kDa. MCAD plus developmental regression had greater reactivity against human fetal brain at 36 and 39 kDa. Data support a possible complex association between genetic/metabolic/environmental factors and the placental transfer of maternal antibodies in autism.

Singer, H. S., C. M. Morris, et al. (2006). "Antibrain antibodies in children with autism and their unaffected siblings." J Neuroimmunol **178**(1-2): 149-55.

Serum autoantibodies to human brain, identified by ELISA and Western immunoblotting, were evaluated in 29 children with autism spectrum disorder (22 with autistic disorder), 9 non-autistic siblings and 13 controls. More autistic subjects than controls had bands at 100 kDa in caudate, putamen and prefrontal cortex ($p<0.01$) as well as larger peak heights of bands at 73 kDa in the cerebellum and cingulate gyrus. Both autistic disorder subjects and their

matched non-autistic siblings had denser bands (peak height and/or area under the curve) at 73 kDa in the cerebellum and cingulate gyrus than did controls ($p<0.01$). Results suggest that children with autistic disorder and their siblings exhibit differences compared to controls in autoimmune reactivity to specific epitopes located in distinct brain regions.

Singh, V. K., S. X. Lin, et al. (2002). "Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism." J Biomed Sci **9**(4): 359-64.

Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73-75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism.

Singh, V. K., S. X. Lin, et al. (1998). "Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism." Clin Immunol Immunopathol **89**(1): 105-8.

Considering an autoimmunity and autism connection, brain autoantibodies to myelin basic protein (anti-MBP) and neuron-axon filament protein (anti-NAFP) have been found in autistic children. In this current study, we examined associations between virus serology and autoantibody by simultaneous analysis of measles virus antibody (measles-IgG), human herpesvirus-6 antibody (HHV-6-IgG), anti-MBP, and anti-NAFP. We found that measles-IgG and HHV-6-IgG titers were moderately higher in autistic children but they did not significantly differ from normal controls. Moreover, we found that a vast majority of virus serology-positive autistic sera was also positive for brain autoantibody: (i) 90% of measles-IgG-positive autistic sera was also positive for anti-MBP; (ii) 73% of measles-IgG-positive autistic sera was also positive for anti-NAFP; (iii) 84% of HHV-6-IgG-positive autistic sera was also positive for anti-MBP; and (iv) 72% of HHV-6-IgG-positive autistic sera was also positive for anti-NAFP. This study is the

first to report an association between virus serology and brain autoantibody in autism; it supports the hypothesis that a virus-induced autoimmune response may play a causal role in autism.

Singh, V. K. and W. H. Rivas (2004). "Prevalence of serum antibodies to caudate nucleus in autistic children." Neurosci Lett **355**(1-2): 53-6.

Autism may involve autoimmunity to brain. We studied regional distribution of antibodies to rat caudate nucleus, cerebral cortex, cerebellum, brain stem and hippocampus. The study included 30 normal and 68 autistic children. Antibodies were assayed by immunoblotting. Autistic children, but not normal children, had antibodies to caudate nucleus (49% positive sera), cerebral cortex (18% positive sera) and cerebellum (9% positive sera). Brain stem and hippocampus were negative. Antibodies to caudate nucleus were directed towards three proteins having 160, 115 and 49 kD molecular weights. Since a significant number of autistic children had antibodies to caudate nucleus, we propose that an autoimmune reaction to this brain region may cause neurological impairments in autistic children. Thus, the caudate nucleus might be involved in the neurobiology of autism.

Singh, V. K., R. Warren, et al. (1997). "Circulating autoantibodies to neuronal and glial filament proteins in autism." Pediatr Neurol **17**(1): 88-90.

Autoimmunity may be a pathogenic factor in autism, a behavioral disorder of early childhood onset. Circulating autoantibodies are produced in organ-specific autoimmunity; therefore, we investigated them in the plasma of autistic subjects, mentally retarded (MR) subjects, and healthy controls. Autoantibodies (IgG isotype) to neuron-axon filament protein (anti-NAFP) and glial fibrillary acidic protein (anti-GFAP) were analyzed by the Western immunoblotting technique. We found a significant increase in incidence of anti-NAFP (P = .004) and anti-GFAP (P = .002) in autistic subjects, but not in MR subjects. Clinically, these autoantibodies may be related to autoimmune pathology in autism.

Singh, V. K., R. P. Warren, et al. (1993). "Antibodies to myelin basic protein in children with autistic behavior." Brain Behav Immun **7**(1): 97-103.

Based on a possible pathological relationship of autoimmunity to autism, antibodies reactive with myelin basic protein (anti-MBP) were investigated in the sera of autistic children. Using a screening serum dilution of 1:400 in the protein-immunoblotting technique, approximately 58% (19 of 33) sera of autistic children (< or = 10 years of age) were found to be positive for anti-MBP. This result in autistics was significantly (p < or = .0001) different from the controls (8 of 88 or only 9% positive), which included age-matched children with normal health, idiopathic mental retardation (MR) and Down syndrome (DS), and normal adults of 20 to 40 years of age. Since autism is a syndrome of unknown etiology, it is possible that anti-MBP antibodies are associated with the development of autistic behavior.

Todd, R. D. and R. D. Ciaranello (1985). "Demonstration of inter- and intraspecies differences in serotonin binding sites by antibodies from an autistic child." Proc Natl Acad Sci U S A **82**(2): 612-6.

Serotonin (5-HT) binding sites from bovine and rat cerebral cortex membranes share pharmacological properties that allow both to be subclassified by the same criteria. We show here that [3H]5-HT binding sites from human cortex also possess pharmacological properties that follow the same subclassification scheme as for bovine and rat cortex. In addition, we show that solubilized 5-HT1 and 5-HT3 sites from all three species have an s20,w value of 3.4. Despite these similar pharmacological and physical characteristics, we can demonstrate antigenic differences between receptor types and species. Human 5-HT1A sites can be distinguished from human 5-HT1B, 5-HT2, and 5-HT3 sites and from equivalent sites in rat and bovine cortex. The anti-human 5-HT1A antibodies were discovered in the blood of an autistic child and may have clinical or etiologic significance for this disorder.

Todd, R. D., J. M. Hickok, et al. (1988). "Antibrain antibodies in infantile autism." Biol Psychiatry **23**(6): 644-7.

Vargas, D. L., C. Nascimbene, et al. (2005). "Neuroglial activation and neuroinflammation in the brain of patients with autism." Ann Neurol **57**(1): 67-81.

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of

autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

Vojdani, A., A. W. Campbell, et al. (2002). "Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and Streptococcus group A." J Neuroimmunol **129**(1-2): 168-77.

We measured autoantibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of preexisting T-cells and central nervous system antigens to immunocompetent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.

Vojdani, A., T. O'Bryan, et al. (2004). "Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism." Nutr Neurosci **7**(3): 151-61.

The mechanisms behind autoimmune reaction to nervous system antigens in autism are not understood. We assessed the reactivity of sera from 50 autism patients and 50 healthy controls to specific peptides from gliadin and the cerebellum. A significant percentage of autism patients showed elevations in antibodies against gliadin and cerebellar peptides simultaneously. For examining cross-reaction between dietary proteins and cerebellar antigens, antibodies were prepared in rabbits, and binding of rabbit anti-gliadin, anti-cerebellar peptides, anti-MBP, anti-milk, anti-egg, anti-soy and anti-corn to either gliadin- or cerebellar-antigen-coated wells was measured. In comparison to anti-gliadin peptide binding to gliadin peptide at 100%, the reaction of anti-cerebellar peptide to gliadin peptide was 22%, whereas the binding of anti-myelin basic protein (MBP), anti-milk, anti-egg and anti-soy to gliadin was less than 10%. Further examination of rabbit anti-gliadin (EQVPLVQQ) and anti-cerebellar (EDVPLLED) 8 amino acid (AA) peptides with human serum albumin (HSA) and an unrelated peptide showed no binding, but the reaction of these antibodies with both the cerebellar and gliadin peptides was greater than 60%. This cross-

reaction was further confirmed by DOT-immunoblot and inhibition studies. We conclude that a subgroup of patients with autism produce antibodies against Purkinje cells and gliadin peptides, which may be responsible for some of the neurological symptoms in autism.

Warren, R. P., P. Cole, et al. (1990). "Detection of maternal antibodies in infantile autism." J Am Acad Child Adolesc Psychiatry **29**(6): 873-7.

Maternal antibodies reactive with antigenic proteins expressed on the cell surface of paternal lymphocytes can be detected in couples with histories of more than one miscarriage or stillbirth. It is possible, but not proven, that these antibodies also react with tissues of the fetus and result in fetal death. Since many mothers of autistic children have a history of pregnancy disorder, antibodies were studied in 11 mothers of autistic children who were 6 years of age or younger. Six of the mothers had antibodies that reacted with lymphocytes of the autistic child. Five of these six mothers had a history of pregnancy disorder. Since antigens expressed on lymphocytes are found on cells of the central nervous system and, perhaps, other tissues of the developing embryo, it is suggested that aberrant maternal immunity may be associated with the development of some cases of infantile autism.

Weizman, A., R. Weizman, et al. (1982). "Abnormal immune response to brain tissue antigen in the syndrome of autism." Am J Psychiatry **139**(11): 1462-5.

Cell-mediated immune response to human myelin basic protein was studied by the macrophage migration inhibition factor test in 17 autistic patients and a control group of 11 patients suffering from other mental diseases included in the differential diagnosis of the syndrome of autism. Of the 17 autistic patients, 13 demonstrated inhibition of macrophage migration, whereas none of the nonautistic patients showed such a response. The results indicate the existence of a cell-mediated immune response to brain tissue in the syndrome of autism.

Zakharenko, O. M., T. P. Kliushnik, et al. (1999). "[Nerve growth factor auto-antibodies in the sera of mothers of schizophrenic children and children from high risk group]." Zh Nevrol Psikhiatr Im S S Korsakova **99**(3): 44-6.

A level of autoantibodies (aAB) to nerve growth factor (NGF) was measured in blood serum of children from 4 groups: 1) schizophrenic patients; 2) children from the families, in which one of the parents suffered with schizophrenia (high risk groups of schizophrenia); 3) children with residual-organic damages of CNS; 4) control group. This index was also determined in their mothers. Significant elevation of a titer of aAB to NGF was observed in blood of children from groups 1 and 2 as well as in their mothers, as compared with 3 and 4 groups. Among the mothers of the children from 1 and 2 groups there were met women with different endogenous mental disorders, with the disorders of personality as well as mentally healthy women. An increase of a level of aAB to NGF was found in all the women from groups 1 and 2, independently of their mental status including

mentally healthy women. Such results allow to consider elevated level of aAB to NGF as a risk factor of mental pathology.

Zerrate, M. C., M. Pletnikov, et al. (2007). "Neuroinflammation and behavioral abnormalities after neonatal terbutaline treatment in rats: implications for autism." J Pharmacol Exp Ther **322**(1): 16-22.

Autism is a neurodevelopmental disorder presenting before 3 years of age with deficits in communication and social skills and repetitive behaviors. In addition to genetic influences, recent studies suggest that prenatal drug or chemical exposures are risk factors for autism. Terbutaline, a beta2-adrenoceptor agonist used to arrest preterm labor, has been associated with increased concordance for autism in dizygotic twins. We studied the effects of terbutaline on microglial activation in different brain regions and behavioral outcomes in developing rats. Newborn rats were given terbutaline (10 mg/kg) daily on postnatal days (PN) 2 to 5 or PN 11 to 14 and examined 24 h after the last dose and at PN 30. Immunohistochemical studies showed that administration of terbutaline on PN 2 to 5 produced a robust increase in microglial activation on PN 30 in the cerebral cortex, as well as in cerebellar and cerebrocortical white matter. None of these effects occurred in animals given terbutaline on PN 11 to 14. In behavioral tests, animals treated with terbutaline on PN 2 to 5 showed consistent patterns of hyper-reactivity to novelty and aversive stimuli when assessed in a novel open field, as well as in the acoustic startle response test. Our findings indicate that beta2-adrenoceptor overstimulation during an early critical period results in microglial activation associated with innate neuroinflammatory pathways and behavioral abnormalities, similar to those described in autism. This study provides a useful animal model for understanding the neuropathological processes underlying autism spectrum disorders.

Zimmerman, A. W., S. L. Connors, et al. (2007). "Maternal antibrain antibodies in autism." Brain Behav Immun **21**(3): 351-7.

Autism is a neurodevelopmental disorder of prenatal onset that is behaviorally defined. There is increasing evidence for systemic and neuroimmune mechanisms in children with autism. Although genetic factors are important, atypical prenatal maternal immune responses may also be linked to the pathogenesis of autism. We tested serum reactivity in 11 mothers and their autistic children, maternal controls, and several groups of control children, to prenatal, postnatal, and adult rat brain proteins, by immunoblotting. Similar patterns of reactivity to prenatal (gestational day 18), but not postnatal (day 8) or adult rat brain proteins were identified in autistic children, their mothers, and children with other neurodevelopmental disorders, and differed from mothers of normal children, normal siblings of children with autism and normal child controls. Specific patterns of antibody reactivity were present in sera from the autism mothers, from 2 to 18 years after the birth of their affected children and were unrelated to birth order. Immunoblotting using specific antigens for myelin

basic protein (MBP) and glial acidic fibrillary protein (GFAP) suggests that these proteins were not targets of the maternal antibodies. The identification of specific serum antibodies in mothers of children with autism that recognize prenatally expressed brain antigens suggests that these autoantibodies could cross the placenta and alter fetal brain development.

Zimmerman, A. W., H. Jyonouchi, et al. (2005). "Cerebrospinal fluid and serum markers of inflammation in autism." Pediatr Neurol **33**(3): 195-201.

Systemic immune abnormalities have no known relevance to brain dysfunction in autism. In order to find evidence for neuroinflammation, we compared levels of sensitive indicators of immune activation: quinolinic acid, neopterin, and biopterin, as well as multiple cytokines and cytokine receptors, in cerebrospinal fluid and serum from children with autism, to control subjects with other neurologic disorders. In cerebrospinal fluid from 12 children with autism, quinolinic acid ($P = 0.037$) and neopterin ($P = 0.003$) were decreased, and biopterin ($P = 0.040$) was elevated, compared with control subjects. In sera from 35 persons with autism, among cytokines, only tumor necrosis factor receptor II was elevated compared with controls ($P < 0.02$). Decreased quinolinic acid and neopterin in cerebrospinal fluid are paradoxical and suggest dysmaturation of metabolic pathways and absence of concurrent infection, respectively, in autism. Alternatively, they may be produced by microglia but remain localized and not expressed in cerebrospinal fluid.

4 - Autism and Treatment of Inflammation (29 citations)

Boris, M., A. Goldblatt, et al. (2005). "Improvement in children with autism treated with intravenous gamma globulin." J Nutritional Environmental Medicine **15**(4): 169-176.

Boris, M., C. C. Kaiser, et al. (2007). "Effect of pioglitazone treatment on behavioral symptoms in autistic children." J Neuroinflammation **4**: 3.

INTRODUCTION: Autism is complex neuro-developmental disorder which has a symptomatic diagnosis in patients characterized by disorders in language/communication, behavior, and social interactions. The exact causes for autism are largely unknown, but is has been speculated that immune and inflammatory responses, particularly those of Th2 type, may be involved. Thiazolidinediones (TZDs) are agonists of the peroxisome proliferator activated receptor gamma (PPARgamma), a nuclear hormone receptor which modulates insulin sensitivity, and have been shown to induce apoptosis in activated T-lymphocytes and exert anti-inflammatory effects in glial cells. The TZD pioglitazone (Actos) is an FDA-approved PPARgamma agonist used to treat type 2 diabetes, with a good safety profile, currently being tested in clinical trials of other neurological diseases including AD and MS. We therefore tested the safety and therapeutic potential of oral pioglitazone in a small cohort of children with diagnosed autism. CASE DESCRIPTION: The rationale and risks of taking pioglitazone were explained to the parents, consent was obtained, and treatment was initiated at either 30 or 60 mg per day p.o. A total of 25 children (average age 7.9 +/- 0.7 year old) were enrolled. Safety was assessed by measurements of metabolic profiles and blood pressure; effects on behavioral symptoms were assessed by the Aberrant Behavior Checklist (ABC), which measures hyperactivity, inappropriate speech, irritability, lethargy, and stereotypy, done at baseline and after 3-4 months of treatment. DISCUSSION AND EVALUATION: In a small cohort of autistic children, daily treatment with 30 or 60 mg p.o. pioglitazone for 3-4 months induced apparent clinical improvement without adverse events. There were no adverse effects noted and behavioral measurements revealed a significant decrease in 4 out of 5 subcategories (irritability, lethargy, stereotypy, and hyperactivity). Improved behaviors were inversely correlated with patient age, indicating stronger effects on the younger patients. CONCLUSION: Pioglitazone should be considered for further testing of therapeutic potential in autistic patients.

Bradstreet, J. J., S. Smith, et al. (2007). "Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders." Med Hypotheses **68**(5): 979-87.

Multiple studies now demonstrate that autism is medically characterized, in part, by immune system dysregulation, including evidence of neuroglial activation and gastrointestinal inflammation. This neuroglial process has further been characterized as neuroinflammation. In addition, a subset of autistic children

exhibit higher than average levels of androgens. Spironolactone is an aldosterone antagonist and potassium-sparing diuretic with a desirable safety profile. It possesses potent anti-inflammatory and immune modifying properties that might make it an excellent medical intervention for autism spectrum disorders. Furthermore, spironolactone demonstrates substantial anti-androgen properties that might further enhance its appeal in autism, particularly in a definable subset of hyperandrogenic autistic children. One case report is briefly reviewed demonstrating objective clinical improvements in an autistic child after spironolactone administration. Additional research in controlled trials is now needed to further define the risks and benefits of spironolactone use in children with autism.

Brudnak, M. A., B. Rimland, et al. (2002). "Enzyme-based therapy for autism spectrum disorders -- is it worth another look?" Med Hypotheses **58**(5): 422-8.

Autism is a developmental disease usually manifesting within the first three years of life. To date, no causative agent has been found. Similarly, treatment options have been limited. Of the treatment options available, a number of them have been nutritionally based in an attempt to address one or more of the theories regarding the etiology of the disease. An example would be enzyme therapy for the digestion of purported offending neuroactive peptides collectively known as exorphins. This paper discusses the exorphin theory of autism and subsequent treatment with dietary enzyme therapy. Novel data are presented in support of the theory that enzymes play a critical role in autism. Forty-six patients between the ages of 5 and 31 were selected for inclusion in the study based on a diagnosis placing them in the category of the autism spectrum disorders (ASD). The diets were supplemented with a novel dietary enzyme formulation, ENZYMAID, for a period of 12 weeks. Progress was tracked according to the Symptom Outcome Survey (SOS) (1) form method of symptom charting and presented in a table for further analysis. The novel enzyme formula, ENZYMAID, beneficially and safely affected all 13 of the parameters measured. Improvements ranged from 50-90%, depending on the parameter measured. Enzyme therapy to treat ASD may indeed a viable option in treatment protocols. These results indicate that further controlled studies are warranted.

Chez, M. G., T. Dowling, et al. (2007). "Elevation of tumor necrosis factor-alpha in cerebrospinal fluid of autistic children." Pediatr Neurol **36**(6): 361-5.

Recent reports implicating elevated cytokines in the central nervous system in a small number of patients studied with autism have reported clinical regression. These studies have not focused on tumor necrosis factor-alpha as a possible marker for inflammatory damage. A series of 10 children with autism had clinical evaluation of their serum and spinal fluid for inflammatory changes and possible metabolic disease as part of their neurological evaluation. Elevation of cerebrospinal fluid levels of tumor necrosis factor-alpha was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in

all of the patients studied. The ratio of the cerebrospinal fluid levels to serum levels averaged 53.7:1. This ratio is significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum tumor necrosis factor-alpha levels have been simultaneously measured. This observation may offer a unique insight into central nervous system inflammatory mechanisms that may contribute to the onset of autism and may serve as a potential clinical marker. More controlled study of this potentially important observation may prove valuable.

Elder, J. H., M. Shankar, et al. (2006). "The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial." J Autism Dev Disord **36**(3): 413-20.

This study tested the efficacy of a gluten-free and casein-free (GFCF) diet in treating autism using a randomized, double blind repeated measures crossover design. The sample included 15 children aged 2-16 years with autism spectrum disorder. Data on autistic symptoms and urinary peptide levels were collected in the subjects' homes over the 12 weeks that they were on the diet. Group data indicated no statistically significant findings even though several parents reported improvement in their children. Although preliminary, this study demonstrates how a controlled clinical trial of the GFCF diet can be conducted, and suggests directions for future research.

Fayad, M. N., R. Choueiri, et al. (1997). "Landau-Kleffner syndrome: consistent response to repeated intravenous gamma-globulin doses: a case report." Epilepsia **38**(4): 489-94.

PURPOSE: Although several treatments have been tried for Landau-Kleffner syndrome (LKS) too many patients are refractory to known therapies. We report an 8-year-old girl who failed other therapies but who had a consistent response after treatment with intravenous (i.v.) gamma-globulin. METHODS: We monitored the girl from the age of 6 years, when she presented with a 6-month history of loss of language with normal hearing, normal brain magnetic resonance imaging (MRI), increased cerebrospinal fluid (CSF) IgG index, and an EEG showing almost continuous, predominantly left-sided spike- and slow-wave complexes. She had no clinical seizures and did not respond to consecutive trials of valproate (VPA), clonazepam (CZP), prednisone, and carbamazepine (CBZ). She received three courses of intravenous (i.v.) gamma-globulin; after each course, clinical and electrographic improvement lasted a few months. After each of the initial two courses, clinical improvement lasted 3-4 months but was followed by recurrence of the spikes on the EEG and by speech deterioration. RESULTS: However, her last remission has been continuous for the past 16 months. Her CSF IgG index became normal after the first i.v. gamma-globulin infusion. CONCLUSIONS: Based on our experience with this patient and on other investigators' experience, we believe that further research into immunologic mechanisms and therapies of this syndrome are warranted.

Feasby, T., B. Banwell, et al. (2007). "Guidelines on the use of intravenous immune globulin for neurologic conditions." Transfus Med Rev **21**(2 Suppl 1): S57-107.

Canada's per capita use of intravenous immune globulin (IVIG) grew by approximately 115% between 1998 and 2006, making Canada one of the world's highest per capita users of IVIG. It is believed that most of this growth is attributable to off-label usage. To help ensure IVIG use is in keeping with an evidence-based approach to the practice of medicine, the National Advisory Committee on Blood and Blood Products (NAC) and Canadian Blood Services convened a panel of national experts to develop an evidence-based practice guideline on the use of IVIG for neurologic conditions. The mandate of the expert panel was to review evidence regarding use of IVIG for 22 neurologic conditions and formulate recommendations on IVIG use for each. A panel of 6 clinical experts, one expert in practice guideline development and 4 representatives from the NAC met to review the evidence and reach consensus on the recommendations for the use of IVIG. The primary sources used by the panel were 2 recent evidence-based reviews. Recommendations were based on interpretation of the available evidence and, where evidence was lacking, consensus of expert clinical opinion. A draft of the practice guideline was circulated to neurologists in Canada for feedback. The results of this process were reviewed by the expert panel, and modifications to the draft guideline were made where appropriate. This practice guideline will provide the NAC with a basis for making recommendations to provincial and territorial health ministries regarding IVIG use management. Recommendations for use of IVIG were made for 14 conditions, including acute disseminated encephalomyelitis, chronic inflammatory demyelinating polyneuropathy, dermatomyositis, diabetic neuropathy, Guillain-Barre syndrome, Lambert-Eaton myasthenic syndrome, multifocal motor neuropathy, multiple sclerosis, myasthenia gravis, opsoclonus-myoclonus, pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, polymyositis, Rasmussen's encephalitis, and stiff person syndrome; IVIG was not recommended for 8 conditions including adrenoleukodystrophy, amyotropic lateral sclerosis, autism, critical illness polyneuropathy, inclusion body, myositis, intractable childhood epilepsy, paraproteinemic neuropathy (IgM variant), and POEMS syndrome. Development and dissemination of evidence-based clinical practice guidelines may help to facilitate appropriate use of IVIG.

Golubchik, P., M. Lewis, et al. (2007). "Neurosteroids in child and adolescent psychopathology." Eur Neuropsychopharmacol **17**(3): 157-64.

Neurosteroids play a significant role in neurodevelopment and are involved in a wide variety of psychopathological processes. There is accumulating evidence on their role in adult psychopathology, including Alzheimer disease, schizophrenia, mood disorder, anxiety disorders and post-traumatic stress disorder. Little is known, however, about the possible role of neurosteroids in child and adolescent psychopathology although there is increasing evidence for their critical role from

the early stages of brain development until adolescence. In this review we focus on the involvement of neurosteroids in neurodevelopment and mental disorders in children and adolescents. Adequate physiological levels protect the developing neural system from insult and contribute to the regulation of brain organization and function. Neurosteroids may be involved in the pathophysiology and pharmacotherapy of a variety of disorders in children and adolescents, including schizophrenia, depression, eating disorders, aggressive behavior and attention deficit. The complex interaction between neurosteroids, neurodevelopment, life-events, genetics and mental disorders in children and adolescents merits further investigation.

Gupta, S. (1999). "Treatment of children with autism with intravenous immunoglobulin." J Child Neurol **14**(3): 203-5.

Gupta, S. (2000). "Immunological treatments for autism." J Autism Dev Disord **30**(5): 475-9.

Several investigators, including ourselves, have reported significant changes in various immune responses in children with autism. These changes demonstrate dysregulation of the immune system (deficiency in some components of the immune system and excesses in others). In addition, certain genes in the major histocompatibility complex (that regulates immune responses) appear to be involved in autism. Based upon immunological abnormalities, various treatment modalities have been applied to children with autism. In this brief review, these immunological changes and various biological therapies are analyzed and summarized.

Gupta, S., S. Aggarwal, et al. (1996). "Dysregulated immune system in children with autism: beneficial effects of intravenous immune globulin on autistic characteristics." J Autism Dev Disord **26**(4): 439-52.

Gupta, S., B. Rimland, et al. (1996). "Pentoxifylline: brief review and rationale for its possible use in the treatment of autism." J Child Neurol **11**(6): 501-4.

Johnson, S. M. and E. Hollander (2003). "Evidence that eicosapentaenoic acid is effective in treating autism." J Clin Psychiatry **64**(7): 848-9.

Knivsberg, A. M., K. L. Reichelt, et al. (2002). "A randomised, controlled study of dietary intervention in autistic syndromes." Nutr Neurosci **5**(4): 251-61.

Impaired social interaction, communication and imaginative skills characterize autistic syndromes. In these syndromes urinary peptide abnormalities, derived from gluten, gliadin, and casein, are reported. They reflect processes with opioid effect. The aim of this single blind study was to evaluate effect of gluten and casein-free diet for children with autistic syndromes and urinary peptide abnormalities. A randomly selected diet and control group with 10 children in

each group participated. Observations and tests were done before and after a period of 1 year. The development for the group of children on diet was significantly better than for the controls.

Knivsberg, A. M., K. L. Reichelt, et al. (1995). "Autistic symptoms and diet: a follow-up study." Scand J Ed Research **39**: 223-236.

Millward, C., M. Ferriter, et al. (2004). "Gluten- and casein-free diets for autistic spectrum disorder." Cochrane Database Syst Rev(2): CD003498.

BACKGROUND: It has been suggested that peptides from gluten and casein may have a role in the origins of autism and that the physiology and psychology of autism might be explained by excessive opioid activity linked to these peptides. Research has reported abnormal levels of peptides in the urine and cerebrospinal fluid of persons with autism. If this is the case, diets free of gluten and /or casein should reduce the symptoms associated with autism. OBJECTIVES: To determine the efficacy of gluten- and/or casein- free diets as an intervention to improve behaviour, cognitive and social functioning in individuals with autism. SEARCH STRATEGY: Electronic searching of abstracts from the Cochrane Library (Issue 3, 2003), PsycINFO (1971- May 2003), EMBASE (1974- May 2003), CINAHL (1982- May 2003), MEDLINE (1986- May 2003), ERIC (1965-2003), LILACS (to 2003) and the specialist register of the Cochrane Complementary Medicine Field (January 2004). Review bibliographies were also examined to identify potential trials. SELECTION CRITERIA: All randomised controlled trials involving programmes which eliminated gluten, casein or both gluten and casein from the diets of individuals diagnosed with autistic spectrum disorder. DATA COLLECTION AND ANALYSIS: Abstracts of studies identified in searches of electronic databases were read and assessed to determine whether they might meet the inclusion criteria. The authors independently selected the relevant studies from the reports identified in this way. As only one trial fitted the inclusion criteria, no meta-analysis is currently possible and data are presented in narrative form. MAIN RESULTS: The one trial included reported results on four outcomes. Unsurprisingly in such a small-scale study, the results for three of these outcomes (cognitive skills, linguistic ability and motor ability) had wide confidence intervals that spanned the line of nil effect. However, the fourth outcome, reduction in autistic traits, reported a significant beneficial treatment effect for the combined gluten- and casein- free diet. REVIEWERS' CONCLUSIONS: This is an important area of investigation and large scale, good quality randomised controlled trials are needed.

Plioplys, A. V. (1998). "Intravenous immunoglobulin treatment of children with autism." J Child Neurol **13**(2): 79-82.

Since autism has been associated with immunologic abnormalities suggesting an autoimmune cause of autistic symptoms in a subset of patients, this study was undertaken to investigate whether intravenous immunoglobulin (i.v.Ig) would

improve autistic symptoms. Ten autistic children with immunologic abnormalities, demonstrated on blood tests, were enrolled in this study. Their ages ranged from 4 to 17 years, with two girls and eight boys. Eight children (1 female and 7 male) historically had undergone autistic regression. Intravenous immunoglobulin, 200 to 400 mg/kg, was administered every 6 weeks for an intended treatment program of four infusions. In five children, there was no detectable change in behavior during the treatment program. In four children, there was a mild improvement noted in attention span and hyperactivity. In none of these children did the parents feel that the improvement was sufficient to warrant further continuation of the infusions beyond the termination of the program. Only in one child was there a very significant improvement, with almost total amelioration of autistic symptoms over the time period of the four infusions. Once the treatment program was completed, this child gradually deteriorated over a 5-month time period and fully reverted to his previous autistic state. In this treatment program, five children had no response to intravenous immunoglobulin. In the four children who showed mild improvements, those improvements may simply have been due to nonspecific effects of physician intervention and parental expectation (ie, placebo effect). However, in one child there was a very significant amelioration of autistic symptoms. There were no distinguishing historic or laboratory features in this child who improved. Given a positive response rate of only 10% in this study, along with the high economic costs of the immunologic evaluations and the intravenous immunoglobulin treatments, the use of intravenous immunoglobulin to treat autistic children should be undertaken only with great caution, and only under formal research protocols.

Plioplys, A. V. (2000). "Intravenous immunoglobulin treatment in autism." J Autism Dev Disord **30**(1): 73-4.

Robinson, P., D. Anderson, et al. (2007). "Evidence-based guidelines on the use of intravenous immune globulin for hematologic and neurologic conditions." Transfus Med Rev **21**(2 Suppl 1): S3-8.

In Canada, intravenous immune globulin (IVIG) use has increased by 115% over the past 7 to 8 years. Given this increased usage, Canadian Blood Services and the National Advisory Committee on Blood and Blood Products for Canada identified the need to develop and disseminate evidence-based guidelines to facilitate appropriate IVIG use. As a result, guidelines for IVIG use in hematologic and neurologic conditions have been developed and are published in this supplement of Transfusion Medicine Reviews. This commentary provides a brief description of the process used to develop these guidelines and includes a summary of the recommendations for IVIG use in the various conditions evaluated.

Rossignol, D. A., L. W. Rossignol, et al. (2007). "The effects of hyperbaric oxygen therapy on oxidative stress, inflammation, and symptoms in children with autism: an open-label pilot study." BMC Pediatr **7**(1): 36.

ABSTRACT: BACKGROUND: Recently, hyperbaric oxygen therapy (HBOT) has increased in popularity as a treatment for autism. Numerous studies document oxidative stress and inflammation in individuals with autism; both of these conditions have demonstrated improvement with HBOT, along with enhancement of neurological function and cognitive performance. In this study, children with autism were treated with HBOT at atmospheric pressures and oxygen concentrations in current use for this condition. Changes in markers of oxidative stress and inflammation were measured. The children were evaluated to determine clinical effects and safety. METHODS: Eighteen children with autism, ages 3-16 years, underwent 40 hyperbaric sessions of 45 minutes duration each at either 1.5 atmospheres (atm) and 100% oxygen, or at 1.3 atm and 24% oxygen. Measurements of C-reactive protein (CRP) and markers of oxidative stress, including plasma oxidized glutathione (GSSG), were assessed by fasting blood draws collected before and after the 40 treatments. Changes in clinical symptoms, as rated by parents, were also assessed. The children were closely monitored for potential adverse effects. RESULTS: At the endpoint of 40 hyperbaric sessions, neither group demonstrated statistically significant changes in mean plasma GSSG levels, indicating intracellular oxidative stress appears unaffected by either regimen. A trend towards improvement in mean CRP was present in both groups; the largest improvements were observed in children with initially higher elevations in CRP. When all 18 children were pooled, a significant improvement in CRP was found ($p = 0.021$). Pre- and post-parental observations indicated statistically significant improvements in both groups, including motivation, speech, and cognitive awareness ($p < 0.05$). No major adverse events were observed. CONCLUSIONS: In this prospective pilot study of children with autism, HBOT at a maximum pressure of 1.5 atm with up to 100% oxygen was safe and well tolerated. HBOT did not appreciably worsen oxidative stress and significantly decreased inflammation as measured by CRP levels. Parental observations support anecdotal accounts of improvement in several domains of autism. However, since this was an open-label study, definitive statements regarding the efficacy of HBOT for the treatment of individuals with autism must await results from double-blind, controlled trials. Trial Registration: clinicaltrials.gov NCT00324909.

Schneider, C. K., R. D. Melmed, et al. (2006). "Oral human immunoglobulin for children with autism and gastrointestinal dysfunction: a prospective, open-label study." J Autism Dev Disord **36**(8): 1053-64.

Immunoglobulin secretion onto mucosal surfaces is a major component of the mucosal immune system. We hypothesized that chronic gastrointestinal (GI) disturbances associated with autistic disorder (AD) may be due to an underlying deficiency in mucosal immunity, and that orally administered immunoglobulin

would be effective in alleviating chronic GI dysfunction in these individuals. In this pilot study, twelve male subjects diagnosed with AD were evaluated using a GI severity index (GSI) while receiving daily dosing with encapsulated human immunoglobulin. Following eight weeks of treatment, 50% of the subjects met prespecified criteria for response in GI signs and symptoms and showed significant behavioral improvement as assessed by the Autism Behavior Checklist and parent and physician rated Clinical Global Impression of Improvement.

Shenoy, S., S. Arnold, et al. (2000). "Response to steroid therapy in autism secondary to autoimmune lymphoproliferative syndrome." J Pediatr **136**(5): 682-7.

We report a child who developed autoimmune lymphoproliferative syndrome (ALPS) secondary to a heterozygous dominant negative mutation in the death domain of the Fas receptor. Previously developmentally normal, he had symptoms of autism with rapid regression in developmental milestones coincident with the onset of lymphoproliferation and autoimmune hemolytic anemia. Low-dose steroid therapy induced early and complete remission in the ALPS phenotype. There was subjective improvement, followed by objective improvement in speech and developmental milestones. We propose that autism may be part of the autoimmune disease spectrum of ALPS in this child, and this case represents a novel manifestation and target organ involvement in this disease.

Singh, V. K., H. H. Fudenberg, et al. (1988). "Immunodiagnosis and immunotherapy in autistic children." Ann N Y Acad Sci **540**: 602-4.

Stefanatos, G. A., W. Grover, et al. (1995). "Case study: corticosteroid treatment of language regression in pervasive developmental disorder." J Am Acad Child Adolesc Psychiatry **34**(8): 1107-11.

The authors describe a child whose language and behavior regressed at 22 months and in whom pervasive developmental disorder was later diagnosed. At 6 years, he displayed a profound receptive-expressive aphasia accompanied by behavioral disturbances characterized by hyperactivity, impaired social interactions, tantrums, gestural stereotypies, and echolalia. A single-photon emission computed tomography scan and steady-state auditory evoked potentials suggested bitemporal and left frontal pathophysiology. The overall profile resembled Landau-Kleffner syndrome, but no electroencephalographic disturbance was evident. Corticosteroid treatment resulted in amelioration of language abilities and behavior. These findings suggest that the factors underlying language regression in pervasive developmental disorder can, in special circumstances, be amenable to pharmacological treatment.

Stubbs, E. G., S. S. Budden, et al. (1980). "Transfer factor immunotherapy of an autistic child with congenital cytomegalovirus." J Autism Dev Disord **10**(4): 451-8.

Tsuru, T., M. Mori, et al. (2000). "Effects of high-dose intravenous corticosteroid therapy in Landau-Kleffner syndrome." Pediatr Neurol **22**(2): 145-7.

Two children with Landau-Kleffner syndrome were successfully treated with antiepileptic drugs and a high-dose intravenous corticosteroid. A combination of valproate and a benzodiazepine (clonazepam or diazepam) ameliorated epileptic seizures and electroencephalographic spikes and waves, but speech disturbances persisted. Both patients were treated with an intravenous infusion of high-dose methylprednisolone sodium succinate (20 mg/kg daily) for 3 consecutive days. This infusion was repeated three times with a 4-day interval between treatments, which resulted in a rapid improvement in speech ability. After intravenous therapy, prednisolone was given orally (2 mg/kg daily for 1 month, then gradually withdrawn), which maintained the clinical improvement in speech.

Wakefield, A. J., J. M. Puleston, et al. (2002). "Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands." Aliment Pharmacol Ther **16**(4): 663-74.

There is growing awareness that primary gastrointestinal pathology may play an important role in the inception and clinical expression of some childhood developmental disorders, including autism. In addition to frequent gastrointestinal symptoms, children with autism often manifest complex biochemical and immunological abnormalities. The gut-brain axis is central to certain encephalopathies of extra-cranial origin, hepatic encephalopathy being the best characterized. Commonalities in the clinical characteristics of hepatic encephalopathy and a form of autism associated with developmental regression in an apparently previously normal child, accompanied by immune-mediated gastrointestinal pathology, have led to the proposal that there may be analogous mechanisms of toxic encephalopathy in patients with liver failure and some children with autism. Aberrations in opioid biochemistry are common to these two conditions, and there is evidence that opioid peptides may mediate certain aspects of the respective syndromes. The generation of plausible and testable hypotheses in this area may help to identify new treatment options in encephalopathies of extra-cranial origin. Therapeutic targets for this autistic phenotype may include: modification of diet and entero-colonic microbial milieu in order to reduce toxin substrates, improve nutritional status and modify mucosal immunity; anti-inflammatory/immunomodulatory therapy; and specific treatment of dysmotility, focusing, for example, on the pharmacology of local opioid activity in the gut.

Zimmerman, A. W. (2000). "Commentary: immunological treatments for autism: in search of reasons for promising approaches." J Autism Dev Disord **30**(5): 481-4.

5 - Autism and Oxidative Stress (70 citations):

Abbott, L. C. and S. S. Nahm (2004). "Neuronal nitric oxide synthase expression in cerebellar mutant mice." Cerebellum **3**(3): 141-51.

Nitric oxide (NO) is a diffusible, multifunctional signaling molecule found in many areas of the brain. NO signaling is involved in a wide array of neurophysiological functions including synaptogenesis, modulation of neurotransmitter release, synaptic plasticity, central nervous system blood flow and cell death. NO synthase (NOS) activity regulates the production of NO and the cerebellum expresses high levels of nitric oxide synthase (NOS) in granule, stellate and basket cells. Cerebellar mutant mice provide excellent opportunities to study changes of NO/NOS concentrations and activities to gain a greater understanding of the roles of NO and NOS in cerebellar function. Here, we have reviewed the current understanding of the functional roles of NO and NOS in the cerebellum and present NO/NOS activities that have been described in various cerebellar mutant mice and NOS knockout mice. NO appears to exert neuroprotective effects at low to moderate concentrations, whereas NO becomes neurotoxic as the concentration increases. Excessive NO production can cause oxidative stress to neurons, ultimately impairing neuronal function and result in neuronal cell death. Based on their genetics and cerebellar histopathology, some of cerebellar mutant mice display similarities with human neurological conditions and may prove to be valuable models to study several human neurological disorders, such as autism and schizophrenia.

Amminger, G. P., G. E. Berger, et al. (2007). "Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study." Biol Psychiatry **61**(4): 551-3.

BACKGROUND: There is increasing evidence that fatty acid deficiencies or imbalances may contribute to childhood neurodevelopmental disorders. METHODS: We conducted a randomized, double-blind, placebo-controlled 6-week pilot trial investigating the effects of 1.5 g/d of omega-3 fatty acids (.84 g/d eicosapentaenoic acid, .7 g/d docosahexaenoic acid) supplementation in 13 children (aged 5 to 17 years) with autistic disorders accompanied by severe tantrums, aggression, or self-injurious behavior. The outcome measure was the Aberrant Behavior Checklist (ABC) at 6 weeks. RESULTS: We observed an advantage of omega-3 fatty acids compared with placebo for hyperactivity and stereotypy, each with a large effect size. Repeated-measures ANOVA indicated a trend toward superiority of omega-3 fatty acids over placebo for hyperactivity. No clinically relevant adverse effects were elicited in either group. CONCLUSIONS: The results of this study provide preliminary evidence that omega-3 fatty acids may be an effective treatment for children with autism.

Anderson, M. P., B. S. Hooker, et al. (2008). "Bridging from cells to cognition in autism pathophysiology: biological pathways to defective brain function and plasticity " American Journal of Biochemistry and Biotechnology **4**(2): 167-176.

We review evidence to support a model where the disease process underlying autism may begin when an in utero or early postnatal environmental, infectious, seizure, or autoimmune insult triggers an immune response that increases reactive oxygen species (ROS) production in the brain that leads to DNA damage (nuclear and mitochondrial) and metabolic enzyme blockade and that these inflammatory and oxidative stressors persist beyond early development (with potential further exacerbations), producing ongoing functional consequences. In organs with a high metabolic demand such as the central nervous system, the continued use of mitochondria with damaged DNA and impaired metabolic enzyme function may generate additional ROS which will cause persistent activation of the innate immune system leading to more ROS production. Such a mechanism would self-sustain and possibly progressively worsen. The mitochondrial dysfunction and altered redox signal transduction pathways found in autism would conspire to activate both astroglia and microglia. These activated cells can then initiate a broad-spectrum proinflammatory gene response. Beyond the direct effects of ROS on neuronal function, receptors on neurons that bind the inflammatory mediators may serve to inhibit neuronal signaling to protect them from excitotoxic damage during various pathologic insults (e.g., infection). In autism, over-zealous neuroinflammatory responses could not only influence neural developmental processes, but may more significantly impair neural signaling involved in cognition in an ongoing fashion. This model makes specific predictions in patients and experimental animal models and suggests a number of targets sites of intervention. Our model of potentially reversible pathophysiological mechanisms in autism motivates our hope that effective therapies may soon appear on the horizon.

Bell, J. G., E. E. MacKinlay, et al. (2004). "Essential fatty acids and phospholipase A2 in autistic spectrum disorders." Prostaglandins Leukot Essent Fatty Acids **71**(4): 201-4.

A health questionnaire based on parental observations of clinical signs of fatty acid deficiency (FAD) showed that patients with autism and Asperger's syndrome (ASP) had significantly higher FAD scores (6.34+/-4.37 and 7.64+/-6.20, respectively) compared to controls (1.78+/-1.68). Patients with regressive autism had significantly higher percentages of 18:0,18:2n-6 and total saturates in their RBC membranes compared to controls, while 24:0, 22:5n-6, 24:1 and the 20:4n-6/20:5n-3 ratio were significantly higher in both regressive autism and ASP groups compared to controls. By comparison, the 18:1n-9 and 20:4n-6 values were significantly lower in patients with regressive autism compared to controls while 22:5n-3, total n-3 and total dimethyl acetals were significantly lower in both regressive autism and ASP groups compared to controls. Storage of RBC at -20 degrees C for 6 weeks resulted in significant reductions in highly unsaturated fatty acid levels in polar lipids of patients with regressive autism, compared to

patients with classical autism or ASP, or controls. Patients diagnosed with both autism and ASP showed significantly increased levels of EPA (approximately 200%) and DHA (approximately 40%), and significantly reduced levels of ARA (approximately 20%), 20:3n-6 and ARA/EPA ratio in their RBC polar lipids, when supplemented with EPA-rich fish oils, compared to controls and non-supplemented patients with autism. Patients with both regressive autism and classical autism/Asperger's syndrome had significantly higher concentrations of RBC type IV phospholipase A2 compared to controls. However, patients with autism/ASP, who had taken EPA supplements, had significantly reduced PLA2 concentrations compared to unsupplemented patients with classical autism or ASP.

Bell, J. G., J. R. Sargent, et al. (2000). "Red blood cell fatty acid compositions in a patient with autistic spectrum disorder: a characteristic abnormality in neurodevelopmental disorders?" Prostaglandins Leukot Essent Fatty Acids **63**(1-2): 21-5.

The fatty acid compositions of red blood cell (RBC) phospholipids from a patient with autistic spectrum disorder (ASD) had reduced percentages of highly unsaturated fatty acids (HUFA) compared to control samples. The percentage of HUFA in the RBC from the autistic patient was dramatically reduced (up to 70%) when the sample was stored for 6 weeks at -20 degrees C. However, only minor HUFA reductions were recorded in control samples stored similarly, or when the autistic sample was stored at -80 degrees C. A similar instability in RBC HUFA compositions upon storage at -20 degrees C has been recorded in schizophrenic patients. In a number of other neurodevelopmental conditions, including attention deficit hyperactivity disorder (ADHD) and dyslexia, reduced concentrations of RBC HUFA have been recorded. The extent and nature of these aberrations require further assessment to determine a possible common biochemical origin of neurodevelopmental disorders in general. To facilitate this, a large scale assessment of RBC fatty acid compositions in patients with ASD, and related disorders, should be performed as a matter of urgency. Supplementing cells in culture with the tryptophan metabolite indole acrylic acid (IAA) affected the levels of cellular HUFA and prostaglandin production. Indole acroyl glycine (IAG), a metabolite of IAA excreted in urine, is found in high concentrations in patients with neurodevelopmental disorders including ASD, ADHD, dyslexia, Asperger's syndrome and obsessive compulsive disorder.

Bello, S. C. (2007). "Autism and environmental influences: review and commentary." Rev Environ Health **22**(2): 139-56.

Progress has been slow in identifying pre- and post-natal environmental exposures that might trigger the features that characterize autism. During the past thirty years, research in the field of autism has been conducted in a setting in which diagnostic criteria for this condition have changed and broadened, and differences of opinion regarding diagnostic issues and diagnostic terminology

continue. The documented prevalence of all forms of autism has increased steadily during this time, suggesting one or more environmental contributors. Not established, however, is whether an increasing incidence of autism is responsible for increasing prevalence. The increase in documented prevalence could result from expanding and changing case definitions and increased reporting due to increased awareness on the part of professionals who work with children and by the public. This review provides a background for the evolving story of autism and describes the research on the relation between autism and the environment, with a particular focus on some of the more recently proposed environmental triggers. Critical analysis of this body of scientific research in a historical framework helps to explain the often controversial nature of the proposed relations between autism and environmental factors, as well as to rationalize some of the pitfalls in research design and in the often questionable interpretation of data so obtained.

Beversdorf, D. Q., S. E. Manning, et al. (2005). "Timing of prenatal stressors and autism." J Autism Dev Disord **35**(4): 471-8.

Recent evidence supports a role for genetics in autism, but other findings are difficult to reconcile with a purely genetic cause. Pathological changes in the cerebellum in autism are thought to correspond to an event before 30-32 weeks gestation. Our purpose was to determine whether there is an increased incidence of stressors in autism before this time period. Surveys regarding incidence and timing of prenatal stressors were distributed to specialized schools and clinics for autism and Down syndrome, and to mothers of children without neurodevelopmental diagnoses in walk-in clinics. Incidence of stressors during each 4-week block of pregnancy was recorded. Incidence of stressors in the blocks prior to and including the predicted time period (21-32 weeks gestation) in each group of surveys was compared to the other prenatal blocks. A higher incidence of prenatal stressors was found in autism at 21-32 weeks gestation, with a peak at 25-28 weeks. This does support the possibility of prenatal stressors as a potential contributor to autism, with the timing of stressors consistent with the embryological age suggested by neuroanatomical findings seen in the cerebellum in autism. Future prospective studies would be needed to confirm this finding.

Blaylock, R. (2003). "Interactions of cytokines, excitotoxins, and reactive nitrogen and oxygen species in autism spectrum disorders." J Amer Nutr Assoc **6**: 21-35.

Boadi, W. Y., L. Thaire, et al. (1991). "Effects of dietary factors on antioxidant enzymes in rats exposed to hyperbaric oxygen." Vet Hum Toxicol **33**(2): 105-9.

To delineate the effect of dietary supplementation with vitamin E (Vit E) alone or in combination with riboflavin (Rib) or selenium (Se) or both, on biological oxidative damage in rat brain and lungs we exposed rats to hyperbaric oxygen (HBO) and measured the activities of glutathione reductase (GSSG-R),

glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G-6-PD) prior to or 48 h after exposure. Rats fed the dietary supplements, and a control group maintained on an unsupplemented diet, for 30 d, were each divided into 2 subgroups, of which 1 was exposed to 4.5 absolute atmospheres (ATA) of 100% oxygen for 30 min, hereafter referred to as "exposed". The remaining subgroups were left unexposed. Pre-exposure GSSG-R activity in brain was elevated in all experimentally fed groups (ranging from 23 to 84%) compared with the unexposed control, whereas GSH-Px, G-6-PD and SOD activities were unchanged. The lungs showed significant increases in pre-exposure GSSG-R, ranging from 15 to 28%, and GSH-Px, ranging from 13 to 23%, activities in all the groups fed the supplemental nutrients, except those on Vit E alone. Increases in G-6-PD activity were observed only in those fed supplements of Rib. In most cases exposure to oxygen caused an increase in GSSG-R, GSH-Px and G-6-PD activities. However the increases were higher in the supplemented groups.(ABSTRACT TRUNCATED AT 250 WORDS)

Boso, M., E. Emanuele, et al. (2006). "Alterations of circulating endogenous secretory RAGE and S100A9 levels indicating dysfunction of the AGE-RAGE axis in autism." Neurosci Lett **410**(3): 169-73.

An excess accumulation of advanced glycation end products (AGEs) has been reported in autism brains. Through their interaction with their putative receptor RAGE, AGEs can promote neuroinflammation, oxidative stress and neuronal degeneration. To shed more light on the possible alterations of the AGEs-RAGE axis in autism, hereto we measured plasma levels of endogenous secretory RAGE (esRAGE) and its proinflammatory ligand S100A9 in 18 young adults with autistic spectrum disorder (ASD) and 18 age- and gender-matched healthy comparison subjects. The Childhood Autism Rating Scale (CARS) was used to assess the severity of autistic symptoms. Significantly reduced levels of esRAGE (P = 0.0023) and elevated concentrations of S100A9 (P = 0.0012) were found in ASD patients as compared to controls. In autistic patients, there was a statistically significant positive correlation between CARS scores and S100A9 levels (r = 0.49, P = 0.035), but no significant correlation was seen between esRAGE and S100A9 values (r = -0.23, P = 0.34). Our results of a significantly reduced peripheral level of esRAGE coupled with elevated S100A9 point to a subtle but definite dysfunction of the AGEs/RAGE axis in autism that could play a role in the pathophysiology of this disorder.

Bransfield, R. C., J. S. Wulfman, et al. (2007). "The association between tick-borne infections, Lyme borreliosis and autism spectrum disorders." Med Hypotheses.

Chronic infectious diseases, including tick-borne infections such as Borrelia burgdorferi may have direct effects, promote other infections and create a weakened, sensitized and immunologically vulnerable state during fetal development and infancy leading to increased vulnerability for developing autism spectrum disorders. A dysfunctional synergism with other predisposing and

contributing factors may contribute to autism spectrum disorders by provoking innate and adaptive immune reactions to cause and perpetuate effects in susceptible individuals that result in inflammation, molecular mimicry, kynurenine pathway changes, increased quinolinic acid and decreased serotonin, oxidative stress, mitochondrial dysfunction and excitotoxicity that impair the development of the amygdala and other neural structures and neural networks resulting in a partial Kluver-Bucy Syndrome and other deficits resulting in autism spectrum disorders and/or exacerbating autism spectrum disorders from other causes throughout life. Support for this hypothesis includes multiple cases of mothers with Lyme disease and children with autism spectrum disorders; fetal neurological abnormalities associated with tick-borne diseases; similarities between tick-borne diseases and autism spectrum disorder regarding symptoms, pathophysiology, immune reactivity, temporal lobe pathology, and brain imaging data; positive reactivity in several studies with autistic spectrum disorder patients for Borrelia burgdorferi (22%, 26% and 20-30%) and 58% for mycoplasma; similar geographic distribution and improvement in autistic symptoms from antibiotic treatment. It is imperative to research these and all possible causes of autism spectrum disorders in order to prevent every preventable case and treat every treatable case until this disease has been eliminated from humanity.

Chauhan, A. and V. Chauhan (2006). "Oxidative stress in autism." Pathophysiology **13**(3): 171-81.

Autism is a severe developmental disorder with poorly understood etiology. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipid peroxidation, detoxifying agents (such as glutathione), and antioxidants involved in the defense system against reactive oxygen species (ROS). Lipid peroxidation markers are elevated in autism, indicating that oxidative stress is increased in this disease. Levels of major antioxidant serum proteins, namely transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), are decreased in children with autism. There is a positive correlation between reduced levels of these proteins and loss of previously acquired language skills in children with autism. The alterations in ceruloplasmin and transferrin levels may lead to abnormal iron and copper metabolism in autism. The membrane phospholipids, the prime target of ROS, are also altered in autism. The levels of phosphatidylethanolamine (PE) are decreased, and phosphatidylserine (PS) levels are increased in the erythrocyte membrane of children with autism as compared to their unaffected siblings. Several studies have suggested alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in autism. Additionally, altered glutathione levels and homocysteine/methionine metabolism, increased inflammation, excitotoxicity, as well as mitochondrial and immune dysfunction have been suggested in autism. Furthermore, environmental and genetic factors may increase vulnerability to oxidative stress in autism. Taken together, these studies suggest increased oxidative stress in autism that

may contribute to the development of this disease. A mechanism linking oxidative stress with membrane lipid abnormalities, inflammation, aberrant immune response, impaired energy metabolism and excitotoxicity, leading to clinical symptoms and pathogenesis of autism is proposed.

Chauhan, A., V. Chauhan, et al. (2004). "Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin--the antioxidant proteins." Life Sci **75**(21): 2539-49.

Autism is a neurological disorder of childhood with poorly understood etiology and pathology. We compared lipid peroxidation status in the plasma of children with autism, and their developmentally normal non-autistic siblings by quantifying the levels of malonyldialdehyde, an end product of fatty acid oxidation. Lipid peroxidation was found to be elevated in autism indicating that oxidative stress is increased in this disease. Levels of major antioxidant proteins namely, transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein) in the serum, were significantly reduced in autistic children as compared to their developmentally normal non-autistic siblings. A striking correlation was observed between reduced levels of these proteins and loss of previously acquired language skills in children with autism. These results indicate altered regulation of transferrin and ceruloplasmin in autistic children who lose acquired language skills. It is suggested that such changes may lead to abnormal iron and copper metabolism in autism, and that increased oxidative stress may have pathological role in autism.

Chauhan, A., A. Sheikh, et al. (2008). "Increased copper-mediated oxidation of membrane phosphatidylethanolamine in autism." American Journal of Biochemistry and Biotechnology **4**(2): 95-100.

We have previously reported that levels of phosphatidylethanolamine (PE) in the erythrocyte membrane and of ceruloplasmin, a copper-transport antioxidant protein, in the serum are lower in children with autism than in control subjects. In the present study, we report that (a) copper oxidizes and reduces the levels of membrane PE and (b) copper-mediated oxidation of PE is higher in lymphoblasts from autistic subjects than from control subjects. The effect of copper was examined on the oxidation of liposomes composed of brain lipids from mice and also on the lymphoblasts from autism and control subjects. Among the various metal cations (copper, iron, calcium, cadmium and zinc), only copper was found to oxidize and decrease the levels of PE. The metal cations did not affect the levels of other phospholipids. The action of copper on PE oxidation was time-dependent and concentration-dependent. No difference was observed between copper-mediated oxidation of diacyl-PE and alkenyl-PE (plasmalogen), suggesting that plasmalogenic and non-plasmalogenic PE are equally oxidized by copper. Together, these studies suggest that ceruloplasmin and copper may contribute to oxidative stress and to reduced levels of membrane PE in autism.

Chauhan, V., A. Chauhan, et al. (2004). "Alteration in amino-glycerophospholipids levels in the plasma of children with autism: a potential biochemical diagnostic marker." Life Sci **74**(13): 1635-43.

Currently, there is no biochemical test to assist in the behavioral diagnosis of autism. We observed that levels of phosphatidylethanolamine (PE) were decreased while phosphatidylserine (PS) were increased in the erythrocyte membranes of children with autism as compared to their non-autistic developmentally normal siblings. A new method using Trinitrobenezene sulfonic acid (TNBS) for the quantification of PE and PS (amino-glycerophospholipids, i.e., AGP) in the plasma of children was developed and standardized. Wavelength scans of TNBS-PE and TNBS-PS complexes gave two peaks at 320 nm and 410 nm. When varying concentrations of PS and PE were used, a linear regression line was observed at 410 nm with TNBS. Using this assay, the levels of AGP were found to be significantly increased in the plasma of children with autism as compared to their non-autistic normal siblings. It is proposed that plasma AGP levels may function as a potential diagnostic marker for autism.

Chez, M. G., C. P. Buchanan, et al. (2002). "Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders." J Child Neurol **17**(11): 833-7.

L-Carnosine, a dipeptide, can enhance frontal lobe function or be neuroprotective. It can also correlate with gamma-aminobutyric acid (GABA)-homocarnosine interaction, with possible anticonvulsive effects. We investigated 31 children with autistic spectrum disorders in an 8-week, double-blinded study to determine if 800 mg L-carnosine daily would result in observable changes versus placebo. Outcome measures were the Childhood Autism Rating Scale, the Gilliam Autism Rating Scale, the Expressive and Receptive One-Word Picture Vocabulary tests, and Clinical Global Impressions of Change. Children on placebo did not show statistically significant changes. After 8 weeks on L-carnosine, children showed statistically significant improvements on the Gilliam Autism Rating Scale (total score and the Behavior, Socialization, and Communication subscales) and the Receptive One-Word Picture Vocabulary test (all $P < .05$). Improved trends were noted on other outcome measures. Although the mechanism of action of L-carnosine is not well understood, it may enhance neurologic function, perhaps in the enterorhinal or temporal cortex.

Corbett, B. A., S. Mendoza, et al. (2006). "Cortisol circadian rhythms and response to stress in children with autism." Psychoneuroendocrinology **31**(1): 59-68.

BACKGROUND: Autism is a severe neurodevelopmental disorder characterized by impairment in communication, social interaction, repetitive behaviors and difficulty adapting to novel experiences. The Hypothalamic-Pituitary-Adrenocortical (HPA) system responds consistently to perceived novel or unfamiliar situations and can serve as an important biomarker of the response to a variety of different stimuli. Previous research has suggested that children with

autism may exhibit dysfunction of the HPA system, but it is not clear whether altered neuroendrocrine regulation or altered responsiveness underlies the differences between children with and without autism. In order to provide preliminary data concerning HPA regulation and responsiveness, we compared circadian rhythms and response to a non-social, environmental stressor in children with and without autism. METHODS: Circadian rhythms of cortisol were estimated in children with (N=12) and without (N=10) autism via analysis of salivary samples collected in the morning, afternoon and evening on 2 consecutive days. HPA responsiveness was assessed by examining the time course of changes in salivary cortisol in response to a mock MRI. RESULTS: Both groups showed expected circadian variation with higher cortisol concentration in morning than in the evening samples. The children with autism, but not typical children, showed a more variable circadian rhythm as well as statistically significant elevations in cortisol following exposure to a novel, nonsocial stimulus. CONCLUSIONS: The results suggest that children with autism process and respond idiosyncratically to novel and threatening events resulting in an exaggerated cortisol response.

Danfors, T., A. L. von Knorring, et al. (2005). "Tetrahydrobiopterin in the treatment of children with autistic disorder: a double-blind placebo-controlled crossover study." J Clin Psychopharmacol **25**(5): 485-9.

Twelve children, all boys, aged 4 to 7 years, with a diagnosis of autistic disorder and low concentrations of spinal 6R-l-erythro-5,6,7,8-tetrahydrobiopterin (tetrahydrobiopterin) were selected to participate in a double-blind, randomized, placebo-controlled, crossover study. The children received a daily dose of 3 mg tetrahydrobiopterin per kilogram during 6 months alternating with placebo. Treatment-induced effects were assessed with the Childhood Autism Rating Scale every third month. The results showed small nonsignificant changes in the total scores of Childhood Autism Rating Scale after 3- and 6-month treatment. Post hoc analysis looking at the 3 core symptoms of autism, that is, social interaction, communication, and stereotyped behaviors, revealed a significant improvement of the social interaction score after 6 months of active treatment. In addition, a high positive correlation was found between response of the social interaction score and IQ. The results indicate a possible effect of tetrahydrobiopterin treatment.

Deth, R., C. Muratore, et al. (2008). "How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis." Neurotoxicology **29**(1): 190-201.

Recently higher rates of autism diagnosis suggest involvement of environmental factors in causing this developmental disorder, in concert with genetic risk factors. Autistic children exhibit evidence of oxidative stress and impaired methylation, which may reflect effects of toxic exposure on sulfur metabolism. We review the metabolic relationship between oxidative stress and methylation, with particular emphasis on adaptive responses that limit activity of cobalamin

and folate-dependent methionine synthase. Methionine synthase activity is required for dopamine-stimulated phospholipid methylation, a unique membrane-delimited signaling process mediated by the D4 dopamine receptor that promotes neuronal synchronization and attention, and synchrony is impaired in autism. Genetic polymorphisms adversely affecting sulfur metabolism, methylation, detoxification, dopamine signaling and the formation of neuronal networks occur more frequently in autistic subjects. On the basis of these observations, a "redox/methylation hypothesis of autism" is described, in which oxidative stress, initiated by environment factors in genetically vulnerable individuals, leads to impaired methylation and neurological deficits secondary to reductions in the capacity for synchronizing neural networks.

Dolske, M. C., J. Spollen, et al. (1993). "A preliminary trial of ascorbic acid as supplemental therapy for autism." Prog Neuropsychopharmacol Biol Psychiatry **17**(5): 765-74.

1. This study presents the results of a 30-week double-blind, placebo-controlled trial exploring the effectiveness of ascorbic acid (8g/70kg/day) as a supplemental pharmacological treatment for autistic children in residential treatment. 2. Residential school children (N = 18) were randomly assigned to either ascorbate-ascorbate-placebo treatment order group or ascorbate-placebo-ascorbate treatment order group. Each treatment phase lasted 10 weeks and behaviors were rated weekly using the Ritvo-Freeman scale. 3. Significant group by phase interactions were found for total scores and also sensory motor scores indicating a reduction in symptom severity associated with the ascorbic acid treatment. 4. These results were consistent with a hypothesized dopaminergic mechanism of action of ascorbic acid.

Evans, T. A., S. L. Siedlak, et al. (2008). "The autistic phenotype exhibits a remarkably localized modification of brain protein by products of free radical-induced lipid oxidation " American Journal of Biochemistry and Biotechnology **4**(2): 61-72.

Oxidative damage has been documented in the peripheral tissues of autism patients. In this study, we sought evidence of oxidative injury in autistic brain. Carboxyethyl pyrrole (CEP) and iso[4]levuglandin (iso[4]LG)E2-protein adducts, that are uniquely generated through peroxidation of docosahexaenoate and arachidonate-containing lipids respectively, and heme oxygenase-1 were detected immunocytochemically in cortical brain tissues and by ELISA in blood plasma. Significant immunoreactivity toward all three of these markers of oxidative damage in the white matter and often extending well into the grey matter of axons was found in every case of autism examined. This striking threadlike pattern appears to be a hallmark of the autistic brain as it was not seen in any control brain, young or aged, used as controls for the oxidative assays. Western blot and immunoprecipitation analysis confirmed neurofilament heavy chain to be a major target of CEP-modification. In contrast, in plasma from 27 autism spectrum disorder patients and 11 age-matched healthy controls

we found similar levels of plasma CEP (124.5 ± 57.9 versus 110.4 ± 30.3 pmol/mL), iso[4]LGE2 protein adducts (16.7 ± 5.8 versus 13.4 ± 3.4 nmol/mL), anti-CEP (1.2 ± 0.7 versus 1.2 ± 0.3) and anti-iso[4]LGE2 autoantibody titre (1.3 ± 1.6 versus 1.0 ± 0.9), and no differences between the ratio of NO2Tyr/Tyr (7.81 E-06 ± 3.29 E-06 versus 7.87 E-06 ± 1.62 E-06). These findings provide the first direct evidence of increased oxidative stress in the autistic brain. It seems likely that oxidative injury of proteins in the brain would be associated with neurological abnormalities and provide a cellular basis at the root of autism spectrum disorders.

Gargus, J. J. and F. Imtiaz (2008). "Mitochondrial energy-deficient endophenotype in autism." American Journal of Biochemistry and Biotechnology **4**(2): 198-207.

While evidence points to a multigenic etiology of most autism, the pathophysiology of the disorder has yet to be defined and the underlying genes and biochemical pathways they subserve remain unknown. Autism is considered to be influenced by a combination of various genetic, environmental and immunological factors; more recently, evidence has suggested that increased vulnerability to oxidative stress may be involved in the etiology of this multifactorial disorder. Furthermore, recent studies have pointed to a subset of autism associated with the biochemical endophenotype of mitochondrial energy deficiency, identified as a subtle impairment in fat and carbohydrate oxidation. This phenotype is similar, but more subtle than those seen in classic mitochondrial defects. In some cases the beginnings of the genetic underpinnings of these mitochondrial defects are emerging, such as mild mitochondrial dysfunction and secondary carnitine deficiency observed in the subset of autistic patients with an inverted duplication of chromosome 15q11-q13. In addition, rare cases of familial autism associated with sudden infant death syndrome (SIDS) or associated with abnormalities in cellular calcium homeostasis, such as malignant hyperthermia or cardiac arrhythmia, are beginning to emerge. Such special cases suggest that the pathophysiology of autism may comprise pathways that are directly or indirectly involved in mitochondrial energy production and to further probe this connection three new avenues seem worthy of exploration: 1) metabolomic clinical studies provoking controlled aerobic exercise stress to expand the biochemical phenotype, 2) high-throughput expression arrays to directly survey activity of the genes underlying these biochemical pathways and 3) model systems, either based upon neuronal stem cells or model genetic organisms, to discover novel genetic and environmental inputs into these pathways.

Jackson, M. J. and P. J. Garrod (1978). "Plasma zinc, copper, and amino acid levels in the blood of autistic children." J Autism Child Schizophr **8**(2): 203-8.

Plasma zinc, copper, and amino acid levels have been measured in a group of autistic children. All three variables were found to be normal. These findings are in disagreement with the previously reported results of some other workers but if

confirmed would indicate that autism cannot simply be attributed to a disorder of zinc metabolism.

James, S. J., P. Cutler, et al. (2004). "Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism." Am J Clin Nutr **80**(6): 1611-7.

BACKGROUND: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism. OBJECTIVE: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism. DESIGN: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children. RESULTS: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children. CONCLUSIONS: An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism.

James, S. J., S. Melnyk, et al. (2006). "Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism." Am J Med Genet B Neuropsychiatr Genet **141**(8): 947-56.

Autism is a behaviorally defined neurodevelopmental disorder usually diagnosed in early childhood that is characterized by impairment in reciprocal communication and speech, repetitive behaviors, and social withdrawal. Although both genetic and environmental factors are thought to be involved, none have been reproducibly identified. The metabolic phenotype of an individual reflects the influence of endogenous and exogenous factors on genotype. As such, it provides a window through which the interactive impact of genes and environment may be viewed and relevant susceptibility factors identified. Although abnormal methionine metabolism has been associated with other neurologic disorders, these pathways and related polymorphisms have not been

evaluated in autistic children. Plasma levels of metabolites in methionine transmethylation and transsulfuration pathways were measured in 80 autistic and 73 control children. In addition, common polymorphic variants known to modulate these metabolic pathways were evaluated in 360 autistic children and 205 controls. The metabolic results indicated that plasma methionine and the ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), an indicator of methylation capacity, were significantly decreased in the autistic children relative to age-matched controls. In addition, plasma levels of cysteine, glutathione, and the ratio of reduced to oxidized glutathione, an indication of antioxidant capacity and redox homeostasis, were significantly decreased. Differences in allele frequency and/or significant gene-gene interactions were found for relevant genes encoding the reduced folate carrier (RFC 80G > A), transcobalamin II (TCN2 776G > C), catechol-O-methyltransferase (COMT 472G > A), methylenetetrahydrofolate reductase (MTHFR 677C > T and 1298A > C), and glutathione-S-transferase (GST M1). We propose that an increased vulnerability to oxidative stress (endogenous or environmental) may contribute to the development and clinical manifestations of autism.

Johannesson, T., J. Kristinsson, et al. (2003). "[Neurodegenerative diseases, antioxidative enzymes and copper. A review of experimental research.]." Laeknabladid **89**(9): 659-671.

Introduction: In almost all degenerative diseases of the brain aggregation of proteins inside neurons or extracellulary, is a common pathological phenomenon regardless of etiology. It is assumed that the biochemical pathways leading to aggregation are more harmful than the aggregations themselves and most likely imply production of free oxygen radicals. This oxidative stress is in the body met by free radical scavengers in the form of specific chemical substances and antioxidative enzymes. It has therefore been postulated that defective free radical defense is a common pathway in most neurodegenerative diseases in humans as well as in other mammals. Material and methods: The concentration of copper and the activity of two antioxidative copper containing enzymes, ceruloplasmin and superoxide dismutase (SOD 1), was analyzed in the blood. A series of case control studies were performed in Alzheimer s disease (AD), Parkinson s disease (PD) and amyotrophic lateral sclerosis (ALS) as well as in Down s syndrome and autism. Furthermore, a study in sheep was conducted in different areas with different risks of infection of scrapie. In that study, in addition, the activity of the selenium-containing enzyme, glutathione peroxidase, was determined as well as the concentration of manganese in blood. Results: The oxidative activity of ceruloplasmin and SOD1 was shown to be significantly lowered in Alzheimer s disease without any signs of copper deficiency. In Parkinson s disease, the oxidative activity of ceruloplasmin was also on the whole shown to be signifcantly lowered, and furthermore, it decreased significantly as well as the SOD1 activity with duration of the disease. In ALS, the means of all of the determinations were shown to be the same, but the equality of variances

differed significantly in the patients compared to their controls. In Down s syndrome past the age of 40, when Alzheimer s type changes appear in the brain, the SOD1 activity and the ceruloplasmin specific oxidative activity (activity in relation to concentration) was significantly lowered compared with the younger patients. In autism, a non-degenerative affection of the central nervous system, there was no difference between patients and their controls. In the sheep, the results indicated a relationship between decreased glutathione peroxidase activity, and possibly also SOD1 activity, and increased susceptibility to scrapie infection. No connection was found between ceruloplasmin oxidative activity and susceptibility to scrapie infection. Susceptibility to scrapie infection was apparantly not conntected with low levels of copper or high levels of manganese in blood of the animals. Discussion: The results indicate that the oxidative defenses in four neurodegenerative diseases with different clinical features are defective as the activity of two copper containing antioxidative enzymes, ceruloplasmin and SOD1, was found defective in all of them. In a developmental syndrome (autism), where neither active degenerative changes nor aggregations are found, no such changes in enzyme activity were detected. The results thus support the idea that deranged oxidative defense is a common denominator in the pathogenesis of these diseases. As far as sheep is concerned, the results also indicate, that there is a defect in oxidative defense connected with increased susceptibility to scrapie infection in the form of lowered glutathione peroxidase activity.

Johnson, S. (2001). "Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson's disease?" Med Hypotheses **56**(5): 641-5.

Zinc has several crucial functions in brain development and maintenance: it binds to p53, preventing it from binding to supercoiled DNA and ensuring that p53 cause the expression of several paramount genes, such as the one that encodes for the type I receptors to pituitary adenine cylase-activator peptide (PACAP), which directs embryonic development of the brain cortex, adrenal glands, etc.; it is required for the production of CuZnSOD and Zn-thionein, which are essential to prevent oxidative damage; it is required for many proteins, some of them with Zn fingers, many of them essential enzymes for growth and homeostasis. For example, the synthesis of serotonin involves Zn enzymes and since serotonin is necessary for melatonin synthesis, a Zn deficiency may result in low levels of both hormones. Unfortunately, Zn levels tend to be low when there is excess Cu and Cd. Moreover, high estrogen levels tend to cause increased absorption of Cu and Cd, and smoking and eating food contaminated with Cd result in high levels of the latter. Furthermore, ethanol ingestion increases the elimination of Zn and Mg (which acts as a cofactor for CuZnSOD).Increased Cu levels may also be found in people with Wilson's disease, which is a rather rare disease. However, the heterozygote form (only one faulty copy of the chromosome) is not so rare. Therefore, the developing fetus of a pregnant women who is low in Zn and high in Cu may experience major difficulties in the early development of the brain,

which may later manifest themselves as schizophrenia, autism or epilepsy. Similarly, a person who gradually accumulates Cu, will tend to experience a gradual depletion of Zn, with a corresponding increase in oxidative damage, eventually leading to Parkinson's disease. Also discussed are the crucial roles of histidine, histamine, vitamin D, essential fatty acids, vitamin E, peroxynitrate, etc. in the possible oxidative damage involved in these mental diseases.

Jory, J. and W. R. McGinnis (2008). "Red-cell trace minerals in children with autism." American Journal of Biochemistry and Biotechnology **4**(2): 101-104.

Abnormalities in mineral-dependent antioxidant enzymes in children with autism raise interest in the determination of trace mineral status in this population. A cross sectional investigation of red cell mineral levels was carried out among 20 children with autism and 15 controls. Children with autism demonstrated significantly lower red cell selenium ($p<0.0006$) and higher molybdenum ($p<0.01$) than the controls. There was a trend toward lower red cell zinc and higher cobalt and vanadium, among the children with autism. There were no differences in red cell levels of chromium, copper, manganese, or magnesium. These findings confirm an earlier report of low red cell selenium in autism and support a role for decreased trace mineral status in oxidative stress in autism through alteration of selenium-dependent antioxidant enzymes and increased lipid peroxidation.

Junaid, M. A., D. Kowal, et al. (2004). "Proteomic studies identified a single nucleotide polymorphism in glyoxalase I as autism susceptibility factor." Am J Med Genet A **131**(1): 11-7.

Autism is a neurodevelopmental disability characterized by deficits in verbal communications, impairments in social interactions, and repetitive behaviors. Several studies have indicated strong involvement of multigenic components in the etiology of autism. Linkage analyses and candidate gene search approaches so far have not identified any reliable susceptibility genes. We are using a proteomic approach to identify protein abnormalities due to aberrant gene expression in autopsied autism brains. In four of eight autism brains, we have found an increase in polarity (more acidic) of glyoxalase I (Glo1) by two-dimensional gel electrophoresis. To identify the molecular change resulting in the shift of Glo1 polarity, we undertook sequencing of GLO1 gene. Direct sequencing of GLO1 gene/mRNA in these brains, has identified a single nucleotide polymorphism (SNP), C419A. The SNP causes an Ala111Glu change in the protein sequence. Population genetics of GLO1 C419A SNP studied in autism (71 samples) and normal and neurological controls (49 samples) showed significantly higher frequency for the A419 (allele frequency 0.6 in autism and 0.4 in controls, one-tailed Fisher's test $P < 0.0079$). Biochemical measurements have revealed a 38% decrease in Glo1 enzyme activity in autism brains (one-tailed t-test $P < 0.026$). Western blot analysis has also shown accumulation of advanced glycation

end products (AGE's) in autism brains. These data suggest that homozygosity for A419 GLO1 resulting in Glu111 is a predisposing factor in the etiology of autism.

Kern, J. K. and A. M. Jones (2006). "Evidence of toxicity, oxidative stress, and neuronal insult in autism." J Toxicol Environ Health B Crit Rev **9**(6): 485-99.

According to the Autism Society of America, autism is now considered to be an epidemic. The increase in the rate of autism revealed by epidemiological studies and government reports implicates the importance of external or environmental factors that may be changing. This article discusses the evidence for the case that some children with autism may become autistic from neuronal cell death or brain damage sometime after birth as result of insult; and addresses the hypotheses that toxicity and oxidative stress may be a cause of neuronal insult in autism. The article first describes the Purkinje cell loss found in autism, Purkinje cell physiology and vulnerability, and the evidence for postnatal cell loss. Second, the article describes the increased brain volume in autism and how it may be related to the Purkinje cell loss. Third, the evidence for toxicity and oxidative stress is covered and the possible involvement of glutathione is discussed. Finally, the article discusses what may be happening over the course of development and the multiple factors that may interplay and make these children more vulnerable to toxicity, oxidative stress, and neuronal insult.

Kinney, D. K., A. M. Miller, et al. (2008). "Autism prevalence following prenatal exposure to hurricanes and tropical storms in Louisiana." J Autism Dev Disord **38**(3): 481-8.

Hurricanes and tropical storms served as natural experiments for investigating whether autism is associated with exposure to stressful events during sensitive periods of gestation. Weather service data identified severe storms in Louisiana from 1980 to 1995 and parishes hit by storm centers during this period. Autism prevalences in different cohorts were calculated using anonymous data on birth dates and parishes of children diagnosed with autism in the state mental health system, together with corresponding census data on all live births in Louisiana. Prevalence increased in dose-response fashion with severity of prenatal storm exposure, especially for cohorts exposed near the middle or end of gestation ($p < 0.001$). Results complement other evidence that factors disrupting development during sensitive gestational periods may contribute to autism.

Kita, T., G. C. Wagner, et al. (2003). "Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption." J Pharmacol Sci **92**(3): 178-95.

Methamphetamine (METH)-induced neurotoxicity is characterized by a long-lasting depletion of striatal dopamine (DA) and serotonin as well as damage to striatal dopaminergic and serotonergic nerve terminals. Several hypotheses regarding the mechanism underlying METH-induced neurotoxicity have been proposed. In particular, it is thought that endogenous DA in the striatum may play an important role in mediating METH-induced neuronal damage. This

hypothesis is based on the observation of free radical formation and oxidative stress produced by auto-oxidation of DA consequent to its displacement from synaptic vesicles to cytoplasm. In addition, METH-induced neurotoxicity may be linked to the glutamate and nitric oxide systems within the striatum. Moreover, using knockout mice lacking the DA transporter, the vesicular monoamine transporter 2, c-fos, or nitric oxide synthetase, it was determined that these factors may be connected in some way to METH-induced neurotoxicity. Finally a role for apoptosis in METH-induced neurotoxicity has also been established including evidence of protection of bcl-2, expression of p53 protein, and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), activity of caspase-3. The neuronal damage induced by METH may reflect neurological disorders such as autism and Parkinson's disease.

Lombard, J. (1998). "Autism: a mitochondrial disorder?" Med Hypotheses **50**(6): 497-500.

Autism is a developmental disorder characterized by disturbance in language, perception and socialization. A variety of biochemical, anatomical and neuroradiographical studies imply a disturbance of brain energy metabolism in autistic patients. The underlying etiology of a disturbed bioenergetic metabolism in autism is unknown. A likely etiological possibility may involve mitochondrial dysfunction with concomitant defects in neuronal oxidative phosphorylation within the central nervous system. This hypothesis is supported by a frequent association of lactic acidosis and carnitine deficiency in autistic patients. Mitochondria are vulnerable to a wide array of endogenous and exogenous factors which appear to be linked by excessive nitric oxide production. Strategies to augment mitochondrial function, either by decreasing production of endogenous toxic metabolites, reducing nitric oxide production, or stimulating mitochondrial enzyme activity may be beneficial in the treatment of autism.

López-Hurtado, E. and J. J. Prieto (2008). "A microscopic study of language-related cortex in autism." American Journal of Biochemistry and Biotechnology **4**(2): 130-145.

Impaired language function is a principle criterion for the diagnosis of autism. The present study of brain from age-matched autistic and control subjects compared brain regions associated with the production and processing of speech. Wernicke's area (Brodmann 22, speech recognition), Broca's area (Brodmann 44, speech production) andthe gyrus angularis (Brodmann 39, reading) from autistic subjects (7-44 years of age) and control subjects (8-56 years of age) were examined microscopically. Striking differences in the density of glial cells, the density of neurons andthe number of lipofuscin-containing neurons were observed in the autistic group compared with the control group. The mean density of glial cells was greater in the autistic cohort than controls in area 22 ($p<0.001$), area 39 ($p<0.01$) andarea 44 ($p<0.05$). The density of neurons was lesser in autism in area 22 ($p<0.01$) and area 39 ($p<0.01$). The autistic group exhibited significantly greater numbers of lipofuscin-containing

cells in area 22 ($p<0.001$) and area 39 ($p<0.01$). The results are consistent with accelerated neuronal death in association with gliosis and lipofuscin accumulation in autism after age seven. Production of lipofuscin (a matrix of oxidized lipid and cross-linked protein more commonly associated with neurodegenerative disease) is accelerated under conditions of oxidative stress. Area 22 in autism evidenced the greatest glial increase, the greatest neuronal decrease and the greatest increase of non-specific cells containing lipofuscin, which itself may contribute to greater free-radical generation in brain.

MacFabe, D. F., D. P. Cain, et al. (2007). "Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders." Behav Brain Res **176**(1): 149-69.

Clinical observations suggest that certain gut and dietary factors may transiently worsen symptoms in autism spectrum disorders (ASD), epilepsy and some inheritable metabolic disorders. Propionic acid (PPA) is a short chain fatty acid and an important intermediate of cellular metabolism. PPA is also a by-product of a subpopulation of human gut enterobacteria and is a common food preservative. We examined the behavioural, electrophysiological, neuropathological, and biochemical effects of treatment with PPA and related compounds in adult rats. Intraventricular infusions of PPA produced reversible repetitive dystonic behaviours, hyperactivity, turning behaviour, retropulsion, caudate spiking, and the progressive development of limbic kindled seizures, suggesting that this compound has central effects. Biochemical analyses of brain homogenates from PPA treated rats showed an increase in oxidative stress markers (e.g., lipid peroxidation and protein carbonylation) and glutathione S-transferase activity coupled with a decrease in glutathione and glutathione peroxidase activity. Neurohistological examinations of hippocampus and adjacent white matter (external capsule) of PPA treated rats revealed increased reactive astrogliosis (GFAP immunoreactivity) and activated microglia (CD68 immunoreactivity) suggestive of a neuroinflammatory process. This was coupled with a lack of cytotoxicity (cell counts, cleaved caspase 3' immunoreactivity), and an increase in phosphorylated CREB immunoreactivity. We propose that some types of autism may be partial forms of genetically inherited or acquired disorders involving altered PPA metabolism. Thus, intraventricular administration of PPA in rats may provide a means to model some aspects of human ASD in rats.

MacFabe, D. F., K. Rodríguez-Capote, et al. (2008). "A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and oxidative stress in discrete regions of adult rat brain " American Journal of Biochemistry and Biotechnology **4**(2): 146-166.

Innate neuroinflammatory changes, increased oxidative stress and disorders of glutathione metabolism may be involved in the pathophysiology of autism spectrum disorders (ASD). Propionic acid (PPA) is a dietary and gut bacterial

short chain fatty acid which can produce brain and behavioral changes reminiscent of ASD following intraventricular infusion in rats. Adult Long-Evans rats were given intraventricular infusions of either PPA (500ug uL-1, 4µl animal-1) or phosphate buffered saline (PBS) vehicle, twice daily for 7 days. Immediately following the second daily infusion, the locomotor activity of each rat was assessed in an automated open field (Versamax) for 30 min. PPA-treated rats showed significant increases in locomotor activity compared to PBS vehicle controls. Following the last treatment day, specific brain regions were assessed for neuroinflammatory or oxidative stress markers. Immunohistochemical analyses revealed reactive astrogliosis (GFAP), activated microglia (CD68, Iba1) without apoptotic cell loss (Caspase 3 and NeuN) in hippocampus and white matter (external capsule) of PPA treated rats. Biomarkers of protein and lipid peroxidation, total glutathione (GSH) as well as the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) were examined in brain homogenates. Some brain regions of PPA treated animals (neocortex, hippocampus, thalamus, striatum) showed increased lipid and protein oxidation accompanied by decreased total GSH in neocortex. Catalase activity was decreased in most brain regions of PPA treated animals suggestive of reduced antioxidant enzymatic activity. GPx and GR activity was relatively unaffected by PPA treatment while GST was increased perhaps indicating involvement of GSH in the removal of PPA or related catabolites. Impairments in GSH and catalase levels may render CNS cells more susceptible to oxidative stress from a variety of toxic insults. Overall, these findings are consistent with those found in ASD patients and further support intraventricular PPA administration as an animal model of ASD.

McGinnis, W. R. (2004). "Oxidative stress in autism." Altern Ther Health Med **10**(6): 22-36; quiz 37, 92.

STATEMENT OF PURPOSE: Indirect markers are consistent with greater oxidative stress in autism. They include greater free-radical production, impaired energetics and cholinergics, and higher excitotoxic markers. Brain and gut, both abnormal in autism, are particularly sensitive to oxidative injury. Higher red-cell lipid peroxides and urinary isoprostanes in autism signify greater oxidative damage to biomolecules. A preliminary study found accelerated lipofuscin deposition--consistent with oxidative injury to autistic brain in cortical areas serving language and communication. Double-blind, placebo-controlled trials of potent antioxidants--vitamin C or carnosine--significantly improved autistic behavior. Benefits from these and other nutritional interventions may be due to reduction of oxidative stress. Understanding the role of oxidative stress may help illuminate the pathophysiology of autism, its environmental and genetic influences, new treatments, and prevention. OBJECTIVES: Upon completion of this article, participants should be able to: 1. Be aware of laboratory and clinical evidence of greater oxidative stress in autism. 2. Understand how gut, brain,

nutritional, and toxic status in autism are consistent with greater oxidative stress. 3. Describe how anti-oxidant nutrients are used in the contemporary treatment of autism.

McGinnis, W. R. (2005). "Oxidative stress in autism." Altern Ther Health Med **11**(1): 19.

McGinnis, W. R. (2007). "Could oxidative stress from psychosocial stress affect neurodevelopment in autism?" J Autism Dev Disord **37**(5): 993-4.

Miller, D. M. and J. S. Woods (1993). "Urinary porphyrins as biological indicators of oxidative stress in the kidney. Interaction of mercury and cephaloridine." Biochem Pharmacol **46**(12): 2235-41.

Reduced porphyrins (hexahydroporphyrins, porphyrinogens) are readily oxidized in vitro by free radicals which are known to mediate oxidative stress in tissue cells. To determine if increased urinary porphyrin concentrations may reflect oxidative stress to the kidney in vivo, we measured the urinary porphyrin content of rats treated with mercury as methyl mercury hydroxide (MMH) or cephaloridine, both nephrotoxic, oxidative stress-inducing agents. Rats exposed to MMH at 5 ppm in the drinking water for 4 weeks showed a 4-fold increase in 24-hr total urinary porphyrin content and a 1.3-fold increase in urinary malondialdehyde (MDA), an established measure of oxidative stress in vivo. Treatment with cephaloridine alone (10-500 mg/kg, i.p.) produced a dose-related increase in urinary MDA and total porphyrin levels up to 1.6 and 7 times control values, respectively. Injection of MMH-treated rats with cephaloridine (500 mg/kg) caused a synergistic (20-fold) increase in urinary porphyrin levels, but an additive (1.9-fold) increase in the MDA concentration. Studies in vitro demonstrated that cephaloridine stimulated the iron-catalyzed H2O2-dependent oxidation of porphyrinogens to porphyrins in the absence of either microsomes or mitochondria. Additionally, porphyrinogens were oxidized to porphyrins in an iron-dependent microsomal lipid peroxidation system. Moreover, porphyrinogens served as an effective antioxidant (EC50 approximately 1-2 microM) to lipid peroxidation. These results demonstrate that MMH and cephaloridine synergistically, as well as individually, promote increased oxidation of reduced porphyrins in the kidney and that this action may be mechanistically linked to oxidative stress elicited by these chemicals. Increased urinary porphyrin levels may, therefore, represent a sensitive indicator of oxidative stress in the kidney in vivo.

Ming, X., M. A. Cheh, et al. (2008). "Evidence of oxidative stress in autism derived from animal models." American Journal of Biochemistry and Biotechnology **4**(2): 218-225.

Autism is a pervasive neurodevelopmental disorder that leads to deficits in social interaction, communication and restricted, repetitive motor movements. Autism is a highly heritable disorder, however, there is mounting evidence to suggest that toxicant-induced oxidative stress may play a role. The focus of this article

will be to review our animal model of autism and discuss our evidence that oxidative stress may be a common underlying mechanism of neurodevelopmental damage. We have shown that mice exposed to either methylmercury (MeHg) or valproic acid (VPA) in early postnatal life display aberrant social, cognitive and motor behavior. Interestingly, early exposure to both compounds has been clinically implicated in the development of autism. We recently found that Trolox, a water-soluble vitamin E derivative, is capable of attenuating a number of neurobehavioral alterations observed in mice postnatally exposed to MeHg. In addition, a number of other investigators have shown that oxidative stress plays a role in neural injury following MeHg exposure both in vitro and in vivo. New data presented here will show that VPA-induced neurobehavioral deficits are attenuated by vitamin E as well and that the level of glial fibrillary acidic protein (GFAP), a marker of astrocytic neural injury, is altered following VPA exposure. Collectively, these data indicate that vitamin E and its derivative are capable of protecting against neurobehavioral deficits induced by both MeHg and VPA. This antioxidant protection suggests that oxidative stress may be a common mechanism of injury leading to aberrant behavior in both our animal model as well as in the human disease state.

Ming, X., T. P. Stein, et al. (2005). "Increased excretion of a lipid peroxidation biomarker in autism." Prostaglandins Leukot Essent Fatty Acids **73**(5): 379-84.

It is thought that autism could result from an interaction between genetic and environmental factors with oxidative stress as a potential mechanism linking the two. One genetic factor may be altered oxidative-reductive capacity. This study tested the hypothesis that children with autism have increased oxidative stress. We evaluated children with autism for the presence of two oxidative stress biomarkers. Urinary excretion of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-isoprostane-F2alpha (8-iso-PGF2alpha) were determined in 33 children with autism and 29 healthy controls. 8-iso-PGF2alpha levels were significantly higher in children with autism. The isoprostane levels in autistic subjects were variable with a bimodal distribution. The majority of autistic subjects showed a moderate increase in isoprostane levels while a smaller group of autistic children showed dramatic increases in their isoprostane levels. There was a trend of an increase in 8-OHdG levels in children with autism but it did not reach statistical significance. There was no significant correlation between the levels of the biomarkers and vitamin intake, dietary supplements, medicine, medical disorders, or history of regression. These results suggest that the lipid peroxidation biomarker is increased in this cohort of autistic children, especially in the subgroup of autistic children.

Mutter, J., J. Naumann, et al. (2005). "Mercury and autism: accelerating evidence?" Neuro Endocrinol Lett **26**(5): 439-46.

The causes of autism and neurodevelopmental disorders are unknown. Genetic and environmental risk factors seem to be involved. Because of an observed

increase in autism in the last decades, which parallels cumulative mercury exposure, it was proposed that autism may be in part caused by mercury. We review the evidence for this proposal. Several epidemiological studies failed to find a correlation between mercury exposure through thimerosal, a preservative used in vaccines, and the risk of autism. Recently, it was found that autistic children had a higher mercury exposure during pregnancy due to maternal dental amalgam and thimerosal-containing immunoglobulin shots. It was hypothesized that children with autism have a decreased detoxification capacity due to genetic polymorphism. In vitro, mercury and thimerosal in levels found several days after vaccination inhibit methionine synthetase (MS) by 50%. Normal function of MS is crucial in biochemical steps necessary for brain development, attention and production of glutathione, an important antioxidative and detoxifying agent. Repetitive doses of thimerosal leads to neurobehavioral deteriorations in autoimmune susceptible mice, increased oxidative stress and decreased intracellular levels of glutathione in vitro. Subsequently, autistic children have significantly decreased level of reduced glutathione. Promising treatments of autism involve detoxification of mercury, and supplementation of deficient metabolites.

Nataf, R., C. Skorupka, et al. (2006). "Porphyrinuria in childhood autistic disorder: implications for environmental toxicity." Toxicol Appl Pharmacol **214**(2): 99-108.

To address a possible environmental contribution to autism, we carried out a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 269 children with neurodevelopmental and related disorders referred to a Paris clinic (2002-2004), including 106 with autistic disorder. Urinary porphyrin levels determined by high-performance liquid chromatography were compared between diagnostic groups including internal and external control groups. Coproporphyrin levels were elevated in children with autistic disorder relative to control groups. Elevation was maintained on normalization for age or to a control heme pathway metabolite (uroporphyrin) in the same samples. The elevation was significant ($P < 0.001$). Porphyrin levels were unchanged in Asperger's disorder, distinguishing it from autistic disorder. The atypical molecule precoproporphyrin, a specific indicator of heavy metal toxicity, was also elevated in autistic disorder ($P < 0.001$) but not significantly in Asperger's. A subgroup with autistic disorder was treated with oral dimercaptosuccinic acid (DMSA) with a view to heavy metal removal. Following DMSA there was a significant ($P = 0.002$) drop in urinary porphyrin excretion. These data implicate environmental toxicity in childhood autistic disorder.

Ng, F., M. Berk, et al. (2008). "Oxidative stress in psychiatric disorders: evidence base and therapeutic implications." Int J Neuropsychopharmacol: 1-26.

Oxidative stress has been implicated in the pathogenesis of diverse disease states, and may be a common pathogenic mechanism underlying many major psychiatric disorders, as the brain has comparatively greater vulnerability to

oxidative damage. This review aims to examine the current evidence for the role of oxidative stress in psychiatric disorders, and its academic and clinical implications. A literature search was conducted using the Medline, Pubmed, PsycINFO, CINAHL PLUS, BIOSIS Previews, and Cochrane databases, with a time-frame extending to September 2007. The broadest data for oxidative stress mechanisms have been derived from studies conducted in schizophrenia, where evidence is available from different areas of oxidative research, including oxidative marker assays, psychopharmacology studies, and clinical trials of antioxidants. For bipolar disorder and depression, a solid foundation for oxidative stress hypotheses has been provided by biochemical, genetic, pharmacological, preclinical therapeutic studies and one clinical trial. Oxidative pathophysiology in anxiety disorders is strongly supported by animal models, and also by human biochemical data. Pilot studies have suggested efficacy of N-acetylcysteine in cocaine dependence, while early evidence is accumulating for oxidative mechanisms in autism and attention deficit hyperactivity disorder. In conclusion, multi-dimensional data support the role of oxidative stress in diverse psychiatric disorders. These data not only suggest that oxidative mechanisms may form unifying common pathogenic pathways in psychiatric disorders, but also introduce new targets for the development of therapeutic interventions.

Padhye, U. (2003). "Excess dietary iron is the root cause for increase in childhood autism and allergies." Med Hypotheses **61**(2): 220-2.

Autism is a profoundly and poorly understood developmental disorder that impairs a person's social and communication abilities. I propose a hypothesis that the excessive dietary iron consumed by today's infants is the root cause of increased cases of Autism, allergies and other childhood diseases. Iron is a powerful immune system modulator. Excess iron causes hyperactive immune system. This hyperactive immune system attacks undigested food peptides. The chemicals released during these intense allergic reactions can damage surrounding tissue. Neurodegeneration is caused by combination of, oxidative stress induced by free iron radicals and intense immune reactions. Iron chelators have shown beneficial results in Autism and allergies.

Pardo, C. A. and C. G. Eberhart (2007). "The neurobiology of autism." Brain Pathol **17**(4): 434-47.

Improving clinical tests are allowing us to more precisely classify autism spectrum disorders and diagnose them at earlier ages. This raises the possibility of earlier and potentially more effective therapeutic interventions. To fully capitalize on this opportunity, however, will require better understanding of the neurobiological changes underlying this devastating group of developmental disorders. It is becoming clear that the normal trajectory of neurodevelopment is altered in autism, with aberrations in brain growth, neuronal patterning and cortical connectivity. Changes to the structure and function of synapses and dendrites have also been strongly implicated in the pathology of autism by

morphological, genetic and animal modeling studies. Finally, environmental factors are likely to interact with the underlying genetic profile, and foster the clinical heterogeneity seen in autism spectrum disorders. In this review we attempt to link the molecular pathways altered in autism to the neurodevelopmental and clinical changes that characterize the disease. We focus on signaling molecules such as neurotrophin, Reelin, PTEN and hepatocyte growth factor, neurotransmitters such as serotonin and glutamate, and synaptic proteins such as neurexin, SHANK and neuroligin. We also discuss evidence implicating oxidative stress, neuroglial activation and neuroimmunity in autism.

Pasca, S. P., B. Nemes, et al. (2006). "High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism." Life Sci **78**(19): 2244-8.

Autism is a behaviorally defined disorder of unknown etiology that is thought to be influenced by genetic and environmental factors. High levels of homocysteine and oxidative stress are generally associated with neuropsychiatric disorders. The purpose of this study was to compare the level of homocysteine and other biomarkers in children with autism to corresponding values in age-matched healthy children. We measured total homocysteine (tHcy), vitamin B(12), paraoxonase and arylesterase activities of human paraoxonase 1 (PON1) in plasma and glutathione peroxidase (GPx) activity in erythrocytes from 21 children: 12 with autism (age: 8.29 +/- 2.76 years) and 9 controls (age: 8.33 +/- 1.82 years). We found statistically significant differences in tHcy levels and in arylesterase activity of PON1 in children with autism compared to the control group: 9.83 +/- 2.75 vs. 7.51 +/- 0.93 micromol/L (P < or =0.01) and 72.57 +/- 11.73 vs. 81.83 +/- 7.39 kU/L (P < or =0.005). In the autistic group there was a strong negative correlation between tHcy and GPx activity and the vitamin B(12) level was low or suboptimal. In conclusion, our study shows that in children with autism there are higher levels of tHcy, which is negatively correlated with GPx activity, low PON1 arylesterase activity and suboptimal levels of vitamin B(12).

Poling, J. S., R. E. Frye, et al. (2006). "Developmental regression and mitochondrial dysfunction in a child with autism." J Child Neurol **21**(2): 170-2.

Autistic spectrum disorders can be associated with mitochondrial dysfunction. We present a singleton case of developmental regression and oxidative phosphorylation disorder in a 19-month-old girl. Subtle abnormalities in the serum creatine kinase level, aspartate aminotransferase, and serum bicarbonate led us to perform a muscle biopsy, which showed type I myofiber atrophy, increased lipid content, and reduced cytochrome c oxidase activity. There were marked reductions in enzymatic activities for complex I and III. Complex IV (cytochrome c oxidase) activity was near the 5% confidence level. To determine the frequency of routine laboratory abnormalities in similar patients, we performed a retrospective study including 159 patients with autism (Diagnostic and Statistical Manual of Mental Disorders-IV and Childhood Autism Rating Scale) not previously diagnosed with metabolic disorders and 94 age-matched controls

with other neurologic disorders. Aspartate aminotransferase was elevated in 38% of patients with autism compared with 15% of controls (P <.0001). The serum creatine kinase level also was abnormally elevated in 22 (47%) of 47 patients with autism. These data suggest that further metabolic evaluation is indicated in autistic patients and that defects of oxidative phosphorylation might be prevalent.

Rose, S., S. Melnyk, et al. (2008). "The frequency of polymorphisms affecting lead and mercury toxicity among children with autism." American Journal of Biochemistry and Biotechnology **4**(2): 85-94.

Individual risk of developmental neurotoxicity with exposure to environmentally relevant levels of lead and mercury is likely to be determined by genetic susceptibility factors as well as additive interactions with other environmental pollutants, cumulative dose, and the developmental stage of exposure. The apparent increase in autism diagnosis over the last 15 years has enhanced interest in the possibility that an environmental trigger may be required to uncover the genetic liability in some cases of autism. The exquisite sensitivity of the developing brain and immune system to very low levels of lead and mercury give this hypothesis biologic plausibility. Delta aminolevulinic acid dehydratase (ALAD) and coproporphyin oxidase (CPOX) are two enzymes inhibited by low levels of lead and mercury, respectively. Common polymorphisms in these genes have been associated with elevated blood levels of lead and mercury and could potentially increase vulnerability to prenatal and/or postnatal developmental neurotoxicity. To explore this possibility, the frequency of the ALAD2 variant and variants in CPOX-4 and CPOX-5 were evaluated in 450 autistic children and 251 unaffected controls. A significant increase in the frequency of the ALAD2 allele was observed; however, contrary to our hypothesis, the frequency of both CPOX variants was significantly lower among the autistic children. Both lead and mercury induce oxidative stress by depleting the major intracellular antioxidant, glutathione. Among 242 autistic children with the variant ALAD2 allele, significant decreases in plasma glutathione and in the glutathione redox ratio were observed. These results suggest that children with autism who inherit the ALAD2 allele with lower glutathione levels may be at increased risk for lead toxicity during prenatal and postnatal neurodevelopment.

Ross, M. A. (2000). "Could oxidative stress be a factor in neurodevelopmental disorders?" Prostaglandins Leukot Essent Fatty Acids **63**(1-2): 61-3.

There is evidence of co-morbidity in the neurodevelopmental disorders and they display depletion of polyunsaturated fatty acids (PUFAs) in their plasma and red cell membranes. This suggests an abnormal fatty acid metabolism, which may affect cell signalling and synthesis of eicosanoids. This common feature in the neurodevelopmental disorders may be genetic in origin: however, oxidative stress may also contribute to decreased PUFAs found in these disorders.

Rossignol, D. A. (2007). "Hyperbaric oxygen therapy might improve certain pathophysiological findings in autism." Med Hypotheses **68**(6): 1208-27.

Autism is a neurodevelopmental disorder currently affecting as many as 1 out of 166 children in the United States. Numerous studies of autistic individuals have revealed evidence of cerebral hypoperfusion, neuroinflammation and gastrointestinal inflammation, immune dysregulation, oxidative stress, relative mitochondrial dysfunction, neurotransmitter abnormalities, impaired detoxification of toxins, dysbiosis, and impaired production of porphyrins. Many of these findings have been correlated with core autistic symptoms. For example, cerebral hypoperfusion in autistic children has been correlated with repetitive, self-stimulatory and stereotypical behaviors, and impairments in communication, sensory perception, and social interaction. Hyperbaric oxygen therapy (HBOT) might be able to improve each of these problems in autistic individuals. Specifically, HBOT has been used with clinical success in several cerebral hypoperfusion conditions and can compensate for decreased blood flow by increasing the oxygen content of plasma and body tissues. HBOT has been reported to possess strong anti-inflammatory properties and has been shown to improve immune function. There is evidence that oxidative stress can be reduced with HBOT through the upregulation of antioxidant enzymes. HBOT can also increase the function and production of mitochondria and improve neurotransmitter abnormalities. In addition, HBOT upregulates enzymes that can help with detoxification problems specifically found in autistic children. Dysbiosis is common in autistic children and HBOT can improve this. Impaired production of porphyrins in autistic children might affect the production of heme, and HBOT might help overcome the effects of this problem. Finally, HBOT has been shown to mobilize stem cells from the bone marrow to the systemic circulation. Recent studies in humans have shown that stem cells can enter the brain and form new neurons, astrocytes, and microglia. It is expected that amelioration of these underlying pathophysiological problems through the use of HBOT will lead to improvements in autistic symptoms. Several studies on the use of HBOT in autistic children are currently underway and early results are promising.

Rossignol, D. A. and J. J. Bradstreet (2008). "Evidence of mitochondrial dysfunction in autism and implications for treatment." American Journal of Biochemistry and Biotechnology **4**(2): 208-217.

Classical mitochondrial diseases occur in a subset of individuals with autism and are usually caused by genetic anomalies or mitochondrial respiratory pathway deficits. However, in many cases of autism, there is evidence of mitochondrial dysfunction (MtD) without the classic features associated with mitochondrial disease. MtD appears to be more common in autism and presents with less severe signs and symptoms. It is not associated with discernable mitochondrial pathology in muscle biopsy specimens despite objective evidence of lowered mitochondrial functioning. Exposure to environ-mental toxins is the likely etiology for MtD in autism. This dysfunction then contributes to a number of diagnostic

symptoms and comorbidities observed in autism including: cognitive impairment, language deficits, abnormal energy metabolism, chronic gastrointestinal problems, abnormalities in fatty acid oxidation, and increased oxidative stress. MtD and oxidative stress may also explain the high male to female ratio found in autism due to increased male vulnerability to these dysfunctions. Biomarkers for mitochondrial dysfunction have been identified, but seem widely under-utilized despite available therapeutic interventions. Nutritional supplementation to decrease oxidative stress along with factors to improve reduced glutathione, as well as hyperbaric oxygen therapy (HBOT) represent supported and rationale approaches. The underlying pathophysiology and autistic symptoms of affected individuals would be expected to either improve or cease worsening once effective treatment for MtD is implemented.

Rossignol, D. A. and L. W. Rossignol (2006). "Hyperbaric oxygen therapy may improve symptoms in autistic children." Med Hypotheses **67**(2): 216-28.

Autism is a neurodevelopmental disorder that currently affects as many as 1 out of 166 children in the United States. Recent research has discovered that some autistic individuals have decreased cerebral perfusion, evidence of neuroinflammation, and increased markers of oxidative stress. Multiple independent single photon emission computed tomography (SPECT) and positron emission tomography (PET) research studies have revealed hypoperfusion to several areas of the autistic brain, most notably the temporal regions and areas specifically related to language comprehension and auditory processing. Several studies show that diminished blood flow to these areas correlates with many of the clinical features associated with autism including repetitive, self-stimulatory and stereotypical behaviors, and impairments in communication, sensory perception, and social interaction. Hyperbaric oxygen therapy (HBOT) has been used with clinical success in several cerebral hypoperfusion syndromes including cerebral palsy, fetal alcohol syndrome, closed head injury, and stroke. HBOT can compensate for decreased blood flow by increasing the oxygen content of plasma and body tissues and can even normalize oxygen levels in ischemic tissue. In addition, animal studies have shown that HBOT has potent anti-inflammatory effects and reduces oxidative stress. Furthermore, recent evidence demonstrates that HBOT mobilizes stem cells from human bone marrow, which may aid recovery in neurodegenerative diseases. Based upon these findings, it is hypothesized that HBOT will improve symptoms in autistic individuals. A retrospective case series is presented that supports this hypothesis.

Rossignol, D. A., L. W. Rossignol, et al. (2007). "The effects of hyperbaric oxygen therapy on oxidative stress, inflammation, and symptoms in children with autism: an open-label pilot study." BMC Pediatr **7**(1): 36.

ABSTRACT: BACKGROUND: Recently, hyperbaric oxygen therapy (HBOT) has increased in popularity as a treatment for autism. Numerous studies document oxidative stress and inflammation in individuals with autism; both of these

conditions have demonstrated improvement with HBOT, along with enhancement of neurological function and cognitive performance. In this study, children with autism were treated with HBOT at atmospheric pressures and oxygen concentrations in current use for this condition. Changes in markers of oxidative stress and inflammation were measured. The children were evaluated to determine clinical effects and safety. METHODS: Eighteen children with autism, ages 3-16 years, underwent 40 hyperbaric sessions of 45 minutes duration each at either 1.5 atmospheres (atm) and 100% oxygen, or at 1.3 atm and 24% oxygen. Measurements of C-reactive protein (CRP) and markers of oxidative stress, including plasma oxidized glutathione (GSSG), were assessed by fasting blood draws collected before and after the 40 treatments. Changes in clinical symptoms, as rated by parents, were also assessed. The children were closely monitored for potential adverse effects. RESULTS: At the endpoint of 40 hyperbaric sessions, neither group demonstrated statistically significant changes in mean plasma GSSG levels, indicating intracellular oxidative stress appears unaffected by either regimen. A trend towards improvement in mean CRP was present in both groups; the largest improvements were observed in children with initially higher elevations in CRP. When all 18 children were pooled, a significant improvement in CRP was found ($p = 0.021$). Pre- and post-parental observations indicated statistically significant improvements in both groups, including motivation, speech, and cognitive awareness ($p < 0.05$). No major adverse events were observed. CONCLUSIONS: In this prospective pilot study of children with autism, HBOT at a maximum pressure of 1.5 atm with up to 100% oxygen was safe and well tolerated. HBOT did not appreciably worsen oxidative stress and significantly decreased inflammation as measured by CRP levels. Parental observations support anecdotal accounts of improvement in several domains of autism. However, since this was an open-label study, definitive statements regarding the efficacy of HBOT for the treatment of individuals with autism must await results from double-blind, controlled trials. Trial Registration: clinicaltrials.gov NCT00324909.

Sajdel-Sulkowska, E. M., B. Lipinski, et al. (2008). "Oxidative stress in autism: elevated cerebellar 3-nitrotyrosine levels." American Journal of Biochemistry and Biotechnology **4**(2): 73-84.

It has been suggested that oxidative stress and/or mercury compounds play an important role in the pathophysiology of autism. This study compared for the first time the cerebellar levels of the oxidative stress marker 3-nitrotyrosine (3-NT), mercury (Hg) and the antioxidant selenium (Se) levels between control and autistic subjects. Tissue homogenates were prepared in the presence of protease inhibitors from the frozen cerebellar tissue of control (n=10; mean age, 15.5 years; mean PMI, 15.5 hours) and autistic (n=9; mean age 12.1 years; mean PMI, 19.3 hours) subjects. The concentration of cerebellar 3-NT, determined by ELISA, in controls ranged from 13.69 to 49.04 pmol g^{-1} of tissue; the concentration of 3-NT in autistic cases ranged from 3.91 to 333.03 pmol g^{-1} of

tissue. Mean cerebellar 3-NT was elevated in autism by 68.9% and the increase was statistically significant (p=0.045). Cerebellar Hg, measured by atomic absorption spectrometry ranged from 0.9 to 35 pmol $g^{-}1$ tissue in controls (n=10) and from 3.2 to 80.7 pmol $g^{-}1$ tissue in autistic cases (n=9); the 68.2% increase in cerebellar Hg was not statistically significant. However, there was a positive correlation between cerebellar 3-NT and Hg levels (r=0.7961, p=0.0001). A small decrease in cerebellar Se levels in autism, measured by atomic absorption spectroscopy, was not statistically significant but was accompanied by a 42.9% reduction in the molar ratio of Se to Hg in the autistic cerebellum. While preliminary, the results of the present study add elevated oxidative stress markers in brain to the growing body of data reflecting greater oxidative stress in autism.

Sierra, C., M. A. Vilaseca, et al. (2001). "Oxidative stress in Rett syndrome." Brain Dev **23 Suppl 1**: S236-9.

The investigation of parameters that might influence the neurological evolution of Rett syndrome might also yield new information about its pathogenic mechanisms. Oxidative stress caused by oxygen free radicals is involved in the neuropathology of several neurodegenerative disorders, as well as in stroke and seizures. To evaluate the free radical metabolism in Rett syndrome, we measured red blood cell antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) and plasma malondialdehyde, as lipid peroxidation marker in a group of patients with Rett syndrome. No significant differences were observed in erythrocyte glutathione peroxidase, glutathione reductase and catalase activities, between the Rett syndrome patients and the control group. Erythrocyte superoxide dismutase activities were significantly decreased in Rett syndrome patients (P<0.001) compared with the control group. Plasma malondialdehyde concentrations were significantly increased in Rett syndrome patients (P<0.001). An unbalanced nutritional status in Rett syndrome might explain the reduced enzyme activity found in these patients. Our results suggest that free radicals generated from oxidation reactions might contribute to the pathogenesis of Rett syndrome. The high levels of malondialdehyde reflect peroxidative damage of biomembranes that may contribute to progressive dementia, impaired motor function, behavioural changes, and seizures, in Rett syndrome. We found a probable relationship between the degree of oxidative stress and the severity of symptoms, which should be further investigated with a larger number of patients in different disease stages.

Sogut, S., S. S. Zoroglu, et al. (2003). "Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism." Clin Chim Acta **331**(1-2): 111-7.

BACKGROUND: There is evidence that oxygen free radicals play an important role in the pathophysiology of many neuropsychiatric disorders. Although it has

not been investigated yet, several recent studies proposed that nitric oxide (NO) and other parameters related to oxidative stress may have a pathophysiological role in autism. METHODS: We assessed the changes in superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and thiobarbituric acid-reactive substances (TBARS) levels in plasma as well as NO levels in red blood cells (RBC) in patients with autism (n=27) compared to age- and sex-matched normal controls (n=30). RESULTS: In the autistic group, increased RBC NO levels ($p<0.0001$) and plasma GSH-Px activity ($p<0.0001$) and unchanged plasma TBARS levels and SOD activity were detected. CONCLUSIONS: These findings indicate a possible role of increased oxidative stress and altered enzymatic antioxidants, both of which may be relevant to the pathophysiology of autism.

Sokol, D. K., D. Chen, et al. (2006). "High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression." J Child Neurol **21**(6): 444-9.

Autism is characterized by restricted, repetitive behaviors and impairment in socialization and communication. Although no neuropathologic substrate underlying autism has been found, the findings of brain overgrowth via neuroimaging studies and increased levels of brain-derived neurotrophic factor (BDNF) in neuropathologic and blood studies favor an anabolic state. We examined acetylcholinesterase, plasma neuronal proteins, secreted beta-amyloid precursor protein (APP), and amyloid-beta 40 and amyloid-beta 42 peptides in children with and without autism. Children with severe autism and aggression expressed secreted beta-amyloid precursor protein at two or more times the levels of children without autism and up to four times more than children with mild autism. There was a trend for children with autism to show higher levels of secreted beta-amyloid precursor protein and nonamyloidogenic secreted beta-amyloid precursor protein and lower levels of amyloid-beta 40 compared with controls. This favors an increased alpha-secretase pathway in autism (anabolic), opposite to what is seen in Alzheimer disease. Additionally, a complex relationship between age, acetylcholinesterase, and plasma neuronal markers was found.

Suh, J. H., W. J. Walsh, et al. (2008). "Altered sulfur amino acid metabolism in immune cells of children diagnosed with autism " American Journal of Biochemistry and Biotechnology **4**(2): 105-113.

Autism Spectrum Disorder (ASD) is a behaviorally defined neurodevelopmental disorder whose etiology is poorly understood. Recent studies have shown that autistic children may be experiencing increased inflammation and oxidative stress. Altered immune regulation may be one contributing factor to inflammation and oxidative stress in autistic children. Sulfur amino acid (SAA) metabolism plays a critical role in regulating blood leukocyte functions and oxidative stress. However, it is not known whether autism impacts SAA metabolism in peripheral immune cells. To address this question, a novel liquid

chromatography linked tandem mass spectrometric (LC/MS/MS) method was used to determine the levels of SAA metabolites in peripheral blood mononuclear cells obtained from 11 healthy controls and 31 autistic children. Improved detection sensitivity and selectivity of the LC/MS/MS method allowed accurate quantification using small samples. Results show that leukocytes from autistic children contained significantly lower concentrations of S-adenosylmethionine (-35%; p = 0.01), and elevated levels of intracellular homocysteine content (+80%; p=0.003). Additionally, the levels of intracellular total cysteine and glutathione (GSH) were reduced by 39% (p=0.004) and 25% (p=0.01), respectively. These autism-associated changes were leukocyte specific in that no significant alterations in SAA metabolite concentrations were detected in the plasma samples. Our results provide novel evidence for altered metabolism in immune cells; furthermore, this data suggest the involvement of inflammation in autism. Dietary differences between controls and patients, however, remain a potential confounder.

Sweeten, T. L., D. J. Posey, et al. (2003). "High blood monocyte counts and neopterin levels in children with autistic disorder." Am J Psychiatry **160**(9): 1691-3.

OBJECTIVE: Leukocyte counts and plasma neopterin levels were determined in autistic children and matched healthy comparison subjects. METHOD: Blood from 31 autistic children and 28 age- and gender-matched healthy comparison subjects was analyzed for numbers of neutrophils, eosinophils, basophils, lymphocytes, monocytes, and total leukocytes and for plasma neopterin levels. RESULTS: The monocyte count and neopterin level were significantly higher in the autistic children than in the comparison subjects. CONCLUSIONS: These results suggest that the immune system may be activated in some children with autism.

Sweeten, T. L., D. J. Posey, et al. (2004). "High nitric oxide production in autistic disorder: a possible role for interferon-gamma." Biol Psychiatry **55**(4): 434-7.

BACKGROUND: Neuroimmune regulation abnormalities have been implicated in the pathophysiology of autistic disorder. Nitric oxide (NO) is involved in immune reactivity and is known to affect brain neurodevelopmental processes. Recent evidence indicates that NO, and cytokines involved in NO production, may be high in children with autism. The purpose of this study was to verify that plasma NO is high in children with autism and determine whether this elevation is related to plasma levels of cytokines involved in NO production. METHODS: The metabolites of NO, nitrite, and nitrate (NOx), along with the cytokines interferon-gamma (IFN-gamma), tumor necrosis factor-alpha, and interleukin-1beta, were measured in plasma of 29 children with autism (mean age +/- SD = 6.1 +/- 2.8 years) and 27 age- and gender-matched healthy comparison subjects using commercially available assay kits. RESULTS: Plasma levels of NOx were significantly higher in the autistic subjects (p =.006); plasma levels of the cytokines did not differ between groups. NOx and IFN-gamma levels were

positively correlated in the autistic subjects (r =.51; p =.005). CONCLUSIONS: These results confirm that plasma NO is high in some children with autism and suggest that this elevation may be related to IFN-gamma activity.

Torsdottir, G., S. Hreidarsson, et al. (2005). "Ceruloplasmin, superoxide dismutase and copper in autistic patients." Basic Clin Pharmacol Toxicol **96**(2): 146-8.

Trushina, E. and C. T. McMurray (2007). "Oxidative stress and mitochondrial dysfunction in neurodegenerative diseases." Neuroscience **145**(4): 1233-48.

In recent years, it has become increasingly clear that mitochondrial dysfunction and oxidative damage are major contributors to neuronal loss. Free radicals, typically generated from mitochondrial respiration, cause oxidative damage of nucleic acids, lipids, carbohydrates and proteins. Despite enormous amount of effort, however, the mechanism by which oxidative damage causes neuronal death is not well understood. Emerging data from a number of neurodegenerative diseases suggest that there may be common features of toxicity that are related to oxidative damage. In this review, while focusing on Huntington's disease (HD), we discuss similarities among HD, Friedreich ataxia and xeroderma pigmentosum, which provide insight into shared mechanisms of neuronal death.

Williams, T. A., A. E. Mars, et al. (2007). "Risk of autistic disorder in affected offspring of mothers with a glutathione S-transferase P1 haplotype." Arch Pediatr Adolesc Med **161**(4): 356-61.

OBJECTIVE: To test whether polymorphisms of the glutathione S-transferase P1 gene (GSTP1) act in the mother during pregnancy to contribute to the phenotype of autistic disorder (AD) in her fetus. DESIGN: Transmission disequilibrium testing (TDT) in case mothers and maternal grandparents. SETTING: Autistic disorder may result from multiple genes and environmental factors acting during pregnancy and afterward. Teratogenic alleles act in mothers during pregnancy to contribute to neurodevelopmental disorders in their offspring; however, only a handful have been identified. GSTP1 is a candidate susceptibility gene for AD because of its tissue distribution and its role in oxidative stress, xenobiotic metabolism, and JNK regulation. PARTICIPANTS: We genotyped GSTP1*G313A and GSTP1*C341T polymorphisms in 137 members of 49 families with AD. All probands received a clinical diagnosis of AD by Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule-Generic testing. MAIN OUTCOME MEASURES: Association of haplotypes with AD was tested by the TDT-Phase program, using the expectation-maximization (EM) algorithm for uncertain haplotypes and for incomplete parental genotypes, with standard measures of statistical significance. RESULTS: The GSTP1*A haplotype was overtransmitted to case mothers (P = .01 [P = .03 using permutation testing]; odds ratio, 2.67 [95% confidence interval, 1.39-5.13]). Results of the combined haplotype and genotype analyses suggest that the GSTP1-313 genotype alone

determined the observed haplotype effect. CONCLUSIONS: Overtransmission of the GSTP1*A haplotype to case mothers suggests that action in the mother during pregnancy likely increases the likelihood of AD in her fetus. If this is confirmed and is a result of a gene-environment interaction occurring during pregnancy, these findings could lead to the design of strategies for prevention or treatment.

Yao, Y., W. J. Walsh, et al. (2006). "Altered vascular phenotype in autism: correlation with oxidative stress." Arch Neurol **63**(8): 1161-4.

BACKGROUND: Autism is a neurologic disorder characterized by impaired communication and social interaction. Results of previous studies showed biochemical evidence for abnormal platelet reactivity and altered blood flow in children with autism. OBJECTIVE: To evaluate the vascular phenotype in children with autism. DESIGN AND MAIN OUTCOME MEASURES: Urinary levels of isoprostane F(2alpha)-VI, a marker of lipid peroxidation; 2,3-dinor-thromboxane B(2), which reflects platelet activation; and 6-keto-prostaglandin F(1alpha), a marker of endothelium activation, were measured by means of gas chromatography-mass spectrometry in subjects with autism and healthy control subjects. SETTING AND SUBJECTS: Children with a clinical diagnosis of autism attending the Pfeiffer Treatment Center. RESULTS: Compared with controls, children with autism had significantly higher urinary levels of isoprostane F(2alpha)-VI, 2,3-dinor-thromboxane B(2), and 6-keto-prostaglandin F(1alpha). Lipid peroxidation levels directly correlated with both vascular biomarker ratios. CONCLUSION: Besides enhanced oxidative stress, platelet and vascular endothelium activation also could contribute to the development and clinical manifestations of autism.

Yorbik, O., C. Akay, et al. (2004). "Zinc status in autistic children." J Trace Elem Exp Med **17**(2): 101-107.

Yorbik, O., A. Sayal, et al. (2002). "Investigation of antioxidant enzymes in children with autistic disorder." Prostaglandins Leukot Essent Fatty Acids **67**(5): 341-3.

Impaired antioxidant mechanisms are unable to inactivate free radicals that may induce a number of pathophysiological processes and result in cell injury. Thus, any abnormality in antioxidant defence systems could affect neurodevelopmental processes and could have an important role in the etiology of autistic disorder. The plasma levels of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), and erythrocyte levels of GSH-Px were investigated in 45 autistic children and compared with 41 normal controls. Levels of erythrocyte SOD, erythrocyte and plasma GSH-Px were assayed spectrophotometrically. Activities of erythrocyte SOD, erythrocyte and plasma GSH-Px in autistic children were significantly lower than normals. These results indicate that autistic children have low levels of activity of blood antioxidant enzyme systems; if similar

abnormalities are present in brain, free radical accumulation could damage brain tissue.

Zoroglu, S. S., F. Armutcu, et al. (2004). "Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism." Eur Arch Psychiatry Clin Neurosci **254**(3): 143-7.

There is great evidence in recent years that oxygen free radicals play an important role in the pathophysiology of many neuropsychiatric disorders. The present study was performed to assess the changes in red blood cells thiobarbituric acid-reactive substances (TBARS) levels, and superoxide dismutase (SOD), catalase (CAT), adenosine deaminase (ADA) and xanthine oxidase (XO) activities in patients with autism (n = 27) compared to age- and sex-matched normal controls (n = 26). In the autistic group, increased TBARS levels ($p < 0.001$) and XO ($p < 0.001$) and SOD ($p < 0.001$) activity, decreased CAT ($p < 0.001$) activity and unchanged ADA activity were detected. It is proposed that antioxidant status may be changed in autism and this new situation may induce lipid peroxidation. These findings indicated a possible role of increased oxidative stress and altered enzymatic antioxidants, both of which may be relevant to the pathophysiology of autism.

Zoroglu, S. S., M. Yurekli, et al. (2003). "Pathophysiological role of nitric oxide and adrenomedullin in autism." Cell Biochem Funct **21**(1): 55-60.

Several studies indicate that nitric oxide (NO) is involved in the aetiopathogenesis of many neuropsychiatric disorders such as schizophrenia, bipolar disorder, depression, Alzheimer's disease, Hungtington disease and stroke. Although it has not been investigated yet, several recent studies proposed that NO may have a pathophysiological role in autism. Adrenomedullin (AM), a recently discovered 52-amino acid peptide hormone, induces vasorelaxation by activating adenylate cyclase and also by stimulating NO release. AM immune reactivity is present in the brain consistent with a role as a neurotransmitter. It has been stated that NO and AM do function in the regulation of many neurodevelopmental processes. We hypothesized that NO and AM activities have been affected in autistic patients and aimed to examine these molecules. Twenty-six autistic patients and 22 healthy control subjects were included in this study. AM and total nitrite (a metabolite of NO) levels have been measured in plasma. The mean values of plasma total nitrite and AM levels in the autistic group were significantly higher than control values, respectively ($p < 0.001$, $p = 0.028$). There is no correlation between total nitrite and AM levels ($r = 0.11$, $p = 0.31$). Certainly, this subject needs much further research investigating autistic patients in earlier periods of life and with subtypes of the disorder.

6- Autism and oxidative stress treatments (5 citations):

Amminger, G. P., G. E. Berger, et al. (2007). "Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study." Biol Psychiatry **61**(4): 551-3.

BACKGROUND: There is increasing evidence that fatty acid deficiencies or imbalances may contribute to childhood neurodevelopmental disorders. METHODS: We conducted a randomized, double-blind, placebo-controlled 6-week pilot trial investigating the effects of 1.5 g/d of omega-3 fatty acids (.84 g/d eicosapentaenoic acid, .7 g/d docosahexaenoic acid) supplementation in 13 children (aged 5 to 17 years) with autistic disorders accompanied by severe tantrums, aggression, or self-injurious behavior. The outcome measure was the Aberrant Behavior Checklist (ABC) at 6 weeks. RESULTS: We observed an advantage of omega-3 fatty acids compared with placebo for hyperactivity and stereotypy, each with a large effect size. Repeated-measures ANOVA indicated a trend toward superiority of omega-3 fatty acids over placebo for hyperactivity. No clinically relevant adverse effects were elicited in either group. CONCLUSIONS: The results of this study provide preliminary evidence that omega-3 fatty acids may be an effective treatment for children with autism.

Chez, M. G., C. P. Buchanan, et al. (2002). "Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders." J Child Neurol **17**(11): 833-7.

L-Carnosine, a dipeptide, can enhance frontal lobe function or be neuroprotective. It can also correlate with gamma-aminobutyric acid (GABA)-homocarnosine interaction, with possible anticonvulsive effects. We investigated 31 children with autistic spectrum disorders in an 8-week, double-blinded study to determine if 800 mg L-carnosine daily would result in observable changes versus placebo. Outcome measures were the Childhood Autism Rating Scale, the Gilliam Autism Rating Scale, the Expressive and Receptive One-Word Picture Vocabulary tests, and Clinical Global Impressions of Change. Children on placebo did not show statistically significant changes. After 8 weeks on L-carnosine, children showed statistically significant improvements on the Gilliam Autism Rating Scale (total score and the Behavior, Socialization, and Communication subscales) and the Receptive One-Word Picture Vocabulary test (all $P < .05$). Improved trends were noted on other outcome measures. Although the mechanism of action of L-carnosine is not well understood, it may enhance neurologic function, perhaps in the enterorhinal or temporal cortex.

Danfors, T., A. L. von Knorring, et al. (2005). "Tetrahydrobiopterin in the treatment of children with autistic disorder: a double-blind placebo-controlled crossover study." J Clin Psychopharmacol **25**(5): 485-9.

Twelve children, all boys, aged 4 to 7 years, with a diagnosis of autistic disorder and low concentrations of spinal 6R-l-erythro-5,6,7,8-tetrahydrobiopterin

(tetrahydrobiopterin) were selected to participate in a double-blind, randomized, placebo-controlled, crossover study. The children received a daily dose of 3 mg tetrahydrobiopterin per kilogram during 6 months alternating with placebo. Treatment-induced effects were assessed with the Childhood Autism Rating Scale every third month. The results showed small nonsignificant changes in the total scores of Childhood Autism Rating Scale after 3- and 6-month treatment. Post hoc analysis looking at the 3 core symptoms of autism, that is, social interaction, communication, and stereotyped behaviors, revealed a significant improvement of the social interaction score after 6 months of active treatment. In addition, a high positive correlation was found between response of the social interaction score and IQ. The results indicate a possible effect of tetrahydrobiopterin treatment.

Dolske, M. C., J. Spollen, et al. (1993). "A preliminary trial of ascorbic acid as supplemental therapy for autism." Prog Neuropsychopharmacol Biol Psychiatry **17**(5): 765-74.

1. This study presents the results of a 30-week double-blind, placebo-controlled trial exploring the effectiveness of ascorbic acid (8g/70kg/day) as a supplemental pharmacological treatment for autistic children in residential treatment. 2. Residential school children (N = 18) were randomly assigned to either ascorbate-ascorbate-placebo treatment order group or ascorbate-placebo-ascorbate treatment order group. Each treatment phase lasted 10 weeks and behaviors were rated weekly using the Ritvo-Freeman scale. 3. Significant group by phase interactions were found for total scores and also sensory motor scores indicating a reduction in symptom severity associated with the ascorbic acid treatment. 4. These results were consistent with a hypothesized dopaminergic mechanism of action of ascorbic acid.

James, S. J., P. Cutler, et al. (2004). "Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism." Am J Clin Nutr **80**(6): 1611-7.

BACKGROUND: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism. OBJECTIVE: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism. DESIGN: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the

autistic children. RESULTS: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children. CONCLUSIONS: An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism.

7 - Autism & Infection – 22 citations

1: J Neurosci Res. 2007 Apr;85(5):1143-8.

Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders.

Nicolson GL, Gan R, Nicolson NL, Haier J.

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We examined the blood of 48 patients from central and southern California diagnosed with autistic spectrum disorders (ASD) by using forensic polymerase chain reaction and found that a large subset (28/48 or 58.3%) of patients showed evidence of Mycoplasma spp. infections compared with two of 45 (4.7%) age-matched control subjects (odds ratio = 13.8, P < 0.001). Because ASD patients have a high prevalence of one or more Mycoplasma spp. and sometimes show evidence of infections with Chlamydia pneumoniae, we examined ASD patients for other infections. Also, the presence of one or more systemic infections may predispose ASD patients to other infections, so we examined the prevalence of C. pneumoniae (4/48 or 8.3% positive, odds ratio = 5.6, P < 0.01) and human herpes virus-6 (HHV-6, 14/48 or 29.2%, odds ratio = 4.5, P < 0.01) coinfections in ASD patients. We found that Mycoplasma-positive and -negative ASD patients had similar percentages of C. pneumoniae and HHV-6 infections, suggesting that such infections occur independently in ASD patients. Control subjects also had low rates of C. pneumoniae (1/48 or 2.1%) and HHV-6 (4/48 or 8.3%) infections, and there were no coinfections in control subjects. The results indicate that a large subset of ASD patients shows evidence of bacterial and/or viral infections (odds ratio = 16.5, P < 0.001). The significance of these infections in ASD is discussed in terms of appropriate treatment. (c) 2007 Wiley-Liss, Inc.

PMID: 17265454 [PubMed - indexed for MEDLINE]

2: Appl Environ Microbiol. 2004 Nov;70(11):6459-65.

Real-time PCR quantitation of clostridia in feces of autistic children.

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Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three Clostridium clusters and one Clostridium species, C. bolteae, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle (CT) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of C. bolteae could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for C. bolteae and the following Clostridium groups were statistically significant: mean counts of C. bolteae and clusters I and XI in autistic children were 46-fold (P = 0.01), 9.0-fold (P = 0.014), and 3.5-fold (P = 0.004) greater than those in control children, respectively, but not for cluster XIVab (2.6 x 10(8) CFU/g in autistic children and 4.8 x 10(8) CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.

Publication Types:
Evaluation Studies
Research Support, U.S. Gov't, Non-P.H.S.

PMID: 15528506 [PubMed - indexed for MEDLINE]

3: J Med Microbiol. 2005 Oct;54(Pt 10):987-91.

Differences between the gut microflora of children with autistic spectrum

disorders and that of healthy children.

Parracho HM, Bingham MO, Gibson GR, McCartney AL.

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Children with autistic spectrum disorders (ASDs) tend to suffer from severe gastrointestinal problems. Such symptoms may be due to a disruption of the indigenous gut flora promoting the overgrowth of potentially pathogenic

micro-organisms. The faecal flora of patients with ASDs was studied and compared with those of two control groups (healthy siblings and unrelated healthy children). Faecal bacterial populations were assessed through the use of a culture-independent technique, fluorescence in situ hybridization, using oligonucleotide probes targeting predominant components of the gut flora. The faecal flora of ASD patients contained a higher incidence of the Clostridium histolyticum group (Clostridium clusters I and II) of bacteria than that of healthy children. However, the non-autistic sibling group had an intermediate level of the C. histolyticum group, which was not significantly different from either of the other subject groups. Members of the C. histolyticum group are recognized toxin-producers and may contribute towards gut dysfunction, with their metabolic products also exerting systemic effects. Strategies to reduce clostridial population levels harboured by ASD patients or to improve their gut microflora profile through dietary modulation may help to alleviate gut disorders common in such patients.

Publication Types:

Comparative Study
Research Support, Non-U.S. Gov't

PMID: 16157555 [PubMed - indexed for MEDLINE]

4: Arch Neurol. 1981 Mar;38(3):191-4.

Acquired reversible autistic syndrome in acute encephalopathic illness in

children.

DeLong GR, Bean SC, Brown FR 3rd.

In seeking the neurologic substrate of the autistic syndrome of childhood, previous studies have implicated the medial temporal lobe or the ring of mesolimbic cortex located in the mesial frontal and temporal lobes. During an acute encephalopathic illness, a clinical picture developed in three children that was consistent with infantile autism. This development was reversible. It was differentiated from acquired epileptic aphasia, and the language disorder was differentiated aphasia. One child has rises in serum herpes simplex titers, and a computerized tomographic (CT) scan revealed an extensive lesion of the temporal lobes, predominantly on the left. The other two, with similar clinical syndromes, had normal CT scans, and no etiologic agent was defined. These cases are examples of an acquired and reversible autistic syndrome in childhood, emphasizing the clinical similarities to bilateral medial temporal lobe disease as described in man, including the Klüver-Bucy syndrome seen in postencephalitic as well as

postsurgical states.

Publication Types:
Case Reports

PMID: 6162440 [PubMed - indexed for MEDLINE]

5: J Neurovirol. 2005 Feb;11(1):1-10.

Autistic disorder and viral infections.

Libbey JE, Sweeten TL, McMahon WM, Fujinami RS.

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Autistic disorder (autism) is a behaviorally defined developmental disorder with

a wide range of behaviors. Although the etiology of autism is unknown, data suggest that autism results from multiple etiologies with both genetic and environmental contributions, which may explain the spectrum of behaviors seen in this disorder. One proposed etiology for autism is viral infection very early in development. The mechanism, by which viral infection may lead to autism, be it through direct infection of the central nervous system (CNS), through infection elsewhere in the body acting as a trigger for disease in the CNS, through alteration of the immune response of the mother or offspring, or through a combination of these, is not yet known. Animal models in which early viral infection results in behavioral changes later in life include the influenza virus model in pregnant mice and the Borna disease virus model in newborn Lewis rats. Many studies over the years have presented evidence both for and against the association of autism with various viral infections. The best association to date has been made between congenital rubella and autism; however, members of the herpes virus family may also have a role in autism. Recently, controversy has arisen as to the involvement of measles virus and/or the measles, mumps, rubella (MMR) vaccine in the development of autism. Biological assays lend support to the association between measles virus or MMR and autism whereas epidemiologic studies show no association between MMR and autism. Further research is needed to clarify both the mechanisms whereby viral infection early in development may lead to autism and the possible involvement of the MMR vaccine in the development of autism.

Publication Types:
Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, P.H.S.
Review

PMID: 15804954 [PubMed - indexed for MEDLINE]

6: Eur Child Adolesc Psychiatry. 2002 Jun;11(3):142-6.

Autistic symptoms following herpes encephalitis.

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Autism is a childhood onset neurodevelopmental disorder characterized by reciprocal social deficits, communication impairment, and rigid ritualistic interests, with the onset almost always before three years of age. Although the etiology of the disorder is strongly influenced by genes, environmental factors are also important. In this context, several reports have described its association with known medical conditions, including infections affecting the central nervous system. In this report, we describe an 11-year-old Asian youngster who developed the symptoms of autism following an episode of herpes encephalitis. In contrast to previous similar reports, imaging studies suggested a predominant involvement of the frontal lobes. At follow-up after three years, he continued to show the core deficits of autism. This case further supports the role of environmental factors, such as infections, in the etiology of autism, and suggests that in a minority of cases, autistic symptoms can develop in later childhood.

Publication Types:
Case Reports
Research Support, Non-U.S. Gov't

PMID: 12369775 [PubMed - indexed for MEDLINE]

7: J Autism Dev Disord. 1992 Mar;22(1):107-13.

Brief report: autism and herpes simplex encephalitis.

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Publication Types:
Case Reports

PMID: 1592760 [PubMed - indexed for MEDLINE]

8: Dev Med Child Neurol. 1991 Oct;33(10):920-4.

Autistic syndrome with onset at age 31 years: herpes encephalitis as a possible model for childhood autism.

Gillberg IC.

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The author describes a previously healthy man who contracted herpes encephalitis at the age of 31 years, and over the following months developed all the symptoms considered diagnostic of autism. This case report casts doubt on the notion of autism as an exclusively developmental disorder. It is suggested that temporal lobe damage may cause autism in some cases.

Publication Types:
Case Reports
Research Support, Non-U.S. Gov't

PMID: 1743418 [PubMed - indexed for MEDLINE]

9: Can J Psychiatry. 1991 Nov;36(9):686-92.

Autism: its primary psychological and neurological deficit.

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Autism is a perplexing condition because of its unique presenting signs and high degree of variability. Evidence is presented that the basic underlying information processing disorder is a dysfunction of the appreciation of the emotional significance of incoming stimuli and attaching motivational value to the stimuli. It is proposed that this dysfunction occurs when the amygdaloid nucleus and/or its connections are disrupted, resulting in the variability of the

presentation of this syndrome among individuals. Herpes simplex encephalitis sometimes results in signs of autism. The virus has a predilection to attack specific areas of the brain, which provides information on the probable underlying neurological dysfunction in autism.

Publication Types:

Review

PMID: 1773407 [PubMed - indexed for MEDLINE]

10: J Autism Dev Disord. 1986 Sep;16(3):369-75.

Onset at age 14 of a typical autistic syndrome. A case report of a girl with herpes simplex encephalitis.

Gillberg C.

Publication Types:
Case Reports

PMID: 3558293 [PubMed - indexed for MEDLINE]

11: Curr Opin Neurol. 2002 Jun;15(3):333-8.

Neurological adverse events associated with vaccination.

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Public tolerance to adverse reactions is minimal. Several reporting systems have been established to monitor adverse events following immunization. The present review summarizes data on neurologic complications following vaccination, and provides evidence that indicates whether they were directly associated with the vaccines. These complications include autism (measles vaccine), multiple sclerosis (hepatitis B vaccine), meningoencephalitis (Japanese encephalitis vaccine), Guillain-Barré syndrome and giant cell arteritis (influenza vaccine), and reactions after exposure to animal rabies vaccine. Seizures and hypotonic/hyporesponsive episodes following pertussis vaccination and potential

risks associated with varicella vaccination, as well as vaccine-associated paralytic poliomyelitis following oral poliovirus vaccination, are also described. In addition, claims that complications are caused by adjuvants, preservatives and contaminants [i.e. macrophagic myofasciitis (aluminium), neurotoxicity (thimerosal), and new variant Creutzfeldt-Jakob disease (bovine-derived materials)] are discussed.

Publication Types:

Review

PMID: 12045734 [PubMed - indexed for MEDLINE]

12: J Autism Dev Disord. 2004 Oct;34(5):583-6.

Brief report: autistic disorder in three children with cytomegalovirus infection.

Sweeten TL, Posey DJ, McDougle CJ.

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Previous research has identified a relationship between autistic disorder (autism) and specific congenital infections. Three cases of congenital or perinatal cytomegalovirus (CMV) infection occurring in association with autism are described. Hypothetical mechanisms relating congenital infection, such as CMV, to the development of autism are discussed. A better understanding of the immunologic response to certain congenital infections may provide important information pertaining to the pathophysiology and etiology of autism in vulnerable individuals.

Publication Types:
Case Reports

Research Support, Non-U.S. Gov't

PMID: 15628611 [PubMed - indexed for MEDLINE]

13: J Autism Dev Disord. 2003 Aug;33(4):455-9.

Possible association between congenital cytomegalovirus infection and autistic

disorder.

Yamashita Y, Fujimoto C, Nakajima E, Isagai T, Matsuishi T.

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We encountered seven children with symptomatic congenital cytomegalovirus (CMV) infection from 1988 to 1995, of whom two (28.6%) developed typical autistic disorder. Case 1: A boy born at 38 weeks' gestation with a birth weight of 3164 g showed generalized petechiae, hepatosplenomegaly, and positive serum CMV-specific IgM antibodies. He was profoundly deaf, mentally retarded, and exhibited a lack of eye contact, stereotyped repetitive play, and hyperactivity. Case 2: A boy delivered at 39 weeks gestation with a birthweight of 2912 g showed non-progressive dilatation of the lateral ventricles observed postnatally. CMV-specific IgM antibodies were positive and CMV-DNA in the urine was confirmed by PCR. The boy was mentally retarded but not deaf. He showed no interest in people and delayed speech development. Subependymal cysts were detected by cranial ultrasound after birth in both patients. This is the first report describing subependymal cysts and the later development of AD. Cranial magnetic resonance imaging revealed an abnormal intensity area in the periventricular white matter suggestive of disturbed myelination; however, no migration disorders were found in our patients. These findings suggest that the timing of injury to the developing brain by CMV may be in the third trimester in some patients with autistic disorder.

Publication Types:
Case Reports

PMID: 12959425 [PubMed - indexed for MEDLINE]

14: Neuropediatrics. 1990 May;21(2):102-3.

Autism as one of several disabilities in two children with congenital cytomegalovirus infection.

Ivarsson SA, Bjerre I, Vegfors P, Ahlfors K.

Department of Pediatric, University of Lund, Malmö General Hospital, Sweden.

Two children with congenital cytomegalovirus (CMV)-infection, severely disabled

where autism was one of the disabilities are described. The characterization of the maternal infection have been possible and the connection between congenital CMV-infection and autism is discussed.

Publication Types:
Case Reports

PMID: 2163029 [PubMed - indexed for MEDLINE]

15: J Autism Dev Disord. 1984 Jun;14(2):183-9.

Autism and congenital cytomegalovirus.

Stubbs EG, Ash E, Williams CP.

Two cases of congenital cytomegalovirus infection associated with autism are reported. The viral hypothesis of autism is discussed along with a brief review of the literature. Suggestions are made for future research.

Publication Types:
Case Reports

PMID: 6086566 [PubMed - indexed for MEDLINE]

16: J Autism Dev Disord. 1983 Sep;13(3):249-53.

Autism in a child with congenital cytomegalovirus infection.

Markowitz PI.

A case is reported of early infantile autism associated with a congenital cytomegalovirus infection. The diagnosis of autism is based on the child's failure to develop good interpersonal relationships, poor eye contact, delayed and deviant use of language, and her rote and nonthematic use of objects and playthings. Resistance to change and self-stimulatory behavior were also present. Onset was before 2 years of age. Congenital cytomegalovirus infection was suggested by the presence of an antibody response to the virus, culture of the virus from the urine, sensorineural hearing loss, and inflammatory damage to the retina of the eye. Although over time improvement was noted, at last examination at the age of 5 years her behavior is still markedly deviant. This and other reported cases suggest that congenital viral infection may be an important cause of infantile autism. It is hypothesized that an ability of the agent to establish

chronic infection may predispose to behavioral aberration.

Publication Types:
Case Reports

PMID: 6315673 [PubMed - indexed for MEDLINE]

17: J Autism Dev Disord. 1980 Dec;10(4):451-8.

Transfer factor immunotherapy of an autistic child with congenital

cytomegalovirus.

Stubbs EG, Budden SS, Burger DR, Vandenbark AA.

Publication Types:
Research Support, U.S. Gov't, Non-P.H.S.
Research Support, U.S. Gov't, P.H.S.

PMID: 6100889 [PubMed - indexed for MEDLINE]

18: J Autism Child Schizophr. 1978 Mar;8(1):37-43.

Autistic symptoms in a child with congenital cytomegalovirus infection.

Stubbs EG.

A case of intrauterine cytomegalovirus infection with onset of autistic symptoms apparently after 6 months of age is reported. Physicians who find autistic symptoms in very young children might include cytomegalovirus in their differential to document the presence or absence of a correlation.

Publication Types:
Case Reports

PMID: 205531 [PubMed - indexed for MEDLINE]

19: Med Hypotheses. 2008;70(5):967-74. Epub 2007 Nov 5.

The association between tick-borne infections, Lyme borreliosis and autism

spectrum disorders.

Bransfield RC, Wulfman JS, Harvey WT, Usman AI.

Department of Psychiatry, Riverview Medical Center, 225 State Route 35, Red Bank, NJ, United States.

Chronic infectious diseases, including tick-borne infections such as Borrelia burgdorferi may have direct effects, promote other infections and create a weakened, sensitized and immunologically vulnerable state during fetal development and infancy leading to increased vulnerability for developing autism spectrum disorders. A dysfunctional synergism with other predisposing and contributing factors may contribute to autism spectrum disorders by provoking innate and adaptive immune reactions to cause and perpetuate effects in susceptible individuals that result in inflammation, molecular mimicry, kynurenine pathway changes, increased quinolinic acid and decreased serotonin, oxidative stress, mitochondrial dysfunction and excitotoxicity that impair the development of the amygdala and other neural structures and neural networks resulting in a partial Klüver-Bucy Syndrome and other deficits resulting in autism spectrum disorders and/or exacerbating autism spectrum disorders from other causes throughout life. Support for this hypothesis includes multiple cases of mothers with Lyme disease and children with autism spectrum disorders; fetal neurological abnormalities associated with tick-borne diseases; similarities between tick-borne diseases and autism spectrum disorder regarding symptoms, pathophysiology, immune reactivity, temporal lobe pathology, and brain imaging data; positive reactivity in several studies with autistic spectrum disorder patients for Borrelia burgdorferi (22%, 26% and 20-30%) and 58% for mycoplasma; similar geographic distribution and improvement in autistic symptoms from antibiotic treatment. It is imperative to research these and all possible causes of autism spectrum disorders in order to prevent every preventable case and treat every treatable case until this disease has been eliminated from humanity.

PMID: 17980971 [PubMed - in process]

20: J Neuroimmunol. 2002 Aug;129(1-2):168-77.

Erratum in:
J Neuroimmunol 2002 Sep;130(1-2):248.

Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and

Streptococcus group A.

Vojdani A, Campbell AW, Anyanwu E, Kashanian A, Bock K, Vojdani E.

Section of Neuroimmunology, Immunosciences Laboratory, Inc., 8693 Wilshire Boulevard, Suite 200, Beverly Hills, CA 90211, USA. immunsci@ix.netcom.com

We measured autoantibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of preexisting T-cells and central nervous system antigens to immunocompetent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.

PMID: 12161033 [PubMed - indexed for MEDLINE]

21: Behav Brain Res. 2007 Jan 10;176(1):141-8. Epub 2006 Jul 21.

Abnormal social behaviors in young and adult rats neonatally infected with Borna disease virus.

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Autism spectrum disorders (ASD) have been the focus of a great deal of research and clinical speculation. This intense interest relates to both the perplexing pathogenesis and devastating consequences of these disorders. One of the

obstacles to understanding the pathogenesis of autism and to developing efficient treatment has been the paucity of animal models that could be used for hypotheses-driven mechanistic studies of abnormal brain and behavior development and for the pre-clinical testing novel pharmacological treatments. In this report, we briefly review our animal model of ASD based on neonatal Borna disease virus (BDV) infection and present new data about abnormal social interaction in adult BDV-infected rats. We found that neonatal BDV infection profoundly affected social behaviors in adult rats. Compared to the control rats, both 90- and 180-day-old infected rats spent less time in active social interaction and more time in following their partners. In the intruder-resident test, the BDV-infected

resident rats exhibited less aggression towards the intruders and showed more the following-the-intruder behavior. The following-the-partner behavior may be an example of "stereotypic" activity due to BDV-induced abnormal social communication between rats. The previously published results and present findings indicate that neonatal BDV infection significantly altered the normal pattern of social interaction in rats. Co-localization of activated microglia and dying

Purkinje cells in BDV-infected rats suggests that the BDV model could be used to study a pathogenic link of Purkinje cell dropout and neuroinflammation to abnormal social behaviors.

PMID: 16860408 [PubMed - indexed for MEDLINE]

22: Front Biosci. 2002 Mar 1;7:d593-607.

Borna disease virus infection of the neonatal rat: developmental brain injury model of autism spectrum disorders.

Pletnikov MV, Moran TH, Carbone KM.

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Autism spectrum disorders (ASD) have been the focus of a great deal of research and clinical speculation. This intense interest relates to both the perplexing pathogenesis and devastating consequences of these disorders. One of the obstacles to understanding the pathogenesis of autism and its efficient treatment has been the paucity of animal models that could be used for hypotheses-driven mechanistic studies of abnormal brain and behavior development and for the pre-clinical testing novel pharmacological treatments. The present review

provides a detailed analysis of a new animal model of ASD. This model utilizes neonatal Borna disease virus (BDV) infection of the rat brain as a unique experimental teratogen to study the pathogenesis of neurodevelopmental damage.

For more than a decade, studies of the BDV animal model have yielded much insight into the pathogenic processes of abnormal brain development and resulting autistic-like behavioral abnormalities in rats. The most recent experiments demonstrate the utility of the BDV model for studying the pathophysiological mechanisms of the gene-environment interaction that determines differential disease outcomes and variability in responses to treatments.

Publication Types:
Research Support, U.S. Gov't, P.H.S.
Review

PMID: 11861216 [PubMed - indexed for MEDLINE]

23: Ann N Y Acad Sci. 2001 Jun;939:318-9.

Rat model of autism spectrum disorders. Genetic background effects on Borna disease virus-induced developmental brain damage.

Pletnikov MV, Jones ML, Rubin SA, Moran TH, Carbone KM.

Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

PMID: 11462786 [PubMed - indexed for MEDLINE]

24: Curr Top Microbiol Immunol. 2001;253:157-77.

Borna disease virus infection of adult and neonatal rats: models for neuropsychiatric disease.

Hornig M, Solbrig M, Horscroft N, Weissenböck H, Lipkin WI.

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Animal models provide unique opportunities to explore interactions between host and environment. Two models have been established based on Borna disease virus

infection that provide new insights into mechanisms by which neurotropic agents and/or immune factors may impact developing or mature CNS circuitry to effect

complex disturbances in movement and behavior. Note in press: Since this chapter was submitted, several manuscripts have been published that extend findings reported here and support the relevance of BDV infections of neonatal Lewis rats as models for investigating mechanisms of neurodevelopmental damage in autism. Behavioral abnormalities, including disturbed play behavior and chronic emotional overactivity, have been described by Pletnikov et al. (1999); inhibition of responses to novel stimuli were described by Hornig et al. (1999); loss of Purkinje cells following neonatal BDV infection has been demonstrated by Eisenman et al. (1999), Hornig et al. (1999), and Weissenböck et al. (2000); and alterations in cytokine gene expression have been reported by Hornig et al. (1999), Plata-Salaman et al. (1999) and Sauder et al. (1999).

PMID: 11417134 [PubMed - indexed for MEDLINE]

25: J Autism Dev Disord. 2006 Nov;36(8):1039-51.

Etiologies of autism in a case-series from Tanzania.

Mankoski RE, Collins M, Ndosi NK, Mgalla EH, Sarwatt VV, Folstein SE.

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Sciences, Boston, MA, USA.

Most autism has a genetic cause although post-encephalitis cases are reported. In a case-series (N = 20) from Tanzania, 14 met research criteria for autism. Three (M:F = 1:2) had normal development to age 22, 35, and 42 months, with onset of autism upon recovery from severe malaria, attended by prolonged high fever, convulsions, and in one case prolonged loss of consciousness. In four other cases (M:F = 3:1), the temporal relationship between onset of autism and severe infection was close, but possibly spurious since malaria is common in Tanzania and there were indications of abnormal development in the child or a family member. In seven cases, (M:F = 6:1) autism onset was unrelated to malaria. The excess of non-verbal cases (N = 10) is related local diagnostic practice.

Publication Types:
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't

PMID: 16897390 [PubMed - indexed for MEDLINE]

26: Lakartidningen. 1992 Jan 29;89(5):279-80.

[Information-based adjustment of sex education clinics for adolescents]

[Article in Swedish]

Ruusuvaara L.

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PMID: 1738248 [PubMed - indexed for MEDLINE]

27: J Autism Child Schizophr. 1977 Mar;7(1):49-55.

Depressed lymphocyte responsiveness in autistic children.

Stubbs EG, Crawford ML.

Although there are associations linking autism with prenatal rubella, cytomegalovirus, syphilis, and varicella, the etiology of the autistic state remains obscure. Host defense against the etiologic agents postulated to be responsible for the autism-associated syndromes is believed to be primarily of the cell-mediated type. In this preliminary study, cellular immune function was assessed in vitro by phytohemagglutinin (PHA) stimulation of lymphocyte cultures. Twelve autistic children and 13 control subjects were compared. The autistic group exhibited a depressed lymphocyte transformation response to PHA when compared to the control subjects (p less than .01).

Publication Types:
- Comparative Study
- In Vitro
- Research Support, U.S. Gov't, Non-P.H.S.

PMID: 139400 [PubMed - indexed for MEDLINE]

28: Curr Psychiatr Ther. 1982;21:225-39.

Psychiatric aspects of infectious diseases.

Schwab JJ.

Publication Types:
Case Reports
Review

PMID: 6761074 [PubMed - indexed for MEDLINE]

29: Med Hypotheses. 1984 Apr;13(4):399-405.

Reptilian behavioural patterns in childhood autism.

Thong YH.

Childhood autism may be caused by damage to three phylogenetically distinct regions of the brain, or their major pathways and connections. Injury to the neocortex results in loss of language and cognitive function, while injury to the limbic cortex results in autistic withdrawal and abolition of play behaviour. Injury to the more primitive striatal complex, mammalian counterpart of the brain of reptiles, results in a bizarre and truncated form of stereotyped and ritualistic behaviour. The causes of brain injury in childhood autism could be those common in the perinatal period including cerebral anoxia, haemorrhage, phenylketonuria, neurolipidoses , meningitis, toxoplasmosis, and congenital rubella. All these conditions have previously been shown to be associated with childhood autism.

PMID: 6727722 [PubMed - indexed for MEDLINE]

30: Curr Neurovasc Res. 2006 May;3(2):149-57.

Autoantibodies associated with psychiatric disorders.

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Growing evidence suggests that autoantibodies to neuronal or endothelial targets in psychiatric disorders exist and may be pathogenic. This review describes and discusses the possible role of autoantibodies related to the psychiatric manifestations in autoimmune diseases, autoantibodies related to the psychiatric disorders present in post-streptococcal diseases, celiac disease, chronic fatigue

syndrome and substance abuse, and autoantibodies related to schizophrenia and autism, disorders now considered of autoimmune origin.

Publication Types:
Review

PMID: 16719797 [PubMed - indexed for MEDLINE]

8- Aluminum Toxicity – 19 citations

1: J Alzheimers Dis. 2006 Nov;10(2-3):179-201.

Aluminum and Alzheimer's disease: a new look.

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Despite the circumstantial and sometimes equivocal support, the hypothetic involvement of aluminum (Al) in the etiology and pathogenesis of Alzheimer's disease (AD) has subsisted in neuroscience. There are very few other examples of scientific hypotheses on the pathogenesis of a disease that have been revisited so many times, once a new method that would allow a test of Al's accumulations in the brain of AD patients or a comparison between Al-induced and AD neuropathological signs has become available. Although objects of methodological controversies for scientists and oversimplification for lay spectators, several lines of evidence have strongly supported the involvement of Al as a secondary aggravating factor or risk factor in the pathogenesis of AD. We review evidence on the similarities and dissimilarities between Al-induced neurofibrillary degeneration and paired helical filaments from AD, the accumulation of Al in neurofibrillary tangles and senile plaques from AD, the neuropathological dissociation between AD and dialysis associated encephalopathy, and the epidemiological relations between Al in drinking water and the prevalence of AD.

We also critically analyze the prospects of Al-amyloid cascade studies and other evolving lines of evidence that might shed insights into the link between Al and AD. The message between the lines of the following article is that the involvement of Al in the pathogenesis of AD should not be discarded, especially in these times when the amyloid dogma of AD etiology shows its myopia.

Publication Types:
Historical Article
Review

PMID: 17119287 [PubMed - indexed for MEDLINE]

2: J Alzheimers Dis. 2006 Nov;10(2-3):223-53.

Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected

to contribute to metal-induced neurodegeneration.

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The etiology of many neurodegenerative diseases has been only partly attributed to acquired traits, suggesting environmental factors may also contribute. Metal dyshomeostasis causes or has been implicated in many neurodegenerative diseases. Metal flux across the blood-brain barrier (the primary route of brain metal uptake) and the choroid plexuses as well as sensory nerve metal uptake from the nasal cavity are reviewed. Transporters that have been described at the blood-brain barrier are listed to illustrate the extensive possibilities for moving substances into and out of the brain. The controversial role of aluminum in Alzheimer's disease, evidence suggesting brain aluminum uptake by transferrin-receptor mediated endocytosis and of aluminum citrate by system Xc;{-} and an organic anion transporter, and results suggesting transporter-mediated aluminum brain efflux are reviewed. The ability of manganese to produce a parkinsonism-like syndrome, evidence suggesting manganese uptake by transferrin- and non-transferrin-dependent mechanisms which may include store-operated calcium channels, and the lack of transporter-mediated manganese brain efflux, are discussed. The evidence for transferrin-dependent and independent mechanisms of brain iron uptake is presented. The copper transporters, ATP7A and ATP7B, and their roles in Menkes and Wilson's diseases, are summarized. Brain zinc uptake is facilitated by L- and D-histidine, but a transporter, if involved, has not been identified. Brain lead uptake may involve a non-energy-dependent process, store-operated calcium channels, and/or an ATP-dependent calcium pump. Methyl mercury can form a complex with L-cysteine that mimics methionine, enabling its transport by the L system. The putative roles of zinc transporters, ZnT and Zip, in regulating brain zinc are discussed. Although brain uptake mechanisms for some metals have been identified, metal efflux from the brain has received little attention, preventing integration of all processes that contribute to brain metal concentrations.

Publication Types:
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, Non-P.H.S.
Review

PMID: 17119290 [PubMed - indexed for MEDLINE]

3: J Alzheimers Dis. 2006 Nov;10(2-3):135-44.

Mechanisms of aluminum-induced neurodegeneration in animals: Implications for

Alzheimer's disease.

Savory J, Herman MM, Ghribi O.

Department of Pathology, University of Virginia, Charlottesville, VA, USA.

For four decades the controversial question concerning a possible role for aluminum neurotoxicity in contributing to the pathogenesis of Alzheimer's disease has been debated, and studies by different investigators have yielded contradictory results. The lack of sensitivity to aluminum neurotoxicity in transgenic mouse models of Alzheimer's disease has not allowed the system to be used to explore important aspects of this toxicity. Rabbits are particularly sensitive to aluminum neurotoxicity and they develop severe neurological changes that are dependent on dose, age and route of administration. The most prominent feature induced by aluminum in rabbit brain is a neurofibrillary degeneration that shares some similarity with the neurofibrillary tangles found in Alzheimer's disease patients. In the present review we discuss data from our laboratory and others, on the effects of aluminum on behaviour, neurologic function and morphology, using aluminum administered to rabbits via different routes. Finally, we will examine data on the possible cellular mechanisms underlying aluminum neurotoxicity, and potential neuroprotective strategies against aluminum toxicity.

Publication Types:
Research Support, U.S. Gov't, Non-P.H.S.

Review

PMID: 17119283 [PubMed - indexed for MEDLINE]

4: Brain Res Rev. 2006 Aug 30;52(1):193-200. Epub 2006 Mar 10.

Some aspects of astroglial functions and aluminum implications for neurodegeneration.

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The present decade had witnessed an unprecedented attention focused on glial cells as a result of their unusual physiological roles that are being unraveled. It is now known that, rather than being a mere supporter of neurons, astroglia are actively involved in their modulation. The aluminum hypothesis seems to have been laid to rest, probably due to contradictory epidemiological reports on it as a causative factor of neurodegenerative diseases. Surprisingly, newer scientific evidences continue to appear and recent findings have implicated astrocytes as the principal target of its toxic action. In view of the likely detrimental effects of the interaction between these two infamous partners in neuroscience on neurons and nervous system, we have reviewed some aspects of glia-neuron interaction and discussed the implications of aluminum-impaired astrocytic functions on neurodegeneration. Because sporadic causes still account for the majority of the neurodegenerative diseases of which Alzheimer's disease is the most prominent, it has been suggested that neurotoxicologists should not relent in screening for the environmental agents, such as aluminum, and that considerable attention should be given to glial cells in view of the likely implications of environmental toxicants on their never-imagined newly reported roles in the central nervous system (CNS).

Publication Types:

Review

PMID: 16529821 [PubMed - indexed for MEDLINE]

5: J Alzheimers Dis. 2005 Nov;8(2):171-82; discussion 209-15.

Effects of aluminum on the nervous system and its possible link with neurodegenerative diseases.

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Aluminum is environmentally abundant, but not an essential element. Aluminum has been associated with several neurodegenerative diseases, such as dialysis

encephalopathy, amyotrophic lateral sclerosis and Parkinsonism dementia in the Kii peninsula and Guam, and in particular, Alzheimer's disease. Although this association remains controversial, there is increasing evidence which suggests the implication of metal homeostasis in the pathogenesis of Alzheimer's disease.

Aluminum, zinc, copper, and iron cause the conformational changes of Alzheimer's amyloid-beta protein. Al causes the accumulation of tau protein and amyloid-beta protein in experimental animals. Aluminum induces neuronal apoptosis in vivo as well as in vitro. Furthermore, a relationship between aluminum and the iron-homeostasis or calcium-homeostasis has been suggested. Based on these findings, the characteristics of aluminum neurotoxicity are reviewed, and the potential link between aluminum and neurodegenerative diseases is reconsidered.

Publication Types:
Research Support, Non-U.S. Gov't
Review

PMID: 16308486 [PubMed - indexed for MEDLINE]

6: Curr Opin Pharmacol. 2005 Dec;5(6):637-40. Epub 2005 Sep 28.

Aluminum: new recognition of an old problem.

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The aluminum problem is now over 25 years old, but has remained a neglected concern. Data indicate that aluminum contaminates much of the raw material used to manufacture solutions used for intravenous nutritional support of hospitalized and ambulatory patients, and that pharmaceutical manufacturers have only recently obtained the technology necessary to detect aluminum contamination of their products. As a result, aluminum bypassed normal barriers and entered the blood, accumulating in tissues such as bone, liver and the central nervous system with toxic consequences. Now that the FDA has finally issued a rule governing aluminum contamination in these solutions, manufacturers will need to develop methods to minimize such contamination; scientists should also realize that when data they obtain indicate a serious problem in the manufacturing sector they should be sure that the problem is properly addressed.

Publication Types:

Review

PMID: 16198633 [PubMed - indexed for MEDLINE]

7: Toxicol Ind Health. 2002 Aug;18(7):309-20.

Aluminum as a toxicant.

Becaria A, Campbell A, Bondy SC.

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Although aluminum is the most abundant metal in nature, it has no known biological function. However, it is known that there is a causal role for aluminum in dialysis encephalopathy, microcytic anemia, and osteomalacia.

Aluminum has also been proposed to play a role in the pathogenesis of Alzheimer's disease (AD) even though this issue is controversial. The exact mechanism of aluminum toxicity is not known but accumulating evidence suggests that the metal can potentiate oxidative and inflammatory events, eventually leading to tissue damage. This review encompasses the general toxicology of aluminum with emphasis on the potential mechanisms by which it may accelerate the progression of chronic age-related neurodegenerative disorders.

Publication Types:
Research Support, U.S. Gov't, P.H.S.
Review

PMID: 15068131 [PubMed - indexed for MEDLINE]

8: Immunol Allergy Clin North Am. 2003 Nov;23(4):699-712.

Aluminum inclusion macrophagic myofasciitis: a recently identified condition.

Gherardi RK, Authier FJ.

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The authors conclude that the persistence of aluminum hydroxide at the site of intramuscular injection is a novel finding which has an exact significance that remains to be established fully. It seems mandatory to evaluate possible long-term adverse effects induced by this compound, because this issue has not been addressed (in the past, aluminum hydroxide was believed to be cleared quickly from the body). If safety concerns about the long-term effects of aluminum hydroxide are confirmed, novel and alternative vaccine adjuvants to rescue vaccine-based strategies should be proposed to ensure the enormous benefit for public health that these vaccines provide worldwide.

Publication Types:
Review

PMID: 14753387 [PubMed - indexed for MEDLINE]

9: Brain Res Bull. 2003 Nov 15;62(1):15-28.

The role of metals in neurodegenerative processes: aluminum, manganese, and zinc.

Zatta P, Lucchini R, van Rensburg SJ, Taylor A.

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Until the last decade, little attention was given by the neuroscience community to the neurometabolism of metals. However, the neurobiology of heavy metals is now receiving growing interest, since it has been linked to major neurodegenerative diseases. In the present review some metals that could possibly be involved in neurodegeneration are discussed. Two of them, manganese and zinc, are essential metals while aluminum is non-essential. Aluminum has long been known as a neurotoxic agent. It is an etiopathogenic factor in diseases related to long-term dialysis treatment, and it has been controversially invoked as an aggravating factor or cofactor in Alzheimer's disease as well as in other neurodegenerative diseases. Manganese exposure can play an important role in causing Parkinsonian disturbances, possibly enhancing physiological aging of the brain in conjunction with genetic predisposition. An increased environmental burden of manganese may have deleterious effects on more sensitive subgroups of the population, with sub-threshold neurodegeneration in the basal ganglia, generating a pre-Parkinsonian condition. In the case of zinc, there has as yet been no evidence that it is involved in the etiology of neurodegenerative

diseases in humans. Zinc is redox-inactive and, as a result of efficient homeostatic control, does not accumulate in excess. However, adverse symptoms in humans are observed on inhalation of zinc fumes, or accidental ingestion of unusually large amounts of zinc. Also, high concentrations of zinc have been found to kill bacteria, viruses, and cultured cells. Some of the possible

mechanisms for cell death are reviewed.

Publication Types:
Review

PMID: 14596888 [PubMed - indexed for MEDLINE]

10: Nutr Rev. 2003 Sep;61(9):306-10.

Parenteral nutrition-associated cholestasis in neonates: the role of aluminum.

Arnold CJ, Miller GG, Zello GA.

College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7M 0Z9.

Parenteral nutrition (PN) is an essential component in the care of premature and ill infants. The incidence of parenteral nutrition-associated cholestasis (PNAC) ranges from 7.4 to 84%. One substance in PN solutions that has been implicated in PNAC is aluminum. Aluminum loading in animals and humans causes hepatic accumulation and damage. The degree of aluminum contamination of PN solutions has decreased over time, but contamination still significantly exceeds levels that are safe for human neonates. Further study into the relationship between aluminum contamination in neonatal PN solutions and the development of PNAC is necessary.

Publication Types:
Review

PMID: 14552065 [PubMed - indexed for MEDLINE]

11: J Inorg Biochem. 2003 Sep 15;97(1):151-4.

Intracellular mechanisms underlying aluminum-induced apoptosis in rabbit brain.

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Loss of neurons is a hallmark of neurodegenerative disorders and there is increasing evidence suggesting that apoptosis is a key mechanism by which neurons die in these diseases. Mitochondrial dysfunction has been implicated in this process of neuronal cell death, but there is a growing body of evidence suggesting also an active role for the endoplasmic reticulum in regulating apoptosis, either independent of mitochondria, or in concert with mitochondrial-initiated pathways. Investigations in our laboratory have focused on neuronal injury resulting from the administration of aluminum maltolate, via the intracisternal route, to New Zealand white rabbits. This treatment induces both mitochondrial and endoplasmic reticulum stress. Agents such as lithium or glial cell-line derived neurotrophic factor (GDNF) have the ability to prevent aluminum-induced neuronal death by interfering with the mitochondrial and/or the endoplasmic reticulum-mediated apoptosis cascade. Cytochrome c release from mitochondria and binding to Apaf-1 initiates the aluminum-induced apoptosis cascade; this is prevented by lithium treatment. GDNF also protects against aluminum-induced apoptosis but by upregulation of Bcl-X(L), thereby preventing the binding of cytochrome c to Apaf-1. This animal model system involving neurotoxicity induced by an aluminum compound provides new information on mechanisms of neurodegeneration and neuroprotection.

Publication Types:
Research Support, Non-U.S. Gov't
Review

PMID: 14507471 [PubMed - indexed for MEDLINE]

12: Vaccine. 2002 May 31;20 Suppl 3:S18-23.

Erratum in:
Vaccine. 2002 Sep 10;20(27-28):3428.

Aluminum salts in vaccines--US perspective.

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Aluminum in the form of aluminum hydroxide, aluminum phosphate or alum has been commonly used as an adjuvant in many vaccines licensed by the US Food and Drug Administration. Chapter 21 of the US Code of Federal Regulations [610.15(a)] limits the amount of aluminum in biological products, including vaccines, to 0.85 mg/dose. The amount of aluminum in vaccines currently licensed in the US ranges from 0.85-0.125 mg/dose. Clinical studies have demonstrated that aluminum enhances the antigenicity of some vaccines such as diphtheria and tetanus toxoids. Moreover, aluminum-adsorbed diphtheria and tetanus toxoids are distinctly more effective than plain fluid toxoids for primary immunization of children. There is little difference between plain and adsorbed toxoids for booster immunization. Aluminum adjuvants have a demonstrated safety profile of over six decades; however, these adjuvants have been associated with severe local reactions such as erythema, subcutaneous nodules and contact hypersensitivity.

Publication Types:
Review

PMID: 12184360 [PubMed - indexed for MEDLINE]

13: Environ Res. 2002 Jun;89(2):101-15.

Aluminum: impacts and disease.

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Aluminum is the most widely distributed metal in the environment and is extensively used in modern daily life. Aluminum enters into the body from the environment and from diet and medication. However, there is no known physiological role for aluminum within the body and hence this metal may produce adverse physiological effects. The impact of aluminum on neural tissues is well reported but studies on extraneural tissues are not well summarized. In this review, the impacts of aluminum on humans and its impact on major physiological systems are summarized and discussed. The neuropathologies associated with high brain aluminum levels, including structural, biochemical, and neurobehavioral changes, have been summarized. In addition, the impact of aluminum on the musculoskeletal system, respiratory system, cardiovascular system, hepatobiliary system, endocrine system, urinary system, and reproductive system are discussed.

Publication Types:
Review

PMID: 12123643 [PubMed - indexed for MEDLINE]

14: J Neurosci Res. 2001 Dec 1;66(5):1009-18.

Aluminum, NO, and nerve growth factor neurotoxicity in cholinergic neurons.

Szutowicz A.

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Several neurotoxic compounds, including Al, NO, and beta-amyloid may contribute to the impairment or loss of brain cholinergic neurons in the course of various neurodegenerative diseases. Genotype and phenotypic modifications of cholinergic neurons may determine their variable functional competency and susceptibility to reported neurotoxic insults. Hybrid, immortalized SN56 cholinergic cells from mouse septum may serve as a model for in vitro cholinotoxicity studies. Differentiation by various combinations of cAMP, retinoic acid, and nerve growth factor may provide cells of different morphologic maturity as well as activities of acetylcholine and acetyl-CoA metabolism. In general, differentiated cells appear to be more susceptible to neurotoxic signals than the non-differentiated ones, as evidenced by loss of sprouting and connectivity, decreases in choline acetyltransferase and pyruvate dehydrogenase activities, disturbances in acetyl-CoA compartmentation and metabolism, insufficient or excessive acetylcholine release, as well as increased expression of apoptosis markers. Each neurotoxin impaired both acetylcholine and acetyl-CoA metabolism of these cells. Activation of p75 or trkA receptors made either acetyl-CoA or cholinergic metabolism more susceptible to neurotoxic influences, respectively. Neurotoxins aggravated detrimental effects of each other, particularly in differentiated cells. Thus brain cholinergic neurons might display a differential susceptibility to Al and other neurotoxins depending on their genotype or phenotype-dependent variability of the cholinergic and acetyl-CoA metabolism. Copyright 2001 Wiley-Liss, Inc.

Publication Types:
Research Support, Non-U.S. Gov't

Review

PMID: 11746431 [PubMed - indexed for MEDLINE]

15: Ann N Y Acad Sci. 1997 Oct 15;825:152-66.
Toxin-induced blood vessel inclusions caused by the chronic administration of aluminum and sodium fluoride and their implications for dementia.

Isaacson RL, Varner JA, Jensen KF.
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Publication Types:

Review

PMID: 9369984 [PubMed - indexed for MEDLINE]

16: J Toxicol Environ Health. 1996 Aug 30;48(6):667-83.

Prevention and treatment of aluminum toxicity including chelation therapy: status and research needs.

Yokel RA, Ackrill P, Burgess E, Day JP, Domingo JL, Flaten TP, Savory J.
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The prevention and treatment of aluminum (Al) accumulation and toxicity are reviewed. Recommendations to further our understanding of desferrioxamine (deferoxamine, DFO) treatment and to develop more effective chelation approaches are provided. Reduction of Al accumulation and toxicity may benefit end-stage renal disease (ESRD) patients and perhaps those suffering from specific neurodegenerative disorders as well as workers with Al-induced neurocognitive disorders. The clearance of Al may be increased by extracorporeal chelation, renal transplantation, perhaps complexation with simple ligands such as silicon (Si), and systemic chelation therapy. The abilities of extracorporeal chelation and Si to reduce Al accumulation require further evaluation. Although it may not be possible to design Al-specific chelators, chelators with greater Al selectivity are desired. Aluminum-selective chelation might be achieved by targeted chelator distribution or by the use of adjuvants with the chelator. The ability of carboxylic acids to facilitate Al elimination, under specific conditions, warrants further study. Desferrioxamine does not produce significant biliary Al excretion. A chelator with this property may be useful in ESRD patients. The necessity for an Al chelator to distribute extravascularly to be effective is unknown and should be determined to guide the selection of alternatives to DFO. The lack of oral efficacy and occasional side effects of DFO

encourage identification of orally effective, safer Al chelators. The bidentate 3-hydroxypyridin-4-ones are currently the most encouraging alternatives to DFO.

They have been shown to increase urinary Al excretion in rats and rabbits, but to have toxicity comparable to, or greater than, DFO. Their toxicity may relate to incomplete metal complexation. The ability of orally effective chelators to increase absorption of chelated metal from the gastrointestinal (Gl) tract needs to be evaluated. Orally effective, safe Al chelators would be of benefit to peritoneal dialysis patients and those with neurodegenerative disorders, if Al chelation therapy is indicated. The reduction of Alzheimer's disease (AD) progression and the reversal of Al-induced behavioral deficits and neurofibrillary tangles by DFO encourage further study of Al chelation therapy for selected neurodegenerative disorders.

Publication Types:
Review

PMID: 8772805 [PubMed - indexed for MEDLINE]

17: J Toxicol Environ Health. 1996 Aug 30;48(6):649-65.

Systemic aluminum toxicity: effects on bone, hematopoietic tissue, and kidney.

Jeffery EH, Abreo K, Burgess E, Cannata J, Greger JL.
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Although the full mechanisms are not yet elucidated, research into the mechanism of toxicity of aluminum (Al) on bone formation and remodeling and on hematopoietic tissue is ongoing. In contrast little information exists on the interactive effects of systemic Al and the kidney. In bone, both clinically and experimentally, high doses of Al inhibit remodeling, slowing both osteoblast and osteoclast activities and producing osteomalacia and adynamic bone disease. In contrast, while very low levels of Al are mitogenic in bones of experimental animals, the effect of low levels of Al in humans is unknown. Aluminum has been shown to have its mitogenic action at the osteoblast, but whether the effect on resorption is viz osteoblast-directed changes in osteoclast activity has not yet been determined. Parathyroid hormone (PTH) levels are disrupted by Al in humans and animals. Whether altered PTH levels play a major or even a minor role in Al-dependent osteotoxicity requires clarification. In hematopoietic tissue, Al causes a microcytic anemia, not reversible by iron. Friend leukemia cells treated with Al have been reported to accumulate excess iron, without incorporating it

into ferritin or heme. It is not yet known which steps in iron metabolism are disrupted by Al, if they involve a single mechanism of action, or even if this disruption in iron metabolism accounts for the anemia seen in Al toxicosis. In kidney, research is needed to evaluate Al nephrotoxicity; there are almost no studies in this area. Furthermore, research is needed to evaluate mechanisms of renal Al excretion, presently shown by one study to occur at the distal tubule. Such studies might well throw light on whether Al plays a role in aggravating renal insufficiency, or whether the role of the kidney in Al toxicosis is limited to the causative effect of renal compromise on Al accumulation. In summary, while a number of mechanisms have been proposed for the toxic action of Al, no single mechanism emerges to explain these diverse effects of systemic Al. Recommendations for future research are presented and summarized in Table 1.

Publication Types:
Review

PMID: 8772804 [PubMed - indexed for MEDLINE]

18: J Toxicol Environ Health. 1996 Aug 30;48(6):585-97.

What we know and what we need to know about developmental aluminum toxicity.

Golub MS, Domingo JL.
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Information concerning developmental aluminum (Al) toxicity is available from clinical studies and from animal testing. An Al toxicity syndrome including encephalopathy, osteomalacia, and anemia has been reported in uremic children receiving dialysis. In addition, some components of the syndrome, particularly osteomalacia, have been reported in non-dialyzed uremic children receiving Al-based phosphate binders, nonuremic infants receiving parenteral nutrition with Al-containing fluids, and nonuremic infants given high doses of Al antacids. The number of children in clinical populations that are at risk of Al toxicity is not known and needs to be determined. Work in animal models (rats, mice, and rabbits) demonstrates that Al is distributed transplacentally and is present in milk. Oral Al administration during pregnancy produces a syndrome including growth retardation, delayed ossification, and malformations at doses that also lead to reduced maternal weight gain. The severity of the effects is highly dependent on the form of Al administered. In the postnatal period, reduced pup weight gain and effects on neuromotor development have been described as a result of developmental exposures. The significance of these findings for human health requires better understanding of the amount and bioavailability of Al in food,

drinking water, and medications and from sources unique to infants and children such as breast milk, soil ingestion, and medications used specifically by pregnant women and children. We also need a better understanding of the unique biological actions of Al that may occur during developmental periods, and unique aspects of the developing organism that make it more or less susceptible to Al toxicity.

Publication Types:
Review

PMID: 8772800 [PubMed - indexed for MEDLINE]

19: J Toxicol Environ Health. 1996 Aug 30;48(6):569-84.

Aluminum toxicokinetics.
Exley C, Burgess E, Day JP, Jeffery EH, Melethil S, Yokel RA.
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In this study of the toxicokinetics of aluminum we have examined some of the fundamental issues that currently define our understanding of the toxicology of aluminum in humans. There is a vast literature on this subject, and it was not our aim to review this literature but to use it to develop our understanding of the toxicokinetics of aluminum and to identify critical and unresolved issues related to its toxicity. In undertaking this task we have chosen to define the term toxicokinetics to encompass those factors that influence both the lability of aluminum in a body and the sites at which aluminum is known to accumulate, with or without consequent biological effect. We have approached our objective from the classical pharmacological approach of ADME: the absorption, distribution, metabolism, and excretion of aluminum. This approach was successful in identifying several key deficits in our understanding of aluminum toxicokinetics. For example, we need to determine the mechanisms by which aluminum crosses epithelia, such as those of the gastrointestinal tract and the central nervous system, and how these mechanisms influence both the subsequent transport and fate of the absorbed aluminum and the concomitant nature and severity of the biological response to the accumulation of aluminum. Our hope in highlighting these unresolved issues (summarized in Table 1) is that they will be addressed in future research.

Publication Types:
Review PMID: 8772799 [PubMed - indexed for MEDLINE]

9 - Dietary Intervention Studies – 102 citations

Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. Neuropsychobiology. 2005;51(2):77-85.

Jyonouchi H, **Geng L**, **Ruby A**, **Zimmerman-Bier B**.

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OBJECTIVE: Our previous study indicated an association between cellular immune reactivity to common dietary proteins (DPs) and excessive proinflammatory cytokine production with endotoxin (lipopolysaccharide, LPS), a major stimulant of innate immunity in the gut mucosa, in a subset of autism spectrum disorder (ASD) children. However, it is unclear whether such abnormal LPS responses are intrinsic in these ASD children or the results of chronic gastrointestinal (GI) inflammation secondary to immune reactivity to DPs. This study further explored possible dysregulated production of proinflammatory and counter-regulatory cytokines with LPS in ASD children and its relationship to GI symptoms and the effects of dietary intervention measures.

METHODS: This study includes ASD children (median age 4.8 years) on the unrestricted (n = 100) or elimination (n = 77) diet appropriate with their immune reactivity. Controls include children with non-allergic food hypersensitivity (NFH; median age 2.9 years) on the unrestricted (n = 14) or elimination (n = 16) diet, and typically developing children (median age 4.5 years, n = 13). The innate immune responses were assessed by measuring production of proinflammatory (TNF-alpha, IL-1beta, IL-6, and IL-12) and counter-regulatory (IL-1ra, IL-10, and sTNFRII) cytokines by peripheral blood mononuclear cells (PBMCs) with LPS. The results were also compared to T-cell responses with common DPs and control T-cell mitogens assessed by measuring T-cell cytokine production.

RESULTS: ASD and NFH PBMCs produced higher levels of TNF-alpha with LPS than controls regardless of dietary interventions. However, only in PBMCs from ASD children with positive gastrointestinal (GI(+)) symptoms, did we find a positive association between TNF-alpha levels produced with LPS and those with cow's milk protein (CMP) and its major components regardless of dietary interventions. In the unrestricted diet group, GI(+) ASD PBMCs produced higher IL-12 than controls and less IL-10 than GI(-) ASD PBMCs with LPS. GI(+) ASD but not GI(-) ASD or NFH PBMCs produced less counter-regulatory cytokines with LPS in the unrestricted diet group than in the elimination diet group. There was no significant difference among the study groups with regard to cytokine production in responses to T-cell mitogens and other recall antigens. Conclusion: Our results revealed that there are findings limited to GI(+) ASD PBMCs in both the unrestricted and elimination diet groups. Thus our findings indicate intrinsic

defects of innate immune responses in GI(+) ASD children but not in NFH or GI(-) ASD children, suggesting a possible link between GI and behavioral symptoms mediated by innate immune abnormalities. Copyright 2005 S. Karger AG, Basel.

Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism. Nutr Neurosci. 2004 Jun;7(3):151-61.

Vojdani A, **O'Bryan T**, **Green JA**, **Mccandless J**, **Woeller KN**, **Vojdani E**, **Nourian AA**, **Cooper EL**.

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The mechanisms behind autoimmune reaction to nervous system antigens in autism are not understood. We assessed the reactivity of sera from 50 autism patients and 50 healthy controls to specific peptides from gliadin and the cerebellum. A significant percentage of autism patients showed elevations in antibodies against gliadin and cerebellar peptides simultaneously. For examining cross-reaction between dietary proteins and cerebellar antigens, antibodies were prepared in rabbits, and binding of rabbit anti-gliadin, anti-cerebellar peptides, anti-MBP, anti-milk, anti-egg, anti-soy and anti-corn to either gliadin- or cerebellar-antigen-coated wells was measured. In comparison to anti-gliadin peptide binding to gliadin peptide at 100%, the reaction of anti-cerebellar peptide to gliadin peptide was 22%, whereas the binding of anti-myelin basic protein (MBP), anti-milk, anti-egg and anti-soy to gliadin was less than 10%. Further examination of rabbit anti-gliadin (EQVPLVQQ) and anti-cerebellar (EDVPLLED) 8 amino acid (AA) peptides with human serum albumin (HSA) and an unrelated peptide showed no binding, but the reaction of these antibodies with both the cerebellar and gliadin peptides was greater than 60%. This cross-reaction was further confirmed by DOT-immunoblot and inhibition studies. We conclude that a subgroup of patients with autism produce antibodies against Purkinje cells and gliadin peptides, which may be responsible for some of the neurological symptoms in autism.

Heat shock protein and gliadin peptide promote development of peptidase antibodies in children with autism and patients with autoimmune disease. Clin Diagn Lab Immunol. 2004 May;11(3):515-24.

Vojdani A, **Bazargan M**, **Vojdani E**, **Samadi J**, **Nourian AA**, **Eghbalieh N**, **Cooper EL**.

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Searching for a mechanism underlying autoimmunity in autism, we postulated that gliadin peptides, heat shock protein 60 (HSP-60), and streptokinase (SK) bind to

different peptidases resulting in autoantibody production against these components. We assessed this hypothesis in patients with autism and in those with mixed connective tissue diseases. Associated with antigliadin and anti-HSP antibodies, children with autism and patients with autoimmune disease developed anti-dipeptidylpeptidase I (DPP I), anti-dipeptidylpeptidase IV (DPP IV [or CD26]) and anti-aminopeptidase N (CD13) autoantibodies. A significant percentage of autoimmune and autistic sera were associated with elevated immunoglobulin G (IgG), IgM, or IgA antibodies against three peptidases, gliadin, and HSP-60. These antibodies are specific, since immune absorption demonstrated that only specific antigens (e.g., DPP IV absorption of anti-DPP IV), significantly reduced IgG, IgM, and IgA antibody levels. For direct demonstration of SK, HSP-60, and gliadin peptide binding to DPP IV, microtiter wells coated with DPP IV were reacted with SK, HSP-60, and gliadin. They were then reacted with anti-DPP IV or anti-SK, anti-HSP, and antigliadin antibodies. Adding SK, HSP-60, and gliadin peptides to DPP IV resulted in 27 to 43% inhibition of the DPP IV-anti-DPP IV reaction, but DPP IV-positive peptides caused 18 to 20% enhancement of antigen-antibody reactions. We propose that (i) superantigens (e.g., SK and HSP-60) and dietary proteins (e.g., gliadin peptides) in individuals with predisposing HLA molecules bind to aminopeptidases and (ii) they induce autoantibodies to peptides and tissue antigens. Dysfunctional membrane peptidases and autoantibody production may result in neuroimmune dysregulation and autoimmunity.

Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders. J Pediatr. 2005 May;146(5):605-10.

Jyonouchi H, Geng L, Ruby A, Reddy C, Zimmerman-Bier B.

Objective To evaluate an association between cytokine production with common dietary proteins as a marker of non-allergic food hypersensitivity (NFH) and gastrointestinal (GI) symptoms in young children with autism spectrum disorders (ASD). Study design Peripheral blood mononuclear cells (PBMCs) were obtained from 109 ASD children with or without GI symptoms (GI [+] ASD, N = 75 and GI (-) ASD, N = 34], from children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. We measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMCs stimulated with whole cow's milk protein (CMP), its major components (casein, beta-lactoglobulin, and alpha-lactoalbumin), gliadin, and soy.

Results PBMCs obtained from GI (+) ASD children produced more tumor necrosis factor-alpha (TNF-alpha)/interleukin-12 (IL-12) than those obtained from control subjects with CMP, beta-lactoglobulin, and alpha-lactoalbumin, irrespective of objective GI symptoms. They also produced more TNF-alpha with gliadin, which was more frequently observed in the group with loose stools. PBMCs obtained from GI (-) ASD children produced more TNF-alpha/IL-12 with CMP than those from control subjects,

but not with beta-lactoglobulin, alpha-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable.

Conclusion A high prevalence of elevated TNF-alpha/IL-12 production by GI (+) ASD PBMCs with CMP and its major components indicates a role of NFH in GI symptoms observed in children with ASD.

Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. J Clin Immunol. 2004 Nov;24(6):664-73.

Ashwood P, Anthony A, Torrente F, Wakefield AJ.

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A lymphocytic enterocolitis has been reported in a cohort of children with autistic spectrum disorder (ASD) and gastrointestinal (GI) symptoms. This study tested the hypothesis that dysregulated intestinal mucosal immunity with enhanced pro-inflammatory cytokine production is present in these ASD children. Comparison was made with developmentally normal children with, and without, mucosal inflammation. Duodenal and colonic biopsies were obtained from 21 ASD children, and 65 developmentally normal paediatric controls, of which 38 had signs of histological inflammation. Detection of CD3+ lymphocyte staining for spontaneous intracellular TNFalpha, IL-2, IL-4, IFNgamma, and IL-10, was performed by multicolor flow cytometry. Duodenal and colonic mucosal CD3+ lymphocyte counts were elevated in ASD children compared with noninflamed controls ($p<0.03$). In the duodenum, the proportion of lamina propria (LP) and epithelial CD3(+)TNFalpha+ cells in ASD children was significantly greater compared with noninflamed controls ($p<0.002$) but not coeliac disease controls. In addition, LP and epithelial CD3(+)IL-2+ and CD3(+)IFNgamma+, and epithelial CD3(+)IL-4+ cells were more numerous in ASD children than in noninflamed controls ($p<0.04$). In contrast, CD3(+)IL-10+ cells were fewer in ASD children than in noninflamed controls ($p<0.05$). In the colon, LP CD3(+)TNFalpha+ and CD3(+)IFNgamma+ were more frequent in ASD children than in noninflamed controls ($p<0.01$). In contrast with Crohn's disease and non-Crohn's colitis, LP and epithelial CD3(+)IL-10+ cells were fewer in ASD children than in nondisease controls ($p<0.01$). There was a significantly greater proportion of CD3(+)TNFalpha+ cells in colonic mucosa in those ASD children who had no dietary exclusion compared with those on a gluten and/or casein free diet ($p<0.05$). There is a consistent profile of CD3+ lymphocyte cytokines in the small and large intestinal mucosa of these ASD children, involving increased pro-inflammatory and decreased regulatory activities. The data provide further evidence of a diffuse mucosal immunopathology in some ASD children and the potential for benefit of dietary and immunomodulatory therapies.

Mechanisms of non-IgE mediated adverse reaction to common Dietary Proteins (DPs) in children with Autism Spectrum Disorders (ASD) February 2004, Supplement • Volume 113 • Number 2

Rationale We have reported elevated IFN-/TNF- production by peripheral blood mononuclear cells (PBMCs) against cow's milk protein (CMP), soy, and gliadin in a substantial number of ASD children (Neuropsychobiology 46:76, 2002). IFN-/TNF- production is partly regulated by IL-10 (negatively) and IL-12 (positively).

Methods We examined IFN-, IL-5, TNF-, IL-10 and IL-12 production by PBMCs against common DPs in 68 ASD children on a regular diet (Median age 5.4 yr): >50% of them had gastrointestinal symptoms. Controls include 11 children with DP intolerance (DPI) (Median age 2.5 yr), and 10 normal children (Median age 3.3 y).

Results ASD and DPI PBMCs produced larger amounts of IFN- and TNF-; against CMP and gliadin than controls ($p<0.01$). IL-12 production against CMP was higher in ASD PBMCs ($p<0.01$). IL-10 production by ASD, DPI, and control PBMCs were equivalent, resulting in higher TNF/IL-10 ratio with CMP/gliadin in ASD and DPI PBMCs ($p<0.01$). IL-12/IL-10 ratios with CMP/gliadin were also higher in ASD PBMCs ($p<0.01$), but not in DPI cells. In 11/11 DPI children, TNF-/IL-10 ratios with CMP and/or gliadin were >0.5 with excellent responses to the appropriate elimination diet. TNF-/IL-10 ratios >0.5 with CMP and gliadin were found in 41/68 and 32/68 ASD children, respectively. In these children, the elimination diet based on immune reactivity helped resolve GI symptoms and attenuate autistic behaviors by parental report.

Conclusions Disregulated production of inflammatory and counter-regulatory cytokines may be associated with non-IgE mediated adverse reaction to common DPs in some ASD children, indicating therapeutic significance of dietary interventions in these children.

Jyonouchi H, Sun S, Le H.: Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J Neuroimmunol 2001 Nov 1;120(1-2):170-9

Abstract:

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We determined innate and adaptive immune responses in children with developmental regression and autism spectrum disorders (ASD, N=71), developmentally normal siblings (N=23), and controls (N=17). With lipopolysaccharide (LPS), a stimulant for innate immunity, peripheral blood mononuclear cells (PBMCs) from 59/71 (83.1%) ASD patients produced >2 SD above the control mean (CM) values of TNF-alpha, IL-1beta,

and/or IL-6 produced by control PBMCs. ASD PBMCs produced higher levels of proinflammatory/counter-regulatory cytokines without stimuli than controls. With stimulants of phytohemagglutinin (PHA), tetanus, IL-12p70, and IL-18, PBMCs from 47.9% to 60% of ASD patients produced >2 SD above the CM values of TNF-alpha depending on stimulants. Our results indicate excessive innate immune responses in a number of ASD children that may be most evident in TNF-alpha production.

White JF.: Intestinal pathophysiology in autism. Exp Biol Med (Maywood). 2003 Jun;228(6):639-49.

Vojdani A, Pangborn JB, Vojdani E, Cooper EL., Infections, toxic chemicals and dietary peptides binding to lymphocyte receptors and tissue enzymes are major instigators of autoimmunity in autism. Int J Immunopathol Pharmacol. 2003 Sep-Dec;16(3):189-99.

Bull G, Shattock P, Whiteley P, Anderson R, Groundwater PW, Lough JW, Lees G.: Indolyl-3-acryloylglycine (IAG) is a putative diagnostic urinary marker for autism spectrum disorders. Med Sci Monit. 2003 Oct;9(10):CR422-5.

Abstract:

Sunderland General Hospital, Sunderland, UK.

BACKGROUND: Autism is a heterogeneous pervasive developmental disorder with a poorly defined aetiology and pathophysiology. There are indications that the incidence of the disease is rising but still no definitive diagnostic biochemical markers have been isolated. Here we have addressed the hypothesis that urinary levels of trans -indolyl-3-acryloylglycine (IAG) are abnormal in patients diagnosed with autism spectrum disorders (ASD) compared to age-matched controls. MATERIAL/METHODS: Urine samples were collected on an opportunistic basis and analysed for IAG concentration (normalised against creatinine content to account for changes in urinary volume) using reversed phase HPLC with UV detection. RESULTS: Statistical analysis (Mann-Whitney tests) showed highly significant increases (p=0.0002) in the levels of urinary IAG in the ASD group (median 942 microV per mmol/L of creatinine [interquartile range 521-1729], n=22) compared to asymptomatic controls (331 [163-456], n=18). Detailed retrospective analysis showed that gender (boys 625 microV per mmol/L of creatinine [294-1133], n=29; girls 460 [282-1193], n=11: P=0.79) and age (control donor median 10 years [8-14], n=15; ASD median 9 years [7-11] n=22: P=0.54) were not significantly correlated with IAG levels in this non-blinded volunteer study. CONCLUSIONS: Our results strongly suggest that urinary titres of IAG may constitute an objective diagnostic indicator for ASD. Mechanisms for the involvement of IAG in ASD are discussed together with future strategies to address its specificity.

Reichelt KL, Knivsberg AM.: Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutr Neurosci. 2003 Feb;6(1):19-28.

Buie T, Winter H, Kushak, R: Preliminary findings in gastrointestinal investigation of autistic patients. 2002.

Summary:

Harvard University and Mass General Hospital, http://www.ladders.org/autism.php

111 patients evaluated, ages 14 Months to 20 Years, all with GI symptoms of pain or diarrhea. Endoscopic findings: Esophagitis in 23 (20%), Gastritis in 14 (12%); 4 had Helicobacter pylori; Duodenitis in 11 (10%); 2 had Celiac Sprue; Eosinophilic Inflammation in 5 (5%). 10 out of 90 tested (11%) had unusually low enzyme activity: 2 with total pancreatic insufficiency and 5 with multiple enzyme defects. Lactase deficiency was found in 55% of ASD children tested, and combined deficiency of disacchraridase enzymes was found in 15%. Enzyme assays correlate well with hydrogen breath tests. Colitis was found in 11 of 89 patients (12%), none with features of Ulcerative Colitis or Crohn's. Histologic (biopsy reviewed) lymphoid nodular hyperplasia was found in 15 of 89 patients (16%). Eosinophilic inflammation was found in 13 of 89 patients (14%); cause or significance is unclear. Conclusions: more than 50% of autistic children appear to have GI symptoms, food allergies, and maldigestion or malabsorption issues. We need large, evidence-based studies need to be done in order to fully understand the gut-brain association in autism.

Krigsman, A, et al: Preliminary data presented at congressional hearing. 2002 Jun.

Summary:

New York University School of Medicine: www.med.nyu.edu

We examined 43 patients with autism, in whom we demonstrated enterocolitis in 65% and terminal ileal LNH in 90%. As of November, 2002, our total patient population now stands at 82, and the percentages of enterocolitis and LNH are essentially unchanged. Additional studies will follow.

Gluten- and casein-free diets for autistic spectrum disorder. Cochrane Database Syst Rev. 2004;(2):CD003498.

Millward C, **Ferriter M**, **Calver S**, **Connell-Jones G**.

BACKGROUND: It has been suggested that peptides from gluten and casein may have a role in the origins of autism and that the physiology and psychology of autism might be explained by excessive opioid activity linked to these peptides. Research has reported

abnormal levels of peptides in the urine and cerebrospinal fluid of persons with autism. If this is the case, diets free of gluten and /or casein should reduce the symptoms associated with autism.

OBJECTIVES: To determine the efficacy of gluten- and/or casein- free diets as an intervention to improve behaviour, cognitive and social functioning in individuals with autism. SEARCH STRATEGY: Electronic searching of abstracts from the Cochrane Library (Issue 3, 2003), PsycINFO (1971- May 2003), EMBASE (1974- May 2003), CINAHL (1982- May 2003), MEDLINE (1986- May 2003), ERIC (1965-2003), LILACS (to 2003) and the specialist register of the Cochrane Complementary Medicine Field (January 2004). Review bibliographies were also examined to identify potential trials. SELECTION CRITERIA: All randomised controlled trials involving programmes which eliminated gluten, casein or both gluten and casein from the diets of individuals diagnosed with autistic spectrum disorder.

DATA COLLECTION AND ANALYSIS: Abstracts of studies identified in searches of electronic databases were read and assessed to determine whether they might meet the inclusion criteria. The authors independently selected the relevant studies from the reports identified in this way. As only one trial fitted the inclusion criteria, no meta-analysis is currently possible and data are presented in narrative form.

MAIN RESULTS: The one trial included reported results on four outcomes. Unsurprisingly in such a small-scale study, the results for three of these outcomes (cognitive skills, linguistic ability and motor ability) had wide confidence intervals that spanned the line of nil effect. However, **the fourth outcome, reduction in autistic traits, reported a significant beneficial treatment effect for the combined gluten- and casein- free diet. REVIEWERS' CONCLUSIONS: This is an important area of investigation and large scale, good quality randomised controlled trials are needed.**

Whiteley P, Shattock P: Biochemical aspects in autism spectrum disorders: updating the opioid-excess theory and presenting new opportunities for biomedical intervention. Expert Opin Ther Targets. 2002 Apr;6(2):175-83.

Abstract:

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Autism is a lifelong condition usually described as affecting social, cognitive and imaginative abilities. For many years, parents and some professionals have observed that in concordance with the behavioural and psychological symptoms of the condition, there are a number of physiological and biochemical correlates which may also be of relevance to the syndrome. One area of interest that encompasses many of these

observations is the opioid-excess theory of autism. The main premise of this theory is that autism is the result of a metabolic disorder. Peptides with opioid activity derived from dietary sources, in particular foods that contain gluten and casein, pass through an abnormally permeable intestinal membrane and enter the central nervous system (CNS) to exert an effect on neurotransmission, as well as producing other physiologically-based symptoms. Numerous parents and professionals worldwide have found that removal of these exogenously derived compounds through exclusion diets can produce some amelioration in autistic and related behaviours. There is a surprisingly long history of research accompanying these ideas. The aim of this paper is to review the accompanying evidence in support of this theory and present new directions of intervention as a result of it.

Vojdani A, Campbell AW, Anyanwu E, Kashanian A, Bock K, Vojdani E: Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and Streptococcus group A. J Neuroimmunol 2002 Aug;129(1-2):168-77.

Abstract:

Section of Neuroimmunology, Immunosciences Laboratory, Inc., 8693 Wilshire Boulevard, Suite 200, Beverly Hills, CA 90211, USA. immunsci@ix.netcom.com

We measured autoantibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of preexisting T-cells and central nervous system antigens to immunocompetent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.

Knivsberg AM, Reichelt KL, Hoien T, Nodland M: A randomised, controlled study of dietary intervention in autistic syndromes. Nutr Neurosci 2002 Sep;5(4):251-61.

Center for Reading Research, Stavanger University College, Norway. ann-mari.knivsberg@slf.his.no

Abstract:

Impaired social interaction, communication and imaginative skills characterize autistic syndromes. In these syndromes urinary peptide abnormalities, derived from gluten, gliadin, and casein, are reported. They reflect processes with opioid effect. The aim of this single blind study was to evaluate effect of gluten and casein-free diet for children with autistic syndromes and urinary peptide abnormalities. A randomly selected diet and control group with 10 children in each group participated. Observations and tests were done before and after a period of 1 year. The development for the group of children on diet was significantly better than for the controls.

Kidd PM.: Autism, an extreme challenge to integrative medicine. Part: 1: The knowledge base. Altern Med Rev. 2002 Aug;7(4):292-316.

Kidd PM.: Autism, an extreme challenge to integrative medicine. Part 2: medical management. Altern Med Rev. 2002 Dec;7(6):472-99.

Hadjivassiliou M, Grunewald RA, Davies-Jones GA: Gluten sensitivity as a neurological illness. J Neurol Neurosurg Psychiatry. 2002 May;72(5):560-3. [No abstract available]

Hadjivassiliou M, Boscolo S, Davies-Jones GA, Grunewald RA, Not T, Sanders DS, Simpson JE, Tongiorgi E, Williamson CA, Woodroofe NM: The humoral response in the pathogenesis of gluten ataxia. Neurology 2002 Apr 23;58(8):1221-6.

Abstract: Department of Clinical Neurology, The Royal Hallamshire Hospital, Sheffield, UK. m.hadjivassiliou@sheffield.ac.uk

OBJECTIVE: To characterize humoral response to cerebellum in patients with gluten ataxia. BACKGROUND: Gluten ataxia is a common neurologic manifestation of gluten sensitivity. METHODS: The authors assessed the reactivity of sera from patients with gluten ataxia (13), newly diagnosed patients with celiac disease without neurologic dysfunction (24), patients with other causes of cerebellar degeneration (11), and healthy control subjects (17) using indirect immunocytochemistry on human cerebellar and rat CNS tissue. Cross-reactivity of a commercial IgG antigliadin antibody with human cerebellar tissue also was studied. RESULTS: Sera from 12 of 13 patients with gluten ataxia stained Purkinje cells strongly. Less intense staining was seen in some but not all sera from patients with newly diagnosed celiac disease without neurologic dysfunction. At high dilutions (1:800) staining was seen only with sera from patients with gluten ataxia but not in control subjects. Sera from patients with gluten ataxia also stained some brainstem and cortical neurons in rat CNS tissue. Commercial anti-gliadin antibody stained human Purkinje cells in a similar manner. Adsorption of the antigliadin antibodies using crude gliadin abolished the staining in patients with celiac disease without neurologic dysfunction, but not in those with gluten ataxia. CONCLUSIONS:

Patients with gluten ataxia have antibodies against Purkinje cells. Antigliadin antibodies cross-react with epitopes on Purkinje cells.

Garvey J: Diet in autism and associated disorders. J Fam Health Care 2002;12(2):34-8.

Abstract: Royal Free Hospital, London.

A dietitian discusses the theory that peptides with opioid activity may cause or trigger autism. The use of an exclusion diet to treat autism is explained, weighing the potential benefits against some of the practical difficulties of keeping to a strict exclusion diet. The use of nutritional supplements is described. An abnormal gut flora has also been implicated in autism and the use of probiotics and prebiotics in improving the integrity of the gut mucosa is also discussed.

Cornish E: Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. J Hum Nutr Diet 2002 Aug;15(4):261-9.

Abstract: Senior Community Dietitian, Community Nutrition Service, South Derbyshire Community Health NHS Trust, Dar es Salaam, Tanzania.

BACKGROUND: There is growing interest in possible dietary involvement in the aetiology and treatment of Autistic Spectrum Disorders (ASD). Research has focused on the physiological and behavioural effects of dietary change but has not examined the effect of exclusion diets on nutritional intake. AIMS: The aim of this study was to examine whether the removal of major dietary staples placed children with autism at risk of nutrient deficiency and compares their food choice with ASD children not following gluten and/or casein free diets. METHODS: A postal questionnaire was sent to parents of children aged 3-16 years, diagnosed with ASD belonging to the National Autistic Society in Leicestershire and southern Derbyshire. Detailed dietary information and a 3-day food diary were collected. The sample size was small: those using gluten/casein free diets (n = 8) and those not following diet (n = 29). RESULTS: Nutrient intakes fell below the Lower Reference Nutrient Intake (LRNI) in 12 children (32%) for zinc, calcium, iron, vitamin A, vitamin B12 and riboflavin in the nondiet group and four children (50%) for zinc and calcium in the diet group. Fruit and vegetable intakes were higher and cereal, bread and potato consumption were lower in those children using gluten and/or casein free diets. CONCLUSION: No significant differences in the energy, protein and micronutrient intakes were found between the two groups of children. A longitudinal prospective study is suggested to examine whether differences in food choice are the result of dietary intervention or the prerequisite for the successful application of diet in this special group of children.

Wakefield AJ, Puleston JM, Montgomery SM, Anthony A, O'Leary JJ, Murch SH: Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands. Aliment Pharmacol Ther. 2002 Apr;16(4):663-74.

Inflammatory Bowel Disease Study Group, Centre for Gastroenterology, Department of Medicine, Royal Free and University College Medical School, London, UK. wakers@aol.com

There is growing awareness that primary gastrointestinal pathology may play an important role in the inception and clinical expression of some childhood developmental disorders, including autism. In addition to frequent gastrointestinal symptoms, children with autism often manifest complex biochemical and immunological abnormalities. The gut-brain axis is central to certain encephalopathies of extra-cranial origin, hepatic encephalopathy being the best characterized. Commonalities in the clinical characteristics of hepatic encephalopathy and a form of autism associated with developmental regression in an apparently previously normal child, accompanied by immune-mediated gastrointestinal pathology, have led to the proposal that there may be analogous mechanisms of toxic encephalopathy in patients with liver failure and some children with autism. Aberrations in opioid biochemistry are common to these two conditions, and there is evidence that opioid peptides may mediate certain aspects of the respective syndromes. The generation of plausible and testable hypotheses in this area may help to identify new treatment options in encephalopathies of extra-cranial origin. Therapeutic targets for this autistic phenotype may include: modification of diet and entero-colonic microbial milieu in order to reduce toxin substrates, improve nutritional status and modify mucosal immunity; anti-inflammatory/immunomodulatory therapy; and specific treatment of dysmotility, focusing, for example, on the pharmacology of local opioid activity in the gut.

Knivsberg AM, Reichelt KL, Nodland M: Reports on dietary intervention in autistic disorders. Nutr Neurosci 2001;4(1):25-37.

Center for Reading Research, Stavanger College, Norway. ann-mari.knivsberg@slf.his.no

Abstract:

Autism is a developmental disorder for which no cure currently exists. Gluten and/or casein free diet has been implemented to reduce autistic behaviour, in addition to special education, since early in the eighties. Over the last twelve years various studies on this dietary intervention have been published in addition to anecdotal, parental reports. The scientific studies include both groups of participants as well as single cases, and beneficial results are reported in all, but one study. While some studies are based on urinary peptide abnormalities, others are not. The reported results are, however, more or less identical; reduction of autistic behaviour, increased social and communicative skills, and reappearance of autistic traits after the diet has been broken.

Hadjivassiliou M, Grunewald RA, Lawden M, Davies-Jones GA, Powell T, Smith CM: Headache and CNS white matter abnormalities associated with gluten sensitivity. Neurology 2001 Feb 13;56(3):385-8.

Abstract: Department of Clinical Neurology, The Royal HallamshireHospital, Sheffield,UK. m.hadjivassiliou@sheffield.ac.uk

The authors describe 10 patients with gluten sensitivity and abnormal MRI. All experienced episodic headache, six had unsteadiness, and four had gait ataxia. MRI abnormalities varied from confluent areas of high signal throughout the white matter to foci of high signal scattered in both hemispheres. Symptomatic response to gluten-free diet was seen in nine patients.

Dubynin VA , Ivleva IuA, Malinovskaia IV, Kamenskii AA, Andreeva LA, Alfeeva LIu, Miasoedov NF: Changes in beta-casomorphine-7 effect on behavior of albino rat pups in postnatal development [Article in Russian]. Zh Vyssh Nerv Deiat Im I P Pavlova 2001 May-Jun;51(3):386-9.

Abstract: Lomonosov State University, Institute of Molecular Genetics, Russian Academy of Sciences, Moscow.

The analgetic effect of heptapeptide beta-casomorphine-7 in newborn albino rats (20 mg/kg, i.p.) was recorded already 14 days after birth in the "hot plate" test. The first signs of a possible influence of the peptide on motor activity were observed only at the age of 28 days. They are expressed in impairment of motor coordination and change in locomotion level ("Opto-Varimex" test). The obtained evidence probably reflect the processes of discrete maturation of different components of the opioid system of the rat brain.

Furlano RI, Anthony A, Day R, Brown A, McGarvey L, Thomson MA, Davies SE, Berelowitz M, Forbes A, Wakefield AJ, Walker-Smith JA, Murch SH: Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism. J Pediatr. 2001 Mar;138(3):366-72.

University Department of Paediatric Gastroenterology, the Inflammatory Bowel Diseases Study Group, Royal Free and University College School of Medicine, London, United Kingdom.

OBJECTIVES: We have reported colitis with ileal lymphoid nodular hyperplasia (LNH) in children with regressive autism. The aims of this study were to characterize this lesion and determine whether LNH is specific for autism. METHODS: Ileo-colonoscopy was performed in 21 consecutively evaluated children with autistic spectrum disorders and

bowel symptoms. Blinded comparison was made with 8 children with histologically normal ileum and colon, 10 developmentally normal children with ileal LNH, 15 with Crohn's disease, and 14 with ulcerative colitis. Immunohistochemistry was performed for cell lineage and functional markers, and histochemistry was performed for glycosaminoglycans and basement membrane thickness. RESULTS: Histology demonstrated lymphocytic colitis in the autistic children, less severe than classical inflammatory bowel disease. However, basement membrane thickness and mucosal gamma delta cell density were significantly increased above those of all other groups including patients with inflammatory bowel disease. CD8(+) density and intraepithelial lymphocyte numbers were higher than those in the Crohn's disease, LNH, and normal control groups; and CD3 and plasma cell density and crypt proliferation were higher than those in normal and LNH control groups. Epithelial, but not lamina propria, glycosaminoglycans were disrupted. However, the epithelium was HLA-DR(-), suggesting a predominantly T(H)2 response. INTERPRETATION: Immunohistochemistry confirms a distinct lymphocytic colitis in autistic spectrum disorders in which the epithelium appears particularly affected. This is consistent with increasing evidence for gut epithelial dysfunction in autism.

Cavataio F, Carroccio A, Iacono G.: Milk-induced reflux in infants less than one year of age. J Pediatr Gastroenterol Nutr 2000;30 Suppl:S36-44

Abstract: 1st Divisione Pediatria, Gastroenterologia, Ospedale dei Bambini G. Di Cristina, Palermo, Italy.

Cow's milk allergy (CMA) and gastroesophageal reflux are considered to be among the most common disturbances in infants less than 1 year of age. In recent years, the relationship existing between these two entities has been investigated and some important conclusions have been reached: In just under half the cases of GER in infants less than 1 year of age there is an association with CMA; in a high proportion of cases, GER is not only CMA-associated but also CMA-induced; the frequency of this association should induce pediatricians to screen for possible concomitant CMA in all infants with GER less than 1 year old; with the exception of some patients with mild typical CMA manifestations (diarrhea, dermatitis, or rhinitis), the symptoms of GER associated with CMA are the same as those observed in primary GER; immunologic tests are useful in a suspected association between GER and CMA; and subjects with GER secondary to CMA show a typical pH-monitoring tracing pattern, characterized by a progressive, slow decrease in esophageal pH between feedings. This article reviews the main features of the two diseases, stressing the aspects in common between them and comments on all the listed points.

NOTE: Reflux appears to be common in infants later dx'd with autism.

Carroccio A, Montalto G, Custro N, Notarbartolo A, Cavataio F, D'Amico D, Alabrese D, Iacono G: Evidence of very delayed clinical reactions to cow's milk in cow's milk-intolerant patients. Allergy 2000 Jun;55(6):574-9.

Abstract: Internal Medicine, University Hospital of Palermo, Italy.

BACKGROUND: In patients with cow's milk protein intolerance (CMPI), delayed clinical reactions to cow's milk (CM) ingestion may be misdiagnosed if the clinical symptoms are not "classical" and there is a long time lapse between ingestion of CM and the clinical reaction. The aim was to evaluate the clinical outcome of CMPI in a cohort of CM-intolerant children, with particular attention to the occurrence of clinical manifestations beyond 72 h after CM challenge. METHODS: Eighty-six consecutive patients (44 boys, 42 girls) with new CMPI diagnoses were enrolled; median age at diagnosis was 4 months. Patients were followed up for a mean period of 40 months. In all patients, CMPI diagnosis was made on the observation of symptoms, their disappearance after elimination diet, and their reappearance on double-blind CM challenge. At CMPI diagnosis, immunologic tests to demonstrate IgE-mediated hypersensitivity were performed. After 12 months of CM-free diet, CM tolerance was re-evaluated with a CM challenge continued at home for up to 30 days, according to a double-blind, placebo-controlled method. Patients who did not achieve CM tolerance continued a CM-free diet and subsequently underwent yearly CM challenge. RESULTS: The percentages of CMPI patients who became CM-tolerant after 1, 2, and 3 years of CM-free diet were 30%, 54.5%, and 70%, respectively. At the end of the follow-up period, 26/86 subjects showed persistent CMPI; these patients had a higher percentage of positivity of total serum IgE ($P<0.05$), RAST ($P<0.01$), and cutaneous prick tests for CM antigens ($P<0.001$) than all the others. At CMPI diagnosis, all patients had a clinical reaction within 72 h from the beginning of the CM challenge; at the subsequent "cure" challenges, we observed patients who first reacted to CM more than 72 h after ingestion. In total, 10 out of 86 patients showed "very delayed reactions"; in these patients, the mean time between the beginning of CM challenge and the onset of a clinical symptom was 13.3 days (range 4-26 days). The number of "very late reactors" increased from the first to the third of the "cure" CM challenges, performed at yearly intervals. The "very delayed" CMPI manifestations in these subjects were constipation (five cases), wheezing (two cases), dermatitis plus constipation (two cases), and dermatitis alone (one case); in 6/10 patients, the symptoms observed at the "cure challenge" were different from those at CMPI onset. CONCLUSIONS: Very delayed clinical reactions to reintroduction of CM in the diet can occur in CMPI patients; thus, accurate follow-up and frequent outpatient observation in patients with a long history of CMPI are probably more useful and safer than prolonged CM challenge.

Cade R, Privette M, Fregly M, Rowland N, Sun Z, Zele V, Wagemaker H, Edlestein C: Autism and schizophrenia: intestinal disorders. Nutritional Neuroscience 3: 57-72, 2000. [No abstract available]

Pedersen OS, Liu Y, Reichelt KL.: Serotonin uptake stimulating peptide found in plasma of normal individuals and in some autistic urines. J Pept Res 1999 Jun;53(6):641-6

Abstract: Research Institute, University of Oslo, Rikshospitalet, Norway.

We have isolated a tripeptide from normal plasma and autistic urines which stimulates the uptake of serotonin (5-HT) into platelets. This peptide was purified by high-performance liquid chromatography (HPLC) and characterized by sequenation and mass-spectrometry. Synthetic peptide showed co-chromatography with the biological sample in the HPLC systems used. Close to 60% of the autistic children diagnosed using the Diagnostic Statistical Manual III-R had an increased HPLC peak eluting like this peptide in their urines compared with controls.

Ek J, Stensrud M, Reichelt KL.: Gluten-free diet decreases urinary peptide levels in children with celiac disease. J Pediatr Gastroenterol Nutr 1999 Sep;29(3):282-5.

Abstract: Department of Pediatrics, Buskerud Central Hospital, Drammen, Norway.

BACKGROUND: Increased urine secretion of peptides has been found in celiac disease, probably resulting from increased intestinal uptake of peptides caused by damage to the small gut mucosa. METHODS: High-performance liquid chromatography of low-molecular-weight peptides in the urine was performed over 6 months, before and after a gluten-free diet was instituted in children who clinically improved while consuming the diet. RESULTS: A significant decrease of peptide levels was observed in children consuming the gluten-free diet. Certain peptide peaks thought to be gluten related decreased the most after the patients began the diet. CONCLUSIONS: Because the peptides decrease in patients consuming a gluten-free diet, it is reasonable to conclude that such peptides have a mostly dietary origin.

Cade JR et al: Autism and schizophrenia linked to malfunctioning enzyme for milk protein digestion. Autism, Mar 1999.

- Sun Z, Cade JR, Fregly MJ, Privette RM. Caesomorphine induces Fos-like reactivity in discrete brain regions relevant to schizophrenia and autism. Autism 1999;3:67-84
- Sun Z, Cade JR. A peptide found in schizophrenia and autism causes behavioral changes in rats. Autism 1999;3:85

Cade JR, Privette RM, Fregly M, Rowland N, Sun Z, Zele V, Wagemaker H Edelstein C: Autism and schizophrenia: Intestinal disorders. Nutritional Neuroscience 1999, 2, 57-72.

Alberti A, Pirrone P, Elia M, Waring RH, Romano C: Sulphation deficit in "low-functioning" autistic children: a pilot study. Biol Psychiatry 1999 Aug 1;46(3):420-4.

Abstract: Department of Pediatrics, Oasi Institute for Research on Mental Retardation and Brain Aging (IRCCS), Troina, Italy.

BACKGROUND: Parents of autistic children and autism support groups often report that autistic episodes are exacerbated when the children eat certain foodstuffs such as dairy products, chocolates, wheat, corn sugar, apples, and bananas. The hypothesis that autistic behavior might be related to metabolic dysfunctions has led us to investigate in a group of "low functioning" autistic children and in an age-matched control group each made up of 20 subjects, the sulphation capacity available. METHODS: Utilizing the biochemical characteristics of paracetamol we evaluated by high performance liquid chromatography, the urine paracetamol-sulfate/paracetamol-glucuronide (PS/PG) ratio in all subjects following administration of this drug. RESULTS: The PS/PG ratio in the group of autistic subjects gave a significantly lower results than the control group with $p < .00002$. CONCLUSIONS: The inability to effectively metabolize certain compounds particularly phenolic amines, toxic for the CNS, could exacerbate the wide spectrum of autistic behavior.

Iacono G, Cavataio F, Montalto G, Florena A, Tumminello M, Soresi M, Notarbartolo A, Carroccio A: Intolerance of cow's milk and chronic constipation in children. New England Journal of Medicine 1998 / 339 (16) / 1100-1104.

Abstract: Divisione di Pediatria, Ospedale G. Di Cristina, Palermo, Italy.

BACKGROUND: Chronic diarrhea is the most common gastrointestinal symptom of intolerance of cow's milk among children. On the basis of a prior open study, we hypothesized that intolerance of cow's milk can also cause severe perianal lesions with pain on defecation and consequent constipation in young children. METHODS: We performed a double-blind, crossover study comparing cow's milk with soy milk in 65 children (age range, 11 to 72 months) with chronic constipation (defined as having one bowel movement every 3 to 15 days). All had been referred to a pediatric gastroenterology clinic and had previously been treated with laxatives without success; 49 had anal fissures and perianal erythema or edema. After 15 days of observation, the patients received cow's milk or soy milk for two weeks. After a one-week washout period, the feedings were reversed. A response was defined as eight or more bowel movements during a treatment period. RESULTS: Forty-four of the 65 children (68 percent) had a response while receiving soy milk. Anal fissures and pain with defecation resolved. None of the children who received cow's milk had a response. In all 44 children with a response, the response was confirmed with a double-blind challenge with cow's milk. Children with a response had a higher frequency of coexistent rhinitis, dermatitis, or bronchospasm than those with no response (11 of 44 children vs. 1 of 21, P=0.05); they were also more likely to have anal fissures and erythema or edema at

base line (40 of 44 vs. 9 of 21, P<0.001), evidence of inflammation of the rectal mucosa on biopsy (26 of 44 vs. 5 of 21, P=0.008), and signs of hypersensitivity, such as specific IgE antibodies to cow's-milk antigens (31 of 44 vs. 4 of 21, P<0.001). CONCLUSIONS: In young children, chronic constipation can be a manifestation of intolerance of cow's milk.

Iacono G, Cavataio F, Montalto G, Soresi M, Notarbartolo A, Carroccio A: Persistent cow's milk protein intolerance in infants: the changing faces of the same disease. Clin Exp Allergy 1998 Jul;28(7):817-23.

Abstract: II Divisione di Pediatria, Ospedale Di Cristina, Universita di Palermo, Italy.

BACKGROUND: Recent research has shown that cow's milk protein intolerance (CMPI) often persists beyond 4 years of age. AIMS: To evaluate the clinical and immunological characteristics of a group of infants with persistent CMPI. PATIENTS AND METHODS: Twelve infants (6 m, 6f) with persistent CMPI were followed up from birth until a median age of 5 years. The patients underwent CMP challenge each year to evaluate CMP-tolerance. As controls we followed 26 infants (12 m, 14 f) with CMPI that resolved within 1-2 years. RESULTS: A family history of atopic disease was found in 10/12 patients with persistent CMPI and in 10/26 controls (P<0.01). Clinical presentation changed over time: at onset symptoms were prevalently gastrointestinal, while at the end of the study there was an increased frequency of wheezing and constipation and a higher frequency of delayed reactions to CMP-challenge than at study commencement (9/12 vs 2/12; P<0.007). 11/12 infants with persistent CMPI and 3/26 controls (P<0.0001) presented multiple food intolerance. During the observation period 9/12 infants with persistent CMPI and 2/26 controls showed atopic disease: asthma, rhinitis, eczema (P < 0.0001). CONCLUSIONS: Persistent CMPI forms are characterized by: (a) considerable importance of familial atopic disease; (b) change in CMPI manifestations over time and more prolonged delay between CMP consumption and manifestation of symptoms; (c) very high frequency of multiple food intolerance and allergic diseases.

Teschemacher, H. et al: Milk protein-derived opioid receptor ligands. Biopolymers. 1997 / 43 (2) / 99-117.

Abstract:

Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat, Giessen, Germany.

Milk is mammalian characteristic and is of particular importance for humans: Mother's milk or its substitutes from cows' milk are absolutely essential nutriments for the neonate and cows' milk also represents a basic foodstuff for adults. However, in addition to their well-known nutritive role, milk constituents apparently are also able to carry specific information from the milk producer's to the milk receiver's organism:

Thus, a number of milk protein fragments has been shown to behave like opioid receptor ligands able to address opioidergic systems in the adult's or in the neonate's organism. With respect to the proteins, which they are derived off these peptides have been named alpha-casein exorphins or casoxin D (alpha-casein), beta-casomorphins or beta-casorphin (beta-casein), casoxin or casoxin A, B, or C (k-casein), alpha-lactorphins (alpha-lactalbumin), beta-lactorphin (beta-lactoglobulin) or lactoferroxins (lactoferrin). Only casoxins and lactoferroxins display antagonistic properties; the other peptides behave like opioid receptor agonists. Most of the information available so far has been collected about beta-casomorphins. These peptides obviously can be released from beta-casein in the adult's or in the neonate's organism, where they might elicit opioid effects in the frame of a regulatory role as "food hormones". Several synthetic beta-casomorphin derivatives have been shown to be highly specific and potent mu-type opioid receptor ligands which frequently have been used as standard tools in opioid research.

Fukudome, S. et al: Release of opioid peptides, gluten exorphins by the action of pancreatic elastase. FEBS Lett. 1997 / 412 (3) / 475-479.

Abstract: Food Research Laboratory, Nisshin Flour Milling Co. Ltd., Saitama, Japan.

The release of opioid peptides, gluten exorphins A, which have been isolated from the pepsin-thermolysin digest of wheat gluten, with gastrointestinal proteases was examined. High levels of gluten exorphin A5 (Gly-Tyr-Tyr-Pro-Thr) immunoreactive materials were detected in the pepsin-pancreatic elastase digest by a competitive ELISA. From this digest, gluten exorphin A5, B5 and B4 were isolated. This means that these peptides are released in the gastrointestinal tracts after ingestion of wheat gluten. The yield of gluten exorphin A5 in the pepsin-elastase digest was larger than that in the pepsin-thermolysin digest. The gluten exorphin A5 sequence is found 15 times in the primary structure of the high molecular weight glutenin. The region from which gluten exorphin A5 was released by the action of pancreatic elastase was identified using synthetic fragment peptides.

Scifo R, Cioni M, Nicolosi A, Batticane N, Tirolo C, Testa N, Quattropani MC, Morale MC, Gallo F, Marchetti B: Opioid-immune interactions in autism: behavioural and immunological assessment during a double-blind treatment with naltrexone. Ann Ist Super Sanita 1996;32(3):351-9.

Abstract: Servizio di Psichiatria, Istituto OASI per lo Studio del Ritardo Mentale e l'Involuzione Cerebrale, Troina (Enna), Italy.

The emerging concept of opioid peptides as a new class of chemical messengers of the neuroimmune axis and the presence of a number of immunological abnormalities in infantile autism prompted us to correlate biological (hormonal and immunological) determinations and behavioural performances during treatment with the potent opiate

antagonist, naltrexone (NAL). Twelve autistic patients ranging from7 to 15 years, diagnosed according to DSM-III-R, entered a double-blind crossover study with NAL at the doses of 0.5, 1.0 and 1.5 mg/kg every 48 hours. The behavioural evaluation was conducted using the specific BSE and CARS rating scales NAL treatment produced a significant reduction of the autistic symptomatology in seven ("responders") out of 12 children. The behavioural improvement was accompanied by alterations in the distribution of the major lymphocyte subsets, with a significant increase of the T-helper-inducers (CD4+CD8-) and a significant reduction of the T-cytotoxic-suppressor (CD4-CD8+) resulting in a normalization of the CD4/CD8 ratio. Changes in natural killer cells and activity were inversely related to plasma beta-endorphin levels. It is suggested that the mechanisms underlying opioid-immune interactions are altered in this population of autistic children and that an immunological screening may have prognostic value for the pharmacological therapy with opiate antagonists.

Hadjivassiliou M, Gibson A, Davies-Jones GA , Lobo AJ, Stephenson TJ, Milford-Ward A: Does cryptic gluten sensitivity play a part in neurological illness? Lancet 1996 Feb 10;347(8998):369-71.

Abstract: Department of Neurology, Royal Hallamshire Hospital, Sheffield, UK.

BACKGROUND: Antigliadin antibodies are a marker of untreated coeliac disease but can also be found in individuals with normal small-bowel mucosa. Because neurological dysfunction is a known complication of coeliac disease we have investigated the frequency of antigliadin antibodies, as a measure of cryptic gluten sensitivity, and coeliac disease in neurological patients. METHODS: Using ELISA, we estimated serum IgG and IgA antigliadin antibodies in 147 neurological patients who were divided into two groups. There were 53 patients with neurological dysfunction of unknown cause despite full investigation (25 ataxia, 20 peripheral neuropathy, 5 mononeuritis multiplex, 4 myopathy, 3 motor neuropathy, 2 myelopathy). The remaining 94 patients were found to have a specific neurological diagnosis (16 stroke, 12 multiple sclerosis, 10 Parkinson's disease, 56 other diagnoses) and formed the neurological control group. 50 healthy blood donors formed a third group. FINDINGS: The proportions of individuals with positive titres for antigliadin antibodies in the three groups were 30/53, 5/94, and 6/50 respectively (57, 5, and 12%). The difference in proportion between group 1 and the combined control groups was 0.49 (95% CI 0.35-0.63). Distal duodenal biopsies in 26 out of 30 antigliadin-positive patients from group 1 revealed histological evidence of coeliac disease in nine (35%), non-specific duodenitis in ten (38%), and no lesion in seven (26%) individuals. INTERPRETATION: Our data suggest that gluten sensitivity is common in patients with neurological disease of unknown cause and may have aetiological significance.

D'Eufemia P., Celli M., Finocchiaro R., Pacifico L., Viozzi L., Zaccagnini M., Cardi E., Giardini O: Abnormal Intestinal Permeability in Children with Autism. Acta Paediatrica,1996; 85: 1076-1079.

Abstract: Institute ofPediatrics, La Sapienza University of Rome, Italy.

We determined the occurrence of gut mucosal damage using the intestinal permeability test in 21 autistic children who had no clinical and laboratory findings consistent with known intestinal disorders. An altered intestinal permeability was found in 9 of the 21 (43%) autistic patients, but in none of the 40 controls. Compared to the controls, these nine patients showed a similar mean mannitol recovery, but a significantly higher mean lactulose recovery (1.64% +/- 1.43 vs 0.38% +/- 0.14; P < 0.001). **We speculate that an altered intestinal permeability could represent a possible mechanism for the increased passage through the gut mucosa of peptides derived from foods with subsequent behavioural abnormalities.**

Lucarelli, S., Frediani, T., Zingoni, A.M., Ferruzzi, F.,Giardini, O., Quintieri, F., Barbato, M., D'Eufemia, P., Cardi, E.: Food allergy and infantile autism, Panminerva Med., 1995 Sep; 37(3): 137-41.

Abstract: Department of Paediatrics, University of Rome La Sapienza, Italy.

The etiopathogenesis of infantile autism is still unknown. Recently some authors have suggested that food peptides might be able to determine toxic effects at the level of the central nervous system by interacting with neurotransmitters. In fact a worsening of neurological symptoms has been reported in autistic patients after the consumption of milk and wheat. The aim of the present study has been to verify the efficacy of a cow's milk free diet (or other foods which gave a positive result after a skin test) in 36 autistic patients. We also looked for immunological signs of food allergy in autistic patients on a free choice diet. We noticed a marked improvement in the behavioural symptoms of patients after a period of 8 weeks on an elimination diet and we found high levels of IgA antigen specific antibodies for casein, lactalbumin and beta-lactoglobulin and IgG and IgM for casein. The levels of these antibodies were significantly higher than those of a control group which consisted of 20 healthy children. Our results lead us to hypothesise a relationship between food allergy and infantile autism as has already been suggested for other disturbances of the central nervous system.

Lensing P, Schimke H, Klimesch W, Pap V, Szemes G, Klingler D, Panksepp J: Clinical case report: opiate antagonist and event-related desynchronization in 2 autistic boys. Neuropsychobiology 1995;31(1):16-23.

Abstract: Department of Physiological Psychology, University of Salzburg, Austria.

Event-related desynchronization and visual orientational behavior were examined in 2 autistic boys to determine if blockade of endogenous opioid activity facilitates cognitive processing at a cortical level. Before naltrexone, the boys showed no selective alpha blocking during exposure to either mother's pictures or white light. Unlike normals, they exhibited strong alpha band enhancement at temporocentral recording sites. Two hours

after administering 0.5 mg/kg naltrexone, mother-as well as light-related alpha blocking appeared at occipital, occipitotemporal, and prefrontal sites. These effects were gone 24 h after dosing in one child, but persisted in the other. A parallel increase in visual pursuit in a social context was observed. These results affirm that autistic gaze aversion can be caused by excessive opioid activity interfering with corticothalamocortical processing of visual stimuli.

Kurek M, Czerwionka-Szaflarska M, Doroszewska G: Pseudoallergic skin reactions to opiate sequences of bovine casein in healthy children. Rocz Akad Med Bialymst 1995;40(3):480-5.

Abstract: Department of Gastroenterology, Academy of Medicine, Gdansk.

Skin tests with opioid peptides naturally occurring in cow's milk: beta-casomorphin-7 and alpha-casein (90-95), were performed in 25 healthy children. Wheal and flare reactions, similiar to histamine and codeine were observed in all children. The area of these reactions was concentration dependent. Pretreatment with H1 antagonist--cetirizine significantly inhibited the skin response to both peptides. Beta-casomorphin-7 and alpha-casein (90-95) are noncytotoxic histamine releasers in humans.

Knivbserg A.M., Reichelt K.L., Nodland M., Hoien T.: Autistic syndromes and diet. A four year follow-up study. Scand J Educat. Res. 1995, 39: 223-236.

Abstract: Dietary intervention was applied to 15 subjects with autistic syndromes, with pathological urine patterns, and increased levels of peptides found in their twenty-four-hour urine samples. The peptides, some of which are probably derived from gluten and casein, are thought to have a negative pharmacological effect on attention, brain maturation, social interaction and learning. Our hypothesis was that a diet without these proteins would facilitate learning. Social behaviour, as well as cognitive and communicative skills, were assessed before diet. The subjects were closely followed for a year, after which their urine was retested blind, and the assessment of behaviors and skills was repeated. Further retesting was made four years after the onset of dietary intervention. Normalization of urine patterns and peptide levels was found after one year. Likewise, a decrease in odd behaviour and an improvement in the use of social, cognitive and communicative skills were registered. This positive development continued through the next three years, though at a lower rate. These promising results encourage further research on the effect of dietary intervention.

Bouvard MP, Leboyer M, Launay JM, Recasens C, Plumet MH, Waller-Perotte D, Tabuteau F, Bondoux D, Dugas M, Lensing P, et al: Low-dose naltrexone effects on plasma chemistries and clinical symptoms in autism: a double-blind, placebo-controlled study. Psychiatry Res 1995 Oct 16;58(3):191-201.

Abstract: Service de Psychopathologie de l'Enfant et de l'Adolescent, Hopital Robert Debre, Paris, France.

The effect of month-long naltrexone (NTX) treatment at a daily oral dose of 0.5 mg/kg/day was contrasted with placebo (PLC) in a double-blind study with conjoint clinical and biochemical evaluations of therapeutic effects. Modest clinical benefits were achieved with both PLC and NTX, with marginally better overall results following NTX, and degree of improvement appeared to be related to plasma chemical profiles. Massively elevated levels of beta-endorphin were observed in all children with assays using C-terminal antibody but not with an N-terminal antibody assay. In addition, 70% of the children exhibited abnormally low levels of adrenocorticotropic hormone, and smaller subsets exhibited elevated norepinephrine (60%), arginine-vasopressin (50%), and serotonin (20%). The best clinical responders exhibited the clearest normalization of the elevated plasma chemistries, especially in C-terminal-beta-endorphin and serotonin. There was some evidence of therapeutic carry-over effects in both clinical and biochemical measures in those children who received NTX before PLC. The results suggest that NTX only benefits a subgroup of autistic children, who may be identified by the presence of certain plasma abnormalities. These results suggest a possible linkage between abnormal plasma chemistries, especially those related to the pro-opiomelanocortin system, and autistic symptoms.

Reichelt K.L. Knivsberg AM, Nodland M, Lind G: Nature and consequences of hyperpeptiduria and bovine casomorphin found in autistic syndromes. Develop Brain Dysfunct. 1994, 7: 71-85. [No abstract available]

Gardner M.L.G.: Absorption of intact proteins and peptides. Physiol of gastrointestinal Tract 3rd edit (edit: LR Johnson) Raven press, New York 1994: 1795-1820.

Leboyer M, Bouvard MP, Launay JM, Recasens C, Plumet MH, Waller-Perotte D, Tabuteau F, Bondoux D, Dugas M: Opiate hypothesis in infantile autism? Therapeutic trials with naltrexone [Article in French]. Encephale 1993 Mar-Apr;19(2):95-102.

Abstract: Service de Psychiatrie Adulte, Hopital Pitie-Salpetriere, Paris.

The opioid hypothesis suggests that childhood autism may result from excessive brain opioid activity during neonatal period which may constitutionally inhibit social motivation, yielding autistic isolation and aloofness (Panksepp, 1979). This hypothesis has now received strong support and is currently based on three types of arguments: (1) similarity between autistic symptomatology and abnormal behaviors induced in young animals by injections of exogenous opioids, such as increasing social aloofness and decreasing social vocalization; (2) direct biochemical evidence of abnormalities of peripheral endogenous opioids being reported in autism and (3) therapeutic effects of the long lasting opioid receptor blocking agent naltrexone in autism. In this article, we

give description of open and double-blind studies of naltrexone in autism. Naltrexone has been tested in several open studies. We performed an open trial with naltrexone in 2 autistic girls, displaying serious self-injurious behavior, reduced crying and a marked preference for salty and spicy foods, symptoms that could be related to a dysfunction of the opioid system. With dosages of 1 mg/kg/day, we observed an immediate reduction of hyperactivity, self-injurious behavior and aggressiveness, while attention improved. In addition, social behaviors, smiling, social seeking behaviors and play interactions increased (Leboyer, Bouvard et Dugas, 1988). Campbell et al. (1988) has also reported a tranquilizing and a stimulating effect in 6 out of 8 children with autism. We did confirm these preliminary results in a double-blind study performed on 4 children with autism. In a cross-over double-blind study, three dosages of naltrexone (0.5, 1 and 2 mg/kg/day) and placebo were compared. (ABSTRACT TRUNCATED AT 250 WORDS)

Fukudome S, Yoshikawa M: Gluten exorphin C, A novel opioid peptide derived from wheat gluten. FEBS 1993; 316: 17-19.

Abstract: Research Control Department, Nisshin Flour Milling Co., Ltd., Nihonbashi, Tokyo, Japan.

A novel opioid peptide, Tyr-Pro-Ile-Ser-Leu, was isolated from the pepsin-trypsin-chymotrypsin digest of wheat gluten. Its IC50 values were 40 microM and 13.5 microM in the GPI and MVD assays, respectively. This peptide was named gluten exorphin C. Gluten exorphin C had a structure quite different from any of the endogenous and exogenous opioid peptides ever reported in that the N terminal Tyr was the only aromatic amino acid. The analogs containing Tyr-Pro-X-Ser-Leu were synthesized to study its structure-activity relationship. Peptides in which X was an aromatic amino acid or an aliphatic hydrophobic amino acid had opioid activity.

Bidet B, Leboyer M, Descours B, Bouvard MP, Benveniste J: Allergic sensitization in infantile autism. J Autism Dev Disord 1993 Jun;23(2):419-20. [No abstract available.]

McLaughlin P.J., Zagon I.S.: Endogenous opiod systems and clinical implications for infantile autism. Proceedings of the International Symposium on Neurobiology of Infantile Autism, Tokyo , 1990, Neurobiology of Infantile Autism, Excerpta Medica 1992.

Lensing P, Klingler D, Lampl C, Leboyer M, Bouvard M, Plumet MH, Panksepp J: Naltrexone open trial with a 5-year-old-boy. A social rebound reaction. Acta Paedopsychiatr 1992;55(3):169-73.

Abstract: School Psychology of Upper Austria, Linz.

The neurobiological rationale for an opiate antagonist pharmacotherapy of autism is presented. Naltrexone efficacy in decreasing autistic behaviour and in increasing social-affiliative behaviour was explored in a 5-year-old autistic boy. Naltrexone (0.5 mg/kg 3

times peer week) was effective in immediately decreasing gross motor activity and stereotyped behaviour and caused a delayed increase of crying, smiling and rough-and-tumble play. This single case presents preliminary evidence that a therapeutically valuable rebound reaction is possible and that the human opioid system modulates social-affective processes. The possibility of psychological factors being instrumental in achieving this effect is discussed as being suitable for future clinical trials.

Kurek M, Przybilla B, Hermann K, Ring J: A naturally occurring opioid peptide from cow's milk, beta-casomorphine-7, is a direct histamine releaser in man.Int Arch Allergy Immunol 1992;97(2):115-20.

Abstract: Department of Dermatology, Ludwig-Maximilian-University, Munich.

beta-Casomorphine-7, a naturally occurring product of cow's milk with opiate-like activity, was studied for possible direct histamine liberation activities in humans. It was found to cause concentration-dependent in vitro histamine release from peripheral leukocytes of healthy adult volunteers. Intradermal injection of beta-casomorphine-7 induced a wheal and flare reaction in the skin similar to histamine or codeine. Oral pretreatment with the H1 antagonist terfenadine significantly inhibited the skin responses to beta-casomorphine-7. The intradermal injection of an opiate receptor antagonist, naloxone, inhibited in vitro histamine release and skin reactions only in a 100-fold excess over beta-casomorphine-7. These findings suggest that beta-casomorphine-7 can be regarded as a noncytotoxic, direct histamine releaser in humans. The clinical relevance of these findings deserves further studies.

Fukudome S, Yoshikawa M: Opioid peptides derived from wheat gluten: their isolation and characterization. Federation of European Biochemical Societies (FEBS) 1992; 296: 107-111.

Abstract:Research Control Department, Nisshin Flour Milling Co., Ltd., Tokyo, Japan.

Four opioid peptides were isolated from the enzymatic digest of wheat gluten. Their structures were Gly-Tyr-Tyr-Pro-Thr, Gly-Tyr-Tyr-Pro,Tyr-Gly-Gly-Trp-Leu and Tyr-Gly-Gly-Trp, which were named gluten exorphins A5, A4, B5 and B4, respectively. The gluten exorphin A5 sequence was found at 15 sites in the primary structure of the high molecular weight glutenin and was highly specific for delta-receptors. The structure-activity relationships of gluten exorphins A were unique in that the presence of Gly at their N-termini increased their activities. Gluten exorphin B5, which corresponds to [Trp4,Leu5]enkephalin, showed the most potent activity among these peptides. Its IC50 values were 0.05 microM and 0.017 microM, respectively, on the GPI and the MVD assays.

Dubynin VA, Maklakova AS, Nezavibat'ko VN, Alfeeva LA, Kamenskii AA, Ashmarin IP: Effects of systemically-administered beta-casomorphin-7 on nociception in rats. [Article in Russian] Biull Eksp Biol Med 1992 Sep;114(9):284-6.

Abstract: The influence of food-derived heptapeptide beta-casomorphin-7 (beta-CM-7) on pain sensibility of white rats was studied by tail flick test. As shown for doses 10 and 20 mg/kg intraperitoneally, injected beta-CM-7 induced significant analgesia; lower peptide concentration (5 mg/kg) was ineffective. As a whole, there is a significant positive correlation between the intensity of analgesia and the quantity of administered exorphine. These changes of pain sensibility were observed for one hour after injection of heptapeptide; further measurements showed no significant difference of time reaction between control and experimental groups of rats. It was found out that animals with high native level of pain sensibility (4-8 sec) made the main contribution to manifestation of analgesia.

Bouvard M.P., Leboyer M., Launay J.M., Kerdelhue B., Dugas M.: The opiod excess hypothesis of autism: A double-blind study of naltrexone. Proc. of the Intern. Symp. on Neurob. of Inf. Autism, 1990, Neurobiology of Infantile Autism, Exerpta Medica 1992.

Teschemacher H, Koch G: Opioids in the milk. Endocr Regul 1991 Sep;25(3):147-50.

Abstract: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Germany.

In various studies, the milk has been screened for the presence of free or precursor-bound opioids. In fact, various opioid receptor ligands with agonistic or even antagonistic activity were found. Besides the alkaloid morphine, peptides derived from alpha-casein (alpha-casein exorphins), beta-casein (beta-casomorphins; beta-casorphin), alpha-lactalbumin (alpha-lactorphins) and beta-lactoglobulin (beta-lactorphin) were among the agonists. In addition, certain peptides derived from k-casein (casoxins) or from lactoferrin (lactoferroxins) were found to behave like opioid antagonists. Although a functional role in the mammalian organism for all of these compounds appears to be well possible, evidence has only been presented for the functional significance of beta-casomorphins, so far. These peptides might play a role in reproduction or nutrition in the female, in the newborn's or in a milk consumer's organism, respectively. Thus, opioids related to milk might represent essential exogenous extensions of the endogenous opiodergic systems.

Risebro, B.: Gluten-free diet in infantile autism. Tidsskr Nor Laegeforen 1991 Jun 10;111(15):1885-6 [Article in Norwegian]

Reichelt K.L., Knivsberg A.M., Lind G., Nodland M.: The probable etiology and possible treatment of childhood autism. Brain Dysfunct. 1991, 4: 308-319. [No abstract available]

Longoni R, Spina L, Mulas A, Carboni E, Garau L, Melchiorri P, Di Chiara G: (D-Ala2)deltorphin II: D1-dependent stereotypies and stimulation of dopamine release in the nucleus accumbens. J Neurosci 1991 Jun;11(6):1565-76.

Abstract:

Institute of Experimental Pharmacology and Toxicology, University of Cagliari, Italy.

In order to investigate the relative role of central delta- and mu-opioid receptors in behavior, the effects of (D-Ala2)deltorphin II, a natural delta-opioid peptide, and PL017, a beta-casomorphin derivative specific for mu receptors, were compared after local intracerebral and intraventricular administration. Intracerebral infusion of the two peptides was done bilaterally in the limbic nucleus accumbens and in the ventral and dorsal caudate putamen of freely moving rats through chronic intracerebral cannulas. After intra-accumbens infusion, the two peptides elicited marked but opposite behavioral effects: while (D-Ala2)deltorphin II evoked dose-dependent motor stimulation characterized by locomotion, sniffing, and oral stereotypies, PL017 elicited motor inhibition with rigidity and catalepsy. These effects were site specific because they could not be evoked from the ventral or from the dorsal caudate. Low doses of naloxone (0.1 mg/kg, s.c.) blocked the effects of PL017 but not those of (D-Ala2)deltorphin II, which instead were reduced by high doses of naloxone (1.0 mg/kg) and by the putative delta-antagonist naltrindole; this drug failed to affect the catalepsy induced by PL017. Therefore, while (D-Ala2)deltorphin II effects were delta-mediated, PL017 effects were mu-mediated. Blockade of dopamine D1 receptors by SCH 23390 abolished (D-Ala2)deltorphin II effects, while blockade of dopamine D2 receptors by raclopride or by haloperidol was without effect. Local application by reverse dialysis of (D-Ala2)deltorphin II (5 microM) to the accumbens resulted in a naloxone-sensitive increase of extracellular dopamine concentrations; these effects could not be evoked from the caudate, nor by PL017 in the accumbens. Intracerebroventricular administration of (D-Ala2)deltorphin II or of PL017 elicited behavioral effects qualitatively similar to those obtained from the accumbens.

If deltorphin II is indeed present in the urine, this may explain why low doses of naloxone are often only moderately effective at reducing autistic behaviors.

Fukudome S.-I. and Yoshikawa M.: Opioid peptides derived from wheat gluten: Their isolation and characterization. FEBS Letters 1991, 296: 107-111.

Abstract: Four opioid peptides were isolated from the enzymatic digest of wheat gluten. Their structures were Gly-Tyr-Tyr-Pro-Thr, Gly-Tyr-Tyr-Pro, Tyr-Gly-Gly-Trp-Leu

and Tyr-Gly-Gly-Trp, which were named gluten exorphins A5, A4, B5 and B4, respectively. The gluten exorphin A5 sequence was found at 15 sites in the primary structure of the high molecular weight glutenin and was highly specific for delta-receptors. The structure-activity relationships of gluten exorphins A were unique in that the presence of Gly at their N-termini increased their activities. Gluten exorphin B5, which corresponds to [Trp4,Leu5]enkephalin, showed the most potent activity among these peptides. Its IC50 values were 0.05 microM and 0.017 microM, respectively, on the GPI and the MVD assays.

Shattock P. Kennedy A, Rowell F, Berney T: Role of neuropeptides in autism and their relationships with classical neurotransmitters. Brain Dysfunct 1990, 3: 328-346. [No abstract available]

Reichelt K.L., Ekrem J., Scott H.: Gluten, milk proteins and autism: Dietary interventions effects on behavior and peptide secretion. J Appl Nutrition 1990, 42: 1-11. [No abstract available]

Marchetti B, Scifo R, Batticane N, Scapagnini U: Immunological Significance of Opioid Peptide Dysfunction in Infantile Autism. Brain Dysfunction,3: 346-354,1990. [No abstract available]

Leboyer M, Bouvard MP, Lensing P, Launay JM, Tabuteau F, Arnaud P, Waller D, Plumet MH, Recasens C, Kerdelhue B, Dugas M, Panksepp J: Opioid Excess Hypothesis of Autism. Brain Dysfunction 1990; 3: 285-298. [No abstract available]

Knivsberg A.M., Wiig K., Lind G., Nodland M., Reichelt K.L.: Dietary intervention in autistic syndromes. Brain Dysfunc. 1990, 3: 315-327. [No abstract available]

Cade R. et al.: The effects of dialysis and diet in schizophrenia. Psychiatry: A World perspective 1990, 3: 494-500. [No abstract available]

Barthelemy C, Bruneau N, Adrien J, Roux S, Lelord G: Clinical, Biological and Therapeutic Applications of the Functional Analysis of Autistic Disorders. Brain Dysfunction, 3: 271-284, 1990. [No abstract available]

Herrera-Marschitz M, Terenius L, Grehn L, Ungerstedt U: Rotational behaviour produced by intranigral injections of bovine and human beta-casomorphins in rats. Psychopharmacology (Berl) 1989;99(3):357-61.

Abstract: Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

The biological activity of beta-casein derived beta-casomorphin peptides was evaluated by injecting bovine beta-casomorphin-5 (Tyr-Pro-Phe-Pro-Gly), the homologous sequence in human beta-casein (Tyr-Pro-Phe-Val-Glu) and the corresponding N-

terminal tetrapeptides into the left substantia nigra of rats. Their ability to produce rotational behaviour was compared to that produced by three reference compounds, morphine, D-ala2D-leu5 enkephalin and U50,488H, ligands for mu, delta and kappa types of opioid receptors, respectively. The relative potencies of beta-casomorphins and morphine were compared to those tested in two in vitro assays for opioid activity: (1) inhibition of the electrically induced contraction of the isolated myenteric plexus-longitudinal muscle of the guinea-pig ileum and (2) displacement of 3H-dihydromorphine binding to brain membranes. The same ranking order of potency was found in all three assays, the peptides from human beta-casein being about 10-fold less potent than those from bovine beta-casein. The effects of both morphine and bovine beta-casomorphin-5 in producing rotational behaviour were antagonized by naloxone; however, approximately 10-fold more naloxone was required to antagonize the beta-casomorphin-5 effect than that of morphine. The present data are discussed in the light of the recent observation that high concentrations of beta-casomorphin-like peptides are found in the cerebrospinal fluid and plasma of women with postpartum psychosis.

Ramabadran K, Bansinath M: Opioid peptides from milk as a possible cause of sudden infant death syndrome. Med Hypotheses 1988 Nov;27(3):181-7.

Abstract: Department of Anesthesiology, New York University Medical Center, NY 10016.

Milk from breast or baby formula is the exclusive source of nutrition for newborn infants. Short chain opioid peptides such as beta-casomorphins have been isolated from breast milk as well as baby formula. These biologically active peptides are absorbed from the gastrointestinal tract. In infants predisposed to respiratory apnea because of abnormal autonomic nervous system development and respiratory control mechanisms, opioid peptides derived from milk might be one of the etiological factors for sudden infant death syndrome and near miss sudden infant death syndrome.

Paroli E: Opioid Peptides from Food (the Exorphins). World Review of Nutrition and Dietetics 1988; 55: 58-97. [No abstract available]

Hole K. et al.: Attention deficit disorders: A study of peptide-containing urinary complexes. J Develop Behav Pediatrics 1988, 9: 205-212.

Abstract: Department of Physiology, University of Bergen, Norway.

In several behavioral disorders, we have observed that abnormal amounts of peptides and protein-associated peptide complexes are excreted in the urine. The gel filtration patterns of these excreted substances have some specificity for the different disorders. The urinary excretion of peptide-containing complexes was studied in 91 boys and 13 girls (mean age 9.4 years, range 1-23) with the clinical diagnosis of attention deficit disorder (ADD), with or without hyperactivity. The gel filtration of urine precipitate

showed patterns in all patients that were different from those seen in 36 normal controls. Sixty-four patients had increased benzoic acid-glycoprotein-peptide complexes in the late peaks. The symptoms of all these patients fit the criteria for diagnosis of attention deficit disorder with hyperactivity (ADDH). Thirty-five patients showed reduced amounts of uric acid complexes in the late peaks. Clinically, this group, with the exception of three patients, fit the criteria for diagnosis of attention deficit disorder without hyperactivity. Five patients showed reduced amounts of all urinary complexes; four of these were hyperactive. Moderate exercise in control children did not change the urinary pattern. One urinary peptide fraction from hyperactive patients, purified to homogeneity, increased the uptake of 14C[5-HT] in platelets. Strict clinical, neuropsychological, and psychophysiological selection of the patients reduced the heterogeneity of the patterns. Although more studies are needed, the findings seem promising for the possibility of developing biochemical tests that may be helpful diagnostically.

Dohan, FC: Genetic hypothesis of idiopathic schizophrenia: its exorphin connection. Schizophr. Bull. 1988 / 14 (4) / 489-494.

Abstract:Medical College of Pennsylvania, Eastern Pennsylvania, Psychiatric Institute, Philadelphia, 19129.

This brief overview proposes a testable oligogenic model of the inheritance of susceptibility to idiopathic schizophrenia: "abnormal" genes at each of a few complementary loci. The model is based on my assumptions as to the likely genetic abnormalities at possibly four or five interacting loci that would permit exorphins, the opioid peptides from some food proteins, especially glutens and possibly caseins, to go from gut to brain and cause symptoms of schizophrenia. Exorphins may reach the brain cerebrospinal fluid (CSF) in harmful amounts because of their genetically increased, receptor-mediated transcellular passage across the gut epithelial barrier plus decreased catabolism by genetically defective enzymes. A schizophrenia-specific, genetically enhanced affinity for exorphins by opioid receptors influencing dopaminergic and other neurons would permit sustained dysfunction at low CSF exorphin concentrations. Tests of each postulated genetic abnormality are suggested. This model is supported by a variety of evidence, including a significant effect of gluten or its absence on relapsed schizophrenic patients, the high correlation of changes in first admission rates for schizophrenia with changes in grain consumption rates, and the rarity of cases of schizophrenia where grains and milk are rare.

Sahley TL, Panksepp J: Brain opioids and autism: an updated analysis of possible linkages. J Autism Dev Disord 1987 Jun;17(2):201-16.

Abstract:

Considerable clinical evidence suggests that autistic children lack the normal ability or desire to engage others socially, as indicated by their poor social skills and inappropriate use of language for communicative purposes. Specifically, these children seem to lack normal amounts of social-emotional interest in other people, leading perhaps to a decreased initiative to communicate. This paper summarizes experimental evidence supporting a neurological theory, which posits that autism, at least partially, represents in the brain, such as brain opioids. These substances modulate social-emotional processes, and the possibility that blockade of opioid activity in the brain may be therapeutic for early childhood autism is discussed.

Kahn A, Rebuffat E, Blum D, Casimir G, Duchateau J, Mozin MJ, Jost R: Difficulty in initiating and maintaining sleep associated with cow's milk allergy in infants. Sleep 1987 Apr;10(2):116-21.

Abstract: To confirm that sleeplessness in infants can be related to an undiagnosed allergy to cow's milk proteins, 71 infants were studied. Group I consisted of 20 infants referred for chronic insomnia that had appeared in the early days of life. Group II was made up of 31 infants admitted for skin or digestive symptoms attributed to cow's milk intolerance; 13 of these infants were shown to sleep as poorly as the infants of group I. Group III consisted of 20 infants with no history of sleep disturbance or milk allergy. The three groups of infants were comparable for sex and age. Laboratory tests revealed immunologic reactions to milk in all the infants in groups I and II. The sleep of the insomniac infants (group I, and the 13 "poor sleepers" in group II) became normal after cow's milk was eliminated from the diet. Insomnia reappeared when the infants in group I were challenged with milk. We conclude that infants with clinically evident milk allergy may suffer from sleeplessness and that when no evident cause for a chronic insomnia can be found in an infant the possibility of milk allergy should be given serious consideration.

Meisel, H: Chemical characterization and opioid activity of an exorphin isolated from in vivo digests of casein. FEBS Lett. 1986 / 196 (2) / 223-227.

Abstract:

The in vivo formation of an opioid peptide (exorphin) derived from beta-casein has been proved for the first time. It was isolated from duodenal chyme of minipigs after feeding with the milk protein casein. The exorphin has been identified as a beta-casein fragment by end-group determinations and qualitative amino acid analysis of the purified peptide. This peptide, named beta-casomorphin-11, displayed substantial opioid activity in an opiate receptor-binding assay.

Alpers DH: Uptake and fate of absorbed amino acids and peptides in the mammalian intestine. Federation Proc. 1986; 45:2261-2267.

Abstract:Intraluminal and brush-border digestion of proteins results in a mixture of amino acids and small peptides. Thirteen brush-border peptidases have been described. Despite all of these enzymes, some peptides escape digestion and are absorbed intact. The assimilated products of protein digestion can follow multiple paths: absorption into the blood as amino acids or small peptides, metabolism within the enterocyte, incorporation into proteins of the enterocyte, and incorporation into proteins to be secreted into plasma. Unlike other tissues, the intestinal mucosa is not very responsive to metabolic regulation as regards amino acid uptake or regulation of protein synthesis. Most effects after dietary manipulation or drug or hormonal stimulation are modes (two-to fivefold increases). This constitutive metabolism of amino acids in the intestinal mucosa is consistent with its essential role in absorption. The mucosa also is a major contributor to apolipoproteins, which are probably the quantitatively most important proteins secreted from the intestine. Alterations in apoprotein secretion have been noted after fat feeding, and are both transcriptionally and translationally regulated. Although the fractional renewal rate of protein in the intestine is the highest of any tissue in the body, the quantitative importance of alterations in protein synthesis or secretion to the fate of intracellular amino acids is not known.

Svedberg, J.et al: Demonstration of beta-casomorphin immunoreactive materials in in vitro digests of bovine milk and in small intestine contents after bovine milk ingestion in adult humans. Peptides 1985 / 6 / pag.825-830.

Abstract: Healthy young volunteers ingested one liter of cows' milk; then the contents of the small intestine were aspirated through an intestinal tube at various times and assayed for the presence of bovine beta-casomorphin immunoreactive materials. Considerable amounts of beta-casomorphin-7, but no beta-casomorphin-5 and only small amounts of beta-casomorphin-4 or -6 immunoreactive materials were found. Chromatographical characterization showed that most of the beta-casomorphin-7 immunoreactive material was not identical with beta-casomorphin-7, whereas the major part of the beta-casomorphin-4 or -6 immunoreactive materials might be identical with their corresponding beta-casomorphins. Analogous results were obtained for in vitro digestion of bovine milk which had been designed as a rough imitation of the gastrointestinal digestion process. A regulatory influence of beta-casomorphins as "food hormones" on intestinal functions is suggested.

Saelid G, et. al.: Peptide-Containing Fractions in Depression. Biol Psychiatry 1985, 20: 245-256.

Abstract: A mixture of peptides and glycoproteins has been found in benzoic acid-precipitable material from urines of psychomotorically agitated and retarded endogenous depressive patients. This complex mixture of compounds is fractionated on a Sephadex G-25 gel, from which the different peaks are further separated on Biogel P2. The G-25 elution profiles ultraviolet absorbance, 280 nm) from depressive patients deviated from the normal pattern. The increase in hydrolyzable ninhydrin-colorable

material of the P2 fractionation step encountered in psychotic depression was several-fold that of the normal population. Neurochemically active peptide-containing fractions were found. As explanation of these findings, it is probable that a genetically determined peptidase insufficiency is present, causing a peptide overflow when the secretion outstrips the breakdown. This model could easily combine more psychodynamic models with the genetic-biological models. The variability of the peptide patterns could possibly reflect the considerable clinical variability of the syndrome. Furthermore, the presence of a group of active compounds with different neuropharmacological activities might reflect the composite nature of the depressive syndrome.

Rix KJ, Ditchfield J, Freed DL, Goldberg DP, Hillier VF: Food antibodies in acute psychoses. Psychol Med 1985 May;15(2):347-54.

Abstract: Antibodies to a variety of foods, and in particular cereals, were measured in serum from 100 patients with acute psychoses and 100 elective surgical patients. For 13 out of 14 foods to which non-IgE antibodies were detected the schizophrenics had slightly more antibodies than the controls. There was an association between a possible secondary mania and the presence of IgE antibodies to wheat or rye. However, neither the schizophrenia nor the mania findings can be regarded as evidence for food allergy causing psychiatric disorder, since the immunological findings in both cases may represent consequences of the illnesses or their treatment, rather than causes of the illness.

Chang, KJ, Su YF, Brent DA, Chang JK: Isolation of a specific mu-opiate receptor peptide, morphiceptin, from an enzymatic digest of milk proteins. J. Biol. Chem. 1985 / 260 (17) / pag. 9706-9712.

Abstract: Specific radioimmunoassays have been developed for the measurement of naturally occurring morphiceptin and beta-casomorphin. These peptides and related exorphins were isolated from an enzymatic digest of caseins by chromatographic techniques including gel filtration, hydrophobic column and multiple-step high pressure liquid chromatography. Three exorphins were purified and characterized in their radioimmunological, biological, and chemical properties. They were identified as morphiceptin, beta-casomorphin, and 8-prolyl-beta-casomorphin. Since morphiceptin is a highly specific mu-agonist and can be derived from a milk protein, it is possible that morphiceptin is an exogenous opioid ligand specific for mu-receptors in the brain and gastrointestinal tract.

Takahashi M, Fukunaga H, Kaneto H, Fukudome S, Yoshikawa M: Behavioral and pharmacological studies on gluten exorphin A5, a newly isolated bioactive food protein fragment, in mice. Jpn J Pharmacol 2000 Nov;1984(3):259-65.

Abstract: Department of Pharmacoinformatics, School of Pharmaceutical Sciences, Nagasaki University, Japan. takahashi@net.nagasaki-u.ac.jp

Central effects of gluten exorphin A5 (Gly-Tyr-Tyr-Pro-Thr), a fragment from wheat gluten, were studied on the pain-inhibitory system, emotionality and learning/memory processes in mice. Orally administered gluten exorphin A5 produced neither an antinociceptive effect nor an effect on morphine analgesia. Intracerebroventricularly (i.c.v.) administered gluten exorphin A5 produced mild but significant antinociception in a dose-depepndent manner, while not affecting the morphine analgesia. On the other hand, oral gluten exorphin A5 suppressed the endogenous pain-inhibitory system, i.e., antinociception induced by socio-psychological- (PSY-) stress (SIA) using a communication box; intraperitoneal gluten exorphin A5 abolished both footshock- (FS-) stress-induced antinociception (SIA) and PSY-SIA; and i.c.v. gluten exorphin A5 suppressed FS-SIA, but rather potentiated PSY-SIA. This peptide given by these routes was without effect on forced swim-SIA. In addition, oral gluten exorphin A5 tended to prolong the retention time on open arms in the elevated plus-maze test. Finally, oral gluten exorphin A5 when given during the post-training period of learning/memory processes significantly increased the latency into the dark compartment in the one-trail step-though type passive avoidance test, indicating that the peptide also facilitates the acquire/consolidation process of learning/memory. Thus, gluten exorphin A5 has been found to produce various effects not only in the peripheral nervous systems but also in the central nervous system.

Pfeiffer CC: Schizophrenia and wheat gluten enteropathy. Biol Psychiatry 1984 Mar;19(3):279-80. [No abstract available]

Lindstrom LH, Nyberg F, Terenius L, Bauer K, Besev G, Gunne LM, Lyrenas S, Willdeck-Lund G, Lindberg B: (1984) CSF and plasma beta-casomorphin-like opioid peptides in post-partum psychosis. Amer. J. Psychiat. 1984, 141: 1059-1066.

Abstract:

The authors measured opioid receptor-active components in the CSF of 11 women with postpartum psychosis, 11 healthy lactating women, and 16 healthy women who were not lactating. Activity that eluted with 0.2 M acetic acid 0.7-0.9 times the total volume of the column (fraction II activity) was significantly higher in the CSF of both healthy and psychotic women in the puerperium than in that of the lactating women. Very high levels of fraction II activity were seen in four psychotic patients. Material from these patients was further characterized by electrophoresis and high-performance liquid chromatography: The material migrated as bovine beta-casomorphin. Receptor-active material with the same characteristics was also found in the plasma of these four patients. The authors conclude that certain cases of postpartum psychosis are associated with the occurrence in plasma and CSF of unique opioid peptides probably related to bovine beta-casomorphin.

Huebner FR, Lieberman KW, Rubino RP, Wall JS: Demonstration of high opioid-like activity in isolated peptides from wheat gluten hydrolysates. Peptides 1984 Nov-Dec;5(6):1139-47.

Abstract: Because of a possible relationship between schizophrenia and celiac disease, a condition in some individuals who are sensitive to wheat gluten proteins in the diet, there has been interest in observations that peptides derived from wheat gluten proteins exhibit opioid-like activity in in vitro tests. To determine the origin of the peptides exhibiting opioid activity, wheat proteins were fractionated by size (gel filtration), by charge differences (ion exchange chromatography) and by differences in hydrophobicity (reversed-phase HPLC). These fractions were hydrolyzed by pepsin or pepsin and trypsin and the resulting peptides separated by gel filtration chromatography. The separated peptides were tested for opioid-like activity by competitive binding to opioid receptor sites in rat brain tissue in the presence of tritium-labeled dihydromorphine. The peptides showed considerable differences in activity; while some peptides exhibited no activity, 0.5 mg of the most active peptides were equivalent to 1 nM of morphine in the binding assay. The most active peptides were derived from the gliadin fraction of the gluten complex.

Dohan et. al: "Is Schizophrenia Rare if Grain is Rare?" Biol Psychiat 1984; 19(3): 385-399.

Abstract: If, as hypothesized, neuroactive peptides from grain glutens are the major agents evoking schizophrenia in those with the genotype(s), it should be rare if grain is rare. To test this, we analyzed the results of our clinical examinations (e.g., kuru) and observations of anthropologists on peoples consuming little or no grain. Only two overtly insane chronic schizophrenics were found among over 65,000 examined or closely observed adults in remote regions of Papua New Guinea (PNG, 1950-1967) and Malaita, Solomon Islands (1980-1981), and on Yap, Micronesia (1947-1948). In preneuroleptic Europe over 130 would have been expected. When these peoples became partially westernized and consumed wheat, barley beer, and rice, the prevalence reached European levels. Our findings agree with previous epidemiologic and experimental results indicating that grain glutens are harmful to schizophrenics.

Loukas, S. et al: Opioid activities and structures of alpha-casein-derived exorphins. Biochemistry 1983 / 22 (19) / 4567-4573.

Gardner MLG: Evidence for, and implications of, passage of intact peptides across the intestinal mucosa. Biochemical Society Transactions 1983; 11: 810-812. [no abstract available]

Morley, JE: Food peptides. A new class of hormones ? J. Am. Med. Assoc. 1982 / 247 (17) / 2379-2380. [No abstract available]

Reichelt KL, Hole K, Hamberger A, Saelid G, Edminson PD, Braestrup CB, Lingjaerde O, Ledaal P, Orbeck H: Biologically active peptide-containing fractions in schizophrenia and childhood autism. Adv Biochem Psychopharmacol 1981; 28: 627-643.

Abstract: It is well documented that peptides have a major role in the effective functioning of higher animals at all levels from enzyme stabilization to homeostatic mechanisms governing essential functions such as eating, sexual behavior, and temperature regulation. The effects of exogenously administered peptides on neurotransmitter release, uptake, metabolism and behavioral consequences are also well established. We have attempted to extend these findings by postulating peptidergic neurons as transducers of multisignal inputs, and that development of pathological states may be due to genetically-determined reduced levels of activity of key peptidases, leading to excretion of regulatory peptides into the circulation. We have been able to demonstrate that, in schizophrenia and autism (in well defined clinical cases), the patterns of peptides and associated proteins from urinary samples differ considerably from each other and from normal controls. In addition to this, further purification of the material obtained has led to the discovery of a number of factors capable of modulating the function of major neurotransmitters. Some of these are in the final stages of characterization as peptides, while the remainder are also probably peptides, as purification has been followed by both biological testing and chemical analysis for peptidic material. We have outlined a number of parameters which we consider relevant in any attempt to put psychiatric disorders on a biological foundation. Any new advances in the neurochemical understanding of such disorders must take into consideration the observations of several different disciplines including genetics and psychology. However, at this stage of research it is far too early to speculate on the relevance of the various biological activities to the etiology and symptomatology of schizophrenia and childhood autism.

Trygstad OE, et al: Patterns of peptides and protein-associated-peptide complexes in psychiatric disorders. Br J Psychiatry 1980 Jan;136:59-72.

Abstract: Peptidic neurones may be considered as multisignal intergrators and transducers. When formation or release of peptide outstrips genetically determined breakdown capacity, overflow of peptides to the body fluids and urine may be expected. In this paper, pathological urinary chromatographic patterns of peptides are shown for genetic, functional and mixed disorders. Part symptoms of the disorders may be induced with the biologically isolated and purified peptides as well as with chemically synthesized peptides.

Ross-Smith, P, Jenner FA: Diet (gluten) and Schizophrenia. J. Hum. Nutr. 1980 / 34 (2) / 107-112.

Four aspects of clinical evidence for an association between gluten and schizophrenia are examined. The scientific evidence for the role of gluten is set out. Finally, reference is made to other dietary approaches.

Zioudrou C, Streaty RA, Klee WA: Opioid peptides derived from food proteins. The exorphins. J. Biol. Chem.1979 / 254 (7) / 2446-2449.

Abstract: Peptides with opioid activity are found in pepsin hydrolysates of wheat gluten and alpha-casein. The opioid activity of these peptides was demonstrated by use of the following bioassays: 1) naloxone-reversible inhibition of adenylate cyclase in homogenates of neuroblastoma X-glioma hybrid cells; 2) naloxone-reversible inhibition of electrically stimulated contractions of the mouse vas deferens; 3) displacement of [3H]dihydromorphine and [3H-Tyr, dAla2]met-enkephalin amide from rat brain membranes. Substances which stimulate adenylate cyclase and increase the contractions of the mouse vas deferens but do not bind to opiate receptors are also isolated from gluten hydrolysates. It is suggested that peptides derived from some food proteins may be of physiological importance.

Panksepp J: A neurochemical theory of autism. Point of View, North-Holland Biomedical Press, Jul 1979.

Hole K, Bergslien AA, Jørgensen H, Berge O-G, Reichelt KL & Trygstad OE:(1979) A peptide containing fraction from schizophrenia which stimulates opiate receptors and inhibits dopamine uptake. Neuroscience, 4, 1139-1147. [No abstract available]

Dohan FC: Schizophrenia and neuroactive peptides from food. Lancet 1979 May 12;1(8124):1031. [No abstract available]

Brantl V, Teschemacher H: Naunyn Schmiedebergs Arch Pharmacol 1979 Apr 30;306(3):301-4. A material with opioid activity in bovine milk and milk products.

Abstract: Chloroform-methanol extracts of lyophilized milk, of commercially available dried milk or baby food and of casein digests were tested for opioid activity on the guinea-pig ileum longitudinal muscle-myenteric plexus preparation. Compounds with opioid activity--which proved to be resistant to peptidases--were detected in certain batches of baby food, casein digest, and cow milk in considerably varying amounts.

Ashkenazi et. al: "Immunologic reaction of psychotic patients to fractions of gluten" Am J Psychiat 1979; 136: 1306-1309.

Abstract: Production of a leukocyte migration inhibition factor by peripheral blood lymphocytes in response to challenge with gluten fractions was studied in hospitalized patients with schizophrenia and other psychoses compared with normal individuals and

with children and adolescents with celiac disease. The schizophrenic and other psychotic patients could be subdivided into two groups, one that responded in the leukocyte migration inhibition factor test as the celiac patients did and one that responded as the normal control subjects did. The psychotic and schizophrenic patients did not show any evidence of malabsorption. The authors speculate that gluten may be involved in biological processes in the brain in certain psychotic individuals.

O'Banion D, Armstrong B, Cummings RA, Stange J.: Disruptive behavior: a dietary approach. J Autism Child Schizophr 1978 Sep;8(3):325-37.

Abstract: The effect of particular foods on levels of hyperactivity, uncontrolled laughter, and disruptive behaviors was studied in an 8-year-old autistic boy. The floor of the child's room was taped off into six equal-sized rectangles to measure general activity level. Frequency data were recorded on screaming, biting, scratching, and object throwing. A time-sample technique was used to record data on laughing. Data were gathered during four phases. During an initial 4-day period the child was fed a normal American diet. A 6-day fasting period followed, during which time only spring water was allowed. The third phase lasted 18 days and involved the presentation of individual foods. During the final phase of the study the child was given only foods that had not provoked a reaction in the third phase. Results showed that foods such as wheat, corn, tomatoes, sugar, mushrooms, and dairy products were instrumental in producing behavioral disorders with this child.

Singh MM, Kay SR: Wheat gluten as a pathogenic factor in schizophrenia. Science 1976 Jan 30;191(4225):401-2.

Abstract: Schizophrenics maintained on a cereal grain-free and milk-free diet and receiving optimal treatment with neuropleptics showed an interruption or reversal of their therapeutic progress during a period of "blind" wheat gluten challenge. The exacerbation of the disease process was not due to variations in neuroleptic doses. After termination of the gluten challenge, the course of improvement was reinstated. The observed effects seemed to be due to a primary schizophrenia-promoting effect of wheat gluten.

Dohan FC, Grasberger JC: Relapsed schizophrenics: earlier discharge from the hospital after cereal-free, milk-free diet. Am J Psychiatry. 1973 Jun;130(6):685-8. [No abstract available]

Goodwin MS, Cowen MA, Goodwin TC: Malabsorption and cerebral dysfunction: a multivariate and comparative study of autistic children. J Autism Child Schizophr 1971 Jan-Mar;1(1):48-62. [No abstract available]

Dohan FC: "Is celiac disease a clue to pathogenesis of schizophrenia?" Mental Hyg 1969; 53: 525-529. [No abstract available]

Dohan FC: Wheat "consumption" and hospital admissions for schizophrenia during World War II. A preliminary report. Am J Clin Nutr. 1966 Jan;18(1):7-10. [No abstract available]

Cooke WT, Smith WT: Neurological disorders associated with adult celiac disease. Brain 1966, 89: 683-722. [No abstract available]

Dohan FC: Cereals and schizophrenia: data and hypothesis. Acta Psychiat Scand 1966; 42: 125-152. [No abstract available]

10- Correcting Nutritional Deficiencies – 81 citations

Adams JB, Holloway C. Pilot study of a moderate dose multivitamin/mineral supplement for children with autistic spectrum disorder. J Altern Complement Med. 2004 Dec;10(6):1033-9.

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OBJECTIVE: Determine the effect of a moderate dose multivitamin/mineral supplement on children with autistic spectrum disorder. DESIGN: Randomized, double-blind, placebo-controlled 3-month study. SUBJECTS: Twenty (20) children with autistic spectrum disorder, ages 3-8 years. RESULTS: A Global Impressions parental questionnaire found that the supplement group reported statistically significant improvements in sleep and gastrointestinal problems compared to the placebo group. An evaluation of vitamin B(6) levels prior to the study found that the autistic children had substantially elevated levels of B6 compared to a control group of typical children (75% higher, $p < 0.0000001$). Vitamin C levels were measured at the end of the study, and the placebo group had levels that were significantly below average for typical children, whereas the supplement group had near-average levels. DISCUSSION: The finding of high vitamin B(6) levels is consistent with recent reports of low levels of pyridoxal-5-phosphate and low activity of pyridoxal kinase (i.e., pyridoxal is only poorly converted to pyridoxal-5-phosphate, the enzymatically active form). This may explain the functional need for high-dose vitamin B(6) supplementation in many children and adults with autism.

PMID: 15673999 [PubMed - indexed for MEDLINE]

Adams JB, George F, Audhya T. Abnormally high plasma levels of vitamin B6 in children with autism not taking supplements compared to controls not taking supplements. J Altern Complement Med. 2006 Jan-Feb;12(1):59-63.

BACKGROUND: There have been many studies of the effect of high-dose supplementation of vitamin B6 on children and adults with autism, with all but one reporting benefits. OBJECTIVE: The aim of this study was to investigate the biochemical basis for vitamin B6 therapy by measuring the level of total vitamin B6 in the plasma of unsupplemented children with autism spectrum disorder compared to unsupplemented control subjects. PARTICIPANTS: Children with autism spectrum disorders (n = 35, age 3-9 years) and unrelated typical children (n = 11, age 6-9 years), all from Arizona, were studied. (This includes the data

from 24 children with autism from our previous study.) METHODOLOGY: A microbiologic assay was used to measure the level of total vitamin B6 (including phosphorylated and unphosphorylated forms), in a blinded fashion. RESULTS: Children with autism had a 75% higher level of total vitamin B6 than the controls (medians of 56 versus 32 ng/mL, respectively, p = 0.00002). Most of the autistic children (77%) had levels that were more than 2 standard deviations above the median value of the controls. The autistic girls (n = 5) also had elevated levels (mean of 54.6 ng/mL, median of 60 ng/mL). DISCUSSION: These results are consistent with previous studies that found that: (1) pyridoxal kinase had a very low activity in children with autism and (2) pyridoxal 5 phosphate (PLP) levels are unusually low in children with autism. Thus, it appears that the low conversion of pyridoxal and pyridoxine to PLP results in low levels of PLP, which is the active cofactor for 113 known enzymatic reactions, including the formation of many key neurotransmitters. CONCLUSIONS: Total vitamin B6 is abnormally high in autism, consistent with previous reports of an impaired pyridoxal kinase for the conversion of pyridoxine and pyridoxal to PLP. This may explain the many published studies of benefits of high-dose vitamin B6 supplementation in some children and adults with autism.

PMID: 16494569 [PubMed - indexed for MEDLINE]

Aldred S, Moore KM, Fitzgerald M, Waring RH. Plasma amino acid levels in children with autism and their families. J Autism Dev Disord. 2003 Feb;33(1):93-7.

Pharmaceutical Sciences Research Institute, Aston University, Birmingham, B4 7ET, United Kingdom.

Plasma amino acid levels were measured in autistic and Asperger syndrome patients, their siblings, and parents. The results were compared with values from age-matched controls. Patients with autism or Asperger syndrome and their siblings and parents all had raised glutamic acid, phenylalanine, asparagine, tyrosine, alanine, and lysine ($p < .05$) than controls, with reduced plasma glutamine. Other amino acids were at normal levels. These results show that children with autistic spectrum disorders come from a family background of dysregulated amino acid metabolism and provide further evidence for an underlying biochemical basis for the condition.

PMID: 12708584 [PubMed - indexed for MEDLINE]

Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K (m)): relevance to genetic disease and polymorphisms. Am J Clin Nutr. 2002 Apr;75(4):616-58.

Amminger GP, Berger GE, Schafer MR, Klier C, Friedrich MH, Feucht M. Omega-3 Fatty Acids Supplementation in Children with Autism: A Double-blind Randomized, Placebo-controlled Pilot Study. Biol Psychiatry. 2006 Aug 22; [Epub ahead of print].

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As many as one-third of mutations in a gene result in the corresponding enzyme having an increased Michaelis constant, or K(m), (decreased binding affinity) for a coenzyme, resulting in a lower rate of reaction. About 50 human genetic diseases due to defective enzymes can be remedied or ameliorated by the administration of high doses of the vitamin component of the corresponding coenzyme, which at least partially restores enzymatic activity. Several single-nucleotide polymorphisms, in which the variant amino acid reduces coenzyme binding and thus enzymatic activity, are likely to be remediable by raising cellular concentrations of the cofactor through high-dose vitamin therapy. Some examples include the alanine-to-valine substitution at codon 222 (Ala222-->Val) [DNA: C-to-T substitution at nucleo-tide 677 (677C-->T)] in methylenetetrahydrofolate reductase (NADPH) and the cofactor FAD (in relation to cardiovascular disease, migraines, and rages), the Pro187-->Ser (DNA: 609C-->T) mutation in NAD(P):quinone oxidoreductase 1 [NAD(P)H dehy-drogenase (quinone)] and FAD (in relation to cancer), the Ala44-->Gly (DNA: 131C-->G) mutation in glucose-6-phosphate 1-dehydrogenase and NADP (in relation to favism and hemolytic anemia), and the Glu487-->Lys mutation (present in one-half of Asians) in aldehyde dehydrogenase (NAD +) and NAD (in relation to alcohol intolerance, Alzheimer disease, and cancer).

PMID: 11916749 [PubMed - indexed for MEDLINE]

Arnold GL, Hyman SL, Mooney RA, Kirby RS. Plasma amino acids profiles in children with autism: potential risk of nutritional deficiencies. J Autism Dev Disord. 2003 Aug;33(4):449-54.

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The plasma amino acid profiles of 36 children with autism spectrum disorders were reviewed to determine the impact of diet on amino acid patterns. Ten of the children were on gluten and casein restricted diets administered by parents, while the other 26 consumed unrestricted diets. No amino acid profile specific to autism was identified. However, children with autism had more essential amino acid deficiencies consistent with poor protein nutrition than an age/gender

matched control group. There was a trend for children with autism who were on restricted diets to have an increased prevalence of essential amino acid deficiencies and lower plasma levels of essential acids including the neurotransmitter precursors tyrosine and tryptophan than both controls and children with autism on unrestricted diets. These data indicate that larger, more focused studies of protein nutrition in children with autism are needed in order to determine the extent to which restricted diets might place the developing brains of children with autism at risk from protein malnutrition. The high rate of tryptophan and tyrosine deficiency in this group is also of concern given their role as neurotransmitter precursors.

PMID: 12959424 [PubMed - indexed for MEDLINE]

Baker SB, Worthley LI. The essentials of calcium, magnesium and phosphate metabolism: part I. Physiology. Crit Care Resusc. 2002 Dec;4(4):301-6.

Department of Critical Care Medicine, Flinders University of South Australia, Adelaide, South Australia. PART I

OBJECTIVE: To review the components of calcium, phosphate and magnesium metabolism that are relevant to the critically ill patient in a two-part presentation. DATA SOURCES: A review of articles reported on calcium, phosphate and magnesium disorders in the critically ill patient. SUMMARY OF REVIEW: Calcium, phosphate and magnesium have important intracellular and extracellular functions with their metabolism often linked through common hormonal signals. A predominant portion of total body calcium is unionised within bone and serves an important structural function. Intracellular and extracellular ionised calcium changes are often linked and have important secretory and excitatory roles. The extracellular ionised calcium is carefully regulated by parathyroid hormone and vitamin D, whereas calcitonin is secreted largely in response to hypercalcaemia. Phosphorous is needed for bone structure although it also has an important role in cell wall structure, energy storage as ATP, oxygen transport and acid-base balance. Ionised calcium, in as far as it controls PTH secretion, indirectly controls urinary phosphate excretion. When plasma phosphate increases, tubular reabsorption also increases up to a maximum (TmPO4), thereafter phosphate is excreted. The minimum oral requirement for phosphate is about 20 mmol/day. Magnesium is a predominantly intracellular ion that acts as a metallo-coenzyme in more than 300 phosphate transfer reactions and thus has a critical role in the transfer, storage and utilisation of energy within the body. Extracellular magnesium concentrations are largely controlled by the kidneys with the renal tubular maximum reabsorption (TmMg) controlling the plasma magnesium concentration. CONCLUSIONS: In the critically ill patient calcium, magnesium

and phosphate metabolism, are often disturbed with an alteration in intake, increased liberation from bone and damaged tissue and reduced excretion (e.g. during renal failure), causing alterations in extracellular concentrations and subsequent disordered organ function.

PMID: 16573443 [PubMed] PART II

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OBJECTIVE: To review the components of calcium, phosphate and magnesium metabolism that are relevant to the critically ill patient, in a two-part presentation. DATA SOURCES: A review of articles reported on calcium, phosphate and magnesium disorders in the critically ill patient. SUMMARY OF REVIEW: Abnormal calcium metabolism in the critically ill patient often presents with an alteration in plasma ionised calcium. The characteristic clinical features of an acute reduction in ionised plasma calcium include tetany, laryngospasm, paraesthesia, confusion, hallucinations, seizures and, rarely, hypotension all of which resolve with intravenous calcium administration. The clinical features of an acute increase in plasma ionised calcium include anorexia, nausea, vomiting, constipation, polyuria, weakness, lethargy, hypotonia and ectopic calcification and, depending on the aetiology, may require intravenous saline, frusemide, diphosphonate, glucocorticoid or calcitonin. Acute hypophosphataemia may present with paraesthasia, confusion, seizures, weakness, hypotension and heart failure and in the critically ill requires intravenous sodium or potassium phosphate. Hyperphosphataemia is often associated with renal failure and if severe usually presents with the clinical features of the associated hypocalcaemia. The clinical features of hypomagnesaemia include confusion, delerium, seizures, weakness, cramps, tetany and tachyarrhythmias, all of which resolve with intravenous magnesium sulphate. Hypermagnesaemia is usually associated with excess magnesium administration in a patient with renal failure and if severe can cause areflexia, hypotonia, respiratory and cardiac arrest. Intravenous calcium chloride will rapidly reverse the cardiovascular abnormalities. CONCLUSIONS: Calcium, phosphate and magnesium functions are closely linked with abnormal plasma levels of these compounds often causing similar cardiovascular and neurological features.

PMID: 16573444 [PubMed]

Barthelemy C, Garreau B, Leddet I, Sauvage D, Domenech J, Muh JP, Lelord G. Biological and clinical effects of oral magnesium and associated magnesium-vitamin B6

administration on certain disorders observed in infantile autism. Therapie. 1980 Sep-Oct;35(5):627-32. [Article in French]

Bell JG, Sargent JR, Tocher DR, Dick JR. Red blood cell fatty acid compositions in a patient with autistic spectrum disorder: a characteristic abnormality in neurodevelopmental disorders? Prostaglandins Leukot Essent Fatty Acids. 2000 Jul-Aug;63(1-2):21-5.

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The fatty acid compositions of red blood cell (RBC) phospholipids from a patient with autistic spectrum disorder (ASD) had reduced percentages of highly unsaturated fatty acids (HUFA) compared to control samples. The percentage of HUFA in the RBC from the autistic patient was dramatically reduced (up to 70%) when the sample was stored for 6 weeks at -20 degrees C. However, only minor HUFA reductions were recorded in control samples stored similarly, or when the autistic sample was stored at -80 degrees C. A similar instability in RBC HUFA compositions upon storage at -20 degrees C has been recorded in schizophrenic patients. In a number of other neurodevelopmental conditions, including attention deficit hyperactivity disorder (ADHD) and dyslexia, reduced concentrations of RBC HUFA have been recorded. The extent and nature of these aberrations require further assessment to determine a possible common biochemical origin of neurodevelopmental disorders in general. To facilitate this, a large scale assessment of RBC fatty acid compositions in patients with ASD, and related disorders, should be performed as a matter of urgency. Supplementing cells in culture with the tryptophan metabolite indole acrylic acid (IAA) affected the levels of cellular HUFA and prostaglandin production. Indole acroyl glycine (IAG), a metabolite of IAA excreted in urine, is found in high concentrations in patients with neurodevelopmental disorders including ASD, ADHD, dyslexia, Asperger's syndrome and obsessive compulsive disorder. Copyright 2000 Harcourt Publishers Ltd.

PMID: 10970708 [PubMed - indexed for MEDLINE]

Boris M, Goldblatt A, Galanko J, James SJ. Association of MTHFR gene variants with autism. J Am Phys Surg. 2004: 9(4):106-108.

Bronner F. Extracellular and intracellular regulation of calcium homeostasis. ScientificWorldJournal. 2001 Dec 22;1:919-25.

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An organism with an internal skeleton must accumulate calcium while maintaining body fluids at a well-regulated, constant calcium concentration. Neither calcium absorption nor excretion plays a significant regulatory role. Instead, isoionic calcium uptake and release by bone surfaces causes plasma calcium to be well regulated. Very rapid shape changes of osteoblasts and osteoclasts, in response to hormonal signals, modulate the available bone surfaces so that plasma calcium can increase when more low-affinity bone calcium binding sites are made available and can decrease when more high-affinity binding sites are exposed. The intracellular free calcium concentration of body cells is also regulated, but because cells are bathed by fluids with vastly higher calcium concentration, their major regulatory mechanism is severe entry restriction. All cells have a calcium-sensing receptor that modulates cell function via its response to extracellular calcium. In duodenal cells, the apical calcium entry structure functions as both transporter and a vitamin D--responsive channel. The channel upregulates calcium entry, with intracellular transport mediated by the mobile, vitamin D-dependent buffer, calbindin D9K, which binds and transports more than 90% of the transcellular calcium flux. Fixed intracellular calcium binding sites can, like the body's skeleton, take up and release calcium that has entered the cell, but the principal regulatory tool of the cell is restricted entry.

PMID: 12805727 [PubMed - indexed for MEDLINE]

Bu B, Ashwood P, Harvey D, King IB, Water JV, Jin LW. Fatty acid compositions of red blood cell phospholipids in children with autism. Prostaglandins Leukot Essent Fatty Acids. 2006 Apr;74(4):215-21.

Department of Pathology, M.I.N.D. Institute, University of California at Davis, 2805 50th Street, Sacramento, CA 95817, USA.

We compared the compositions of fatty acids including n-3, n-6 polyunsaturated fatty acids, trans- and cis-monounsaturated fatty acids, and saturated fatty acids in the red blood cell membranes of 40 children with autism (20 with early onset autism and 20 with developmental regression) and age-matched, 20 typically developing controls and 20 subjects with non-autistic developmental disabilities. The main findings include increased levels of eicosenoic acid (20:1n9) and erucic acid (22:1n9) in autistic subjects with developmental regression when compared with typically developing controls. In addition, an increase in 20:2n6 and a decrease in 16:1n7t were observed in children with clinical regression compared to those with early onset autism. Our results do not provide strong evidence for the hypothesis that abnormal fatty acid metabolism plays a role in the pathogenesis of autism spectrum disorder, although they suggest some metabolic or dietary abnormalities in the regressive form of autism.

PMID: 16581239 [PubMed - indexed for MEDLINE]

Calingasan NY, Huang PL, Chun HS, Fabian A, Gibson GE. Vascular factors are critical in selective neuronal loss in an animal model of impaired oxidative metabolism. J Neuropathol Exp Neurol. 2000 Mar;59(3):207-17.

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Thiamine deficiency (TD) models the cellular and molecular mechanisms by which chronic oxidative deficits lead to death of select neurons in brain. Region- and cell-specific oxidative stress and vascular changes accompany the TD-induced neurodegeneration. The current studies analyzed the role of oxidative stress in initiating these events by testing the role of intercellular adhesion molecule-1 (ICAM-1) and endothelial nitric oxide synthase (eNOS) in the selective neuronal loss that begins in the submedial thalamic nucleus of mice. Oxidative stress to microvessels is known to induce eNOS and ICAM-1. TD increased ICAM-1 immunoreactivity in microvessels within the submedial nucleus and adjacent regions 1 day prior to the onset of neuronal loss. On subsequent days, the pattern of ICAM-1 induction overlapped that of neuronal loss, and of induction of the oxidative stress marker heme oxygenase-1 (HO-1). The intensity and extent of ICAM-1 and HO-1 induction progressively spread in parallel with the neuronal death in the thalamus. Targeted disruption of ICAM-1 or eNOS gene, but not the neuronal NOS gene, attenuated the TD-induced neurodegeneration and HO-1 induction. TD induced ICAM-1 in eNOS knockout mice, but did not induce eNOS in mice lacking ICAM-1. These results demonstrate that in TD, an ICAM-1-dependent pathway of eNOS induction leads to oxidative stress-mediated death of metabolically compromised neurons. Thus, TD provides a useful model to help elucidate the role of ICAM-1 and eNOS in the selective neuronal death in diseases in which oxidative stress is implicated.

PMID: 10744059 [PubMed - indexed for MEDLINE]

Chez MG, Buchanan CP, Aimonovitch MC, Becker M, Schaefer K, Black C, Komen J. Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders. J Child Neurol. 2002 Nov;17(11):833-7.

Research Division, Autism and Epilepsy Specialty Services of Illinois, Ltd, Lake Bluff, IL 60044, USA. mchezmd@interaccess.com

L-Carnosine, a dipeptide, can enhance frontal lobe function or be neuroprotective. It can also correlate with gamma-aminobutyric acid (GABA)-

homocarnosine interaction, with possible anticonvulsive effects. We investigated 31 children with autistic spectrum disorders in an 8-week, double-blinded study to determine if 800 mg L-carnosine daily would result in observable changes versus placebo. Outcome measures were the Childhood Autism Rating Scale, the Gilliam Autism Rating Scale, the Expressive and Receptive One-Word Picture Vocabulary tests, and Clinical Global Impressions of Change. Children on placebo did not show statistically significant changes. After 8 weeks on L-carnosine, children showed statistically significant improvements on the Gilliam Autism Rating Scale (total score and the Behavior, Socialization, and Communication subscales) and the Receptive One-Word Picture Vocabulary test (all $P < .05$). Improved trends were noted on other outcome measures. Although the mechanism of action of L-carnosine is not well understood, it may enhance neurologic function, perhaps in the enterorhinal or temporal cortex.

PMID: 12585724 [PubMed - indexed for MEDLINE]

Chugani DC, Sundram BS, Behen M, Lee ML, Moore GJ. Evidence of altered energy metabolism in autistic children. Prog Neuropsychopharmacol Biol Psychiatry. 1999 May;23(4):635-41.

Department of Pediatrics, Wayne State University School of Medicine, Children's Hospital of Michigan, Detroit, USA. dchugani@pet.wayne.edu

1. In this pilot study, the authors investigated the hypotheses there are increased concentrations of lactate in brain and plasma and reduced brain concentrations of N-acetyl-aspartate (NAA) in autistic children. 2. NAA and lactate levels in the frontal lobe, temporal lobe and the cerebellum of 9 autistic children were compared to 5 sibling controls using MRS. Plasma lactate levels were measured in 15 autistic children compared to 15 children with epilepsy. 3. Preliminary results show lower levels of NAA cerebellum in autistic children ($p = 0.043$). Lactate was detected in the frontal lobe in one autistic boy, but was not detected any of the other autistic subjects or siblings. 4. Plasma lactate levels were higher in the 15 autistic children compared to 15 children with epilepsy ($p = 0.0003$). 5. Higher plasma lactate in the autistic group is consistent with metabolic changes in some autistic children. The findings of altered brain NAA and lactate in autistic children suggest that MRS may be useful characterizing regional neurochemical and metabolic abnormalities in autistic children.

PMID: 10390722 [PubMed - indexed for MEDLINE]

Coleman M, Steinberg G, Tippett J, Bhagavan HN, Coursin DB, Gross M, Lewis C, DeVeau L. A preliminary study of the effect of pyridoxine administration in a subgroup of hyperkinetic children: a double-blind crossover comparison with methylphenidate. Biol Psychiatry. 1979 Oct;14(5):741-51.

A small sample of six patients with the putative "hyperkinetic syndrome" participated in a research protocol comparing administration of pyridoxine, methylphenidate, and placeboes. The children had had low whole blood serotonin levels and a history of previous responsiveness to methylphenidate. The results of the double-blind clinical evaluation showed trends suggesting that both pyridoxine and methylphenidate were more effective than placebo in suppressing the symptoms of hyperkinesis. Pyridoxine elevated whole-blood serotonin levels, methylphenidate did not. Clinical and laboratory evidence indicated that the pyridoxine effects persisted after the 3-week period when the vitamin had been given in this experimental design.

PMID: 497303 [PubMed - indexed for MEDLINE]

Deluca HF. The vitamin D system: a view from basic science to the clinic. Clin Biochem. 1981 Oct;14(5):213-22.

Vitamin D produced in the skin and absorbed in the small intestine must be modified metabolically before it can function. It is ultimately converted to a hormone in the kidney that stimulates intestine, bone and kidney to mobilize calcium and phosphorus. This results in normal bone development and normal neuromuscular function. The vitamin D hormone appears to act by a nuclear mechanism to facilitate a target organ response. Finally the vitamin D hormone is produced in response to the need for calcium and phosphorus. The calcium need is interpreted by the parathyroid gland that in turn secretes parathyroid hormone. The parathyroid hormone stimulates production of the vitamin D hormone. This constitutes the vitamin D endocrine system that plays an important role not only in calcium homeostasis but in phosphate homeostasis and in calcium economy of the body. A number of disease states including hypoparathyroidism, pseudohypoparathyroidism, renal osteodystrophy, certain types of vitamin D-resistant rickets and osteoporosis can in part be related to disturbance in the vitamin D endocrine system. Thus measurement of the vitamin D hormone and its precursor will be of great value in diagnosis of metabolic bone disease and most importantly, the availability of new vitamin D compounds will play an important role in the treatment of these bone diseases.

PMID: 7037225 [PubMed - indexed for MEDLINE]

Dhawan M, Kachru DN, Tandon SK. Influence of thiamine and ascorbic acid supplementation on the antidotal efficacy of thiol chelators in experimental lead intoxication. Arch Toxicol. 1988;62(4):301-4.

Industrial Toxicology Research Centre, Lucknow, India.

The influence of the administration of thiamine (vitamin B1), ascorbic acid (vitamin C) or their combination on the efficacy of two thiol metal chelators, viz. alpha-mercapto-beta-(2-furyl) acrylic acid (MFA) and 2,3-dimercaptosuccinic acid (DMS), in counteracting lead (Pb) toxicity was investigated in rats. Ascorbic acid or its combination with thiamine enhanced the urinary elimination of Pb, reduced the hepatic and renal burden of Pb, and reversed the Pb-induced inhibition of the activity of blood delta-aminolevulinic acid dehydratase (delta-ALA-D). All these effects were more evident in DMS- than in MFA-treated rats. The combination of MFA and DMS treatments further improved the performance of the animals in enhancing urinary Pb excretion and in reducing Pb hepatic levels.

PMID: 3240094 [PubMed - indexed for MEDLINE]

Dickinson VA, Block G, Russek-Cohen E. Supplement use, other dietary and demographic variables, and serum vitamin C in NHANES II. J Am Coll Nutr. 1994 Feb;13(1):22-32.

Nutritional Sciences Program, University of Maryland.

OBJECTIVE: Our objective was to evaluate the effect of regular use of nutritional supplements on serum vitamin C levels in a multivariable regression model, taking into account other dietary and demographic variables which may affect nutritional status. METHODS: We analyzed NHANES II data for subjects age 3 to 74. Analysis was limited to regular supplement users and nonusers, excluding irregular users. Multivariable regression analysis was performed with SUDAAN, incorporating sample weights and accounting for the complex survey design. RESULTS: Regular supplement users had substantially higher serum vitamin C levels than nonusers ($p < 0.001$). The magnitude of the effect of supplement use on serum vitamin C was 0.23-0.33 mg/dL in children and teens, and 0.36-0.46 mg/dL in adults. In adults who smoked, bottom quartile vitamin C levels were 0.3 mg/dL in men and 0.4 mg/dL in women who did not use supplements, compared to 0.9 and 1.1 mg/dL in regular supplement users. There was a significant interaction of smoking and supplement use in men ($p < 0.001$). CONCLUSION: Regular supplement use has a strong impact on serum vitamin C levels, independent of other dietary and demographic characteristics of supplement users which may favor improved nutritional status.

PMID: 8157850 [PubMed - indexed for MEDLINE]

Dolske MC, Spollen J, McKay S, Lancashire E, Tolbert L. A preliminary trial of ascorbic acid as supplemental therapy for autism. Prog Neuropsychopharmacol Biol Psychiatry. 1993 Sep;17(5):765-74.

Department of Psychiatry, University of Alabama at Birmingham.

1. This study presents the results of a 30-week double-blind, placebo-controlled trial exploring the effectiveness of ascorbic acid (8g/70kg/day) as a supplemental pharmacological treatment for autistic children in residential treatment. 2. Residential school children (N = 18) were randomly assigned to either ascorbate-ascorbate-placebo treatment order group or ascorbate-placebo-ascorbate treatment order group. Each treatment phase lasted 10 weeks and behaviors were rated weekly using the Ritvo-Freeman scale. 3. Significant group by phase interactions were found for total scores and also sensory motor scores indicating a reduction in symptom severity associated with the ascorbic acid treatment. 4. These results were consistent with a hypothesized dopaminergic mechanism of action of ascorbic acid.

PMID: 8255984 [PubMed - indexed for MEDLINE]

Fernstrom JD. Can nutrient supplements modify brain function? Am J Clin Nutr. 2000 Jun;71(6 Suppl):1669S-75S.

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Over the past 40 y, several lines of investigation have shown that the chemistry and function of both the developing and the mature brain are influenced by diet. Examples are the effect of folate deficiency on neural tube development during early gestation, the influence of essential fatty acid deficiency during gestation and postnatal life on the development of visual function in infants, and the effects of tryptophan or tyrosine intake (alone or as a constituent of dietary protein) on the production of the brain neurotransmitters derived from them (serotonin and the catecholamines, respectively). Sometimes the functional effects are clear and the underlying biochemical mechanisms are not (as with folate and essential fatty acids); in other cases (such as the amino acids tyrosine and tryptophan), the biochemical effects are well understood, whereas the effect on brain function is not. Despite the incomplete knowledge base on the effects of such nutrients, investigators, physicians, and regulatory bodies have promoted the use of these nutrients in the treatment of disease. Typically, these nutrients have been given in doses above those believed to be required for normal health; after they have been given in pure form, unanticipated adverse effects have

occasionally occurred. If this pharmacologic practice is to continue, it is important from a public safety standpoint that each nutrient be examined for potential toxicities so that appropriate purity standards can be developed and the risks weighed against the benefits when considering their use.

PMID: 10837313 [PubMed - indexed for MEDLINE]

Filipek PA, Juranek J, Nguyen MT, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. J Autism Dev Disord. 2004 Dec;34(6):615-23.

Department of Pediatrics, College of Medicine, University of California, Irvine, CA, USA. filipek@uci.edu

A random retrospective chart review was conducted to document serum carnitine levels on 100 children with autism. Concurrently drawn serum pyruvate, lactate, ammonia, and alanine levels were also available in many of these children. Values of free and total carnitine ($p < 0.001$), and pyruvate ($p = 0.006$) were significantly reduced while ammonia and alanine levels were considerably elevated ($p < 0.001$) in our autistic subjects. The relative carnitine deficiency in these patients, accompanied by slight elevations in lactate and significant elevations in alanine and ammonia levels, is suggestive of mild mitochondrial dysfunction. It is hypothesized that a mitochondrial defect may be the origin of the carnitine deficiency in these autistic children.

PMID: 15679182 [PubMed - indexed for MEDLINE]

Friedman SD, Shaw DW, Artru AA, Richards TL, Gardner J, Dawson G, Posse S, Dager SR. Regional brain chemical alterations in young children with autism spectrum disorder. Neurology. 2003 Jan 14;60(1):100-7.

Department of Radiology, University of Washington School of Medicine, Seattle 98105-6099, USA.

OBJECTIVE: The authors evaluated regional brain chemistry for evidence of increased neuronal packing density in autism. METHODS: Forty-five 3- to 4-year-old children with autism spectrum disorder (ASD), 13 children with typical development (TD), and 15 children with delayed development (DD) were studied using dual-echo proton echoplanar spectroscopic imaging (32 x 32 matrix-1 cm(3) voxels) to measure brain chemical concentrations and relaxation times. Chemical quantification was corrected for tissue partial volume and relative measures of chemical relaxation (T(2r)) were calculated from the paired echoes. Measures from averaged and individual regions were compared using analysis of variance corrected for multiple comparisons. RESULTS: ASD subjects demonstrated reduced N-acetylaspartate (NAA) (-10%), creatine (Cre) (-8%),

and myo-inositol (-13%) concentrations compared to TD controls and prolonged NAA T(2r) relative to TD (7%) and DD (9%) groups. Compared to DD subjects, children with ASD also demonstrated prolonged T(2r) for choline (10%) and Cre (9%). Regional analyses demonstrated subtle patterns of chemical alterations in ASD compared to the TD and DD groups. CONCLUSIONS: Brain chemical abnormalities are present in ASD at 3 to 4 years of age. However, the direction and widespread distribution of these abnormalities do not support hypothesis of diffuse increased neuronal packing density in ASD.

PMID: 12525726 [PubMed - indexed for MEDLINE]

Gaull GE. Taurine in pediatric nutrition: review and update. Pediatrics. 1989 Mar;83(3):433-42.

Department of Pediatrics, Northwestern University School of Medicine, Chicago.

Taurine was long considered an end product of the metabolism of the sulfur-containing amino acids, methionine and cyst(e)ine. Its only clearly recognized biochemical role had been as a substrate in the conjugation of bile acids. Taurine is found free in millimolar concentrations in animal tissues, particularly those that are excitable, rich in membranes, and generate oxidants. Various lines of evidence suggest one major nutritional role as protecting cell membranes by attenuating toxic substances and/or by acting as an osmoregulator. The totality of evidence suggests that taurine is nonessential in the rodent, it is an essential amino acid in the cat, and it is conditionally essential in man and monkey. Absence from the diet of a conditionally essential nutrient does not produce immediate deficiency disease but, in the long term, can cause problems. Taurine is now added to many infant formulas as a measure of prudence to provide improved nourishment with the same margin of safety for its newly identified physiologic functions as that found in human milk. Such supplementation can be justified by the finding of improved fat absorption in preterm infants and in children with cystic fibrosis, as well as by salutary effects on auditory brainstem-evoked responses in preterm infants. Experimental findings in animal models and in human cell models provide further justification for taurine supplementation of infant formulas.

PMID: 2645571 [PubMed - indexed for MEDLINE]

Geoghegan M, McAuley D, Eaton S, Powell-Tuck J. Selenium in critical illness. Curr Opin Crit Care. 2006 Apr;12(2):136-41.

Department of Adult and Paediatric Gastroenterology, St Bartholomew's and the Royal London Hospital School of Medicine and Dentistry, Queen Mary University of London, London, UK. mgeoghegan@qmul.ac.uk

PURPOSE OF REVIEW: Selenium is a trace element essential to human health. Critical illness is associated with the generation of oxygen free radicals resulting in a condition of oxidative stress. Supplementing critically ill patients with antioxidant nutrients may improve survival. Selenium levels can be low due to redistribution to high-priority organs and dilution associated with aggressive resuscitation of the patient. The purpose of this review is to investigate the benefit of selenium supplementation in critically ill patients. RECENT FINDINGS: Most of the selenium-supplementation trials were performed in relatively small patient populations presenting with trauma, sepsis, burns and adult respiratory distress syndrome. Widely varying doses of selenium of between 200 and 1000 microg were used, either alone or in combination with other antioxidants. Significant improvements have been demonstrated in length of hospital stay, rate of infection and need for haemodialysis in these patients. However, no trial has demonstrated a statistically significant improvement in mortality. Two recent meta-analyses suggest a trend towards reduced mortality with selenium supplementation. SUMMARY: Selenium, by supporting antioxidant function, may be associated with a reduction in mortality. To demonstrate this large, well-designed randomized trials are required.

PMID: 16543790 [PubMed - indexed for MEDLINE]

Goebel L, Driscoll H. Scurvy. www.emedicine.com. 7-15-05.

Grattan-Smith PJ, Wilcken B, Procopis PG, Wise GA. The neurological syndrome of infantile cobalamin deficiency: developmental regression and involuntary movements. Mov Disord. 1997 Jan;12(1):39-46.

Department of Paediatric Neurology, Westmead Hospital, Sydney, Australia.

Developmental regression is the presenting symptom of most infants with cobalamin (Vitamin B12) deficiency. We present a report of three infants with cobalamin deficiency in which the infants also developed a movement disorder. In each case the mother was a vegetarian and the infant was exclusively breast-fed. In two of the infants, a striking movement disorder consisting of a combination of tremor and myoclonus particularly involving face, tongue, and pharynx appeared 48 h after the initiation of treatment with intramuscular

cobalamin. This was associated with marked changes in plasma amino acid levels. Paradoxically, the onset of the movement disorder coincided with overall neurological improvement. The third infant had a persistent focal tremor, which appeared before the commencement of treatment. The movements slowly abated during a 3-6 week period. The presence of a movement disorder in cobalamin deficiency has received less attention than other features, but in a mild form is probably common. It may offer an early clue to the diagnosis before the onset of profound neurological deterioration. The cause of the severe movement disorder that can appear after treatment is not known.

PMID: 8990052 [PubMed - indexed for MEDLINE]

Horvath K, Perman JA. Autistic disorder and gastrointestinal disease. Curr Opin Pediatr. 2002 Oct;14(5):583-7

Hunt C, Chakravorty NK, Annan G, Habibzadeh N, Schorah CJ. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. Int J Vitam Nutr Res. 1994;64(3):212-9.

Food & Nutrition Department, Huddersfield University.

A randomised double-blind trial involving vitamin C/placebo supplementation was conducted on 57 elderly patients admitted to hospital with acute respiratory infections (bronchitis and bronchopneumonia). Patients were assessed clinically and biochemically on admission and again at 2 and 4 weeks after admission having received either 200 mg vitamin C per day, or placebo. This relatively modest oral dose led to a significant increase in plasma and white cell vitamin C concentration even in the presence of acute respiratory infection. Using a clinical scoring system based on major symptoms of the respiratory condition, patients supplemented with the vitamin fared significantly better than those on placebo. This was particularly the case for those commencing the trial most severely ill, many of whom had very low plasma and white cell vitamin C concentrations on admission. Various mechanisms by which vitamin C could assist this type of patient are discussed.

PMID: 7814237 [PubMed - indexed for MEDLINE]

Imura K, Okada A. Amino acid metabolism in pediatric patients. Nutrition. 1998 Jan;14(1):143-8.

Department of Pediatric Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health, Japan.

As with energy requirements, protein requirements are relatively much greater in infants and decline progressively with age. Amino acid metabolism in pediatric patients is characterized by the following differences. The requirement for essential amino acids in neonates is larger than that in adults. Because of low activity of phenylalanine hydroxylase and cystathionase, hyperphenylalaninemia and hypermethioninemia tend to occur, whereas tyrosine and cysteine tend to be deficient. In addition to cysteine and tyrosine, histidine, lysine, arginine and taurine are considered as semiessential amino acids. Nowadays there are different kinds of amino acid formulas to satisfy these specific requirements, and most of these formulas are intended to normalize the plasma aminogram. However, the nutritional benefit of these formulas for growth and development is still not completely proven, and the pharmacological use for specific diseases is expected with some modification of these formulas.

PMID: 9437700 [PubMed - indexed for MEDLINE]

James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr. 2004 Dec;80(6):1611-7.

Department of Pediatrics, University of Arkansas for Medical Sciences, and the Arkansas Children's Hospital Research Institute, Little Rock, AR 72202, USA. jamesjill@uams.edu

BACKGROUND: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism. OBJECTIVE: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism. DESIGN: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children. RESULTS: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation

(significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children. CONCLUSIONS: An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism.

PMID: 15585776 [PubMed - indexed for MEDLINE]

Johnson S. Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson's disease? Med Hypotheses. 2001 May;56(5):641-5.

Zinc has several crucial functions in brain development and maintenance: it binds to p53, preventing it from binding to supercoiled DNA and ensuring that p53 cause the expression of several paramount genes, such as the one that encodes for the type I receptors to pituitary adenine cylase-activator peptide (PACAP), which directs embryonic development of the brain cortex, adrenal glands, etc.; it is required for the production of CuZnSOD and Zn-thionein, which are essential to prevent oxidative damage; it is required for many proteins, some of them with Zn fingers, many of them essential enzymes for growth and homeostasis. For example, the synthesis of serotonin involves Zn enzymes and since serotonin is necessary for melatonin synthesis, a Zn deficiency may result in low levels of both hormones. Unfortunately, Zn levels tend to be low when there is excess Cu and Cd. Moreover, high estrogen levels tend to cause increased absorption of Cu and Cd, and smoking and eating food contaminated with Cd result in high levels of the latter. Furthermore, ethanol ingestion increases the elimination of Zn and Mg (which acts as a cofactor for CuZnSOD).Increased Cu levels may also be found in people with Wilson's disease, which is a rather rare disease. However, the heterozygote form (only one faulty copy of the chromosome) is not so rare. Therefore, the developing fetus of a pregnant women who is low in Zn and high in Cu may experience major difficulties in the early development of the brain, which may later manifest themselves as schizophrenia, autism or epilepsy. Similarly, a person who gradually accumulates Cu, will tend to experience a gradual depletion of Zn, with a corresponding increase in oxidative damage, eventually leading to Parkinson's disease. Also discussed are the crucial roles of histidine, histamine, vitamin D, essential fatty acids, vitamin E, peroxynitrate, etc. in the possible oxidative damage involved in these mental diseases. Copyright 2001 Harcourt Publishers Ltd.

PMID: 11388783 [PubMed - indexed for MEDLINE]

Johnston CS, Thompson LL. Vitamin C status of an outpatient population. J Am Coll Nutr. 1998, 17(4)366-370.

Department of Family Resources and Human Development, Arizona State University, Tempe 85287-2502, USA.

OBJECTIVE: To determined the prevalence of vitamin C deficiency (plasma vitamin C concentrations less than 11.4 mumol/L) and vitamin C depletion (plasma vitamin C concentrations from 11.4 to less than 28.4 mumol/L) in an outpatient population. SUBJECTS AND METHODS: A consecutive sample of patients presenting at a health maintenance organization laboratory for outpatient procedures was utilized. Plasma vitamin C concentrations were determined in 350 females and 144 males, aged 6 to 92 years (mean +/- SD: 46.7 +/- 18.7 years). RESULTS: The mean plasma vitamin C concentration for all subjects was 32.4 +/- 13.6 mumol/L. Mean plasma vitamin C did not vary by sex, race, or fasted state. Diabetics had a significantly lower mean plasma vitamin C concentration (25.6 +/- 10.8 mumol/L) compared to patients presenting for general check-up/gynecological exams (33.5 +/- 14.8 mumol/L) or pregnancy exams (32.4 +/- 9.7 mumol/L). Six percent of subjects had plasma vitamin C concentrations indicative of vitamin C deficiency (n = 31), and 30.4% of the sample were vitamin C depleted (n = 150). The prevalence of vitamin C deficiency or vitamin C depletion did not differ by race or visit category. CONCLUSIONS: Surprisingly high rates of vitamin C deficiency and vitamin C depletion were evident among generally healthy, middle class patients visiting a health care facility for routine health exams, gynecological exams, and pregnancy exams.

PMID: 9710847 [PubMed - indexed for MEDLINE]

Jonas C, Etienne T, Barthelemy C, Jouve J, Mariotte N. Clinical and biochemical value of Magnesium + vitamin B6 combination in the treatment of residual autism in adults. Therapie. 1984 Nov-Dec;39(6):661-9. Article in French

Kidd PM. Autism, an extreme challenge to integrative medicine. Part 2: medical management. Altern Med Rev. 2002 Dec;7(6):472-99.

Kleijnen J, Knipschild P. Niacin and vitamin B6 in mental functioning: a review of controlled trials in humans. Biol Psychiatry. 1991 May 1;29(9):931-41.

Department of Epidemiology/Health Care Research, University of Limburg, The Netherlands.

Fifty-three controlled trials of the effects of niacin, vitamin B6, and multivitamins on mental functions are reviewed. The results are interpreted with emphasis on the methodological quality of the trials. It turns out that virtually all trials show serious short-comings: in the number of participants, the presentation of baseline characteristics and outcomes, and the description of changes in concomitant treatments. Only in autistic children are some positive results are found with very high dosages of vitamin B6 combined with magnesium, but further evidence is needed before more definitive conclusions can be drawn. For many other indications (hyperactive children, children with Down's syndrome, IQ changes in healthy schoolchildren, schizophrenia, psychological functions in healthy adults and geriatric patients) there is no adequate support from controlled trials in favor of vitamin supplementation.

PMID: 1828703 [PubMed - indexed for MEDLINE]

Kornreich L, Bron-Harlev E, Hoffmann C, Schwarz M, Konen O, Schoenfeld T, Straussberg R, Nahum E, Ibrahim AK, Eshel G, Horev G. Thiamine deficiency in infants: MR findings in the brain. Am J Neuroradiol. 2005 Aug;26(7):1668-74.

Department of Imaging, Schneider Children's Medical Center of Israel, Petah Tiqva.

BACKGROUND AND PURPOSE: Thiamine deficiency is extremely rare in infants in developed countries. To our knowledge, its MR findings in the brain have not been reported. The purpose of this study was to investigate the brain MR findings in infants with encephalopathy due to thiamine deficiency. METHODS: The study group included six infants aged 2-10 months with encephalopathy who had been fed with solely soy-based formula devoid of thiamine from birth. All underwent MR evaluation at admission and follow-up (total of 14 examinations). In one patient, MR spectroscopy (MRS) was performed. RESULTS: In five patients T2-weighted, fluid-attenuated inversion recovery, or proton-attenuated sequences showed bilateral and symmetric hyperintensity in the periaqueductal area, basal ganglia and thalami. Five had lesions in the mammillary bodies, and three, in the brain stem. In all six patients, the frontal region (cortex and white matter) was clearly involved. At presentation, MRS of the periaqueductal area showed a lactate doublet. On long-term follow-up, three of four patients had severe frontal damage; in two, this occurred as part of diffuse parenchymal loss, and in one, it was accompanied by atrophy of the basal ganglia and thalami. CONCLUSION: Thiamine deficiency in infants is characterized by involvement of the frontal lobes and basal ganglia, in addition to the lesions in the periaqueductal region, thalami, and the mammillary bodies described in adults. MRS demonstrates a characteristic lactate peak.

PMID: 16091511 [PubMed - indexed for MEDLINE]

Kozielec T, Starobrat-Hermelin B. Assessment of magnesium levels in children with attention deficit hyperactivity disorder (ADHD). Magnes Res. 1997 Jun;10(2):143-8.

Department of Family Medicine, Pomeranian Medical Academy, Szczecin, Poland.

A positive influence of magnesium in the prevention and treatment of hyperactivity in children is more and more frequently raised in the literature. The aim of our work was to estimate magnesium contents in children with attention deficit hyperactivity disorder, (ADHD). The investigations comprised 116 children (94 boys and 20 girls), aged 9-12 years, with recognized ADHD. In 68 out of 116 patients examined ADHD occurred with other coexisting disorders specific to the developmental age and in the remaining 48 patients it occurred together with disruptive behaviour. Magnesium levels have been determined in blood serum, red blood cells and in hair with the aid of atomic absorption spectroscopy. Magnesium deficiency was found in 95 per cent of those examined, most frequently in hair (77.6 per cent), in red blood cells (58.6 per cent) and in blood serum (33.6 per cent) of children with ADHD. The conclusion from the investigations is that magnesium deficiency in children with ADHD occurs more frequently than in healthy children. Analysis of the material indicated the correlation between levels of magnesium and the quotient of development to freedom from distractibility.

PMID: 9368235 [PubMed - indexed for MEDLINE]

Kushak R, Winter W, Farber N, Buie T. Gastrointestinal symptoms and intestinal disaccharidase activities in children with autism. J Pediatr Gastroenterol Nutr. 2005 Oct: 41(4):508.

Lelord G, Muh JP, Barthelemy C, Martineau J, Garreau B, Callaway E. Effects of pyridoxine and magnesium on autistic symptoms—initial observations. J Autism Dev Disord. 1981 Jun;11(2):219-30.

In an open trial, a heterogeneous group of 44 children with autistic symptoms were treated with large doses of vitamin B6 and magnesium. Clinical improvement with worsening on termination of the trial was observed in 15 children. Thirteen responders and 8 nonresponders were retested in a 2-week, crossover, double-blind trial, and the responses to the open trial were confirmed.

PMID: 6765503 [PubMed - indexed for MEDLINE]

Lelord G, Callaway E, Muh JP. Clinical and biological effects of high doses of vitamin B6 and magnesium on autistic children. Acta Vitaminol Enzymol. 1982;4(1-2):27-44.

In 1973 Rimland reported that some autistic children responded favorably to high doses of vitamin B6. Since this finding, different studies were performed to identify apparently B6 responsive subjects and to critically evaluate clinical and biological B6 responsiveness. Magnesium was included because large doses of B6 might increase irritability. 44 patients (mean age 9.3 years) were examined. All selected children had marked autistic symptoms. The children received a complete diagnostic work-up, including psychiatric, psychological, neurological and medical evaluation. Clinical data were scored using an estimate of global clinical state and numerical rating on a 18 item scale (Behavior Summarized Evaluation). In a first open trial 15 out of 44 children exhibited moderate clinical improvement with worsening on termination of the trial. Thirteen responders and 8 non responders were re-tested in a 2-week crossover, double-blind trial and the responses to the open trial were confirmed. Biochemical data analysis revealed that a significant decrease in urinary homovanillic acid (HVA) levels was observed during B6-Mg administration. During B6-Mg treatment, middle latency evoked potentials exhibited a significant increase of amplitude.

PMID: 7124567 [PubMed - indexed for MEDLINE]

Levy J. Immunonutrition: the pediatric experience. Nutrition. 1998 Jul-Aug;14(7-8):641-7.

Children's Digestive Health Center, Columbia University College of Physicians and Surgeons, New York, New York 10032-3784, USA.

The health benefits of specific nutrients in the diet are reviewed as they pertain to the pediatric population and its unique needs. Secretory immunoglobulins, lysozyme, interferon, and growth factors, among others, are known to confer immunological advantages to breast milk. Inhibition of bacterial pathogens, as well as permissive growth of a protective colonic ecoflora occur as a result of various cellular and biochemical mechanisms at play. The immunomodulatory properties of minerals such as iron, zinc, and selenium, are presented and the newly recognized protective role of vitamin A and its importance in developing countries and in conditions of compromised nutrition are discussed. The review also covers the role of arginine, glutamine, and nucleotides in adaptive responses

of the developing gut and in pathologic states such as necrotizing enterocolitis, short bowel syndrome, and inflammatory bowel disease. Probiotics (specific microbial feeds with potential benefits to the host), and prebiotics (dietary components such as complex carbohydrates able to change the colonic microenvironment fostering colonization with non-enteropathogens) are areas of current interest because they offer alternatives for the management of the growing problem of multiple antibiotic resistance and overwhelming infections in the hospitalized patient.

PMID: 9684269 [PubMed - indexed for MEDLINE]

Li J, Lin JC, Wang H, Peterson JW, Furie BC, Furie B, Booth SL, Volpe JJ, Rosenberg PA. Novel role of vitamin K in preventing oxidative injury to developing oligodendrocytes and neurons. J Neuroscience. 2003 Jul: 23(13):5816-5826.

Department of Neurology, Division of Neuroscience, Children's Hospital, Boston, MA 02115, USA.

Oxidative stress is believed to be the cause of cell death in multiple disorders of the brain, including perinatal hypoxia/ischemia. Glutamate, cystine deprivation, homocysteic acid, and the glutathione synthesis inhibitor buthionine sulfoximine all cause oxidative injury to immature neurons and oligodendrocytes by depleting intracellular glutathione. Although vitamin K is not a classical antioxidant, we report here the novel finding that vitamin K1 and K2 (menaquinone-4) potently inhibit glutathione depletion-mediated oxidative cell death in primary cultures of oligodendrocyte precursors and immature fetal cortical neurons with EC50 values of 30 nm and 2 nm, respectively. The mechanism by which vitamin K blocks oxidative injury is independent of its only known biological function as a cofactor for gamma-glutamylcarboxylase, an enzyme responsible for posttranslational modification of specific proteins. Neither oligodendrocytes nor neurons possess significant vitamin K-dependent carboxylase or epoxidase activity. Furthermore, the vitamin K antagonists warfarin and dicoumarol and the direct carboxylase inhibitor 2-chloro-vitamin K1 have no effect on the protective function of vitamin K against oxidative injury. Vitamin K does not prevent the depletion of intracellular glutathione caused by cystine deprivation but completely blocks free radical accumulation and cell death. The protective and potent efficacy of this naturally occurring vitamin, with no established clinical side effects, suggests a potential therapeutic application in preventing oxidative damage to undifferentiated oligodendrocytes in perinatal hypoxic/ischemic brain injury.

PMID: 12843286 [PubMed - indexed for MEDLINE]

Liebscher DH, Liebscher DE. About the misdiagnosis of magnesium deficiency. J Am Coll Nutr. 2004 Dec;23(6):730S-1S.

Self-Help Organisation on Mineral Imbalances, Task Force Magnesium-Deficiency Tetany, Rummelsburger Str. 13, D-10315 Berlin, Germany. dierck-h.liebscher@magnesiumhilfe.de

The experience of our self-help organisation shows that the reason patients with symptoms of magnesium (Mg) deficiency do not get Mg therapy is acceptance of an inappropriate lower limit of the reference values for serum Mg concentration. The commonly designated low limit of the normal range seems to have been selected from values obtained for symptomatic patients. It is below levels that exist in patients with marginal deficiencies that can predispose to development of pathologic findings, so that the prevalence and importance of this disease is insufficiently considered. The lower reference limit of the normal population is erroneously regarded as a diagnostic criterion that excludes Mg deficiency when the serum level is even slightly above the reference limit that only excludes normality at lower levels. It is a statistical error to use the confidence limits of the normal population as the exclusion limit for those with abnormal Mg status.

PMID: 15637222 [PubMed - indexed for MEDLINE]

Litov RE, Combs GF Jr. Selenium in pediatric nutrition. Pediatrics. 1991 Mar;87(3):339-51.

Mead Johnson Research Center, Bristol-Myers Squibb Company, Evansville, Indiana 47721-0001.

Se is an essential nutrient that provides antioxidant protection in concert with vitamin E. Several selenoproteins have been identified, but only one, SeGSHpx, has a known function, that of neutralizing toxic hydroperoxides. Plasma Se concentration, being responsive to changes in Se intake, is the most practical and widely used measure of nutritional Se status. The plasma Se concentrations of the majority of healthy infants and children fall within the range of 50 to 150 micrograms/L. Although SeGSHpx activity measures the metabolically functional form of Se, the lack of a standardized analytical method has limited its usefulness as an index of nutritional Se status. Se deficiency was first observed in animals, but it is now recognized to occur in humans. Two human diseases associated with severe nutritional Se deficiency have been reported from China: a juvenile cardiomyopathy named Keshan disease and a chondrodystrophy

named Kaschin-Beck disease. Long-term TPN, which provides negligible amounts of intrinsic Se, has been demonstrated in some cases to result in biochemical and clinical impairment. Although there are no consistent signs and symptoms characteristic of TPN-associated Se deficiency in addition to the low blood selenium levels, some patients will experience leg muscle pain and altered serum transaminase and creatine kinase activities. These manifestation of Se deficiency usually take years to develop. Recent information about the amount of dietary Se needed to maximize plasma SeGSHpx activity in adult men has allowed for better estimates of the Se requirement for humans. Recommended daily dietary allowances published recently by the National Academy of Sciences have been revised for infants and children in this paper by making appropriate adjustments for the protein requirements of these age-groups. These recommended intakes for Se can generally be met by consuming adequate amounts of cereals, meat, eggs, dairy products, human milk, and infant formula, which are good sources of highly available Se and are of low risk of providing excess amounts of Se. Suboptimal Se intakes by pregnant women may predispose their infants to low Se status at birth, which in turn may affect the infants ability to maintain adequate Se status during the first few months of life. In those situations where protein intake is restricted, such as in phenylketonuria and maple syrup urine disease, Se-supplemented formulas should be used. The most critical situation for Se supplementation is in pediatric patients receiving long-term TPN therapy.(ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 2000274 [PubMed - indexed for MEDLINE]

Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: a pilot study. Neuro Endocrinol Lett. 2002 Aug;23(4):303-8.

Preventive Medicine Group, 24700 Center Ridge Road, Westlake, OH 44145, USA. dlonsdale@pol.net

OBJECTIVES: In a Pilot Study, the clinical and biochemical effects of thiamine tetrahydrofurfuryl disulfide (TTFD) on autistic spectrum children were investigated. SUBJECTS AND METHODS: Ten children were studied. Diagnosis was confirmed through the use of form E2, a computer assessed symptom score. For practical reasons, TTFD was administered twice daily for two months in the form of rectal suppositories, each containing 50 mg of TTFD. Symptomatic responses were determined through the use of the computer assessed Autism Treatment Evaluation Checklist (ATEC) forms. The erythrocyte transketolase (TKA) and thiamine pyrophosphate effect (TPPE), were measured at outset and on completion of the study to document intracellular thiamine deficiency. Urines

from patients were examined at outset, after 30 days and after 60 days of treatment and the concentrations of SH-reactive metals, total protein, sulfate, sulfite, thiosulfate and thiocyanate were determined. The concentrations of metals in hair were also determined. RESULTS: At the beginning of the study thiamine deficiency was observed in 3 out of the 10 patients. Out of 10 patients, 6 had initial urine samples containing arsenic in greater concentration than healthy controls. Traces of mercury were seen in urines from all of these autistic children. Following administration of TTFD an increase in cadmium was seen in 2 children and in lead in one child. Nickel was increased in the urine of one patient during treatment. Sulfur metabolites in urine did not differ from those measured in healthy children. CONCLUSIONS: Thiamine tetrahydrofurfuryl disulfide appears to have a beneficial clinical effect on some autistic children, since 8 of the 10 children improved clinically. We obtained evidence of an association of this increasingly occurring disease with presence of urinary SH-reactive metals, arsenic in particular.

PMID: 12195231 [PubMed - indexed for MEDLINE]

Mahadik SP, Scheffer RE. Oxidative injury and potential use of antioxidants in schizophrenia. Prostaglandins Leukot Essent Fatty Acids. 1996 Aug;55(1-2):45-54.

Department of Psychiatry and Health Behavior, Medical College of Georgia, USA.

There is increasing evidence that oxidative injury contributes to pathophysiology of schizophrenia, indicated by the increased lipid peroxidation products in plasma and CSF, and altered levels of both enzymatic and non-enzymatic antioxidants in chronic and drug-naive first-episode schizophrenic patients. The increased plasma lipid peroxidation is also supported by concomitant lower levels of esterified polyunsaturated essential fatty acids of red blood cell plasma membrane phospholipids. Because membrane phospholipids play a critical role in neuronal signal transduction, oxidative damage of these lipids may contribute to the proposed altered neurotransmitter receptor-mediated signal transduction and thereby alter information processing in schizophrenia. Adjunctive treatment with antioxidants (e.g. vitamins E and C, beta-carotene and quinones) at the initial stages of illness may prevent further oxidative injury and thereby ameliorate and prevent further possible deterioration of associated neurological and behavioral deficits in schizophrenia.

PMID: 8888122 [PubMed - indexed for MEDLINE]

Martineau J, Barthelemy C, Garreau B, Lelord G. Vitamin B6, magnesium, and combined B6-Mg: therapeutic effects in childhood autism. Biol Psychiatry. 1985 May;20(5):467-78.

> This article reports the behavioral, biochemical, and electrophysiological effects of four therapeutic crossed-sequential double-blind trials with 60 autistic children: Trial A--vitamin B6 plus magnesium/magnesium; Trial B--vitamin B6 plus magnesium; Trial C--magnesium; and Trial D--vitamin B6. Therapeutic effects were controlled using behavior rating scales, urinary excretion of homovanillic acid (HVA), and evoked potential (EP) recordings. The behavioral improvement observed with the combination vitamin B6-magnesium was associated with significant modifications of both biochemical and electrophysiological parameters: the urinary HVA excretion decreased, and EP amplitude and morphology seemed to be normalized. These changes were not observed when either vitamin B6 or magnesium was administered alone.
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> PMID: 3886023 [PubMed - indexed for MEDLINE]

Megson MN. Is autism a G-alpha protein defect reversible with natural vitamin A? Med Hypotheses. 2000 Jun;54(6):979-83.

> Pediatric and Adolescent Ability Center, Richmond, VA 23226, USA.
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> Autism may be a disorder linked to the disruption of the G-alpha protein, affecting retinoid receptors in the brain. A study of 60 autistic children suggests that autism may be caused by inserting a G-alpha protein defect, the pertussis toxin found in the DPT vaccine, into genetically at-risk children. This toxin separates the G-alpha protein from retinoid receptors. Those most at risk report a family history of at least one parent with a pre-existing G-alpha protein defect, including night blindness, pseudohypoparathyroidism or adenoma of the thyroid or pituitary gland.Natural vitamin A may reconnect the retinoid receptors critical for vision, sensory perception, language processing and attention. Autism spectrum disorders have increased from 1 in 10 000 in 1978 to 1 in 300 in some US communities in 1999. Recent evidence indicates that autism is a disorder of the nervous system and the immune system, affecting multiple metabolic pathways.
>
> PMID: 10867750 [PubMed

Moon J. The role of vitamin D in toxic metal absorption: a review. J Am Coll Nutr. 1994 Dec;13(6):559-64.

National College of Naturopathic Medicine, Portland Oregon 97216.

Vitamin D increases intestinal calcium and phosphate absorption. Not so well known, however, is that vitamin D stimulates the co-absorption of other essential minerals like magnesium, iron, and zinc; toxic metals including lead, cadmium, aluminum, and cobalt; and radioactive isotopes such as strontium and cesium. Vitamin D may contribute to the pathologies induced by toxic metals by increasing their absorption and retention. Reciprocally, lead, cadmium, aluminum, and strontium interfere with normal vitamin D metabolism by blocking renal synthesis of 1,25-dihydroxyvitamin D. This is the first review of the role of the vitamin D endocrine system in metal toxicology.

PMID: 7706586 [PubMed - indexed for MEDLINE]

Moretti R, Torre P, Antonello RM, Cattaruzza T, Cazzato G, Bava A. Vitamin B12 and folate depletion in cognition: a review. Neurol India. 2004 Sep;52(3):310-8.

Dipartimento Fisiologia e Patologia, Università degli Studi, Trieste, Italy. moretti@univ.trieste.it.

In cross-sectional studies, low levels of folate and B12 have been shown to be associated with cognitive decline and dementia Evidence for the putative role of folate, vitamin B12 in neurocognitive and other neurological functions comes from reported cases of severe vitamin deficiencies, particularly pernicious anemia, and homozygous defects in genes that encode for enzymes of one-carbon metabolism. The neurological alterations seen in these cases allow for a biological role of vitamins in neurophysiology. Results are quite controversial and there is an open debate in literature, considering that the potential and differential role of folate and B12 vitamin in memory acquisition and cognitive development is not completely understood or accepted. What is not clear is the fact that vitamin B12 and folate deficiency deteriorate a pre-existing not overt pathological situation or can be dangerous even in normal subjects. Even more intriguing is the interaction between B12 and folate, and their role in developing hyperhomocysteinemia. The approach to the rehabilitation of the deficiency with adequate vitamin supplementation is very confusing. Some authors suggest it, even in chronic situations, others deny any possible role. Starting from these quite confusing perspectives, the aim of this review is to report and categorize the data obtained from the literature. Despite the plausible biochemical mechanism, further studies, based on clinical, neuropsychological, laboratory and (lastly) pathological features will be necessary to better understand this fascinating biochemical riddle.

PMID: 15472418 [PubMed - indexed for MEDLINE]

Mousain-Bosc M, Roche M, Polge A, Pradal-Prat D, Rapin J, Bali JP. Improvement of neurobehavioral disorders in children supplemented with magnesium-vitamin B6. II. Pervasive developmental disorder-autism. Magnes Res. 2006 Mar;19(1):53-62.

Explorations Fonctionnelles du Système Nerveux, Centre Hospitalier Universitaire Carémeau, Nîmes, France.

Previous studies reported positive results with the use of Mg-vitamin B6 in autism. Despite these reports, this intervention remains controversial. In order to study relationships between changes in clinical symtoms and biological parameters, 33 children (mean age: 4 [1-10] years old) with clinical symptoms of pervasive developmental disorder or autism (PDD, as defined in DSM-IV) were followed for at least 6 months; another group of 36 children (same age) devoided of any known pathology was used as control. All PDD children received a magnesium-vit B6 (Mg-B6) regimen (6 mg/kg/d Mg and 0.6 mg/kg/d vit B6). Intraerythrocyte Mg2+ (Erc-Mg), serum Mg2+ (s-Mg) and blood ionized Ca2+ (i-Ca) were measured before and after treatment. Clinical symptoms of PDD were scored (0 to 4). In contrast to s-Mg or i-Ca, PDD children exhibited significantly lower Erc-Mg values than controls (2.17 +/- 0.4 versus 2.73 +/- 0.23 mmol/L; 16/33). The Mg-B6 regimen led to an increase in Erc-Mg values (2.42 +/- 0.41 (after) versus 2.17 +/- 0.4 mmol/l (before), 11/17) and this supplementation improved PDD symptoms in 23/33 children ($p < 0.0001$) with no adverse effects: social interactions (23/33), communication (24/33), stereotyped restricted behavior (18/33), and abnormal/delayed functioning (17/33); 15/33 children were improved in the first three groups of symptoms. When the Mg-B6 treatment was stopped, PDD symtoms reappeared in few weeks. A statistically significant relationship was found in Erc-Mg values from children before treatment and their mothers. In conclusion, this study suggests that the behavioral improvement observed with the combination vitamin B6-magnesium in PDD/autism is associated with concomitant modifications of Erc-Mg values.

PMID: 16846101 [PubMed - indexed for MEDLINE]

Mousain-Bosc M, Roche M, Rapin J, Bali JP. Magnesium VitB6 intake reduces central nervous system hyperexcitability in children. J Am Coll Nutr. 2004 Oct;23(5):545S-548S.

Department of Pediatry, CHU Nimes, 30029 Nimes Cedex, France.

OBJECTIVE: Ionic magnesium (Mg(2+)) depletion has long been known to cause hyperexcitability with convulsive seizures in rodents, effects that have been reversed by treatment with magnesium (Mg). Metabolic disorders and genetic alterations are suspected in this pathology, in which Mg(2+) transport and intracellular distribution may be reduced without change in serum Mg(2+) concentrations. We evaluated the effects of Mg(2+)/vitamin B6 regimen on the behavior of 52 hyperexcitable children (under 15 years of age) and their families. METHODS: To assess intracellular Mg(2+), we measured intra-erthrocyte Mg(2+) levels (ERC-Mg). Our reference values for normal subjects were 2.46 to 2.72 mmol/L. In 30 of the 52 hyperactive children, there were low ERC-Mg values: 2.041 +/- 0.279 mmol/L). Combined Mg(2+)/vitamin B6 intake (100 mg/day) for 3 to 24 weeks restored normal ERC-Mg values (2.329 +/- 0.386 mmol/L). RESULTS: In all patients, symptoms of hyperexcitability (physical aggressivity, instability, scholar attention, hypertony, spasm, myoclony) were reduced after 1 to 6 months treatment. Other family members shared similar symptoms, had low ERC-Mg values, and also responded clinically to increased Mg(2+)/vitamin B6 intakes. Two typical families are described. CONCLUSION: This open study indicates that hyperexcitable children have low ERC-Mg with normal serum Mg(2+) values, and that Mg(2+)/vitamin B6 supplementation can restore normal ERC-Mg levels and improve their abnormal behavior.

PMID: 15466962 [PubMed - indexed for MEDLINE]

Ohsaki Y, Shirakawa H, Hiwatashi K, Furukawa Y, Mizutani T, Komai M. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci Biotechnol Biochem. 2006 Apr;70(4):926-32.

Laboratory of Nutrition, Department of Science of Food Function and Health, Graduate School of Agricultural Science, Tohoku University , Sendai, Japan.

Vitamin K (K) is essential for blood coagulation and bone metabolism in mammals. K acts as a cofactor in the posttranslational synthesis of gamma-carboxyglutamic acid from glutamic acid residues. In addition to the liver and bone, K is found in the brain, heart, kidney and gonadal tissue. However, the physiological role of K in these various organs is not yet fully understood. It is likely that K has functions other than its role as a cofactor of protein gamma-glutamyl carboxylation. We used in this study the DNA microarray technique to identify the effect of K status on gene expression in the rat liver. The expression of genes involved in the acute inflammation response was enhanced in rats fed with a K-deficient diet relative to the control and K1-supplemented diet groups. Moreover, dietary supplementation with K1 suppressed the inflammation induced

by lipopolysaccharide administration. These results indicate that orally administrated K1 suppressed inflammation in the rat.

PMID: 16636460 [PubMed - indexed for MEDLINE]

Olmez A, Yalcin S, Yurdakok K, Coskun T. Serum selenium levels in acute gastroenteritis of possible viral origin. J Trop Pediatr. 2004 Apr;50(2):78-81.

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Selenium, as an essential micronutrient, is required for the proper functioning of the immune system and its deficiency affects the occurrence, virulence, or disease progression of some viral infections. We conducted a study to determine the serum selenium levels of children with acute gastroenteritis of possible viral origin and the effect of the serum selenium levels on the severity and the morbidity of the disease. The study was performed prospectively on 109 children aged 2-24 months with diarrhea of less than 8 days' duration admitted to the Diarrheal Disease Training and Treatment Unit. Blood samples were taken for selenium measurement on admission and 7-10 days after the end of the disease. Forty-three healthy children formed the control group. The mean serum selenium level on admission (62.41 +/- 13.06 microg/dl) was significantly lower than the mean of the second samples 7-10 days after the end of the diarrhea (81.73 +/- 17.10 microg/dl). The mean of the control group was 74.36 +/- 10.75 microg/dl, which was lower than the mean of the second samples but higher than the first sample. The frequency of vomiting and purging on admission and at the control visit, duration of diarrhea on admission, total duration of diarrhea, dehydration, breastfeeding, sex of the patients, and severity score of the disease did not alter the serum selenium levels. No correlation was detected between serum selenium levels and the parameters above. Further studies about the changes in selenium status during infectious diseases and the effect of selenium status on related mortality and morbidity are required to determine if there is need for supplementation.

PMID: 15088795 [PubMed - indexed for MEDLINE]

Pangborn J, Baker SM. Autism: Effective Biomedical Treatments (Have We Done Everything We Can For This Child? Individuality In An Epidemic). San Diego: Autism Research Institute; 2nd Edition Sept. 2005:232-235.

Pfeiffer CC, Braverman ER. Zinc, the brain and behavior. Biol Psychiatry. 1982 Apr;17(4):513-32.

> The total content of zinc in the adult human body averages almost 2 g. This is approximately half the total iron content and 10 to 15 times the total body copper. In the brain, zinc is with iron, the most concentrated metal. The highest levels of zinc are found in the hippocampus in synaptic vesicles, boutons, and mossy fibers. Zinc is also found in large concentrations in the choroid layer of the retina which is an extension of the brain. Zinc plays an important role in axonal and synaptic transmission and is necessary for nucleic acid metabolism and brain tubulin growth and phosphorylation. Lack of zinc has been implicated in impaired DNA, RNA, and protein synthesis during brain development. For these reasons, deficiency of zinc during pregnancy and lactation has been shown to be related to many congenital abnormalities of the nervous system in offspring. Furthermore, in children insufficient levels of zinc have been associated with lowered learning ability, apathy, lethargy, and mental retardation. Hyperactive children may be deficient in zinc and vitamin B-6 and have an excess of lead and copper. Alcoholism, schizophrenia, Wilson's disease, and Pick's disease are brain disorders dynamically related to zinc levels. Zinc has been employed with success to treat Wilson's disease, achrodermatitis enteropathica, and specific types of schizophrenia.
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> PMID: 7082716 [PubMed - indexed for MEDLINE]

Raiten DJ, Massaro T. Perspectives on the nutritional ecology of autistic children. J Autism Dev Disord. 1986 Jun;16(2):133-43.

> Dietary intake was assessed in a sample population of 40 autistic and 34 control children with a 7-day diet record kept by the parent or primary caregiver. A questionnaire was completed by each participant to obtain descriptive data on nutrition and health issues, attitudes and beliefs about nutrition, and nutrition knowledge. The autistic children had significantly greater intake of all nutrients with the exception of vitamins A and C, and fat; overall adequacy of diets was similar for both groups. Parent/primary caregivers of autistic children reported a more positive belief in the relationship between diet and behavior, and a more positive attitude about the importance of nutrition. A higher incidence of food cravings, pica, and perceived eating problems were reported by the parent/caregivers of autistic children.
>
> PMID: 3722115 [PubMed - indexed for MEDLINE]

Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hopper C, Harris M, Hodges SJ. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine. 1995 Apr;7(3):287-90.

Department of Oral and Maxillofacial Surgery, Eastman Dental Institute for Oral Healthcare Sciences, London.

Naphthoquinone vitamins (vitamins K) are widely recognized for their role in the gamma-carboxylation of specific glutamyl residues in coagulation, anti-coagulation and extra-hepatic proteins. Recently, however, there have been reports that these compounds can exert actions other than those normally associated with protein gamma-carboxylation. These observations suggest that naphthoquinones may have effects on the production of inflammatory mediators including cytokines. Fibroblasts are now recognized as a rich source of cytokines and we have examined the effect of various naphthoquinones on the production of interleukin 6 (IL-6) by lipopolysaccharide-stimulated human gingival fibroblasts. Compounds examined in this study include: phylloquinone (K1), menaquinone-4 (K2), menadione (K3), 2,3-dimethoxy-1,4-naphthoquinone (DMK) and a synthetic product of vitamin K catabolism, 2-methyl, 3-(2'methyl)-hexanoic acid-1,4-naphthoquinone (KCAT). All of these compounds are capable of inhibiting IL-6 production with a rank order of potency: KCAT > K3 > DMK > K2 > K1. The most potent compound, KCAT, inhibited IL-6 production with an IC50 of 3 x 10(-7)M. The mechanism of action of these naphthoquinones on fibroblast IL-6 production is unknown. Given that K3 and KCAT are inactive in the gamma-carboxylation reaction, we suggest that this activity is not essential for the inhibition of IL-6 production and that activity may be related to the redox capacity of these naphthoquinones.

PMID: 7640347 [PubMed - indexed for MEDLINE]

Richardson AJ. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. Int Rev Psychiatry. 2006 Apr;18(2):155-72.

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Omega-3 fatty acids are dietary essentials, and are critical to brain development and function. Increasing evidence suggests that a relative lack of omega-3 may contribute to many psychiatric and neurodevelopmental disorders. This review focuses on the possible role of omega-3 in attention-deficit/hyperactivity disorder (ADHD) and related childhood developmental disorders, evaluating the existing evidence from both research and clinical perspectives. Theory and experimental

evidence support a role for omega-3 in ADHD, dyslexia, developmental coordination disorder (DCD) and autism. Results from controlled treatment trials are mixed, but the few studies in this area have involved different populations and treatment formulations. Dietary supplementation with fish oils (providing EPA and DHA) appears to alleviate ADHD-related symptoms in at least some children, and one study of DCD children also found benefits for academic achievement. Larger trials are now needed to confirm these findings, and to establish the specificity and durability of any treatment effects as well as optimal formulations and dosages. Omega-3 is not supported by current evidence as a primary treatment for ADHD or related conditions, but further research in this area is clearly warranted. Given their relative safety and general health benefits, omega-3 fatty acids offer a promising complementary approach to standard treatments.

PMID: 16777670 [PubMed - indexed for MEDLINE]

Rimland B. Controversies in the treatment of autistic children: vitamin and drug therapy. J Child Neurol. 1988;3 Suppl:S68-72.

Institute for Child Behavior Research, San Diego, CA 92116.

A survey of approximately 4,000 questionnaires completed by parents of autistic children provided ratings on a variety of treatments and interventions. Among the biomedical treatments, the use of high-dosage vitamin B6 and magnesium (n = 318) received the highest ratings, with 8.5 parents reporting behavioral improvement to every one reporting behavioral worsening. Deanol (n = 121) was next most highly rated, with 1.8 parents reporting improvement to each one reporting worsening. Fenfluramine (n = 104) was third, with a ratio of 1.5:1. Thioridazine hydrochloride (Mellaril), by far the most often used drug on the list (n = 724), was fourth with a helped-worsened ratio of 1.4:1. The research literature on the use of vitamin B6-magnesium is briefly reviewed, and mention is made of recent findings regarding high-dosage folic acid in autism and biotin in Rett syndrome.

PMID: 3058789 [PubMed - indexed for MEDLINE]

Rimland, B. High dosage levels of certain vitamins in the treatment of children with severe mental disorders. In D. Hawkins & L. Pauling (Eds.), Orthomolecular Psychiatry . 1973 (pp. 513-538).

Rimland B, Callaway E, Dreyfus P. The effect of high doses of vitamin B6 on autistic children: a double-blind crossover study. Am J Psychiatry. 1978 Apr;135(4):472-5.

The authors used data from an earlier nonblind study to identify 16 autistic-type child outpatients who had apparently improved when given vitamin B6 (pyridoxine). In a double-blind study each child's B6 supplement was replaced during two separate experimental trial periods with either a B6 supplement or a matched placebo. Behavior was rated as deteriorating significantly during the B6 withdrawal.

PMID: 345827 [PubMed - indexed for MEDLINE]

Rosenberg IH. Folic acid and neural-tube defects—time for action? N Engl J Med. 1992 Dec 24;327(26):1875-7.

Department of Human Genetics and Teratology, National Institute of Hygiene, Budapest, Hungary.

BACKGROUND. The risk of recurrent neural-tube defects is decreased in women who take folic acid or multivitamins containing such during the periconceptional period. The extent to which folic acid supplementation can reduce the first occurrence of defects is not known. METHODS. We conducted a randomized, controlled trial of periconceptional multivitamin supplementation to test the efficacy of this treatment in reducing the incidence of a first occurrence of neural-tube defects. Women planning a pregnancy (in most cases their first) were randomly assigned to receive a single tablet of a vitamin supplement (containing 12 vitamins, including 0.8 mg of folic acid; 4 minerals; and 3 trace elements) or a trace-element supplement (containing copper, manganese, zinc, and a very low dose of vitamin C) daily for at least one month before conception and until the date of the second missed menstrual period or later. RESULTS. Pregnancy was confirmed in 4753 women. The outcome of the pregnancy (whether the fetus or infant had a neural-tube defect or congenital malformation) was known in 2104 women who received the vitamin supplement and in 2052 who received the trace-element supplement. Congenital malformations were significantly more prevalent in the group receiving the trace-element supplement than in the vitamin-supplement group (22.9 per 1000 vs. 13.3 per 1000, P = 0.02). There were six cases of neural-tube defects in the group receiving the trace-element supplement, as compared with none in the vitamin-supplement group (P = 0.029). The prevalence of cleft lip with or without cleft palate was not reduced by periconceptional vitamin supplementation. CONCLUSIONS. Periconceptional vitamin use decreases the incidence of a first occurrence of neural-tube defects.

PMID: 1307234 [PubMed - indexed for MEDLINE]

Schectman G, Byrd JC, Hoffmann R. Ascorbic acid requirements for smokers: analysis of a population survey. Am J Clin Nutr. 1991 Jun;53(6):1466-70.

Division of General Internal Medicine, Medical College of Wisconsin, Milwaukee 53226.

The recommended dietary allowance (RDA) of ascorbic acid for smokers was recently increased from 60 to 100 mg. To determine whether this new RDA for smokers is sufficient to reduce the risk of low serum ascorbic acid (AA) concentrations (LoC) to the same concentration as nonsmokers, we analyzed the dietary intakes and serum concentrations of AA in 11,582 adult respondents in the National Health and Nutrition Examination Survey (1976-1980). Serum AA concentrations and the risk of LoC (serum ascorbic acid levels less than 23 mumol/L) for smokers consuming different amounts of AA were compared with those for nonsmokers whose AA intake exceeded the RDA (60 mg). Serum AA concentrations were reduced, and risk of LoC increased, in smokers maintaining AA intakes greater than 60, 100, and 150 mg. Only smokers consuming greater than 200 mg AA/d had serum ascorbate concentrations and risk of LoC equivalent to nonsmokers meeting the RDA.

PMID: 2035475 [PubMed - indexed for MEDLINE

Schoon EJ, Muller MC, Vermeer C, Schurgers LJ, Brummer RJ, Stockbrugger RW. Low serum and bone vitamin K status in patients with longstanding Crohn's disease: another pathogenetic factor of osteoporosis in Crohn's disease? Gut. 2001 Apr;48(4):473-7.

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BACKGROUND: A high prevalence of osteoporosis is reported in Crohn's disease. The pathogenesis is not completely understood but is probably multifactorial. Longstanding Crohn's disease is associated with a deficiency of fat soluble vitamins, among them vitamin K. Vitamin K is a cofactor in the carboxylation of osteocalcin, a protein essential for calcium binding to bone. A high level of circulating uncarboxylated osteocalcin is a sensitive marker of vitamin K deficiency. AIMS: To determine serum and bone vitamin K status in patients with Crohn's disease and to elucidate its relationship with bone mineral density. METHODS: Bone mineral density was measured in 32 patients with longstanding

Crohn's disease and small bowel involvement, currently in remission, and receiving less than 5 mg of prednisolone daily. Serum levels of vitamins D and K, triglycerides, and total immunoreactive osteocalcin, as well as uncarboxylated osteocalcin ("free" osteocalcin) were determined. The hydroxyapatite binding capacity of osteocalcin was calculated. Data were compared with an age and sex matched control population. RESULTS: Serum vitamin K levels of CD patients were significantly decreased compared with normal controls ($p<0.01$). "Free" osteocalcin was higher and hydroxyapatite binding capacity of circulating osteocalcin was lower than in matched controls ($p<0.05$ and $p<0.001$, respectively), indicating a low bone vitamin K status in Crohn's disease. In patients, an inverse correlation was found between "free" osteocalcin and lumbar spine bone mineral density ($r=-0.375$, $p<0.05$) and between "free" osteocalcin and the z score of the lumbar spine ($r=-0.381$, $p<0.05$). Multiple linear regression analysis showed that "free" osteocalcin was an independent risk factor for low bone mineral density of the lumbar spine whereas serum vitamin D was not. CONCLUSIONS: The finding that a poor vitamin K status is associated with low bone mineral density in longstanding Crohn's disease may have implications for the prevention and treatment of osteoporosis in this disorder.

PMID: 11247890 [PubMed - indexed for MEDLINE]

Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH, Sanderson MJ, Bodenham A. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am J Clin Nutr. 1996 May;63(5):760-5.

Division of Clinical Sciences, University of Leeds, United Kingdom.

Plasma concentrations of the antioxidant vitamin ascorbic acid were measured by high-performance liquid chromatography in critically ill patients in whom the excessive generation of reactive oxygen species could compromise antioxidant defense mechanisms. Median concentrations of both total vitamin C (ascorbic acid and dehydroascorbic acid) and ascorbic acid in these patients were < 25% ($P < 0.001$) of the values found in healthy control subjects and in subjects in two other disease groups (diabetes, gastritis) in which reactive oxygen species are reported to be increased. The low values could not be explained by age, sex, intake, or treatment differences, but were associated with the severity of the illness and were not prevented by the use of parenteral nutrition containing ascorbic acid. In addition, the vitamin was less stable in blood samples taken from critically ill patients than in similar samples from subjects in the other groups. The findings indicate that antioxidant defenses could be considerably compromised in these very sick patients. If this reduces the patient's capacity to

scavenge reactive species, then the potential of these species to damage DNA and lipid membranes could be increased and compromise recovery.

PMID: 8615361 [PubMed - indexed for MEDLINE]

Singh RB, Niaz MA, Agarwal P, Begom R, Rastogi SS. Effect of antioxidant-rich foods on plasma ascorbic acid, cardiac enzyme, and lipid peroxide levels in patients hospitalized with acute myocardial infarction. J Am Diet Assoc. 1995 Jul;95(7):775-80.

Heart Research Laboratory, Medical Hospital, Moradabad, UP, India.

OBJECTIVE: To determine whether a fat- and energy-reduced diet rich in antioxidant vitamins C and E, beta carotene, and soluble dietary fiber reduces free-radical stress and cardiac enzyme level and increases plasma ascorbic acid level 1 week after acute myocardial infarction. DESIGN: Randomized, single blind, controlled study. SETTING: Primary- and secondary-care research center for patients with myocardial infarction. SUBJECTS: All subjects with suspected acute myocardial infarction (n = 505) were considered for entry to the study. Subjects with definite or possible acute myocardial infarction and unstable angina (according to World Health Organization criteria) were assigned to either an intervention diet (n = 204) or a control diet (n = 202) within 48 hours of symptoms of infarction. INTERVENTIONS: Intervention and control groups were advised to consume a fat-reduced, oil-substituted diet. The intervention group was also advised to eat more fruits, vegetable soup, pulses, and crushed almonds and walnuts mixed with skim milk. MAIN OUTCOME MEASURES: Reduction in plasma lipid peroxide and lactate dehydrogenase cardiac enzyme levels, increase in plasma ascorbic acid level, and compliance with diet, especially with vitamin C intake as determined by chemical analysis. STATISTICAL ANALYSIS: A two-sample t test using one-way analysis of variance for comparison of data. RESULTS: Plasma lipid peroxide level decreased significantly in the intervention group compared with the control group (0.59 pmol/L in the intervention group and 0.10 pmol/L in the control group; 95% confidence interval of difference = 0.19 to 0.83). Lactate dehydrogenase level increased less in the intervention group than in the control group (427.7 vs 561.2 U/L; confidence interval of difference = 82.9 to 184.7). Plasma ascorbic acid level increased more in the intervention group than in the control group (23.38 vs 7.95 mumol/L; confidence interval of difference = 12.85 to 26.13). APPLICATIONS/CONCLUSIONS: Consumption of an antioxidant-rich diet may reduce the plasma levels of lipid peroxide and cardiac enzyme and increase the plasma level of ascorbic acid. Antioxidant-rich foods may reduce myocardial necrosis and reperfusion injury induced by oxygen free radicals.

PMID: 7797808 [PubMed - indexed for MEDLINE]

Sogut S, Zoroglu SS, Ozyurt H, Yilmaz HR, Ozugurlu F, Sivasli E, Yetkin O, Yanik M, Tutkun H, Savas HA, Tarakcioglu M, Akyol O. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clin Chim Acta. 2003 May;331(1-2):111-7.

Department of Biochemistry, Faculty of Medicine, Inönü University, Pasakosku Mahallesi 11, Sok. Ozkaracalar Apt. No: 42/4, Malatya 44200, Turkey.

BACKGROUND: There is evidence that oxygen free radicals play an important role in the pathophysiology of many neuropsychiatric disorders. Although it has not been investigated yet, several recent studies proposed that nitric oxide (NO) and other parameters related to oxidative stress may have a pathophysiological role in autism. METHODS: We assessed the changes in superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and thiobarbituric acid-reactive substances (TBARS) levels in plasma as well as NO levels in red blood cells (RBC) in patients with autism (n=27) compared to age- and sex-matched normal controls (n=30). RESULTS: In the autistic group, increased RBC NO levels ($p<0.0001$) and plasma GSH-Px activity ($p<0.0001$) and unchanged plasma TBARS levels and SOD activity were detected. CONCLUSIONS: These findings indicate a possible role of increased oxidative stress and altered enzymatic antioxidants, both of which may be relevant to the pathophysiology of autism.

PMID: 12691871 [PubMed - indexed for MEDLINE]

Starobrat-Hermelin B, Kozielec T. The effects of magnesium physiological supplementation on hyperactivity in children with attention deficit hyperactivity disorder (ADHD). Positive response to magnesium oral loading test. Magnes Res. 1997 Jun;10(2):149-56.

Department of Family Medicine, Pomeranian Medical Academy, Szczecin, Poland.

Children with ADHD are 'a group at risk' as far as their further emotional and social development and educational possibilities are concerned, and the consequences of the lack of an appropriate therapy appears to be serious. Some of these children do not respond to prevailing therapy methods. It is reported that dietetic factors can play a significant role in the etiology of ADHD syndrome, and magnesium deficiency can help in revealing hyperactivity in children. The aim of our work was to assess the influence of magnesium supplementation on

hyperactivity in patients with ADHD. The examination comprised 50 hyperactive children, aged 7-12 years, who fulfilled DSM IV criteria for ADHD syndrome, with recognized deficiency of magnesium in the blood (blood serum and red blood cells) and in hair using atomic absorption spectroscopy. In the period of 6 months those examined regularly took magnesium preparations in a dose of about 200 mg/day. 30 of those examined with ADHD showed coexisting disorders specific to developmental age, and 20 of them showed disruptive behaviour. The control group consisted of 25 children with ADHD and magnesium deficiency, who were treated in a standard way, without magnesium preparations. 15 members of this group showed coexisting disorders specific for developmental age, and 10 members showed disruptive behaviour. Hyperactivity was assessed with the aid of psychometric scales: the Conners Rating Scale for Parents and Teachers, Wender's Scale of Behavior and the Quotient of Development to Freedom from Distractibility. In the group of children given 6 months of magnesium supplementation, independently of other mental disorders coexisting with hyperactivity, an increase in magnesium contents in hair and a significant decrease of hyperactivity of those examined has been achieved, compared to their clinical state before supplementation and compared to the control group which had not been treated with magnesium.

PMID: 9368236 [PubMed - indexed for MEDLINE]

Sturman JA, Chesney RW. Taurine in pediatric nutrition. Pediatr Clin North Am. 1995 Aug;42(4):879-97.

Department of Developmental Biochemistry, Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA.

The past 20 years have seen the status of taurine change from an end product of methionine and cysteine metabolism and substance conjugated to bile acids to that of an important, and sometimes essential, nutrient. It is now added to most synthetic human infant formulas and pediatric parenteral solutions throughout the world. This article describes the research that led to this end.

PMID: 7610018 [PubMed - indexed for MEDLINE]

Sugiura I, Furie B, Walsh CT, Furie BC. Propeptide and glutamate-containing substrates bound to the vitamin K-dependent carboxylase convert its vitamin K epoxidase function from an inactive to an active state. Proc Natl Acad Sci U S A. 1997 Aug 19;94(17):9069-74.

Center for Hemostasis and Thrombosis Research, Tufts University School of Medicine, Boston, MA 02111, USA.

The vitamin K-dependent gamma-glutamyl carboxylase catalyzes the posttranslational conversion of glutamic acid to gamma-carboxyglutamic acid in precursor proteins containing the gamma-carboxylation recognition site (gamma-CRS). During this reaction, glutamic acid is converted to gamma-carboxyglutamic acid while vitamin KH2 is converted to vitamin K 2,3-epoxide. Recombinant bovine carboxylase was purified free of gamma-CRS-containing propeptide and endogenous substrate in a single-step immunoaffinity procedure. We show that in the absence of gamma-CRS-containing propeptide and/or glutamate-containing substrate, carboxylase has little or no epoxidase activity. Epoxidase activity is induced by Phe-Leu-Glu-Glu-Leu (FLEEL) (9.2 pmol per min per pmol of enzyme), propeptide, residues -18 to -1 of proFactor IX (3.4 pmol per min per pmol of enzyme), FLEEL and propeptide (100 pmol per min per pmol of enzyme), and proPT28 (HVFLAPQQARSLLQRVRRANTFLEEVRK, residues -18 to +10 of human acarboxy-proprothrombin), (5.3 pmol per min per pmol of enzyme). These results indicate that in the absence of propeptide or glutamate-containing substrate, oxygenation of vitamin K by the carboxylase does not occur. Upon addition of propeptide or glutamate-containing substrate, the enzyme is converted to an active epoxidase. This regulatory mechanism prevents the generation of a highly reactive vitamin K intermediate in the absence of a substrate for carboxylation.

PMID: 9256436 [PubMed - indexed for MEDLINE

Tchantchou F, Graves M, Shea TB. Expression and activity of methionine cycle genes are altered following folate and vitamin E deficiency under oxidative challenge: modulation by apolipoprotein E-deficiency. Nutr Neurosci. 2006 Feb-Apr;9(1-2):17-24.

Department of Biological Sciences, Center for Cellular Neurobiology and Neurodegeneration Research, University of Massachusetts Lowell, Lowell, MA 01854, USA.

Folate deficiency increases neuronal oxidative damage and potentiates the deleterious effects of apolipoprotein E (ApoE) deficiency. Mice lacking ApoE (ApoE -/- mice) upregulate the expression and activity of another enzyme, glutathione synthase (GS), when deprived of folate, in an apparent attempt to compensate for increased oxidative damage. Herein, we examined the influence of ApoE and folate deficiency on expression and activity of several enzymes of the methionine cycle. Expression and activity of methylene tetrahydrofolate reductase was increased in the order ApoE +/+ < ApoE +/- < ApoE -/- in

response to folate deprivation. Expression of cystathione beta synthase followed a similar pattern. By contrast, expression and activity of methionine synthase decreased following folate deprivation in the order ApoE +/+ < ApoE +/- < ApoE -/-. These studies demonstrate that folate deficiency induces compensatory regulation of methionine cycle genes, and that these effects are potentiated by ApoE deficiency in a gene-dosage manner. They further support the notion that latent genetic deficiencies, including those of methionine cycle, may contribute to Alzheimer's disease, especially in concert with age-related nutritional deficiencies.

PMID: 16910166 [PubMed - indexed for MEDLINE]

Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci. 2001 Apr;22(4):195-201.

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The enzyme methylenetetrahydrofolate reductase (MTHFR) directs folate species either to DNA synthesis or to homocysteine (Hcy) remethylation. The common MTHFR C677T polymorphism affects the activity of the enzyme and hence folate distribution. Under conditions of impaired folate status, the homozygous TT genotype has been regarded as harmful because it is associated with a high concentration of plasma total Hcy, increased risk of neural tube defects and colorectal neoplasias, and can also predispose individuals to adverse effects from drugs with antifolate effects. The MTHFR C677T polymorphism shows no consistent correlation with cardiovascular risk and longevity but, in combination with positive folate balance, the TT genotype is associated with decreased risk of colorectal neoplasias. Because of the high prevalence of this polymorphism in most populations, the TT variant might represent an ancestral genetic adaptation to living constraints (tissue injury or unbalanced vitamin intake) that has become a determinant of disease profiles in modern times.

PMID: 11282420 [PubMed - indexed for MEDLINE]

Vancassel S, Durand G, Barthelemy C, Lejeune B, Martineau J, Guilloteau D, Andres C, Chalon S. Plasma fatty acid levels in autistic children. Prostaglandins Leukot Essent Fatty Acids. 2001 Jul;65(1):1-7.

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Phospholipid fatty acids are major structural components of neuronal cell membranes, which modulate membrane fluidity and hence function. Evidence from clinical and biochemical sources have indicated changes in the metabolism of fatty acids in several psychiatric disorders. We examined the phospholipid fatty acids in the plasma of a population of autistic subjects compared to mentally retarded controls. Our results showed a marked reduction in the levels of 22: 6n-3 (23%) in the autistic subjects, resulting in significantly lower levels of total (n-3) polyunsaturated fatty acids (PUFA) (20%), without significant reduction in the (n-6) PUFA series, and consequently a significant increase in the (n-6)/(n-3) ratio (25%). These variations are discussed in terms of potential differences in PUFA dietary intake, metabolism, or incorporation into cellular membranes between the two groups of subjects. These results open up interesting perspectives for the investigation of new biological indices in autism. Moreover, this might have new therapeutic implications in terms of child nutrition. Copyright 2001 Harcourt Publishers Ltd.

PMID: 11487301 [PubMed - indexed for MEDLINE]

Van Gelder NM, Sherwin AL, Sacks C, Anderman F. Biochemical observations following administration of taurine to patients with epilepsy. Brain Res. 1975 Aug 29;94(2):297-306.

Amino acid analysis of plasma and urine obtained from 12 patients with epilepsy indicated that the plasma concentrations of taurine and glutamic acid were much higher than might have been expected. Glutamic acid in urine was also increased in these patients. Oral administration of taurine did not appreciably affect the levels of amino acids in plasma or urine with the exception of that of glutamic acid. In patients with an abnormal plasma concentration of glutamic acid, the administration of taurine caused glutamic acid levels to change in the direction of normal values along with a decrease in the urinary excretion of this amino acid. This action of taurine was independent of either its initial or final plasma concentration. Amino acid concentrations in the CSF were within normal range and were not influenced by taurine administration. The selective elevation of both taurine and glutamic acid in the plasma, combined with previous findings of a deficiency of these same amino acids in human and experimental epileptogenic brain, implies that some patients with epilepsy may suffer from an aberration in taurine and glutamic acid metabolism. Taurine administration appears to partially correct these biochemical abnormalities. Theoretically, such normalization of the amino acid profile in epileptogenic brain may be beneficial, but clinical signs of

improvement may only become apparent after a long delay. The present study was designed to determine only the biochemical parameters implicated in taurine administration and no definite conclusions can be drawn as to the clinical efficacy of the amino acid in epilepsy. However, this study suggests that in future clinical trials investigating the potential use of taurine as an antiepileptic agent, the oral dose of taurine should not exceed 1.0 g/day and optimal doses may be as low as 0.1-0.5 g/day. In one patient who received 2.0-2.5 g of taurine/day for 2 weeks, a generalized amino aciduria occurred.

PMID: 807299 [PubMed - indexed for MEDLINE]

Vervoort LM, Ronden JE, Thijssen HH. The potent antioxidant activity of the vitamin K cycle in microsomal lipid peroxidation. Biochem Pharmacol. 1997 Oct 15;54(8):871-6.

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In the vitamin K cycle, vitamin K-hydroquinone, the active cofactor for gamma-glutamylcarboxylase, is continuously regenerated. The successive pathways contain oxidation of the hydroquinone to the epoxide, followed by reduction to the quinone and reduction to the hydroquinone. Vitamin K-hydroquinone is a potent radical scavenging species (Mukai et al., J Biol Chem 267: 22277-22281, 1992). We tested the potential antioxidant activity of the vitamin K cycle in lipid peroxidation reactions (thiobarbituric acid reactive substances, TBARS) in rat liver microsomes. As prooxidant we used Fe2+/ascorbate, NADPH-Fe3+/ATP, and NADPH/CCl4. Vitamin K (< or = 50 microM) on its own did not influence the formation of TBARS. In combination with 1 mM dithiothreitol (DTT), the reductive cofactor for the microsomal enzyme vitamin K epoxide reductase, vitamin K suppressed lipid peroxidation with a concentration that blocked the maximal response by 50% (IC50) of ca. 0.2 microM. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinone-4) were equally active. Warfarin (5 microM) and chloro-vitamin K (50 microM), inhibitors of vitamin K epoxide reductase and gamma-glutamylcarboxylase, respectively, were able to completely abolish the antioxidant effect. Lipid peroxidation was inversely related to the amount of vitamin K hydroquinone in the reaction. Vitamin K epoxide reductase seemed sensitive to lipid peroxidation, with half of the activity being lost within 10 min during oxidation with NADPH/CCl4. The inactivation could be attenuated by antioxidants such as vitamin E, reduced glutathione, and menadione and also by a K vitamin in combination with DTT, but not by superoxide dismutase and catalase. The results show that the vitamin K cycle could act as a potent antioxidant, that the active species in all probability is vitamin K-hydroquinone, and that the primary reaction product is the semiquinone. The results also show

that the reaction product is processed in the vitamin K cycle to regenerate vitamin K-hydroquinone.

PMID: 9354587 [PubMed - indexed for MEDLINE]

Wakefield AJ, Murch SH, Anthony A, Linnell J, Casson DM, Malik M, Berelowitz M, Dhillon AP, Thomson MA, Harvey P, Valentine A, Davies SE, Walker-Smith JA. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet. 1998 Feb 28;351(9103):637-41.

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BACKGROUND: We investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder. METHODS: 12 children (mean age 6 years [range 3-10], 11 boys) were referred to a paediatric gastroenterology unit with a history of normal development followed by loss of acquired skills, including language, together with diarrhoea and abdominal pain. Children underwent gastroenterological, neurological, and developmental assessment and review of developmental records. Ileocolonoscopy and biopsy sampling, magnetic-resonance imaging (MRI), electroencephalography (EEG), and lumbar puncture were done under sedation. Barium follow-through radiography was done where possible. Biochemical, haematological, and immunological profiles were examined. FINDINGS: Onset of behavioural symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children, with measles infection in one child, and otitis media in another. All 12 children had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. Histology showed patchy chronic inflammation in the colon in 11 children and reactive ileal lymphoid hyperplasia in seven, but no granulomas. Behavioural disorders included autism (nine), disintegrative psychosis (one), and possible postviral or vaccinal encephalitis (two). There were no focal neurological abnormalities and MRI and EEG tests were normal. Abnormal laboratory results were significantly raised urinary methylmalonic acid compared with age-matched controls ($p=0.003$), low haemoglobin in four children, and a low serum IgA in four children. INTERPRETATION: We identified associated gastrointestinal disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers.

PMID: 9500320 [PubMed - indexed for MEDLINE]

Walsh WJ, Glab LB, Haakenson ML. Reduced violent behavior following biochemical therapy. Physiol Behav. 2004 Oct 15;82(5):835-9.

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Reduced violent behavior following biochemical therapy. We conducted an outcome study to measure the effectiveness of biochemical therapy for 207 consecutive patients presenting with a diagnosed behavior disorder. The treatment protocols were based on clinical evaluation and our past experience in the treatment of 8000 patients with behavior disorders at the Pfeiffer Treatment Center (PTC) over a 10-year period. Each test subject was screened for chemical imbalances previously found in high incidence in this population, including metal-metabolism disorders, methylation abnormalities, disordered pyrrole chemistry, heavy-metal overload, glucose dyscontrol, and malabsorption. The clinical procedure included a medical history, assay of 90 biochemical factors, and a physical examination. Standardized treatment protocols were applied for each imbalance that was identified. The frequencies of physical assaults and destructive episodes were determined using a standardized behavior scale before and after treatment, with follow-up ranging from 4 to 8 months. RESULTS: Seventy-six percent of the test subjects achieved compliance during the treatment period. The remaining 24% were reported to have discontinued the therapy. A reduced frequency of assaults was reported by 92% of the compliant assaultive patients, with 58% achieving elimination of the behavior. A total of 88% of compliant destructive patients exhibited a reduced frequency of destructive incidents and 53% achieved elimination of the behavior. Statistical significance was found for reduced frequency of assaults ($t=7.74$, $p<0.001$) and destructive incidents ($t= 8.77$, $p<0.001$). The results of this outcome study strongly suggest that individualized biochemical therapy may be efficacious in achieving behavioral improvements in this patient population.

PMID: 15451647 [PubMed - indexed for MEDLINE]

Waring RH, Klovrza LV. Sulphur metabolism in autism. J Nutr Env Med. 2000:10:25-35.

White JF. Intestinal pathophysiology in autism. Exp Biol Med (Maywood). 2003 Jun;228(6):639-49.

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Autism is a life-long developmental disorder affecting as many as 1 in 500 children. The causes for this profound disorder are largely unknown. Recent research has uncovered pathology in the gastrointestinal tract of autistic children. The pathology, reported to extend from the esophagus to the colon, is described here along with other studies pointing to a connection between diet and the severity of symptoms expressed in autism. The evidence that there is impaired intestinal permeability in autism is reviewed, and various theories are discussed by which a leaky gut could develop. Lastly, some possible ways in which impaired gastrointestinal function might influence brain function are discussed.

PMID: 12773694 [PubMed - indexed for MEDLINE]

Whiteley P, Waring R, Williams L, Klovrza L, Nolan F, Smith S, Farrow M, Dodou K, Lough WJ, Shattock P. Spot urinary creatinine excretion in pervasive developmental disorders. Pediatr Int. 2006 Jun;48(3):292-7.

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BACKGROUND: Excretion of creatinine in urine represents the end-point of endogenous energy transfer from stored adenosine triphosphate in skeletal and cardiac muscle. Measurement of urinary creatinine is commonly used to correct for total urine concentration. Various quantitative measures of compounds suspected to be either pathological to, or indicative of, possible therapeutic interventions for Pervasive Developmental Disorders (PDD) have relied extensively on spot creatinine as a ratio quantity, although this important metabolite has not been exclusively studied within this population. METHODS: Levels of urinary creatinine in spot urine samples were analyzed for a group of children diagnosed with PDD (n=24; median age, 75 months; range, 39-137 months) and a control group (n=50; median age, 109 months; range, 59-140 months). Diagnosis of PDD was confirmed using the Autism Diagnostic Interview-Revised. Samples were collected and analyzed blind for creatinine content using an improved Jaffe's reaction method. RESULTS: Controlling for sample pH and body mass index, a significant decrease in urinary creatinine concentration was found in the PDD group compared to controls using a Mann-Whitney two-tailed ranks test (P=0.001). CONCLUSION: Further studies of protein catabolism and renal function in autism are required to ascertain the relevance of decreased spot urinary creatinine excretion identified in this preliminary study. Issues regarding the use of single urine creatinine measurements and associated confounding variables are discussed in light of the findings, together with recommendations to

use other internal or external standards for the quantification of urinary compounds in PDD research.

PMID: 16732798 [PubMed - indexed for MEDLINE]

Whiting SJ, Calvo MS. Dietary recommendations for vitamin D: a critical need for functional end points to establish an estimated average requirement. J Nutr. 2005 Feb;135(2):304-9.

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From its inaugural value in 1941, the Recommended Dietary Allowance (RDA) for adults for vitamin D has remained close to 400 IU (10 microg) level. This original recommended intake was based on the observation that the amount of vitamin D activity in a teaspoon of cod liver oil was sufficient to prevent rickets in infants. Since that time until 1997, determination of vitamin D requirements and status was more conjecture than science. In 1997, when the recommended intake level of vitamin D was set as an adequate intake value rather than an RDA, much has been learned about metabolism of vitamin D. The circulating metabolite 25-hydroxyvitamin D is the major static indicator of vitamin D status. Using its response to diet in the absence of sun exposure, a dose-response study suggests a mean requirement of at least 500 IU (12.5 microg) from which an RDA could be set. Other factors may need adjustment, such as sun exposure and body fat. However, functional indicators of status are needed. The role of vitamin D in calcium metabolism (i.e., calciotropic functions) is better understood; bone turnover and parathyroid hormone are potential indicators. Vitamin D has noncalciotropic functions arising from extrarenal synthesis of the active metabolite 1,25 dihydroxyvitamin D involving cell proliferation and immunity, from which function indicators of status may be derived. Despite gaps in our knowledge, there are data from which new dietary reference intake values for vitamin D may be set.

PMID: 15671232 [PubMed - indexed for MEDLINE]

Young G, Conquer J. Omega-3 fatty acids and neuropsychiatric disorders. Reprod Nutr Dev. 2005 Jan-Feb;45(1):1-28.

Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada.

Epidemiological evidence suggests that dietary consumption of the long chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), commonly found in fish or fish oil, may modify the risk for certain neuropsychiatric disorders. As evidence, decreased blood levels of omega-3 fatty acids have been associated with several neuropsychiatric conditions, including Attention Deficit (Hyperactivity) Disorder, Alzheimer's Disease, Schizophrenia and Depression. Supplementation studies, using individual or combination omega-3 fatty acids, suggest the possibility for decreased symptoms associated with some of these conditions. Thus far, however, the benefits of supplementation, in terms of decreasing disease risk and/or aiding in symptom management, are not clear and more research is needed. The reasons for blood fatty acid alterations in these disorders are not known, nor are the potential mechanisms by which omega-3 fatty acids may function in normal neuronal activity and neuropsychiatric disease prevention and/or treatment. It is clear, however, that DHA is the predominant n-3 fatty acid found in the brain and that EPA plays an important role as an anti-inflammatory precursor. Both DHA and EPA can be linked with many aspects of neural function, including neurotransmission, membrane fluidity, ion channel and enzyme regulation and gene expression. This review summarizes the knowledge in terms of dietary omega-3 fatty acid intake and metabolism, as well as evidence pointing to potential mechanisms of omega-3 fatty acids in normal brain functioning, development of neuropsychiatric disorders and efficacy of omega-3 fatty acid supplementation in terms of symptom management.

PMID: 15865053 [PubMed - indexed for MEDLINE]

Zitterman A. Effects of vitamin K on calcium and bone metabolism. Curr Opin Clin Nutr Metab Care. 2001 Nov 4(6):483-487.

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The K vitamins, a group of napthoquinones, are required for the carboxylation of a limited number of proteins including the bone matrix protein osteocalcin. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinones), differ regarding food source (green vegetables and fermented products, respectively), bioavailability and intermediate metabolism. Epidemiological studies provide evidence for an association between a low vitamin K intake and an enhanced osteoporotic fracture risk. Doses of vitamin K1 up to 15 times the current recommended dietary allowance have successfully been used to reduce the percentage of undercarboxylated osteocalcin in the circulation. Studies demonstrating clear beneficial effects on bone health, however, are still lacking.

In contrast, therapy with very high pharmacological doses of the vitamin K2 menatetrenone has impressively been used to prevent further bone mineral loss and fracture risk in osteoporotic patients.

PMID: 11706280 [PubMed - indexed for MEDLINE]

Zoroglu SS, Yurekli M, Meram I, Sogut S, Tutkun H, Yetkin O, Sivasli E, Savas HA, Yanik M, Herken H, Akyol O. Pathophysiological role of nitric oxide and adrenomedullin in autism. Cell Biochem Funct. 2003 Mar;21(1):55-60.

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Several studies indicate that nitric oxide (NO) is involved in the aetiopathogenesis of many neuropsychiatric disorders such as schizophrenia, bipolar disorder, depression, Alzheimer's disease, Hungtington disease and stroke. Although it has not been investigated yet, several recent studies proposed that NO may have a pathophysiological role in autism. Adrenomedullin (AM), a recently discovered 52-amino acid peptide hormone, induces vasorelaxation by activating adenylate cyclase and also by stimulating NO release. AM immune reactivity is present in the brain consistent with a role as a neurotransmitter. It has been stated that NO and AM do function in the regulation of many neurodevelopmental processes. We hypothesized that NO and AM activities have been affected in autistic patients and aimed to examine these molecules. Twenty-six autistic patients and 22 healthy control subjects were included in this study. AM and total nitrite (a metabolite of NO) levels have been measured in plasma. The mean values of plasma total nitrite and AM levels in the autistic group were significantly higher than control values, respectively ($p < 0.001$, $p = 0.028$). There is no correlation between total nitrite and AM levels ($r = 0.11$, $p = 0.31$). Certainly, this subject needs much further research investigating autistic patients in earlier periods of life and with subtypes of the disorder. Copyright 2002 John Wiley & Sons, Ltd.

PMID: 12579522 [PubMed - indexed for MEDLINE]

11- Immune Dysregulation in Autism – 71 citations

Ahlsen G, Rosengren L, Belfrage M, Palm A, Haglid K, Hamberger A, Gillberg C. Glial fibrillary acidic protein in the cerebrospinal fluid of children with autism and other neuropsychiatric disorders. Biol Psychiatry. 1993 May 15;33(10):734-43.

Department of Child Neuropsychiatry, University of Göteborg, Sweden.

The cerebrospinal fluid (CSF) of 47 children and adolescents with autism was analyzed for the contents of two astroglial proteins, the glial fibrillary acidic protein (GFA) and S 100. The results were contrasted with those obtained in similarly aged cases with other neuropsychiatric disorders (n = 25) and in normal children (n = 10). S-100 did not discriminate the groups from each other. However, GFA in autism and autistic-like conditions was at a level almost three times that in the normal group. The results could implicate gliosis and unspecific brain damage in autism. An alternative model would be increased synapse turnover regardless of underlying cause.

PMID: 8353169 [PubMed - indexed for MEDLINE]

Ashwood P, Anthony A, Pellicer AA, Torrente F, Walker-Smith JA, Wakefield AJ. Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology. J Clin Immunol. 2003 Nov;23(6):504-17.

The Inflammatory Bowel Disease Study Group, and Centre for Paediatric Gastroenterology, Royal Free and University College, Medical School, London, United Kingdom. pashwood@ucdavis.edu

Inflammatory intestinal pathology has been reported in children with regressive autism (affected children). Detailed analysis of intestinal biopsies in these children indicates a novel lymphocytic enterocolitis with autoimmune features; however, links with cognitive function remain unclear. To characterize further, the nature and extent of this disease we examined the mucosal infiltrate using flow cytometry. Duodenal, ileal, and colonic biopsies were obtained from 52 affected children, 25 histologically normal, and 54 histologically inflamed, developmentally normal controls. Epithelial and lamina propria lymphocyte populations were isolated and examined by multicolor flow cytometry. Adjacent biopsies were assessed by semiquantitative histopathology. At all sites, CD3(+) and CD3(+)CD8(+) IEL as well as CD3(+) LPL were significantly increased in affected children compared with developmentally normal noninflamed control groups ($p<0.01$) reaching levels similar to inflamed controls. In addition, two populations--CD3(+)CD4(+) IEL and LP CD19(+) B cells--were significantly

increased in affected children compared with both noninflamed and inflamed control groups including IBD, at all sites examined ($p<0.01$). Histologically there was a prominent mucosal eosinophil infiltrate in affected children that was significantly lower in those on a gluten- and casein-free diet, although lymphocyte populations were not influenced by diet.The data provide further evidence of a pan-enteric mucosal immunopathology in children with regressive autism that is apparently distinct from other inflammatory bowel diseases.

PMID: 15031638 [PubMed - indexed for MEDLINE]

Ashwood P, Anthony A, Torrente F, Wakefield AJ. Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. J Clin Immunol. 2004 Nov;24(6):664-73.

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A lymphocytic enterocolitis has been reported in a cohort of children with autistic spectrum disorder (ASD) and gastrointestinal (GI) symptoms. This study tested the hypothesis that dysregulated intestinal mucosal immunity with enhanced pro-inflammatory cytokine production is present in these ASD children. Comparison was made with developmentally normal children with, and without, mucosal inflammation. Duodenal and colonic biopsies were obtained from 21 ASD children, and 65 developmentally normal paediatric controls, of which 38 had signs of histological inflammation. Detection of CD3+ lymphocyte staining for spontaneous intracellular TNFalpha, IL-2, IL-4, IFNgamma, and IL-10, was performed by multicolor flow cytometry. Duodenal and colonic mucosal CD3+ lymphocyte counts were elevated in ASD children compared with noninflamed controls ($p<0.03$). In the duodenum, the proportion of lamina propria (LP) and epithelial CD3(+)TNFalpha+ cells in ASD children was significantly greater compared with noninflamed controls ($p<0.002$) but not coeliac disease controls. In addition, LP and epithelial CD3(+)IL-2+ and CD3(+)IFNgamma+, and epithelial CD3(+)IL-4+ cells were more numerous in ASD children than in noninflamed controls ($p<0.04$). In contrast, CD3(+)IL-10+ cells were fewer in ASD children than in noninflamed controls ($p<0.05$). In the colon, LP CD3(+)TNFalpha+ and CD3(+)IFNgamma+ were more frequent in ASD children than in noninflamed controls ($p<0.01$). In contrast with Crohn's disease and non-Crohn's colitis, LP and epithelial CD3(+)IL-10+ cells were fewer in ASD children than in nondisease controls ($p<0.01$). There was a significantly greater proportion of CD3(+)TNFalpha+ cells in colonic mucosa in those ASD children who had no dietary exclusion compared with those on a gluten and/or casein

free diet ($p<0.05$). There is a consistent profile of CD3+ lymphocyte cytokines in the small and large intestinal mucosa of these ASD children, involving increased pro-inflammatory and decreased regulatory activities. The data provide further evidence of a diffuse mucosal immunopathology in some ASD children and the potential for benefit of dietary and immunomodulatory therapies.

PMID: 15622451 [PubMed - indexed for MEDLINE]

Ashwood P, Van de Water J. Is autism an autoimmune disease? Autoimmun Rev. 2004 Nov;3(7-8):557-62.

Department of Internal Medicine, Division of Rheumatology, and UC Davis M.I.N.D. Institute, University of California, Davis, CA 95616, USA.

Autism spectrum disorder (ASD) is a spectrum of behavioral anomalies characterized by impaired social interaction and communication, often accompanied by repetitive and stereotyped behavior. The condition manifests within the first 3 years of life and persists into adulthood. There are numerous hypotheses regarding the etiology and pathology of ASD, including a suggested role for immune dysfunction. However, to date, the evidence for involvement of the immune system in autism has been inconclusive. While immune system abnormalities have been reported in children with autistic disorder, there is little consensus regarding the nature of these differences which include both enhanced autoimmunity and reduced immune function. In this review, we discuss current findings with respect to immune function and the spectrum of autoimmune phenomena described in children with ASD.

PMID: 15546805 [PubMed - indexed for MEDLINE]

Ashwood P, Willis S, Van de Water J. The immune response in autism: a new frontier for autism research. J Leuk Biol. 2006 Jul:80;1-15.

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Autism spectrum disorders (ASD) are part of a broad spectrum of neurodevelopmental disorders known as pervasive developmental disorders, which occur in childhood. They are characterized by impairments in social interaction, verbal and nonverbal communication and the presence of restricted and repetitive stereotyped behaviors. At the present time, the etiology of ASD is

largely unknown, but genetic, environmental, immunological, and neurological factors are thought to play a role in the development of ASD. Recently, increasing research has focused on the connections between the immune system and the nervous system, including its possible role in the development of ASD. These neuroimmune interactions begin early during embryogenesis and persist throughout an individual's lifetime, with successful neurodevelopment contingent upon a normal balanced immune response. Immune aberrations consistent with a dysregulated immune response, which so far, have been reported in autistic children, include abnormal or skewed T helper cell type 1 (T(H)1)/T(H)2 cytokine profiles, decreased lymphocyte numbers, decreased T cell mitogen response, and the imbalance of serum immunoglobulin levels. In addition, autism has been linked with autoimmunity and an association with immune-based genes including human leukocyte antigen (HLA)-DRB1 and complement C4 alleles described. There is potential that such aberrant immune activity during vulnerable and critical periods of neurodevelopment could participate in the generation of neurological dysfunction characteristic of ASD. This review will examine the status of the research linking the immune response with ASD.

PMID: 16698940 [PubMed - indexed for MEDLINE]

Bach JF. Infections and autoimmune diseases. J Autoimmun. 2005;25 Suppl:74-80.

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The high percentage of disease-discordant pairs of monozygotic twins demonstrates the central role of environmental factors in the etiology of autoimmune diseases. Efforts were first focussed on the search for triggering factors. The study of animal models has clearly shown that infections may trigger autoimmune diseases, as in the case of Coxsackie B4 virus in type I diabetes and the encephalomyocarditis virus in autoimmune myositis, two models in which viruses are thought to act by increasing immunogenicity of autoantigens secondary to local inflammation. The induction of a Guillain-Barré syndrome in rabbits after immunization with a peptide derived from Campylobacter jejuni is explained by mimicry between C. jejuni antigens and peripheral nerve axonal antigens. Other models involve chemical modification of autoantigens, as in the case of iodine-induced autoimmune thyroiditis. These mechanisms have so far only limited clinical counterparts (rheumatic fever, Guillain-Barré syndrome and drug-induced lupus or myasthenia gravis) but one may assume that unknown viruses may be at the origin of a number of chronic autoimmune diseases, such as type I diabetes and multiple sclerosis) as illustrated by the convergent data incriminating IFN-alpha in the pathophysiology of type I diabetes and systemic

lupus erythematosus. Perhaps the difficulties met in identifying the etiologic viruses are due to the long lag time between the initial causal infection and onset of clinical disease. More surprisingly, infections may also protect from autoimmune diseases. Western countries are being confronted with a disturbing increase in the incidence of most immune disorders, including autoimmune and allergic diseases, inflammatory bowel diseases, and some lymphocyte malignancies. Converging epidemiological evidence indicates that this increase is linked to improvement of the socio-economic level of these countries, posing the question of the causal relationship and more precisely the nature of the link. Epidemiological and clinical data support the hygiene hypothesis according to which the decrease of infections observed over the last three decades is the main cause of the incessant increase in immune disorders. The hypothesis does not exclude an etiological role for specific pathogens in a given immune disorder as might notably be the case in inflammatory bowel diseases. Even in this setting, infections could still have a non-specific protective role. Independently of the need for confirmation by epidemiological prospective studies, the hygiene hypothesis still poses numerous questions concerning the nature of protective infectious agents, the timing of their involvement with regard to the natural history of immune diseases and, most importantly, the mechanisms of protection. Four orders of mechanisms are being explored. Antigenic competition is the first hypothesis (immune responses against pathogens compete with autoimmune and allergic responses). This is probably an important mechanism but its modalities are still elusive in spite of considerable experimental data. Its discussion in the context of homeostatic regulation of lymphocyte pools has shed new light on this hypothesis with possible competition for self MHC peptide recognition and interleukin-7. Another hypothesis deals with immunoregulation. Infectious agents stimulate a large variety of regulatory cells (Th2, CD25+, Tr1, NKT, ...) whose effects extend to other specificities than those which triggered their differentiation (bystander suppression). Infectious agents may also intervene through components which are not recognized as antigens but bind to specific receptors on cells of the immune system. Major attention has recently been drawn to Toll receptors (expressed on macrophages and possibly on regulatory T cells) and TIM proteins present on Th cells, which may express the function of the virus receptor (as in the case of the Hepatitis A virus and Tim-1). Experimental data will be presented to support each of these hypotheses. In any event, the final proof of principle will be derived from therapeutic trials where the immune disorders in question will be prevented or better cured by products derived from protective infectious agents. Numerous experimental data are already available in several models. Preliminary results have also been reported in atopic dermatitis using bacterial extracts and probiotics.

PMID: 16278064 [PubMed - indexed for MEDLINE]

Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S, Elia M, Schneider C, Melmed R, Sacco R, Persico AM, Levitt P. A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci U S A. 2006 Oct 19.

Department of Pharmacology, Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN 37203, USA.

There is strong evidence for a genetic predisposition to autism and an intense interest in discovering heritable risk factors that disrupt gene function. Based on neurobiological findings and location within a chromosome 7q31 autism candidate gene region, we analyzed the gene encoding the pleiotropic MET receptor tyrosine kinase in a family based study of autism including 1,231 cases. MET signaling participates in neocortical and cerebellar growth and maturation, immune function, and gastrointestinal repair, consistent with reported medical complications in some children with autism. Here, we show genetic association (P = 0.0005) of a common C allele in the promoter region of the MET gene in 204 autism families. The allelic association at this MET variant was confirmed in a replication sample of 539 autism families (P = 0.001) and in the combined sample (P = 0.000005). Multiplex families, in which more than one child has autism, exhibited the strongest allelic association (P = 0.000007). In case-control analyses, the autism diagnosis relative risk was 2.27 (95% confidence interval: 1.41-3.65; P = 0.0006) for the CC genotype and 1.67 (95% confidence interval: 1.11-2.49; P = 0.012) for the CG genotype compared with the GG genotype. Functional assays showed that the C allele results in a 2-fold decrease in MET promoter activity and altered binding of specific transcription factor complexes. These data implicate reduced MET gene expression in autism susceptibility, providing evidence of a previously undescribed pathophysiological basis for this behaviorally and medically complex disorder.

PMID: 17053076 [PubMed - indexed for MEDLINE]

Chess S, Fernandez P, Korn S. Behavioral consequences of congenital rubella. J Pediatr. 1978 Oct;93(4):699-703.

Psychiatric and behavioral consequences of congenital rubella are reported for 243 children studies during the preschool period, and for 205 of these who were re-examined at ages 8 to 9. At preschool 37% were retarded, with the skew toward severe and profound; 15% had reactive behavior disorder and 7% had autism. At school age retardation diminished to 25%, but neurotic problems and behavioral pathology due to neurologic damage both increased. There were two remissions and three new instances of autism.

PMID: 702254 [PubMed - indexed for MEDLINE]

Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. J Child Neurol. 1999 Jun;14(6):388-94. Nov;112(5):e420.

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Autism is an age-dependent neurologic disorder that is often associated with autoimmune disorders in the patients' relatives. To evaluate the frequency of autoimmune disorders, as well as various prenatal and postnatal events in autism, we surveyed the families of 61 autistic patients and 46 healthy controls using questionnaires. The mean number of autoimmune disorders was greater in families with autism; 46% had two or more members with autoimmune disorders. As the number of family members with autoimmune disorders increased from one to three, the risk of autism was greater, with an odds ratio that increased from 1.9 to 5.5, respectively. In mothers and first-degree relatives of autistic children, there were more autoimmune disorders (16% and 21%) as compared to controls (2% and 4%), with odds ratios of 8.8 and 6.0, respectively. The most common autoimmune disorders in both groups were type 1 diabetes, adult rheumatoid arthritis, hypothyroidism, and systemic lupus erythematosus. Forty-six percent of the autism group reported having relatives with rheumatoid diseases, as compared to 26% of the controls. Prenatal maternal urinary tract, upper respiratory, and vaginal infections; asphyxia; prematurity, and seizures were more common in the autistic group, although the differences were not significant. Thirty-nine percent of the controls, but only 11% of the autistic, group, reported allergies. An increased number of autoimmune disorders suggests that in some families with autism, immune dysfunction could interact with various environmental factors to play a role in autism pathogenesis.

PMID: 10385847 [PubMed - indexed for MEDLINE]

Connolly AM, Chez MG, Pestronk A, Arnold ST, Mehta S, Deuel RK. Serum autoantibodies to brain in Landau-Kleffner variant, autism, and other neurologic disorders. J Pediatr. 1999 May;134(5):607-13.

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OBJECTIVE: Etiologically unexplained disorders of language and social development have often been reported to improve in patients treated with immune-modulating regimens. Here we determined the frequency of autoantibodies to brain among such children. DESIGN: We collected sera from a cohort of children with (1) pure Landau-Kleffner syndrome (n = 2), (2) Landau-Kleffner syndrome variant (LKSV, n = 11), and (3) autistic spectrum disorder (ASD, n = 11). None had received immune-modulating treatment before the serum sample was obtained. Control sera (n = 71) were from 29 healthy children, 22 with non-neurologic illnesses (NNIs), and 20 children with other neurologic disorders (ONDs). We identified brain autoantibodies by immunostaining of human temporal cortex and antinuclear autoantibodies using commercially available kits. RESULTS: IgG anti-brain autoantibodies were present in 45% of sera from children with LKSV, 27% with ASD, and 10% with ONDs compared with 2% from healthy children and control children with NNIs. IgM autoantibodies were present in 36% of sera from children with ASD, 9% with LKSV, and 15% with ONDs compared with 0% of control sera. Labeling studies identified one antigenic target to be endothelial cells. Antinuclear antibodies with titers >/=1:80 were more common in children with ASD and control children with ONDs. CONCLUSION: Children with LKSV and ASD have a greater frequency of serum antibodies to brain endothelial cells and to nuclei than children with NNIs or healthy children. The presence of these antibodies raises the possibility that autoimmunity plays a role in the pathogenesis of language and social developmental abnormalities in a subset of children with these disorders.

PMID: 10228297 [PubMed - indexed for MEDLINE]

Croen LA, Najjar DV, Ray GT, Lotspeich L, Bernal P. A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. Pediatrics. 2006 Oct;118(4):e1203-11.

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OBJECTIVE: Data on the current costs of medical services for children with autism spectrum disorders are lacking. Our purpose for this study was to compare health care utilization and costs of children with and without autism spectrum disorders in the same health plan. PATIENTS AND METHODS: Participants included all 2- to 18-year-old children with autism spectrum disorders (n = 3053) and a random sample of children without autism spectrum disorders (n = 30529) who were continuously enrolled in the Kaiser Permanente Medical Care Program in northern California between July 1, 2003, and June 30,

2004. Data on health care utilization and costs were derived from health plan administrative databases. MAIN OUTCOME MEASURES: Outcome measures included mean annual utilization and costs of health services per child. RESULTS: Children with autism spectrum disorders had a higher annual mean number of total clinic (5.6 vs 2.8), pediatric (2.3 vs 1.6), and psychiatric (2.2 vs 0.3) outpatient visits. A higher percentage of children with autism spectrum disorders experienced inpatient (3% vs 1%) and outpatient (5% vs 2%) hospitalizations. Children with autism spectrum disorders were nearly 9 times more likely to use psychotherapeutic medications and twice as likely to use gastrointestinal agents than children without autism spectrum disorders. Mean annual member costs for hospitalizations (550 dollars vs 208 dollars), clinic visits (1373 dollars vs 540 dollars), and prescription medications (724 dollars vs 96 dollars) were more than double for children with autism spectrum disorders compared with children without autism spectrum disorders. The mean annual age- and gender-adjusted total cost per member was more than threefold higher for children with autism spectrum disorders (2757 dollars vs 892 dollars). Among the subgroup of children with other psychiatric conditions, total mean annual costs were 45% higher for children with autism spectrum disorders compared with children without autism spectrum disorders; excess costs were largely explained by the increased use of psychotherapeutic medications. CONCLUSIONS: The utilization and costs of health care are substantially higher for children with autism spectrum disorders compared with children without autism spectrum disorders. Research is needed to evaluate the impact of improvements in the management of children with autism spectrum disorders on health care utilization and costs.

PMID: 17015508 [PubMed - indexed for MEDLINE]

Croonenberghs J, Wauters A, Devreese K, Verkerk R, Scharpe S, Bosmans E, Egyed B, Deboutte D, Maes M. Increased serum albumin, gamma globulin, immunoglobulin IgG, and IgG2 and IgG4 in autism. Psychol Med. 2002 Nov;32(8):1457-63.

University Center of Child and Adolescent Psychiatry, A. Z. M. and Department of Medical Biochemistry, University of Antwerp, Wilrijk, Clinical Laboratory A. Z. Middelheim, Antwerp, The Netherlands.

BACKGROUND: Research on the biological pathophysiology of autism has found some evidence that immune alterations may play a role in the pathophysiology of that illness. As a consequence we expected to find that autism is accompanied by abnormalities in the pattern obtained in serum protein electrophoresis and in the serum immunoglobulin (Ig) and IgG subclass profile. METHOD: We examined whether subjects with autism showed changes in total serum protein (TSP) and the serum concentrations of albumin, alpha1 globulin, alpha2 globulin, beta

globulin and gamma globulins, IgA, IgM and IgG and the IgG subclasses IgG 1, IgG2, IgG3 and IgG4, compared with normal controls. RESULTS: We found significantly increased concentrations of TSP in autistic subjects, which were attributable to increased serum concentrations of albumin and gamma globulin. Serum IgG, IgG2 and IgG4 were also significantly raised. In autism there were significant and positive correlations between social problems and TSP and serum gamma globulin and between withdrawal symptoms and TSP and serum albumin and IgG. CONCLUSIONS: The results suggest that autism is characterized by increased TSP, a unique pattern obtained in serum protein electrophoresis, i.e. increased serum albumin and IgG, and by a specific IgG subclass profile, i.e. increased serum IgG2 and IgG4. The increased serum concentrations of IgGs in autism may point towards an underlying autoimmune disorder and/or an enhanced susceptibility to infections resulting in chronic viral infections, whereas the IgG subclass skewing may reflect different cytokine-dependent influences on autoimmune B cells and their products.

PMID: 12455944 [PubMed - indexed for MEDLINE]

Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, Styles P, Vincent A. Maternal neuronal antibodies associated with autism and a language disorder. Ann Neurol. 2003 Apr;53(4):533-7.

Neurosciences Group, Department of Clinical Neurology, University of Oxford, Oxford, United Kingdom.

Neurodevelopmental disorders could be caused by maternal antibodies or other serum factors. We detected serum antibodies binding to rodent Purkinje cells and other neurons in a mother of three children: the first normal, the second with autism, and the third with a severe specific language disorder. We injected the serum (0.5-1.0 ml/day) into pregnant mice during gestation and found altered exploration and motor coordination and changes in cerebellar magnetic resonance spectroscopy in the mouse offspring, comparing with offspring of mice injected with sera from mothers of healthy children. This evidence supports a role for maternal antibodies in some forms of neurodevelopmental disorder.

PMID: 12666123 [PubMed - indexed for MEDLINE]

DeLong GR, Bean SC, Brown FR 3rd. Acquired reversible autistic syndrome in acute encephalopathic illness in children. Arch Neurol. 1981 Mar;38(3):191-4.

In seeking the neurologic substrate of the autistic syndrome of childhood, previous studies have implicated the medial temporal lobe or the ring of mesolimbic cortex located in the mesial frontal and temporal lobes. During an acute encephalopathic illness, a clinical picture developed in three children that was consistent with infantile autism. This development was reversible. It was differentiated from acquired epileptic aphasia, and the language disorder was differentiated aphasia. One child has rises in serum herpes simplex titers, and a computerized tomographic (CT) scan revealed an extensive lesion of the temporal lobes, predominantly on the left. The other two, with similar clinical syndromes, had normal CT scans, and no etiologic agent was defined. These cases are examples of an acquired and reversible autistic syndrome in childhood, emphasizing the clinical similarities to bilateral medial temporal lobe disease as described in man, including the Klüver-Bucy syndrome seen in postencephalitic as well as postsurgical states.

PMID: 6162440 [PubMed - indexed for MEDLINE]

Denney DR, Frei BW, Gaffney GR. Lymphocyte subsets and interleukin-2 receptors in autistic children. J Autism Dev Disord. 1996 Feb;26(1):87-97.

Department of Psychology, University of Kansas, Lawrence 66045, USA.

Blood samples were obtained from 10 male autistic children ages 7-15 years and 10 age-matched, male, healthy controls. Lymphocyte subsets (helper-inducer, suppressor-cytotoxic, total T, and total B cells) were enumerated using monoclonal antibodies and flow cytometry. Bound and soluble interleukin-2 receptors were assayed in unstimulated blood samples and in cell cultures following 72-hour stimulation with phytohemagglutinin. The children with autism had a lower percentage of helper-inducer cells and a lower helper:suppressor ratio, with both measures inversely related to the severity of autistic symptoms (r = - .56 and - .68, respectively). A lower percentage of lymphocytes expressing bound interleukin-2 receptors following mitogenic stimulation was also noted, and this too was inversely related to the severity of autistic symptoms.

PMID: 8819772 [PubMed - indexed for MEDLINE]

Engstrom HA, Ohlson S, Stubbs EG, Maciulis A, Caldwell V, Odell JD, Torres A.R. Decreased Expression of CD95 (FAS/APO-1) on CD4+ T-lymphocytes from Participants with Autism. J Dev Phys Disabil. 2003 Jun 15;2:155-163(9).

Fallon J. Could one of the most widely prescribed antibiotics amoxicillin/ clavulanate "augmentin" be a risk factor for autism? Med Hypotheses. 2005;64(2):312-5.

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Autism is an ever increasing problem in the United States. Characterized by multiple deficits in the areas of communication, development, and behavior; autistic children are found in every community in this country and abroad. Recent findings point to a significant increase in autism which can not be accounted for by means such as misclassification. The state of California recently reported a 273% increase in the number of cases between 1987 and 1998. Many possible causes have been proposed which range from genetics to environment, with a combination of the two most likely. Since the introduction of clavulanate/amoxicillin in the 1980s there has been the increase in numbers of cases of autism. In this study 206 children under the age of three years with autism were screened by means of a detailed case history. A significant commonality was discerned and that being the level of chronic otitis media. These children were found to have a mean number 9.96 bouts of otitis media (with a standard error of the mean of +/-1.83). This represents a sum total for all 206 children of 2052 bouts of otitis media. These children received a mean number of 12.04 courses of antibiotics (standard error of the mean of +/-.125). The sum total number of courses of antibiotics given to all 206 children was 2480. Of those 893 courses were Augmentin. with 362 of these Augmentin courses administered under the age of one year. A proposed mechanism whereby the production of clavulanate may yield high levels of urea/ammonia in the child is presented. Further an examination of this mechanism needs to be undertaken to determine if a subset of children are at risk for neurotoxicity from the use of clavulanic acid in pharmaceutical preparations.

PMID: 15607562 [PubMed - indexed for MEDLINE]

Ferrante P, Saresella M, Guerini FR, Marzorati M, Musetti MC, Cazzullo AG. Significant association of HLA A2-DR11 with CD4 naive decrease in autistic children. Biomed Pharmacother. 2003 Oct;57(8):372-4.

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Nine autistic children and 37 ethnically homogenous controls were enrolled in the study to assess their human leukocyte antigen (HLA) pattern, and eight healthy children were studied to define their peripheral blood cell subsets. We observed a significant decrease in CD4+ naive and an increase in CD4+ memory T cells in

autistic children. These differences were significantly more pronounced in the autistic children bearing the HLA A2 and DR11 alleles. These data support the hypothesis that autism could be due to an immune imbalance occurring in genetically predisposed children.

PMID: 14568232 [PubMed - indexed for MEDLINE]

Fiumara A, Sciotto A, Barone R, D'Asero G, Munda S, Parano E, Pavone L. Peripheral lymphocyte subsets and other immune aspects in Rett syndrome. Pediatr Neurol. 1999 Sep;21(3):619-21.

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A possible role of the immune system in the pathogenesis of some neurologic disorders, including infantile autism, was recently postulated. This observation prompted the authors to investigate some immunologic aspects in a group of patients with Rett syndrome, a disorder still not completely clarified but with some points of commonality with infantile autism. Humoral and cell-mediated immunity were investigated in 20 females with Rett syndrome. Peripheral lymphocyte subsets revealed a reduced percentage of CD8+ suppressor-cytotoxic cells in all of the patients with Rett syndrome, resulting in an increased CD4+/CD8+ ratio. In addition, 15 (75%) of the 20 patients had low levels of natural killer cells. Soluble interleukin-2 receptor was elevated in the youngest patients. Antineuronal and antimyelin ganglioside antibodies were absent, as were antinuclear antibodies, antistriated muscle antibodies, and antismooth muscle antibodies. Immunoglobulin fractions and complement were normal for age in all of the patients.

PMID: 10513687 [PubMed - indexed for MEDLINE]

Furlano RI, Anthony A, Day R, Brown A, McGavery L, Thomson MA, Davies SE, Berelowitz M, Forbes van Gent T, Heijnen CJ, Treffers PD. Autism and the immune system. J Child Psychol Psychiatry. 1997 Mar;38(3):337-49.

Gupta S, Aggarwal S, Rashanravan B, Lee T. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol. 1998 May 1;85(1):106-9.

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Th1-like (IL-2, IFN-gamma) and Th2-like (IL-4, IL-6, and IL-10) cytokines were examined in CD4+ and CD8+ T cells in children with autism. Intracellular cytokines were measured using specific antibodies to various cytokines and anti-CD4 or anti-CD8 monoclonal antibodies by FACScan. Proportions of IFN-gamma+CD4+ T cells and IL-2+CD4+ T cells (Th1), and IFN-gamma+CD8+ and IL-2+CD8+ T cells (TC1) were significantly lower in autistic children as compared to healthy controls. In contrast, IL-4+CD4+ T cells (Th2) and IL-4+CD8+ T cells (TC2) were significantly increased in autism. The proportions of IL-6+ CD4+, IL-6+CD8+ and IL-10+CD4+, IL-10+CD8+ T cells were comparable in autism and control group. These data suggest that an imbalance of Th1- and Th2-like cytokines in autism may play a role in the pathogenesis of autism.

PMID: 9627004 [PubMed - indexed for MEDLINE]

Gurney JG, McPheeters ML, Davis MM. Parental report of health conditions and health care use among children with and without autism: National Survey of Children's Health. Arch Pediatr Adolesc Med. 2006 Aug;160(8):825-30.

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OBJECTIVE: To compare parent-reported prevalence of health conditions and health care use between children with and without autism. DESIGN: Cross-sectional analysis of the 2003 to 2004 National Survey of Children's Health. SETTING: Population-based sample across the United States. PARTICIPANTS: More than 100 000 parents. The main exposure was "autism" (not further defined), from response to the question: "Has a doctor or health professional ever told you that your child has autism?" MAIN OUTCOME MEASURES: Medical and mental health conditions and measures of health care use. RESULTS: Autism prevalence among children aged 3 to 17 years was 53 per 10 000 (95% confidence interval, 45-61 per 10,000), equating to a national estimate of 324 000 children (95% confidence interval, 274,000-375,000 children). Children with autism had a significantly (P<.001) higher prevalence of depression or anxiety problems (38.9% vs 4.2%) and behavioral or conduct problems (58.9% vs 5.2%) than children without autism. Respiratory, food, and skin allergies were reported by parents more often for children with autism, with food allergies having the strongest relative difference between the groups (odds ratio, 4.5; 95% confidence interval, 3.0-7.0). Children with autism had significantly (P<.001) higher mean physician visits over 12 months for preventive care, nonemergency care, and hospital emergency care, and were far more likely than children without autism to receive physical, occupational, or speech therapy (76.0% vs 6.3%), to need treatment or counseling for an emotional,

developmental, or behavioral problem (75.4% vs 7.0%), and, among those taking a prescribed medication, to be using a medication long-term (51.4% vs 14.5%). CONCLUSION: We found markedly higher reports of concurrent conditions and health care use associated with childhood autism in this study.

PMID: 16894082 [PubMed - indexed for MEDLINE]

James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr. 2004 Dec;80(6):1611-7.

Department of Pediatrics, University of Arkansas for Medical Sciences, and the Arkansas Children's Hospital Research Institute, Little Rock, AR 72202, USA. jamesjill@uams.edu

BACKGROUND: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism. OBJECTIVE: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism. DESIGN: Plasma concentrations of methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children. RESULTS: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children. CONCLUSIONS: An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism.

PMID: 15585776 [PubMed - indexed for MEDLINE]

Jyonouchi H, Geng L, Ruby A, Reddy C, Zimmerman-Bier B. Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders. J Pediatr. 2005 May;146(5):605-10.

Department of Pediatrics, Division of Pulmonary, Allergy/Immunology, and Infectious Diseases, New Jersey Medical School/UMDNJ, 185 South Orange Avenue, Newark, NJ 07101-1709, USA. jyanouha@umdnj.edu

OBJECTIVE: To evaluate an association between cytokine production with common dietary proteins as a marker of non-allergic food hypersensitivity (NFH) and gastrointestinal (GI) symptoms in young children with autism spectrum disorders (ASD). STUDY DESIGN: Peripheral blood mononuclear cells (PBMCs) were obtained from 109 ASD children with or without GI symptoms (GI [+] ASD, N = 75 and GI (-) ASD, N = 34], from children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. We measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMCs stimulated with whole cow's milk protein (CMP), its major components (casein, beta-lactoglobulin, and alpha-lactoalbumin), gliadin, and soy. RESULTS: PBMCs obtained from GI (+) ASD children produced more tumor necrosis factor-alpha (TNF-alpha)/interleukin-12 (IL-12) than those obtained from control subjects with CMP, beta-lactoglobulin, and alpha-lactoalbumin, irrespective of objective GI symptoms. They also produced more TNF-alpha with gliadin, which was more frequently observed in the group with loose stools. PBMCs obtained from GI (-) ASD children produced more TNF-alpha/IL-12 with CMP than those from control subjects, but not with beta-lactoglobulin, alpha-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable. CONCLUSION: A high prevalence of elevated TNF-alpha/IL-12 production by GI (+) ASD PBMCs with CMP and its major components indicates a role of NFH in GI symptoms observed in children with ASD.

PMID: 15870662 [PubMed - indexed for MEDLINE]

Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J Neuroimmunol. 2001 Nov 1;120(1-2):170-9.

Department of Pediatrics, University of Minnesota, MMC 610 FUMC, 420 Delaware Street SE, Minneapolis, MN 55455, USA. jyono001@tc.umn.edu

We determined innate and adaptive immune responses in children with developmental regression and autism spectrum disorders (ASD, N=71), developmentally normal siblings (N=23), and controls (N=17). With lipopolysaccharide (LPS), a stimulant for innate immunity, peripheral blood mononuclear cells (PBMCs) from 59/71 (83.1%) ASD patients produced >2 SD above the control mean (CM) values of TNF-alpha, IL-1beta, and/or IL-6 produced by control PBMCs. ASD PBMCs produced higher levels of proinflammatory/counter-regulatory cytokines without stimuli than controls. With stimulants of phytohemagglutinin (PHA), tetanus, IL-12p70, and IL-18, PBMCs from 47.9% to 60% of ASD patients produced >2 SD above the CM values of TNF-alpha depending on stimulants. Our results indicate excessive innate immune responses in a number of ASD children that may be most evident in TNF-alpha production.

PMID: 11694332 [PubMed - indexed for MEDLINE]

Jyonouchi H, Sun S, Itokazu N. Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. Neuropsychobiology. 2002;46(2):76-84.

Department of Pediatrics, University of Minnesota, Minneapolis, Minn, USA.

OBJECTIVES: Children with autism spectrum disorder (ASD) frequently reveal various gastrointestinal (GI) symptoms that may resolve with an elimination diet along with apparent improvement of some of the behavioral symptoms. Evidence suggests that ASD may be accompanied by aberrant (inflammatory) innate immune responses. This may predispose ASD children to sensitization to common dietary proteins (DP), leading to GI inflammation and aggravation of some behavioral symptoms. METHODS: We measured IFN-gamma, IL-5, and TNF-alpha production against representative DPs [gliadin, cow's milk protein (CMP), and soy] by peripheral blood mononuclear cells (PBMCs) from ASD and control children [those with DP intolerance (DPI), ASD siblings, and healthy unrelated children]. We evaluated the results in association with proinflammatory and counter-regulatory cytokine production with endotoxin (LPS), a microbial product of intestinal flora and a surrogate stimulant for innate immune responses. RESULTS: ASD PBMCs produced elevated IFN-gamma and TNF-alpha, but not IL-5 with common DPs at high frequency as observed in DPI PBMCs. ASD PBMCs revealed increased proinflammatory cytokine responses with LPS at high frequency with positive correlation between proinflammatory cytokine production with LPS and IFN-gamma and TNF-alpha production against DPs. Such correlation was less evident in DPI PBMCs. CONCLUSION: Immune reactivity to DPs may be associated with apparent DPI and GI inflammation in ASD children

that may be partly associated with aberrant innate immune response against endotoxin, a product of the gut bacteria. Copyright 2002 S. Karger AG, Basel

PMID: 12378124 [PubMed - indexed for MEDLINE]

Jyonouchi H, Geng L, Ruby A, Zimmerman-Bier B. Dysregulated innate immune responses in young children with autism spectrum disorders: their relationship to gastrointestinal symptoms and dietary intervention. Neuropsychobiology. 2005;51(2):77-85.

Konstantareas MM, Homatidis S. Ear infections in autistic and normal children. J Autism Dev Disord. 1987 Dec;17(4):585-94.

Clarke Institute of Psychiatry, Toronto, Ontario, Canada.

The frequency of ear infections, ear tube drainage, and deafness was examined through parental reports in autistic and yoke-matched, normal children. For the autistic group these difficulties were additionally examined as a function of the children's cognitive and communication abilities, verbal versus nonverbal status, sex, and degree of autistic symptomatology. Autistic children had a greater incidence of ear infections than matched normal peers. Lower-functioning children had an earlier onset of ear infections than their higher-functioning autistic peers. Ear infections coexisted with low-set ears, and with a higher autistic symptomatology score. The findings are discussed in terms of greater CNS vulnerability in the autistic children, which is likely present since embryogenesis. The possible adverse consequences of intermittent hearing loss on language, cognitive, and socioaffective development are considered.

PMID: 3680158 [PubMed - indexed for MEDLINE]

Korvatska E, Van de Water J, Anders TF, Gershwin ME. Genetic and immunologic considerations in autism. Neurobiol Dis. 2002 Mar;9(2):107-25.

Division of Rheumatology, Allergy, and Clinical Immunology, University of California at Davis, Davis, California 95616, USA.

According to recent epidemiological surveys, autistic spectrum disorders have become recognized as common childhood psychopathologies. These life-lasting conditions demonstrate a strong genetic determinant consistent with a polygenic mode of inheritance for which several autism susceptibility regions have been

identified. Parallel evidence of immune abnormalities in autistic patients argues for an implication of the immune system in pathogenesis. This review summarizes advances in the molecular genetics of autism, as well as recently emerging concerns addressing the disease incidence and triggering factors. The neurochemical and immunologic findings are analyzed in the context of a neuroimmune hypothesis for autism. Studies of disorders with established neuroimmune nature indicate multiple pathways of the pathogenesis; herein, we discuss evidence of similar phenomena in autism. (c)2002 Elsevier Science (USA).

PMID: 11895365 [PubMed - indexed for MEDLINE]

Krause I, He XS, Gershwin ME, Shoenfeld Y. Brief report: immune factors in autism: a critical review. J Autism Dev Disord. 2002 Aug;32(4):337-45.

Research Unit of Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, and the Sackler Faculty of Medicine, Tel-Aviv University, Israel.

Pervasive developmental disorders represent a group of neurodevelopmental disorders that affect children early in their development. Autistic disorder is the best described of these disorders, yet even this term covers a broad group of clinical presentations. Various immune system abnormalities, including autoimmunity and defects in different subsets of immune cells, have been reported in children with autistic disorder, suggesting that immune factors may play a role in the development of autism. Based on anecdotal observation, vaccination was proposed to cause autism in some children, but several controlled studies have failed to support this claim. Intravenous immunoglobulin infusions has been tested as immunotherapy for autism, although the preliminary results are inconclusive and there is a risk of potentially fatal transmission of blood-borne pathogens. To examine this issue, intensive well-controlled epidemiological and bench studies need to be carried out in defined and carefully controlled study subjects to establish the cellular and molecular basis of autism, against which the effects of each proposed immune factor can be examined.

PMID: 12199139 [PubMed - indexed for MEDLINE]

Libbey JE, Sweeten TL, McMahon WM, Fujinami RS. Autistic disorder and viral infections. J Neurovirol. 2005 Feb;11(1):1-10.

Department of Neurology, University of Utah, Salt Lake City, Utah 84132-2305, USA.

Autistic disorder (autism) is a behaviorally defined developmental disorder with a wide range of behaviors. Although the etiology of autism is unknown, data suggest that autism results from multiple etiologies with both genetic and environmental contributions, which may explain the spectrum of behaviors seen in this disorder. One proposed etiology for autism is viral infection very early in development. The mechanism, by which viral infection may lead to autism, be it through direct infection of the central nervous system (CNS), through infection elsewhere in the body acting as a trigger for disease in the CNS, through alteration of the immune response of the mother or offspring, or through a combination of these, is not yet known. Animal models in which early viral infection results in behavioral changes later in life include the influenza virus model in pregnant mice and the Borna disease virus model in newborn Lewis rats. Many studies over the years have presented evidence both for and against the association of autism with various viral infections. The best association to date has been made between congenital rubella and autism; however, members of the herpes virus family may also have a role in autism. Recently, controversy has arisen as to the involvement of measles virus and/or the measles, mumps, rubella (MMR) vaccine in the development of autism. Biological assays lend support to the association between measles virus or MMR and autism whereas epidemiologic studies show no association between MMR and autism. Further research is needed to clarify both the mechanisms whereby viral infection early in development may lead to autism and the possible involvement of the MMR vaccine in the development of autism.

PMID: 15804954 [PubMed - indexed for MEDLINE]

Lopez-Pison J, Rubio-Rubio R, Urena-Hornos T, Omenaca-Teres M, Sans A, Cabrerizo de Diago R, Pena-Segura JL. Retrospective diagnosis of congenital infection by cytomegalovirus in the case of one infant.

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INTRODUCTION: 10-15% of asymptomatic congenital infections by cytomegalovirus (CMV) in the neonatal period develop persistent problems with varying degrees of severity, fundamentally involving neurological disorders, neurosensory hypoacusis and hypovision, which appear from the age of 6-9 months onwards, when a diagnosis is no longer possible. The PCR (polymerase chain reaction) technique can detect DNA of CMV in blood samples on filter

paper used for screening hypothyroidism and metabolic pathologies that were kept from the neonatal period. CASE REPORT: A child aged 3 years and 8 months with delayed intrauterine growth, autism, mental retardation, microcephalus and neurosensory hypoacusis; periventricular calcifications, leukoencephalopathy and bilateral malformation of the temporal lobe; and a diagnosis of congenital CMV confirmed by detection of DNA by PCR in the blood sample on filter paper saved from the neonatal period. CONCLUSIONS: The retrospective study of congenital infection by CMV should be considered when faced with severity and varying association of delayed intrauterine growth, microcephalus, neurosensory hypoacusis, chorioretinitis, mental retardation, autism or other behavioural disorders, intracranial calcifications, encephaloclastic alterations, leukoencephalopathy, cortical dysplasia and malformations of the temporal lobe and the hippocampus. Since the filter papers from neonatal screening are not kept for ever, perhaps the idea of doing so ought to be considered, given the possibilities they offer for retrospective studies.

PMID: 15973639 [PubMed - indexed for MEDLINE]

Lucarelli S, Frediani T, Zingoni AM, Ferruzzi F, Giardini O, Quintieri F, Barbato M, D'Eufemia P, Cardi E. Food allergy and infantile autism. Panminerva Med. 1995 Sep;37(3):137-41.

Department of Paediatrics, University of Rome La Sapienza, Italy.

The etiopathogenesis of infantile autism is still unknown. Recently some authors have suggested that food peptides might be able to determine toxic effects at the level of the central nervous system by interacting with neurotransmitters. In fact a worsening of neurological symptoms has been reported in autistic patients after the consumption of milk and wheat. The aim of the present study has been to verify the efficacy of a cow's milk free diet (or other foods which gave a positive result after a skin test) in 36 autistic patients. We also looked for immunological signs of food allergy in autistic patients on a free choice diet. We noticed a marked improvement in the behavioural symptoms of patients after a period of 8 weeks on an elimination diet and we found high levels of IgA antigen specific antibodies for casein, lactalbumin and beta-lactoglobulin and IgG and IgM for casein. The levels of these antibodies were significantly higher than those of a control group which consisted of 20 healthy children. Our results lead us to hypothesise a relationship between food allergy and infantile autism as has already been suggested for other disturbances of the central nervous system.

PMID: 8869369 [PubMed - indexed for MEDLINE]

Mehler MF, Kessler JA. Cytokines in brain development and function. Adv Protein Chem. 1998;52:223-51.

Department of Neurology, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Albert Einstein College of Medicine, Bronx, New York 10461, USA.

PMID: 9917922 [PubMed - indexed for MEDLINE]

Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, Yee BK, Feldon J. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J Neurosci. 2006 May 3;26(18):4752-62.

Laboratory of Behavioural Neurobiology, Swiss Federal Institute of Technology Zurich, CH-8603 Schwerzenbach, Switzerland.

Disturbance to early brain development is implicated in several neuropsychiatric disorders including autism, schizophrenia, and mental retardation. Epidemiological studies have indicated that the risk of developing these disorders is enhanced by prenatal maternal infection, presumably as a result of neurodevelopmental defects triggered by cytokine-related inflammatory events. Here, we demonstrate that the effects of maternal immune challenge between middle and late gestation periods in mice are dissociable in terms of fetal brain cytokine responses to maternal inflammation and the pathological consequences in brain and behavior. Specifically, the relative expression of pro- and anti-inflammatory cytokines in the fetal brains in response to maternal immune challenge may be an important determinant among other developmental factors for the precise pathological profile emerging in later life. Thus, the middle and late gestation periods correspond to two windows with differing vulnerability to adult behavioral dysfunction, brain neuropathology in early adolescence, and of the acute cytokine responses in the fetal brain.

PMID: 16672647 [PubMed - indexed for MEDLINE]

Molloy C, Morrow A, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, Altaye M, Wills-Karp M. Elevated cytokine levels in children with autism spectrum disorder. J Neuroimmunology. 2006;172:198-205.

Center for Epidemiology and Biostatistics, Cincinnati Children's Hospital Medical Center, and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229-3039, United States. cynthia.molloy@cchmc.org

This study compared production of IL-2, IFN-gamma, IL-4, IL-13, IL-5 and IL-10 in peripheral blood mononuclear cells from 20 children with autism spectrum disorder to those from matched controls. Levels of all Th2 cytokines were significantly higher in cases after incubation in media alone, but the IFN-gamma/IL-13 ratio was not significantly different between cases and controls. Cases had significantly higher IL-13/IL-10 and IFN-gamma/IL-10 than controls. Conclusion: Children with ASD had increased activation of both Th2 and Th1 arms of the adaptive immune response, with a Th2 predominance, and without the compensatory increase in the regulatory cytokine IL-10.

PMID: 16360218 [PubMed - indexed for MEDLINE]

Murch SH, Walker-Smith JA. Nutrition in inflammatory bowel disease. Baillieres Clin Gastroenterol. 1998 Dec;12(4):719-38.

University Department of Paediatric Gastroenterology, Royal Free Hospital, London, UK.

Nutrition is clearly disturbed by active intestinal inflammation. Appetite is reduced, yet energy substrates are diverted into the inflammatory process, and thus weight loss is characteristic. The nutritional disturbance represents part of a profound defect of somatic function. Linear growth and pubertal development in children are notably retarded, body composition is altered, and there may be significant psychosocial disturbance. Macrophage products such as tumour necrosis factor-alpha and interleukins-1 and 6 may be the central molecules that link the inflammatory process to this derangement of homeostasis. Intriguingly, there is also increasing evidence that an aggressive nutritional programme may in itself be sufficient to reduce the mucosal inflammatory response. Recent evidence suggests that enteral nutrition alone may reduce many pro-inflammatory cytokines to normal and allow mucosal healing. In addition, specific nutritional components, such as n-3 polyunsaturated fatty acids, may have an anti-inflammatory effect as they may alter the pattern of leukotrienes generated during the immune response. The recent discovery of the specific molecular mediators of appetite and body composition, such as leptin and myostatin, may allow increased therapeutic specificity and further improvement in the nutritional treatment of the inflammatory bowel diseases.

PMID: 10079904 [PubMed - indexed for MEDLINE]

Niehus R, Lord C. Early medical history of children with autism spectrum disorders. J Dev Behav Pediatr. 2006 Apr;27(2 Suppl):S120-7.

University of Michigan Autism and Communication Disorders Center University of Michigan, Ann Arbor, Michigan 48109, USA.

Previous studies have suggested that children with autism spectrum disorders (ASD) may have different medical histories than nonspectrum children in several areas: their reactions to vaccinations, number of ear infections, chronic gastrointestinal problems, and use of antibiotics. Furthermore, some studies have found associations between regressive autism and gastrointestinal (GI) symptoms. The present study analyzes the medical records from birth to the age of 2 years of 99 children (24 typically developing; 75 with ASD, of whom 29 had parent-reported regression). Data were coded in the following areas: frequency and purpose of pediatrician visits, frequency and type of illnesses and medications, type and chronicity of GI complaints, date of vaccinations, growth data, and whether the pediatrician noted behaviors indicative of an ASD before the age of 2 years. Children with ASD were found to have significantly more ear infections than the typically developing children as well as to use significantly more antibiotics. Typically developing children had significantly more illness-related fevers. There was a nonsignificant trend toward the ASD group having more chronic gastrointestinal problems. There were no significant differences between the groups for the age of vaccination or for number of pediatrician visits. Finally, pediatricians noted symptoms of onset of possible autism, including language delay, for 44 of the 75 children with ASD and 2 of the 24 typical children. Results are discussed in terms of needs for future research.

PMID: 16685178 [PubMed - indexed for MEDLINE]

Okada K, Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, Suda S, Suzuki K, Sugihara GI, Matsuzaki H, Sugiyama T, Kawai M, Minabe Y Takei N, Mori N. Decreased serum levels of transforming growth factor-beta1 in patients with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2006 Oct 5.

Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka 431-3192, Japan.

BACKGROUND: The neurobiological basis for autism remains poorly understood. Given the key role of transforming growth factor-beta1 (TGF-beta1) in brain development, we hypothesized that TGF-beta1 plays a role in the

pathophysiology of autism. In this study, we studied whether serum levels of TGF-beta1 are altered in patients with autism. METHODS: We measured serum levels of TGF-beta1 in 19 male adult patients with autism and 21 age-matched male healthy subjects using enzyme-linked immunosorbent assay (ELISA). RESULTS: The serum levels (7.34+/-5.21 ng/mL (mean+/-S.D.)) of TGF-beta1 in the patients with autism were significantly ($z=-5.106$, $p<0.001$) lower than those (14.48+/-1.64 ng/mL (mean+/-S.D.)) of normal controls. However, there were no marked or significant correlations between serum TGF-beta1 levels and other clinical variables, including Autism Diagnostic Interview-Revised (ADI-R) scores, Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), aggression, Theory of Mind, and Intellectual Quotient (IQ) in patients. CONCLUSIONS: These findings suggest that decreased levels of TGF-beta1 may be implicated in the pathophysiology of autism.

PMID: 17030376 [PubMed - indexed for MEDLINE]

Pardo CA, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. Int Rev Psychiatry. 2005 Dec;17(6):485-95.

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Autism is a complex neurodevelopmental disorder of early onset that is highly variable in its clinical presentation. Although the causes of autism in most patients remain unknown, several lines of research support the view that both genetic and environmental factors influence the development of abnormal cortical circuitry that underlies autistic cognitive processes and behaviors. The role of the immune system in the development of autism is controversial. Several studies showing peripheral immune abnormalities support immune hypotheses, however until recently there have been no immune findings in the CNS. We recently demonstrated the presence of neuroglial and innate neuroimmune system activation in brain tissue and cerebrospinal fluid of patients with autism, findings that support the view that neuroimmune abnormalities occur in the brain of autistic patients and may contribute to the diversity of the autistic phenotypes. The role of neuroglial activation and neuroinflammation are still uncertain but could be critical in maintaining, if not also in initiating, some of the CNS abnormalities present in autism. A better understanding of the role of neuroinflammation in the pathogenesis of autism may have important clinical and therapeutic implications.

PMID: 16401547 [PubMed - indexed for MEDLINE]

Pletnikov MV, Jones ML, Rubin SA, Moran TH, Carbone KM. Rat model of autism spectrum disorders. Genetic background effects on Borna disease virus-induced developmental brain damage. Ann N Y Acad Sci. 2001 Jun;939:318-9.

Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

PMID: 11462786 [PubMed - indexed for MEDLINE]

Plioplys AV. Autism: electroencephalogram abnormalities and clinical improvement with valproic acid. Arch Pediatr Adolesc Med. 1994 Feb;148(2):220-2.

Plioplys AV.

Division of Neurology, Mercy Hospital and Medical Center, Chicago, IL 60616.

PMID: 8118547 [PubMed - indexed for MEDLINE]

Plioplys AV, Greaves A. Yoshida W. Anti-CNS antibodies in childhood neurologic diseases. Neuropediatrics. 1989;20:93.

Department of Pediatrics, Hospital for Sick Children, University of Toronto, Ontario, Canada.

To study the incidence of circulating anti-CNS antibodies in childhood neurologic diseases, a population study was undertaken. Serum samples were obtained from a total of 348 children and stored at -80 degrees C until being studied. The samples were collected when routine blood tests were being performed. In all cases informed consent was obtained. This study was approved by hospital ethics review committees. One hundred and ninety-nine of the samples were from children with no known neurologic illnesses and served as the control group. One hundred and twenty-one of the samples were from children with epilepsy and the remaining 28 from a number of different neurologic conditions. The serum samples were screened against normal, adult, autopsy-derived cerebellar and frontal cortex tissue sections and Western blots. Serum immunoreactivity was revealed using HRP-conjugated anti-human IgG. Significant findings included: (1) patients with epilepsy had an increased incidence of anti-CNS reactivity as revealed on frontal cortex immunoblots (p less

than 0.05) but not on cerebellar immunoblots; (2) there was an increase in the incidence of immunoblot reactivity with age in the controls and the neurology cases; (3) there was an increased incidence of immunoblot reactivity in those cases with a presumed inflammatory central or peripheral neurologic disease; (4) in six additional cases with opsoclonus-myoclonus there was cerebellar-specific immunoreactivity with identified antigenic molecular weights of 27 and 35, and 62 kDaltons; (5) in 31 additional cases of systemic lupus erythematosus there was significant immunoblot reactivity (p less than 0.001) when compared to a subset of age-matched controls. There was no difference in immunoreactivity between males and females. There was no significant increase in immunoreactivity in those children with cognitive disturbances including developmental delay and mental retardation.

PMID: 2739881 [PubMed - indexed for MEDLINE]

Rumsey JM, Ernst M. Functional neuroimaging of autistic disorders. Ment Retard Dev Disabil Res Rev. 2000;6(3):171-9.

Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland 20892, USA. jrumsey@box-jonih.gov

Functional neuroimaging methods hold promise for elucidating the neurobiology of autistic disorders, yet they present difficult practical and scientific challenges when applied to these complex and heterogeneous syndromes. Single-state studies of brain metabolism and blood flow thus far have failed to yield consistent findings, but suggest considerable variability in regional patterns of cerebral synaptic activity. Patients with idiopathic autism are less likely to show abnormalities than are patients with comorbid illness or epilepsy. Activation studies have begun to suggest alterations in brain organization for language and cognition. Neurotransmitter studies using positron emission tomography (PET) suggest abnormalities of serotonergic and dopaminergic function. Studies using magnetic resonance spectroscopy (MRS) have begun to document metabolic deficits in the frontal cortex and cerebellum. A single study using magnetoencephalography suggests a high incidence of epileptiform activity in children with autistic regression. Research needs include well-controlled developmental studies, particularly of young subjects and relatively homogeneous subgroups, which balance scientific rigor with ethical constraints. Investigations of the serotonergic and dopaminergic systems, limbic-based memory and emotional systems, and the role of epileptiform activity in autism represent priorities for future research.

PMID: 10982494 [PubMed - indexed for MEDLINE]

Silva SC, Correia C, Fesel C, Barreto M, Coutinho AM, Marques C, Miguel TS, Ataide A, Bento C, Borges L, Oliveira G, Vicente AM. Autoantibody repertoires to brain tissue in autism nuclear families. J Neuroimmunol. 2004 Jul;152(1-2):176-82.

Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2781-196 Oeiras, Portugal.

The hypothesis of an immune dysfunction in autism spectrum disorders has previously been put forward without, however, compelling evidence of a direct relation to its etiology or pathogenesis. To further understand if autoimmunity could play a significant role in autism, we analyzed autoantibody repertoires to brain tissue extract in the plasma of 171 autism children, their parents, and 54 controls, by quantitative immunoblotting. Multiparametric analysis revealed significant differences between patients and controls, and showed that one single reactivity in Section 32 of the blot had the most power to discriminate between these samples. Family correlation coefficients and heritability estimates did not provide any evidence that this reactivity was genetically determined. While the molecular weight of the target protein suggested that it might be an isoform of Myelin Basic Protein (MBP), inhibition assays with human MBP argued against this hypothesis. The study evidences the widespread occurrence of autoreactivities to brain tissue in autism patients, which may represent the immune system's neuroprotective response to a previous brain injury occurred during neurodevelopment. The molecular identification of the target protein in Section 32 will contribute to the understanding of the role of immune responses against brain antigens in autistic patients.

PMID: 15223250 [PubMed - indexed for MEDLINE]

Singer HS, Morris CM, Williams PN, Yoon DY, Hong JJ, Zimmerman AW. Antibrain antibodies in children with autism and their unaffected siblings. J Neuroimmunol. 2006 Sep;178(1-2):149-155.

Department of Neurology, Johns Hopkins University School of Medicine, Jefferson Street Building 124, 600 N. Wolfe Street, Baltimore, MD 21287-1000, USA. hsinger@jhmi.edu

Serum autoantibodies to human brain, identified by ELISA and Western immunoblotting, were evaluated in 29 children with autism spectrum disorder (22 with autistic disorder), 9 non-autistic siblings and 13 controls. More autistic subjects than controls had bands at 100 kDa in caudate, putamen and prefrontal

cortex ($p<0.01$) as well as larger peak heights of bands at 73 kDa in the cerebellum and cingulate gyrus. Both autistic disorder subjects and their matched non-autistic siblings had denser bands (peak height and/or area under the curve) at 73 kDa in the cerebellum and cingulate gyrus than did controls ($p<0.01$). Results suggest that children with autistic disorder and their siblings exhibit differences compared to controls in autoimmune reactivity to specific epitopes located in distinct brain regions.

PMID: 16842863 [PubMed - indexed for MEDLINE]

Singh VK. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. J Neuroimmunol. 1996 May;66(1-2):143-5.

Department of Psychiatry, University of Michigan, School of Medicine, Ann Arbor 48109-0656, USA.

Immune factors such as autoimmunity have been implicated in the genesis of autism, a neurodevelopmental disorder. Since autoimmune response involves immune activation, the plasma levels of interferon-alpha (IFN-alpha), interferon-gamma (IFN-gamma), interleukin-12 (IL-12), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and soluble intercellular adhesion molecule-1 (sICAM-1) were measured in autistic patients and age-matched normal controls. The levels of IL-12 and IFN-gamma were significantly ($P < \text{or} = 0.05$) higher in patients as compared to controls. However, IFN-alpha, IL-6, TNF-alpha, and sICAM-1 levels did not significantly differ between the two groups. Because macrophage-derived IL-12 is known to selectively induce IFN-gamma in T helper type-1 (Th-1) cells, it is suggested that IL-12 and IFN-gamma increases may indicate antigenic stimulation of Th-1 cells pathogenetically linked to autoimmunity in autism.

PMID: 8964908 [PubMed - indexed for MEDLINE]

Singh VK. Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol. 1998 May 1;85(1):106-9.

Department of Medicine, University of California, Irvine 92697-4075, USA. sgupta@uci.edu

Th1-like (IL-2, IFN-gamma) and Th2-like (IL-4, IL-6, and IL-10) cytokines were examined in CD4+ and CD8+ T cells in children with autism. Intracellular

cytokines were measured using specific antibodies to various cytokines and anti-CD4 or anti-CD8 monoclonal antibodies by FACScan. Proportions of IFN-gamma+CD4+ T cells and IL-2+CD4+ T cells (Th1), and IFN-gamma+CD8+ and IL-2+CD8+ T cells (TC1) were significantly lower in autistic children as compared to healthy controls. In contrast, IL-4+CD4+ T cells (Th2) and IL-4+CD8+ T cells (TC2) were significantly increased in autism. The proportions of IL-6+ CD4+, IL-6+CD8+ and IL-10+CD4+, IL-10+CD8+ T cells were comparable in autism and control group. These data suggest that an imbalance of Th1- and Th2-like cytokines in autism may play a role in the pathogenesis of autism.

PMID: 9627004 [PubMed - indexed for MEDLINE]

Singh VK, Warren R, Averett R, Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. Pediatr Neurol. 1997 Jul;17(1):88-90.

Department of Psychiatry, University of Michigan, Ann Arbor 48109-1065, USA.

Autoimmunity may be a pathogenic factor in autism, a behavioral disorder of early childhood onset. Circulating autoantibodies are produced in organ-specific autoimmunity; therefore, we investigated them in the plasma of autistic subjects, mentally retarded (MR) subjects, and healthy controls. Autoantibodies (IgG isotype) to neuron-axon filament protein (anti-NAFP) and glial fibrillary acidic protein (anti-GFAP) were analyzed by the Western immunoblotting technique. We found a significant increase in incidence of anti-NAFP (P = .004) and anti-GFAP (P = .002) in autistic subjects, but not in MR subjects. Clinically, these autoantibodies may be related to autoimmune pathology in autism.

PMID: 9308986 [PubMed - indexed for MEDLINE]

Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. Brain Behav Immun. 1993 Mar;7(1):97-103.

Biomedical Division, Center for Persons with Disabilities, Logan, Utah.

Based on a possible pathological relationship of autoimmunity to autism, antibodies reactive with myelin basic protein (anti-MBP) were investigated in the sera of autistic children. Using a screening serum dilution of 1:400 in the protein-immunoblotting technique, approximately 58% (19 of 33) sera of autistic children (< or = 10 years of age) were found to be positive for anti-MBP. This result in autistics was significantly (p < or = .0001) different from the controls (8

of 88 or only 9% positive), which included age-matched children with normal health, idiopathic mental retardation (MR) and Down syndrome (DS), and normal adults of 20 to 40 years of age. Since autism is a syndrome of unknown etiology, it is possible that anti-MBP antibodies are associated with the development of autistic behavior.

PMID: 7682457 [PubMed - indexed for MEDLINE]

Singh VK, Singh EA, Warren RP. Hyperserotoninemia and serotonin receptor antibodies in children with autism but not mental retardation. Biol Psychiatry. 1997 Mar 15;41(6):753-5.

Veterans Administration West Los Angeles Medical Center, California 90073.

This study examined the linkage between elevated blood serotonin in autism and the presence of circulating autoantibodies against the serotonin 5HT1A receptor. Information was also obtained on the diagnostic and receptor specificity of these autoantibodies. Blood serotonin was measured as was inhibition of serotonin binding to human cortical membranes by antibody-rich fractions of blood from controls and from patients with childhood autism, schizophrenia, obsessive-compulsive disorder, Tourette's, and multiple sclerosis. The results showed elevated blood serotonin was not closely related to inhibition of serotonin binding by antibody-rich blood fractions. Inhibition of binding was highest for patients with multiple sclerosis and was not specific to the 5HT1A receptor as currently defined. Although inhibition was not specific to autism, the data were insufficient to establish if people with autism differed from normal controls on this measure.

PMID: 1375597 [PubMed - indexed for MEDLINE]

Singh VK, Rivas WH. Prevalence of serum antibodies to caudate nucleus in autistic children. Neurosci Lett. 2004 Jan 23;355(1-2):53-6.

Department of Biology, Biotechnology Center Building, Utah State University, 4700 Old Main Hill, Logan, UT 84322-4700, USA. singhvk@cc.usu.edu

Autism may involve autoimmunity to brain. We studied regional distribution of antibodies to rat caudate nucleus, cerebral cortex, cerebellum, brain stem and hippocampus. The study included 30 normal and 68 autistic children. Antibodies were assayed by immunoblotting. Autistic children, but not normal children, had antibodies to caudate nucleus (49% positive sera), cerebral cortex (18% positive

sera) and cerebellum (9% positive sera). Brain stem and hippocampus were negative. Antibodies to caudate nucleus were directed towards three proteins having 160, 115 and 49 kD molecular weights. Since a significant number of autistic children had antibodies to caudate nucleus, we propose that an autoimmune reaction to this brain region may cause neurological impairments in autistic children. Thus, the caudate nucleus might be involved in the neurobiology of autism.

PMID: 14729233 [PubMed - indexed for MEDLINE]

Stubbs EG, Crawford ML. Depressed lymphocyte responsiveness in autistic children. J Autism Child Schizophr. 1977 Mar;7(1):49-55.

Although there are associations linking autism with prenatal rubella, cytomegalovirus, syphilis, and varicella, the etiology of the autistic state remains obscure. Host defense against the etiologic agents postulated to be responsible for the autism-associated syndromes is believed to be primarily of the cell-mediated type. In this preliminary study, cellular immune function was assessed in vitro by phytohemagglutinin (PHA) stimulation of lymphocyte cultures. Twelve autistic children and 13 control subjects were compared. The autistic group exhibited a depressed lymphocyte transformation response to PHA when compared to the control subjects (p less than .01).

PMID: 139400 [PubMed - indexed for MEDLINE]

Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics. 2003 Sweeten TL, Posey DJ, McDougle CJ. High blood monocyte counts and neopterin levels in children with autistic disorder. Am J Psychiatry. 2003 Sep;160(9):1691-3.

Department of Psychiatry, Indiana University School of Medicine, and James Whitcomb Riley Hospital for Children Indianapolis 46202-4800, USA.

OBJECTIVES: Increased prevalence of familial autoimmune disease is a common finding among probands with various autoimmune disorders. Autistic disorder (autism) is a highly genetic disorder with known immune and immunogenetic abnormalities. Previous research has found an increased frequency of autoimmune disorders in families with autistic probands. We further investigated this association by determining the frequency of autoimmune disorders in

families that have probands with pervasive developmental disorders (PDDs), including autism, compared with 2 control groups. METHODS: Three well-defined study groups, including 1) families that have a child with a PDD, 2) families that have a child with an autoimmune disorder, and 3) families with a healthy control child, constituted the sample. A questionnaire inquiring about which first- and second-degree family members had received a diagnosis of having specific autoimmune disorders was completed by 101 families in each group. RESULTS: The frequency of autoimmune disorders was significantly higher in families of the PDD probands compared with families of both the autoimmune and healthy control probands. Autoimmunity was highest among the parents of PDD probands compared with parents of the healthy control subjects. Hypothyroidism/Hashimoto's thyroiditis and rheumatic fever were significantly more common in families with PDD probands than in the healthy control families. CONCLUSIONS: Autoimmunity was increased significantly in families with PDD compared with those of healthy and autoimmune control subjects. These preliminary findings warrant additional investigation into immune and autoimmune mechanisms in autism.

PMID: 14595086 [PubMed - indexed for MEDLINE]

Sweeten TL, Posey DJ, McDougle CJ. Brief report: autistic disorder in three children with cytomegalovirus infection. J Autism Dev Disord. 2004 Oct;34(5):583-6.

Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46202-4800, USA.

Previous research has identified a relationship between autistic disorder (autism) and specific congenital infections. Three cases of congenital or perinatal cytomegalovirus (CMV) infection occurring in association with autism are described. Hypothetical mechanisms relating congenital infection, such as CMV, to the development of autism are discussed. A better understanding of the immunologic response to certain congenital infections may provide important information pertaining to the pathophysiology and etiology of autism in vulnerable individuals.

PMID: 15628611 [PubMed - indexed for MEDLINE]

Sweeten TL, Posey DJ, Shankar S, McDougle CJ. High nitric oxide production in autistic disorder: a possible role for interferon-gamma. Bio Psychiatry. 2004 Feb 15:55(4):434-7.

Department of Psychiatry, Indiana University School of Medicine, 1111 W. 10th Street, Indianapolis, IN 46202-4800, USA.

BACKGROUND: Neuroimmune regulation abnormalities have been implicated in the pathophysiology of autistic disorder. Nitric oxide (NO) is involved in immune reactivity and is known to affect brain neurodevelopmental processes. Recent evidence indicates that NO, and cytokines involved in NO production, may be high in children with autism. The purpose of this study was to verify that plasma NO is high in children with autism and determine whether this elevation is related to plasma levels of cytokines involved in NO production. METHODS: The metabolites of NO, nitrite, and nitrate (NOx), along with the cytokines interferon-gamma (IFN-gamma), tumor necrosis factor-alpha, and interleukin-1beta, were measured in plasma of 29 children with autism (mean age +/- SD = 6.1 +/- 2.8 years) and 27 age- and gender-matched healthy comparison subjects using commercially available assay kits. RESULTS: Plasma levels of NOx were significantly higher in the autistic subjects (p =.006); plasma levels of the cytokines did not differ between groups. NOx and IFN-gamma levels were positively correlated in the autistic subjects (r =.51; p =.005). CONCLUSIONS: These results confirm that plasma NO is high in some children with autism and suggest that this elevation may be related to IFN-gamma activity.

PMID: 14960298 [PubMed - indexed for MEDLINE]

Swisher CN, Swisher L. Letter: Congenital rubella and autistic behavior. N Engl J Med. 1975 Jul 24;293(4):198.

Swisher CN, **Swisher L**.

PMID: 1134536 [PubMed - indexed for MEDLINE]

Todd RD, Hickok JM, Anderson GM, Cohen DJ. Antibrain antibodies in infantile autism. Biol Psychiatry. 1988 Mar 15;23(6):644-7.

Todd RD, **Hickok JM**, **Anderson GM**, **Cohen DJ**.

Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

PMID: 3355880 [PubMed - indexed for MEDLINE]

Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A, Davies SE, Wakefield AJ, Thomson MA, Walker-Smith JA, Murch SH. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatry. 2002;7(4):375-82, 334.

Centre for Paediatric Gastroenterology, Royal Free & University College Medical School, London, UK.

We have reported lymphocytic colitis in children with regressive autism, with epithelial damage prominent. We now compare duodenal biopsies in 25 children with regressive autism to 11 with coeliac disease, five with cerebral palsy and mental retardation and 18 histologically normal controls. Immunohistochemistry was performed for lymphocyte and epithelial lineage and functional markers. We determined the density of intraepithelial and lamina propria lymphocyte populations, and studied mucosal immunoglobulin and complement C1q localisation. Standard histopathology showed increased enterocyte and Paneth cell numbers in the autistic children. Immunohistochemistry demonstrated increased lymphocyte infiltration in both epithelium and lamina propria with upregulated crypt cell proliferation, compared to normal and cerebral palsy controls. Intraepithelial lymphocytes and lamina propria plasma cells were lower than in coeliac disease, but lamina propria T cell populations were higher and crypt proliferation similar. Most strikingly, IgG deposition was seen on the basolateral epithelial surface in 23/25 autistic children, co-localising with complement C1q. This was not seen in the other conditions. These findings demonstrate a novel form of enteropathy in autistic children, in which increases in mucosal lymphocyte density and crypt cell proliferation occur with epithelial IgG deposition. The features are suggestive of an autoimmune lesion.

PMID: 11986981 [PubMed - indexed for MEDLINE]

Trajkovski V, Ajdinski L, Spiroski M. Plasma concentration of immunoglobulin classes and subclasses in children with autism in the Republic of Macedonia: retrospective study. Croat Med J. 2004 Dec;45(6):746-9.

Institute of Immunobiology and Human Genetics, Medical Faculty, PO Box 60, 1109 Skopje, Republic of Macedonia.

AIM: To examine plasma concentration of IgA, IgM, IgG classes, and IgG1, IgG2, IgG3, and IgG4 subclasses in children with autism. METHODS: Infantile autism was diagnosed by the Diagnostic and Statistical Manual for Mental Disorders

(DSM)-IV and the International Classification of Diseases (ICD)-10 criteria. Plasma samples were collected from 35 autistic subjects, and their 21 siblings (biological brothers and sisters) who served as healthy controls. Plasma samples were separated by centrifugation and stored at -20 degrees C until the determination. Plasma immunoglobulin classes (IgM, IgA, IgG) and subclasses (IgG1, IgG2, IgG3, IgG4) were determined using a nephelometer. RESULTS: Plasma concentrations (mean+/-standard deviation) of IgM and IgG in autistic children (1.36+/-0.31 g/L and 13.14+/-1.27 g/L, respectively) were significantly higher (p=0.031 and p=0.023, respectively) in comparison with their healthy brothers or sisters (1.20+/-0.15 g/L and 12.39+/-0.96 g/L, respectively). Children with autism had significantly higher plasma concentrations of IgG4 (p<0.001) compared to their siblings (healthy brothers or sisters). Plasma concentration of IgA, IgG1, IgG2, and IgG3 were similar in autistic children and their healthy brothers or sisters. Increased plasma concentration of IgG1 was found (p=0.027) in autistic males (8.06+/-2.40), as compared with their healthy brothers (5.24+/-4.13 g/L). Plasma concentrations of IgG (14.28+/-3.66 g/L), and IgG1 (9.41+/-2.20 g/L) in autistic females were increased (p=0.012 and p=0.021, respectively) in comparison with IgG (11.07+/-2.07) and IgG1 (6.37+/-3.38 g/L) in their healthy sisters. CONCLUSION: Children with autism have increased plasma concentration of immunoglobulines. Increased immunoglobulines in children with autism could be a result of impaired development of the immune system, and/or genetic factors connected with defense mechanism in these children.

PMID: 15578810 [PubMed - indexed for MEDLINE]

Tuchman RF, Rapin I, Shinnar S. Autistic and dysphasic children. I: Clinical characteristics. Pediatrics. 1991 Dec;88(6):1211-8.

Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, Bronx, New York.

Autism and dysphasia are behaviorally defined disorders of higher cerebral function which in preschool children share the common core symptom of impairment of language. In this study we describe the clinical characteristics of 314 autistic and 237 dysphasic nonautistic children evaluated by one child neurologist. There was no significant difference between autistic and dysphasic children in gestational age, birth weight, or prevalence of associated medical disorders, all of which were infrequent, although a positive history of resuscitation or ventilatory support was more common in dysphasic than autistic children (P = .03). As a group autistic children are more likely than dysphasic children to have language subtypes affecting central processing and formulation,

a family history of psychiatric disorders and autism, and a history of regression of language and behavior. After excluding 12 girls with autistic symptoms who met the clinical criteria for Rett syndrome, we found that there was no significant difference in the number of autistic and dysphasic children with an abnormal sensorimotor examination. Girls with autism were more likely than boys to have severe mental deficiency (38% of autistic girls vs 23% of boys) (P = 0.012) and a motor deficit (27% vs 11%) (P = .0009).

PMID: 1956739 [PubMed - indexed for MEDLINE]

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005 Jan;57(1)67-81.

Department of Neurology, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA.

Autism is a neurodevelopmental disorder characterized by impaired communication and social interaction and may be accompanied by mental retardation and epilepsy. Its cause remains unknown, despite evidence that genetic, environmental, and immunological factors may play a role in its pathogenesis. To investigate whether immune-mediated mechanisms are involved in the pathogenesis of autism, we used immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent assays to study brain tissues and cerebrospinal fluid (CSF) from autistic patients and determined the magnitude of neuroglial and inflammatory reactions and their cytokine expression profiles. Brain tissues from cerebellum, midfrontal, and cingulate gyrus obtained at autopsy from 11 patients with autism were used for morphological studies. Fresh-frozen tissues available from seven patients and CSF from six living autistic patients were used for cytokine protein profiling. We demonstrate an active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of autistic patients. Immunocytochemical studies showed marked activation of microglia and astroglia, and cytokine profiling indicated that macrophage chemoattractant protein (MCP)-1 and tumor growth factor-beta1, derived from neuroglia, were the most prevalent cytokines in brain tissues. CSF showed a unique proinflammatory profile of cytokines, including a marked increase in MCP-1. Our findings indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients, suggesting that future therapies might involve modifying neuroglial responses in the brain.

PMID: 15546155 [PubMed - indexed for MEDLINE]

Vojdani A, Campbell AW, Anyanwu E, Kashanian A, Bock K, Vojdani E. Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, Chlamydia pneumoniae and Streptococcus group A. J Neuroimmunol. 2002 Aug;129(1-2):168-77.

Section of Neuroimmunology, Immunosciences Laboratory, Inc., 8693 Wilshire Boulevard, Suite 200, Beverly Hills, CA 90211, USA. immunsci@ix.netcom.com

We measured autoantibodies against nine different neuron-specific antigens and three cross-reactive peptides in the sera of autistic subjects and healthy controls by means of enzyme-linked immunosorbent assay (ELISA) testing. The antigens were myelin basic protein (MBP), myelin-associated glycoprotein (MAG), ganglioside (GM1), sulfatide (SULF), chondroitin sulfate (CONSO4), myelin oligodendrocyte glycoprotein (MOG), alpha,beta-crystallin (alpha,beta-CRYS), neurofilament proteins (NAFP), tubulin and three cross-reactive peptides, Chlamydia pneumoniae (CPP), streptococcal M protein (STM6P) and milk butyrophilin (BTN). Autistic children showed the highest levels of IgG, IgM and IgA antibodies against all neurologic antigens as well as the three cross-reactive peptides. These antibodies are specific because immune absorption demonstrated that only neuron-specific antigens or their cross-reactive epitopes could significantly reduce antibody levels. These antibodies may have been synthesized as a result of an alteration in the blood-brain barrier. This barrier promotes access of preexisting T-cells and central nervous system antigens to immunocompetent cells, which may start a vicious cycle. These results suggest a mechanism by which bacterial infections and milk antigens may modulate autoimmune responses in autism.

PMID: 12161033 [PubMed - indexed for MEDLINE]

Wakefield AJ, Walker-Smith JA, Murch SH. Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism. Pediatrics 2001;138:366-72.

University Department of Paediatric Gastroenterology, the Inflammatory Bowel Diseases Study Group, Royal Free and University College School of Medicine, London, United Kingdom.

OBJECTIVES: We have reported colitis with ileal lymphoid nodular hyperplasia (LNH) in children with regressive autism. The aims of this study were to characterize this lesion and determine whether LNH is specific for autism. METHODS: Ileo-colonoscopy was performed in 21 consecutively evaluated

children with autistic spectrum disorders and bowel symptoms. Blinded comparison was made with 8 children with histologically normal ileum and colon, 10 developmentally normal children with ileal LNH, 15 with Crohn's disease, and 14 with ulcerative colitis. Immunohistochemistry was performed for cell lineage and functional markers, and histochemistry was performed for glycosaminoglycans and basement membrane thickness. RESULTS: Histology demonstrated lymphocytic colitis in the autistic children, less severe than classical inflammatory bowel disease. However, basement membrane thickness and mucosal gamma delta cell density were significantly increased above those of all other groups including patients with inflammatory bowel disease. CD8(+) density and intraepithelial lymphocyte numbers were higher than those in the Crohn's disease, LNH, and normal control groups; and CD3 and plasma cell density and crypt proliferation were higher than those in normal and LNH control groups. Epithelial, but not lamina propria, glycosaminoglycans were disrupted. However, the epithelium was HLA-DR(-), suggesting a predominantly T(H)2 response. INTERPRETATION: Immunohistochemistry confirms a distinct lymphocytic colitis in autistic spectrum disorders in which the epithelium appears particularly affected. This is consistent with increasing evidence for gut epithelial dysfunction in autism.

PMID: 11241044 [PubMed - indexed for MEDLINE]

Warren RP, Margaretten NC, Pace NC, Foster A. Immune abnormalities in patients with autism. J Autism Dev Disord. 1986 Jun;16(2):189-97.

We have begun an investigation on the immune systems of patients with autism in attempt to determine if immune mechanisms are involved in the development of this severe developmental disorder. A study of 31 autistic patients has revealed several immune-system abnormalities, including reduced responsiveness in the lymphocyte blastogenesis assay to phytohemagglutinin, concanavalin A, and pokeweed mitogen; decreased numbers of T lymphocytes; and an altered ratio of helper to suppressor T cells. Immune-system abnormalities may be directly related to underlying biologic processes of autism, or these changes may be an indirect reflection of the actual pathologic mechanism.

PMID: 2941410 [PubMed - indexed for MEDLINE]

Warren RP, Cole P, Odell JD, Pingree CB, Warren WL, White E, Yonk J, Singh VK. Detection of maternal antibodies in infantile autism. J Am Acad Child Adolesc Psychiatry. 1990 Nov;29(6):873-7.

Developmental Center for Handicapped Persons, Utah State University, Logan 84322.

Maternal antibodies reactive with antigenic proteins expressed on the cell surface of paternal lymphocytes can be detected in couples with histories of more than one miscarriage or stillbirth. It is possible, but not proven, that these antibodies also react with tissues of the fetus and result in fetal death. Since many mothers of autistic children have a history of pregnancy disorder, antibodies were studied in 11 mothers of autistic children who were 6 years of age or younger. Six of the mothers had antibodies that reacted with lymphocytes of the autistic child. Five of these six mothers had a history of pregnancy disorder. Since antigens expressed on lymphocytes are found on cells of the central nervous system and, perhaps, other tissues of the developing embryo, it is suggested that aberrant maternal immunity may be associated with the development of some cases of infantile autism.

PMID: 2273013 [PubMed - indexed for MEDLINE]

Warren RP, Foster A, Margaretten NC. Reduced natural killer cell activity in autism. J Am Acad Child Adolesc Psychiatry. 1987 May;26(3):333-5.

Warren RP, **Foster A**, **Margaretten NC**.

PMID: 3597287 [PubMed - indexed for MEDLINE]

Warren RP, Singh VK, Averett RE, Odell JD, Maciulis A, Burger RA, Daniels WW, Warren WL. Immunogenetic studies in autism and related disorders. Mol Chem Neuropathol. 1996 May-Aug;28(1-3):77-81.
Utah State University, Logan 84322, USA.

The major histocompatibility complex comprises a number of genes that control the function and regulation of the immune system. One of these genes, the C4B gene, encodes a product that is involved in eliminating pathogens such as viruses and bacteria from the body. We previously reported that a deficient form of the C4B gene, termed the C4B null allele (no C4B protein produced) had an increased frequently in autism. In this study we attempted to confirm the

increased incidence of the C4B null allele in autism and investigated the presence of a C4B null allele in two other childhood disorders, attention-deficit hyperactivity disorder and dyslexia (reading disability). In addition, we explored the relationship of autism to the DR beta 1 gene, a gene located close to the C4B in autism. We confirmed the finding of an increased frequency of the C4B null allele in autism and found that the related disorders also had an increased frequency of this null allele. In addition, two alleles of the DR beta 1 gene also had significantly increased representation in the autistic subjects.

PMID: 8871944 [PubMed - indexed for MEDLINE]

Yonk LJ, Warren RP, Burger RA, Cole P, Odell JD, Warren WL, White E, Singh VK. CD4+ helper T cell depression in autism. Immunol Lett. 1990 Sep;25(4):341-5.

Developmental Center for Handicapped Persons, Utah State University, Logan 84322-6800.

CD4+ (helper) T cells are a heterogenous population of lymphocytes including at least two distinct subpopulations. To investigate the possibility that immune abnormalities in some subjects with autism may involve abnormal distributions of CD4+ and/or CD8+ cells, (suppressor) T cells, peripheral blood lymphocytes of 25 autistic subjects were characterized with monoclonal antibodies and flow cytometry. The autistic subjects had a significantly lower percentage and number of CD4+ cells, a lower number of T cells (CD2+ cells) and B cells (CD20+ cells), and a lower percentage and number of total lymphocytes than siblings and normal subjects. The level of blood values for female subjects appeared lower than those for males as compared to normal subjects of the same sex. These results suggest that a decrease in CD4+ cells is associated with autism.

PMID: 1979061 [PubMed - indexed for MEDLINE]

Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA, Pearce DA. Maternal antibrain antibodies in autism. Brain Behav Immun. 2006 Oct 5.

Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, 707 North Broadway, Baltimore, MD 21205, USA. zimmerman@kennedykrieger.org

Autism is a neurodevelopmental disorder of prenatal onset that is behaviorally defined. There is increasing evidence for systemic and neuroimmune

mechanisms in children with autism. Although genetic factors are important, atypical prenatal maternal immune responses may also be linked to the pathogenesis of autism. We tested serum reactivity in 11 mothers and their autistic children, maternal controls, and several groups of control children, to prenatal, postnatal, and adult rat brain proteins, by immunoblotting. Similar patterns of reactivity to prenatal (gestational day 18), but not postnatal (day 8) or adult rat brain proteins were identified in autistic children, their mothers, and children with other neurodevelopmental disorders, and differed from mothers of normal children, normal siblings of children with autism and normal child controls. Specific patterns of antibody reactivity were present in sera from the autism mothers, from 2 to 18 years after the birth of their affected children and were unrelated to birth order. Immunoblotting using specific antigens for myelin basic protein (MBP) and glial acidic fibrillary protein (GFAP) suggests that these proteins were not targets of the maternal antibodies. The identification of specific serum antibodies in mothers of children with autism that recognize prenatally expressed brain antigens suggests that these autoantibodies could cross the placenta and alter fetal brain development.

PMID: 17029701 [PubMed - indexed for MEDLINE]

Zimmerman AW, Jyonouchi H, Comi AM, Connors SL, Milstien S, Varsou A, Heyes MP. Cerebrospinal fluid and serum markers of inflammation in autism. Pediatr Neurol. 2005 Sep;33(3):195-201.

Department of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore Maryland 21205, USA.

Systemic immune abnormalities have no known relevance to brain dysfunction in autism. In order to find evidence for neuroinflammation, we compared levels of sensitive indicators of immune activation: quinolinic acid, neopterin, and biopterin, as well as multiple cytokines and cytokine receptors, in cerebrospinal fluid and serum from children with autism, to control subjects with other neurologic disorders. In cerebrospinal fluid from 12 children with autism, quinolinic acid ($P = 0.037$) and neopterin ($P = 0.003$) were decreased, and biopterin ($P = 0.040$) was elevated, compared with control subjects. In sera from 35 persons with autism, among cytokines, only tumor necrosis factor receptor II was elevated compared with controls ($P < 0.02$). Decreased quinolinic acid and neopterin in cerebrospinal fluid are paradoxical and suggest dysmaturation of metabolic pathways and absence of concurrent infection, respectively, in autism. Alternatively, they may be produced by microglia but remain localized and not expressed in cerebrospinal fluid.

PMID: 16139734 [PubMed - indexed for MEDLINE]

12 - Environmental Toxicities (4 citations)

Pesticide Toxicity and the Developing Brain

Brenda Eskenazi, Lisa G. Rosas, Amy R. Marks, Asa Bradman, Kim Harley, Nina Holland, Caroline Johnson, Laura Fenster and Dana B. Barr

Basic & Clinical Pharmacology & Toxicology, Volume 102, Issue 2, Page 228-236, Feb 2008, doi: 10.1111/j.1742-7843.2007.00171.x

- Abstract
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Developmental Origins of Health and Disease: New Insights

Mark A. Hanson and Peter D. Gluckman

Basic & Clinical Pharmacology & Toxicology, Volume 102, Issue 2, Page 90-93, Feb 2008, doi: 10.1111/j.1742-7843.2007.00186.x

- Abstract
- | References
- | Full Text HTML
- | Full Text PDF (114 KB)

Assessing Developmental Toxicant Exposures via Biomonitoring

Larry L. Needham, Antonia M. Calafat and Dana B. Barr

Basic & Clinical Pharmacology & Toxicology, Volume 102, Issue 2, Page 100-108, Feb 2008, doi: 10.1111/j.1742-7843.2007.00185.x

- Abstract
- | References
- | Full Text HTML
- | Full Text PDF (206 KB)

Consequences of Exposure to Carcinogens Beginning During Developmental Life

Morando Soffritti, Fiorella Belpoggi, Davide Degli Esposti, Laura Falcioni and Luciano Bua

Basic & Clinical Pharmacology & Toxicology, Volume 102, Issue 2, Page 118-124, Feb 2008, doi: 10.1111/j.1742-7843.2007.00200.x

- Abstract
- | References
- | Full Text HTML
- | Full Text PDF (128 KB)

13 – Thimerosal and Toxicity

A comprehensive review of mercury provoked autism.

Geier DA, **King PG**, **Sykes LK**, **Geier MR**.

The Institute of Chronic Illnesses, Silver Spring, MD, USA.

Emerging evidence supports the theory that some autism spectrum disorders (ASDs) may result from a combination of genetic/biochemical susceptibility, specifically a reduced ability to excrete mercury (Hg), and exposure to Hg at critical developmental periods. Elemental/inorganic Hg is released into the air/water where it becomes methylated and accumulates in animal tissues. The US population is primarily exposed to methyl-Hg by fish consumption. In addition, many pharmaceuticals have been, and some continue to be, a ubiquitous source of danger because they contain mercurials. Mercurials may be found in drugs for the eye, ear, nose, throat, and skin; in bleaching creams; as preservatives in cosmetics, tooth pastes, lens solutions, vaccines, allergy test and immunotherapy solutions; in antiseptics, disinfectants, and contraceptives; in fungicides and herbicides; in dental fillings and thermometers; and many other products. Hg has been found to cause immune, sensory, neurological, motor, and behavioural dysfunctions similar to traits defining/associated with ASDs, and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Furthermore, a review of molecular mechanisms indicates that Hg exposure can induce death, disorganization and/or damage to selected neurons in the brain similar to that seen in recent ASD brain pathology studies, and this alteration may likely produce the symptoms by which ASDs are diagnosed. Finally, a review of treatments suggests that ASD patients who undergo protocols to reduce Hg and/or its effects show significant clinical improvements in some cases. In conclusion, the overwhelming preponderance of the evidence favours acceptance that Hg exposure is capable of causing some ASDs.

PMID: 19106436 [PubMed - in process] Indian J Med Res. 2008 Oct;128(4):383-411.

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- A case series of children with apparent mercury toxic encephalopathies manifesting with clinical symptoms of regressive autistic disorders. [J Toxicol Environ Health A. 2007]
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- ReviewMercury exposure in children: a review. [Toxicol Appl Pharmacol. 2004]
- ReviewThe genetics of autism. [Pediatrics. 2004]
- Application of a latent variable model for a multicenter study on early effects due to mercury exposure. [Neurotoxicology. 2003]

1:

Effects of chelators on mercury, iron, and lead neurotoxicity in cortical culture.

Rush T, Hjelmhaug J, Lobner D.

Neurotoxicology. 2008 Nov 5. [Epub ahead of print]

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Feeding mice with diets containing mercury-contaminated fish flesh from French Guiana: a model for the mercurial intoxication of the Wayana Amerindians.

Bourdineaud JP, **Bellance N**, **Bénard G**, **Brèthes D**, **Fujimura M**, **Gonzalez P**, **Marighetto A**, **Maury-Brachet R**, **Mormède C**, **Pédron V**, **Philippin JN**, **Rossignol R**, **Rostène W**, **Sawada M**, **Laclau M**.

Université de Bordeaux 1-CNRS UMR 5805, Station Marine d'Arcachon, place du Docteur Peyneau, Arcachon, France. jp.bourdineaud@epoc.u-bordeaux1.fr

BACKGROUND: In 2005, 84% of Wayana Amerindians living in the upper marshes of the Maroni River in French Guiana presented a hair mercury concentration exceeding the limit set up by the World Health Organization (10 microg/g). To determine whether this mercurial contamination was harmful, mice have been fed diets prepared by incorporation of mercury-polluted fish from French Guiana. METHODS: Four diets containing 0, 0.1, 1, and 7.5% fish flesh, representing 0, 5, 62, and 520 ng methylmercury per g, respectively, were given to four groups of mice for a month. The lowest fish regimen led to a mercurial contamination pressure of 1 ng mercury per day per g of body weight, which is precisely that affecting the Wayana Amerindians. RESULTS: The expression of several genes was modified with mercury intoxication in liver, kidneys, and hippocampus, even at the lowest tested fish regimen. A net genetic response could be observed for mercury concentrations accumulated within tissues as weak as 0.15 ppm in the liver, 1.4 ppm in the kidneys, and 0.4 ppm in the hippocampus. This last value is in the range of the mercury concentrations found in the brains of chronically exposed patients in the Minamata region or in brains from heavy fish consumers. Mitochondrial respiratory rates showed a 35-40% decrease in respiration for the three contaminated mice groups. In the muscles of mice fed the lightest fish-containing diet, cytochrome c oxidase activity was decreased to 45% of that of the control muscles. When mice behavior was assessed in a cross maze, those fed the lowest and mid-level fish-containing diets developed higher anxiety state behaviors compared to mice fed with control diet. CONCLUSION: We conclude that a vegetarian diet containing as little as 0.1% of mercury-contaminated fish is able to trigger in mice, after only one month of exposure, disorders presenting all the hallmarks of mercurial contamination.

PMID: 18959803 [PubMed - in process] Environ Health. 2008 Oct 29;7:53

Hepatitis B triple series vaccine and developmental disability in US children aged 1–9 years

Carolyn Gallagher* and Melody Goodman
Graduate Program in Public Health, Stony Brook University Medical Center, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York, USA
(Final version received 14 November 2007)
This study investigated the association between vaccination with the Hepatitis B triple series vaccine prior to 2000 and developmental disability in children aged 1–9 years (n¼1824), proxied by parental report that their child receives early intervention or special education services (EIS). National Health and Nutrition Examination Survey 1999–2000 data were analyzed and adjusted for survey design by Taylor Linearization using SAS version 9.1 software, with SAS callable SUDAAN version 9.0.1. The odds of receiving EIS were approximately nine times as great for vaccinated boys (n¼46) as for unvaccinated boys (n¼7), after adjustment for confounders. This study found statistically significant

evidence to suggest that boys in United States who were vaccinated with the triple series Hepatitis B vaccine, during the time period in which vaccines were manufactured with thimerosal, were more susceptible to developmental disability than were unvaccinated boys.

Toxicological & Environmental Chemistry
Vol. 90, No. 5, September–October 2008, 997–1008

14: Binstock Citations

Controversies: thimerosal and MMR – cites 1-36

1: Parker SK et al. Thimerosal-containing vaccines and autistic spectrum disorder: a critical review of published original data. Pediatrics. 2004 Sep;114(3):793-804.

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8a. CDC quote from p22: Bernard/Safeminds presentation to IOM, Oct 21, 2004
http://www.safeminds.org/iomvsd21oct04presentation.pdf

8b. Summary of CDC 1999 findings, p96-7 in: Neurodevelopmental disorders following thimerosal-containing childhood vaccines... Geier DA, Geier MR, in Defeat Autism Now! Conference Proceedings, Fall 2004, p95-101.

In 1999, the CDC intiated study designed to review the medical records of hundreds of thousands of children in the CDC's Vaccine Safety Datalink (VSD). The VSD is a massive databse that tracks the medical records of hundreds of thousands of patients belonging to seven major health maintenance organizations.

"In the initial analysis of the VSD database conducted by Dr. Thomas Verstraeten, [then] a CDC researcher, in the fall of 1999, showed statistically significantly large

increased risks for neurodevelopmental disorders following additional doses of thimerosal... The following are [sic] a brief sampling of some of effects observed:"

autism = 7.62 (95% Confidence Interval (CI) = 1.84-31.5)
autism = 11.35 (95% CI = 2.70-47.76)
specific disorders of sleep of non-organic origin = 4.98 (95% CI = 1.55-15.94)
specific disorders of sleep of non-organic origin = 4.64 (95% CI = 1.12-19.25)
phase-disruption of 24-hour sleep-wake cycle = 53.64 (95% CI = 3.23-892.10)
somnambulism or night terrors = 5.76 (95% CI = 1.38-24.05)
attention deficit without mention of hyperactivity = 6.38 (95% CI = 1.56-26.09)
attention deficit with mention of hyperactivity = 8.29 (95% CI = 2.03-33.89)
developmental speech or language disorder = 2.09 (95% CI = 1.08-4.03)
other developmental speech or language delay = 2.32 (95% CI = 1.20-4.48)
unspecified delay in development = 2.08 (95% CI = 1.03-4.19)

[the above data] among children receiving > 25 micrograms ethylmercury from thimerosal at age 1 month in comparison to children receiving 0 micrograms of ethylmercury at age 1 month;

attention deficit disorder = 2.88 (95% CI = 1.05-7.88) and
attention deficit disorder = 2.84 (95% CI = 1.03-7.85), and
coordination disorder = 18.26 (95% CI = 5.65-59.01)

among children receiving > 75 micrograms of ethylmercury from thimerosal in comparison to children receiving < 12.5 microgram from thimerosal at age 3 months; and

autism = 2.15 (95% CI = 1.04-4.43) and

among children receiving increases of 7.5-10 micrograms of thimerosal over 1 month, in comparison to children receiving less than 5 micrograms of ethylmercury from thimerosal over 1 month [9-10].

"Additionally, studies were conducted in 2000 by CDC to evaluate the dose-response effects of thimerosal on childhood neurodevelopmental disorders based upon evaluation of the VSD database [11]. It was found that there were **statistically significant relationships between increasing exposures to thimerosal and the following outcomes**, including:
(1) for two months of age, an unspecified developmental delay, which has its own ICD-9 code.
(2) Exposure at three months of age, Tics.
(3) Exposure at six months of age, language and speech delays, which are two separate ICD-9 codes.
(4) Exposure at one, three and six months of age, the entire category of

neurodevelopmental delays, which includes all of these plus a number of other disorders (i.e., including autism)."

[9.] Email from Thomas Verstraeten to Robert Davis and Frank Destefano. Nov 29, 1999. Obtained under FOIA by SafeMinds.
[10.] Email from Thomas Verstraeten to Robert Davis and Frank Destefano.Dec 17, 1999. Obtained under FOIA by SafeMinds.

8c. The Truth behind the Vaccine Coverup. Russell Blaylock MD. Sep 12 2004

http://sydney.indymedia.org/front.php3?article_id=45874&group=webcast

Excerpt: "It all started when a friend of mind sent me a copy of a letter from Congressman David Weldon, M.D. to the director of the CDC, Dr Julie L. Gerberding, in which he alludes to a study by a Doctor Thomas Verstraeten, then representing the CDC, on the connection between infant exposure to thimerosal-containing vaccines and neurodevelopmental

injury. In this shocking letter Congressman Weldon referrers to Dr. Verstraeten's study which looked at the data from the Vaccine Safety Datalink and found a significant correlation between thimerosal exposure via vaccines and several neurodevelopmental disorders including tics, speech and language delays, and possibly to ADD.

"Congressman Weldon questions the CDC director as to why, following this meeting, Dr. Verstraeten published his results, almost four years later, in the journal Pediatrics to show just the opposite, that is, that there was no correlation to any neurodevelopmental problems related to thimerosal exposure in infants…"

8d. Original in-house CDC study is online. Verstraeten et al 2000, unpublished; obtained via FOIA

http://factsformedia.com/factsformedia/thimerosalstudy.pdf

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9b. Blaylock R, MD. The thimerosal coverup – a thorough delineation of CDC, FDA knowledge about thimerosal circa 1999.
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10a. House Subcommittee Hearing transcripts: Truth Revealed: New Scientific Discoveries Regarding Mercury in Medicine and Autism. September 08, 2004

http://reform.house.gov/WHR/Hearings/EventSingle.aspx?EventID=1311

10b. Testimony of Lyn Redwood, RN, MSN; President; Safeminds

http://reform.house.gov/UploadedFiles/Testimony Redwood.pdf

10c. Analysis and critique of the CDC's handling of the thimerosal exposure assessment based on Vaccine Safety Datalink (VSD) information. Safeminds, 2003.

http://www.momsonamissionforautism.org/index/VSD.SafeMinds.critique.pdf

10d. NoMercury.org – an information resource about thimerosal

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11. Verstraeten T, Davis RL, DeStefano F et al. Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. Pediatrics. 2003 Nov;112(5):1039-48. Erratum in: Pediatrics. 2004 Jan;113(1):184.

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"We were initially highly skeptical that differences in the concentrations of thimerosal in vaccines would have any effect on the incidence rate of neurodevelopmental disorders after childhood immunization. This study presents the first epidemiologic evidence, based upon tens of millions of doses of vaccine administered in the United States, that associates increasing thimerosal from vaccines with neurodevelopmental disorders."

13. Geier DA, Geier MR. An assessment of the impact of thimerosal on childhood neurodevelopmental disorders. Pediatr Rehabil. 2003 6(2):97-102 PMID 14534046

"The [thimerosal] dose-response curves showed increases in odds ratios of neurodevelopmental disorders from both the VAERS and US Department of Education data closely linearly correlated with increasing doses of mercury from thimerosal-containing childhood vaccines and that for overall odds ratios statistical significance was achieved. Similar slopes and linear regression coefficients for autism odds ratios in VAERS and the US Department of Education data help to mutually validate each other."

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[An excellent summary & rebuttal of pro-thimerosal articles.]

17. Baskin DS et al. Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts. Toxicol Sci. 2003 Aug;74(2):361-8. Epub 2003 May 28. PMID: 12773768

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"Interpretation: Administration of vaccines containing thiomersal does not seem to raise blood concentrations of mercury above safe values in infants." [But see cite 20]

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"Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury [at nanomolar levels described by Pichichero et al), aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins."

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28. Holmes AS, Blaxill MF, Haley BE. Reduced levels of mercury in first baby haircuts of autistic children. Int J Toxicol. 2003 Jul-Aug;22(4):277-85. PMID: 12933322

"Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively."

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34. Excerpts from CDC's in-house conference: Thimerosal sequelae

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35. Congressman, Dr. Weldon's letter to the CDC director, available at: http://momsonamissionforautism.org/Autism_Central/Dr_Weldon_Responds.shtml

36a. IOM presentation of Congressman Dave Weldon, M.D. http://www.nationalautismassociation.org/pdf/Weldon.pdf

36b. Doctors must prescribe without all the facts. Dr. Darshak Sanghavi, Children's Hospital and Harvard Medical School. sanghavi@post.harvard.edu October 12, 2004 http://www.boston.com/news/globe/health_science/articles/2004/10/12/doctors_must_prescribe_without_all_the_facts/

Intestinal pathologies

37. D'Eufemia P et al. Abnormal intestinal permeability in children with autism. Acta Paediatr. 1996 Sep;85(9):1076-9. PMID: 8888921

"We determined the occurrence of gut mucosal damage using the intestinal permeability test in 21 autistic children who had no clinical and laboratory findings

consistent with known intestinal disorders. An altered intestinal permeability was found in 9 of the 21 (43%) autistic patients, but in none of the 40 controls."

38. Reichelt KL, Knivsberg AM. Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutr Neurosci. 2003 Feb;6(1):19-28. PMID: 12608733

39. Mercer ME, Holder MD. Food cravings, endogenous opioid peptides, and food intake: a review. Appetite. 1997 Dec;29(3):325-52. PMID: 9468764

40a. Lucarelli S et al. Food allergy and infantile autism. Panminerva Med. 1995 Sep;37(3):137-41. PMID: 8869369

"The aim of the present study has been to verify the efficacy of a cow's milk free diet (or other foods which gave a positive result after a skin test) in 36 autistic patients. We also looked for immunological signs of food allergy in autistic patients on a free choice diet. We noticed a marked improvement in the behavioural symptoms of patients after a period of 8 weeks on an elimination diet and we found high levels of IgA antigen specific antibodies for casein, lactalbumin and beta-lactoglobulin and IgG and IgM for casein. The levels of these antibodies were significantly higher than those of a control group which consisted of 20 healthy children."

40b. Iacono G et al. Chronic constipation as a symptom of cow milk allergy. J Pediatr. 1995 Jan;126(1):34-9. PMID: 7815220

"Twenty-seven consecutive infants (mean age, 20.6 months) with chronic "idiopathic" constipation were studied to investigate the possible relation between constipation and cow milk protein allergy (CMPA). The infants were initially observed on an unrestricted diet, and the number of stools per day was recorded. Subsequently the infants were put on a diet free of cow milk protein (CMP) for two periods of 1 month each, separated by two challenges with CMP. During the CMP-free diet, there was a resolution of symptoms in 21 patients; during the two consecutive challenges, constipation reappeared within 48 to 72 hours. In another six patients the CMP-free diet did not lead to improvement of constipation. Only four of the patients who improved on the CMP-free diet had concomitant symptoms of suspected CMPA, but a medical history of CMPA was found in 15 of the 21 patients cured and in only one of the six patients whose condition had not improved ($p < 0.05$); in addition, in 15 of the 21 cured patients, results of one or more laboratory tests (specific IgE, IgG, anti-beta-lactoglobulin, circulating eosinophils) were positive at the time of diagnosis, indicating hypersensitivity, compared with one of the six patients whose condition did not improve ($p < 0.05$). The endoscopic and histologic findings at the time of diagnosis showed proctitis with monocytic infiltration in two patients cured with the CMP-free diet; after 1 month on this diet, they were completely normal. We conclude that constipation in infants may have an allergic pathogenesis."

40c. Iacono G et al. Intolerance of cow's milk and chronic constipation in children. N Engl J Med. 1998 Oct 15;339(16):1100-4. PMID: 9770556

BACKGROUND: Chronic diarrhea is the most common gastrointestinal symptom of intolerance of cow's milk among children. On the basis of a prior open study, we hypothesized that intolerance of cow's milk can also cause severe perianal lesions with pain on defecation and consequent constipation in young children. METHODS: We performed a double-blind, crossover study comparing cow's milk with soy milk in 65 children (age range, 11 to 72 months) with chronic constipation (defined as having one bowel movement every 3 to 15 days). All had been referred to a pediatric gastroenterology clinic and had previously been treated with laxatives without success; 49 had anal fissures and perianal erythema or edema. After 15 days of observation, the patients received cow's milk or soy milk for two weeks. After a one-week washout period, the feedings were reversed. A response was defined as eight or more bowel movements during a treatment period. RESULTS: Forty-four of the 65 children (68 percent) had a response while receiving soy milk. Anal fissures and pain with defecation resolved. None of the children who received cow's milk had a response. In all 44 children with a response, the response was confirmed with a double-blind challenge with cow's milk. Children with a response had a higher frequency of coexistent rhinitis, dermatitis, or bronchospasm than those with no response (11 of 44 children vs. 1 of 21, P=0.05); they were also more likely to have anal fissures and erythema or edema at base line (40 of 44 vs. 9 of 21, P<0.001), evidence of inflammation of the rectal mucosa on biopsy (26 of 44 vs. 5 of 21, P=0.008), and signs of hypersensitivity, such as specific IgE antibodies to cow's-milk antigens (31 of 44 vs. 4 of 21, P<0.001). CONCLUSIONS: In young children, chronic constipation can be a manifestation of intolerance of cow's milk.

41. Arnold GL et al. Plasma amino acids profiles in children with autism: potential risk of nutritional deficiencies. J Autism Dev Disord 2003 33(4):449-54. PMID: 12959424

"No amino acid profile specific to autism was identified. However, children with autism had more essential amino acid deficiencies consistent with poor protein nutrition than an age/gender matched control group. There was a trend for children with autism who were on restricted diets to have an increased prevalence of essential amino acid deficiencies and lower plasma levels of essential acids including the neurotransmitter precursors tyrosine and tryptophan than both controls and children with autism on unrestricted diets."

42. Chauhan V et al. Alteration in amino-glycerophospholipids levels in the plasma of children with autism: a potential biochemical diagnostic marker. Life Sci 2004 Feb 13;74(13):1635-43. PMID: 14738907

"the levels of AGP [amino-glycerophospholipids] were found to be significantly increased in the plasma of children with autism as compared to their non-autistic normal siblings."

43. Knivsber AM et al. Reports on dietary intervention in autistic disorders. Nutr Neurosci. 2001;4(1):25-37. PMID: 11842874

"…Gluten and/or casein free diet has been implemented to reduce autistic behaviour, in addition to special education, since early in the eighties. Over the last twelve years various studies on this dietary intervention have been published in addition to anecdotal, parental reports. The scientific studies include both groups of participants as well as single cases, and beneficial results are reported in all, but one study. While some studies are based on urinary peptide abnormalities, others are not. The reported results are, however, more or less identical; reduction of autistic behaviour, increased social and communicative skills, and reappearance of autistic traits after the diet has been broken."

44. Karyn Seroussi -- Dietary Intervention for Autism Defeat Autism Now! 2003 Philadelphia

http://64.202.182.52/powerpoint/dan2003/KarynSeroussi.htm

45. ARI's Parent Ratings Data

Parent Ratings for Autism HTML

Parent Ratings for Autism PDF

Parent Ratings for Aspergers HTML

Parent Ratings for Aspergers PDF

46: Wakefield AJ et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet. 1998 28;351(9103):637-41. PMID: 9500320

47: Ashwood P et al. Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology. J Clin Immunol. 2003 Nov;23(6):504-17. PMID: 15031638

"At all sites, CD3(+) and CD3(+)CD8(+) IEL as well as CD3(+) LPL were significantly increased in affected children compared with developmentally normal noninflamed control groups ($p<0.01$) reaching levels similar to inflamed controls. In addition, two populations--CD3(+)CD4(+) IEL and LP CD19(+) B cells--were significantly increased in

affected children compared with both noninflamed and inflamed control groups including IBD, at all sites examined ($p<0.01$). Histologically there was a prominent mucosal eosinophil infiltrate in affected children that was significantly lower in those on a gluten- and casein-free diet, although lymphocyte populations were not influenced by diet.The data provide further evidence of a pan-enteric mucosal immunopathology in children with regressive autism that is apparently distinct from other inflammatory bowel diseases."

48: Wakefield AJ. Enterocolitis, autism and measles virus. Mol Psychiatry. 2002;7 Suppl 2:S44-6. PMID: 12142948

49: Wakefield AJ. The gut-brain axis in childhood developmental disorders. J Pediatr Gastroenterol Nutr. 2002 May-Jun;34 Suppl 1:S14-7. PMID: 12082381

50. Binstock T. Anterior insular cortex: linking intestinal pathology and brain function in autism-spectrum subgroups. Med Hypotheses 2001 57(6):714-7. PMID: 11918432

"Numerous parents and some physicians report that an autistic child's attention and language improve in response to treatments which eliminate certain dietary antigens and/or which improve intestinal health. For at least some autism-spectrum children, the link between intestinal pathology, attention, and language may derive from shared neuroanatomic pathways within the anterior insular cortex (aIC); from a neurotrophic virus such as herpes simplex (HSV) migrating within afferents to the insular cortex; and/or from synaptic exhaustion in the aIC as induced by chronically inappropriate neuronal activity in the enteric nervous system and/or its vagal efferents."

51: Torrente F et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatry. 2002;7(4):375-82, 334.

PMID: 11986981

"Most strikingly, IgG deposition was seen on the basolateral epithelial surface in 23/25 autistic children, co-localising with complement C1q. This was not seen in the other conditions. These findings demonstrate a novel form of enteropathy in autistic children, in which increases in mucosal lymphocyte density and crypt cell proliferation occur with epithelial IgG deposition. The features are suggestive of an autoimmune lesion."

52. Uhlmann V et al. Potential viral pathogenic mechanism for new variant inflammatory bowel disease. Mol Pathol. 2002 Apr;55(2):84-90. PMID: 11950955

"AIMS: A new form of inflammatory bowel disease (ileocolonic lymphonodular hyperplasia) has been described in a cohort of children with developmental disorder. This study investigates the presence of persistent measles virus in the intestinal tissue of these patients (new variant inflammatory bowel disease) and a series of controls by

molecular analysis… RESULTS: Seventy five of 91 patients with a histologically confirmed diagnosis of ileal lymphonodular hyperplasia and enterocolitis were positive for measles virus in their intestinal tissue compared with five of 70 control patients. Measles virus was identified within the follicular dendritic cells and some lymphocytes in foci of reactive follicular hyperplasia. The copy number of measles virus ranged from one to 300,00 copies/ng total RNA. CONCLUSIONS: The data confirm an association between the presence of measles virus and gut pathology in children with developmental disorder."

53. Wakefield AJ et al. Review article: the concept of entero-colonic encephalopathy, autism and opioid receptor ligands.Aliment Pharmacol Ther 2002 16(4):663-74. PMID 11929383

54. Furlano RI et al. Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism. J Pediatr. 2001 Mar;138(3):366-72. PMID: 11241044

"OBJECTIVES: We have reported colitis with ileal lymphoid nodular hyperplasia (LNH) in children with regressive autism. The aims of this study were to characterize this lesion and determine whether LNH is specific for autism. METHODS: Ileo-colonoscopy was performed in 21 consecutively evaluated children with autistic spectrum disorders and bowel symptoms. Blinded comparison was made with 8 children with histologically normal ileum and colon, 10 developmentally normal children with ileal LNH, 15 with Crohn's disease, and 14 with ulcerative colitis. Immunohistochemistry was performed for cell lineage and functional markers, and histochemistry was performed for glycosaminoglycans and basement membrane thickness. RESULTS: Histology demonstrated lymphocytic colitis in the autistic children, less severe than classical inflammatory bowel disease. However, basement membrane thickness and mucosal gamma delta cell density were significantly increased above those of all other groups including patients with inflammatory bowel disease. CD8(+) density and intraepithelial lymphocyte numbers were higher than those in the Crohn's disease, LNH, and normal control groups; and CD3 and plasma cell density and crypt proliferation were higher than those in normal and LNH control groups. Epithelial, but not lamina propria, glycosaminoglycans were disrupted. However, the epithelium was HLA-DR(-), suggesting a predominantly T(H)2 response. INTERPRETATION: Immunohistochemistry confirms a distinct lymphocytic colitis in autistic spectrum disorders in which the epithelium appears particularly affected. This is consistent with increasing evidence for gut epithelial dysfunction in autism."

55. O'Leary JJ et al. Measles virus and autism. Lancet. 2000 Aug 26;356(9231):772. PMID: 11085720

56. Wakefield AJ et al. Enterocolitis in children with developmental disorders. Am J Gastroenterol. 2000 Sep;95(9):2285-95. PMID: 11007230

"OBJECTIVE: Intestinal pathology, i.e., ileocolonic lymphoid nodular hyperplasia (LNH) and mucosal inflammation, has been described in children with developmental disorders. This study describes some of the endoscopic and pathological characteristics in a group of children with developmental disorders (affected children) that are associated with behavioral regression and bowel symptoms, and compares them with pediatric controls. METHODS: Ileocolonoscopy and biopsy were performed on 60 affected children (median age 6 yr, range 3-16; 53 male). Developmental diagnoses were autism (50 patients), Asperger's syndrome (five), disintegrative disorder (two), attention deficit hyperactivity disorder (ADHD) (one), schizophrenia (one), and dyslexia (one). Severity of ileal LNH was graded (0-3) in both affected children and 37 developmentally normal controls (median age 11 yr, range 2-13 yr) who were investigated for possible inflammatory bowel disease (IBD). Tissue sections were reviewed by three pathologists and scored on a standard proforma. Data were compared with ileocolonic biopsies from 22 histologically normal children (controls) and 20 children with ulcerative colitis (UC), scored in an identical manner. Gut pathogens were sought routinely. RESULTS: Ileal LNH was present in 54 of 58 (93%) affected children and in five of 35 (14.3%) controls ($p < 0.001$). Colonic LNH was present in 18 of 60 (30%) affected children and in two of 37 (5.4%) controls ($p < 0.01$). Histologically, reactive follicular hyperplasia was present in 46 of 52 (88.5%) ileal biopsies from affected children and in four of 14 (29%) with UC, but not in non-IBD controls ($p < 0.01$). Active ileitis was present in four of 51 (8%) affected children but not in controls. Chronic colitis was identified in 53 of 60 (88%) affected children compared with one of 22 (4.5%) controls and in 20 of 20 (100%) with UC. Scores of frequency and severity of inflammation were significantly greater in both affected children and those with UC, compared with controls ($p < 0.001$). CONCLUSIONS: A new variant of inflammatory bowel disease is present in this group of children with developmental disorders."

60. Wakefield AJ, Montgomery SM. Autism, viral infection and measles-mumps-rubella vaccination. Isr Med Assoc J. 1999 Nov;1(3):183-7. PMID: 10731332

61. Wakefield AJ. MMR vaccination and autism. Lancet. 1999 Sep 11;354(9182):949-50. PMID: 10489978

62a. Horvath K et al. Gastrointestinal abnormalities in children with autistic disorder. J Pediatr. 1999 Nov;135(5):559-63. PMID: 10547242

"OBJECTIVES: Our aim was to evaluate the structure and function of the upper gastrointestinal tract in a group of patients with autism who had gastrointestinal symptoms. STUDY DESIGN: Thirty-six children (age: 5.7 +/- 2 years, mean +/- SD) with autistic disorder underwent upper gastrointestinal endoscopy with biopsies, intestinal and pancreatic enzyme analyses, and bacterial and fungal cultures. The most frequent gastrointestinal complaints were chronic diarrhea, gaseousness, and abdominal discomfort and distension. RESULTS: Histologic examination in these 36

children revealed grade I or II reflux esophagitis in 25 (69.4%), chronic gastritis in 15, and chronic duodenitis in 24. The number of Paneth's cells in the duodenal crypts was significantly elevated in autistic children compared with non-autistic control subjects. Low intestinal carbohydrate digestive enzyme activity was reported in 21 children (58.3%), although there was no abnormality found in pancreatic function… CONCLUSIONS: Unrecognized gastrointestinal disorders, especially reflux esophagitis and disaccharide malabsorption, may contribute to the behavioral problems of the non-verbal autistic patients…"

62b. Horvath K, Perman JA. Autism and gastrointestinal symptoms. Curr Gastroenterol Rep. 2002 Jun;4(3):251-8. PMID: 12010627

63. Horvath K, Perman JA. Autistic disorder and gastrointestinal disease. Curr Opin Pediatr. 2002 Oct;14(5):583-7. PMID: 12352252

"High prevalence of histologic abnormalities in the esophagus, stomach, small intestine and colon, and dysfunction of liver conjugation capacity and intestinal permeability were reported. Three surveys conducted in the United States described high prevalence of gastrointestinal symptoms in children with autistic disorder. Treatment of the digestive problems may have positive effects on their behavior."

64. Quigley EM, Hurley D. Autism and the gastrointestinal tract. Am J Gastroenterol. 2000 Sep;95(9):2154-6. PMID: 11007210

65. Tim Buie, M.D. Presentation at 2003 Defeat Autism Now!, Philadelphia.

http://64.202.182.52/powerpoint/dan2003/TimothyBuie.htm

MV in PBMCs, as variant SSPE

66. Kawashima H et al. Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. Dig Dis Sci. 2000 Apr;45(4):723-9. PMID 10759242

"One of eight patients with Crohn disease, one of three patients with ulcerative colitis, and three of nine children with autism, were positive. Controls were all negative. The sequences obtained from the patients with Crohn's disease shared the characteristics with wild-strain virus. The sequences obtained from the patients with ulcerative colitis and children with autism were consistent with being vaccine strains. The results were concordant with the exposure history of the patients. Persistence of measles virus was confirmed in PBMC in some patients with chronic intestinal inflammation."

67. Jeff Bradstreet, M.D. A Case-control Study of Mercury Burden in Children with Autistic Disorders and Measles Virus Genomic RNA in Cerebrospinal Fluid in Children with Regressive Autism. IOM presentation, Feb 9, 2004.

Slides: http://www.iom.edu/view.asp?id=18578

Audio: http://www.iom.edu/view.asp?id=19130

68. 48a: Valsamakis A et al. Altered virulence of vaccine strains of measles virus after prolonged replication in human tissue. J Virol. 1999 73(10): 8791-7. PMID 10482633

http://jvi.asm.org/cgi/reprint/73/10/8791.pdf

69. Binstock T. Intra-monocyte pathogens delineate autism subgroups. Med Hypotheses. 2001 Apr;56(4):523-31. PMID 11339860

70. Garg RK. Subacute sclerosing panencephalitis.Postgrad Med J. 2002 Feb;78(916):63-70. PMID 11807185.

71. Neuroprogressive disease of post-infectious origin: a review of a resurging subacute sclerosing panencephalitis (SSPE). Ment Retard Dev Disabil Res Rev. 2001;7(3):217-25. PMID: 11553938

72. Gascon GG. Subacute sclerosing panencephalitis. Semin Pediatr Neurol. 1996 Dec;3(4):260-9. PMID: 8969008

MMR additional miscellany

73. Geier DA, Geier MR. A comparative evaluation of the effects of MMR immunization and mercury doses from thimerosal-containing childhood vaccines on the population prevalence of autism. Med Sci Monit. 2004 Mar;10(3):PI33-9. Epub 2004 Mar 01. PMID: 14976450

"These studies have shown that there is biological plausibility and epidemiological evidence showing a direct relationship between increasing doses of mercury from thimerosal-containing vaccines and neurodevelopmental disorders, and measles-containing vaccines and serious neurological disorders. It is recommended that thimerosal be removed from all vaccines, and additional research be undertaken to produce a MMR vaccine with an improved safety profile."

74. Vijendra K. Singh, Ph.D. Autism, Vaccines, and Immune Reactions. IOM presentation, Feb 9, 2004.

Audio only: http://www.iom.edu/view.asp?id=19132

75. Singh VK, Jensen RL. Elevated levels of measles antibodies in children with autism. Pediatr Neurol. 2003 Apr;28(4):292-4. PMID: 12849883

"Virus-induced autoimmunity may play a causal role in autism. To examine the etiologic link of viruses in this brain disorder, we conducted a serologic study of measles virus, mumps virus, and rubella virus. Viral antibodies were measured by enzyme-linked immunosorbent assay in the serum of autistic children, normal children, and siblings of autistic children. The level of measles antibody, but not mumps or rubella antibodies, was significantly higher in autistic children as compared with normal children (P = 0.003) or siblings of autistic children (P <or= 0.0001). Furthermore, immunoblotting of measles vaccine virus revealed that the antibody was directed against a protein of approximately 74 kd molecular weight. The antibody to this antigen was found in 83% of autistic children but not in normal children or siblings of autistic children. Thus autistic children have a hyperimmune response to measles virus, which in the absence of a wild type of measles infection might be a sign of an abnormal immune reaction to the vaccine strain or virus reactivation."

76. Singh VK et al. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. J Biomed Sci. 2002 Jul-Aug;9(4):359-64. PMID 12145534

"Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73-75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism."

77. Singh VK et al. Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism. Clin Immunol Immunopathol 1998 89(1):105-8. PMID: 9756729

"Considering an autoimmunity and autism connection, brain autoantibodies to myelin basic protein (anti-MBP) and neuron-axon filament protein (anti-NAFP) have been found

in autistic children. In this current study, we examined associations between virus serology and autoantibody by simultaneous analysis of measles virus antibody (measles-IgG), human herpesvirus-6 antibody (HHV-6-IgG), anti-MBP, and anti-NAFP. We found that measles-IgG and HHV-6-IgG titers were moderately higher in autistic children but they did not significantly differ from normal controls. Moreover, we found that a vast majority of virus serology-positive autistic sera was also positive for brain autoantibody: (i) 90% of measles-IgG-positive autistic sera was also positive for anti-MBP; (ii) 73% of measles-IgG-positive autistic sera was also positive for anti-NAFP; (iii) 84% of HHV-6-IgG-positive autistic sera was also positive for anti-MBP; and (iv) 72% of HHV-6-IgG-positive autistic sera was also positive for anti-NAFP. This study is the first to report an association between virus serology and brain autoantibody in autism; it supports the hypothesis that a virus-induced autoimmune response may play a causal role in autism."

vMV, MV & immunity

78. Hussey GD et al. The effect of Edmonston-Zagreb and Schwarz measles vaccines on immune response in infants. J Infect Dis. 1996 Jun; 173(6): 1320-6 PMID: 8648203

The effects of measles immunization on immune responses in infants and the roles of vaccine strain and age of immunization are not known. Eighty-eight children were immunized at 6 or 9 months of age with the Edmonston-Zagreb (EZ) or Schwarz (SW6, SW9) strain of measles vaccine… Therefore, measles immunization resulted in suppression of lymphoproliferation, which was most evident in infants with the highest antibody responses and most immune activation."

79. Auwaerter PG et al. Changes within T cell receptor V beta subsets in infants following measles vaccination. John Hopkins University School of Medicine, Baltimore, MD 21287, USA. Clin Immunol Immunopathol 1996 79(2): 163-70. PMID: 8620622

"Measles produces immune suppression which contributes to an increased susceptibility to other infections. These data suggest that [vaccinal and wild-type] measles virus may affect immune responses in part by altering the T cell receptor repertoire."

80. Schneider-Schaulies S, ter Meulen V. Triggering of and interference with immune activation: interactions of measles virus with monocytes and dendritic cells. Viral Immunol. 2002;15(3):417-28. PMID: 12479392

81. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. J Exp Med 1997;186:813-23

82. Sonoda S, Nakayama T. Detection of measles virus genome in lymphocytes from asymptomatic healthy children. J Med Virol 2001 65(2):381-7 PMID: 11536248

"In 83 individuals immunized with measles vaccine, the vaccine strain genome was detected in 10 (71.4%) of 14 recipients whose PBMC were obtained within 2 months of vaccination."

83. Valsamakis A et al. Strains of measles vaccine differ in their ability to replicate in an damage human thymus. J Infect Dis. 2001 Feb 1; 183(3): 498-502. Johns Hopkins University, Baltimore, Maryland, USA. PMID: 11133383

[Question: How would the thymic-damage findings be exacerbated if tested tissues were selected to represent humans with excessively increased susceptibility, eg, an infant with persisting colic and/or persisting otitis?]

MV & Vitamin A

84. Yalcin SS et al. The effect of live measles vaccines on serum vitamin A levels in healthy children. Acta Paediatr Jpn. 1998 Aug; 40(4): 345-9. PMID: 9745778

"Serum retinol levels have been shown to be depressed during measles infection. This study aims to demonstrate whether there is any decrease in serum vitamin A level following immunization with live viral vaccine and its relation with vaccine seroconversion in children with measles. Since many children receive measles vaccine alone or in combination with measles-mumps-rubella vaccine, we studied serum vitamin A levels and antibody levels in healthy, well-nourished children before and after immunization with monovalent and combined live attenuated measles vaccine… CONCLUSION: Serum vitamin A levels are reduced following vaccination with monovalent and combined live attenuated measles vaccines."

85. Vitamin A administered with measles vaccine to nine-month-old infants does not reduce vaccine immunogenicity. J Nutr. 1999 Aug; 129(8): 1569-73. PMID 10419992 http://www.nutrition.org/cgi/reprint/129/8/1569.pdf

"Among malnourished infants, the geometric mean titer was significantly greater in the vitamin A group compared to the placebo group (ratio of geometric means, 1.57; 95% confidence interval, 1. 18-2.0), but seroconversion rates did not differ."

[Comment: note the theoretical implication that malourished children may have lower cell-mediated immunity and thus generate increased antibody immunity. This is consistent with immunity lab-data in many autistic children.]

86. Yalcin SS, Yurdakok K. Sex-specific differences in serum vitamin A values after measles immunization. Pediatr Infect Dis J. 1999 Aug; 18(8): 747-8. PMID 10462357

87. Semba RD. Vitamin A and immunity to viral, bacterial and protozoan infections. Proc Nutr Soc 1999 58(3): 719-27. PMID 10604208

"…vitamin A and related retinoids play a major role in immunity, including expression of mucins and keratins, lymphopoiesis, apoptosis, cytokine expression, production of antibody, and the function of neutrophils, natural killer cells, monocytes or macrophages, T lymphocytes and B lymphocytes. Recent clinical trials suggest that vitamin A supplementation reduces morbidity and mortality in different infectious diseases, such as measles, diarrhoeal disease, measles-related pneumonia, human immunodeficiency virus infection and malaria. Immune responses vary considerably during different infections, and the available data suggest that the modulation of immune function by vitamin A may also vary widely, depending on the type of infection and immune responses involved."

88. Molina EL, Patel JA. A to Z: vitamin A and zinc, the miracle duo. Indian J Pediatr. 1996 63(4): 427-31. PMID 10832460

"Dietary micronutrients such as vitamins and trace minerals are known modulators of host immune responses against common pathogens. In this respect, vitamin A and zinc have recently received increased attention. Several in vivo and in vitro studies suggest that vitamin A may be a critical player in the mucosal immune responses in the respiratory and gastrointestinal tracts, particularly in undernourished children. The effect may be mediated primarily by stabilization of the membrane of mucosal epithelial cells, as well as enhanced leukocyte functions. The beneficial effect of vitamin A therapy in reducing measles-associated morbidity and mortality suggests its crucial role in defenses against viral pathogens. Zinc is also known affect leukocyte functions such as phagocytosis and T-lymphocyte-mediated immune responses… Dietary supplementation or therapeutic treatment with vitamin A and zinc may be a cheap yet effective means of preventing or treating infections in highly susceptible populations. Additional studies, however, are required to better define the types of pathogens and the specific human populations that may benefit from such therapy."

88b. Chandra RK, Wadhwa M. Nutritional modulation of intestinal mucosal immunity. Immunol Invest. 1989 Jan-May;18(1-4):119-26. PMID: 2659508

"Protein-energy malnutrition results in an increased risk of gastrointestinal infection. This can be attributed in part to impaired immune responses. Cell-mediated immunity is decreased as judged by reduced number and function of thymus-dependent lymphocytes, impaired delayed cutaneous hypersensitivity reactions, and decreased production of lymphokines. Concentration of secretory IgA is reduced and there are fewer intraepithelial lymphocytes. Antibody responses following viral vaccine administration are reduced and there is decrease in natural killer cell activity. In addition, the number of bacteria binding to epithelial cells is increased. These changes are observed also in certain selected nutrient deficiencies, such as that of vitamin A. It

is suggested that impaired systemic and mucosal immunity contributes to the increased frequency and severity of intestinal infections seen in undernourished individuals."

88c. Lie C et al. Impact of large-dose vitamin A supplementation on childhood diarrhoea, respiratory disease and growth. Eur J Clin Nutr. 1993 Feb;47(2):88-96. PMID: 8436094

"One hundred and seventy-two 0.5-3.0-year-old children in a mountainous area of northern Hebei Province of China were randomly assigned to a vitamin A supplementation group (n = 98) or a control group (n = 74) for a 1 year double-blind study. Capsules containing 200,000 IU vitamin A and 40 IU vitamin E were given to the children in the experimental group 3 and 9 months after baseline examination. During the 12 month study period, there was a significant reduction in the incidence of diarrhoea ($P < 0.01$) and respiratory disease ($P < 0.01$) in the children of the experimental group compared to the control. Risk of diarrhoea and respiratory disease were respectively 2.5 and 3.4 times higher in the control children. Serum retinol and IgA levels of the treatment group were significantly higher than that of control group ($P < 0.01$) 7 weeks after first supplementation. There was no significant difference in saliva IgA level between groups. No significant differences in growth were observed. It was concluded that supplementation with large doses of vitamin A decreased the incidence and severity of diarrhoea and respiratory disease in these children, possibly through enhanced activity of the immune system, but had no effect on growth over 1 year."

88d. Sarkar J et al. Vitamin A is required for regulation of polymeric immunoglobulin receptor (pIgR) expression by interleukin-4 and interferon-gamma in a human intestinal epithelial cell line. J Nutr. 1998 Jul;128(7):1063-9. PMID: 9649586

"The secretory immunoglobulin A (IgA) antibody response to infections of mucosal surfaces requires transport of IgA from the basal to apical surface of mucosal epithelial cells by a specific transport protein, the polymeric immunoglobulin receptor (pIgR). We have tested the hypothesis that the vitamin A metabolite all-trans retinoic acid (RA) is required for the regulation of pIgR expression by the cytokines interleukin-4 (IL-4) and interferon-gamma (IFN-gamma) in HT-29 cells… These data indicate that RA strongly interacts with IL-4 and IFN-gamma to regulate pIgR expression in HT-29 cells, suggesting that vitamin A may be required for proper in vivo regulation of IgA transport in response to mucosal infections."

88e. Nikawa T et al. Vitamin A prevents the decline in immunoglobulin A and Th2 cytokine levels in small intestinal mucosa of protein-malnourished mice. J Nutr 1999 129(5):934-41. PMID: 10222382

"These results suggest that large oral supplements of vitamin A may preserve mucosal IgA level during protein malnutrition, possibly by stimulating Th2 cytokine production and thereby, inducing resistance against infection."

88f. Aukrust P et al. pal.aukrust@klinmed.uio.no Decreased vitamin A levels in common variable immunodeficiency: vitamin A supplementation in vivo enhances immunoglobulin production and downregulates inflammatory responses. Eur J Clin Invest. 2000 30(3):252-9. PMID: 10692003

"BACKGROUND: Vitamin A has a broad range of immunological effects, and vitamin A deficiency is associated with recurrent infections. Common variable immunodeficiency (CVI) is a group of B-cell deficiency syndromes with impaired antibody production and recurrent bacterial infections as the major manifestations, but the immunological dysfunctions may also include T cells and macrophages. In the present study we examined the possible role of vitamin A deficiency in CVI… CONCLUSION: A considerable subgroup of CVI patients appears to be characterized by low vitamin A levels. Our findings support a possible role for vitamin A supplementation in CVI, perhaps resulting in enhanced immunoglobulin synthesis and downregulated inflammatory responses.

88g. Bjersing JL et al. jan.bjersing@immuno.gu.se Loss of ileal IgA+ plasma cells and of CD4+ lymphocytes in ileal Peyer's patches of vitamin A deficient rats. Clin Exp Immunol. 2002 Dec;130(3):404-8. PMID: 12452829

"Child mortality in diarrhoeal disease is increased significantly by vitamin A deficiency in poor countries. The pathological mechanisms are not known in detail. However, in this paper we report that vitamin A-deficient Wistar rats had much reduced IgA+ plasma cells in the ileal lamina propria (eightfold reduction from 470 cells/mm(2), P = 0.009), as well as a prominent reduction of CD4+ cells in the parafollicular regions of ileal Peyer's patches (reduction from 7200 to 105 cells/mm(2), P = 0.009). IL-2Ralpha-chain (CD25) positive lymphocytes in the ileal Peyer's patches were also reduced significantly in vitamin A deficiency (from 1400 to 300 cells/mm(2), P = 0.009). The density of CD8 cells tended to be increased relative to the control animals (from 5100 to 6000 cells/mm(2), not statistically significant). In conclusion, the marked decrease of lamina propria IgA+ plasma cells may be one cause of the high diarrhoeal mortality in vitamin A deficiency. This, in turn, appears to be related to reduced numbers of activated or regulatory CD4+ T cells in Peyer's patches.

88h. Kim JY, Chung BH. Effects of combination dietary conjugated linoleic acid with vitamin A (retinol) and selenium on the response of the immunoglobulin production in mice. J Vet Sci. 2003 Apr;4(1):103-8. PMID: 12819373

"The dietary effect of conjugated linoleic acid (CLA) on the response of the immunoglobulin (serum and tissue) production in Balb/C mice was examined at three

doses: 0 %(control), 0.5% and 1.5%. The combination effects of CLA with vitamin ADE or selenium also were investigated. CLA at 0.5% increased serum immunoglobulin A, G, mesenteric lymph node (MHN) and gut luminal IgA (secretory IgA) levels. However, 1.5% CLA decreased SIgG slightly. CLA both alone and combined with vitamin ADE and selenium did not affect serum IgE. The levels of immunoglobulin concentration in the 0.5% CLA group were higher than those in the 1.5% CLA group. The level of serum IgG in 1.5% CLA combined with selenium was maintained at the same level as that of control. It is considered that overdoses of CLA (1.5%) even depressed the production of immunoglobulin but selenium and/or vitamin inhibited this activity to a certain extent.In this study, dietary CLA increased immunoglobulin production in a dose-dependent manner. Vitamin ADE and Selenium combined with CLA also increased the immunoglobulin production response except serum IgE.

89. D'Souza RM, D'Souza R. Vitamin A for preventing secondary infections in children with measles—a systematic review. J Trop Pediatr. 2002 48(2):72-7. PMID 12022432

90. D'Souza RM, D'Souza R. Vitamin A for treating measles in children. Cochrane Database Syst Rev. 2002;(1):CD001479. PMID 11869601

"REVIEWER'S CONCLUSIONS: Although we did not find evidence that a single dose of 200,000 IU of vitamin A per day was associated with reduced mortality among children with measles, there was evidence that the same dose given for two days was associated with a reduced risk of overall mortality and pneumonia specific mortality. The effect was greater in children under the age of two years."

91. Madhulika et al. Vitamin A supplementation in post-measles complications. J Trop Pediatr. 1994 Oct;40(5):305-7. PMID 7807628.

The case fatality rate was 16 per cent in those who received VIT.A, while the same was 32 per cent in those who did not receive Vit.A ($P < 0.02$)."

92. Hussey GD, Klein M. Routine high-dose vitamin A therapy for children hospitalized with measles. J Trop Pediatr. 1993 39(6):342-5. PMID 8133555

Measles is without specific therapy and remains important globally as a cause of childhood death. In controlled studies, high-dose vitamin A therapy (Hi-VAT)—with 400,000 IU vitamin A--has been demonstrated to markedly reduce measles-associated morbidity and mortality."

93. Butler JC et al. Measles severity and serum retinol (vitamin A) concentration among children in the United States. Pediatrics. 1993 Jun;91(6):1176-81. PMID 8502524

94. Bluhm DP, Summers RS. Plasma vitamin A levels in measles and malnourished pediatric patients and their implications in therapeutics. J Trop Pediatr 1993 39(3):179-82. PMID 8326539

This study has shown that there is a high incidence of baseline hyporetinaemia in these patients. The mean retinol plasma levels return to within normal limits after 8 days of either routine treatment or vitamin A supplementation."

95. Ogaro FO et al. Effect of vitamin A on diarrhoeal and respiratory complications of measles. Trop Geogr Med. 1993;45(6):283-6. PMID 8116059

"These findings, along with those from three other trials in Africa, suggest that high dose vitamin A reduces the severity of complications during measles."

96. Coutsoudis A et al. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. Pediatr Infect Dis J. 1992 11(3):203-9. PMID 1565535

These findings reinforce results from animal studies that show that the pathways of vitamin A activity in decreasing morbidity and mortality are partly founded on selective immunopotentiation."

97. Frieden TR et al. Vitamin A levels and severity of measles. New York City. Am J Dis Child. 1992 Feb;146(2):182-6. PMID 1285727

Recent studies show that vitamin A levels decrease during measles and that vitamin A therapy can improve measles outcome in children in the developing world. Vitamin A levels of children with measles have not been studied in developed countries. We therefore measured vitamin A levels in 89 children with measles younger than 2 years and in a reference group in New York City, NY. Vitamin A levels in children with measles ranged from 0.42 to 3.0 mumol/L; 20 (22%) were low. Children with low levels were more likely to have fever at a temperature of 40 degrees C or higher (68% vs 44%), to have fever for 7 days or more (54% vs 23%), and to be hospitalized (55% vs 30%). Children with low vitamin A levels had lower measles-specific antibody levels. No child in the reference group had a low vitamin A level. Our data show that many children younger than 2 years in New York City have low vitamin A levels when ill with measles, and that such children seem to have lower measles-specific antibody levels and increased morbidity. Clinicians may wish to consider vitamin A therapy for children younger than 2 years with severe measles…"

Autism, malnutrition, flora

98: Susan Owens, Defeat Autism Now! Think-tank presentation. Philadelphia 2003.

99. Finegold SM et al. Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 2002 35(Suppl 1):S6-S16 PMID 12173102

"Some cases of late-onset (regressive) autism may involve abnormal flora because oral vancomycin, which is poorly absorbed, may lead to significant improvement in these children. Fecal flora of children with regressive autism was compared with that of control children, and clostridial counts were higher. The number of clostridial species found in the stools of children with autism was greater than in the stools of control children. Children with autism had 9 species of Clostridium not found in controls, whereas controls yielded only 3 species not found in children with autism. In all, there were 25 different clostridial species found. In gastric and duodenal specimens, the most striking finding was total absence of non-spore-forming anaerobes and microaerophilic bacteria from control children and significant numbers of such bacteria from children with autism. These studies demonstrate significant alterations in the upper and lower intestinal flora of children with late-onset autism and may provide insights into the nature of this disorder."

100. Bendel CM. Colonization and epithelial adhesion in the pathogenesis of neonatal candidiasis. Semin Perinatol. 2003 Oct;27(5):357-64. PMID 14626499

"C albicans is the most commonly isolated species in colonized or infected infants. Over the past decade the incidence of both colonization and infection with other Candida species, particularly C parapsilosis, has risen dramatically… Microbial factors also augment colonization, including the ability of Candida to adhere to human epithelium."

101. Walker WA. Role of nutrients and bacterial colonization in the development of intestinal host defense. J Pediatr Gastroenterol Nutr. 2000;30 Suppl 2:S2-7. PMID 10749395

102. Dai D, Walker WA. Protective nutrients and bacterial colonization in the immature human gut. Adv Pediatr. 1999;46:353-82. PMID 10645469

"The normal human microflora is a complex ecosystem that is in part dependent on enteric nutrients for establishing colonization. The gut microbiota are important to the host with regard to metabolic functions and resistance to bacterial infections. At birth, bacterial colonization of a previously germ-free human gut begins. Diet and environmental conditions can influence this ecosystem. A breast-fed, full-term infant has a preferred intestine microbiota in which bifidobacteria predominate over potentially harmful bacteria, whereas in formula-fed infants, coliforms, enterococci, and bacteroides predominate. The pattern of bacterial colonization in the premature

neonatal gut is different from that in the healthy, full-term infant gut… Probiotics and prebiotics modulate the composition of the human intestinal microflora to the benefit of the host. These beneficial effects may result in the suppression of harmful microorganisms, the stimulation of bifidobacterial growth, or both. In the future, control and manipulation of the bacterial colonization… may be a new approach to the prevention and treatment of intestinal infectious diseases of various etiologies."

103. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breastfed infants. Acta Paediatr Suppl. 1999 Aug;88(430):47-57. PMID 10569223

"This article summarizes the published data on the intestinal microflora in breastfed infants published during the last 15 y. Enterobacteria and enterococci are found in high numbers in most infants during the first week of life. Bifidobacteria and Bacteroides spp. are found in increasing numbers at the following weeks. The intestinal microflora in breastfed infants can also be followed by different biochemical parameters. Acetic acid is found in higher concentrations in breastfed than in formula-fed infants. Degradation of mucin starts later in breastfed than in formula-fed infants. The conversion of cholesterol to coprostanol is also delayed by breastfeeding. Geographical differences in the composition of the intestinal microflora in infants have been reported, i.e. enterobacteria, enterococci, bifidobacteria, lactobacilli and bacteroides show different occurrences in developed and developing countries. There are minor differences in the infant's intestinal microflora due to breastfeeding or/and formula feeding."

104. Belley A et al. Intestinal mucins in colonization and host defense against pathogens. Am J Trop Med Hyg. 1999 Apr;60(4 Suppl):10-5 PMID 10344672

"Intestinal mucins are key components of the first line of host defense against intestinal pathogens. These large glycoconjugates secreted by specialized exocrine goblet cells form viscous gels that trap microorganisms and irritants and limit their diffusion to the intestinal epithelium. Moreover, they allow for colonization by indigenous bacterial flora that prevents attachment of pathogenic microbes. The interaction between microbes and mucins involves mucin carbohydrate side chains and microbial adhesin molecules. Certain microorganisms and disease states may alter mucin biochemistry or expression…"

105. Jarvis WR. The epidemiology of colonization. Infect Control Hosp Epidemiol. 1996 Jan;17(1):47-52. PMID 8789688

"Colonization is the presence of a microorganism in or on a host, with growth and multiplication but without any overt clinical expression or detected immune response in the host at the time it is isolated. Normal colonization in humans begins during the birth process and through subsequent contacts with the inanimate or animate environments until a delicately balanced "normal" flora is established; subsequently, the precise

components of this flora evolve. This normal flora, such as coagulase-negative Staphylococcus or Staphylococcus aureus on the skin or Candida albicans in the gastrointestinal tract, vagina, or perineal area, can result in infection when normal body defenses are impaired through underlying disease, immunomodulating therapy, or the use of invasive devices, or when the delicate balance of the normal flora is altered through antimicrobial therapy…"

106. Fitzgerald JF. Colonization of the gastrointestinal tract. Mead Johnson Symp Perinat Dev Med. 1977;(11):35-8. PMID 347190

"The alimentary tract is sterile at birth but colonic colonization is relatively complete by the end of the first week of life. The upper alimentary tract is colonized by microorganisms normally inhabiting the oral cavity. The colon on the other hand appears to be colonized by microorganisms originating in the maternallower alimentary tract. The colonic flora of infants is affected by diet (breast or formula feedings). The presence of a "fecal-type" flora in the proximal small bowel should be considered abnormal…"

Gastrointestinal miscellany

107. Edelson SB, Cantor DS. Autism: xenobiotic influences. Toxicol Ind Health. 1998 Jul-Aug;14(4):553-63. PMID 9664646

"The advances in medical technology during the last four decades has provided evidence for an underlying neurological basis for autism. The etiology for the variations of neurofunctional anomalies found in the autistic spectrum behaviors appears inconclusive as of this date but growing evidence supports the proposal that chronic exposure to toxic agents, i.e., xenobiotic agents, to a developing central nervous system may be the best model for defining the physiological and behavioral data found in these populations. A total of 20 subjects (15 males and 5 females) who received a formal diagnosis of autism by a developmental pediatrician, pediatric neurologist, or licensed psychologist were included. The mean age for the sample was 6.35 yrs offnge = 3-12 years)… It is most noteworthy that of the 20 cases examined for this study, 100% of the cases showed liver detoxication profiles outside of normal. An examination of 18 autistic children in blood analyses that were available showed that 16 of these children showed evidence of levels of toxic chemicals exceeding adult maximum tolerance. [Chelation challenge is more accurately instructive.] In the two cases where toxic chemical levels were not found, there was abnormal D-glucaric acid findings suggesting abnormal xenobiotic influences on liver detoxication processes. A proposed mechanism for the interaction of xenobiotic toxins with immune system dysfunction and continuous and/or progressive endogenous toxicity is presented as it relates to the development of behaviors found in the autistic spectrum.

108. Thony B et al. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J. 2000 347 Pt 1:1-16. PMID 10727395

109. Cohen BI. The significance of ammonia/gamma-aminobutyric acid (GABA) ratio for normality and liver disorders. Med Hypotheses. 2002 Dec;59(6):757-8. PMID 12445521

110. Kidd PM Autism, an extreme challenge to integrative medicine. Part: 1: The knowledge base. Altern Med Rev. 2002 Aug;7(4):292-316. PMID: 12197782

"Autism, archetype of the autistic spectrum disorders (ASD), is a neurodevelopmental disorder characterized by socially aloof behavior and impairment of language and social interaction. Its prevalence has surged in recent years. Advanced functional brain imaging has confirmed pervasive neurologic involvement. Parent involvement in autism management has accelerated understanding and treatment. Often accompanied by epilepsy, cognitive deficits, or other neurologic impairment, autism manifests in the first three years of life and persists into adulthood. Its etiopathology is poorly defined but likely multifactorial with heritability playing a major role. Prenatal toxic exposures (teratogens) are consistent with autism spectrum symptomatology. Frequent vaccinations with live virus and toxic mercurial content (thimerosal) are a plausible etiologic factor. Autistic children frequently have abnormalities of sulfoxidation and sulfation that compromise liver detoxification, which may contribute to the high body burden of xenobiotics frequently found. Frequent copper-zinc imbalance implies metallothionein impairment that could compound the negative impact of sulfur metabolism impairments on detoxification and on intestinal lining integrity. Intestinal hyperpermeability manifests in autistic children as dysbiosis, food intolerances, and exorphin (opioid) intoxication, most frequently from casein and gluten. Immune system abnormalities encompass derangement of antibody production, skewing of T cell subsets, aberrant cytokine profiles, and other impairments consistent with chronic inflammation and autoimmunity. Coagulation abnormalities have been reported."

111. Kidd PM. Autism, an extreme challenge to integrative medicine. Part 2: medical management. Altern Med Rev. 2002 Dec;7(6):472-99. PMID 12495373

"Autism and allied autistic spectrum disorders (ASD) present myriad behavioral, clinical, and biochemical abnormalities. Parental participation, advanced testing protocols, and eclectic treatment strategies have driven progress toward cure. Behavioral modification and structured education are beneficial but insufficient. Dietary restrictions, including removal of milk and other casein dairy products, wheat and other gluten sources, sugar, chocolate, preservatives, and food coloring are beneficial and prerequisite to benefit from other interventions. Individualized IgG or IgE testing can identify other troublesome foods but not non-immune mediated food sensitivities. Gastrointestinal improvement rests on controlling Candida, [parasites and pathogenic bacteria], and using probiotic bacteria and nutrients to correct dysbiosis and decrease gut

permeability. Detoxification of mercury and other heavy metals by DMSA/DMPS chelation can have marked benefit. Documented sulfoxidation-sulfation inadequacies call for sulfur-sulfhydryl repletion and other liver p450 support. Many nutrient supplements are beneficial and well tolerated, including dimethylglycine (DMG) and a combination of pyridoxine (vitamin B6) and magnesium, both of which benefit roughly half of ASD cases. Vitamins A, B3, C, and folic acid; the minerals calcium and zinc; cod liver oil; and digestive enzymes, all offer benefit… Current pharmaceuticals fail to benefit the primary symptoms and can have marked adverse effects. Individualized, in-depth clinical and laboratory assessments and integrative parent-physician-scientist cooperation are the keys to successful ASD management."

112. Kidd PM. An approach to the nutritional management of autism. Altern Ther Health Med. 2003 Sep-Oct;9(5):22-31 PMID 14526708

Glutathione

113. Wang XF, Cynader MS. Astrocytes provide cysteine to neurons by releasing glutathione. J Neurochem. 2000 74(4):1434-42. PMID 10737599

"Cysteine is the rate-limiting precursor of glutathione synthesis. Evidence suggests that astrocytes can provide cysteine and/or glutathione to neurons. However, it is still unclear how cysteine is released and what the mechanisms of cysteine maintenance by astrocytes entail. In this report, we analyzed cysteine, glutathione, and related compounds in astrocyte conditioned medium using HPLC methods. In addition to cysteine and glutathione, cysteine-glutathione disulfide was found in the conditioned medium. In cystine-free conditioned medium, however, only glutathione was detected. These results suggest that glutathione is released by astrocytes directly and that cysteine is generated from the extracellular thiol/disulfide exchange reaction of cystine and glutathione: glutathione + cystine<-->cysteine + cysteine-glutathione disulfide. Conditioned medium from neuron-enriched cultures was also assayed in the same way as astrocyte conditioned medium, and no cysteine or glutathione was detected. This shows that neurons cannot themselves provide thiols but instead rely on astrocytes. We analyzed cysteine and related compounds in rat CSF and in plasma of the carotid artery and internal jugular vein. Our results indicate that cystine is transported from blood to the CNS and that the thiol/disulfide exchange reaction occurs in the brain in vivo. Cysteine and glutathione are unstable and oxidized to their disulfide forms under aerobic conditions. Therefore, constant release of glutathione by astrocytes is essential to maintain stable levels of thiols in the CN*S*."

114. Fonnum F, Lock EA. The contributions of excitotoxicity, glutathione depletion and DNA repair in chemically induced injury to neurones: exemplified with toxic effects on cerebellar granule cells. J Neurochem. 2004 Feb;88(3):513-31. PMID: 14720201

"Six chemicals, 2-halopropionic acids, thiophene, methylhalides, methylmercury, methylazoxymethanol (MAM) and trichlorfon (Fig. 1), that cause selective necrosis to the cerebellum, in particular to cerebellar granule cells, have been reviewed… All six compounds decrease cerebral glutathione (GSH), due to conjugation with the xenobiotic, thereby reducing cellular antioxidant status and making the cells more vulnerable to reactive oxygen species. 2-Halopropionic acids and methylmercury appear to also act via an excitotoxic mechanism leading to elevated intracellular Ca2+, increased reactive oxygen species and ultimately impaired mitochondrial function… We propose that a combination of reduced antioxidant status plus excitotoxicity or DNA damage is required to cause cerebellar neuronal cell death with these chemicals. The small size of cerebellar granule cells, the unique subunit composition of their N-methyl-d-aspartate (NMDA) receptors, their low DNA repair ability, low levels of calcium-binding proteins and vulnerability during postnatal brain development and distribution of glutathione and its conjugating and metabolizing enzymes are all important factors in determining the sensitivity of cerebellar granule cells to toxic compounds."

115. Ehrhart J, Zeevalk GD. Cooperative interaction between ascorbate and glutathione during mitochondrial impairment in mesencephalic cultures. J Neurochem 2003 86(6):1487-97. PMID: 12950457

"These findings indicate that ascorbate contributes to the maintenance of GSSG/GSH status during oxidative stress through scavenging of radical species, attenuation of GSH efflux and redistribution of GSSG to the formation of mixed disulfides. It is speculated that these events are linked by glutaredoxin, an enzyme shown to contain both dehydroascorbate reductase as well as glutathione thioltransferase activities."

116. Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biol Chem. 2003 384(4):505-16. PMID: 12751781

"The antioxidant glutathione (GSH) is essential for the cellular detoxification of reactive oxygen species in brain cells. A compromised GSH system in the brain has been connected with the oxidative stress occuring in neurological diseases. Recent data demonstrate that besides intracellular functions GSH has also important extracellular functions in brain. In this respect astrocytes appear to play a key role in the GSH metabolism of the brain, since astroglial GSH export is essential for providing GSH precursors to neurons. Of the different brain cell types studied in vitro only astrocytes release substantial amounts of GSH. In addition, during oxidative stress astrocytes efficiently export glutathione disulfide (GSSG)…. This review focuses on recent results on the export of GSH and GSSG from brain cells as well as on the functions of extracellular GSH in the brain. In addition, implications of disturbed GSH pathways in brain for neurodegenerative diseases will be discussed."

117. Pastore A et al. Analysis of glutathione: implication in redox and detoxification. Clin Chim Acta. 2003 Jul 1;333(1):19-39. PMID: 12809732

"BACKGROUND: Glutathione is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydroperoxides… Glutathione is a critical factor in protecting organisms against toxicity and disease. This review may turn useful for analysing the glutathione homeostasis, whose impairment represents an indicator of tissue oxidative status in human subjects."

118. Sheehan D et al. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J. 2001 Nov 15;360(Pt 1):1-16. PMID: 11695986

"The glutathione transferases (GSTs; also known as glutathione S-transferases) are major phase II detoxification enzymes found mainly in the cytosol. In addition to their role in catalysing the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also carry out a range of other functions."

119. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology. 2000 Sep;61(3):154-66. PMID: 10971201

"Two supergene families encode proteins with glutathione S-transferase (GST) activity: the family of soluble enzymes comprises at least 16 genes; the separate family of microsomal enzymes comprises at least 6 genes. These two GST families are believed to exert a critical role in cellular protection against oxidative stress and toxic foreign chemicals. They detoxify a variety of electrophilic compounds, including oxidized lipid, DNA and catechol products generated by reactive oxygen species-induced damage to intracellular molecules. An increasing number of GST genes are being recognized as polymorphic. Certain alleles, particularly those that confer impaired catalytic activity (e.g. GSTM1(*)0, GSTT1(*)0), may be associated with increased sensitivity to toxic compounds…"

120. Droge W, Breitkreutz R. Glutathione and immune function. Proc Nutr Soc. 2000 Nov;59(4):595-600. PMID: 11115795

"The immune system works best if the lymphoid cells have a delicately balanced intermediate level of glutathione. Even moderate changes in the intracellular glutathione level have profound effects on lymphocyte functions. Certain functions, such as the DNA synthetic response, are exquisitely sensitive to reactive oxygen intermediates and, therefore, are favoured by high levels of the antioxidant glutathione. Certain signal pathways, in contrast, are enhanced by oxidative conditions and favoured by low intracellular glutathione levels. The available evidence suggests that the lymphocytes from healthy human subjects have, on average, an optimal glutathione level. There is no indication that immunological functions such as resistance to infection or the response to vaccination may be enhanced in healthy human subjects by administration of glutathione or its precursor amino acid cysteine. However,

immunological functions in diseases that are associated with a cysteine and glutathione deficiency may be significantly enhanced and potentially restored by cysteine supplementation..."

121. Functions of glutathione and glutathione disulfide in immunology and immunopathology. FASEB J. 1994 Nov;8(14):1131-8. PMID: 7958618

"Even a moderate increase in the cellular cysteine supply elevates the intracellular glutathione (GSH) and glutathione disulfide (GSSG) levels and potentiates immunological functions of lymphocytes…"

122. Enhancement of tissue glutathione for antioxidant and immune functions in malnutrition. Biochem Pharmacol. 1994 Jun 15;47(12):2113-23. PMID: 8031307

123. Fernandez-Checa JC et al. Oxidative stress: role of mitochondria and protection by glutathione. Biofactors. 1998;8(1-2):7-11. PMID: 9699001

124. N-acetylcysteine. Altern Med Rev. 2000 Oct;5(5):467-71. PMID: 11056417 [No authors listed]

"N-acetylcysteine (NAC) is the acetylated precursor of both the amino acid L-cysteine and reduced glutathione (GSH). Historically it has been used as a mucolytic agent in chronic respiratory illnesses as well as an antidote for hepatotoxicity due to acetaminophen overdose. More recently, animal and human studies of NAC have shown it to be a powerful antioxidant and a potential therapeutic agent in the treatment of cancer, heart disease, HIV infection, heavy metal toxicity, and other diseases characterized by free radical oxidant damage. NAC has also been shown to be of some value in treating Sjogren's syndrome, smoking cessation, influenza, hepatitis C, and myoclonus epilepsy."

125. Cai J et al. Inhibition of influenza infection by glutathione. Free Radic Biol Med. 2003 Apr 1;34(7):928-36. PMID: 12654482

"Infection by RNA virus induces oxidative stress in host cells. Accumulating evidence suggests that cellular redox status plays an important role in regulating viral replication and infectivity. In this study, experiments were performed to determine whether the thiol antioxidant glutathione (GSH) blocked influenza viral infection in cultures of Madin-Darby canine kidney cells or human small airway epithelial cells. Protection against production of active virus particles was observed at a low (0.05-0.1) multiplicity of infection (MOI). GSH inhibited expression of viral matrix protein and inhibited virally induced caspase activation and Fas upregulation. In BALB/c mice, inclusion of GSH in the drinking water decreased viral titer in both lung and trachea homogenates 4 d after intranasal inoculation with a mouse-adapted influenza strain A/X-31. Together, the data suggest that the thiol antioxidant GSH has an anti-influenza activity in vitro and in vivo.

Oxidative stress or other conditions that deplete GSH in the epithelium of the oral, nasal, and upper airway may, therefore, enhance susceptibility to influenza infection.

Tylenol depletes GSH

126. Slattery JT et al. Dose-dependent pharmacokinetics of acetaminophen: evidence of glutathione depletion in humans. Clin Pharmacol Ther 1987 41(4):413-8 PMID 3829578

127. Lauterburg BH, Mitchell JR. Therapeutic doses of acetaminophen stimulate the turnover of cysteine and glutathione in man. J Hepatol. 1987 Apr;4(2):206-11. PMID 3584929

"The data indicate that therapeutic doses of acetaminophen markedly stimulate the rate of turnover of the pool of cysteine available for the synthesis of GSH, most likely due to an increased rate of synthesis of GSH which is required to detoxify the toxic metabolite of acetaminophen. Patients who are not able to respond to a similar demand on their stores of GSH by increasing the synthesis of GSH may be at higher risk of developing hepatic injury from drugs that require GSH for their detoxification."

128. Spielberg SP. Acetaminophen toxicity in lymphocytes heterozygous for glutathione synthetase deficiency. Can J Physiol Pharmacol 1985 63(5):468-71 PMID 4041989

Heterozygous cells failed to use N-acetylcysteine as efficiently to resynthesize glutathione, and the cells were not protected from acetaminophen toxicity. Heterozygotes may be at increased risk of toxicity from drugs whose metabolites are detoxified by glutathione conjugation."

129. Depletion of hepatic glutathione in rats impairs phagocytosis in vivo. Arch Toxicol Suppl 1989;13:326-9 PMID 2774956

GSH & thimerosal

130. Homozygous gene deletions of the glutathione S-transferases M1 and T1 are associated with thimerosal sensitization. Int Arch Occup Environ Health 2000 73(6):384-8 PMID 11007341

131. Muller M et al. Inhibition of the human erythrocytic glutathione-S-transferase T1 (GST T1) by thimerosal. Int J Hyg Environ Health 2001 203(5-6):479-81. PMID 11556154

132. Corrales F et al. Inhibition of glutathione synthesis in the liver leads to S-adenosyl-L-methionine synthetase reduction. Hepatology 1991 14(3):528-33. PMID 1874498

[Note etiologic connection with thimerosal and methionine synthase, cite 20]

133. Pajares MA et al. Modulation of rat liver S-adenosylmethionine synthetase activity by glutathione. J Biol Chem 1992 267(25):17598-605. PMID 1517209

http://www.jbc.org/cgi/reprint/267/25/17598.pdf

[Note etiologic connection with thimerosal and methionine synthase, cite 20]

134a. Meister A et al. Intracellular cysteine and glutathione delivery systems. J Am Coll Nutr. 1986;5(2):137-51. PMID 3722629

134b. Jill James & colleagues. Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors. Neurotoxicology, in press 2004.

Chorioamnionitis, fetal, placental

135. Abruptio placentae and chorioamnionitis-microbiological and histologic correlation. Acta Obstet Gynecol Scand. 1999 May;78(5):363-6 PMID 10326877

"Conclusion: The incidence of silent chorioamnionitis (placental membrane culture positivity) is higher in the abruptio placentae."

136. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics. 2002 Oct;110(4):673-80 PMID 12359779

137. Fetal endothelial cells express vascular cell adhesion molecule in the setting of chorioamnionitis. Am J Reprod Immunol 2000 43(5):259-63 PMID 12359779

138. Chorioamnionitis and uterine function. Obstet Gynecol 2000; 95:909-12 PMID: 10831982

"Several small studies have suggested that chorioamnionitis has an inhibitory effect upon labor, characterized by decreased uterine contractility, decreased sensitivity to oxytocin stimulation, and subnormal cervical dilation." [3 cites]

139. Effect of amniotic fluid bacteria on the course of labor in nulliparous women at term

Obstet Gynecol 68:587-592 1986 PMID: 3763067

[Nulliparous – no prior live births]

"Patients with intraamniotic infection have an increased rate of cesarean delivery… These results support a causal relationship between high-virulence bacteria in the amniotic fluid and poor cervical dilation response to oxytocin…"

"Friedman… studied nulliparous patients with 'amniotic infection syndrome' and found that 70.5% had labor dysfunction. More recent reports have confirmed this association and have also identified an increased frequency of cesarean delivery among these women."

"Koh et al… reported a 43% cesarean section rate in 140 patients with clinical 'chorioamnionitis.' "

"Two-thirds of the cesarean sections were performed because of poor progress in labor despite the use of oxytocin…"

140. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. Am J Obstet Gynecol. 1998 Jul;179(1):186-93

141. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. Am J Pathol 1999 155(1):293-301 PMID 10393861

142. Maternal periodontal disease is associated with an increased risk for preeclampsia.

Obstet Gynecol. 2003 Feb;101(2):227-31 PMID 12576243

Colic, cow's milk allergy in breast fed infants

143. Colic in breast-milk-fed infants: treatment by temporary substitution of neocate infant formula. Acta Paediatr 2000 Jul;89(7):795-802

144. Development of cow's milk allergy in breast-fed infants. Clin Exp Allergy 2001 Jul;31(7):978-87

145. Cow's milk allergy in infancy. Curr Opin Allergy Clin Immunol. 2002 2(3):217-25

146. Cow's milk allergy presented with bloody stools from day 1 of life. Eur J Pediatr. 2003 Mar;162(3):214-5

147. Host A. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol. 2002 Dec;89(6 Suppl 1):33-7. PMID: 12487202

148. Bahna SL. Cow's milk allergy versus cow milk intolerance. Ann Allergy Asthma Immunol. 2002 Dec;89(6 Suppl 1):56-60. PMID: 12487206

149. Magazzu G, Scoglio R. Gastrointestinal manifestations of cow's milk allergy. Ann Allergy Asthma Immunol. 2002 Dec;89(6 Suppl 1):65-8. PMID: 12487208

149b. Iacono G et al. Severe infantile colic and food intolerance: a long-term prospective study. J Pediatr Gastroenterol Nutr. 1991 Apr;12(3):332-5.

"To determine the relationship between infantile colic and cow's milk protein intolerance (CMPI) in formula-fed infants, 70 infants (38 male, 32 female) were selected, with mean age 30.2 ± 21.4 days, with severe colic (duration of crying greater than 4 h per day for 5 days per week). In 50 of the infants in the study group (71.4%) there was a remission of symptoms when cow's milk protein (CMP) was eliminated from the diet. Two successive challenges caused the return of symptoms in all these 50 infants. There was a positive anamnesis for atopy in 9 of 50 of the patients with CMP-related colic and in 1 of 20 of those with non-CMP-related colic (p greater than 0.05). A follow-up period of 18 months' mean duration showed that 22 of 50 (44%) of the infants with CMP-related colic and 1 of 20 (5%) of those with non-CMP-related colic developed an overt alimentary intolerance (p less than 0.02). We conclude that a considerable percentage of the infants with severe colic also have CMPI and that in these cases, dietetic treatment should be the first therapeutic approach."

Chronic Diarrhea of Infancy

150. Chronic protracted diarrhea of infancy: a nutritional disease. Pediatrics. 1983 Dec;72(6):786-800. PMID 6417622

151. Pathogenesis of small-intestinal mucosal lesions in chronic diarrhea of infancy: I. A light microscopic study. J Pediatr Gastroenterol Nutr. 1990 Nov;11(4):455-63. PMID: 2262834

152. Pathogenesis of small-intestinal mucosal lesions in chronic diarrhea of infancy: II. An electron microscopic study. J Pediatr Gastroenterol Nutr 1990 11(4):464-80 PMID 2262835

153. Mehta DI, Blecker U. Chronic diarrhea in infancy and childhood. J La State Med Soc. 1998 Sep;150(9):419-29. PMID 9785754

Recurrent otitis

154. Recent advances in otitis media. 6. Microbiology and immunology. Ann Otol Rhinol Laryngol Suppl. 2002 188:62-81. PMID 11968862

155. Viral-Bacterial Synergy in Otitis Media: Implications for Management. Curr Infect Dis Rep. 2000 Apr;2(2):154-159. PMID: 11095851

156. Chonmaitree T et al. Presence of cytomegalovirus and herpes simplex virus in middle ear fluids from children with acute otitis media. Clin Infect Dis 1992 15(4):650-3 PMID 1330014

157. The common mucosal immune system and current strategies for induction of immune responses in external secretions. J Clin Immunol. 1987 Jul;7(4):265-76. PMID: 3301884

158. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a common mucosal immune system in humans. Proc Natl Acad Sci U S A. 1987 Apr;84(8):2449-53. PMID: 3470804

159. Management of chronic otitis media with effusion: the role of glutathione. Laryngoscope. 2001 Aug;111(8):1486-9. PMID: 11568588

"BACKGROUND: The inflammatory cells documented in chronic otitis media with effusion (OME) spontaneously release oxidants which can induce middle ear (ME) epithelial cell damage. Glutathione (GSH), a major extracellular antioxidant in humans, plays a central role in antioxidant defense. PURPOSE: To evaluate the effects of GSH treatment on chronic otitis media with effusion (OME). SUBJECTS AND INTERVENTION: Sixty children with chronic OME were enrolled, 30 of whom were randomly assigned to the treatment group and 30 to the placebo group. Patients in the treatment group received 600 mg glutathione in 4 mL saline per day subdivided into five 2-minute administrations given by nasal aerosol every 3 or 4 waking hours for 2 weeks. Patients in the control group received 4 mL saline per day following the same procedure as for GSH treatment. RESULTS: Three months after therapy improvement had occurred in 66.6% of patients in the GSH-treated group and in 8% of the control subjects (P <.01). CONCLUSION: On the basis of these results, GSH treatment could be considered for the nonsurgical management of chronic OME."

160. Cow's milk allergy is associated with recurrent otitis media during childhood. Acta Otolaryngol. 1999;119(8):867-73. PMID 10728925

Gluten hypersensitivity

161. Frick TJ, Olsen WA. Celiac disease and the spectrum of gluten sensitivity. Gastroenterologist. 1994 Dec;2(4):285-92. PMID: 7866735

"Celiac disease is a well-known entity in which intolerance to wheat gluten and related proteins from barley, rye, and oats (collectively known as prolamins) damage intestinal mucosa. New insights into the pathology of the celiac intestinal lesion point to a wider spectrum of gluten sensitivity than previously thought. Recent advances in immunology and genetics have shed light on the underlying mechanisms and risks associated with the disease. Although the classical manifestations are well known, the wide variety of

clinical presentations make celiac disease often difficult to diagnose, and the ubiquitous presence of prolamins in the Western diet make treatment challenging.

162. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology 2001 120(3):636-51. PMID: 11179241

"Celiac disease (CD) is a syndrome characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects. The presence of gluten in these subjects leads to self-perpetuating mucosal damage, whereas elimination of gluten results in full mucosal recovery. The clinical manifestations of CD are protean in nature and vary markedly with the age of the patient, the duration and extent of disease, and the presence of extraintestinal pathologic conditions. In addition to the classical gastrointestinal form, a variety of other clinical manifestations of the disease have been described, including atypical and asymptomatic forms. Therefore, diagnosis of CD is extremely challenging and relies on a sensitive and specific algorithm that allows the identification of different manifestations of the disease. Serologic tests developed in the last decade provide a noninvasive tool to screen both individuals at risk for the disease and the general population…"

163. Catassi C, Fabiani E. The spectrum of coeliac disease in children. Baillieres Clin Gastroenterol. 1997 Sep;11(3):485-507. PMID: 9448912

"Coeliac disease is the life-long intolerance to dietary gluten, usually characterized by severe damage to the small-intestinal mucosa. The widespread use of sensitive diagnostic tools, such as the serum anti-gliadin and the anti-endomysial antibodies, has shown not only that coeliac disease is one of the commonest disorders in Western countries but also that this condition is characterized by a higher degree of clinical variability than previously thought (typical, atypical and silent forms). The existence of a latent-potential coeliac disease and even a gluten-sensitive disease with immunological activation of an otherwise normal small-intestinal mucosa has recently been postulated. An increased prevalence of coeliac disease in a number of other disorders has also been reported in both children and adults. The reasons for such a wide clinical heterogeneity are still poorly understood but are likely to depend on both genetic and environmental factors. Further investigations are required to evaluate the impact of undiagnosed, clinically milder forms of coeliac disease on the well-being of the population."

164. Murray JA. The widening spectrum of celiac disease. Am J Clin Nutr. 1999 Mar;69(3):354-65. http://www.ajcn.org/cgi/reprint/69/3/354.pdf

165. Sollid LM, Gray GM. A role for bacteria in celiac disease? Am J Gastroenterol. 2004 May;99(5):905-6. {Comment on: Am J Gastroenterol. 2004 May;99(5):894-904.} PMID: 15128358

"The finding of rod-shaped bacteria attached to the small intestinal epithelium of some untreated and treated celiac-disease patients, but not to the epithelium of healthy controls, ignites the notion that bacteria may be involved in the pathogenesis of celiac disease. This editorial discusses this possibility in relation to the current understanding of the molecular basis of this disorder.

166: Forsberg G et al. Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease. Am J Gastroenterol. 2004 May;99(5):894-904. PMID: 15128357

167. Tursi A et al. High prevalence of small intestinal bacterial overgrowth in celiac patients with persistence of gastrointestinal symptoms after gluten withdrawal. Am J Gastroenterol. 2003 Apr;98(4):839-43. PMID: 12738465

"OBJECTIVE: Celiac disease is a gluten-sensitive enteropathy with a broad spectrum of clinical manifestation, and most celiac patients respond to a gluten-free diet (GFD). However, in some rare cases celiacs continue to experience GI symptoms after GFD, despite optimal adherence to diet. The aim of our study was to evaluate the causes of persistence of GI symptoms in a series of consecutive celiac patients fully compliant to GFD. METHODS: We studied 15 celiac patients (five men, 10 women, mean age 36.5 yr, range 24-59 yr) who continued to experience GI symptoms after at least 6-8 months of GFD (even if of less severity). Antigliadin antibody (AGA) test, antiendomysial antibody (EMA) test, and sorbitol H2-breath test (H2-BT), as well as sophagogastroduodenoscopy (EGD) with histological evaluation, were performed before starting GFD. Bioptic samples were obtained from the second duodenal portion during EGD, and histopathology was expressed according to the Marsh classification. To investigate the causes of persistence of GI symptoms in these patients, we performed AGA and EMA tests, stool examination, EGD with histological examination of small bowel mucosa, and sorbitol-, lactose-, and lactulose H2-breath tests. RESULTS: Histology improved in all patients after 6-8 months of GFD; therefore, refractory celiac disease could be excluded. One patient with Marsh II lesions was fully compliant to his diet but had mistakenly taken an antibiotic containing gluten. Two patients showed lactose malabsorption, one patient showed Giardia lamblia and one patient Ascaris lumbricoides infestation, and 10 patients showed small intestinal bacterial overgrowth (SIBO) by lactulose H2-BT. We prescribed a diet without milk or fresh milk-derived foods to the patient with lactose malabsorption; we treated the patients with parasite infestation with mebendazole 500 mg/day for 3 days for 2 consecutive wk; and we treated the patients with SIBO with rifaximin 800 mg/day for 1 wk. The patients were re-evaluated 1 month after the end of drug treatment (or after starting lactose-free diet); at this visit all patients were symptom-free. CONCLUSIONS: This study showed that SIBO affects most celiacs with persistence of GI symptoms after gluten withdrawal.

168. Wheat allergy: clinical and laboratory findings. Int Arch Allergy Immunol. 2004 Feb;133(2):168-73. PMID: 14764944

169a. Hadjivassiliou M et al. Does cryptic gluten sensitivity play a part in neurological illness? Lancet. 1996 Feb 10;347(8998):369-71. PMID: 8598704

BACKGROUND: Antigliadin antibodies are a marker of untreated coeliac disease but can also be found in individuals with normal small-bowel mucosa. Because neurological dysfunction is a known complication of coeliac disease we have investigated the frequency of antigliadin antibodies, as a measure of cryptic gluten sensitivity, and coeliac disease in neurological patients. METHODS: Using ELISA, we estimated serum IgG and IgA antigliadin antibodies in 147 neurological patients who were divided into two groups. There were 53 patients with neurological dysfunction of unknown cause despite full investigation (25 ataxia, 20 peripheral neuropathy, 5 mononeuritis multiplex, 4 myopathy, 3 motor neuropathy, 2 myelopathy). The remaining 94 patients were found to have a specific neurological diagnosis (16 stroke, 12 multiple sclerosis, 10 Parkinson's disease, 56 other diagnoses) and formed the neurological control group. 50 healthy blood donors formed a third group. FINDINGS: The proportions of individuals with positive titres for antigliadin antibodies in the three groups were 30/53, 5/94, and 6/50 respectively (57, 5, and 12%). The difference in proportion between group 1 and the combined control groups was 0.49 (95% CI 0.35-0.63). Distal duodenal biopsies in 26 out of 30 antigliadin-positive patients from group 1 revealed histological evidence of coeliac disease in nine (35%), non-specific duodenitis in ten (38%), and no lesion in seven (26%) individuals. INTERPRETATION: Our data suggest that gluten sensitivity is common in patients with neurological disease of unknown cause and may have aetiological significance.

169b. Dietary treatment of gluten ataxia. J Neurol Neurosurg Psychiatry. 2003 Sep;74(9):1221-4. PMID: 12933922 m.hadjivassiliou@sheffield.ac.uk

http://jnnp.bmjjournals.com/cgi/reprint/74/9/1221.pdf
BACKGROUND: Gluten ataxia is an immune mediated disease, part of the spectrum of gluten sensitivity, and accounts for up to 40% of cases of idiopathic sporadic ataxia. No systematic study of the effect of gluten-free diet on gluten ataxia has ever been undertaken. OBJECTIVE: To study the effect of gluten-free diet on patients presenting with ataxia caused by gluten sensitivity. METHODS: 43 patients with gluten ataxia were studied. All were offered a gluten-free diet and monitored every six months. All patients underwent a battery of tests to assess their ataxia at baseline and after one year on diet. Twenty six patients (treatment group) adhered to the gluten-free diet and had evidence of elimination of antigliadin antibodies by one year. Fourteen patients refused the diet (control group). Three patients had persistently raised antigliadin antibodies despite adherence to the diet and were therefore excluded from the analysis. RESULTS: After one year there was improvement in ataxia reflected in all of the ataxia tests in the treatment group. This was significant when compared with the control group. The diet associated improvement was apparent irrespective of the presence of an enteropathy. CONCLUSIONS: Gluten ataxia responds to a strict gluten-free diet even in the absence

of an enteropathy. The diagnosis of gluten ataxia is vital as it is one of the very few treatable causes of sporadic ataxia.

170. Food allergy to wheat: identification of immunogloglin E and immunoglobulin

G-binding proteins with sequential extracts and purified proteins from wheat flour. Clin Exp Allergy. 2003 Jul;33(7):962-70. PMID: 12859454

171. Update on wheat hypersensitivity. Curr Opin Allergy Clin Immunol. 2003 Jun;3(3):205-9. PMID: 12840704

Gluten neuropathologies

172: Gabrielli M et al. Association between migraine and Celiac disease: results from a preliminary case-control and therapeutic study. Am J Gastroenterol. 2003 Mar;98(3):625-9. PMID: 12650798

"OBJECTIVES: Subclinical celiac disease (CD) has been associated with various neurological disorders, the most common being neuropathy and cerebellar ataxia. The aims of the present study were to assess the following: 1) the prevalence of CD in patients affected by migraine; 2) whether there are regional cerebral blood flow abnormalities in migraine patients with CD compared to migraine patients without CD; and 3) the effects of a gluten free diet in migraine patients with CD. METHODS: A total of 90 patients affected by idiopathic migraine were enrolled, and 236 blood donors were used as controls. Serum IgG antitransglutaminase (TgA) and IgA antiendomysial (EmA) were measured. In positive cases, diagnosis was confirmed endoscopically. A gluten free diet was started in the patients diagnosed with CD, who were followed for 6 months. A single photon emission CT brain study was performed before and after a gluten free diet. RESULTS: Four of 90 (4.4%; 95% CI = 1.2-11.0) migraine patients were found to have CD compared with 0.4% (95% CI = 0.01-2.3) blood donor controls ($p < 0.05$). During the 6 months of gluten free diet, one of the four patients had no migraine attacks, and the remaining three patients experienced an improvement in frequency, duration, and intensity of migraine. Single photon emission CT studies showed a regional baseline reduction in brain tracer uptake in all four patients. Such reduction in uptake completely resolved at follow-up. CONCLUSIONS: Our results suggest that a significant proportion of patients with migraine may have CD, and that a gluten free diet may lead to a improvement in the migraine in these patients.

173. Gluten sensitivity as a neurological illness. J Neurol Neurosurg Psychiatry. 2002 May;72(5):560-3. PMID: 11971034

http://jnnp.bmjjournals.com/cgi/reprint/72/5/560.pdf

174. Headache and CNS white matter abnormalities associated with gluten sensitivity. Neurology 2001 13;56(3):385-8

"The authors describe 10 patients with gluten sensitivity and abnormal MRI. All experienced episodic headache, six had unsteadiness, and four had gait ataxia. MRI abnormalities varied from confluent areas of high signal throughout the white matter to foci of high signal scattered in both hemispheres. Symptomatic response to gluten-free diet was seen in nine patients."

175. De Santis A et al. Schizophrenic symptoms and SPECT abnormalities in a coeliac patient: regression after a gluten-free diet. J Intern Med. 1997 Nov;242(5):421-3. PMID: 9408073

"A 33-year-old patient, with pre-existing diagnosis of 'schizophrenic' disorder, came to our observation for severe diarrhoea and weight loss. Use of single photon emission computed tomography, (99mTc)HMPAO SPECT, demonstrated hypoperfusion of the left frontal brain area, without evidence of structural cerebral abnormalities. Jejunal biopsy showed villous atrophy. Antiendomysial antibodies were present. A gluten-free diet was started, resulting in a disappearence of psychiatric symptoms, and normalization of histological duodenal findings and of (99mTc)HMPAO SPECT pattern. This is the first case in which, in an undiagnosed and untreated coeliac patient with psychiatric manifestations, the (99mTc)HMPAO SPECT demonstrated a dysfunction of frontal cortex disappearing after a gluten-free diet.

176. Usai P et al. Frontal cortical perfusion abnormalities related to gluten intake and associated autoimmune disease in adult coeliac disease: 99mTc-ECD brain SPECT study. Dig Liver Dis. 2004 Aug;36(8):513-8. PMID: 15334770

OBJECTIVE: Since brain perfusion abnormalities have been described by single-photon emission computed tomography in some autoimmune diseases, the aim of the present study was to evaluate the incidence of perfusion abnormalities by brain single-photon emission computed tomography in a group of coeliac disease patients, and to investigate whether gluten intake and associated autoimmune diseases may be considered risk factors in causing cerebral impairment. METHODS: Thirty-four adult coeliac patients (16 on a gluten-free diet and 18 on a gluten-containing diet, 18 (53%) with autoimmune diseases) underwent 99mTc-ethyl cysteinate dimer brain single-photon emission computed tomography and qualitative evaluation of brain perfusion was performed together with a semiquantitative estimation using the asymmetry index. Ten subjects on our database, matched for sex, age and ethnic group, who were proved normal by histology of jejunal mucosa (four males and six females; median age 39 years, range 27-55 years), were included as control group. RESULTS: Twenty-four out of 34 patients (71%) showed brain single-photon emission computed tomography abnormalities confirmed by abnormal regional asymmetry index (>5%; range 5.8-18.5%). Topographic comparison of the brain areas showed that the more significant

abnormalities were localised in frontal regions, and were significantly different from controls only in coeliac disease patients on unrestricted diet. The prevalence of single-photon emission computed tomography abnormalities was similar in coeliac disease patients with (74%) and without (69%) associated autoimmune disease. CONCLUSIONS: Abnormalities of brain perfusion seem common in coeliac disease. This phenomenon is similar to that previously described in other autoimmune diseases, but does not appear to be related to associated autoimmunity and, at least in the frontal region, may be improved by a gluten-free diet.

Gluten immunologics

177. The humoral response in the pathogenesis of gluten ataxia. Neurology 2002 Apr 23;58(8):1221-6

"The authors assessed the reactivity of sera from patients with gluten ataxia (13), newly diagnosed patients with celiac disease without neurologic dysfunction (24), patients with other causes of cerebellar degeneration (11), and healthy control subjects (17).

"Sera from 12 of 13 patients with gluten ataxia stained Purkinje cells strongly. Less intense staining was seen in some but not all sera from patients with newly diagnosed celiac disease without neurologic dysfunction. At high dilutions (1:800) staining was seen only with sera from patients with gluten ataxia but not in control subjects. Sera from patients with gluten ataxia also stained some brainstem and cortical neurons in rat CNS tissue. Commercial anti-gliadin antibody stained human Purkinje cells in a similar manner… Patients with gluten ataxia have antibodies against Purkinje cells. Antigliadin antibodies cross-react with epitopes on Purkinje cells."

178. Jarvinen TT et al. Intraepithelial lymphocytes in celiac disease. Am J Gastroenterol. 2003 Jun;98(6):1332-7. PMID: 12818278

OBJECTIVE: The aim of this study was to investigate the value of immunohistochemical characterization of different intraepithelial lymphocytes (IELs) in the diagnostic workup of celiac disease (CD). METHODS: The study involved 928 consecutive adult patients undergoing endoscopy undertaken on suspicion of CD or to ascertain the dietary compliance; the control group consisted of 59 adults who underwent endoscopy because of indigestion. Small bowel mucosal morphology, CD3+, alphabeta+, and gammadelta+ IELs were determined. RESULTS: CD was detected in 138 and excluded in 545 adults. CD3+ and gammadelta+ IELs both showed a sensitivity of 93% for CD; specificity was 73% and 88%, respectively. For alphabeta+ cells, the sensitivity was 83% and specificity, 66%. The mucosal morphology recovered on a gluten-free diet and the densities of different IELs, even gammadelta+ cells, decreased. Only the density of gammadelta+ cells remained elevated compared with controls. CONCLUSIONS: Counting of IELs is recommended in borderline cases where the

histology is difficult to interpret. An increase especially in gammadelta+ cells strengthens the probability of CD. However, IELs are not invariably increased in CD.

179. Cataldo F et al. Cytokine genotyping (TNF and IL-10) in patients with celiac disease and selective IgA deficiency. Am J Gastroenterol. 2003 Apr;98(4):850-6. PMID: 12738467

"OBJECTIVE: Selective IgA deficiency (IgAD) and celiac disease (CD) are frequently associated and share the ancestral haplotype human leukocyte antigen (HLA)-8.1, which is characterized by a peculiar cytokine profile. The aim of this study was to evaluate the role of tumor necrosis factor (TNF) and interleukin (IL)-10 alleles in CD and CD-IgAD… CONCLUSIONS: Genetically determined increased production of TNF-alpha and reduction of IL-10 may be relevant for susceptibility to CD, mainly in IgAD, as the different allele expression at TNF and IL-10 loci seems to influence cytokine production profile.

180. Esposito C et al. Expression and enzymatic activity of small intestinal tissue transglutaminase in celiac disease. Am J Gastroenterol. 2003 Aug;98(8):1813-20. PMID: 12907337

"Tissue transglutaminase is more expressed and active in defined areas of the small intestinal mucosa from patients with CD. The presence in the celiac mucosa of proteins able to act as amine-donor substrates suggests that tissue transglutaminase-mediated post-translational modification of proteins cross-linked with gliadin peptides may represent a pathogenic mechanism of CD."

181. Liu E et al. Fluctuating transglutaminase autoantibodies are related to histologic features of celiac disease. Clin Gastroenterol Hepatol. 2003 Sep;1(5):356-62. PMID: 15017653

"BACKGROUND & AIMS: Asymptomatic children at risk for celiac disease (CD) and seropositive for immunoglobulin A anti-TG autoantibodies (TGAA) may lack small intestinal mucosal changes characteristic of CD. We have followed a group of children with serial testing for TGAA… CONCLUSIONS: In children with TGAA seropositivity, the TGAA level varied over time and a higher titer predicted an abnormal biopsy characteristic of CD. A threshold for biopsy for diagnosis of CD could be set higher for screening-identified cases than for clinically identified cases to decrease the frequency of performing "normal" biopsies.

Chelation

182: Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: a pilot study. Neuroendocrinol Lett. 2002 Aug;23(4):303-8. PMID: 12195231 dlonsdale@pol.net

"OBJECTIVES: In a Pilot Study, the clinical and biochemical effects of thiamine tetrahydrofurfuryl disulfide (TTFD) on autistic spectrum children were investigated. SUBJECTS AND METHODS: Ten children were studied. Diagnosis was confirmed through the use of form E2, a computer assessed symptom score. For practical reasons, TTFD was administered twice daily for two months in the form of rectal suppositories, each containing 50 mg of TTFD. Symptomatic responses were determined through the use of the computer assessed Autism Treatment Evaluation Checklist (ATEC) forms. The erythrocyte transketolase (TKA) and thiamine pyrophosphate effect (TPPE), were measured at outset and on completion of the study to document intracellular thiamine deficiency. Urines from patients were examined at outset, after 30 days and after 60 days of treatment and the concentrations of SH-reactive metals, total protein, sulfate, sulfite, thiosulfate and thiocyanate were determined. The concentrations of metals in hair were also determined. RESULTS: At the beginning of the study thiamine deficiency was observed in 3 out of the 10 patients. Out of 10 patients, 6 had initial urine samples containing arsenic in greater concentration than healthy controls. Traces of mercury were seen in urines from all of these autistic children. Following administration of TTFD an increase in cadmium was seen in 2 children and in lead in one child. Nickel was increased in the urine of one patient during treatment. Sulfur metabolites in urine did not differ from those measured in healthy children. CONCLUSIONS: Thiamine tetrahydrofurfuryl disulfide appears to have a beneficial clinical effect on some autistic children, since 8 of the 10 children improved clinically. We obtained evidence of an association of this increasingly occurring disease with presence of urinary SH-reactive metals, arsenic in particular."

182. Lonsdale presentation to Defeat Autism Now! 2003, Philadelphia

http://64.202.182.52/powerpoint/dan2003/Lonsdale.htm

183. A. Holmes, S. Cave, and J.M. El-Dahr. OPEN TRIAL OF CHELATION WITH MES0-2,3-DIMERCAPTO SUCCINIC ACID (DMSA) AND LIPOIC ACID (LA) IN CHILDREN WITH AUTISM. As submitted to IMFAR, June 2, 2001.

"Over 400 patients with autism are currently undergoing treatment for removal of heavy metals. Patients are treated with DMSA alone at doses of 10 mg/kg/dose 3 times a day for 3 days in a row (shorter duration than lead protocol to decrease side effects) with 11 days "off" to allow metals to re-equilibrate. After at least 2 rounds of DMSA alone, the thiol antioxidant lipoic acid (hypothesized to aide in removal of heavy metals across the BBB)is added to each dose of DMSA at 2-3mg/kg/dose. In general, noticeable improvements in language, self-help skills, interaction, and core autistic features are not seen until the patient has been on DMSA with LA for 2-3 months.

"Of patients who have been on DMSA/LA for at least 4 months, these results have been noted on general global assessment by parents, teachers, and MDs: age 1-5yrs(n=40): marked improvement 35%, moderate 39%, slight 15%, none 11%; age 6-12yrs

(n=25): marked 4%, moderate 28%, slight 52%, none 16%; age 13-17 (n=16): moderate 6%, slight 68%, none 26%; age 18+ (n=4): slight 25%, none 75%. For example, a boy 5yr 5mo scored in the average range on a one word expressive vocabulary test 10/00 and at age equivalent 8yr 2mo in 3/01 with no change in education or medication other than starting DMSA/LA.

"The majority of children excrete mercury, lead, and other metals, suggesting that there may be a generalized problem with metal metabolism. Side effects include transient increases in hyperactivity, self-stimulatory behavior, and loose stools. Younger children in particular respond well to this therapy with significant improvement in function."

184. DMSA Chelation efficacy PPT presentation by Jane El-Dahr, M.D.

185. Excellent Defeat Autism Now! PPT Presentations online http://www.autism.com/dan/index.htm

186. Lead poisoning treatment--a continuing need (commentary). J Toxicol Clin Toxicol. 2001;39(7):661-3. PMID: 11778663

187. Lightening the lead load in children. Am Fam Physician 2000 62(3):545-54, 559-60 PMID: 10950212

188. Mercury poisoning. Curr Probl Pediatr. 2000 Mar;30(3):91-9. PMID: 10742922

189. Lead poisoning in children. Curr Opin Pediatr. 1997 Apr;9(2):173-7. PMID: 9204246

190. Lead intoxication in children with pervasive developmental disorders. J Toxicol Clin Toxicol. 1996;34(2):177-81. PMID: 8618251

191. Pediatric arsenic ingestion. Am J Emerg Med. 1995 Jul;13(4):432-5. PMID: 7605532

192. Oral chelators for childhood lead poisoning. Pediatr Ann. 1994 23(11):616-9, 623-6. PMID: 7838614

193. Succimer: the first approved oral lead chelator.Am Fam Physician. 1993 Dec;48(8):1496-502. PMID: 8249780

194. The current role of 2,3-dimercaptosuccinic acid (DMSA) in the management of childhood lead poisoning.Drug Saf. 1993 Aug;9(2):85-92. PMID: 8397892

Viruses in autism

195. Ghaziuddin M et al. Autistic symptoms following herpes encephalitis. Eur Child Adolesc Psychiatry. 2002 Jun;11(3):142-6. PMID: 12369775

"Autism is a childhood onset neurodevelopmental disorder characterized by reciprocal social deficits, communication impairment, and rigid ritualistic interests, with the onset almost always before three years of age. Although the etiology of the disorder is strongly influenced by genes, environmental factors are also important. In this context, several reports have described its association with known medical conditions, including infections affecting the central nervous system. In this report, we describe an 11-year-old Asian youngster who developed the symptoms of autism following an episode of herpes encephalitis. In contrast to previous similar reports, imaging studies suggested a predominant involvement of the frontal lobes. At follow-up after three years, he continued to show the core deficits of autism. This case further supports the role of environmental factors, such as infections, in the etiology of autism, and suggests that in a minority of cases, autistic symptoms can develop in later childhood.

196. Gillberg IC. Autistic syndrome with onset at age 31 years: herpes encephalitis as a possible model for childhood autism. Dev Med Child Neurol. 1991 Oct;33(10):920-4. PMID: 1743418

197. DeLong GR et al. Acquired reversible autistic syndrome in acute encephalopathic illness in children. Arch Neurol. 1981 Mar;38(3):191-4. PMID: 6162440

"In seeking the neurologic substrate of the autistic syndrome of childhood, previous studies have implicated the medial temporal lobe or the ring of mesolimbic cortex located in the mesial frontal and temporal lobes. During an acute encephalopathic illness, a clinical picture developed in three children that was consistent with infantile autism. This development was reversible. It was differentiated from acquired epileptic aphasia, and the language disorder was differentiated aphasia. One child has rises in serum herpes simplex titers, and a computerized tomographic (CT) scan revealed an extensive lesion of the temporal lobes, predominantly on the left. The other two, with similar clinical syndromes, had normal CT scans, and no etiologic agent was defined. These cases are examples of an acquired and reversible autistic syndrome in childhood, emphasizing the clinical similarities to bilateral medial temporal lobe disease as described in man, including the Kluver-Bucy syndrome seen in postencephalitic as well as postsurgical states.

198. Gillberg C. Onset at age 14 of a typical autistic syndrome. A case report of a girl with herpes simplex encephalitis. J Autism Dev Disord. 1986 Sep;16(3):369-75. PMID: 3558293

199. Stubbs EG et al. Autism and congenital cytomegalovirus. J Autism Dev Disord. 1984 Jun;14(2):183-9. PMID: 6086566

200. Ivarsson SA et al. Autism as one of several disabilities in two children with congenital cytomegalovirus infection. Neuropediatrics. 1990 May;21(2):102-3. PMID: 2163029

201. McLachlan RS et al. Treatment of Rasmussen's syndrome with ganciclovir. Neurology. 1996 Oct;47(4):925-8. PMID: 8857720

"Since cytomegalovirus (CMV) has been implicated in the pathogenesis of Rasmussen's syndrome, we treated four patients with ganciclovir, a potent anti-CMV drug. A 7-year-old girl with seizures escalating to 60/day over 3 months despite triple antiepileptic drug therapy became seizure-free 5 days after initiation of treatment with no recurrence at 1.5 years follow-up. Focal neurologic signs, cognitive function, and the EEG returned to normal. Two patients treated 34 and 72 months after disease onset in association with epilepsy surgery had a reduction in seizures and one had no response. CMV genome was detected in the brains of two of the three patients in whom it was assessed. The response to antiviral therapy supports a viral etiology for chronic encephalitis of Rasmussen. If the disease is suspected, treatment with ganciclovir should be considered as early as possible.

202. Domachowske JB et al. Acute manifestations and neurologic sequelae of Epstein-Barr virus encephalitis in children. Pediatr Infect Dis J. 1996 Oct;15(10):871-5. PMID: 8895918

BACKGROUND: Complications of Epstein-Barr virus (EBV) infection are diverse and include a number of neurologic manifestations such as meningitis, meningoencephalitis, cerebellitis, cranial neuritis and others. In general encephalitis caused by EBV in pediatric patients has been considered a self-limited illness with few or no sequelae. METHODS: Charts were reviewed from all patients < 18 years of age admitted to or discharged from the State University of New York Health Science Center at Syracuse between 1982 and 1992 with a diagnosis of encephalitis or meningo- encephalitis. Eleven cases of EBV encephalitis diagnosed during a 10-year period were reviewed to characterize the clinical and laboratory findings in the acute setting and the extent of neurologic sequelae on follow-up. RESULTS: Acute neurologic manifestations were diverse and included combative behavior (55%), seizures (36%), headache (36%) and evidence of focal involvement (27%). Classic findings of infectious mononucleosis were noted infrequently; 18% each had pharyngitis, adenopathy, positive heterophile antibody tests or atypical lymphocytosis. Two patients (18%) had abnormal neuroimaging studies, one in the acute stage and the other at the time of follow-up. Seven patients (64%) had abnormal electroencephalograms (EEGs) in the acute setting; of these three had persistent abnormalities on follow-up. Forty percent developed persistent neurologic abnormalities including global impairment,

perseverative autistic-like behavior and persistent left upper extremity paresis. CONCLUSIONS: Classic signs, symptoms and laboratory findings in infectious mononucleosis may be absent in Epstein-Barr virus encephalitis. Neurologic sequelae occur in a substantial number of patients.

203. Caruso JM et al. Persistent preceding focal neurologic deficits in children with chronic Epstein-Barr virus encephalitis. J Child Neurol. 2000 Dec;15(12):791-6. PMID: 11198493

"Epstein-Barr virus encephalitis is a self-limiting disease with few sequelae. Persistence of neurologic deficits prior to and after the acute illness has yet to be described in children. We describe five children with persistent cognitive and focal neurologic deficits due to chronic Epstein-Barr virus encephalitis with various T2-weighted magnetic resonance imaging abnormalities. Clinical features were a 9-year-old boy with aphasia and apraxia, an 11-year-old girl with impulsivity and inappropriate behavior, a 17-year-old boy with deterioration of cognitive skills and judgment, a 5-year-old boy with complex-partial seizures, and a 6-year-old girl with obsessive-compulsive behavior. All patients had elevated serum Epstein-Barr virus titers for acute infection, with cerebrospinal fluid polymerase chain reaction positive for Epstein-Barr virus in four patients. Three children were treated with methylprednisolone with minimal improvement without changes on magnetic resonance imaging. Epstein-Barr virus encephalitis can present with chronic and insidious neurologic symptoms and should be considered in the differential diagnosis of children with acute or chronic neurologic illness of unknown etiology."

Probiotics

204. Erdeve O et al. The probiotic effect of Saccharomyces boulardii in a pediatric age group. J Trop Pediatr. 2004 Aug;50(4):234-6. PMID: 15357564

"The aim of this study was to determine the efficacy of S. boulardii in diarrhea associated with commonly used antibiotics such as sulbactam-ampicillin (SAM) and azithromycin (AZT). Four hundred and sixty-six patients were assigned to four different groups as follows: group 1:117 patients receiving SAM alone; group 2:117 patients receiving SAM and S. boulardii, group 3:105 patients receiving AZT alone; group 4:127 patients receiving AZT and S. boulardii. Antibiotic-associated diarrhea was seen in 42 of the 222 patients (18.9 per cent) receiving an antibiotic without the probiotic, and in 14 of the 244 patients (5.7 per cent) who received both the probiotic and the antibiotic ($p < 0.05$). In the group receiving SAM where S. boulardii use was found to be significant, the use of S. boulardii decreased the diarrhea rate from 32.3 to 11.4 per cent in the 1-5 years age group ($p < 0.05$). This is a pioneering study investigating combined antibiotic and probiotic use in pediatric diarrhea patients."

205. Gill HS, Guarner F. Probiotics and human health: a clinical perspective. Postgrad Med J. 2004 Sep;80(947):516-26. PMID: 15356352

"There is unequivocal evidence that administration of probiotics could be effective in the treatment of acute infectious diarrhoea in children and the prevention of antibiotic associated diarrhoea and nosocomial/community acquired diarrhoea. Encouraging evidence is also emerging for the effectiveness of probiotics in the prevention and management of pouchitis and paediatric atopic diseases, and the prevention of postoperative infections. There is also strong evidence that certain probiotic strains are able to enhance immune function, especially in subjects with less than adequate immune function such as the elderly. Efficacy of probiotics in the prevention of traveller's diarrhoea, sepsis associated with severe acute pancreatitis, and cancers, the management of ulcerative colitis, and lowering of blood cholesterol remains unproven. In addition to firm evidence of efficacy (for a range of conditions), major gaps exist in our knowledge regarding the mechanisms by which probiotics modulate various physiological functions and the optimum dose, frequency, and duration of treatment for different probiotic strains."

206. Kruis W. Antibiotics and probiotics in inflammatory bowel disease. Aliment Pharmacol Ther. 2004 Oct;20 Suppl 4:75-8. PMID: 15352898

"Summary Treatment with antibiotics in inflammatory bowel disease has a long tradition and is widely used. The indications for antibiotic therapy are wide ranging, from specific situations such as abscesses or fistulae, to patients with severe disease (as an unspecific 'protective' measure), and to address the hypothesis that the enteric flora as a whole, or specific microorganisms such as mycobacteria, are involved in the pathogenesis of inflammatory bowel disease. The best-studied single antibiotic compound is metronidazole. However, overall, the scientific basis for the use of antibiotics is limited, which may reflect a lack of interest from sponsors within the pharmaceutical industry. Despite this weak evidence base, antibiotics are a globally established therapeutic tool in inflammatory bowel disease. Growing evidence from human and animal studies points towards a pivotal pathogenetic role of intestinal bacteria in inflammatory bowel disease. In view of these experimental findings, clinical trials have been undertaken to elucidate the therapeutic effects of probiotics in inflammatory bowel disease. Probiotics are viable nonpathogenic microorganisms which confer health benefits to the host by improving the microbial balance of the indigenous microflora. So far, of the many candidates, one specific strain (Escherichia coli Nissle 1917) and a mixture of eight different bacteria have demonstrated convincing therapeutic efficacy in controlled studies. Maintenance therapy in ulcerative colitis and prevention therapy, as well as the treatment of pouchitis, have emerged as areas in which probiotic therapy offers a valid therapeutic alternative to current treatments. Further investigations may detect additional clinically effective probiotics and other clinical indications."

207. Fedorak RN, Madsen KL. Probiotics and the management of inflammatory bowel disease. Inflamm Bowel Dis. 2004 May;10(3):286-99. PMID: 15290926

"The demonstration that immune and epithelial cells can discriminate between different microbial species has extended our understanding of the actions of probiotics beyond simple barrier and antimicrobial concepts. Several probiotic mechanisms of action, relative to inflammatory bowel disease, have been elucidated: (1) competitive exclusion, whereby probiotics compete with microbial pathogens for a limited number of receptors present on the surface epithelium; (2) immunomodulation and/or stimulation of an immune response of gut-associated lymphoid and epithelial cells; (3) antimicrobial activity and suppression of pathogen growth; (4) enhancement of barrier function; and (5) induction of T cell apoptosis in the mucosal immune compartment. The unraveling of these mechanisms of action has led to new support for the use of probiotics in the management of clinical inflammatory bowel disease. Though level 1 evidence now supports the therapeutic use of probiotics in the treatment of postoperative pouchitis, only levels 2 and 3 evidence is currently available in support of the use of probiotics in the treatment of ulcerative colitis and Crohn's disease. Nevertheless, one significant and consistent finding has emerged during the course of research in the past year: not all probiotic bacteria have similar therapeutic effects. Rigorously designed, controlled clinical trials are vital to investigate the unresolved issues related to efficacy, dose, duration of use, single or multi-strain formulation, and the concomitant use of prebiotics, synbiotics, or antibiotics."

208. Nardone G, Rocco A. Probiotics: a potential target for the prevention and treatment of steatohepatitis. J Clin Gastroenterol. 2004 Jul;38(6 Suppl):S121-2. PMID: 15220676

"The accumulation of fat in hepatocytes with a necroinflammatory component-steatohepatitis-that may or may not have associated fibrosis is becoming a frequent lesion. Although steatohepatitis is currently recognized to be a leading cause of cryptogenic cirrhosis, the pathogenesis has not been fully elucidated. Among the various factors implicated, intestinal bacterial overgrowth may play a role. Indeed, various rat models of intestinal bacterial overgrowth have been associated with liver lesions similar to NASH, and bacterial overgrowth has been observed significantly more often in patients with NASH compared with control subjects. The authors discuss the relationship among intestinal bacterial overgrowth, steatohepatitis development, and probiotic treatment."

209. Saggioro A. Probiotics in the treatment of irritable bowel syndrome. J Clin Gastroenterol. 2004 Jul;38(6 Suppl):S104-6. PMID: 15220671

"Irritable Bowel Syndrome (IBS) may be diagnosed on the presence of symptoms, according to Rome II criteria and some studies have shown that abnormal colonic fermentation may be an important factor in the development of symptoms in some

patients with IBS. Since the fermentations of substrates by the intestinal flora may play a key role in the use of probiotics in the treatment of IBS, fifty patients (24 males, 26 females), mean age 40 years (range = 26-64 years) with IBS, according to Rome II criteria, were enrolled into the study after informed consensus. Patients were randomly assigned to receive either the active preparation containing Lactobacillus Plantarum LP0 1 and Bifidocterium Breve BR0 both at a concentration of 5 x 10 CFU/ml, or placebo powder containing starch identical to the study product, for 4 weeks. To evaluate treatment efficacy two different scores were considered: Pain score in different abdominal locations after treatment decreased in probiotics group of 38% versus 18% ($P < 0.05$) of placebo group after 14 days and of 52% versus 11% ($P < 0.001$) after 28 days. The severity score of characteristic IBD symptoms significantly decreased in probiotic group versus placebo group after 14 days 49.6% versus 9.9% ($P < 0.001$) and these data were confirmed after 28 days (44.4% versus 8.5%, $P < 0.001$). In conclusion, short-term therapy with Lactobacillus PlantarumLP0 1 and Bifidocterium Breve BR0 may be considered a promising approach to the therapy for IBS."

210. Di Stefano M et al. Probiotics and functional abdominal bloating. J Clin Gastroenterol. 2004 Jul;38(6 Suppl):S102-3. PMID: 15220670

"Functional abdominal bloating is a condition dominated by a feeling of abdominal fullness or bloating and without sufficient criteria for another functional gastrointestinal disorder. The currently used therapeutic approaches aim to reduce the volume of intestinal gas, thus increasing intestinal gas elimination or reducing its production. Some promising results have been obtained by the use of prokinetics, such as tegaserod and Prostigmine, and by the use of nonabsorbable antibiotics, such as rifaximin. Another therapeutic approach is represented by the administration of probiotics to modify the composition of colonic flora and thus the production of intestinal gas. The authors recently studied the effect of LGG, which proved to be more effective than placebo in reducing the severity of symptoms."

211. Isolauri E. Dietary modification of atopic disease: Use of probiotics in the prevention of atopic dermatitis. Curr Allergy Asthma Rep. 2004 Jul;4(4):270-5. PMID: 15175140

"The increased prevalence of atopic diseases, atopic dermatitis, allergic rhinitis, and asthma has been described as an epidemic. New approaches in the fight against allergic diseases are called for, the target being the persistence of the atopic T helper 2-skewed immune responder pattern beyond infancy. Atopic dermatitis, the earliest of these conditions, might act as a portal for the development of IgE-mediated atopic manifestations. Abundant evidence implies that specific strains selected from the healthy gut microbiota exhibit powerful antipathogenic and anti-inflammatory capabilities, and several targets for the probiotic approach have emerged in atopic dermatitis: degradation/structural modification of enteral antigens, normalization of the properties of aberrant indigenous microbiota and of gut barrier functions, regulation of

the secretion of inflammatory mediators, and promotion of the development of the immune system. Better understanding of the effects of different probiotic strains and deeper insight into the mechanisms of the heterogeneous manifestations of atopic disease are needed for the validation of specific strains carrying anti-allergic potential."

212. Cross ML. Immune-signalling by orally-delivered probiotic bacteria: effects on common mucosal immunoresponses and protection at distal mucosal sites. Int J Immunopathol Pharmacol. 2004 May-Aug;17(2):127-34. PMID: 15171813

"Probiotics--orally-delivered preparations of non-pathogenic bacterial cells--have been reported to increase anti-microbial protection in the gastrointestinal tract environment, and offer a safe and effective non-pharmaceutical means for combating infectious diseases and certain other pathologies. There is also an increasing body of evidence to suggest that immunostimulation by probiotic bacteria in the gut can enhance immune protection at distal mucosal sites, such as the urogenital and respiratory tracts. This review summarises the current information, from both clinical and animal model studies, of a role for orally-delivered probiotics in modulating mucosal immunoresponses and protection at distal sites. While it is clear that probiotics hold promise in this area, research that is targeted toward identifying the mechanism driving stimulation of the common mucosal immune system, as well as patterns of mucosal tissue homing by immunocytes following probiotic-mediated signalling in the gut, is strongly encouraged."

213. Drakes M et al. Bacterial probiotic modulation of dendritic cells. Infect Immun. 2004 Jun;72(6):3299-309 PMID: 15155633

"Intestinal dendritic cells are continually exposed to ingested microorganisms and high concentrations of endogenous bacterial flora. These cells can be activated by infectious agents and other stimuli to induce T-cell responses and to produce chemokines which recruit other cells to the local environment. Bacterial probiotics are of increasing use against intestinal disorders such as inflammatory bowel disease. They act as nonpathogenic stimuli within the gut to regain immunologic quiescence. This study was designed to determine the ability of a bacterial probiotic cocktail VSL#3 to alter cell surface antigen expression and cytokine production in bone marrow-derived dendritic cell-enriched populations. Cell surface phenotype was monitored by monoclonal fluorescent antibody staining, and cytokine levels were quantitated by enzyme-linked immunosorbent assay. High-dose probiotic upregulated the expression of C80, CD86, CD40, and major histocompatibility complex class II I-Ad. Neither B7-DC or B7RP-1 was augmented after low-dose probiotic or Lactobacillus casei treatment, but B7RP-1 showed increased expression on dendritic cells stimulated with the gram-negative bacterium Escherichia coli. Functional studies showed that probiotic did not enhance the ability of dendritic cells to induce allogeneic T-cell proliferation, as was observed for E. coli. Substantial enhancement of interleukin-10 release was observed in dendritic cell-enriched culture supernatants after 3 days of probiotic stimulation. These results

demonstrate that probiotics possess the ability to modulate dendritic cell surface phenotype and cytokine release in granulocyte-macrophage colony-stimulating factor-stimulated bone marrow-derived dendritic cells. Regulation of dendritic cell cytokines by probiotics may contribute to the benefit of these molecules in treatment of intestinal diseases."

Immune impairments in autism

214. Warren RP et al. Brief report: immunoglobulin A deficiency in a subset of autistic subjects. J Autism Dev Disord. 1997 Apr;27(2):187-92. PMID: 9105969

215. Strong association of the third hypervariable region of HLA-DR beta 1 with autism. J Neuroimmunol. 1996 Jul;67(2):97-102. PMID: 8765331

216. Immunogenetic studies in autism and related disorders.Mol Chem Neuropathol. 1996 May-Aug;28(1-3):77-81. PMID: 8871944

217. Elevated serotonin levels in autism: association with the major histocompatibility complex. Neuropsychobiology. 1996;34(2):72-5. PMID: 8904735

218. Increased frequency of the extended or ancestral haplotype B44-SC30-DR4 in autism. Neuropsychobiology. 1995;32(3):120-3. PMID: 8544967

219. DR-positive T cells in autism: association with decreased plasma levels of the complement C4B protein. Neuropsychobiology. 1995;31(2):53-7. PMID: 7760985

220. Decreased plasma concentrations of the C4B complement protein in autism. Arch Pediatr Adolesc Med. 1994 Feb;148(2):180-3. PMID: 8118537

221. Increased frequency of the null allele at the complement C4b locus in autism. Clin Exp Immunol. 1991 Mar;83(3):438-40. PMID: 2004485

222. Reduced natural killer cell activity in autism. J Am Acad Child Adolesc Psychiatry. 1987 May;26(3):333-5. PMID: 3597287

223. Immune abnormalities in patients with autism. J Autism Dev Disord. 1986 Jun;16(2):189-97. PMID: 2941410

Methylcobalamin miscellany

225. Intestinal absorption and concurrent chemical changes of methylcobalamin. J Lab Clin Med. 1973 Apr;81(4):557-67. PMID: 4696188

226. Detection of malabsorption of vitamin B12 due to gastric or intestinal dysfunction. Semin Nucl Med. 1972 Jul;2(3):220-34. PMID: 4625601

227. Vitamin B 12 malabsorption in chronic pancreatic insufficiency. N Engl J Med 1971 Mar 25;284(12):627-32. PMID: 5547614

228. Complex of intrinsic factor and B12 in human ileum during vitamin B12 absorption. Am J Physiol. 1968 Apr;214(4):832-5. PMID: 5642944

229. The binding of methylcobalamin and vitamin-B 12 coenzyme. S Afr Med J. 1970 May 2;44(18):537-9. PMID: 5445924

230. Competition between bacteria and intrinsic factor for vitamin B 12: implications for vitamin B 12 malabsorption in intestinal bacterial overgrowth. Gastroenterology. 1972 Feb;62(2):255-60. PMID: 4629318

231. Effect of small intestinal bacteria on intrinsic factor and the vitamin B 12-intrinsic factor complex. Scand J Gastroenterol. 1971;6(8):707-13.

232. Correction of cobalamin malabsorption in pancreatic insufficiency with a cobalamin analogue that binds with high affinity to R protein but not to intrinsic factor. In vivo evidence that a failure to partially degrade R protein is responsible for cobalamin malabsorption in pancreatic insufficiency. J Clin Invest. 1978 Jun;61(6):1628-34. PMID: 659618

233. Absorption studies in patients with Crohn's disease and in patients with ulcerative colitis. Acta Med Scand. 1971 Nov;190(5):407-10. PMID: 5149268

234. Production of vitamin B 12 analogues in patients with small-bowel bacterial overgrowth. Ann Intern Med. 1977 Nov;87(5):546-51. PMID: 921081

Thus bacterial production of cobamides, both de novo and from ingested CN-Cbl bound to intrinsic factor, occurs in humans with bacterial overgrowth states and results in a significant loss of vitamin B12 to the host."

[B12 lab result may indicate extreme high, a false positive in some cases]

235. Current concepts of cobalamin (vitamin B12) absorption and malabsorption. J Clin Gastroenterol. 1980 Sep;2(3):287-97. PMID: 7005313

236. The effect of intrinsic factor proteins on the methylating activity of CH3-B12 coenzyme. Bull Acad Pol Sci Biol. 1975;23(6):361-4. PMID: 1164687

237. James Neubrander, M.D. -- Biochemical Context and Clinical Use of Methyl B12 Defeat Autism Now! 2003 Philadelphia
http://64.202.182.52/powerpoint/dan2003/Neubrander.htm

238. James Neubrander, M.D. -- Case presentation of Children with Autism Spectrum Disorder Defeat Autism Now! 2003 Philadelphia
http://64.202.182.52/powerpoint/dan2003/JamesNeubrander.htm

239. James Neubrander, M.D. -- Biochemical Context And Clinical Use Of Vitamin B12. Defeat Autism Now! 2004, Washington, D.C.

240. Walsh WJ et al. Reduced violent behavior following biochemical therapy. Physiol Behav. 2004 Oct 15;82(5):835-9. Pfeiffer Treatment CenterWarrenville, IL 60555 PMID: 15451647

"Reduced violent behavior following biochemical therapy. We conducted an outcome study to measure the effectiveness of biochemical therapy for 207 consecutive patients presenting with a diagnosed behavior disorder. The treatment protocols were based on clinical evaluation and our past experience in the treatment of 8000 patients with behavior disorders at the Pfeiffer Treatment Center (PTC) over a 10-year period. Each test subject was screened for chemical imbalances previously found in high incidence in this population, including metal-metabolism disorders, methylation abnormalities, disordered pyrrole chemistry, heavy-metal overload, glucose dyscontrol, and malabsorption. The clinical procedure included a medical history, assay of 90 biochemical factors, and a physical examination. Standardized treatment protocols were applied for each imbalance that was identified. The frequencies of physical assaults and destructive episodes were determined using a standardized behavior scale before and after treatment, with follow-up ranging from 4 to 8 months. RESULTS: Seventy-six percent of the test subjects achieved compliance during the treatment period. The remaining 24% were reported to have discontinued the therapy. A reduced frequency of assaults was reported by 92% of the compliant assaultive patients, with 58% achieving elimination of the behavior. A total of 88% of compliant destructive patients exhibited a reduced frequency of destructive incidents and 53% achieved elimination of the behavior. Statistical significance was found for reduced frequency of assaults ($t=7.74$, $p<0.001$) and destructive incidents ($t= 8.77$, $p<0.001$). The results of this outcome study strongly suggest that individualized biochemical therapy may be efficacious in achieving behavioral improvements in this patient population.

Epileptiform pattern in autism

241: Nasr JT et al. The Electroencephalogram in Children with Developmental Dysphasia. Epilepsy Behav. 2001 Apr;2(2):115-118. PMID: 12609193

Speech and language delay is a common developmental or acquired disorder. It can be a feature of the autistic spectrum, and if regression of language coincides with epilepsy, the diagnosis of Landau-Kleffner syndrome is considered. Slow acquisition of language without regression is called developmental dysphasia. A retrospective review of clinical and electroencephalographic (including video electroencephalographic) data on 138 children with speech/language delay, seen in a year's time, is presented. The electroencephalogram (EEG) was abnormal in 61% of children with a history of language regression. The EEG was abnormal in
only 15% of children with developmental language disorder, most of whom also had clinical seizures. The difference between the two groups was highly significant (P = 0.004). Therefore obtaining an EEG in children with regression of language, especially if a history of clinical seizures is elicited, is indicated.

242. Wheless JW et al. Language dysfunction in epileptic conditions. Semin Pediatr Neurol. 2002 Sep;9(3):218-28. PMID: 12350043

Epilepsy may disrupt brain functions necessary for language development by its associated intellectual disabilities or directly as a consequence of the seizure disorder. Additionally, in recent years, there has been increasing recognition of the association of epileptiform electroencephalogram (EEG) abnormalities with language disorders and autism spectrum disorders. Any process that impairs language function has long-term consequences for academic, social, and occupational adjustments in children and adolescents with epilepsy. Furthermore, impairments in specific language abilities can impact memory and learning abilities. This article reviews interictal language function in children and adults with epilepsy; epilepsy surgery and language outcome; and language disorders associated with abnormal EEGs. The relationship between epilepsy and language function is complicated as the neuroanatomic circuits common to both overlap. We demonstrate how magnetoencephalography (MEG) offers the ability to analyze the relationship of language, EEG abnormalities, and epilepsy.

243. Hrdlicka M et al. Not EEG abnormalities but epilepsy is associated with autistic regression and mental functioning in childhood autism. Eur Child Adolesc Psychiatry. 2004 13(4):209-13.

The aim of the study was to investigate the potential association of epilepsy and EEG abnormalities with autistic regression and mental retardation. We examined a group of 77 autistic children (61 boys, 16 girls) with an average age of 9.1 +/- 5.3 years. Clinical interview, neurological examination focused on the evaluation of epilepsy, IQ testing, and 21-channel EEG (including night sleep EEG recording) were performed. Normal EEGs were observed in 44.4% of the patients, non-epileptiform abnormal EEGs in 17.5%, and abnormal EEGs with epileptiform discharges in 38.1% of the patients. Epilepsy was found in 22.1% of the subjects. A history of regression was reported in 25.8% of the patients, 54.8% of the sample had abnormal development during the first year of life, and 79.7% of the patients were mentally retarded. Autistic regression was

significantly more frequent in patients with epilepsy than in non-epileptic patients (p = 0.003). Abnormal development during the first year of life was significantly associated with epileptiform EEG abnormalities (p = 0.014). Epilepsy correlated significantly with mental retardation (p = 0.001). Although the biological basis and possible causal relationships of these associations remain to be explained, they may point to different subgroups of patients with autistic spectrum disorders.

244. McVicar KA, Shinnar S. Landau-Kleffner syndrome, electrical status epilepticus in slow wave sleep, and language regression in children. Ment Retard Dev Disabil Res Rev. 2004;10(2):144-9. PMID: 15362173

The Landau-Kleffner syndrome (LKS) and electrical status epilepticus in slow wave sleep (ESES) are rare childhood-onset epileptic encephalopathies in which loss of language skills occurs in the context of an epileptiform EEG activated in sleep. Although in LKS the loss of function is limited to language, in ESES there is a wider spectrum of cognitive impairment. The two syndromes are distinct but have some overlap. The relationship between the epileptiform EEG abnormalities and the loss of cognitive function remains controversial, even in LKS which is the most widely accepted as an acquired epileptic aphasia. Language regression also occurs in younger children, frequently in the context of a more global autistic regression. Many of these children have epileptiform EEGs. The term autistic regression with epileptiform EEG has been proposed for these children. Whether these children are part of an extended LKS spectrum is very controversial, because there are differences in age of onset, clinical phenotype, and EEG findings. An understanding of the available data on clinical characteristics, EEG findings, pathology, prognosis, and treatment of these syndromes is essential for further progress in this area.

245. Tharp BR. Epileptic encephalopathies and their relationship to developmental disorders: Do spikes cause autism? Ment Retard Dev Disabil Res Rev. 2004;10(2):132-4.

Epileptic encephalopathies are progressive clinical and electroencephalographic syndromes where deterioration is thought to be caused by frequent seizures and abundant EEG epileptiform activity. Seizures occur in approximately 10-15% of children with pervasive developmental disorders (PDD) and 8-10% have epileptiform EEG abnormalities without seizures. Thirty percent of children with PDD have regression of social behavior and language at 2-3 years of age. Some authors speculate that the regression is caused by epileptiform activity even in the absence of overt clinical seizures ("autism with epileptic regression") and suggest that elimination of the epileptiform activity, either medically or surgically, should lead to improvement in behavior. This review examines the data showing that interictal epileptiform discharges are associated with transient clinical dysfunction and discusses the implications of these observations for autistic behavioral abnormalities. The results of resective surgery, vagal nerve stimulation, and multiple subpial transaction on children with autism and

epileptiform EEG abnormalities are also discussed. I conclude that there is no evidence that interictal discharges per se cause (or contribute to) the complex behavioral phenotype of autism. There is no justification to support the use of anticonvulsant medication or surgery in children with PDD
without seizures; that is, there is no evidence that treatment to eliminate EEG spikes will have a therapeutic effect on the behavioral abnormalities of PDD and autism.

246. Chez MG et al. Frequency of EEG abnormalities in age-matched siblings of autistic children with abnormal sleep EEG patterns. Epilepsy Behav. 2004 Apr;5(2):159-62. PMID: 15123015

Epileptiform activity in sleep has been described even in the absence of clinical seizures in 43-68% of patients with autistic spectrum disorders (ASDs). Genetic factors may play a significant role in the frequency of epilepsy, yet the frequency in normal age-matched controls is unknown. We studied overnight ambulatory electroencephalograms (EEGs) in 12 nonepileptic, nonautistic children with a sibling with both ASDs and an abnormal EEG. EEG studies were read and described independently by two pediatric epileptologists; 10 were normal studies and 2 were abnormal. The occurrence of abnormal EEGs in our sample (16.6%) was lower than the reported occurrence in children with ASDs. Further, the two abnormal EEGs were of types typically found in childhood and were different from those found in the ASD-affected siblings. The lack of similarity between sibling EEGs suggests that genetic factors alone do not explain the higher frequency of EEG abnormalities reported in ASDs.

Amino acids & epileptiform activity

247. Park YD. The effects of vagus nerve stimulation therapy on patients with intractable
seizures and either Landau-Kleffner syndrome or autism. Epilepsy Behav. 2003 Jun;4(3):286-90. PMID: 12791330

Acquired and developmental comorbid conditions, including language and behavioral disorders, are often associated with epilepsy. Although the relationship between these disorders is not fully understood, their close association may indicate that they share common features, suggesting that these conditions may respond to the same therapies. Not only has vagus nerve stimulation (VNS) therapy been proven to reduce the frequency of pharmacoresistant seizures in epilepsy patients, but preliminary studies also indicate that VNS therapy may improve neurocognitive performance. On the basis of these findings, we hypothesized that VNS therapy would improve the quality of life of patients with either Landau-Kleffner syndrome (LKS) or autism, independent of its effects on seizures. Data were retrospectively queried from the VNS therapy patient outcome registry (Cyberonics, Inc; Houston, TX, USA). A constant cohort of 6 LKS patients and 59 autistic patients were identified. Among the LKS patients, 3 patients at 6 months experienced at least a 50% reduction in seizure frequency as compared with

baseline. Physicians reported quality-of-life improvements in all areas assessed for at least 3 of the 6 children. More than half of the patients with autism (58%) experienced at least a 50% reduction in seizure frequency at 12 months. Improvements in all areas of quality of life monitored were reported for most patients, particularly for alertness (76% at 12 months). Although these preliminary findings are encouraging, a prospective study using standardized measurement tools specific to these disorders and a longer-term follow-up are necessary to better gauge the efficacy of VNS therapy among these patient populations.

248. El Idrissi A et al. Prevention of epileptic seizures by taurine. Adv Exp Med Biol. 2003;526:515-25. PMID: 12908638

249. Dufour F et al. Modulation of absence seizures by branched-chain amino acids: correlation with brain amino acid concentrations. Neurosci Res. 2001 Jul;40(3):255-63. PMID: 11448517

250. Borowicz KK et al. Two essential amino acids, L-lysine and L-histidine, in five types of experimental seizures. Pol J Pharmacol. 2000 Sep-Oct;52(5):345-52. PMID: 11334226

L-Lysine (250-2,000 mg/kg) and L-histidine (1,000-2,000 mg/kg) significantly raised the electroconvulsive threshold. D-Histidine (1,000 mg/kg) was completely ineffective in this regard. Both amino acids were generally inactive in pentetrazole-, picrotoxin- and aminophylline-induced seizures, though L-histidine (2,500 mg/kg) significantly reduced the number of mice with clonic convulsions in the pentetrazole test. Also, L-lysine (2,500 and 3,000 mg/kg) significantly diminished mortality rate in aminophylline-induced seizures. In addition, L-lysine (2,500-3,000 mg/kg) and L-histidine (2,000-2,500 mg/kg) delayed the onset of aminophylline- and picrotoxin-evoked convulsions. L-Lysine and L-histidine (both up to 1,000 mg/kg) did not affect amygdala-kindled seizures in rats. The results indicate that some of indispensable amino acids may play a role in the inhibitory transmission in the central nervous system. A possibility arises that appropriate diet may be an important supportive factor in the treatment of some epileptic patients, probably suffering from generalized tonic-clonic seizures.

251. Kirchner A et al. Effects of taurine and glycine on epileptiform activity induced by removal of Mg2+ in combined rat entorhinal cortex-hippocampal slices. Epilepsia. 2003 Sep;44(9):1145-52. PMID: 12919385

PURPOSE: The imbalance between neuronal inhibition and excitation contributes to epileptogenesis. Inhibition in the central nervous system (CNS) is mediated by gamma-aminobutyric acid (GABA) and glycine. Recent studies indicate the expression of glycine receptor (GlyR) in hippocampus and neocortex. However, the function of GlyR in these regions is not clarified completely. The aim of this study was to investigate whether the GlyR agonists glycine and taurine promote an anticonvulsive effect…. Likewise glycine,

after an initial proconvulsant effect, suppressed epileptiform discharges. CONCLUSIONS: These findings show that GlyR agonists, in particular taurine, could serve as potential anticonvulsants and suggest an important role of GlyR in cortical function and dysfunction.

252. Gietzen DW et al. Indispensable amino acid deficiency and increased seizure susceptibility in rats. Am J Physiol. 1996 Jul;271(1 Pt 2):R18-24. PMID: 8760199

Repeated subthreshold stimulation of limbic brain areas increases seizure susceptibility in experimental models of epilepsy. In addition, acute dietary indispensable amino acid (IAA) deficiency activates the anterior piriform cortex (APC), a seizure-prone limbic brain area in the rat. Based on these two findings, we hypothesized that activation of the APC by chronic exposure to IAA-deficient diets might increase seizure susceptibility. Several nonessential amino acid neurotransmitters are important in seizures, but deficiencies of nontransmitter IAAs have not been well studied in seizure models. In four trials, we made injections of pentylenetetrazole intraperitoneally or of bicuculline into the APC in histidine-, isoleucine-, or threonine-deficient rats and controls. Increased susceptibility to seizures in the deficient animals was observed as increased severity of the seizures, decreased threshold for the dose of the chemostimulant and time to seizure, or a combination thereof. Pair-fed controls showed that this effect was not due to an energy deficit. This novel but robust finding suggests that IAA deficiency may increase vulnerability to seizures by repeated activation of the APC.

253. Hammen A et al. A paradoxical rise of neonatal seizures after treatment with vitamin B6. Eur J Paediatr Neurol. 1998;2(6):319-22. PMID: 10727199

We report the case of a newborn with intractable epileptic seizures developing a paradoxical rise of seizure frequency and electroencephalogram alterations after administration of vitamin B6. We have been unable to determine the aetiology of this disorder. In a newborn presenting with drug-resistant epileptic seizures, the first therapeutic option remains the application of intravenous pyridoxine, but the physician should be aware of the risk of an increase in seizure frequency.

Autism Treatment Evaluation Checklist (ATEC)

254. Autism Treatment Evaluation Checklist (ATEC)
Internet Scoring Program

http://www.autismeval.com/ari-atec/index.html

New Autism Epidemiology Study

whole article free online

http://www.publichealthreports.org/article/PIIS0033354904001347/abstract

http://download.journals.elsevierhealth.com/pdfs/journals/0033-3549/PIIS0033354904001347.pdf

255. Blaxil MF. What's going on? The question of time trends in autism. Public Health Reports 119.6. 536-551 (November 2004)

Synopsis: Increases in the reported prevalence of autism and autistic spectrum disorders in recent years have fueled concern over possible environmental causes. The author reviews the available survey literature and finds evidence of large increases in prevalence in both the United States and the United Kingdom that cannot be explained by changes in diagnostic criteria or improvements in case ascertainment. Incomplete ascertainment of autism cases in young child populations is the largest source of predictable bias in prevalence surveys; however, this bias has, if anything, worked against the detection of an upward trend in recent surveys. Comparison of autism rates by year of birth for specific geographies provides the strongest basis for trend assessment. Such comparisons show large recent increases in rates of autism and autistic spectrum disorders in both the U.S. and the U.K. Reported rates of autism in the United States increased from ,3 per 10,000 children in the 1970s to .30 per 10,000 children in the 1990s, a 10-fold increase. In the United Kingdom, autism rates rose from ,10 per 10,000 in the 1980s to roughly 30 per 10,000 in the 1990s. Reported rates for the full spectrum of autistic disorders rose from the 5 to 10 per 10,000 range to the 50 to 80 per 10,000 range in the two countries. A precautionary approach suggests that the rising incidence of autism should be a matter of urgent public concern.

15 - Which Vaccines Contain Thimerosal of March 2008

Reference: http://www.fda.gov/cber/vaccine/thimerosal.htm#t3

Table 3: Thimerosal and Expanded List of Vaccines - *(updated 3/14/2008)*
Thimerosal Content in Currently Manufactured U.S. Licensed Vaccines

Vaccine	Trade Name	Manufacturer	Thimerosal Concentration[1]	Mercury
Anthrax	Anthrax vaccine	BioPort Corporation	0	0
DTaP	Tripedia[2]	Sanofi Pasteur, Inc	≤ 0.00012%	≤ 0.3 µg/0.5 mL dose
	Infanrix	GlaxoSmithKline Biologicals	0	0
	Daptacel	Sanofi Pasteur, Ltd	0	0
DTaP-HepB-IPV	Pediarix	GlaxoSmithKline Biologicals	0	0
DT	No Trade Name	Sanofi Pasteur, Inc	< 0.00012% (single dose)	< 0.3 µg/0.5mL dose
		Sanofi Pasteur, Ltd[3]	0.01%	25 µg/0.5 mL dose
Td	No Trade Name	Mass Public Health	0.0033%	8.3 µg/0.5 mL dose
	Decavac	Sanofi Pasteur, Inc	≤ 0.00012%	≤ 0.3 µg mercury/0.5 ml dose

	No Trade Name	Sanofi Pasteur, Ltd	0	0
Tdap	Adacel	Sanofi Pasteur, Ltd	0	0
	Boostrix	GlaxoSmithKline Biologicals	0	0
TT	No Trade Name	Sanofi Pasteur, Inc	0.01%	25 µg/0.5 mL dose
Hib	ActHIB/OmniHIB[4]	Sanofi Pasteur, SA	0	0
	HibTITER	Wyeth Pharmaceuticals, Inc.	0	0
	PedvaxHIB liquid	Merck & Co, Inc	0	0
Hib/HepB	COMVAX[5]	Merck & Co, Inc	0	0
Hepatitis B	Engerix-B Pediatric/adolescent Adult	GlaxoSmithKline Biologicals	 0 0	 0 0
	Recombivax HB Pediatric/adolescent Adult (adolescent) Dialysis	Merck & Co, Inc	 0 0 0	 0 0 0
Hepatitis A	Havrix	GlaxoSmithKline Biologicals	0	0

	Vaqta	Merck & Co, Inc	0	0
HepA/HepB	Twinrix	GlaxoSmithKline Biologicals	< 0.0002%	< 1 µg/1mL dose
IPV	IPOL	Sanofi Pasteur, SA	0	0
	Poliovax	Sanofi Pasteur, Ltd	0	0
Influenza	Afluria	CSL Limited	0 (single dose) 0.01% (multidose)	0/0.5 mL (single dose) 24.5 µg/0.5 mL (multidose)
	Fluzone[6]	Sanofi Pasteur, Inc	0.01%	25 µg/0.5 mL dose
	Fluvirin	Novartis Vaccines and Diagnostics Ltd	0.01%	25 µg/0.5 ml dose
	Fluzone (no thimerosal)	Sanofi Pasteur, Inc	0	0
	Fluvirin (Preservative Free)	Novartis Vaccines and Diagnostics Ltd	< 0.0004%	< 1 µg/0.5 mL dose
	Fluarix	GlaxoSmithKline Biologicals	< 0.0004%	< 1 µg/0.5 ml dose
	FluLaval	ID Biomedical Corporation of Quebec	0.01%	25 µg/0.5 ml dose

Influenza, live	FluMist	MedImmune Vaccines, Inc	0	0
Japanese Encephalitis[7]	JE-VAX	Research Foundation for Microbial Diseases of Osaka University	0.007%	35 µg/1.0mL dose 17.5 µg/0.5 mL dose
MMR	MMR-II	Merck & Co, Inc	0	0
Meningococcal	Menomune A, C, AC and A/C/Y/W-135	Sanofi Pasteur, Inc	0.01% (multidose) 0 (single dose)	25 µg/0.5 dose 0
	Menactra A, C, Y and W-135	Sanofi Pasteur, Inc	0	0
Pneumococcal	Prevnar (Pneumo Conjugate)	Wyeth Pharmaceuticals Inc	0	0
	Pneumovax 23	Merck & Co, Inc	0	0
Rabies	IMOVAX	Sanofi Pasteur, SA	0	0
	Rabavert	Novartis Vaccines and Diagnostics	0	0
Smallpox (Vaccinia), Live	ACAM2000	Acambis, Inc.	0	0
Typhoid Fever	Typhim Vi	Sanofi Pasteur, SA	0	0

	Vivotif	Berna Biotech, Ltd	0	0
Varicella	Varivax	Merck & Co, Inc	0	0
Yellow Fever	Y-F-Vax	Sanofi Pasteur, Inc	0	0

Table Footnotes

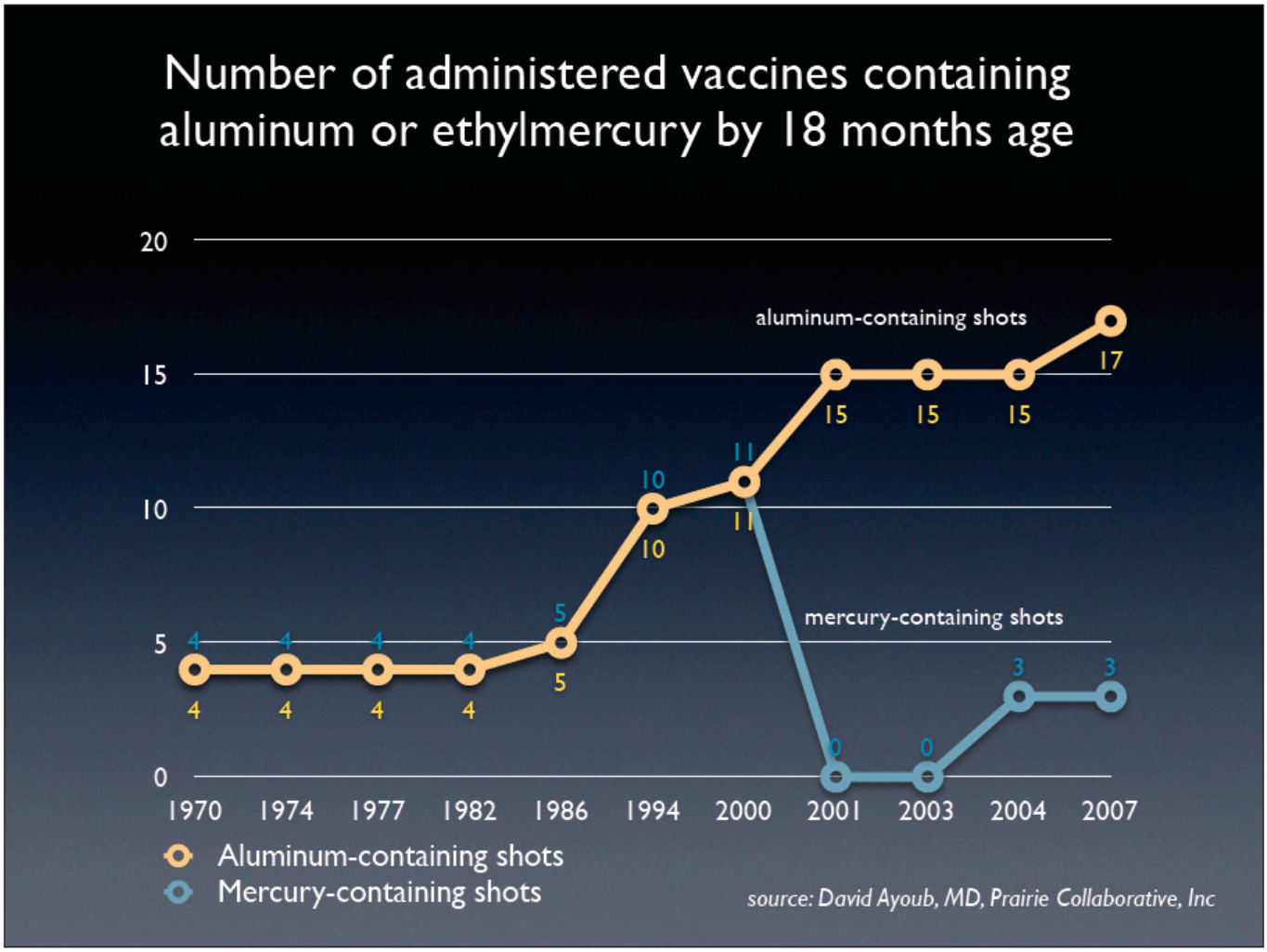
1. Thimerosal is approximately 50% mercury (Hg) by weight. A 0.01% solution (1 part per 10,000) of thimerosal contains 50 µg of Hg per 1 ml dose or 25 µg of Hg per 0.5 ml dose.
2. Sanofi Pasteur's Tripedia may be used to reconstitute ActHib to form TriHIBit. TriHIBit is indicated for use in children 15 to 18 months of age.
3. This vaccine is not marketed in the US.
4. OmniHIB is manufactured by Sanofi Pasteur but distributed by GlaxoSmithKline.
5. COMVAX is not licensed for use under 6 weeks of age because of decreased response to the Hib component.
6. Children under 3 years of age receive a half-dose of vaccine, i.e., 0.25 mL (12.5 µg mercury/dose.)
7. JE-VAX is distributed by Aventis Pasteur. Children 1 to 3 years of age receive a half-dose of vaccine, i.e., 0.5 mL (17.5 µg mercury/dose).

Updated March 2008

Important Note:

EPA recommended daily mercury exposure be limited to: .4 mcg
http://www.epa.gov/teach/chem_summ/mercury_org_summary.pdf

However there are no safety studies outlining any mercury exposure to be safe.



Calculating Aluminum in Vaccines
Here are the current levels of aluminum per shot of the following vaccines, as listed on each vaccine's packaging:

- HIB - PedVax – 225 mcg Aluminum
- PC (Pneumococcal) Vaccine – 125 mcg Aluminum
- DTaP – Taptacel Brand (Sanofi Pasteur) – 330 mcg Aluminum
- DTaP – Tripedia Brand (Sanofi Pasteur) – 170 mcg Aluminum
- DTap – Infanrix Brand (GlaxoSMithKline) – 625 mcg Aluminum
- DT (Sanofi Pasteur) – 170 mcg Aluminum

- dT – Decavac (Sanofi Pasteur) – 280 mcg Aluminum
- Heb P – Recombivax (Merck) – 250 mcg Aluminum
- Hep B – Engerix-B (GlaxoSMithKline) – 250 mcg Aluminum
- Hepatitis A – 250 mcg Aluminum
- HPV – Gardasil – 225 mcg Aluminum

Combination Vaccines

- Comvax (hep B and HIB) – 225 mcg Aluminum
- Pentacel (DTaP, HIB and Polio) – 330 mcg Aluminum

In other words, a newborn who gets a Hepatitis B injection on day one of life would receive 250 mcg of aluminum. This would be repeated at one month with the next Hep B shot. When, at two months, a baby gets its first big round of shots, the total dose of aluminum could vary from 295 mcg (if a non-aluminum HIB and the lowest-aluminum brand of DTaP are used) to a whopping 1225 mcg (if the Hep B vaccine is given along with the brands with the highest aluminum contents). These doses are repeated at four and six months. With most subsequent rounds of shots, a child would continue to get some aluminum throughout the first two years. **But the FDA recommends that premature babies, and anyone with impaired kidney function, receive no more than 10 to 25 mcg of injected aluminum at any one time.**

Source: The Vaccine Book, Dr. Bob Sears

16 - How Much Money is Spent on Vaccine Promotion & Safety Studies

Source [stuart.burns@mail.house.gov]

VACCINE SAFETY SPENDING

Centers for Disease Control (CDC) - Federal spending on vaccine safety is predominantly within the CDC.

• Base funding within the CDC for vaccine safety research in 2007 was $21.6 million. *(I am still awaiting a breakdown from CDC as to how much of this $21.6 million is spent on actual studies relating to vaccine safety, as opposed to simply paying HMOs to provide CDC with raw data.)*

• Of the $21.6 million:

- $16.1 million was spent on the Vaccine Safety Datalink (VSD) and the Clinical Immunization Safety Assessment (CISA)
- $2.7 million was spent on the Vaccine Adverse Events Reporting System (VAERS)
- $2.8 million was spent on an international collaboration known as the Brighton Collaboration.

•Of the $21.6 million outlined above, only a small percentage is actually spent investigating adverse reactions, the bulk of the funding is spent on data collection.

•Also, CDC's vaccine safety research mostly consist of epidemiology (statistical) studies as opposed to looking at specific potential biological mechanisms. Even Dr. Gerberding and top NIH officials acknowledged in an October 2004 Congressional Appropriations Committee hearing that if there is a subset of children who have a genetic susceptibility, the type of statistical (epidimological) studies that the CDC conducts would not detect a vaccine/autism association.

•Only the CDC's CISA program looks at biological mechanisms and focuses on understanding specific ways in which a vaccine may cause a certain reaction. But, CISAs represent a very small amount of spending (roughly $2-$3 million range the last time I looked) and even then the CISAs are not looking at many of the issues that are being raised by parents in America today.

National Institutes of Health (NIH)- The NIH will fund a vaccine safety proposal if it gets through peer review and is in the top 20% of grants when scored. Very few vaccine safety studies reach this threshold, because it is not a priority within NIH. The NIH has no coordinated effort to consider vaccines safety issues. If NIH funds any such studies it is haphazard.

Food and Drug Administration (FDA) – The FDA runs the Vaccine Adverse Events Reporting System (VAERS) in conjunction with the CDC. The FDA does a few studies within VAERS, but the CDC generally takes the lead.

VACCINE PROMOTION SPENDING BY CDC

It would be safe to say that the CDC spends tens of millions of dollars on vaccine promotion. Here is why:

- It is difficult to get a specific dollar figure from the CDC on how much they spend on vaccine promotion. Funding dedicated to vaccine promotion is not broken out separately but is within the $500+ million vaccine operations budget. (The operations budget includes a number of activities including: delivery of vaccines, training of nurses etc.., front line public health professionals, infrastructure support, immunization registries, producing and distributing consumer information (promotion materials), and school-based and community-based delivery programs.

- In addition to any "vaccine promotion" spending in the above, the CDC will spend over $19 million to encourage more Americans to get the annual flu vaccine. ($19 million just for promoting the flu vaccine is roughly the same the CDC spends for all vaccine safety research.)

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17 - Studies Against Vaccines & Autism Relationship with rebuttals

Source: www.safeminds.org (links directly to all the rebuttals)

STUDY ONE AGAINST: IOM 2004 REPORT FINDS NO LINK BETWEEN AUTISM & VACCINES

Study link http://www.fda.gov/fdac/features/2004/504_iom.html

REBUTTAL - Why the 2004 IOM Report against vaccines and autism is WRONG

Source http://www.safeminds.org/pressroom/response/

The data BEHIND the study
http://www.nomercury.org/science/documents/ThimerosalVSDstudy_2-29-00.pdf

SafeMinds Press Room - Failed IOM Report

In response to the failure of the Institute of Medicine-Vaccine Safety Committee to fulfill their duty to protect America's children, SafeMinds will be posting several press releases, studies and comments to highlight the flaws and failures of the committees report. Please feel free to search through the rest of this website to educate yourself to the scientifically proven dangers of Thimerosal/mercury in medical products, especially vaccines, and learn how your voice can be made heard to help remove this threat to America's children.

Comments, commentaries and postings will be frequent. Please check often for the latest updates.

May 25, 2004
Collusion Seen After Release of Flawed Vaccine-Autism Report

SafeMinds...has posted the results of an investigative analysis of several authors relied upon for the flawed Institute of Medicine (IOM) report issued last week attempting to purport a lack of

evidence to the mercury-vaccine-autism link.

May 20, 2004
Office of Special Counsel Forwards Public Health Concerns On Vaccines To Congress

The Office of Special Counsel (OSC) today forwarded to Congress hundreds of disclosures alleging public health and safety concerns about childhood vaccines that include a mercury-based preservative known as thimerosal, and its possible link to neurological disorders, including autism.

May 25, 2004 - Blaxill
"Something Is Rotten In Denmark" - (PowerPoint Version)

An Analysis of the Failures and Conflicts of Interest in Several Studies Used by IOM Vaccine Safety Committee Report

May 19, 2004
SafeMinds Analysis of IOM Vaccine Report: The Failures, the Flaws and the Conflicts of Interest

Washington, D.C. - In the first of a series of forthcoming analyses, SafeMinds...issued the following assessment of the Institute of Medicine (IOM) Vaccine Safety Committee's report on the link between childhood autism and mercury-containing vaccines.

May 18, 2004
SafeMinds Outraged That IOM Report Fails American Public

SafeMinds...issued the following statements in response to the release of the Institute of Medicine (IOM) Vaccine Safety Committee report on the link between mercury-containing vaccines and autism.

Other Responses to Failed IOM Report

May 19, 2004
Research, not arguments, is the answer

Editorial by Nancy Blackmon of the Star News

May 18, 2004
Weldon Calls IOM Conclusions Premature and Hastily Drawn

Statement of Congressman Dave Weldon, MD (FL-15) Regarding the IOM Report on Mercury-Vaccine-Autism Link

May 18, 2004
IOM Chooses Poor Math Over Good Science To Clear Thimerosal

NoMercury.org... issued the following statement in response to the release of the Institute of Medicine (IOM) Immunization Safety Review Committe report on the link between vaccines containing Thimerosal and autism.

May 18, 2004
IOM and CDC Cover-Up: How Far Will They Go To Protect Toxic Vaccines?

Riddled with conflicts of interest, Institute of Medicine releases report derived from flawed data, says National Autism Association.

STUDY TWO AGAINST: Denmark Verstraeten's Study

The Study saying NO LINK
http://www.nomercury.org/science/documents/Verstraeten_VSD_Pediatrics_10-03_Abstract.pdf

More from Verstraeten (Now working at Glaxosmith Kline)
http://www.nomercury.org/science/documents/verstraeten_letter_peds_2004.pdf

REBUTTALL Safe Minds Analysis of Verstraeten's Original Paper FINDS THERE IS A RELATIVE RISK OF 11X's!!

http://www.safeminds.org/Generation%20Zero%20Syn.pdf

http://www.safeminds.org/Generation%20Zero%20Pres.pdf

Congressional Rep Dave Weldon's "Something is Wrong in Denmark"
http://www.nomercury.org/science/documents/AutismOne_Weldon_Remarks.pdf

STUDY THREE AGAINST CDCS Study of the Vaccine Data link: NO LINK BETWEEN AUTISM & VACCINES

http://www.cdc.gov/nip/vacsafe/concerns/thimerosal/researchQAs.htm#vsdres

Transcript of the "Simpsonwood Meeting" discussing this study
http://www.nomercury.org/science/documents/Simpsonwood_Transcript.pdf

STUDY FOUR AGAINST: THIMEROSAL EXPOSURE IN INFANTS AND DEVELOPMENTAL DISORDERS

J Heron etal

Study Link http://pediatrics.aappublications.org/cgi/content/abstract/114/3/577

This is my personal favorite cause they describe " ..additional thimerosal early in a childs life seemed to correlate with improved IQ & Speech development"

WTF!

STUDY FIVE AGAINST: IARGRONIC EXPOSURE TO MERCURY AFTER HEP B VACCINATION IN PRETERM INFANTS

G Stajich etal

Study link: http://www.vaccinesafety.edu/thim-update.htm

Another great quote "…thimerosal was metabolized fairly quickly in the human body.." - maybe for SOME humans – not all

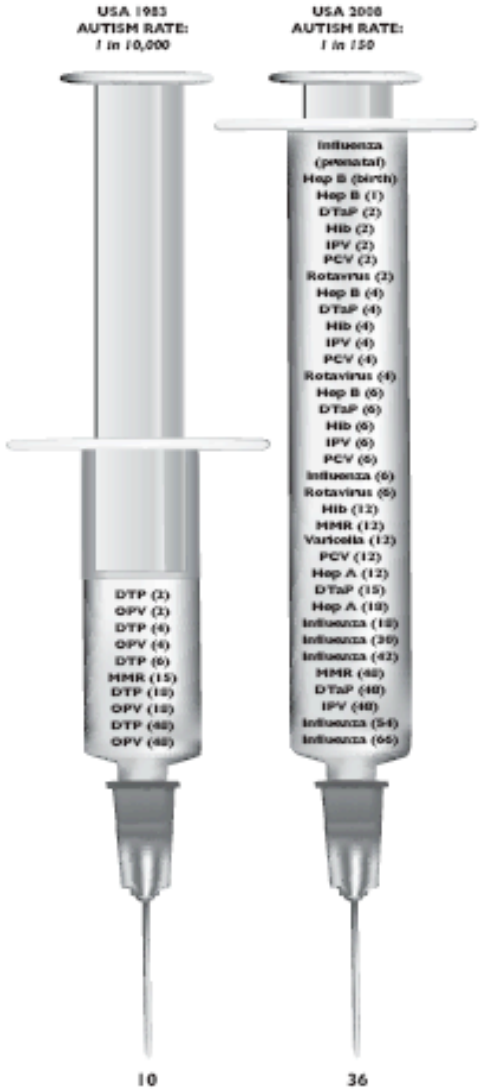
Great Article MERCK MISLED ON VACCINES http://www.nvic.org/Issues/LATimesHg.htm

18 -Difference between Number of Vaccines Given in 1983 vs. 2008

ARE WE POISONING OUR KIDS

IN THE NAME OF PROTECTING THEIR HEALTH?

COMPARISON OF CDC MANDATORY SCHEDULE
Children birth to six years (recommended month)



Green our vaccines. And administer them with greater care.

Mercury. Aluminum. Formaldehyde. Ether. Antifreeze. Not exactly what you'd expect—or want—to find in your child's vaccinations. Vaccines that are supposed to safeguard their health yet, according to our studies, can also do harm to some children.

The statistics speak for themselves. Since 1983, the number of vaccines the CDC recommends we give to our kids has gone from 10 to 36, a whopping increase of 260%. And, with it, the prevalence of neurological disorders like autism and ADHD has grown exponentially as well.

Just a coincidence? We don't think so. Thousands of parents believe their child's regression into autism was triggered, if not caused, by over-immunization with toxic ingredients and live viruses found in vaccines. The Centers for Disease Control and the American Academy of Pediatrics dispute this but independent research and the first-hand accounts of parents tell a different story.

Why are we giving our children so many more vaccines so early in life?
Why do we only test vaccines individually and never consider the combination risk of vaccines administered together? Given the dramatic rise of autism to epidemic levels, isn't it time for the scientific community to seriously consider the anecdotal evidence of so many parents? We urge the CDC and AAP to help us find the answers to these questions and learn why the increase in the number and composition of so many vaccinations has led to a surge in neurodevelopmental disorders. Our children deserve no less.

GENERATION RESCUE
www.generationrescue.org

We want to thank Jim Carrey and Jenny McCarthy for their generous support of Generation Rescue and their never-ending commitment to solving the growing challenges of autism.

Source: www.generationrescue.com

CITATIONS NOTES:

1) Some of studies provided in this document are refenced twice. This is due to a small percentage of studies reflecting information in multiple areas.

2) The studies are available in their entirety on PubMed for a fee at the following link http://www.ncbi.nlm.nih.gov/sites/entrez/ . We reference the studies in their abstract form or listing only. Full studies are available on a fee basis.

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Dan Rossignol MD FAAFP
International Child Development Resource Center www.icdrc.og

Generation Rescue

TACA – www.tacanow.org

Teresa Binstock

Without the help from these individuals this reference would not be possible.

This document is updated and maintained by TACA. Original resources provided references March 2008.

Thank you.

Updated: January 2009