EVALUATION OF AN ASSOCIATION BETWEEN GASTROINTESTINAL SYMPTOMS AND CYTOKINE PRODUCTION AGAINST COMMON DIETARY PROTEINS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

HARUMI JYONOUCHI, MD, LEE GENG, PHD, AGNES RUBY, BS, CHITRA REDDY, MD, AND BARBIE ZIMMERMAN-BIER, MD

Objective To evaluate an association between cytokine production with common dietary proteins as a marker of non-allergic food hypersensitivity (NFH) and gastrointestinal (GI) symptoms in young children with autism spectrum disorders (ASD).

Study design Peripheral blood mononuclear cells (PBMCs) were obtained from 109 ASD children with or without GI symptoms (GI [+] ASD, N = 75 and GI (−) ASD, N = 34], from children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. We measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMCs stimulated with whole cow's milk protein (CMP), its major components (casein, β-lactoglobulin, and α-lactoalbumin), gliadin, and soy.

Results PBMCs obtained from GI (+) ASD children produced more tumor necrosis factor-α (TNF-α)/interleukin-12 (IL-12) than those obtained from control subjects with CMP, β-lactoglobulin, and α-lactoalbumin, irrespective of objective GI symptoms. They also produced more TNF-α with gliadin, which was more frequently observed in the group with loose stools. PBMCs obtained from GI (−) ASD children produced more TNF-α/IL-12 with CMP than those from control subjects, but not with β-lactoglobulin, α-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable.

Conclusion A high prevalence of elevated TNF-α/IL-12 production by GI (+) ASD PBMCs with CMP and its major components indicates a role of NFH in GI symptoms observed in children with ASD. *(J Pediatr 2005;146:605-10)*

Autism spectrum disorders (ASDs) are complex developmental disorders with unknown etiology and no known curative measures. Many parents thus turn to therapeutic measures of complementary and alternative medicine. However, these measures often lack rigorous scientific validation for their efficacy/safety and could be potentially hazardous. Among such measures, a casein-free, gluten-free diet has been very popular, partly because of the high prevalence of gastrointestinal (GI) symptoms (cramping, diarrhea, constipation alternating with diarrhea, gastroesophageal reflux, bloating, and loose/undigested stool) in children with ASD. Moreover, parents/therapists/caretakers frequently report resolution of GI symptoms with the casein-free, gluten-free diet in children with ASD.

Immunoglobulin E (IgE)-mediated food allergy accounts for only a small portion of adverse reaction to dietary proteins (DPs; 2%-3%). Instead, cell-mediated immunity plays a vital role in non-allergic food hypersensitivity (NFH), with major causative DPs being cow's milk protein (CMP), soy, and wheat.[1,2] Tumor necrosis factor-α (TNF-α) production in response to CMP appears closely associated with GI inflammation and clinical features of NFH, and the elimination diet leads to a decline in TNF-α production

See editorial, p 582.

ASD	Autism spectrum disorders	IL	Interleukin
CMP	Cow's milk protein	NFH	Non-allergic food hypersensitivity
CM	Control mean	PBMC	Peripheral blood mononuclear cell
DP	Dietary protein	PDD-NOS	Pervasive developmental disorder not otherwise specified
GI	Gastrointestinal		
GI (+) ASD children	Children with ASD who have positive GI symptoms	Th1 and Th2 cells	Type 1 and type 2 T-helper cells
IFN-γ	Interferon-γ	TNF-α	Tumor necrosis factor-α

From the Department of Pediatrics, New Jersey Medical School, UMDNJ, Newark, New Jersey.

Supported by the Jonty Foundation (St. Paul, Minnesota).

Submitted for publication May 21, 2004; last revision received Sep 27, 2004; accepted Jan 12, 2005.

Reprint requests: Harumi Jyonouchi, MD, Division of Pulmonary, Allergy/Immunology, and Infectious Diseases, Department of Pediatrics, New Jersey Medical School, UMDNJ, 185 South Orange Ave, PO Box 1709, Newark, NJ, 07101-1709. E-mail: jyanouha@umdnj.edu.

0022-3476/$ - see front matter

10.1016/j.jpeds.2005.01.027

Table I. Demographics of the study subjects

Study group	Age (years)	Subject number (Male:Female)	Atopic disorders
GI (+) ASD*	4.7 (1.8−10.6)*	75 (61:14)	19/75 (25.3%)
GI (−) ASD*	5.4 (2.1−10.2)	34 (32:2)	12/34 (35.3%)
NFH	2.8 (1.3−7.8)	15 (10:6)	2/15 (13.3%)
Controls	3.8 (1.0−9.0)	19 (11:8)	unknown

*The results are expressed as the median (range).

with CMP.[3-5] Cell-mediated immune reactions take place several hours and even 1 to 2 days after the intake of reactive DPs. In the absence of commercially available diagnostic laboratory measures, the gold standard of NFH diagnosis is a resolution of GI symptoms with the elimination diet and their recurrence with the challenge of causative DPs. Such clinical features of NFH may make diagnosis more challenging, especially in children who are developmentally delayed.[6]

Our previous study revealed that peripheral blood mononuclear cells (PBMCs) from a number of children with ASD produced elevated levels of TNF-α and interferon-γ (IFN-γ), with CMP as observed in NFH.[1-2,5,7] In that study, there was no increase in atopic disorders or IgE-mediated food allergy.[7] These findings indicate that GI symptoms observed in children with ASD are partly associated with NFH to common DPs, and this may partly explain the apparent favorable effects of the casein-free, gluten-free diet in children with ASD.

However, it is unknown how frequently children with ASD exhibit such cellular reactivity to DPs commonly associated with NFH in comparison with GI symptoms.

METHODS

Study Subjects

The study subjects included children (aged 1-10 years) in Tanner stage 1. Children with ASD were recruited from those referred to the Autism Center at the New Jersey Medical School, University of Medicine and Dentistry of NJ, Newark, NJ. ASD diagnosis was made or ascertained by means of the Diagnostic and Statistical Manual of Mental Disorders-IV, the International Classification of Diseases-10 criteria, or both, the Autism Diagnostic Interview-Revised, and Autism Diagnostic Observational Schedules. Children with NFH and typically developing control children were recruited from those seen in the Allergy/Immunology Clinic and General Pediatrics Clinic at the New Jersey Medical School. We excluded subjects taking neuropsychiatric medications and those with known immunodeficiency, metabolic disorders, genetic diseases, and illnesses involving major organs. Blood samples were collected after institutional review board-approved signed consent forms were obtained. At the time of venipuncture, all study subjects were on an unrestricted diet, not febrile, and had no evidence of acute microbial illnesses.

Children with ASD were subdivided into 2 groups by the presence or absence of GI symptoms, GI (+) or GI (−) (Table I). We defined GI symptoms as vomiting, diarrhea, chronic loose stool, colic and GI cramping, and constipation (often alternating with diarrhea) reported by parents/caretakers/physicians. Of the GI (+) ASD children, autism was diagnosed in 36 and pervasive developmental disorder NOS (PDD-NOS) was diagnosed in 27. Of the GI (−) ASD children, autism was diagnosed in 14 and PDD-NOS was diagnosed in 1. ASD not falling into clear diagnostic criteria of autism or PDD-NOS because of young age was diagnosed in the remaining children. Diagnosis of atopic asthma, allergic rhinitis, and allergic dermatitis was made by the presence of clinical features of these disorders with positive skin test reactivity, the presence of allergen-specific IgE against common airborne and food allergens. The prevalence of atopic disorders in children with ASD was similar to that reported in general population (Table I).[8] Atopic dermatitis was diagnosed in 5 of the 75 GI (+) ASD children (6.7%)and 3 of the 34 GI (−) ASD children (8.8 %), and their skin symptoms were affected by the intake of causative food allergens (mainly milk, egg, and peanut). Asthma and allergic rhinitis symptoms were not associated with intake of food allergens in our study subjects.

GI (+) ASD and NFH children were further subdivided into diarrhea, loose stool, and constipation groups on the basis of features of stool specimen examined as a part of the routine workup for NFH. In GI (+) ASD children with constipation, 4 of 32 were reported to have diarrhea alternating with constipation by parents/caretakers. In NFH children with diarrhea, 2 of 4 were documented as having occasional constipation. Gastroesophageal reflux disease in infancy was reported in 7 of 75 GI (+) ASD children, 1 in the diarrhea group, 3 in the loose stool group, and 3 in the constipation group.

Assessment of Immune Reactivity Against Dietary Proteins

PBMCs (10^6 cells/mL) were cultured in the presence of common DPs for 4 to 5 days, and levels of IFN-γ, TNF-α, Interleukin-5 (IL-5), IL-10, and IL-12p40 in the culture supernatant were determined as a measure of assessing cellular immune reactivity. IFN-γ and IL-5 were selected as representative type 1 and type 2 T-helper (Th1 and Th2) cytokines, respectively. A significant increase in TNF-α production by PBMCs with CMP has been reported in patients with non-allergic CMP hypersensitivity.[3,5] IL-10 was measured as a representative regulatory cytokine produced by T cells and other lineage cells; dysregulated IL-10 production can be associated with various inflammatory and autoimmune conditions.[9] IL-12p40 is a degraded product of biologically functional IL-12p70 that promotes Th1 responses. We also measured IL-4, transforming growth factor-β, and IL-2 in a few study and control subjects. However, we observed little production of these cytokines with DPs, as reported before, and thus these cytokines were not measured in the rest of the study subjects.[7]

Among DPs, we tested reactivity to crude cow's milk and soy protein extracts (provided by Ross Products Division/Abbott Laboratories, Columbus, Ohio), gliadin (a major wheat protein; Sigma, St. Louis, Mo), and major components of

CMP, bovine casein, α-lactoalbumin, and β-lactoglobulin (Sigma). These DPs were major causes of NFH in children.[7] Concentrations of these DPs used were 100 μg/mL for CMP and soy and 10 μg/mL for gliadin and major components of CMP. We detected <1 ng/mL of endotoxin in these DPs (1 mg/mL solution; Endotoxin kit, Sigma). We used recall Ags (tetanus toxoid and dust mite extract) and T cell mitogens (phytohemagglutinin [10 μg/mL] and concanavalin A [1μg/mL]) as positive control stimuli to ensure normal immune reactivity to control stimuli in the study subjects. Cytokine levels in the culture supernatants were measured by an enzyme-linked immunosorbent assay, using OptEIA Reagent Sets (BD Pharmingen, San Diego, Calif). Intra- and inter-variations of cytokine levels were less than 5%.[7,10]

Statistical Analysis

Equality of 2 sets of data values was evaluated with the Mann-Whitney test (independent samples) or Wilcoxon weighed ranks test (related samples). Multiple sets of values were evaluated with the Kruskal-Wallis test. Correlation was assessed with the 2-tailed Kendalls τ test. Differences with a *P* value <.05 were considered to be significant.

RESULTS

Cytokine Production in Response to Common DPs

Our findings in cytokine production with a stimulus of each DP tested were:

1) CMP: PBMCs from both GI (+) and GI (−) ASD children produced more TNF-α and IL-12 than those from control subjects. NFH PBMCs produced more IFN-γ, TNF-α, IL-10, and IL-12 than those from control subjects (Figure 1*A*).
2) Casein: Cytokine production by PBMCs was minimal and did not differ among the study groups (data not shown).
3) β-Lactoglobulin: PBMCs from GI (+) ASD children, but not from GI (−) children, produced more TNF-α and IL-12 than those from control subjects (Figure 1*B*). These levels are also higher than those produced by GI (−) ASD PBMCs (*P* <.05). NFH PBMCs produced the highest levels of IFN-γ, TNF-α, and IL-12 among the study groups (Figure 1*B*).
4) α-Lactoalbumin: GI (+) but not GI (−) ASD PBMCs produced more TNF-α and IL-12 than those from control subjects (Figure 1*C*). IL-12 production by GI (+) ASD PBMCs was also higher than that by GI (−) ASD PBMCs (*P* <.02). NFH PBMCs also produced more TNF-α and IL-12 than those from control subjects (Figure 1*C*), and their TNF-α production was the highest among the study groups (*P* <.005).
5) Gliadin: GI (+) but not GI (−) ASD PBMCs produced more TNF-α than those from control subjects (Figure 1*D*), and TNF-α levels produced were also higher than those produced by GI (−) ASD PBMCs (*P* <.05). NFH PBMCs also produced more TNF-α than those from control subjects.

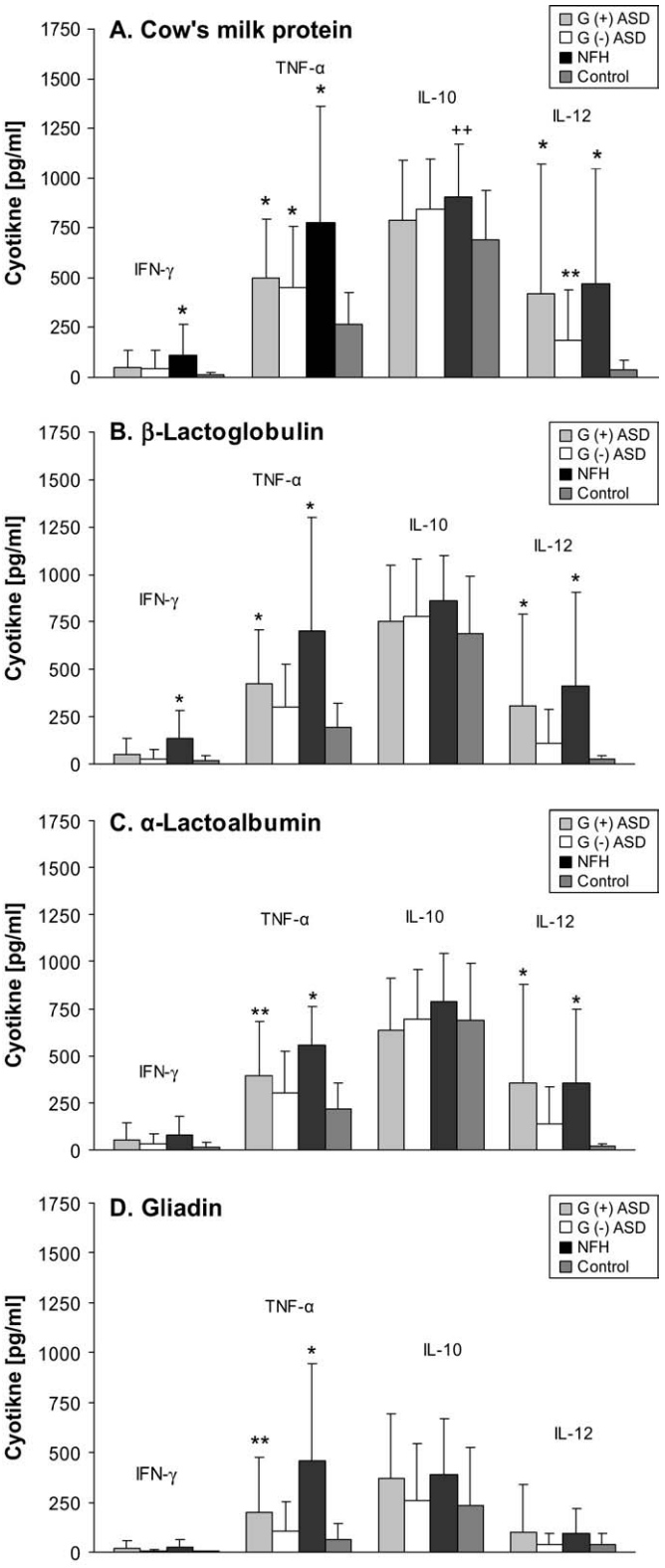

Figure. Production of IFN-γ, TNF-α, IL-10, and IL-12 by PBMCs from GI (+) ASD, G (−) ASD, NFH and control children with stimuli of CMP (**A**), β-lactoglobulin (**B**), α-lactoalbumin (**C**), and gliadin (**D**). The results are expressed as control mean values + 1SD. Marked values are higher than controls by means of the Mann-Whitney test. **P* <.005; ***P* <.02; ++*P* <.05.

Table II. Relationship between gastrointestinal symptoms and tumor necrosis factor-α production by peripheral blood mononuclear cells with cow's milk protein, β-lactoglobulin, and gliadin

Stimulants	CMP (>CM + 1SD)	β-lactoglobulin (>CM + 1SD)	Gliadin (>CM+1SD)
GI (−) ASD (N = 34)	17 (50%)	13 (38.2%)	8 (23.5%)
GI (+) ASD (N = 75)	39 (52.0%)	44 (58.7%)	31 (41.3%)
Diarrhea (N = 8)	4 (50.0%)	4 (50.0%)	1 (12.5%)
Loose stool (N = 39)	21 (53.8%)	25 (64.1%)	23 (59.0%)
Constipation (N = 28)	15 (53.4%)	15 (53.4%)	8 (28.6%)
NFH (N = 15)	13 (86.7%)	13 (86.7%)	10 (66.7%)
Diarrhea (N = 5)	4 (80.0%)	5 (100%)	3 (100%)
Loose stool (N = 10)	9 (90.0%)	8 (80.0%)	7 (70.0%)

Table III. Relationship between gastrointestinal symptoms and interleukin-12 production by peripheral blood mononuclear cells with cow's milk protein, β-lactoglobulin, and gliadin

Stimulants	CMP (>CM + 1SD)	β-lactoglobulin (>CM + 1SD)	Gliadin (>CM+1SD)
GI (−) ASD (N = 34)	15 (44.1%)	10 (29.4%)	4 (11.8%)
GI (+) ASD (N = 75)	42 (56.0%)	40 (53.3%)	12 (16.0%)
Diarrhea (N = 8)	4 (50.0%)	4 (50.0%)	1 (12.5%)
Loose stool (N = 39)	25 (64.1%)	24 (61.5%)	7 (17.9%)
Constipation (N = 28)	12 (42.9%)	13 (46.4%)	4 (14.3%)
NFH (N = 15)	9 (60.0%)	13 (86.7%)	4 (26.7%)
Diarrhea (N = 3)	4 (8.0%)	5 (100%)	0
Loose stool (N = 10)	5 (50.0%)	8 (80.0%)	4 (40.0%)

6) Soy: Cytokine production by PBMCs was minimal in most of the study subjects and did not differ among the study groups. However, elevated levels of IFN-γ production (>100 pg/mL) with soy was found in 7 of the 75 GI (+) ASD children and 4 of the 15 NFH children.

IL-5 production was low and did not differ among the study groups, irrespective of the stimuli (data not shown). Without stimuli, cytokine production by PBMCs was less than the detectable level in most of the subjects. PBMCs from both ASD and NFH subjects produced comparable levels of these cytokines with mitogens and recall antigens as those from control subjects. All NFH children responded well to the elimination diet, with resolution of GI symptoms within 4 months, and had documented recurrence of GI symptoms with re-exposure to causative DPs.

Relationship of GI Symptoms and DP-Induced Cytokine Production in ASD Children

We also analyzed the relationship between elevated TNF-α and IL-12 production (>CM + 1SD) with DPs (CMP, β-lactoglobulin, and gliadin) and objective GI symptoms in ASD and NFH children, because subjective GI symptoms were difficult to assess in children with ASD because of their poor expressive languages. PBMCs that produced >CM + 1SD TNF-α, IL-12, or IFN-γ with gliadin also produced >CM + 1SD of these cytokines with CMP, β-lactoglobulin, or both without exception. The results with α-lactoalbumin were similar to those obtained with β-lactoglobulin.

1) GI (+) ASD group: The most common GI symptoms reported were loose stool and constipation (Table II). GI (+) ASD PBMCs produced >CM + 1SD TNF-α and IL-12 with CMP and β-lactoglobulin at high frequency irrespective of objective GI symptoms. In contrast, >CM + 1SD TNF-α production with gliadin was more frequently found in the loose stool group (Table II). Elevated IL-12 production was less frequently found with a stimulus of gliadin, irrespective of GI symptoms (Table III). A positive correlation was observed between TNF-α and IL-12 levels produced with β-lactoglobulin, α-lactoalbumin, and gliadin ($P < .005$), but not with CMP. In summary, with stimuli of CMP and or β-lactoglobulin, >CM + 1SD TNF-α production, IL-12 production, or both is frequently seen in PBMCs obtained from GI (+) ASD children (55/75; 73.3%).

Among 20 GI (+) ASD children without elevated TNF-α production, IL-12 production, or both with DPs, 7 of 20 had moderate to heavy growth *of Candida albicans* in their stool, 1 in the diarrhea group, 2 in the loose stool group, and 4 in the constipation group. Another 2 subjects had atopic dermatitis with elevated IgE antibodies against common food allergens. Another 5 subjects had a history of chronic or recurrent otitis media and sinusitis requiring frequent antibiosis, 3 in the loose stool group and 2 in the constipation group.

2) GI (−) ASD Group: PBMCs from GI (−) ASD children produced >CM + 1SD TNF-α and IL-12 with CMP at high frequency, but less frequently with β-lactoglobulin or gliadin (Tables II and III). TNF-α and IL-12 levels with CMP, but not with β-LG or gliadin, were positively correlated ($P < .005$). In summary, with stimuli of CMP, β-lactoglobulin, or both, GI (−) ASD PBMCs produced

>CM + 1SD TNF-α more frequently (17/34; 50%) than IL-12 (8/34; 23.5%).

3) NFH group: The most common GI symptoms were loose stool in NFH children (Table III). Most NFH PBMCs produced >CM + 1SD TNF-α and IL-12 with CMP, β-lactoglobulin, or both, irrespective of GI symptoms. With gliadin, >CM + 1SD IL-12 production was only seen in NFH children with loose stool (Table III). TNF-α and IL-12 production were positively correlated in NFH PBMCs with stimuli of β-lactoglobulin and gliadin ($P < .05$).

DISCUSSION

Our results revealed a high prevalence (>70%) of cellular immune reactivity to CMP and its major components in GI (+) ASD children when positive reactivity is defined as >CM + 1SD production of TNF-α, IL-12, or both with CMP, β-lactoglobulin (a major component of CMP), or both. Such cellular reactivity was less remarkable with gliadin. Our findings indicate a possible role of NFH against CMPs in GI symptoms observed in children with ASD.

GI symptoms are frequently observed in children with ASD, with evidence of GI inflammation by means of imaging and endoscopic examinations.[11] Other authors also reported non-specific colitis with ileal-lymphoid nodular hyperplasia accompanied by colonic CD8 and TCRγδ T cell infiltration and prominent epithelial cell damage in regressive autism.[12] In 24 children with regression autism, pathological findings included epithelial IgG and complement deposition with infiltration of enterocytes and lymphocytes in epithelium and lamina propria of duodenum.[13] Other authors also reported an increase in T cells in intestinal epithelium and increased T and B cells in lamina propria in children with ASD who had a resolution of GI symptoms with the implementation of the casein-free, gluten-free diet.[14] These findings indicate a role of T cell mediated, cellular immune responses in the GI inflammation observed in children with ASD.

As noted in the introductory section, cellular immune reactivity to DPs plays a vital role in NFH.[1] Increase in TNF-α production by PBMCs with CMP appear closely associated with clinical features of NFH in children who are reactive to CMP.[2,3,5] In this study, we observed marked elevation of IFN-γ, TNF-α, and IL-12, but not IL-5 or IL-10, in most NFH children when stimulated with CMP and its major components (β-lactoglobulin and α-lactoalbumin). We obtained a similar but less remarkable result in GI (+) ASD PBMCs. Namely, 55 of 75 GI (+) ASD PBMCs (73.3%) produced >CM + 1SD TNF-α/IL-12 with CMP, β-lactoglobulin, or both. Prevalence of AD with or without food allergy and other atopic disorders was equivalent among the study groups and similar to that in the general population. We also obtained similar results with α-lactoalbumin in ASD PBMCs. Taken together, our results indicate that GI symptoms found in children with ASD are partly attributed to NFH to CMP and its 2 major components, α-lactoalbumin and β-lactoglobulin. All the GI (+) ASD children with elevated (>CM + 1SD) TNF-α/IL-12 production responded favorably to the elimination diet per parental report, and we are in the process of conducting a prospective study in children with ASD who have positive or negative cellular immune reactivity to CMP, β-lactoglobulin, or both, as defined as aforementioned.

Our study also revealed that β-lactoglobulin and α-lactoalbumin, but not casein, are major CMP components inducing significant TNF-α, IL-12, and IFN-γ production in GI (+) ASD and NFH subjects. However, in children with ASD who have atopy, especially atopic dermatitis (AD), we observed an increase in IL-5 production with casein (unpublished observation). Our results provide practical information that children with ASD who have NFH to CMP but do not have atopic disorders may have a good probability of tolerating casein-containing processed food, making the implementation of a diary-free diet easier. An increase in TNF-α/IL-12 production to gliadin, a major wheat protein, was less frequently found in GI (+) ASD children, especially those with diarrhea or constipation. Our finding indicates that in GI (+) ASD children, especially those with diarrhea or constipation, a gluten-free diet may not be required for resolution of GI symptoms. These questions need to be addressed further in a prospective study of children with ASD enrolled in a trial of the elimination diet on the basis of defined cellular immune reactivity.

Another notable finding in this study is that 17 of 34 GI (−) ASD PBMCs produced >CM + 1SD TNF-α/IL-12 with CMP, β-lactoglobulin, or both. Among them, 8 subjects also produced >CM + 1SD TNF-α/IL-12 with gliadin. Eleven of these 17 children underwent a trial of the casein-free diet or casein-free, gluten-free diet on the basis of cellular immune reactivity; soy products were not substituted as dairy products in these children, because children with NFH to CMP are at a high risk of developing NFH to soy when it is substituted.[1,2] Parents of all 11 of these children reported more regular bowel movements with less hard stool and an improvement of behavioral symptoms (less irritability, less hyperactivity, less stimulatory behaviors; unpublished observation). These preliminary findings suggest that certain GI symptoms, such as GI cramping because of NFH, may be under-appreciated in children with ASD, most likely because of their poor expressive language. Delayed implementation of intervention measures for NFH could aggravate behavioral symptoms in these children with ASD. Even in children with normal cognitive activity, non-IgE mediated NFH can be frequently under-diagnosed.[6] Further addressing this possibility will require a prospective study examining changes in GI and behavioral symptoms in children with ASD enrolled in a trial of elimination diets on the basis of defined cellular immune reactivity to DPs.

Another notable finding in this study is that 20 of the 75 of GI (+) ASD children did not have elevated immune reactivity against DPs. As described in the Results section, 2 of these 20 children had IgE-mediated food allergy. In the remaining 18 subjects, GI symptoms appeared not to be associated with the NFH to common DPs tested in this study.[1,2] Five of these 18 subjects had a history of frequent

antibiosis caused by recurrent otitis media and sinusitis, and 7 of them had evidence of candida overgrowth on stool cultures. These findings might be associated with their GI symptoms, but further evaluation will be required in these children, and we cannot completely rule out NFH to other DPs in these children.

In addition to IFN-γ and TNF-α that are implicated with the pathogenesis of NFH, we also assessed regulatory cytokine production (IL-10 and IL-12) by PBMCs when stimulated with common DPs. IL-12 produced by innate immune cells promotes Th1 responses that are characterized by Th1 cytokine (IFN-γ and TNF-α) production and potent cellular immune reactivity.[15] PBMCs from GI (+) ASD and NFH children produced more IL-12 with CMP/β-lactoglobulin/α-lactoalbumin than those from control subjects. Even GI (−) ASD PBMCs produced more IL-12 with CMP than those from control subjects. Moreover, IL-12 production was positively correlated with TNF-α production with these stimuli in PBMCs from both ASD and NFH children. Thus, IL-12 production in our assay system likely related to activation of DP-specific T cells, because T cell cytokines promote the production of IL-12 by monocytes.

In contrast to IL-12 production, IL-10 production did not differ among the study groups. IL-10 is one of the major cytokines produced by regulatory T cells and exerts inhibitory actions on monocytes and T cells, partly suppressing production of IFN-γ and TNF-α.[9,16,17] Our results revealed that only NFH PBMCs produced more IL-10 with CMP, pointing out a possibility that the production of regulatory cytokines such as IL-10 might be less in children with ASD who have NFH to CMP. A prospective study evaluating the effects of the elimination diet in ASD children with or without positive cellular immune reactivity to CMP and other common DPs is needed to address this possibility.

REFERENCES

1. Sampson HA. Update of food allergy. J Clin Immunol Allergy 2004; 113:805-19.

2. Sampson HA, Anderson JA. Summary and recommendations: classification of gastrointestinal manifestations due to immunologic reactions to foods in infants and young children. J Pediatr Gastroenterol Nutr 2000;30: S87-94.

3. Benlounes N, Candalh C, Matarazzo P, Dupont C, Heyman M. The time-course of milk antigen-induced TNF-α secretion differs according to the clinical symptoms in children with cow's milk protein. J Allergy Clin Immunol 1999;104:863-9.

4. Sicherer SH. Food protein-induced enterocolitis syndrome: clinical perspectives. J Pediatr Gastroenterol Nutr 2000;30:S45-9.

5. Motrich RD, Gottero C, Rezzonico C, Rezzonico C, Riera CM, Rivero V. Cow's milk stimulated lymphocyte proliferation and TNF-α secretion in hypersensitivity to cow's milk protein. Clin Immunol 2003;109:203-11.

6. Latcham F, Merino F, Lang Al, Garvey J, Thomson MA, Walker-Smith JA, et al. A consistent pattern of minor immunodeficiency and subtle enteropathy in children with multiple food allergy. J Pediatr 2003;143:193-8.

7. Jyonouchi H, Sun S, Itokazu N. Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. Neuropsychobiology 2002; 46:76-84.

8. Ono SJ. Molecular genetics of allergic diseases. Annu Rev Immunol 2000;8:347-66.

9. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683-765.

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

11. Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tioson JT. Gastrointestinal abnormalities in children with autistic disorder. J Pediatr 1999;135:559-63.

12. Furlano R, Anthony A, Day R, Brown A, McGarvey L, Thomson MA, et al. Colonic CD8 and γδ T-cell infiltration with epithelial damage in children with autism. J Pediatr 2001;138:3663-72.

13. Torrente F, Ashwood P, Day R, Machado N, Furlano RI, Anthony A, et al. Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Mol Psychiatry 2002;7: 375-82.

14. Ashwood P, Anthony A, Pellicer AA, Torrente F, Walker-Smith JA, Wakefield AJ. Intestinal lymphocyte population in children with regressive autism: evidence for extensive mucosal immunopathology. J Clin Immunol 2003;23:504-17.

15. Agnello D, Lankford CS, Bream J, Morinobu A, Gadina M, O'Shea JJ, et al. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immuunol 2003;23: 147-61.

16. Fehérvari Z, Sakaguchi S. Development and function of CD25+ CD4+ regulatory T cells. Curr Opin Immunol 2004;16:203-8.

17. Glavin M, Rudensky A. Control of immune homeostasis by naturally arising regulatory CD4+ T cells. Curr Opi Immunol 2003;15:690-6.