overview [immunology | generalist]

Vaccines and Autism

Bernard Rimland, PhD, Woody McGinnis, MD
Autism Research Institute, San Diego, CA

- Autism research is characterized by diverse findings.
- There is no consensus about the biological determinants of autism.
- This paper examines the autistic immune profile and the possible role of vaccines in autism.

Vaccinations may be one of the triggers for autism. Substantial data demonstrate immune abnormality in many autistic children consistent with impaired resistance to infection, activation of inflammatory response, and autoimmunity. Impaired resistance may predispose to vaccine injury in autism.

A mercurial preservative in childhood vaccines, thimerosal, may cause direct neurotoxic, immunodepressive, and autoimmune injury and contribute to early-onset and regressed autism. Live viruses in measles, mumps, and rubella (MMR) may result in chronic infection of the gut and trigger regressed autism. Thimerosal injection may potentiate MMR injury.

Consideration of vaccine etiology must include recognition of compromised gut and nutrition in most autistic children. An integrated view of the underlying biological problems in autistic children serves our understanding of the possible role of vaccines. Development of screening methods for deferral of vaccines in at-risk children is a worthy goal.

Background

The psychiatric model for autism has been replaced by the concept of biological causation, but there is no scientific consensus about the biological determinants. The clinical expression of autistic spectrum disorders is heterogenous, and it is likely that multiple predispositions and triggers exist for the illness. An increasing number of people, including many physician-parents of autistic children, suspect that vaccinations may be one of the triggers.

Long-term prospective studies of the behavioral and neurodevelopmental effects of vaccination do not exist. There is controversy surrounding the mechanism, accuracy, and interpretation of epidemiological studies examining the possible association of autism and vaccinations. The existing body of relevant laboratory and pathological data in autism is therefore of particular interest.

A host of data reflect abnormal immune patterns in autism, consistent with impaired resistance to infection and activation of the inflammatory response. These laboratory studies do not distinguish early-onset and late-onset (regressed) autism. Impaired resistance to infection and autoimmune diathesis may provide fertile ground for vaccine injury, and these conditions may exist prior to both vaccination and the onset of autism.

A shift in the age of onset suggests an operative environmental factor. Thousands of parent reports collected during nearly 40 years by the Autism Research Institute demonstrate a reversal in the relative proportions of early versus regressed autism. General trends, rather than precise inflection points, are derived from this parental data. It is clear that the proportion of autistic children who enjoyed normal neurobehavioral development and then regressed, usually in the second year of life, has been on the rise for about 2 decades. New vaccines, including combined MMR, hepatitis B, and *Haemophilus influenza* are new environmental factors that were introduced during this period of changing onset.

A mercurial preservative, thimerosal, is a vestige of 1930s vaccine technology and lacks full safety testing. Thimerosal-containing vaccines include: Rh-immunoglobulin during gestation; hepatitis B (Hep-B) at birth; diptheria, and tetanus toxoids with acellular pertussis (DTaP) at 2 month intervals after birth; and *Haemophilus influenza* type b

(Hib), often given in combination with HepB and/or diphtheria-pertussis-tetanus (DPT). Due to recent safety concerns, thimerosal-free alternatives for each of these vaccines have become available. No thimerosal-free version of the influenza vaccine, often recommended for children, is yet available.

We postulate that thimerosal in vaccines may cause direct neurotoxic, immunodepressive, and autoimmune injury resulting in either early-onset or regressed autism. Further, we submit that MMR (usually at 15 months) may result in chronic infection of the gut by vaccinial measles, and trigger regressed autism. Thimerosal injections in series prior to or at the time of MMR may potentiate injury by MMR.

Chronic measles infection from MMR is suggested by studies that demonstrate: 1) chronic vaccinial measles infection of the peripheral monocytes of autistic children with enterocolitis; 2) genomic material consistent with chronic measles infection in intestinal biopsies of regressed autistic children with enterocolitis; and 3) presence in the majority of autistic children of a unique anti-MMR antibody highly correlated with a marker for nervous system autoimmunity. Autoimmune injury to both gut and brain is suggested in autism.

An injured, inflamed gut emerges from the current literature as a dominant theme in autism, and both thimerosal and MMR may be contributors. An integrated approach to autism considers the nutritional and toxic implications of gut dysfunction. Many autistic children demonstrate low nutrients and elevated urinary peptides and organic acids with toxic properties. The field of clinical pathology will continue to provide crucial data in understanding autism and the possible role of vaccines, as well as perhaps develop criteria for the deferral of vaccines in certain children at risk.

The Autistic Immune Profile

Laboratory studies of autistic children demonstrate 1) decreased immune indices, 2) activation of the inflammatory immune system, and 3) increased markers for autoimmunity. Laboratory studies in these areas have not been obtained in children prior to autistic regression. Clinicians do suspect more upper respiratory and gastrointestinal infections in children prior to autistic regression. Increased genotypic markers exist in subsets of the autistic population for both impaired resistance to infection and autoimmune diathesis.

A Pattern of Depressed Resistance in Autism

T lymphocytes (T cells) are abnormal in many autistic children. Cytokines from T cells regulate the full spectrum of antibody and cell-mediated response, the latter being particularly important in resistance to viral infections. Both numeric and functional T-cell deficiencies are demonstrated in autistic children. Autistic children have significantly reduced total T-cell and CD4+ lymphocyte subset counts compared with controls.[1-3] Low numbers of CD4+ cells are found in 28% of autistic children, and 32% have low proportions of CD4+.[4] Functional deficits in T cells may be even more significant than low numbers. T cells from autistic children have diminished function,[5] with extremely poor blastogenic response to multiple mitogens [$P<.001$],[2] perhaps due to a CD4+ deficit.[4]

B-lymphocyte (B cell) deficits are quite common in autism, with low numbers of CD20+ in 48% of autistic children.[4] Effective antibody formation by B cells is particularly important in resistance to bacteria, mycoplasmas, and enteroviruses. Low IgG subclass levels are reported in 20% of autistic children,[1] and low IgA levels are reported in 20% of autistic children in 2 independent studies.[4,6] Intravenous immunoglobulin treatments have benefited autistic children both with and without IgG subclass deficiency, perhaps by countering infections or autoimmune processes.[4]

Natural killer (NK) cell abnormality is found in autism. Natural killer cells are specialized lymphocytes that act against infected or otherwise defective host cells. Low numbers of CD3-CD16+ NK cells are found in 24% of autistic children, with decreased proportions of CD3-CD16+ NK cells in 45%.[4] Seventy-five percent of children with Rett syndrome, very similar to autism, have low numbers of NK cells.[7] Forty percent of autistic subjects have low NK-cell cytotoxic function.[8]

Lower C4 complements levels are found in autism and this is consistent with a higher frequency of C4A null allele in the autistic population (58%) compared with controls (27%).[9] The C4A null allele is associated with increased viral and bacterial infections[6] and autoimmune disease.[9]

Combined immune defects are common in autism. Sixty-four percent of autistic children had measurable deficit in at least 1 of 3 cell lines (CD4+ T cells, CD20+ B cells, CD16+ NK cells).[4] A better understanding of the role of clustered immune deficits in autism is needed, and future research in this area should probably differentiate early-onset and regressed subsets.

Absent antibody response to prior vaccination is reported by many clinicians. One study documented absent antibody to rubella in 5 of 13 previously vaccinated autistic children, versus no such absence in the control group.[10] Both T cells and B cells contribute to generation of antibodies after vaccination. Numbers and function of CD4+ T cells are in question in autism, and it is the CD4+ cell that activates B cell production of antibodies to vaccines.[4] A summary is shown in **T1**.

A Pattern of Inflammatory Activation in Autism

In autism, there is clear-cut evidence of activation of the immune response system, which may be due to innate, toxic, or infectious influences - or some combination of these factors. Viral infection may underlie the immune activation. Alpha-interferon, a cytokine from monocytes, acts on cells throughout the body to inhibit viral replication, so elevations are consistent with response to viral infection. Alpha interferon is elevated in autism ($P<.001$), and as an extremely potent analgesic that produces marked social withdrawal and speech loss in large doses when used as treatment for cancer, it may also explain the character-

Immune Impairment in Groups of Mixed Regressed and Early-Onset Children With Autism **T1**

Immune Element	Finding in Autism	Reference
T lymphocytes	Low CD4+ counts 28% Poor response to mitogen *P*<.001	(4) (2)
B lymphocytes	Low CD20+ counts 48% Low IgA 20% / low IgG sub 20%	(4) (4,6) / (1)
NK cells	Low cytotoxic function 40%	(8)
C4A null allele	58% versus 27% controls	(9)
Negative postvaccination titers	38% versus 0% controls	(10)

Inflammatory Activation in Groups of Mixed Regressed and Early-Onset Children With Autism **T2**

Inflammatory Marker	Finding in Autism	Reference
Higher interferon alpha	*P*<.001	(11)
Higher interferon gamma	*P*<.05; <.02	(12,13)
Elevated neopterin	[urine] x 10 control	(14)
Elevated interleukins	High IL-2 / IL-12 x 20	(13,15)

istic social withdrawal, communication deficit, and high pain threshold in autistic children.[11] Higher IL-1RA, also from monocytes, is reported in autism (P <.01) and may also reflect monocytic response to viral infection.[12]

Gamma-interferon, a pro-inflammatory cytokine from T-helper 1 CD4+ lymphocytes, exerts strong antiviral influences via multiple mechanisms, favors IgG production by B cells, and helps activate cytotoxic cells. Gamma-interferon is elevated in autistic children compared with matched controls in 2 studies (P<.05 and P<.02).[12,13] Neopterin levels are clinically useful markers for activation of cellular immunity in a broad range of illnesses. Urinary neopterin levels are 10 times greater in autistic children, likely reflecting higher gamma-interferon.[14]

Interleukins (ILs), a class of pro-inflammatory cytokines, are selectively elevated in autism. Interleukin-2, produced mostly by T helper 1 cells, is a primary marker for immune activation, and is higher in children with autism.[15] Interleukin-12, released from T cells, B cells, NK cells, and monocytes, stimulates cytotoxic cells and promotes differentiation toward T-helper 1 cells. Interleukin-12 is also elevated in children with autism.[15]

Lagging NK-cell numbers and function and the elevation of 3 primary activators of NK cells (interferon gamma, IL-12, and IL-2) may reflect a key problem such as chronic viral infection in autism. Interferon gamma increases NK adherence and lytic capability; IL-2 stimulates greater numbers of NK cells and increases lysis; and IL-12 is the most potent NK-cell activator of all.[16] A summary is shown in **T2**.

A Pattern of Autoimmunity in Autism

Markers for autoimmunity are predominate in autism, and autoimmunity is one of the conditions associated with activation of the inflammatory response. Occurrence of autoimmune illness is 8 times higher in mothers of autistic children.[17] Major histocompatibility class (MHC) proteins are important modulators of the immune response, and in animals MHC subtype determines susceptibility to autoimmune response to antigens such as mercury.[18] Major histocompatibility class haplotype B44-SC30-DR4 is grossly overrepresented in recognized autoimmune disorders such as rheumatoid arthritis. The incidence of this specific haplotype is 40% in autistic children or their mothers, versus 2% of a control group.[19]

Antibodies to central nervous system antigens are common in autism. One study finds 58% of autistic children positive for antibody to myelin basic protein (MBP), versus only 8% in a mixed comparison group comprised of normal and mentally retarded and Down syndrome children.[20] Another study demonstrates nearly 70% of autistic children positive for anti-MBP.[21] Markedly higher levels of autoantibody to neurofilament protein (NFP) and glial fibrillary acidic protein (GFAP) are found in autistic children compared with normal subjects and subjects who are mentally retarded, with anti-NFP in 55% and anti-GFAP in 32% of the autistic group.[22] Serum levels of brain-binding antibodies to nuclear antigen and brain endothelium are higher in autistic children, including IgM autoantibodies in 36%.[23] Macrophage migration inhibition by MBP, implicated in the pathophysiology of other neurological disorders, is demonstrated in 77% of autistic children but none of a control group.[24]

Macrophage dysregulation is inherent in autoimmune disease. Interleukin-12 from macrophages selectively induces interferon gamma in T helper 1 cells. Elevated IL-12 and interferon gamma in autism are consistent with an active autoimmune process. Interleukin-12 is known to initiate organ-specific autoimmunity by activation of T Helper 1 cells.[21] Interferon gamma, in turn, activates IL-12 and appears to be the primary stimulus for autoantibody production in response to mercury.[25] An interferon gamma/IL-12/T helper 1 mechanism is suggested for autoimmune reactivity to measles as well.[21] Normal values of T helper-2 cytokines IL-4, IL-5, and IL-6 in autism[12] are not inconsistent with this mechanism for autoimmune activation in autism.

Recent evidence suggests that an autoimmune lesion exists in the autistic gut. Complement C1q and IgG deposition in the basolateral epithelium and basement membrane in the duodenum of 23 of 25

autistic children referred for gastrointestinal symptoms was also associated with increased epithelial infiltration by CD8+ lymphocytes.[26] Complement and IgG deposition was not seen in controls. A summary of autoimmunity is given in **T3**.

Mercurials[27,28] and infectious agents[12,29] may trigger autoimmune disease. As a source of both mercury (Rh-immunoglobulin, DTaP, HepB, Hib, influenza) and live virus (MMR), vaccines may play independent or combined roles in the physical illness underlying autism. Many parents report onset of autistic symptoms in their children shortly after 1 or more of these vaccines.[21]

T3

Autoimmunity in Groups of Mixed Regressed and Early-Onset Children with Autism

Autoimmune Marker	Finding in Autism	Reference
Familial tendency autoimmune disease	Mothers x 8	(17,19)
Elevated anti-MBP	58% / 70%	(20,21)
Elevated anti NFP / GFAP	55% / 32%	(22)
Elevated Ab to brain nucleus and endothelium	IgM 36%	(23)
Macrophage migration inhibition by MBP`	77% versus 0% controls	(24)
Elevated interferon gamma	*P*<.05; <.02	(12,13)
Elevated IL-12	x 20 controls	(13)
Duodenal IgG / complement / CD8+ infiltration*	92% versus 0% controls	(26)

**This group included only regressed autistic children with GI symptoms.*

T4

Case Study: Autistic Child C.M. DOB 12-1-94

Age Vaccinated	Vaccine(s)	Parenteral Mercury Dosage
2 weeks	Hep-B	12.5 μg Hg as Thimerosal
2 months	Hep-B, DTaP, Hib	12.5, 25, 25 μg Hg
4 months	DTaP, Hib	25, 25 μg Hg
6 months	H-B, DTaP, Hib	12.5, 25, 25 μg Hg
15 months	MMR	Live vaccine, no Thimerosal
18 months	DTaP	25 μg Hg

**Patient of author.*

The Case Against Vaccines Containing Thimerosal

As a class, organic mercurials are renowned for neurotoxic effects at very low doses, and sensitivity to mercurials is highly variable. Thimerosal, a form of ethylmercury, is also called "merthiolate." Topical merthiolate in infants with omphalocele[30] and merthiolate ear irrigations[31] have resulted in significant toxicity. Methylmercury and thimerosal are very similar chemically, but more health data are available for methylmercury. The National Academy of Sciences stated that methylmercury exposure alone may cause neurological problems in an estimated 60,000 children born in the United States every year.[32] Studies in animals and human subjects demonstrate that thimerosal is taken up by the brain.[33] Over many decades the safety of thimerosal in humans has not been studied thoroughly.

T cells are particularly sensitive to methylmercury, and both T-cell and NK-cell activity significantly decrease with chronic exposure.[28] Depressed antibody production is seen in experimental organic mercury exposure.[28] Antibodies to central nervous system proteins are common in human and experimental methylmercury exposure, and imply one of many mechanisms for neurotoxicity.[27] Allergenicity of certain contact lens solutions containing thimerosal is also known. In vitro studies suggest potent toxicity of thimerosal at cellular and enzyme levels.[33,34]

Mercury exposure from vaccines is surprisingly high. General concern about thimerosal in vaccines was stimulated by the relatively recent realization that infants receiving these vaccines were getting bolus amounts of injected organic mercury which, even averaged over time, could exceed the Environmental Protection Agency (EPA) concern level for total average mercury exposure from all sources. A trend towards earlier and higher dosing of injected thimerosal with the successive introduction of new vaccines has reversed only in the past 2 years, as many physicians and medical institutions shift to thimerosal-free alternative vaccines.

Autistic histories are often positive for high thimerosal doses. **T4** shows the vaccination schedule of a regressed autistic child with normal development until 18 to 20 months, then loss of speech, eye contact, social interaction, and attentiveness.

The EPA concern level for total mercury exposure from all sources (0.1 m g/kg/day) is for ongoing exposure from all sources, and did not specifically consider large injected boluses of mercury. Application of the criterion to vaccines is therefore disputed. Some experts suggest that exposure calculations for mercury from thimerosal injection should be averaged over time, while others contend that looking at single-day exposure is more accurate because it is less likely to underestimate heightened risk from episodic large boluses.

Child C.M. exceeded the concern level by either calculation. Over the first 6 months, assuming a generous average weight of 7 kg, concern level would be 126 m g total Hg exposure from all

sources, versus 182.5 m g from thimerosal injection alone. Conversely, applying the EPA concern level on a daily basis would be even more worrisome - at 2 months of age if the child weighed 5 kg, the single-day concern level for the day of the vaccinations would be 0.5 m g, versus actual injection of 62.5 m g of mercury as thimerosal.

There exist striking similarities between autism and mercury poisoning, a full listing of which[36,37] falls outside the scope of this article. One of the more compelling comparisons is a historic illness dubbed Pink Disease (acrodynia), which resulted from mercurial teething powders, lotions, and diaper powders eliminated from usage by the 1950s. In its behavioral aspect, Pink Disease was similar to autism.[37]

Corresponding changes in the appearance of intestinal Paneth cells[38] are seen in experimental methyl mercury exposure[39] and biopsies from autistic children.[40] Mercury may interfere with Paneth cell release of defensins for local immunity to infection from viruses, bacteria, and yeast.

The profile of immune depression in mercury exposure parallels specific abnormalities in many autistic children. T-cell and NK-cell activity significantly decrease with chronic methylmercury exposure.[28] Depressed antibody production is seen in experimental organic mercury exposure.[28]

Mercury exposure produces the same set of nervous system autoantibodies seen in autism. Experimental animal exposure and human industrial exposure to mercury produces levels of anti-MBP, anti-NF, and anti-GFAP which correlate with exposure level and degree of clinical symptoms in mercury poisoning.[27] A relatively new immunoassay, Memory Lymphocyte Immunosensitivity Assay (MELISA by copyright) measures the reactivity of hapten-specific lymphocytes to various allergens, including heavy metals.[41-45] Allergic reactivity to metals measured by MELISA varies markedly among individuals and is specific for different forms of mercury.[46]

Parathyroid hypertensive factor (PHF) is a circulating hormone measurable in the blood. Parathyroid hypertensive factor is known to open calcium L-channels[47] and stimulate phosphodiesterase (PDE),[48] while mercurials are renowned for L-channel blockade and PDE inhibition. Mercury inhibits the rod light response by inhibiting PDE,[49] and poor rod light response is demonstrated in half of autistic children on electroretinograms.[50]

A large unpublished government-sponsored thimerosal study is not comforting. The study considered correlation between diagnoses on HMO charts and total thimerosal received during infancy. Initial screening analysis of more than 110,000 charts revealed correlations between amount of thimerosal injected in the first 6 months and problems reminiscent of autism, but not autism per se: speech delay $P<.0001$, neurodevelopmental disorder $P<.01$, and attention deficit $P<.06$.

There is no question the initial large screening study suggested a problem with thimerosal. At the time these data were presented, the lead investigator asserted "a possible association between certain neurological disorders and exposure to mercury from thimerosal-containing vaccines before the age of 6 months."[51] A year later, after inclusion of more charts from a second HMO and various statistical and methodological treatments, government agencies made reassuring pronouncements about no link between autism and thimerosal. We contend that the study may have provided an initial glimpse of a problem, but otherwise should be discounted, owing to a flawed methodology which renders it as a non-sensitive method.

Sickly infants (premature infants, infants with more serious illness, infants requiring longer hospitalizations) were excluded from this toxicological assessment. This exclusion criterion overlooks the likelihood that physically weaker subjects are more sensitive to the injurious effects of toxins. More specifically, exclusion of children with certain pathologies may overlook the very mechanisms inherent to toxicity. For example, exclusion of infants with neonatal jaundice (bilirubin >16mg/dL in 13% of autistic children vs 3% in controls)[52] would effectively remove a subgroup arguably most prone to problems with organic mercury excretion, which in all humans studied is primarily biliary-fecal. Sickly infants with immunodeficiency were undoubtedly excluded from the study, yet mercury itself is a strong immunosuppressant, etc.

Minds are still open on this subject. After hearings, the Institute of Medicine acknowledged in the summer of 2001 that a link between vaccinial thimerosal and autism is "biologically plausible."[34] In the autumn of 2001, The National Institute for Environmental Health Safety (NIEHS) committed major university funding to investigate the role of mercury in autism. The FDA has discouraged the production of thimerosal-containing scheduled childhood vaccines but has not withdrawn existing stocks of such vaccines from the market.

The Case against MMR: Chronic Infection and Autoimmunity

The manufacturer of MMR vaccine specifies the following contraindications in the 2002 *Physician's Desk Reference*: primary and acquired immunodeficiency states, or cellular immune deficiencies, or hypogammaglobulinemic states

Measles infection from live virus in MMR is a real possibility in the immunosuppressed host, sometimes in unusual locations. For instance, necrotizing measles lymphadenitis after vaccination was reported in a child with familial cellular immunodeficiency.[53] We hypothesize increased risk of chronic measles infection complicating MMR in a subgroup of children with common variable immunodeficiency or selective deficits, particularly if these immune deficits are superimposed on subclinical nutritional deficiencies or other vulnerabilities. Recognition of such a vaccine-injured subset of children would be more difficult if the infection is latent, or in the case of a neurobehavioral syndrome such as autism, outside the brain.

A spectrum of recognized adverse reactions to vaccines is associated with immune deficits. Low titers of specific antibodies to tetanus toxoid are found in 80% of a subgroup of children with abnormal reaction to measles vaccination,[54] which parallels the previously-described negative rubella titers in vaccinated autistic children. The same study demonstrates that reactions to another live vaccine, oral polio,

were associated with low numbers of T lymphocytes as assessed by E-rosette formation in 50% of a subgroup of children. Identical methodology demonstrated decreased T cells in autism as well.[2] Again, the same study demonstrates low IgA levels 5 times more commonly in children with severe reactions to DPT than in a comparison group of children being evaluated for clinical suspicion of immune deficiency (17% vs 3.3%), also of interest in the context of lower IgA levels in autism.[54]

Either the poor immunity seen in children with more severe recognized reactions to vaccines is an antecedent marker for such reactions, or immune impairment follows vaccination. Neither possibility is comforting when we know that immune impairment of autistic children exists after vaccination.

After several years of investigation, we now understand that the autistic gut is extensively inflamed. Reflux esophagitis (69%), chronic gastritis (42%), and chronic duodenitis (67%) are found in the subgroup of autistic children with gastrointestinal symptoms, irritability, or sleeplessness.[40]

Enterocolitis with lymphonodular hyperplasia (LNH) is common in post-MMR regressions. Initial colonoscopic evaluation of a small group of regressed children with gastrointestinal symptoms revealed ileal LNH in 7/12, and patchy chronic colitis in 11/12 autistic children.[55] Subsequent study of a larger group with regression and bowel symptoms found ileal LNH in 89% and colitis in 88%.[56]

Some experts are of the opinion that the ileal LNH and nonspecific enterocolitis found in the regressed autistic group are not classical inflammatory bowel disease.[57] The histology of children regressed after MMR may be sufficiently distinctive to warrant the label, "autistic enterocolitis."[58]

Suggestion of Chronic Vaccinial Measles Infection from MMR

Chronic vaccinial measles infection of mononuclear cells is suggested by 1 published study. Measles RNA was found in the peripheral mononuclear cells in 3/9 regressed autistic children with enterocolitis. Differentiation of wild virus from vaccinial virus was not definitive, but presumptive wild measles RNA from 1/8 patients with Crohn's Disease, presumptive vaccinial measles from 1/3 ulcerative colitis patients, and presumptive vaccinial measles RNA from all 3 of the autistic children were consistent with exposure history.[59]

Small intestinal measles infection has been found in autism. Reverse transcription polymerase chain reaction found measles RNA in distal ileal biopsies of 75/91 (87%) of regressed autistic children with ileal lymphoid hyperplasia versus 5/70 controls (7%). The measles RNA was primarily localized in dendritic cells and lymphocytes in reactive follicular hyperplastic centers.[60] The study does not distinguish vaccinial from wild measles virus. It does pose a most insistent question about the role of MMR.

One study presents serological evidence pointing to vaccinial measles from MMR. An unusual antibody to MMR was found in 75/125 (60%) of autistic children not selected for regression, versus none of 92 controls. Monoclonal antibody studies strongly suggest that this unusual MMR antibody is related only to the measles component of the MMR vacine, not other contents of the MMR vaccine. Greater than 90% of the sera with the unusual MMR antibody are positive for anti-MBP, suggesting a causal relationship between MMR vaccination and autoimmunity in autism, conceivably related to atypical measles infection.[61] In the same study, autistic children were found to have significantly higher ($P<.001$) than normal levels of antibody to both measles virus and MMR antibodies, but not to separate mumps or rubella. Viral infections do induce autoantibodies during the course of infection.[62]

Measles virus protein and human intermediate filament protein are antigenically cross-reactive in monoclonal antibody studies, and subjects with wild measles infection are known to generate antibody to cytoskeletal intermediate filaments.[62] Neurofilament and glial filament in the brain are intermediate filament proteins, as is keratin, which stabilizes gut epithelial cells. Viral-effected molecular mimicry offers a possible explanation for antibody to nervous system.[21] In autism, MHC DR3+ lymphocyte expression is elevated.[63,64] Elevated MHC DR+ activated lymphocytes in autism increase brain permeability to autoantibody by interacting with MHC class 1 expression.[22] Organic mercurials are also known to permeate the blood-brain barrier.[28]

Characteristics of wild measles infection increase MMR doubts. Direct infection of the brain by wild measles virus occurs 1 to 7 months after acute measles and is characterized as measles inclusion body encephalitis (MIBE). Measles inclusion body encephalitis in an apparently healthy child after MMR was confirmed secondary to vaccinial measles virus.[65] MMR-induced subacute sclerosing panencephalitis is well-documented.[66,67] Wild measles delayed encephalopathy without evidence of direct viral invasion of the brain but with clinical features of demyelination is associated with a lymphocytic proliferative response to MBP in over half the affected children, many of whom suffer long-standing neurological deficits ranging from behavioral abnormalities to seizures to persistent motor deficits.[68] It is unclear whether any of these children satisfied the diagnosis of autism.

View of Vaccines From a Broader Perspective on Autism

We do not suspect thimerosal and MMR to be the only triggers for autism. In our clinical experience, nutritional status, food intolerances, concomitant infections and other toxic influences appreciably affect the symptoms of autism, and we think these same factors may potentiate vaccine insult.

Suboptimal nutritional status is demonstrated in most autistic children when sensitive measurements are used. Scores of physicians are finding low intracellular or functional measurements of nutrients such as zinc, magnesium, vitamin B6, and fatty acids in the majority of autistic children, and report excellent improvement with supplementation of these nutrients. This clinical observation needs confirmation with large studies using well-matched controls.

Vitamin A, dubbed the "anti-infective" vitamin in the pre-antibiotic era, definitely trends lower in autism and is of

particular interest in the context of suspected chronic measles infection of the gut after MMR. Vitamin A is critically important in resistance to measles infection, membrane protection, and vision. High doses of vitamin A are indicated treatment for severe measles, and low serum vitamin A measurements correlate directly with severity of illness from measles.[69] Both vitamin A[70,71] and IgA levels[72] are depressed in wild measles infection. One pilot study found low vitamin A levels in 40/65 autistic children,[73] attesting to an earlier published claim of low or borderline serum retinol levels in autism.[74] This is plausible because generally, young children are suspected to have widespread subclinical vitamin A deficiency.[75]

Visual problems in autism such as lateral gaze,[76] poor depth perception, and poor facial recognition may reflect poor rod function related to vitamin A. Most mothers of autistic children have mild to severe night vision deficits,[74] and as discussed previously in the context of elevated PHF in autism, many autistic children are known to have abnormal retinograms consistent with rod dysfunction.[50] Abnormal rod function on retinogram post-MMR is reported in a child with selective CD4+ deficiency.[77]

Zinc is especially important in cell-mediated immunity. Low NK-cell activity,[78] low CD4+ counts, and anergy to skin-testing[79] improve significantly with zinc supplementation of non-autistic children. Brush border enzyme activity and secretory antibodies improve with zinc supplementation,[80] as does diarrheal disease, which is known to accelerate zinc loss.[75] Intracellular assay suggests zinc deficiency in at least half of the autistic children.[73] Administration of zinc to autistic children with low intracellular or lower plasma zinc levels often provides dramatic improvement in bowel and behavior.

A gut weakened by nutritional factors is potentially more prone to injury by vaccines. Zinc deficiency, vitamin A deficiency, and immunoglobulin-mediated food intolerances are recognized causes of gastrointestinal inflammation. Even mild depletion of vitamin A reduces protective mucus and predisposes to microbial attack.[75] Mercurial compounds have a direct, potent inflammatory effect on the gut,[38] and vaccinial infection of gut tissue may be highly injurious. The precise interplay and predominance of these factors is not yet defined.

Studies suggest intestinal protein loss[81] and malabsorption in many autistic children.[82] Secretin, produced by the intestinal brush-border, is important in digestion and support of the bowel membranes, and perhaps also at binding sites within the brain. Circulating secretin levels are often measurably low in autism, at least against an adult reference range.[83] Other deficient products of the brush border include the disaccharidases (lactase, sucrase, or maltase), low in 58% of autistic children with gastrointestinal complaints.[40] Inadequate digestion of disaccharides may favor overgrowth of detrimental flora in the autistic gut.

Increased intestinal permeability (leaky gut) is suspected in the majority of autistic children, and is even found in 43% of the subgroup of autistic children without overt signs or symptoms of gut disease, versus 0/40 controls.[84] A leaky autistic gut is the suspected vehicle for multiple pathological mechanisms in autism. Very high urinary levels of toxic organic acids from fungal and anaerobic organisms are found in autistic children, and these are probably absorbed from the gut.[85] Peptides from inadequate digestion of casein and gluten apparently are absorbed in excess by autistic children, as reflected by very high levels of urinary peptides.[86,87] Poor peptidase production may combine with leaky gut to produce the marked elevation of urinary peptides.

Excessive peptides from undigested casein and gluten are suspected to exert significant toxicity. Large numbers of parents and clinicians[88] report improvement of autistic children on gluten- and casein-free diets, the full benefit of which may not be evident for many months of dietary exclusion. Peptides from undigested casein and gluten are known to have potent opioid activity,[89-91] and opioid peptides can adversely effect brain development.[92,93]

In the context of thimerosal, it should be noted that the converting enzyme for casein and gluten, Dipeptidyl Peptidase IV (DPPIV), is inhibited by mercury at very low concentrations.[94] Similarly, mercury in nanomolar concentrations totally inhibits the intestinal pyridoxal kinase,[95] activator of Vitamin B6, which has been found to benefit children with autism in 18 published studies.[96]

The answers in autism will derive from an integrated approach, which considers the definitively close relationships of brain, immunity, gut, and environmental influences such as nutrition, toxins, and infectious agents. A particularly intriguing discussion currently centers on CD26, which epitomizes the closeness of seemingly disparate systems. CD26 is a T-Helper 1 marker, a T-cell activation antigen that acts as the co-stimulatory activation molecule on memory CD4+ cells and controls many aspects of lymphocyte function, presumably including modulation of the response to vaccines. Interferon, IL-12, and vitamin A all tend to upregulate CD26 gene expression. CD26 is a receptor for adenosine deaminase, low levels of which are associated with impaired cellular immunity.[97] Adenosine deaminase is decreased in autism.[98] CD26 is very interesting from the immune perspective, but truly exciting with an additional realization: CD26 and enzyme DPPIV (gluten/casein digestion) are one and the same.[99] As a key element in both the immune and digestive systems, with seeming pertinence to neurobehavior, this molecule can model the unified thinking we need in autism.

Conclusion

Depressed immunity, autoimmunity, and inflammatory activation are common features in autism. Impaired resistance to infection may predispose to chronic measles infection of the autistic gut by MMR vaccine. Thimerosal-containing vaccine during infancy may depress immunity and lower the threshold for chronic vaccinial measles infection. Thimerosal and MMR may induce autoimmunity to elements of the CNS individually or additively and thus contribute to the pathophysiology of autism.

Significant anatomic and functional gut abnormality is a prevailing theme in

autism, and may be aggravated by injury from MMR and thimerosal or predispose to such injury. Much of the clinical knowledge about nutritional aspects of autism, such as low zinc and vitamin A status, help explain a weakened autistic immunity and gut as well as vulnerability to vaccine injury. Ingress of toxins from the gut reflects gut injury and appears significant.

We are far from certain that vaccines help trigger autism, but we are farther still from certain they do not. Given current available data, thimerosal would stand no chance of approval as a new injectable medication by modern standards, and because thimerosal alternatives exist for all the scheduled childhood vaccines, we call for its summary removal and safe disposal from every repository in this country. We also encourage an intensive effort to find economical thimerosal-free childhood vaccines for the rest of the world.

In many respects, the autistic immune profile fits the diagnostic category of common variable immunodeficiency (CVID). Common variable immunodeficiency is strongly associated with gastrointestinal disease, including LNH, in multiple studies.[100-103] Autism may very well be nature's way of demonstrating a subgroup of CVID children vulnerable to vaccine injury. Current official vaccination guidelines do not exclude CVID children from usual vaccination, but we think this needs refinement. We call for well-funded prospective studies by individuals without conflicts of interest to determine immune, autoimmune, gastrointestinal, and long-term neurobehavioral effects of vaccination, particularly in relation to immune, gut, and nutritional status before and after vaccination. Development of screening tests to identify children with higher risks of any negative effects of MMR should be a high priority. Such screening might include skin-testing for anergy, dietary; family questionnaires to identify possible low vitamin A levels; tetanus titers for anergy; or immunoglobulin and T-cell counts in special cases.

In this period of major uncertainty over MMR and autism, the thoughtful physician would be counseled to temper existing institutional and corporate vaccine guidelines with clinical judgment. In fact, independent thinking may be the only way to resolve certain institutional contradictions, as in the conflicting recommendations pertaining to administration of MMR to children with febrile illness.[67] As many of our colleagues have already determined, the respect for our vaccination program is not lessened if the physician decides to wait a reasonable period after diarrhea or other illness has abated, or advise cod liver oil prophylaxis before vaccination.[99]

Published science and clinical experience are converging rapidly to form a more accurate image of autism. We are learning that autism implies a physically ill child with associated immune, gut, and nutritional problems. Besides helping target biological interventions for autism, understanding the underlying physical problems enhances our grasp of the possible role of vaccines.

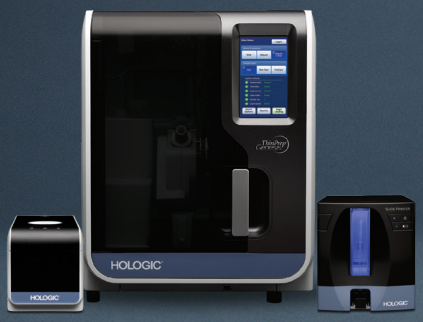
1. Yonk LJ, Warren RP, Burger RA, et al. CD4+ helper T cell depression in autism. *Immunol Lett* 1990;25:341-346.
2. Warren RP, Foster A, Margaretten C, et al. Immune abnormalities in patients with autism. *J Autism Develop Dis* 1986;16:189-197.
3. Warren RP, Yonk LJ, Burger RA, et al. Deficiency of suppressor-inducer (CD4+CD45RA+) T cells in autism. *Immunol Invest*. 1990;19:245-251.
4. Gupta S, Aggarwal S, Heads C. Dysregulated immune system in children with autism: beneficial effects of intravenous immune globulin on autistic characteristics. *J Autism Devel Dis.* 1996;26:439-452.
5. Stubbs EG, Crawford ML. Depressed lymphocyte responsiveness in autistic children. *J Autism Child Schizophr*. 1977;7:49-55.
6. Warren RP, Odell JD, Warren WL, et al. Immunoglobulin A deficiency in a subset of autistic subjects. *J Autism Develop Dis*. 1997;27:187-192.
7. Fiumara A, Sciotto A, Barone R. Peripheral lymphocyte subsets and other immune aspects in Rett syndrome. *Pediatr Neurol*. 1999;21:619-621.
8. Warren RP, Foster A, Margaretten NC. Reduced natural killer cell activity in autism. *J Am Acad Chld Adolesc Psych*. 1987;26:333-335.
9. Warren RP, Singh VK, Cole P. Increased frequency of the null allele at the complement C4b locus in autism. *Clin Exp Immunol.* 1991;83:438-440.
10. Stubbs EG. Autistic children exhibit undetectable hemagglutination-inhibition antibody titers despite previous rubella vaccination. *J Autism and Child Schizophr*. 1976;6:269-274.
11. Stubbs EG. Interferonemia and autism. *J Autism Develop Dis*. 1995;25:71-73.
12. Croonenberghs J, Bosmans E, Deboutte D, et al. Activation of the inflammatory response system in autism. *Neuropsychobiology*. 2002;45:1-6.
13. Singh VK. Plasma increase of interleukin-12 and interferon-gamma: pathological significance in autism. *J Neuroimmunol*. 1996;66:143-145.
14. Messahel S, Pheasant AE, Pall H, et al. Urinary levels of neopterin and biopterin in autism. *Neurosci Lett*. 1998:241:17-20.
15. Singh VJ, Warren RP, Odell JD, et al. Changes of soluble interleukin-2, interleukin-2 receptor, T8 antigen, and interleukin-1 in the serum of autistic children. *Clin Immunol Immunopath*. 1991;61:448-455.
16. Peakman M, Verani D. *Basic and clinical immunology*. New York, NY: Churchill Livingston; 1997:108.
17. Comi AM, Zimmerman AW, Frye VH, et al. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *J Child Neurol*. 1999;14:388-394.
18. Kono DH, Park MS, Szydlik A, et al. Resistance to xenobiotic-induced autoimmunity maps to chromosome 1. *J Immunol*. 2001;167:2396-2403.
19. Daniels WP, Warren RP, Odell JD, et al. Increased frequency of the extended or ancestral haplotype B44-SC30-DR4 in autism. *Neuropsychobiol*. 1995;32:120-123.
20. Singh VK, Warren RP, Odell JD, et al. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun*. 1993;7:97-103.
21. Singh VK, Lin SX, Yang VC. Serological association of measles virus and human herpes virus-6 with brain autoantibodies in autism. *Clin Immunol Immunopathol.* 1998;89:105-108.
22. Singh VK, Warren R, Averett R, et al. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatr Neurol*. 1997;17:88-90.
23. Connolly AM, Chez MG, Pestronk A, et al. Serum autoantibodies to brain in Laundau-Kleffner variant, autism, and other neurological disorders. *J Peds*. 1999;134:607-613.
24. Weizman A, Weizman R, Szekely GA, et al. Abnormal immune response to brain tissue antigen in the syndrome of autism. *Am J Psychiat*. 1982;139:1462-1465.
25. Kono DW, Balomenos D, Pearson DL, et al. The prototypic Th2 autoimmunity induced by mercury is dependent on IFN-Gamma and not Th1/Th2 imbalance. *J Immunol*. 1998;161:234-240.
26. Torrente F, Ashwood P, Day R, et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. *Molec Psych*. 2002;7:375-382.
27. El-Fawal H, Waterman SJ, De Feo A, et al. Neuroimmunotoxicology: humoral assessment of neurotoxicity and autoimmune mechanisms. *Environ Hlth Persp*. 1999;107:767-775.
28. Chang KW ed. *Toxicology of Metals*. Boca Raton, FL: CRC Press; 1996:842-845.
29. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest*. 2001;108:1097-1104.
30. Fagan DG, Pritchard JS, Clarkson TW, et al. Organ mercury levels in infants with omphaloceles treated with organic mercurial antiseptic. *Arch Dis Child*. 1977;52:962-964.
31. Rohyans J, Walson PD, Wood GA. Mercury toxicity following merthiolate ear irrigations. *J Pediatr*. 1984;104:311-313.
32. National Academy of Sciences press release, July 21, 2000.
33. van der Laan JW, de Waal E. *Safety working party assessment of the toxicity of thimerosal in relation to its use in medicinal products*. The European Agency for the Evaluation of Medicinal Products; London: 8 September 1998: B04210.
34. Stratton K, Gable A, McCormick MC, eds. *Immunization safety review: thimerosal-containing vaccines and neurodevelopmental disorders.* Immunization safety review committee, board on health promotion and disease prevention, the Institute of Medicine. 2001 Available at: www.iom.edu/imsafety. Accessed on August 7, 2002.
35. TEST Foundation. Available at: www.altcorp.com/testfoundation.htm Accessed on August 7, 2002.

36. Bernard S, Enayati A, Binstock T, et al. Autism: A unique type of mercury poisoning. Cranford, NJ: ARC Research; 2000: 1-76. Available at: www.autism.com/ari/mercurylong.html. Accessed August 7, 2002.
37. SafeMinds Website Available at: www.dmcreative.tmp.com/safeminds/board.htm. Accessed June 24, 2002.
38. McGinnis WR. Mercury and autistic gut disease. *Environ Hlth Persp*. 2001;109:303-304.
39. Chen W, Body RL, Mottet NK. Biochemical and morphological studies of monkeys chronically exposed to methylmercury. *J Toxicol Environ Hlth*. 1983;12:407-416.
40. Horvath K, Papadimitriou JC, Rabsztyn A, et al. Gastrointestinal abnormalities in children with autistic disorder. *J Pediatr*. 1999;135:559-563.
41. Stejskal VD, Forsback M, Nilsson R. Lymphocyte transformation test for diagnosis of isothiazoline allergy in man. *J Invest Derm*. 1990;94:798-802.
42. Stejskal VD, Cederbrandt K, Lindvall A, et al. MELISA-an in vitro tool for the study of metal allergy. *Toxicol in Vitro*. 1994;8:991-1000.
43. Stejskal VD, Forsbeck M, Cederbrant KE. Mercury-specific lymphocytes: An indication of mercury allergy in man. *J Clin Immunol*. 1996;16:31-40.
44. Tibbling L, Thuomas KA, Lenkei R, et al. Immunological and brain MRI changes in patients with suspected metal intoxication. *Int J Occupat Med Toxicol*. 1995;4.
45. Stejskal VD, Danersund A, Lindvall A, et al. Metal-specific lymphocytes: Risk factors in CFS and other related diseases. *Neuroendocrinol Lett*. 1999;20:289-298.
46. Stejskal VD. Human hapten-specific lymphocytes: Biomarkers of allergy in man. *Drug Info J*. 1997;31:1379-1382.
47. Pang PK, Shan JJ, Lewanczuk RZ, et al. Parathyroid hypertensive factor and intracellular calcium regulation. *J Hypertens*. 1996;14:1053-1060.
48. Lewanczuk RZ, Benishin CG, Shan J, et al. Clinical aspects of parathyroid hypertensive factor. *J Cardiovasc Pharmacol*. 1994;23:23-26
49. Tessier-Lavigne M, Mobbs P, Attwell D. Lead and mercury toxicity and the rod light response. *Invest Opthalmol Vis Sci*. 1985;26:1117-1123.
50. Ritvo ER, Creel D, Realmuto G, et al. Electroretinograms in autism: A pilot study of b-wave amplitudes. *Am J Psychiat*. 1988;145:1085-1086.
51. Verstraeten T, David R, Destefano F. Risk of neurological and renal impairment associated with thimerosal-containing vaccines. Report to the Advisory Committee on Immunization Practices; Atlanta GA: June 21, 2000.
52. Finegan J, Quarrington B. Pre-, peri-, and neonatal factors and infantile autism. *J Child Psych Psychiatr*. 1979;20:119-128.
53. Stejskal J. Measles lymphadenopathy. *Ultrastruct Pathol*. 1980;1:243-247.
54. Dankova E, Kasal P, Bergmannova V, et al. Immunological findings in children with abnormal reactions after immunization. *Cesk Pediat*. 1993;48:9-12.
55. Wakefield AJ, Murch SH, Linnell J, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet*. 1998;351:637-641.
56. Wakefield AJ, Anthony M, Murch SH, et al. Enterocolitis in children with developmental disorders. *Am J Gastroenterol*. 2000;95:2285-2295.
57. Walker-Smith J. Letter: Autism, bowel inflammation, and measles. *Lancet*. 2002;359:705-706.
58. Furlano RI, Anthony A, Day R, et al. Colonic CD8 and gamma delta T-cell infiltration with epithelial damage in children with autism. *J Pediatr*. 2001;138:366-372.
59. Kawashima H, Mori T, Kashiwagi Y, et al. Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. *Digest Dis Sci*. 2000;45:723-729.
60. Uhlmann V, Martin CM, Sheils O, et al. Potential viral pathogenic mechanism for new variant inflammatory bowel disease. *J Clin Pathol Mol Pathol*. 2002;55:1-6.
61. Singh VK. Abnormal measles serology and autoimmunity in autistic children. *J Allergy Clin Immunol*. 2002;109:S232.
62. Fujinami RS, Oldstone MB, Wroblewska Z, et al. Molecular mimicry in virus infection: Crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. *Proc Nat Acad Sci USA*. 1983;80:2346-2350.
63. Warren RP, Odell JD, Warren WL, et al. Strong association of the third hypervariable region of HLA-DR Beta-1 with autism. *J Neuroimmunol*. 1996;67:97-102.
64. Plioplys AV, Greaves A, Kazemi K, et al. Lymphocyte function in autism and Rett's syndrome. *Neuropsychobiol*. 1994;7:12-16.
65. Bitnun A, Shannon P, Durward A, et al. Measles inclusion-body encephalitis caused by the vaccine strain of measles virus. *Clin Infec Dis*. 1999;29:855-861.
66. Belgamwar RB, Prasad S, Appaya P. Measles, mumps, rubella vaccine induced subacute sclerosing panencephalitis. *J Indian Med Assoc*. 1997;95:594.
67. *Physician's Desk Reference 2002*. Montvale NJ: Medical Economics Company; 2002:2120.
68. Johnson RT, Griffin DE, Hirsch RL, et al. Measles encephalomyelitis- clinical and immunologic studies. *N Engl J Med*. 1984;310:137-141.
69. Caballero B, Rice A. Low serum retinol is associated with increased severity of measles in New York City children. *Nutr Rev*. 1992;50:191-192.
70. Yalcin SS, Yurdakok K, Ozalp I, et al. The effect of live measles vaccines on serum vitamin A levels in healthy children. *Acta Paediatr Jpn*. 1998;40:345-349.
71. Butler JC, Havens PL, Sowell AL, et al. Measles severity and serum retinol (vitamin A) concentration among children in the United States. *Pediatrics*. 1993;91:1176-1181.
72. Gupta PC, Dutta AK, Khare S, et al. Immunoglobulins profile of measles. *Indian Pediatr*. 1989;26:780-784.
73. Audhya T. *Abnormalities and dysfunction of vitamins and fatty acids in autism*. Defeat Autism Now Symposium, Phoenix, AZ, July 2000.
74. Megson MN. Is autism a G-alpha protein defect reversible with natural vitamin A? *Med Hypoth*. 2000;54:979-983.
75. Bhaskaram P. Immunobiology of mild micronutrient deficiencies. *Br J Nutr*. 2001;85:S75-S80.
76. Richer JM, Cross RG. Gaze aversion in autistic and normal children. *Acta Psychiatr Scand*. 1976;53:193-210.
77. Schuil J, van de Putte EM, Zwaan CM, et al. Retinopathy following measles, mumps, and rubella vaccination in an immuno-incompetent girl. *Int Opthalmol*. 1998;22:345-347.
78. Fan PC, Teug RJ, Chou CC, et al. Impaired immune function in a premature infant with zinc deficiency after total parenteral nutrition. *Chung Hua Min Kwo Hsiao Erh Kol Hsueh HUI Tsa Chih*. 1996;37:64-69.
79. Sazawal S, Jalla S, Mazumder S, et al. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. *Indian Pediatr*. 1997;34:589-597.
80. Folwaczny C. Zinc and diarrhea in infants. *J Trace Elem Med Biol*. 1997;11:116-122.
81. Walker-Smith JA, Andrews J. Alpha-antitrypsin, autism and celiac disease. *Lancet*. 1972;2:883-884.
82. Goodwin MS, Cowen MA, Goodwin TC. Malabsorption and cerebral dysfunction: A multivariate and comparative study of autistic children. *J Autism Child Schizophr*. 1971;1:48-62.
83. Horvath K, Tildon J. The role of secretin in autistic spectrum disorder. *Int Rev Research Mental Retard*. 2001;23:33-54.
84. D'Eufemia P, Celli M, Finocchiaro R, et al. Abnormal intestinal permeability in children with autism. *Acta Paediatr*. 1996;85:1076-1079.
85. Shaw W, Kassen E, Chaves E. Increased urinary excretion of analogues of Krebs cycle metabolites and arabinose in two brothers with autistic features. *Clin Chem*. 1995;41:1094-1104.
86. Reichelt KL. Biochemistry and psychophysiology of autistic syndromes. *Tidsskr Nor Laegeforen*. 1994;114:1432-1434.
87. Shattock P, Kennedy A, Rowell F, et al. Role of neuropeptides in autism and their relationships with classical neurotransmitters. *Brain Dysfunction*. 1991;3:328-325.
88. Knivsberg AM, Reichelt KL, Nodland M. Reports on dietary intervention in autistic disorders. *Nutr Neurosci*. 2001;4:25-37.
89. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. The exorphins. *J Biol Chem*. 1979;254:2446-2449.
90. Fukudome S, Yoshikawa M. Gluten exorphin c. A novel opioid peptide derived from wheat gluten. *FEBS Lett*. 1993;316:17-19.
91. Chabance B, Marteau P, Rambaud JC. Casein peptide release and passage to the blood in humans during digestion of milk or yogurt. *Biochime*. 1998;80:155-165.
92. Zagon IS, McLaughlin PJ. Endogenous opioid systems regulate cell proliferation in the developing rat brain. *Brain Res*. 1987;412:68-72.
93. Hauser KF, McLaughlin PJ, Zagon IS. Endogenous opioid systems and the regulation of dendritic growth and spine formation. *J Comp Neurol*. 1989;281:13-22.
94. Barrett AJ, ed. *Handbook of proteolytic enzymes*. Academic Press; 1998:379-382.
95. Srikantaiah MV, Radhakrishnan AN. Studies on the metabolism of vitamin B6 in the small intestine: Part III-purification and properties of monkey intestinal pyridoxal kinase. *Indian J Biochem*. 1970;7:151-156.
96. Rimland B. High dose vitamin B6 and magnesium in treating autism: Response to study by Findling et al. *J Autism Dev Disord*. 1998;28:581-582.
97. Scriver CR, Beaudet AL, Sly WS, et al, eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill; 1995:1725-1768.
98. Stubbs G, Litt M, Lis E, et al. Adenosine deaminase activity decreased in autism. *J Amer Acad Chld Psychiatr*. 1982;21:71-74.
99. Pangborn JB, Baker SM. *Biomedical assessment options for children with autism and related problems*. San Diego, CA: Autism Research Institute;2001:54.
100. Lai Ping So A, Mayer L. Gastrointestinal manifestations of primary immunodeficiency disorders. *Semin Gastroinstest Dis*. 1997;8:22-32.
101. Washington K, Stenzel TT, Buckley RH, et al. Gastrointestinal pathology in patients with common variable immunodeficiency and X-linked agammaglobulinemia. *Am J Surg Pathol*. 1996; 20(10):1240-1252.
102. Ojuawo A, Milla PJ, Lindley KJ. Non infective colitis in infancy: evidence in favor of minor immunodeficiency in its pathogenesis. *East Afr Med J*. 1997;74:233-236.
103. Klein N, Jack D. Immunodeficiency and the gut: clues to the role of the immune system in gastrointestinal disease. *Ital J Gastroenterol Hepatol*. 1999;31:802-806.

HOLOGIC®

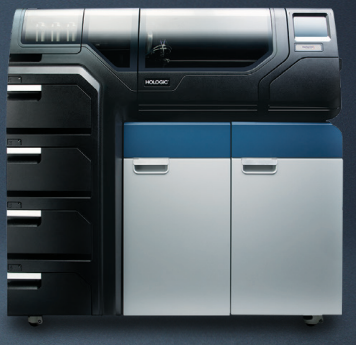
ThinPrep® Processors

Meeting the Demands of Your Lab



THINPREP® GENESIS™ PROCESSOR

THINPREP® 5000 PROCESSOR



THINPREP® 5000 AUTOLOADER

ThinPrep® processors are versatile and scalable solutions for laboratories of all volumes.

The portfolio offers reliable, best-in-class systems that help cytology labs automate their processes – decreasing the need for hands-on labor and increasing laboratory efficiencies.

Explore the portfolio

ThinPrep®