



ARISTO VOJDANI, Ph.D., M.Sc., M.T.

Dr. Aristo Vojdani obtained his PhD in the field of microbiology and clinical immunology with postdoctoral studies in tumor immunology. He is the CEO and Technical Director of Immunosciences Lab., Inc. in Los Angeles, CA; member of the editorial board of three scientific journals; and has published more than 100 articles in scientific journals. He is noted for his papers on autoimmunity and neuroimmunological diseases. In 2006, Dr. Vojdani was presented with the prestigious Herbert J. Rinkel Award by the American Academy of Environmental Medicine (AAEM) for excellence in teaching the techniques of environmental medicine.



JAMA LAMBERT
Immunosciences Lab, Inc.

Jama Lambert earned her Bachelor of Arts and Sciences degree at the University of Oregon. In 2005, she came under the tutelage of Aristo Vojdani, PhD, and she attributes her immune expertise to him. Jama is a technical and fictional freelance writer, who makes her home in Los Angeles, California.

A GUT FEELING FOR IMMUNE DYSREGULATION & NEUROINFLAMMATION IN AUTISM

INTRODUCTION

The genetic aspect of autism is receiving a plethora of media attention in the US. Indeed, genetics play a role in determining an individual's susceptibility to autism spectrum disorders (ASD), celiac, cancer or other complex diseases, but it is the environment that triggers the genes, which culminates in the disorder. The pathophysiology of dysregulation in ASD can be simplified into four understandable steps. First, environmental triggers, such as viral infection, toxicants and/or dietary proteins and peptides, contribute to gut inflammation. This results in step two, enhanced intestinal permeability, or "leaky gut." This state of intestinal dysfunction brings on step three, immune dysregulation, which manifests itself in the nervous system. In step four, the patient has neuroinflammation. Before an environmental factor triggers dysfunction in the body, however, it must first infiltrate a network of immune defenses.

BASICS OF IMMUNOLOGY

Working in concert, molecules, cells and tissues defend the body against non-self antigens and pathogens. (References for this section, except where otherwise noted, come from *Basic Concepts in Immunology: A Student's Survival Guide* by John Clancy, Jr., Ph.D., an easy to comprehend guide to the immune system as a whole.) Microbes attempting an invasion must first get through the skin or mucosal layer, which is composed of scavenger cells and IgA antibodies. If the microbe is successful in penetrating the body's protective shielding or epithelial

surfaces, it must still deal with two distinct but complementary defense systems: innate immunity and adaptive immunity.

INNATE (NON-SPECIFIC) AND ADAPTIVE (ACQUIRED OR SPECIFIC) IMMUNITY

Innate immunity is composed of a series of nonspecific defenses and substances that attack all invaders. It is so-called because this kind of immunity is present in the body from birth. It is also called non-specific because it does not need to be activated by a specific antigen, but will send out specialized cells to engulf and destroy anything it doesn't recognize as being part of the "self." Components of innate immunity include phagocytes, granulocytes, natural killer (NK) cells and the complement system, a biochemical cascade that helps clear pathogens from an organism. Considered the first line of active defense, the innate immune system acts immediately upon attack. If the innate defense fails against the microbe, the adaptive immune system comes into play. It is also called specific immunity because, completely opposite to the innate system, it must be triggered by specific antigens, and then confronts the aliens with specific antibodies and cytotoxic T-cells tailored to effectively destroy the microbes. It is also unlike innate immunity in that there is a certain delay in the adaptive system's response. This is where the adaptive system gets the "acquired" part of its name. While it takes its time to assemble a specific adaptive response to a particular antigen, adaptive immunity's big gun is its immunological memory. It "remembers" having previously

encountered an invading organism and reacts more rapidly and efficiently the next time, thus “acquiring” immunity to that particular pathogen. This immunity can be gained after recovering from most pathogenic attacks and is generally lifelong, although it does not preclude being subject to infection by previously unencountered pathogens.

NK CELLS

As part of innate immunity, NK cells have fascinated immunologists since their discovery thirty years ago. These cells mediate early non-adaptive responses against viruses, intracellular bacteria, parasite-infected cells and malignancies. They mediate these effects through the production of cytokines and the direct killing of transformed, or infected, cells by granule release as shown in Figure 1. Due to their involvement in regulating immune responses, NK cells can be associated with autoimmune disorders. As regulators of adaptive immune responses, NK cells inhibit autoreactive T-cells to curb neuroinflammation. To illustrate adaptive immunity, imagine that a gluten peptide, or *streptokinase* (a bacterial antigen), penetrates the mucosal layer, passes through the intestinal barrier through open tight junctions and enters general circulation. As shown in Figure 2, a patrolling antigen-presenting cell (APC) such as a monocyte, macrophage or dendritic cell will either process or present the already processed antigen to receptors on T-cells. This begins an orchestrated defense.

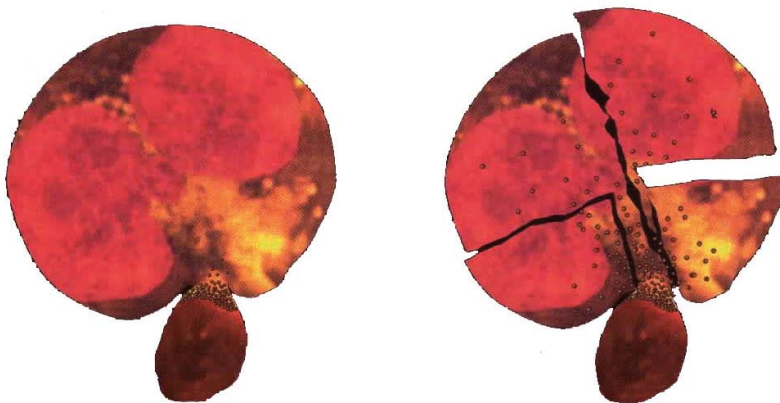


Figure 1: NK Cell Targeting Tumor Cell - On the left, the smaller NK cell binds to a very large tumor cell. To the right, the NK cell has released its granules, which kill the tumor cell by degrading the cell’s membrane.

NK CELLS IN AUTISM

Although immune system abnormalities have been previously implicated in autism and reported in many articles, NK cell activity has only been examined in one study that found reduced activity in 12 of 31 patients. However, this study did not provide evidence for the mechanism responsible for reduced NK cell activity. Thus, we explored the measurement of NK cell activity in 1027 blood samples from autistic children obtained from ten clinics operated by Defeat Autism Now! practitioners and compared the results to 113 healthy controls. The results of this study were recently published in *Journal of Neuroimmunology* [Vojdani, 2008C]. We found that NK cell activity was low in 41-81% of the patients from the different clinics. This same low NK cell activity was found in only 8% of healthy subjects. Vojdani and colleagues concluded that 45% of a subgroup of children with autism suffers from low NK cell activity and that low intracellular levels of glutathione, interleukin (IL)-2 and IL-15 may be responsible. From this study we learn that low NK cell activity is the most prominent immunological abnormality in children with autism, which may be due to suboptimal cytokine production and low cellular level of glutathione. However, it remains to be proven whether or not supplementation with glutathione and inducers of IL-2 and IL-15 will be able to restore low NK cell activity in children with autism to normal levels. In an earlier study [Heuser, 1997], it was shown that NK cell activity could be reversed by

vitamin C supplementation in almost 80% of adult patients who suffered from chronic illnesses; this approach may also be considered for patients with autism. Although NK cell activity is a vital immune marker, it remains to be investigated whether or not reversal of low NK cell activity can contribute to improved clinical manifestations presented in patients with autism.

THE T-CELL FAMILY

Working in concert with NK cells in the killing of targeted cells are the cytotoxic T-cells. T-cells are cells generated in the thymus. The T-helper (Th) cells are mature CD4 T-cells that provide helper functions maximizing the capabilities of the immune system and assist B-cells in humoral immune responses; CD8 cells are cytotoxic T-cells (CTLs) that are involved in recognizing foreign cells. The binding of APCs to CD4 or CD8 T-cells facilitates the activation of T-cells. In the primary response, effector or activated T-cells and memory T-cells are generated. These activated T-cells will die by apoptosis, programmed cell death, within 2 to 3 days after primary response. However, memory cells live months to years and respond fervently to the same antigen in subsequent antigenic challenges known as the secondary response. Activated T-cells will produce cytokines, chemical messengers which activate additional cells. NK and CTL cells are alerted to attack the antigen. Upon a signal from the T-helper cell, the B-cell, which is generated in bone marrow, makes immunoglobulin (Ig) to tag the antigen. In other words, the B-cell places a bullseye on the antigen so that killer cells know what to target in their attack. When the battle has been won, the T-suppressor cell calms the activities of the T-helper, thereby quelling the attack. The cell keeping a precarious balance between T-helper and T-suppressor is the regulatory T-cell (T_{reg}), or T-helper-3 (Th3).

The mysterious operations of T_{regs} have not been fully elucidated. Researchers know T_{regs} are the major arbiters of immune responses, mediating actions through the suppression of inflammatory and destructive immune reactions by balancing Th1, cell-mediated, and

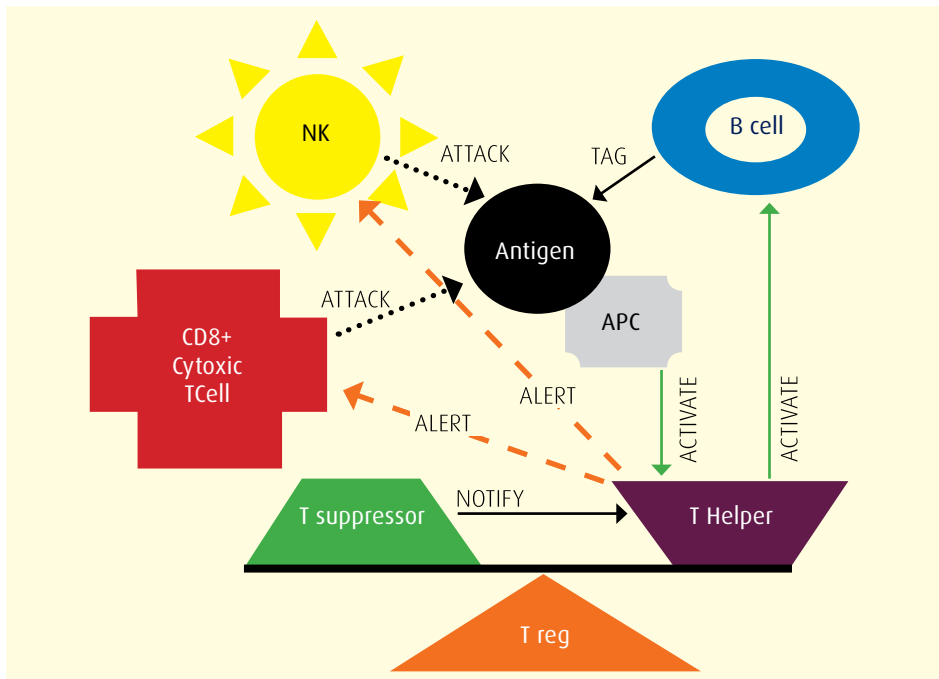


Figure 2: Immune Response - The antigen, a foreign molecule, enters circulation where an antigen-presenting cell (APC) picks it up and takes it to, and activates, the T cell. The T helper activates the B cell to produce a specific antibody against the antigen, and which tags the antigen for recognition. The T helper also alerts the cytotoxic T cell and the natural killer (NK) cell, both of which attack the foreign material. The T suppressor cell notifies the T helper when the battle is over and thus shuts down the immune response. The regulatory T cell (Treg) keeps a balance between the T suppressor and the T helper to maintain homeostasis.

Th2, humoral-mediated immunity. Inappropriate T_{reg} cell functionality potentiates the pathogenesis of diseases with ranging magnitudes of severity. Lack of suppressive capability hinders restraint on immune responses involved in autoimmunity, while suppressive capacity effectively blocks processes necessary for tumor destruction (Vojdani, 2006A, 2006B, 2006C). In a subgroup of ASD patients, the important balance of Th1/Th2 is abnormal, and thus contributes to immune dysfunction associated with the spectrum disorder.

Cellular-mediated immunity involves macrophages, T-cells, natural killer cells and cytokine production. Cytokines are produced by various immune cells, upon activation, in order to communicate with other cells. Th1 cytokines include IL-2, interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α). IL-2 and IFN- γ are the two major cytokines produced by Th1 cells. The Th1 cells, NK cells and macrophages secrete cytokines that are apparently well-coordinated to induce a number of

cytotoxic and inflammatory functions. NK cells secrete IFN- γ ; Th1 cells secrete IFN- γ , lymphotoxin and TNF- α ; and macrophages secrete TNF- β . These cytokines synergize to induce effective lysis, destruction by disruption of the membrane, of target cells, particularly virus-infected cells. The same cytokines activate both macrophages and granulocytes for increased killing function. There is a yin-yang dynamic between agonist and antagonist cytokines, which must be kept in balance for optimal immune reactions.

T-HELPER-1 VERSUS T-HELPER-2

Countering Th1 is Th2 and its humoral immune properties (see Figure 3). Humoral immunity refers to antibody production and the accessory processes that accompany it. Components of this system include secretory IgA, a complex of polymeric IgA and epithelial cell-derived secretory component, immunoglobulins, complement cascade, which is set off when circulating complement encounters an antigen-

antibody complex and each molecule of the complement performs a specialized job to destroy the target cell, and immune complexes, aggregates composed of antigens and immunoglobulins, which activate complement cascade and block cell-mediated immunity. Humoral-mediated immunity also uses cytokines to communicate. Th2 cytokines include IL-4, IL-5, IL-10 and IL-13. Th2 cytokines have the ability to enhance allergic responses. IL-4 is a major switch factor that induces heavy-chain class switching to IgE production by B-cells. As a result of Th2 cell activation and the secretion of the characteristic Th2 cytokine pattern, mast cells and eosinophils become activated. Upon activation (see Figure 4), these cells bind IgE molecules, respond to antigens, and release various mediators. IL-4 also enhances the differentiation of Th2 cells and inhibits differentiation of Th1 cells, thus encouraging the maintenance of a Th2 response and continued enhancement of allergy. In contrast to all these positive influences of Th2 cells on the allergic process, Th1 cells secrete IFN- γ , which inhibits the induction of IgE switching by IL-4 in B-cells, the activation of mast cells, the activation of eosinophils, and the proliferation of Th2 cells. Enhancement of the Th1 response to balance Th1/Th2 is implied in allergy treatment.

Evidence has been accumulating to suggest that the pathogenesis of some human diseases may be classified in terms of type-1 and type-2 T-helper cell cytokine profiles. For example, multiple sclerosis, type-1 diabetes, and arthritis are Th1 phenotypes, while allergy, chemical sensitivity, parasitic infection and lupus erythematosus are Th2 phenotypes (see Table 1). Scientists theorize that at birth the immune system is immature and is skewed toward Th2 cytokine production. Certain stimuli such as infections and viruses usually contracted from older siblings, or peers in day care centers, can help immunological development toward a healthy balance of Th1 and Th2 responses. Children without such exposures, living in a relative "sterile" environment, may perpetuate the Th2 dominance resulting in increased risk of asthma, other atopic diseases and

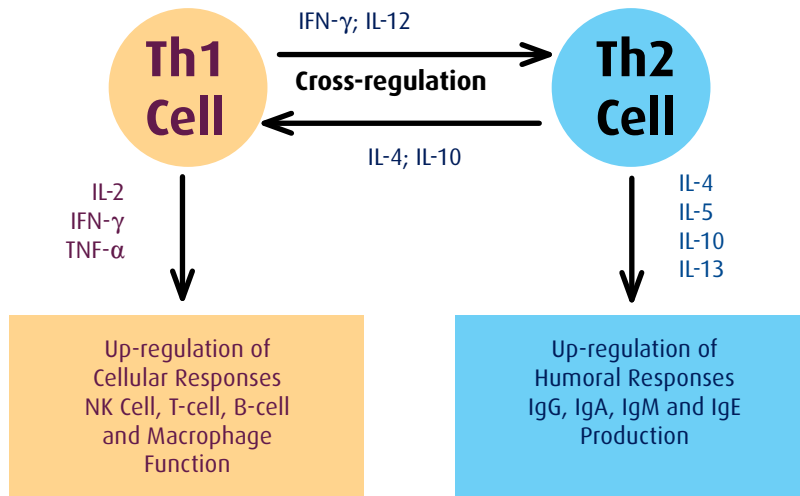


Figure 3: Th1 Versus Th2 Regulation and Cross-Regulation of Cell-Mediated Immunity and Humoral Immune Response - In the presence of different cytokines, T-helper cells up-regulate the cellular and humoral immune responses. T-helper cells also utilize different cytokines to communicate between Th1 and Th2 cells.

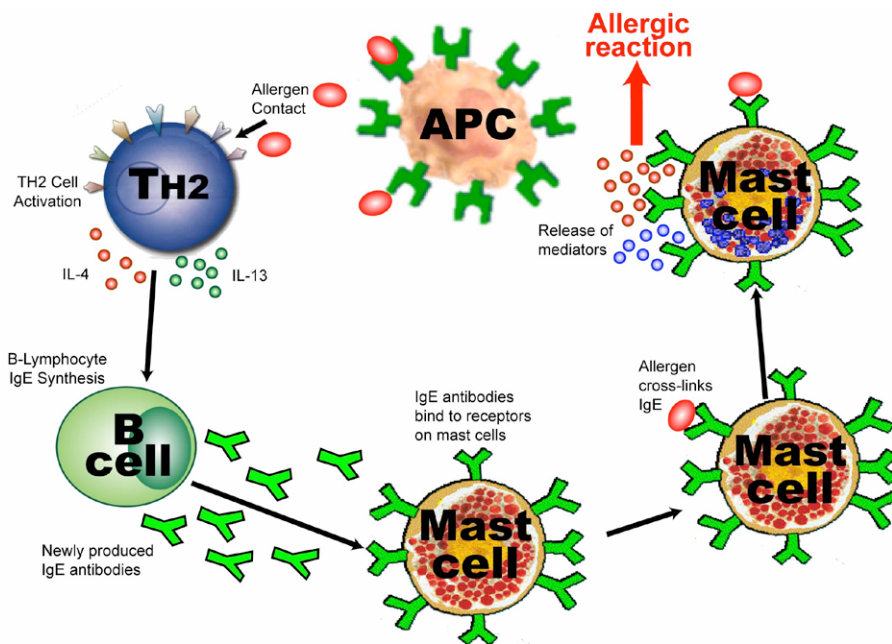


Figure 4: Pathway to Allergy - Antigen presenting cells (APCs) recognize and process allergens, resulting in Th2 stimulation and the production of IL-4 and IL-13. This leads to IgE synthesis by B-cells. The newly secreted IgE antibodies bind to mast cells via specific Fc receptors. The subsequent cross-linking of receptor-bound IgE by allergen triggers the release of mediators and the onset of allergy.

hypersensitivities to chemicals and foods. However, this theory is contradicted by observations that the prevalence of Th1-autoimmune diseases, in which the immune system's recognition ability breaks down and the body begins to manufacture antibodies and T-cells directed against the body's own cells and

organs, is also increasing. Additionally, Th2-skewed parasitic worm infections are not associated with allergy. Elevations of anti-inflammatory cytokines, such as IL-10, that occur during long-term parasitic worm infections, have been shown to be inversely correlated with allergy. The induction of a robust anti-inflammatory

regulatory network by persistent immune challenges offers a unifying explanation for the observed inverse association of many infections with allergic disorders. T_{reg} immunoregulation (see Figure 5) is the dominant means utilized by the immune system to reach an orchestrated harmony between reciprocal response processes in order to ensure adequate host defense with minimal host detriment.

CYTOKINES, THE IMMUNE MESSENGERS

Cytokines, as chemical messengers of the immune system, operate much like hormones and neurotransmitters of the endocrine and nervous systems respectively. These messengers are not exclusive to individualized systems; rather, they affect and modulate each other across systems via shared receptors. For example, the hormone cortisol and chatecholamine neurotransmitters affect the output of secretory IgA, the vital immune component of mucosal linings. Additionally, the gastrointestinal (GI) tract is home to nearly 80% of the immune system and at the same time produces 90% of serotonin. The gut, therefore is the logical starting point for many neurodegenerative disorders. It begins with imbalanced gut microflora, which releases copious amounts of lipopolysaccharide (LPS). The abundant LPS endotoxin induces up-regulation of proinflammatory cytokines TNF-α and IL-1β, resulting in the opening of tight junctions. This is followed by inflammation in the blood stream which travels to the blood-brain barrier (BBB). At the BBB, the inflammation opens the BBB causing neuro-infiltration, neuro-inflammation, neuro-autoimmunity and finally, neurodegeneration. Figure 6 represents the pathophysiology leading to neurodegeneration; if a person's intestinal barrier dysfunction is not addressed, he could develop neuroinflammation and possible neurodegeneration over time.

Spectrum disorders have multiple triggers, symptoms, and system dysfunctions. In cases of ASD, where many of the individuals produce high levels of antibodies to LPS and to BBB protein, the immune and nervous systems are each involved. Therefore, the common

ground for these two systems is the GI tract, which has been addressed by many investigators.

THE GUT

A mucosal layer consisting of beneficial bacteria, or microflora, and secretory IgA, acts as a first line of defense against invasion by preventing antigen adhesion and eventual penetration of the gut's intrinsic layer [Fasano, 2005; Walker, 2004]. Epithelial cells, held together by tight junctions, make up the intrinsic layer, or intestinal barrier, of the GI tract. The system works like window blinds. When the blinds are closed, small shafts of light still penetrate between the slats. The intestinal barrier, even when functioning optimally, allows small molecules to pass into general circulation. This important mechanism builds immunity and primes the systemic immune response [Fasano, 2005; Walker, 2004]. Oral tolerance may occur, diminishing mucosal layer effectiveness. This may lead to an accumulation

Fasano concluded that the loss of intestinal barrier function is the major contributor to autoimmune pathogenesis.

of dietary proteins and/or spawn a microflora imbalance during which normal GI bacteria will release LPS endotoxins [Fasano, 2005]. A store of food products or LPS will up-regulate proinflammatory cytokines, opening tight junctions, and spewing significant amounts of unwanted dietary proteins, bacterial antigens and xenobiotics into circulation, which challenge the immune system [Fasano, 2005]. Fasano concluded that the loss of intestinal barrier function is the major contributor to autoimmune pathogenesis.

GI INFLAMMATION

Many combinations of environmental factors may ignite gut inflammation [Fasano, 2005; Gardner, 2002]. Difficult to digest wheat (gluten, gliadin, gluteomorphin) and dairy (milk, egg white, casein, casomorphin, milk butyrophilin) are notorious instigators

of gut dysfunction [Gardner, 2002; Vojdani, 2004]. These dietary proteins and peptides can stimulate T-cells, induce peptide-specific T-cell responses, and abnormal levels of cytokine production, which may result in inflammation and autoimmune reactions. Acting like opioids, these peptides can enter through an enhanced BBB, compete with opioid receptors, and disrupt neuroimmune communications and behavior [Gardner, 2002]. This is the classic gut to brain connection.

Modern, industrialized societies expose the immune system to untold toxins from pollution, heavy metals, chemicals, and pharmaceuticals. These xenobiotics can produce an inflammatory autoimmune reaction by binding to a carrier protein, which then interacts with gut-associated lymphoid tissues (GALT) [Vojdani, 2003]. Metal complexes, including ethyl mercury from vaccines, possess increased complex stability due to a central metal ion binding capacity to four different amino acids, while other xenobiotics can bind to a single amino acid. Vojdani *et al.* [Vojdani, 2003] found that bacterial antigens and ethyl mercury in a sub group of children with ASD bound to CD26, a cell adhesion molecule, or CD69, an early activation marker, on T-cells and NK cells, and induced antibodies against these molecules as well as to lymphocyte receptors and tissue antigens, resulting in an autoimmune reaction (see Figure 7). Additionally, many infectious agents such as *streptococcus*, measles, Rubella, *Varicellar zoster* and others have been suspected as etiological factors in ASD [Vojdani, 2005]. These agents may bind to certain peptidases like dipeptylpeptidase-IV (DPP-IV), a master enzyme involved in digestion [Vojdani, 2005]. Binding of xenobiotics to enzymes equals autoimmunity. Indeed, xenobiotic exposure can accelerate spontaneous systemic autoimmunity; in addition, low-level xenobiotic exposure enhances susceptibility to systemic autoimmunity in genetically predisposed individuals [Pollard, 2001].

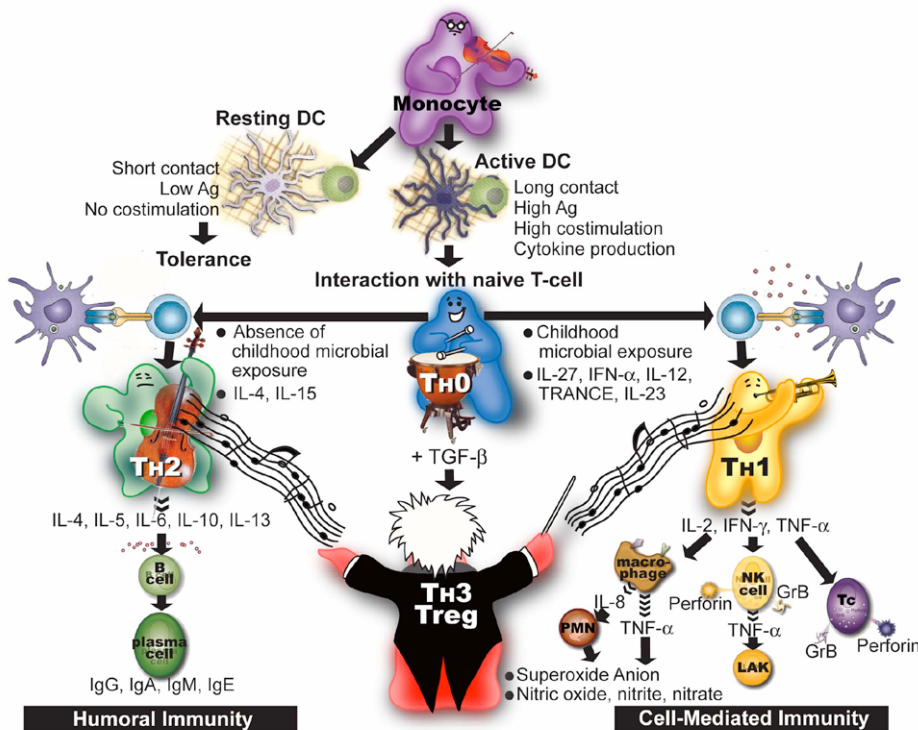


Figure 5: Regulation of the Immune System by T_{reg} Cells and Regulatory Cytokines
TGF-β and IL-10 in Combating Th1/Th2 Imbalance - T_{reg} acts like a conductor of an orchestra, making sure the instruments are making harmony and thus performing the appropriate immune reactions from T-helper cell subsets. If in disaccord, suppressed Th1 development and function may lead to allergic hypersensitivity, while suppressed Th2 development can result in autoimmune disorders.

T-helper -1 Phenotype

- Multiple Sclerosis
- Diabetes
- Arthritis
- Uveitis
- Lyme Disease
- Mercury-induced Autoimmunity
- Atopic Dermatitis (Initiation Phase)
- Inflammatory Bowel Disease
- Bacterial Infection
- Viral Infection
- Mold Infection

T-helper-2 Phenotype

- Lupus Erythematosus
- Lead-induced Autoimmunity
- Allergy
- Asthma
- Chemical Sensitivity
- Parasitic Infection
- Atopic Dermatitis (Inflammatory Phase)
- Inflammatory Bowel Disease
- Bacterial Infection
- Malignancy

Table 1: T-Helper Subset Dependent Cell – Mediated Allergies and Autoimmunities

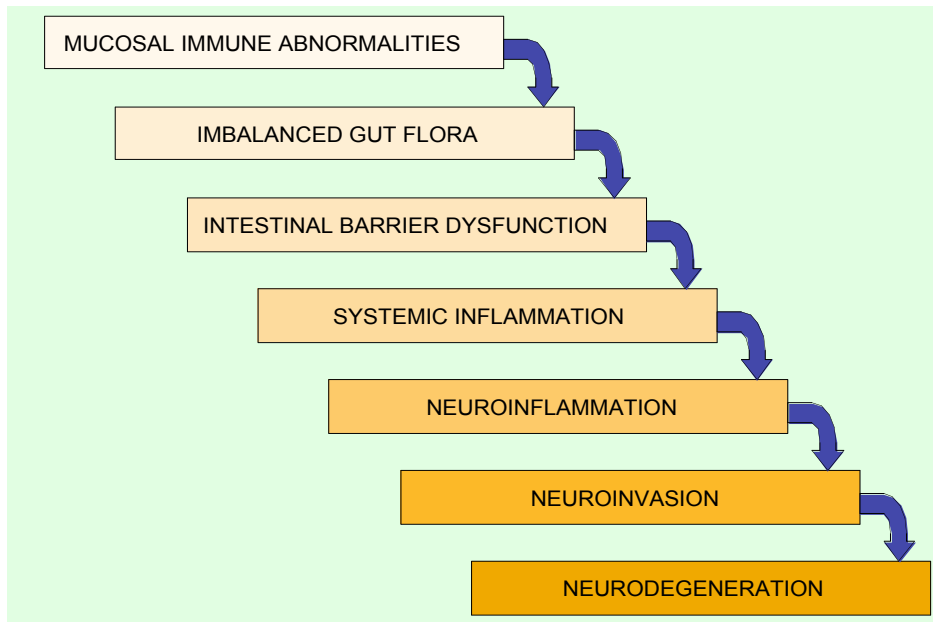


Figure 6: From Gut to Brain Dysfunction - Loss of mucosal tolerance, if unmanaged, can trigger a cascade that induces intestinal barrier dysfunction, systemic inflammation, neuroinflammation, neuroinvasion, and neurodegeneration.

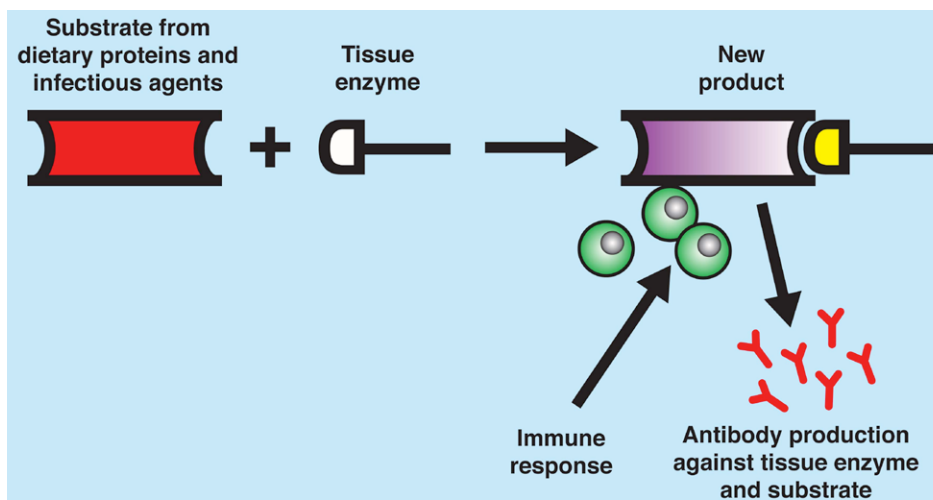


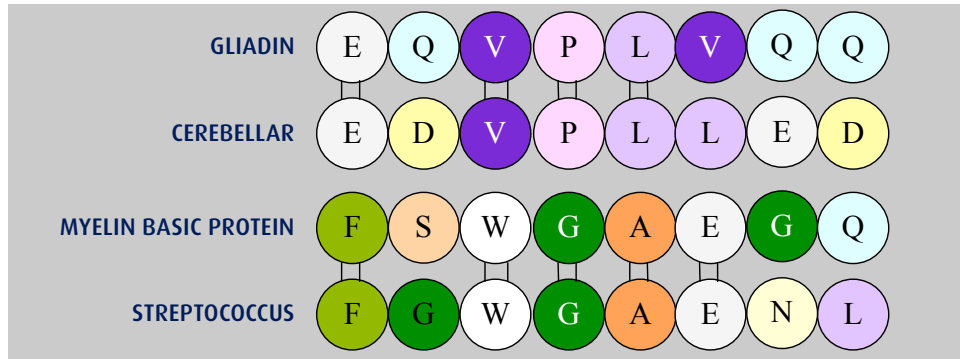
Figure 7: Binding to Enzymes Substrates from dietary proteins and infectious agents or their peptides bind to different tissue enzymes, resulting in antibody production against the enzyme and substrate. The induction of peptide and tissue-specific antibodies may result in autoimmunity.

THE BIRTH OF AUTOIMMUNITY

Researchers study celiac disease to understand more about autoimmune disorders. Celiac’s known inappropriate immune response is to wheat gliadin and related proteins; failure to maintain a strict gluten-free diet may result in immune attack against not only the gut, but also brain, skin, joints, heart, thyroid, and other tissues [Alaedini, 2007; Frustaci, 2002; Vojdani, 2008B; Westall, 2006; Zandoni, 2006]. For some sufferers, the trigger is rotavirus infection [Vojdani, 2008A; Zandoni, 2006]. It is through a mechanism of molecular mimicry (see Figure 8) that autoimmunity of celiac disease reaches beyond the gut [Zandoni, 2006]; the viral protein VP-7 shares homology with celiac peptide, tissue transglutaminase (tTg), heatshock protein (HSP60), toll-like receptor 4 (TLR4) and myotubularin-related protein 2 (MTMR2). Zandoni *et al.* found that these antibodies increased the permeability of the gut intrinsic layer by interacting with the self-antigen desmoglein 1. This protein plays a role in making impermeable seals between the cells that line the gut so that food proteins and other antigens cannot enter circulation where they may be detected by the immune system. Furthermore, defects in MTMR2, part of the protein-tyrosine phosphatase family, are linked to recessive demyelinating neuropathy; and activation of APCs via innate immune receptors such as TLR4 can break self-tolerance and trigger the development of autoimmunity [Zandoni, 2006].

Inflammation associated with ASD, whether leading to autoimmunity or not, can be triggered by a single or a combination of the aforementioned environmental factors. These important cytokines associated with GI inflammation are IL-1 β , TNF- α , and IL-6 [Fasano, 2005]. Upon the induction of mucosal immune dysregulation, cytokines, the immune system’s messenger molecules, are called into action. In reaction to stress and infection, IL-1 is up-regulated to mobilize neutrophils, to facilitate active T-cells, and to stimulate antigen-presenting cells to be more effective [Clancy, 1998]. Working with IL-1, IL-6 acts to increase circulating adrenocorticotrophic hormone (ACTH) levels, while other duties of IL-6

Figure 8: Cross-Reactive Amino Acid Sequences - If amino acids were strung like the beads of a necklace, gliadin and cerebellar protein would look like the above representation, with commonalities, while myelin basic protein shares similarities with streptococcus. When sequence chains mimic each other (molecular mimicry), there is a chance for the production of cross-reactive antibodies, in the process of which the body's own immune systems may attack itself (friendly fire).



include inducing B-cell differentiation and T-cell activation [Clancy, 1998]. Stimulating the acute phase immune reaction, TNF- α activates endothelium, increases permeability, fever and shock, and depletes the glutathione level of cells [Clancy, 1998]. While proinflammatory cytokines are necessary in immune reactions, an overabundance of them or an unchecked response can lead to immune dysfunction and autoimmunity [Clancy, 1998; Fasano, 2005].

NEURODEGENERATION

In most cases, the process of neurodegeneration takes years. It can begin with a seemingly harmless event of oral tolerance, which as explained above, leads to imbalanced microflora and subsequent intestinal barrier dysfunction. The systemic inflammation that ensues triggers neuroinflammation [Vojdani, 2008A; Vojdani, 2008B]. Opening of the other then allows neuroinvasion of molecules such as heavy metals, infectious agents and other toxins circulating in the blood [Vojdani, 2003]. As these toxicants overwhelm the CNS, neurodegeneration occurs [Takeuchi, 1995; Wulferink, 2001]. Indeed, environmental chemicals play a role in the development of nervous system autoimmune disease and/or the progression of neuropathy [El-Fawal, 1999]. Neurons control and regulate body function. Neuroglia, astrocytes, oligodendrocytes, Schwann cells, and microglia provide protection and influence electrical conductive properties of the neuronal axons and dendrites. The blood-brain barrier (BBB), consisting of specialized endothelial cells with tight junctions, pericytes and bone marrow-derived perivascular elements, gives

the nervous system immunoprivileged status by excluding potentially harmful elements, including immune cells. In the presence of chemical-induced nervous system degeneration and continued exposure to the chemicals, lymphocytes can be stimulated to increase autoantibody production to myelin basic protein (MBP) and neurofilament proteins [El-Fawal, 1999]. This can lead to neuroautoimmunity. Fortunately, modern science provides laboratory assessments, or biomarkers, to identify these events. If discovered at an early stage, the progression of degeneration can be slowed, stopped, or even reversed.

BIOMARKERS

Gut function is vital for optimal health. To properly assess intestinal barrier function, antibodies to large molecules found within the GI tract can be measured. Such molecules include dietary proteins and

peptides, yeast, and bacteria antigens including LPS [Vojdani, 1999]. Systemic antibodies detected to these substances indicate enhanced intestinal permeability. Additionally, for patients on the spectrum suffering from food allergy or intolerance, serum IgG and IgA antibodies can be measured to assess delayed food sensitivities, which are more common than IgE-mediated allergy [Sicherer, 2006]. Salivary IgA and IgM can also be measured to assess the breakdown of oral tolerance to food proteins and peptides [Brandtzaeg, 2007; Rumbo, 1998].

Immune assays (see Table 2) may include total immunoglobulins, or functional assessments like cytokine production or natural killer cell activity. Clarification of natural killer cell measurements is warranted here. NK cell count is a computation of the number of killer cells in a given volume of whole blood. Although this assessment holds

Classification	Mucosal		Systemic	
	Humoral	Humoral	Humoral	Cell-Mediated
Biomarkers	<ul style="list-style-type: none"> Secretory IgA (SIgA) Intestinal Barrier Function (Antibodies against large molecules) Cytokine Levels 	<ul style="list-style-type: none"> Total Immunoglobulins Antigen & Tissue Specific Antibodies IgG Subclasses Complement Cascade Immune Complexes Cytokine Levels 	<ul style="list-style-type: none"> Lymphocyte Subset T & B Cell Function NK Cell Activity Regulatory T-Cells T-helper 17 Pro- and Anti Inflammatory Cytokines Th1/Th2 Cytokine Production 	

Table 2: Immune Responses and Evaluations

...environmental triggers, enhanced intestinal permeability, immune dysregulation, and neurodegeneration are the four connected areas to be assessed in ASD.

value, it is the functional assay of NK cell activity that provides the most clinically relevant information. In this test, killer cells are harvested from a specimen and are introduced to a tumor cell. A technician measures the effectiveness of the collective NK cell fight against the tumor cell resulting in tumor cell death. To illustrate NK cell count versus activity, think of a city work crew. At a worksite, there are seven men in hard hats and orange vests, five of whom are "supervising," while two are productively laboring. The worksite is the unit of whole blood, the seven men is the equivalent of an NK cell count and labor being performed by the two equals the NK cell activity. Immunologists know that a patient may have many NK cells and still have immune dysfunction if those cells behave like work site "supervisors," and, on the other hand, another patient may have a low NK count, but exhibit no immune dysregulation because the few cells are fully functional.

Nervous system measurements can be performed on BBB, neurofilament and myelin basic protein antibodies. Abnormal results may signal enhanced BBB permeability, neuronal degeneration, and neurological inflammation respectively [Vojdani, 2008A]. Cellular levels of neurotransmitters may provide information regarding behavioral and cognitive disturbances [Lowen, 1997].

Infectious agents common in ASD include Varicella zoster, cytomegalovirus, Epstein-barr, Human Herpes type-1, -2 and -6 viruses. Measure IgG antibodies for previous exposures and for chronic infection; measure IgM antibodies for recent exposures.

Toxicity issues may be evaluated via glutathione, fibrillarlin, and antibodies to heavy metals. Many forms of glutathione can be measured. The important ratio to know is oxidized (GSSG) to reduced (GSH). Greater than 90% of total glutathione is composed of GSH, which is the antioxidant form that protects cells from toxins, while less than 10% of total glutathione is GSSG, which GSH

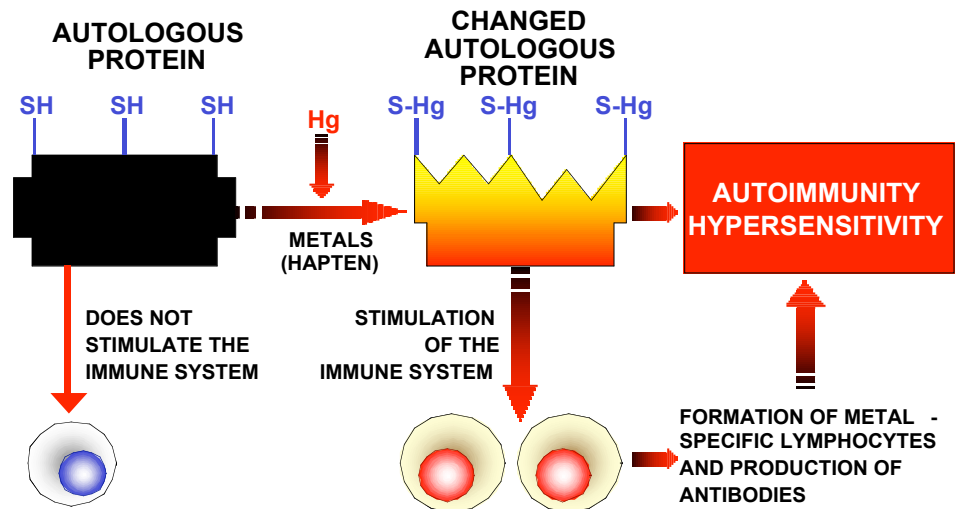


Figure 9: Metals Bind to Tissue - Upon invasion, heavy metals bind to human tissues rather than remain in circulation. As a result, heavy metals can remain in the body for years triggering, or exacerbating, conditions of autoimmunity, or hypersensitivity.

becomes after processing free radicals [Dröge, 1994]. An increased GSSG-to-GSH ratio is indicative of oxidative stress. Fibrillarlin is a nuclear protein to which haptenic chemicals, heavy metals, and infectious agent antigens bind. Once bound to fibrillarlin or other body tissue as shown in Figure 9, these antigens can remain in the body for years inducing inflammation and causing autoimmunity [Tacheuchi, 1995]. The body burden of heavy metals can be evaluated through antibody testing against heavy metals, fibrillarlin, neurofilament and myelin basic protein. Heavy metal levels obtained from blood, urine, or hair are not indicative of body burden because metals bind to tissue rather than remain in circulation [Tacheuchi, 1995; Vojdani, 2003]. Indeed, tissue levels of mercury have been shown to be associated with exacerbated systemic autoimmunity [Pollard, 2001]. El-Fawal's review of neurotoxicity assessments shows that autoantibody assays exhibit promising association between the appearance of autoantibody titers against nervous system proteins and exposure to subclinical levels of known neurotoxins. Even if these humoral responses prove to be an epiphenomenon, secondary to nervous system injury, they

would be useful indices of this injury [El-Fawal, 1999]. Furthermore, studies show that low levels, considered safe, may be highly toxic to some people [Heyer, 2004], while conversely, high levels considered unhealthy may not pose a health risk for others [Davidson, 1998; McKelvey, 2007; Weil, 2005]. Antibodies to heavy metals nucleoproteins, neurofilaments, fibrillarlin and myelin basic protein will provide a better indication of the individual's body burden and immune response to the toxin.

REPAIRING DAMAGED BARRIERS

The road to fixing a leaky BBB or GI tract begins with the gut. Fasano outlines the importance of the intestinal barrier very elegantly, *"The classical paradigm of autoimmune pathogenesis involving a specific genetic makeup and exposure to environmental triggers has been challenged by the addition of a third element: the loss of intestinal barrier function. Genetic predisposition, miscommunication between innate and adaptive immunity, exposure to environmental triggers, and loss of the intestinal barrier function secondary to dysfunction of intercellular tight junctions, seem to*

all be key ingredients involved in the pathogenesis of autoimmune diseases. This new theory implies that, once the autoimmune process is activated, it is not self-perpetuating; rather, it can be modulated or even reversed by preventing the continuous interplay between genes and environment. As tight junction dysfunction allows this interaction, new therapeutic strategies aimed at re-establishing the intestinal barrier function offer innovative, unexplored approaches for the treatment of these devastating diseases. To repair a compromised intestinal barrier in ASD, remember that diet is essential. Eliminate wheat, dairy, sugar, and food additives and colorings. Unprocessed or natural foods are better choices. A yeast protocol may be necessary for those with Candida overgrowth. This protocol may include a sugar-, carbohydrate-free diet, nystatin and the use of pro-

and pre-biotics. Digestive enzymes may be required for better nutrient absorption and reducing inflammatory molecules. To calm inflammation, helpful supplementation may include alpha-lipoic acid and resveratrol. If the BBB has been breached, additional supplementation with omega-3 and -6 oils is beneficial. Effective therapy to reduce neuronal inflammation may include stress reducing techniques, moderate exercise, balancing of neurotransmitters, and the elimination of grains in the diet.

CONCLUSION

The human body is a glorious, mysterious entity. Scientists have not yet uncovered all of its secrets, but have brought to light many important concepts that can assist us in bettering the lives of persons affected by autism spectrum disorders. With unlimited combinations of environmental triggers and the ways in which they contribute to body

dysfunction, each person's ASD is unique. The trick is to discover the particular imbalances of the individual. In spectrum disorders, the important question to continually ask is "Why?" ***Why does the patient perform rituals before eating? Why can't my child tolerate protein? Why does the individual get sleepy after meals?*** There are answers to these questions. As outlined above, environmental triggers, enhanced intestinal permeability, immune dysregulation, and neurodegeneration are the four connected areas to be assessed in ASD. Many biomarkers have been studied and established to help pinpoint effective tailored treatment protocols for persons on the spectrum. Armed with biological knowledge and tenacity, the autism community is able to turn the tide of ASD to where more and more people are living well-rounded, healthy lives, and parents are reconnecting with their children who were once lost to autism.

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