

Food Allergy and Autism Spectrum Disorders: Is There a Link?

Harumi Jyonouchi, MD

Corresponding author

Harumi Jyonouchi, MD
Division of Allergy/Immunology and Infectious Diseases,
Pediatrics, University of Medicine and Dentistry of New Jersey,
New Jersey Medical School, 185 South Orange Avenue,
F570A, MSB, Newark, NJ 07101, USA.
E-mail: jyanouha@umdnj.edu

Current Allergy and Asthma Reports 2009, 9:194–201
Current Medicine Group LLC ISSN 1529-7322
Copyright © 2009 by Current Medicine Group LLC

Gastrointestinal (GI) symptoms are common comorbidities in children with autism spectrum disorders (ASDs). Parents often attribute these GI symptoms to food allergy (FA), although an evaluation for IgE-mediated FA is often unrevealing. Our previous studies indicated a high prevalence of non-IgE-mediated FA in young children with ASDs. Therefore, non-IgE-mediated FA may account for some but not all GI symptoms observed in children with ASDs. This raises the question of what treatment measures are applicable to ASD children with GI symptoms. A wide variety of dietary supplements and dietary intervention measures for ASD children have been promoted by medical professionals practicing complementary and alternative medicine despite the lack of rigorous scientific validation in most instances. This review summarizes possible (or proposed) etiologies of GI symptoms in ASD children and discusses risks and possible benefits of intervention measures promoted by complementary and alternative practitioners, with emphasis on FA.

Introduction

Autism spectrum disorders (ASDs) are complex developmental disorders with largely unknown etiologies. Although recent progress in genetics has defined various genetic diseases that manifest autistic features, this group only accounts for up to 10% to 15% of ASDs, which usually manifest as severe forms of autism [1,2•]. For the remaining ASD patients, the diagnosis of ASD is based solely on subjective behavioral symptoms that can vary markedly over time and during development. Experts generally agree that there are at least two types of ASDs

in terms of development: abnormal cognitive development evident from birth (classical autism) and developmental regression, usually between 18 and 24 months of age following apparently normal development (regressive autism) [3]. In cases of regressive autism, parents often report an apparent temporal association between onset of regression and immune insults such as microbial infection or adverse reactions to medications.

Apart from behavioral symptoms, certain medical conditions (eg, gastrointestinal [GI] symptoms) are present in many but not all ASD children [4]. The presence of comorbidities also affects the behavioral symptoms. Because of the high prevalence of GI symptoms and the apparent clinical improvement by dietary intervention frequently reported by parents, a link between GI abnormalities and the onset and development of ASDs has been posited. Improvement of behavioral symptoms is most commonly reported with a dairy- and wheat-free diet (the so-called casein-free, gluten-free [cf/gf] diet), leading to speculation about a high prevalence of food allergy (FA) in ASD children.

In allergy/immunology practice, it is not unusual to be consulted for the evaluation of FA in an ASD child. In most such cases, parents have many questions regarding laboratory testing and various intervention measures. These ASD patients are challenging for practicing allergists/immunologists. First, due in part to their limited expressive language and other behavioral symptoms, it is more difficult to obtain a detailed history for FA and to perform a physical examination on children with ASD as opposed to normally developing children. It is also difficult to provide medically sound and up-to-date information regarding treatment measures promoted by complementary and alternative (CAM) practitioners, which often lack rigorous scientific validation, especially when the parents are desperate for answers.

This review summarizes previously proposed theories of GI symptoms found in ASD children and examines the supporting evidence. Intervention measures promoted by CAM practitioners are also discussed from the point of view of evidence-based medicine. A major focus is the possible contribution of FA to GI symptoms observed in ASD children. This review aims to provide practical information for practicing allergists/immunologists for evaluating GI symptoms and possible FA in ASD children.

Postulated Etiology of GI Symptoms in ASD Children

Defects of gut mucosa and gut mucosal immune system

Leaky gut hypothesis: defective intestinal permeability

Gut epithelial cells not only serve as an epithelial barrier but also play an important role in the gut mucosal innate immunity, partly by producing multiple mediators. Antimicrobial peptides produced mainly by Paneth cells clustered in the bottom of the intestinal crypts serve as broad-spectrum antibiotics that kill gram-positive and gram-negative bacteria [5]. Colonic epithelial cells are also thought to contribute to the maintenance of homeostasis in the colon, in which microbes cohabit at high concentrations. Colonic epithelium was shown to express Toll-like receptor 9 (TLR9) on the cell surface in rodents [6,7]. TLR9 expressed apically in the epithelium is reported to compromise the inflammatory cascade induced by several TLRs expressed basolaterally [6,7]. Therefore, impairment of epithelial functions can significantly affect barrier functions and intestinal immune homeostasis.

Dysfunction of the intestinal epithelial barrier has been hypothesized in ASD children and is referred to as the *leaky gut hypothesis* [8]. This hypothesis postulates that impaired gut permeability in autistic children permits entry of macromolecules such as milk protein, causing sensitization of the gut mucosal immune system and subsequent FA. It is also postulated that such macromolecules enter the bloodstream, affecting the central nervous system (CNS) directly. Two studies have reported increased intestinal permeability at high frequency (43% and 75%) in ASD children with GI symptoms, as evidenced by altered lactulose to mannitol recovery ratio in the serum [9,10]. However, a recent study of 14 autistic children with current or previous GI complaints found no evidence of altered intestinal permeability [11•]. It should be noted that in these three studies, patients were not evaluated for IgE-mediated FA or non-IgE-mediated FA (NFA).

It is not known whether impaired gut permeability reported in ASD children is an intrinsic defect of the barrier or a result of gut mucosal inflammation caused by FA or other means. Abnormal intestinal permeability has been reported in children with IgE-mediated FA and NFA, and such abnormalities may resolve after implementation of a restricted diet [12–14]. Likewise, chronic gut mucosal inflammation such as inflammatory bowel disease (IBD) can cause increased intestinal permeability. Upregulated expression of protease-activated receptors 1 and 2 was implicated with increased colonic mucosal permeability in patients with Crohn's disease, augmenting transepithelial migration of inflammatory cells [15]. Currently, not enough scientific evidence exists to support a leaky gut hypothesis.

Dysbiosis

The GI tract is inhabited by vast groups of bacterial and fungal species, often called *commensal flora*, that compete with each other for nutrients and space. In humans, commensal flora provide another layer of defense against

invading pathogenic microbes. Intestinal commensal flora also play a role in maintaining intestinal homeostasis. This action is partially attributed to microbial byproducts sensed by pattern-recognition receptors (PRRs), such as TLRs, expressed by intestinal epithelial cells [16].

Many dendritic cells (DCs) reside in the intestinal mucosa and serve as professional antigen-presenting cells. Intestinal DCs extend their dendrites into the intestinal lumen through the tight junction of intestinal epithelial cells, sensing microbial byproducts as well as sampling luminal antigens [17]. Passage of antigen across the gut epithelial barrier to the Peyer's patches is also conducted by microfold cells rich in the follicle-associated epithelium. Antigen transfer via microfold cells appears to be associated with induction of oral tolerance [18]. In rodent models, macrophage migration inhibitory factor produced by DCs with *in vivo* bacterial challenge is reported to augment microfold cell-mediated antigen transport [19]. Through interactions with DCs and other cells, commensal flora appear to preferentially induce T-regulatory cells (Tregs) that depend on a concentration of retinoic acid, a vitamin A metabolite, instead of inducing proinflammatory T-helper (Th) 17 cells [20••,21,22]. Although it is not well understood how intestinal DCs differentiate commensal flora from pathogenic bacteria, induction of Tregs is likely important for maintaining immune homeostasis in the gut mucosa [23].

In addition to anti-inflammatory effects mediated by the host-immune system, commensal flora can exert direct anti-inflammatory actions. *Bacteroides thetaiotaomicron* was shown to restrict the signaling induced by flagellin, a TLR5 agonist and a product of flagellated pathogens. These microbes block downstream signaling associated with nuclear factor- κ B activation by promoting the nuclear export of transcriptionally active RelA [24]. This effect of *B. thetaiotaomicron* may partially explain why the gut tolerates large amounts of flagellated, potentially inflammatory commensal bacteria.

There have been anecdotal reports of the onset of autism after administration of antibiotics and subsequent appearance of GI symptoms. It was hypothesized that antibiotic administration led to disruption of commensal flora and colonization of bacteria-producing neurotoxin. In an open-label trial, administration of oral vancomycin in 10 children with regressive autism resulted in short-term improvement in their behavioral symptoms, indicating a potential effect of dysbiosis in ASD children [25].

Two studies that examined the constitution of gut microflora in ASD children reported differences from normal controls. One study included seven children with regressive autism and documented GI symptoms and four control children. The results revealed significant alterations in the upper and lower intestinal flora [26]. Another study examined 58 ASD children, 15 normal siblings, and 10 unrelated healthy children. The authors reported a higher incidence of *Clostridium histolyticum* in the ASD children, most of whom had a history of multiple anti-

biosis and significant GI symptoms [27]. Environmental factors resulting from altered commensal flora may be a contributing factor in these studies. That is, the results may be affected by frequent antibiotics and the restricted diet on which many ASD children already had been placed at the time of study entry. Careful selection of the study participants with appropriate case controls may shed light on whether dysbiosis has any role in GI symptoms observed in ASD children. In summary, dysbiosis could be a factor in ASD GI symptoms, but it is not yet known whether dysbiosis is secondary to intrinsic defects in the gut mucosa in ASD children or secondary to other medical conditions such as frequent antibiotics or FA.

Shultz et al. [28••] recently reported impaired social behaviors in rats after intracerebroventricular injection of propionic acid (PPA), a metabolic end product of enteric bacteria and an intermediary of fatty acid metabolism. PPA is also commonly used as a food preservative. Investigators have reported that the brains of the animals injected with PPA revealed astrogliosis—evidence of neuroinflammation via activation of CNS innate immune cells [28••]. Previous research from the same group also revealed induction of behavioral symptoms often seen in ASD children in rats by injecting PPA intravenicularly [29]. PPA production is likely to increase with disruption of commensal flora triggered by prolonged antibiotics, severe FA, and viral gastroenteritis. Although most PPA produced remains in the gut, it can cross the intact blood–brain barrier and affect the CNS directly, as revealed in animal models [28••,29]. The effects of PPA may explain the effects of dysbiosis in ASD children with a predisposition such as altered fatty acid metabolism. However, interactions between commensal flora and the gut immune system are very complex, and multiple factors likely contribute to dysbiosis and, possibly, the resultant behavioral changes in ASD children. Further studies are required to address the role of dysbiosis in ASD children. Nevertheless, treatment with probiotics in ASD children with suspected dysbiosis may be justified because of the safety and known efficacy (as discussed subsequently).

Autism colitis

Because of a high prevalence of GI symptoms in ASD children, abnormalities of the GI mucosa have been implicated in the development of autism. Macroscopic (ileocolonic lymphoid nodular hyperplasia [LNH]) and histologic findings indicating mild GI mucosal inflammation have been reported in ASD children with GI symptoms [30–32]. Immunohistochemical studies of biopsy specimens from ASD children revealed higher numbers of CD3⁺CD8⁺ T cells in the epithelium and higher numbers of CD3⁺ T cells and CD19⁺ B cells in the lamina propria compared with biopsy specimens from normal controls [33]. In children with regressive autism, Torrente et al. [34] reported lymphocytic colitis characterized by epithelial IgG and complement deposition. It should be noted that LNH can be observed in children without autism, and the signifi-

cance of “autism colitis” remains controversial. However, one study indicated significantly higher prevalence of LNH in the ileum and colon in ASD children than in controls. LNH appeared to be unaffected by diet or age at the time of colonoscopy in this study [32].

The same research group also reported upregulation of proinflammatory cytokines in the intestinal mucosa of ASD children with GI symptoms [35•,36]. These authors reported that lamina propria CD3⁺ T cells in the duodenum expressed higher interleukin (IL)-2, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ but less IL-10, while lamina propria CD3⁺ T cells in the colon expressed higher TNF- α and IFN- γ in ASD children with GI symptoms than in controls [35•,36]. In 18 ASD children with GI symptoms, higher expression of TNF- α and IFN- γ and less expression of IL-10 by CD3⁺ T cells in both intestinal mucosa and peripheral blood were reported [35•]. However, when gut inflammation was assessed by measuring concentration of proinflammatory cytokines (IL-6, IL-8, and IL-1 β) in the gut mucosal tissue and stool concentration of calprotectin and rectal nitric oxide, no difference between ASD children and controls was observed [37].

Such conflicting results may be partially attributed to the small numbers of study participants and the heterogeneity of causes of GI symptoms in the study participants. It remains to be seen whether “autism colitis” is present or if GI inflammation is associated with other immune abnormalities, such as FA. Unfortunately, in all these studies, the presence of IgE-mediated FA or NFA was not evaluated. Likewise, dietary intervention measures at the time of study were not addressed carefully.

If “autism colitis” is not associated with secondary causes such as FA, are there any mechanisms predisposing ASD children to chronic colitis? Innate immunity is crucial for maintaining immune homeostasis. Polymorphisms of several signaling molecules in innate immunity have been implicated in the risk of development of Crohn’s disease [38,39]. If genetically predetermined innate immune abnormalities exist in the gut mucosa, these ASD children may be more vulnerable to GI inflammation triggered by immune insults as postulated in IBD patients [39]. However, such a genetic mutation or polymorphism has not been documented in ASD children with GI symptoms. Further studies are indicated to address a role of “autism colitis” in ASD. As IBD patients are known to manifest Th17-skewed responses, we evaluated Th17 responses in ASD children with GI symptoms but found no evidence of Th17-skewed responses (unpublished observation).

Systemic Abnormalities Affecting GI Mucosa Abnormal neuronal signaling

Autism was once thought to be a static encephalopathy caused by structural and genetic abnormalities. However, a high frequency of comorbidities such as GI symptoms and sleep disturbance may indicate a chronic inflammatory condition involving other organ systems in some

autism patients. Various researchers reported evidence of ongoing inflammation and chronic oxidative stress not only in the CNS but in the periphery in ASD children [40–45,46•,47]. In cases of autism in which no clearly defined metabolic/genetic causes are found, chronic multisystem disease(s) under the influence of multiple genetic and environmental factors may need to be considered as the etiology. It has been proposed that GI symptoms observed in autistic children are associated with defects affecting the CNS and the gut caused by abnormal neuronal signaling. It was also proposed that abnormal neuronal signaling occurs upon exposure to environmental factors such as neurotoxins at the “right time” in genetically predisposed individuals.

Cholinergic and γ -aminobutyric acid neurotransmission

Altered signaling in cholinergic neurotransmission and γ -aminobutyric acid (GABA) transmission has been reported in ASD patients [48–50]. Altered cholinergic nerve regulation can cause dysregulated inflammatory responses, as cholinergic signaling is important in down-regulating inflammatory responses. Altered GABA transmission affects endocrine and immune systems and could yield GI symptoms. The possible effects of altered neurotransmission in autism have attracted considerable attention from the view of neurotoxicology, especially because various environmental toxins can affect GABA signaling pathways. Autistic children may be genetically more vulnerable to environmental toxins. In addition, behaviors commonly seen in these children (eg, pica) may predispose them to exposure to environmental toxins. However, no evidence currently supports a direct causal relationship between environmental toxin exposure and development of autism and/or GI symptoms.

A redox/methylation hypothesis

Evidence of chronic oxidative stress has been described repeatedly in ASD children [46•]. Altered activity of methionine synthase is effective in defense mechanisms against oxidative stress in the acute stage but not in the chronic stage, causing DNA methylation and dopamine receptor phospholipid methylation. It was hypothesized that given evidence of chronic oxidative stress, such mechanisms may be associated with the neurocognitive defects seen in autism [46•]. In this hypothesis, an increased risk of autism may result from environmental exposure to heavy metals and xenobiotics in genetically susceptible individuals. Xenobiotics also can affect the immune system and alter gene expression by inhibiting DNA methylation. Thus, it is hypothesized that exposure to xenobiotics in the gut mucosa is associated with frequent GI symptoms observed in ASD children. However, it is not well understood how chronic environmental exposure to xenobiotics at low doses impacts the immune system, if this is the case. Epidemiologic studies failed to reveal a causal association between early exposure to mercury, a heavy metal implicated in autism, from thimerosal-containing vaccines

and immunoglobulins and deficits in neuropsychological functioning at the age of 7 to 10 years [51]. Toxic effects of xenobiotics may be significant only in individuals with defined genetic susceptibility.

It remains to be seen whether the inflammatory condition observed in some autistic children is associated with genetic vulnerability plus environmental xenobiotics exposure. Certain neurotropic medications prescribed for autistic children also may be associated with increased oxidative stresses. For example, valproic acid, which is widely used as an antiseizure medication and for controlling behavioral symptoms in ASD children, interferes with β -oxidation in mitochondria and impairs oxidative stress responses [52]. Careful characterization of ASD study participants will likely shed light on a role for xenobiotics in GI symptoms and, possibly, the pathogenesis of autism in ASD children.

Food Allergy IgE-mediated FA

Young children are more vulnerable to sensitization to common food proteins because of an immature gut mucosal immune system. Because of the high frequency of GI symptoms in ASD children, the presence of FA has been widely speculated. Previously reported immunologic abnormalities such as a higher production of Th2 cytokines without stimuli and an increased frequency of Th cells expressing Th2 cytokines indicated a higher prevalence of atopy in ASD children [53,54]. However, to my knowledge, no report has clearly documented a high prevalence of atopy in ASD children. One group examining 30 autistic children reported a higher frequency of skin prick test reactivity in its study population [55]. However, in this study, it is unclear whether these children demonstrated clinical features indicating atopy. In addition, the authors reported normal IgE levels and no asthma symptoms in their autistic study participants [55].

In 325 ASD children evaluated in the Pediatric Allergy/Immunology clinic at our institution, the prevalence of atopic disorders was equivalent to that in the general population (Table 1). These ASD children were evaluated by standard diagnostic measures for atopy, including the measurement of allergen-specific IgE and/or skin prick testing. The prevalence of atopy appeared to be higher in patients diagnosed with Asperger’s syndrome, but this may be secondary to the small number of participants (Table 1). Likewise, we did not find a high frequency of IgE-mediated FA in ASD children evaluated in our clinic (Table 1). Our results make it unlikely that a high prevalence of atopy is associated with the GI symptoms frequently seen in ASD children.

Non-IgE-mediated food allergy

In our previous studies, we hypothesized that GI symptoms frequently seen in ASD children could be partially explained by NFA. We also hypothesized that in ASD

Table 1. Prevalence of atopic disorders in ASD children evaluated in the Pediatric Allergy and Immunology Clinic at the University of Medicine and Dentistry of New Jersey

Diagnosis*	Allergic rhinitis	Atopic dermatitis	Atopic asthma	IgE-mediated FA
ASD	11/44 (25.0%)	3/44 (6.8%)	2/44 (4.5%)	3/44 (6.8%)
Autism	40/163 (24.5%)	9/163 (5.5%)	13/163 (8.0%)	2/163 (1.2%)
PDD-NOS	21/108 (19.4%)	9/108 (8.3%)	13/108 (12.0%)	3/108 (2.8%)
Asperger's syndrome	5/10 (50.0%)	2/10 (20.0%)	1/10 (10.0%)	1/10 (10.0%)
Total ASD children	77/325 (23.7%)	23/325 (7.1%)	29/325 (8.9%)	9/325 (2.8%)

*Median age for children: ASD (4.1 y), autism (6.6 y), PDD-NOS (4.8 y), Asperger's syndrome (6 y).
ASD—autism spectrum disorder; FA—food allergy; PDD-NOS—pervasive developmental disorder, not otherwise specified.

Table 2. Production of counterregulatory cytokines (TGF- β and IL-10) in response to β -lactoglobulin and *Candida* antigen

Study group	Cells stimulated with*		
	Medium alone	β -lactoglobulin	<i>Candida</i> antigen
TGF-β			
Control ($n = 23$), pg/mL	776.8 \pm 481.0	958.3 \pm 620.2	890.2 \pm 64.5
ASD/non-NFA ($n = 27$), pg/mL	588.5 \pm 427.3	669.7 \pm 526.8	603.2 \pm 483.4
ASD/NFA ($n = 21$), pg/mL	496.6 \pm 345.7 [†]	541.4 \pm 278.8 [†]	511.9 \pm 445.8 [†]
ASD/NFA/diet ($n = 52$), pg/mL	654.8 \pm 426.2	802.4 \pm 629.3	776.6 \pm 655.0
IL-10			
Control ($n = 23$), pg/mL	21.6 \pm 20.5	952.3 \pm 391.4	48.9 \pm 70.5
ASD/non-NFA ($n = 27$), pg/mL	43.3 \pm 68.9	950.8 \pm 390.0	71.3 \pm 113.7
ASD/NFA ($n = 21$), pg/mL	36.8 \pm 92.0	1116.6 \pm 358.1	63.1 \pm 110.9
ASD/NFA/diet ($n = 52$), pg/mL	50.0 \pm 111.9	935.6 \pm 478.2	74.3 \pm 138.7

*Peripheral blood mononuclear cells were incubated for 4 d with medium alone, β -lactoglobulin (10 μ g/mL), or *Candida* antigen, and TGF- β and IL-10 levels in the culture supernatant were measured by enzyme-linked immunosorbent assay, as reported by Jyonouchi et al. [57].
[†]TGF- β levels produced were lower than controls ($P < 0.05$) by Wilcoxon signed-rank test.
ASD—autism spectrum disorder; ASD/NFA/diet—ASD/NFA on the restrictive diet with resolution of gastrointestinal symptoms; IL—interleukin; NFA—non-IgE-mediated food allergy; TGF- β —transforming growth factor- β .

children with NFA (ASD/NFA), immune reactivity to food proteins could be detected by measuring the production of TNF- α and other inflammatory cytokines by peripheral blood mononuclear cells (PBMCs) when PBMCs were stimulated with food proteins, as demonstrated in children with NFA to milk proteins [56]. Our results suggested the presence of cellular immune reactivity to common dietary proteins (mainly milk protein) in young ASD children with GI symptoms [57]. However, NFA may be playing a lesser role in GI symptoms in older ASD children, as most children likely outgrow NFA with maturation of the gut immune system and the establishment of oral tolerance [58]. We also observed a lower prevalence of NFA in older (age > 6 years) ASD children (unpublished observation).

To address the role of counterregulatory cytokines, we recently assessed the production of two representative regulatory cytokines by PBMCs obtained from individuals with ASD/NFA ($n = 21$), ASD/NFA on the restrictive diet with resolution of GI symptoms (ASD/NFA/diet, $n = 52$), ASD/non-NFA ($n = 27$), and controls without ASD ($n = 23$). We measured production of IL-10 and transforming

growth factor- β (TGF- β) without stimuli or with β -lactoglobulin, a major milk protein, or *Candida* antigen. We found spontaneous production of TGF- β in all study groups, but production of IL-10 appeared to be more milk protein specific (Table 2). We did not find a significant difference in IL-10 production with β -lactoglobulin or *Candida* antigen among the study groups, whereas TGF- β production was lower in all the settings in ASD/NFA children (Table 2 and Fig. 1).

In NFA children reactive to milk proteins, the presence of milk protein-specific Tregs producing IL-10 and TGF- β has been reported [56]. However, a large amount of spontaneous production of TGF- β in our results may also indicate the presence of antigen-nonspecific Tregs (natural Tregs) [59•]. Our results also indicate that TGF- β -mediated tolerance may be impaired in ASD/NFA children. However, as indicated previously, TGF- β can promote inflammatory reactions by promoting differentiation of Th17 cells [20••,21,22]. Interestingly, in these ASD/NFA patients, we found minimal IL-17 production with these stimuli, not indicating skewed Th17 responses

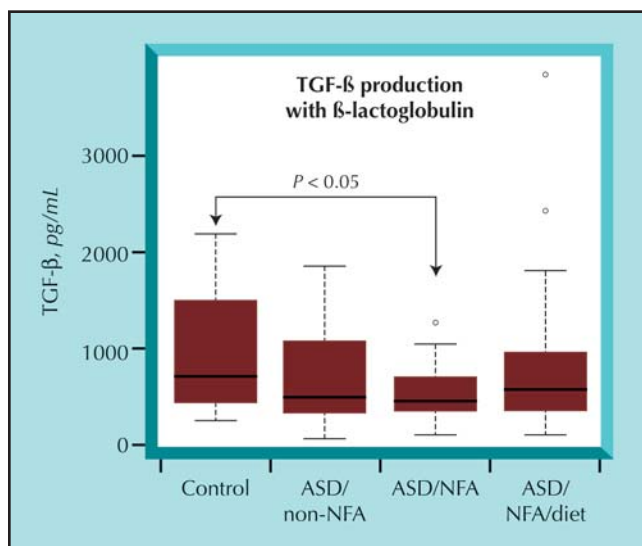


Figure 1. Transforming growth factor- β (TGF- β) production by peripheral blood mononuclear cells (PBMCs) obtained from controls, autism spectrum disorder (ASD) children without non-IgE-mediated food allergy (ASD/non-NFA), ASD children with NFA (ASD/NFA), and ASD/NFA children on the restricted diet with resolution of gastrointestinal symptoms (ASD/NFA/diet) when cells were incubated with β -lactoglobulin. The "I" bar marks the range of TGF- β and interleukin-10 levels; the shaded region marks the median. The box illustrates where the interquartile range falls. Outliers are marked separately as "o." TGF- β production by PBMCs from ASD/NFA children was lower than controls (Wilcoxon signed-rank test).

(unpublished observation). Given our findings, aberrant innate immune responses may provoke undesired immune reactivity against commensal flora in ASD children as observed in IBD patients. Taken together, our data indicate a possible role for NFA (mainly against milk protein) in GI symptoms in some ASD children.

Dietary intervention

Various dietary intervention measures have been promoted by parents and CAM-practicing medical professionals based on anecdotal reports despite the lack of rigorous scientific validation. Among such dietary intervention measures, a *cf/gf* diet appears to be most popular, with beneficial effects frequently reported by parents. Several attempts have been made to determine the effects of a *cf/gf* diet, but most studies were not well designed [60]. Only two prospective studies have addressed the effects of the *cf/gf* diet. These studies examined a small number of children and revealed conflicting results. One study reported beneficial effects in behavioral symptoms in 10 autistic children [61], while another reported no benefits in 15 ASD children [62•]. These studies appeared to have been formulated to test the leaky gut hypothesis, and study participants were not evaluated for IgE-mediated FA or NFA. Neither study addressed GI symptoms in its participants. Young ASD children with clear evidence of IgE-mediated FA or NFA may experience improvement of behavioral symptoms due to resolution of GI discomfort with implementation of a *cf/gf* diet. In such a scenario,

a milk- and soy-free diet may be sufficient, as the most common causative proteins inducing NFA in infants are soy and milk.

There has been a concern that implementing a *cf/gf* diet may lead to nutritional deprivation. One study evaluated the nutritional status of ASD children on a *cf/gf* diet and found no evidence of nutritional deprivation in eight autistic children on the diet compared with 29 ASD controls without dietary intervention [63]. It remains to be seen whether the frequently reported therapeutic effects of a *cf/gf* diet can be attributed solely to FA or any other mechanism.

Other types of dietary intervention measures, such as a specific carbohydrate diet, have been promoted for ASD children, but few scientific data support such measures. Multiple dietary supplements also have been promoted for ASD children by CAM practitioners, but again, scientific evidence has been scant. For example, despite many anecdotal reports of beneficial effects of dimethylglycine, two double-blind, placebo-controlled studies failed to reveal any benefits in 8 and 37 ASD children, respectively [64,65]. Most studies addressing the effects of nutritional supplements involved small numbers of study participants. It remains to be seen whether any dietary supplements provide significant beneficial effects in ASD children. However, considering the skewed dietary habits often seen in ASD children, providing vitamin supplementation and/or other nutritional supplements at optimal but not excessive levels under the guidance of skilled dietitians is reasonable to ensure adequate nutrition.

Probiotics

With the notion of dysbiosis in ASD children, therapeutic use of probiotics has been popular among parents rearing autistic children. As previously noted, probiotics play a role in maintaining intestinal mucosal immune homeostasis [23]. In IBD patients, the efficacy of probiotics, by restoring commensal flora and attenuating local and systemic inflammation, has been well documented [66]. The potential therapeutic effects of probiotics in children with FA have been explored [67,68]. Sound scientific evidence seems to support their potential preventive and therapeutic effects, although no consensus has been reached on which strains to use and how much to supplement [67,68]. The potential beneficial effects of probiotics for treating dysbiosis in ASD children have been speculated but not substantiated in rigorous prospective studies. However, given the scientific evidence produced in other patients, the use of probiotics in ASD children with significant GI symptoms may be justified.

Conclusions

In summary, convincing data support the presence of chronic GI inflammation in ASD children, but the etiology of this GI inflammation is not well understood and is likely affected by multiple genetic and environmental factors. NFA can partially explain the GI symptoms and apparent benefi-

cial effects of dietary interventions in some ASD children, especially young ASD children. Apparent effects of oral vancomycin and altered commensal flora reported in ASD children may be explained partially by dysbiosis, which is likely associated with multiple environmental and, possibly, genetic factors. Further studies are required to understand the etiology of GI symptoms observed in ASD children.

Disclosure

No potential conflict of interest relevant to this article was reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Hertz-Picciotto I, Croen LA, Hansen R, et al.: **The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism.** *Environ Health Perspect* 2006, **114**:1119–1125.
- 2.• Abrahams BS, Geschwind DH: **Advances in autism genetics: on the threshold of a new neurobiology.** *Nat Rev Genet* 2008, **9**:341–355.

This article summarizes recent findings of genetic mutations that can manifest autistic symptoms. This field is expanding very rapidly.

3. Lainhart JE, Ozonoff S, Coon H, et al.: **Autism, regression, and the broader autism phenotype.** *Am J Med Genet* 2002, **113**:231–237.
4. Horvath K, Perman JA: **Autism and gastrointestinal symptoms.** *Curr Gastroenterol Rep* 2002, **4**:251–258.
5. Porter EM, Bevins CL, Ghosh D, Ganz T: **The multifaceted Paneth cell.** *Cell Mol Life Sci* 2002, **59**:156–170.
6. Lee J, Gonzales-Navajas JM, Raz E: **The “polarizing-tolerizing” mechanism of intestinal epithelium: its relevance to colonic homeostasis.** *Semin Immunopathol* 2008, **30**:3–9.
7. Lee J, Mo JH, Shen C, et al.: **Toll-like receptor signaling in intestinal epithelial cells contributes to colonic homeostasis.** *Curr Opin Gastroenterol* 2007, **23**:27–31.
8. White JF: **Intestinal pathophysiology in autism.** *Exp Biol Med (Maywood)* 2003, **228**:639–649.
9. D’Eufemia P, Celli M, Finocchiaro R, et al.: **Abnormal intestinal permeability in children with autism.** *Acta Paediatr* 1996, **85**:1076–1079.
10. Horvath K, Collins RM, Rabsztyan A, et al.: **Secretin improves intestinal permeability in autistic children [abstract].** *J Pediatr Gastroenterol Nutr* 2000, **S31**:31.
- 11.• Robertson MA, Sigalet DL, Holst JJ, et al.: **Intestinal permeability and glucagon-like peptide-2 in children with autism: a controlled pilot study.** *J Autism Dev Disord* 2008, **38**:1066–1071.

This study indicates that an intrinsic defect of gut permeability is unlikely to be associated with GI symptoms in children with ASD.

12. Dupont C, Heyman M: **Food protein-induced enterocolitis syndrome: laboratory perspectives.** *J Pediatr Gastroenterol Nutr* 2000, **30**(Suppl):S50–S57.
13. Heyman M: **Gut barrier dysfunction in food allergy.** *Eur J Gastroenterol Hepatol* 2005, **17**:1279–1285.
14. Ventura MT, Polimeno L, Amoroso AC, et al.: **Intestinal permeability in patients with adverse reactions to food.** *Dig Liver Dis* 2006, **38**:732–736.
15. Chin AC, Lee WY, Nusrat A, et al.: **Neutrophil-mediated activation of epithelial protease-activated receptors-1 and -2 regulates barrier function and transepithelial migration.** *J Immunol* 2008, **181**:5702–5710.

16. Magalhaes JG, Tattoli I, Girardin SE: **The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens.** *Semin Immunol* 2007, **19**:106–115.
17. Rescigno M, Urbano M, Valzasina B, et al.: **Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria.** *Nat Immunol* 2001, **2**:361–367.
18. Chehade M, Mayer L: **Oral tolerance and its relation to food hypersensitivities.** *J Allergy Clin Immunol* 2005, **115**:3–12; quiz 13.
19. Man AL, Lodi F, Bertelli E, et al.: **Macrophage migration inhibitory factor plays a role in the regulation of microfold (M) cell-mediated transport in the gut.** *J Immunol* 2008, **181**:5673–5680.
- 20.•• Mucida D, Park Y, Kim G, et al.: **Reciprocal Th17 and regulatory T cell differentiation mediated by retinoic acid.** *Science* 2007, **317**:256–260.

This paper indicated for the first time that differentiation of Th17 cells and Tregs in the gut is reciprocally regulated by concentrations of cytokines (mainly IL-6) and retinoic acid, which is important for understanding immune homeostasis in the gut mucosa.

21. Sun CM, Hall JA, Blank RB, et al.: **Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid.** *J Exp Med* 2007, **204**:1775–1785.
22. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, et al.: **A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism.** *J Exp Med* 2007, **204**:1757–1764.
23. Burks AW, Laubach S, Jones SM: **Oral tolerance, food allergy, and immunotherapy: implications for future treatment.** *J Allergy Clin Immunol* 2008, **121**:1344–1350.
24. Kelly D, Campbell JI, King TP, et al.: **Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA.** *Nat Immunol* 2004, **5**:104–112.
25. Sandler RH, Finegold SM, Bolte ER, et al.: **Short-term benefit from oral vancomycin treatment of regressive-onset autism.** *J Child Neurol* 2000, **15**:429–435.
26. Finegold SM, Molitoris D, Song Y, et al.: **Gastrointestinal microflora studies in late-onset autism.** *Clin Infect Dis* 2002, **35**:S6–S16.
27. Parracho HM, Bingham MO, Gibson GR, McCartney AL: **Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children.** *J Med Microbiol* 2005, **54**:987–991.
- 28.•• Shultz SR, MacFabe DF, Ossenkopp KP, et al.: **Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism.** *Neuropharmacology* 2008, **54**:901–911.

This study indicated a possible mechanism of dysbiosis that can affect CNS-inducing autistic behaviors, though the study was done in rodents.

29. MacFabe DF, Cain DP, Rodriguez-Capote K, et al.: **Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders.** *Behav Brain Res* 2007, **176**:149–169.
30. 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.
31. Wakefield AJ, Anthony A, Murch SH, et al.: **Enterocolitis in children with developmental disorders.** *Am J Gastroenterol* 2000, **95**:2285–2295.
32. Wakefield AJ, Ashwood P, Limb K, Anthony A: **The significance of ileo-colonic lymphoid nodular hyperplasia in children with autistic spectrum disorder.** *Eur J Gastroenterol Hepatol* 2005, **17**:827–836.
33. Ashwood P, Anthony A, Pellicer AA, et al.: **Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology.** *J Clin Immunol* 2003, **23**:504–517.

34. Torrente F, Ashwood P, Day R, et al.: Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. *Mol Psychiatry* 2002, 7:375–382, 334.
35. Ashwood P, Wakefield AJ: Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *J Neuroimmunol* 2006, 173:126–134.
- This study compared changes in peripheral blood and mucosal lymphocyte cytokine profiles in ASD children with GI symptoms and revealed evidence of inflammatory changes in the GI tract.
36. Ashwood P, Anthony A, Torrente F, Wakefield AJ: Spontaneous mucosal lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms: mucosal immune activation and reduced counter regulatory interleukin-10. *J Clin Immunol* 2004, 24:664–673.
37. DeFelice ML, Ruchelli ED, Markowitz JE, et al.: Intestinal cytokines in children with pervasive developmental disorders. *Am J Gastroenterol* 2003, 98:1777–1782.
38. Lala S, Ogura Y, Osborne C, et al.: Crohn's disease and the NOD2 gene: a role for Paneth cells. *Gastroenterology* 2003, 125:47–57.
39. Cho JH: The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol* 2008, 8:458–466.
40. Vargas DL, Nascimbene C, Krishnan C, et al.: Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* 2005, 57:67–81.
41. Pardo CA, Vargas DL, Zimmerman AW: Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psychiatry* 2005, 17:485–495.
42. Zimmerman AW, Jyonouchi H, Comi AM, et al.: Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatr Neurol* 2005, 33:195–201.
43. Pardo CA, Eberhart CG: The neurobiology of autism. *Brain Pathol* 2007, 17:434–447.
44. Ashwood P, Kwong C, Hansen R, et al.: Brief report: plasma leptin levels are elevated in autism: association with early onset phenotype? *J Autism Dev Disord* 2008, 38:169–175.
45. Chauhan A, Chauhan V, Brown WT, Cohen I: Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin—the antioxidant proteins. *Life Sci* 2004, 75:2539–2549.
46. Deth R, Muratore C, Benzecry J, et al.: How environmental and genetic factors combine to cause autism: a redox/methylation hypothesis. *Neurotoxicology* 2008, 29:190–201.
- This is a good summary of a redox/methylation hypothesis in development of autism.
47. James SJ, Melnyk S, Jernigan S, et al.: Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuro-psychiatr Genet* 2006, 141B:947–956.
48. Martin-Ruiz CM, Lee M, Perry RH, et al.: Molecular analysis of nicotinic receptor expression in autism. *Brain Res Mol Brain Res* 2004, 123:81–90.
49. Ashley-Koch AE, Mei H, Jaworski J, et al.: An analysis paradigm for investigating multi-locus effects in complex disease: examination of three GABA receptor subunit genes on 15q11–q13 as risk factors for autistic disorder. *Ann Hum Genet* 2006, 70:281–292.
50. Vincent JB, Horike SI, Choufani S, et al.: An inversion inv(4)(p12–p15.3) in autistic siblings implicates the 4p GABA receptor gene cluster. *J Med Genet* 2006, 43:429–434.
51. Thompson WW, Price C, Goodson B, et al.: Early thimerosal exposure and neuropsychological outcomes at 7 to 10 years. *N Engl J Med* 2007, 357:1281–1292.
52. Silva MF, Aires CC, Luis PB, et al.: Valproic acid metabolism and its effects on mitochondrial fatty acid oxidation: a review. *J Inherit Metab Dis* 2008 Apr 4 (Epub ahead of print).
53. Gupta S, Aggarwal S, Rashanravan B, Lee T: Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. *J Neuroimmunol* 1998, 85:106–109.
54. Molloy CA, Morrow AL, Meinzen-Derr J, et al.: Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol* 2006, 172:198–205.
55. Bakkaloglu B, Anlar B, Anlar FY, et al.: Atopic features in early childhood autism. *Eur J Paediatr Neurol* 2008, 12:476–479.
56. Karlsson MR, Rugtveit J, Brandtzaeg P: Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004, 199:1679–1688.
57. Jyonouchi H, Geng L, Ruby A, et al.: Evaluation of an association between gastrointestinal symptoms and cytokine production against common dietary proteins in children with autism spectrum disorders. *J Pediatr* 2005, 146:605–610.
58. Hwang JB, Sohn SM, Kim AS: Prospective follow up-oral food challenge in food protein-induced enterocolitis syndrome. *Arch Dis Child* 2008 Oct 1 (Epub ahead of print).
59. Horwitz DA, Zheng SG, Gray JD: Natural and TGF-beta-induced Foxp3(+)/CD4(+)/CD25(+) regulatory T cells are not mirror images of each other. *Trends Immunol* 2008, 29:429–435.
- This is a good review regarding naturally occurring and TGF-beta-induced Tregs with peripheral tolerance.
60. Christison GW, Ivany K: Elimination diets in autism spectrum disorders: any wheat amidst the chaff? *J Dev Behav Pediatr* 2006, 27:S162–S171.
61. Knivsberg AM, Reichelt KL, Høien T, Nodland M: A randomised, controlled study of dietary intervention in autistic syndromes. *Nutr Neurosci* 2002, 5:251–261.
62. Elder JH, Shankar M, Shuster J, et al.: The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial. *J Autism Dev Disord* 2006, 36:413–420.
- This study was designed to implement a well-controlled dietary intervention. The results may indicate that nonrandom implementation of the c/f/gf diet in autistic children irrespective of GI symptoms is unlikely to induce symptomatic improvement.
63. Cornish E: Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. *J Hum Nutr Diet* 2002, 15:261–269.
64. Bolman WM, Richmond JA: A double-blind, placebo-controlled, crossover pilot trial of low dose dimethylglycine in patients with autistic disorder. *J Autism Dev Disord* 1999, 29:191–194.
65. Kern JK, Miller VS, Cauller PL, et al.: Effectiveness of N,N-dimethylglycine in autism and pervasive developmental disorder. *J Child Neurol* 2001, 16:169–173.
66. Chapman TM, Plosker GL, Figgitt DP: VSL#3 probiotic mixture: a review of its use in chronic inflammatory bowel diseases. *Drugs* 2006, 66:1371–1387.
67. Isolauri E, Salminen S: Probiotics: use in allergic disorders: a Nutrition, Allergy, Mucosal Immunology, and Intestinal Microbiota (NAMI) Research Group Report. *J Clin Gastroenterol* 2008, 42(Suppl 2):S91–S96.
68. Savilahti E, Kuitunen M, Vaarala O: Pre and probiotics in the prevention and treatment of food allergy. *Curr Opin Allergy Clin Immunol* 2008, 8:243–248.