Human milk–specific mucosal lymphocytes of the gastrointestinal tract display a T_H2 cytokine profile

Kirsten Beyer, MD,[a] Russell Castro, BS,[a] Audrey Birnbaum, MD,[b] Keith Benkov, MD,[b] Nanci Pittman, MD,[b] and Hugh A. Sampson, MD[a] *New York, NY*

Background: A number of gastrointestinal disorders, including allergic eosinophilic gastroenteritis and food protein–induced enteropathy, have been associated with milk hypersensitivity. The immunologic reactions appear to involve T cells that are activated by specific food proteins.

Objective: The present study was performed to examine the cytokine profiles of milk-specific lymphocytes from the duodenal lamina propria from children with milk-induced gastrointestinal diseases.

Methods: Duodenal biopsy specimens obtained from 10 patients with allergic eosinophilic gastroenteritis, food protein–induced enteropathy, or both and 12 control subjects were mechanically minced and cultured with either mitogens (ie, polyclonal T-cell expansion) or milk proteins (ie, milk-specific T-cell expansion). By using flow cytometry, expanded T cells were phenotyped with anti-CD4, anti-CD8, anti-IL-4, anti-IL-5, and anti-IFN-γ mAbs. The milk specificity of the lines was evaluated by means of the lymphocyte proliferation assay. In addition, the release of T_H1, T_H2, and T_H3 cytokines was determined after restimulation.

Results: In patients and control subjects polyclonal expansion of mucosal lymphocytes resulted in predominantly T_H1 cells. Milk-specific mucosal T-cell lines could be established in 60% of the patients but in none of the control subjects. In contrast to the polyclonal expansion of T cells, the milk-specific expansion of mucosal T cells showed a clear T_H2 cytokine profile. On restimulation with milk protein, these cells showed a high proliferative response. They released T_H2 cytokines, predominately IL-13, but failed to release T_H3 cytokines important in the development of oral tolerance.

Conclusion: The release of T_H2 cytokines after stimulation of milk-specific mucosal T cells may play a pathogenic role in the inflammatory changes seen in milk-induced gastrointestinal disorders. (J Allergy Clin Immunol 2002;109:707-13.)

Key words: *Gut, food allergy, IL-4, IL-5, IL-13, transforming growth factor β, IL-10*

A variety of gastrointestinal disorders in infancy and childhood are due to milk-induced allergic reactions. IgE- and non–IgE-mediated hypersensitivity mechanism each account for about one half of milk allergy–related disorders.[1] A number of clinical syndromes have been described that include symptoms of abdominal pain, vomiting, diarrhea, gross or occult blood in stool, and poor growth. Significant inflammatory changes are seen in endoscopic biopsy samples, which have been associated with immunologic reactions to particular food proteins. Examples of these illnesses include milk-induced allergic eosinophilic gastroenteritis (AEG) and food protein–induced enteropathy.[2] It appears that the immunologic reactions provoking these illnesses involve T cells that are activated by specific food proteins and that elaborate cytokines, chemokines, or both responsible for the inflammatory changes.

Food protein–induced enteropathy is a symptom complex of malabsorption, failure to thrive, diarrhea, emesis, and hypoproteinemia in infants that is usually related to an immunologic reaction to cow's milk protein.[2,3] Patchy villous atrophy with cellular infiltrate on biopsy is characteristic. Diagnosis is based on the combined findings from endoscopies-biopsies, allergen elimination, and challenge.

AEG generally presents with postprandial nausea and vomiting, abdominal pain, diarrhea, early satiety or food refusal, and failure to thrive in children or weight loss in adults.[4,5] The disease is characterized by infiltration of the intestinal walls with eosinophils, gastroesophageal reflux, peripheral eosinophilia, and absence of vasculitis.[5] The immunopathogenic mechanism or mechanisms responsible for this disease are still unclear. In a subset of this disorder, a food-induced IgE-mediated mechanism has been implicated. However, many patients do not have IgE antibodies to the foods that provoke positive food challenge responses.

The gut-associated lymphoid tissues include organized lymphoid tissues, such as mesenteric lymph nodes, Peyer's patches, and lymphoid follicles within the lami-

From [a]the Division of Pediatric Allergy and Immunology and Jaffe Institute for Food Allergy and [b]the Division of Pediatric Gastroenterology, The Mount Sinai School of Medicine, New York.

Supported in part by a grant from the National Center of Research Resources-National Institutes of Health (MO1 RR-00071) awarded to the Mt Sinai School of Medicine, in part by a grant from the National Institute of Allergy and Infectious Diseases—NIH (AI44236), and in part by a grant from the Allergy and Immunology Institute of the International Life Science Institute (ILSI) Research Foundation. The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI.

Received for publication September 24, 2001; revised December 20, 2001; accepted for publication December 27, 2001.

Reprint requests: Kirsten Beyer, MD, The Mount Sinai School of Medicine, Division of Pediatric Allergy and Immunology, Box-No. 1198, One Gustave L. Levy Place, New York, NY 10029-6574.

0091-6749/2002 $35.00 + 0 **1/87/122503**

doi:10.1067/mai.2002.122503

Abbreviations used
AEG: Allergic eosinophilic gastroenteritis
DC: Dendritic cell
PMA: Phorbol 12-myristate 13-acetate
TGF-β: Transforming growth factor β

na propria. In addition, lymphocytes are distributed diffusely within the lamina propria and the epithelium. T cells are the major regulatory component of the immune system, and much of this regulatory function is mediated through the elaboration of cytokines.[6] Unfortunately, little is known about T cell–generated cytokine responses in gastrointestinal food allergy. Although it has been shown that peripheral blood T cells from patients with AEG secrete higher amounts of IL-4 and IL-5 compared with those from control subjects,[7] the immunopathology of this disorder is poorly understood. In the mouse model it was shown that mesenteric T cells from oral antigen–challenged mice cultured with antigen produced high amounts of IL-4 and IL-5 and no detectable IFN-γ.[8] Much less is known about the site of inflammation in human subjects. In patients with food allergy with immediate-type reactions, such as urticaria, angioedema, and anaphylaxis, elevated levels of IL-5 were found in the gut mucosa by means of immunohistochemical staining.[9] In addition, in patients with eosinophilic gastroenteritis, increased levels of IL-3, IL-5, and GM-CSF were found by using the same method.[10] The present study reports for the first time the successful culture of allergen-specific lymphocytes from the site of inflammation in children with milk-induced gastrointestinal disorders. These allergen-specific lymphocytes show a clear T_H2 phenotype and the inability to release T_H3 cytokines important in the development of oral tolerance.

METHODS

Study population

Duodenal biopsy specimens were obtained from 22 children undergoing scheduled endoscopies for the diagnosis or follow-up of a variety of gastrointestinal complaints. Ten patients had AEG, food protein–induced enteropathy, or both (Table I), and 12 children were recruited as control subjects. All patients had milk-induced symptoms (Table I). Four of the control children had primary reflux esophagitis, 2 were given a diagnosis of dyspepsia, 1 had *Helicobacter pylori*–induced gastritis, 1 had irritable bowel syndrome, 1 had a trachea-esophageal fistula, 1 was treated for coin ingestion, 1 had Crohn's colitis, and 1 had abdominal pain without pathologic findings. Informed consent was obtained, and the study was approved by the Mount Sinai School of Medicine Institutional Review Board.

Preparation of lymphocytes

Duodenal biopsy specimens were placed into sterile collection medium (calcium- and magnesium-free HBSS [Sigma]) with 5% AB serum [Gemini-Bio-Products]) and penicillin plus streptomycin (Gibco). The specimens were washed 3 times with collection medium and twice with serum-free culture medium (AIM V plus glutamine and streptomycin [all Gibco]), minced into small fragments, and placed in 24-well plates in culture media for polyclonal or milk-specific T-cell expansion (37°C, 5% CO_2). For polyclonal expansion, one half of the minced biopsy specimens were placed in AIM V culture media containing recombinant (r)IL-2 (20 IU/mL, Sigma) and rIL-4 (5 ng/mL) at 37°C in a 5% CO_2 atmosphere, allowing the lymphocytes to migrate out of the tissue. After 1 to 2 days, some lymphocytes began to migrate out of the tissue. At this time, mitogens (2 μg/mL PHA and 1 ng/mL phorbol 12-myristate 13-acetate [PMA], Sigma) and irradiated (3000 rad) feeder mix (PBMCs mixed from 3 unrelated donors) were added to the wells to expand the total T-cell population. For milk-specific expansion, the other half of the minced biopsy specimens were placed in AIM V culture media containing only rIL-2 (20 IU/mL, Sigma) and milk proteins (200 μg/mL total; 50 μg/mL each of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin; Sigma). No irradiated PBMC and PHA/PMA stimulation were used.

Flow cytometry

After 3 to 4 weeks, lymphocytes (1×10^6/mL) were stimulated with 300 ng/mL PMA and 1.5 μg/mL calcium ionophore (Sigma) in the presence of a protein transport inhibitor (Golgi Stop, PharMingen). After 6 hours, cells were harvested, washed with PBS, and incubated with allophycocyanin-labeled anti-CD4, anti-CD8 mAb, or isotype control (PharMingen) for 30 minutes at 4°C in the dark. After washing, cells were fixed with paraformaldehyde (Cytofix/Cytoperm, PharMingen) and permeabilized with monensin (Perm/Wash Buffer, PharMingen). Intracellular cytokine staining was performed by means of flow cytometry with FITC-labeled anti-IFN-γ or phycoerythrin-labeled anti-IL-4 or anti-IL-5 and the corresponding isotype control (PharMingen). By using a FACSCalibur flow cytometer and the CELLQUEST software (Becton Dickinson), 1×10^4 cells were analyzed per sample, with a gate for lymphocytes on the basis of size and density of the cells in the forward and sideward scatter.

Lymphocyte proliferation test

Rested lymphocytes were cultured after 3 to 4 weeks in triplicate with or without milk proteins (200 μg/mL total; 50 μg/mL each of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin) in 96-well flat-bottom plates. Each well contained 1×10^5 mucosal T cells in AIM V medium. After 5 days, cells were pulsed with 1 μCi of tritiated thymidine (ICN) per well, harvested 16 hours later, and counted in a liquid scintillation counter.

Cytokine release

Cytokines secreted into the cell-culture media were measured after 3 days of milk-specific restimulation. Therefore rested lymphocytes were cultured with or without milk proteins (200 μg/mL total; 50 μg/mL each of α-casein, β-casein, α-lactalbumin, and β-lactoglobulin) in 24-well plates. Each well contained 1×10^6 mucosal T cells in AIM V medium. After 3 days, supernatants were harvested, placed in aliquots, and frozen at –80°C. IL-4, IL-5, IL-13, IL-10, IFN-γ, and transforming growth factor (TGF) β levels were measured by means of ELISA according to the manufacturer's instructions (Pharmingen). In addition, IL-10 and TGF-β levels were also determined in the supernatants of mitogen-expanded mucosa T cells without allergen-specific stimulation.

Statistical analysis

All analyses of data were performed by using nonparametric tests (Mann-Whitney *U* test for comparison between groups and Wilcoxon signed-rank test for comparing the cytokines in supernatant between milk-stimulated T cells and T cells cultured in medium alone) with Prism 2.01 software. Differences associated with *P* values of less than .05 were considered significant.

TABLE I. Clinical characterization of patients with milk-induced gastrointestinal disorders

Patient identifier	Age (y)	Sex	Disease: Clinical symptoms under milk ingestion	Specific IgE (kU/L)	Milk-specific cell growth
A	1	M	AEG, enteropathy, AD: bloody stool, hypoalbuminemia	<0.35	Yes
B	17	F	AEG, AD: vomiting, diarrhea, abdominal pain	<0.35	Yes
C	6	M	AEG: bloody stool, reflux, abdominal pain	<0.35	No
D	17	F	AEG: abdominal pain, vomiting, constipation	<0.35	Yes
E	5	F	Enteropathy: vomiting, diarrhea, hypoalbuminemia	0.8	Yes
F	1	F	AEG, AD: vomiting, diarrhea, failure to thrive	32.6	Yes
G	4	M	AEG: urticaria, failure to thrive, no other specific GI symptoms after milk	11.3	No
H	2	M	AEG, AD: vomiting, diarrhea, eczema	1.34	No
I	9	M	AEG, AD: abdominal pain, vomiting, retrosternal burning	<0.35	No
J	2	M	AEG, enteropathy: bloody stool, edema, hypoalbuminemia	0.65	Yes

All patients showed an improvement with dietary intervention.
AD, Atopic dermatitis; *GI*, gastrointestinal.

RESULTS

Mucosal lymphocytes in children with milk-induced gastrointestinal disorders and control subjects were compared with regard to milk specificity and cytokine profile. The clinical characteristics of the children with milk-induced symptoms are shown in Table I. In both children with milk-induced gastrointestinal disorders and control subjects, lymphocytes could be obtained in 75% to 90% of the biopsy specimens when expanded nonspecifically with mitogens (Table II). In contrast to this, only in children with milk-induced gastrointestinal disorders could lymphocytes be expanded during milk-specific stimulation (Table II).

In both the milk-specific and polyclonal expanded mucosal lymphocytes, intracellular cytokines were determined by means of flow cytometry. The majority of milk-specific mucosal lymphocytes were $CD4^+$ (median, 93%); only a few $CD8^+$ cells were found (median, 0.7%). The milk-specific mucosal lymphocytes showed a predominately T_H2 cytokine profile, with significantly more T_H2 cells ($P < .002$, Fig 1) and fewer T_H1 cells than polyclonal expanded lymphocytes. This was the case despite the presence of IL-4 in the culture medium for the polyclonal expanded cells, which might be expected to promote a T_H2 phenotype. T_H2 cells were defined as $CD4^+$ cells producing IL-4 but no IFN-γ, T_H1 cells as $CD4^+$ cells producing IFN-γ but no IL-4, and T_H0 cells as $CD4^+$ cells producing both cytokines. Milk-specific expanded cells also contained significantly larger amounts of intracellular IL-5 than polyclonal expanded cells ($P < .0005$, Fig 2).

A lymphocyte proliferation test was performed to verify that the lymphocytes expanded with the addition of milk protein to the culture medium were milk specific. The results are shown in Fig 3. In addition, cytokine secretion into the cell-culture media was measured after 3 days of milk-specific restimulation. In all patients the cytokine release showed a similar pattern, with milk-specific release of IL-5 and IL-13 ($P < .05$, Fig 3). Although it is known that activated T cells of the human intestinal lamina propria are in general high producers of IL-10, almost no IL-10 could be measured in the activated milk-specific T-cell lines (Fig 3). A similar low release was found for TGF-β (Fig 3). In contrast, IL-10 and TGF-β were found in the supernatants of polyclonal expanded mucosal T cells from the control patients in significantly higher amounts (IL-10: 5.9 ng/mL [0.08-16.6 ng/mL], $P < .007$; TGF-β: 1.5 ng/mL [0.006-2.5 ng/mL], $P < .02$; data not shown).

DISCUSSION

A number of gastrointestinal disorders have been associated with food hypersensitivity. These gastrointestinal disorders appear to involve T cells that are activated by specific food proteins. In the present study we were able, for the first time, to culture milk-specific lymphocytes from the site of inflammation in children with milk-induced gastrointestinal diseases. We developed a method to culture allergen-specific lymphocytes from the lamina propria. With this method, sufficient cell numbers were obtained to determine their phenotype and to perform functional studies. Using this culture method, we were able to move from studies on peripheral blood lymphocytes to studies of T cells directly involved in the allergic reaction at the site of inflammation. Although in vitro studies in human subjects are generally limited because cofounding factors may be excluded, important knowledge can be added to in vivo findings in animal models mimicking human disease.

Interestingly, allergen-induced lymphocyte proliferation could not be generated in any of the control children, whereas nonspecific polyclonal expansion was observed in 9 of 12 of the control children. Three biopsy specimens of control children showed no lymphocyte growth at all. This is most likely because of an insufficient number of lymphocytes in uninflamed tissue or because of biopsy specimens that were too superficial. In contrast to the polyclonally expanded cells, the milk-specific T cells displayed a clear T_H2 phenotype in children with milk-induced gastrointestinal disorders. It is of interest to note that milk-specific mucosal T cells contain large amounts

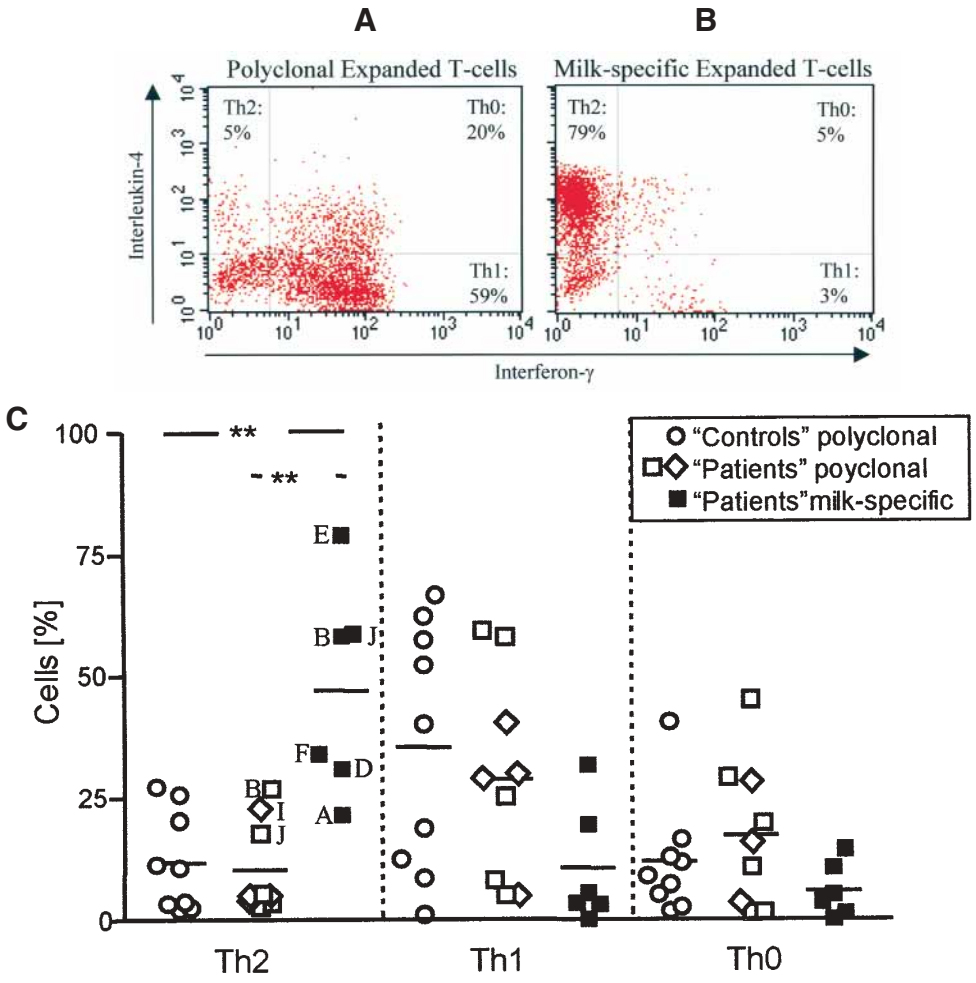

FIG 1. Flow cytometric analysis of CD4+ cells for the intracellular cytokines IL-4 and IFN-γ in polyclonal **(A)** and milk-specific **(B)** expanded mucosal T cells in one of the patients with milk-induced gastrointestinal symptoms is shown. **C** shows the scattergram of individual patients. Polyclonally expanded mucosal T cells of the control group *(open circles)* and polyclonal *(open squares and diamonds)* and milk-specific *(filled squares)* expansion of mucosal T cells from patients with milk-induced gastrointestinal diseases are shown. The *open diamonds* represent the patients who did not show T-cell growth after milk-specific stimulation. The *bar* represents the median. For easier intraindividual comparison, patient identifiers are shown for T_H2 cytokines. $^{**}P < .002$.

of intracellular IL-4 and IL-5. However, only IL-5 (and IL-13) levels in the supernatants increased significantly after milk-specific stimulation; IL-4 levels did not. This may be explained by the different stimulation of the cells. For the measurement of intracellular cytokines, milk-specific mucosal T-cell lines were maximally stimulated for 6 hours with mitogens in the presence of a protein transport inhibitor. This is a standard procedure for the measurement of intracellular cytokines. Under this maximal stimulation, milk-specific T cells show a clear T_H2 phenotype with the presence of intracellular IL-4 and IL-5. The milk-specific mucosal lymphocytes were restimulated only with milk protein and without mitogens to determine which cytokines are released into the supernatant after allergen-specific stimulation. The fact that milk-specific mucosal T-cell lines released predominately IL-5 and IL-13 after allergen-specific stimulation suggests the importance of these cytokines in food allergen–induced gastrointestinal disorders. Both cytokines have important effects on eosinophils.

Tissue eosinophilia is a hallmark of allergic inflammation. Accumulation of eosinophils in the gut is a common feature in food-induced gastrointestinal diseases that is regulated through a complex molecular network involving various cytokines and chemokines. IL-5, which regulates the growth, differentiation, and activation of eosinophils, plays a central role in the orchestration of this response.[11,12] In the present study IL-5 was only present in milk-specific, but not in polyclonal expanded, mucosal T cells and was released on restimulation with milk protein.

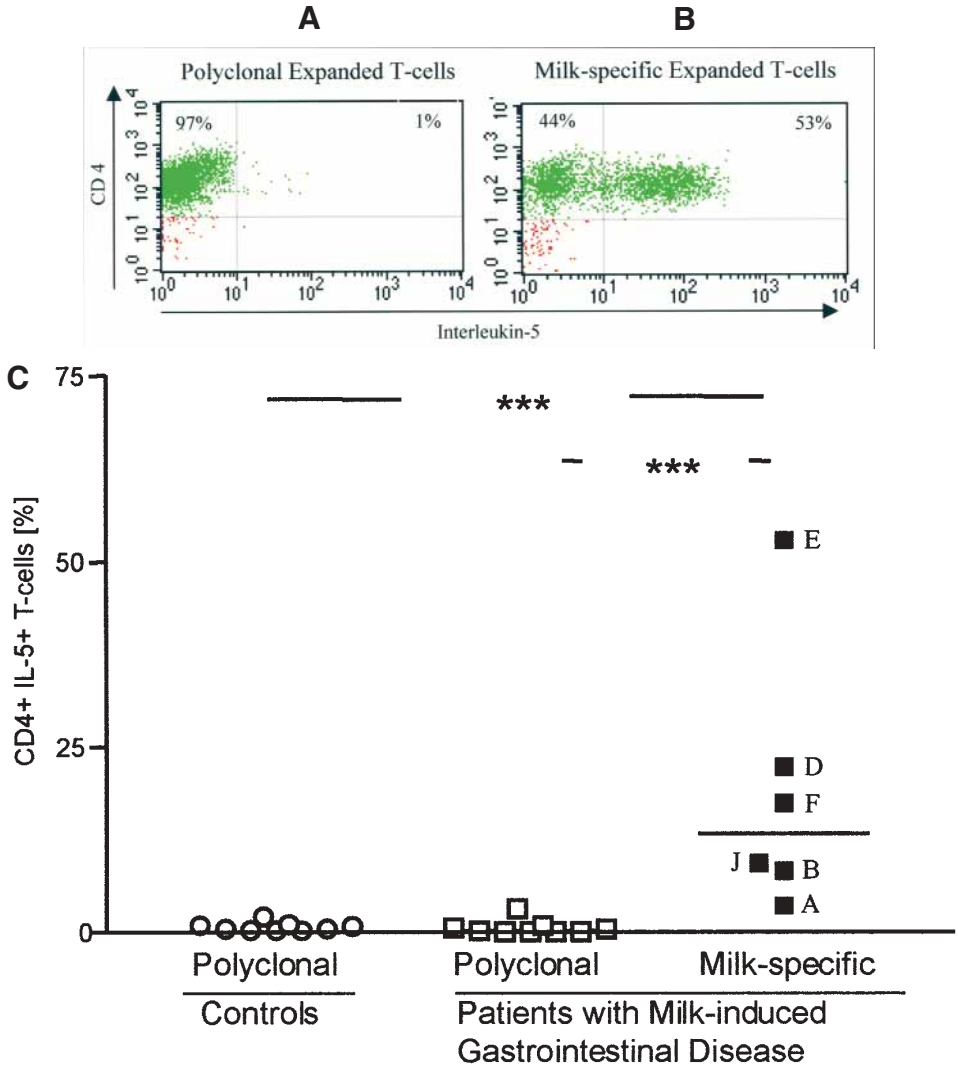

FIG 2. Flow cytometric analysis for CD4 and intracellular IL-5 in polyclonal **(A)** and milk-specific **(B)** expanded mucosal T cells in one of the patients with milk-induced gastrointestinal symptoms is shown. **C** shows the scattergram of individual patients, and the *bar* represents the median. For easier intraindividual comparison between T_H2 cytokines, patient identifiers are shown. ***$P < .0005$.

In addition to IL-5, IL-13 was released in high amounts. IL-13 shares many biologic activities with IL-4 and IL-5.[13-15] IL-13 was found to be upregulated in the skin of patients with atopic dermatitis, as well as in the lungs of patients with asthma. IL-13 has many biologic functions. It enhances the expression of class II MHC antigen, as well as CD80 and CD86. It also induces the low-affinity receptor for IgE (CD23). IL-13 also acts directly on eosinophils. Like IL-5, it prolongs eosinophil survival and thereby probably enhances the pathologic effects of these cells at the site of inflammation.[13-15] In addition, it induces vascular cell adhesion molecule 1 expression on endothelial cells, which leads to the recruitment of T cells, monocytes, and eosinophils.

In contrast to the clear T_H2 response of the milk-specific mucosal T cells in our study, Nagata et al[16] found that lymphocytes from the Peyer's patches display a T_H1 cytokine profile after in vitro stimulation with β-lactoglobulin from cow's milk. However, in contrast to our study, the subjects in Nagata's study were children and adults without milk-induced gastrointestinal diseases. The majority of endoscopies in their study were conducted to investigate suspected inflammatory bowl disease or rectal bleeding but also included patients with hemorrhoids, polyps, or cystic fibrosis. In all cases the ileum was macroscopically and histologically normal. Similar results are known from studies on peripheral blood lymphocytes. Although lymphocytes from patients

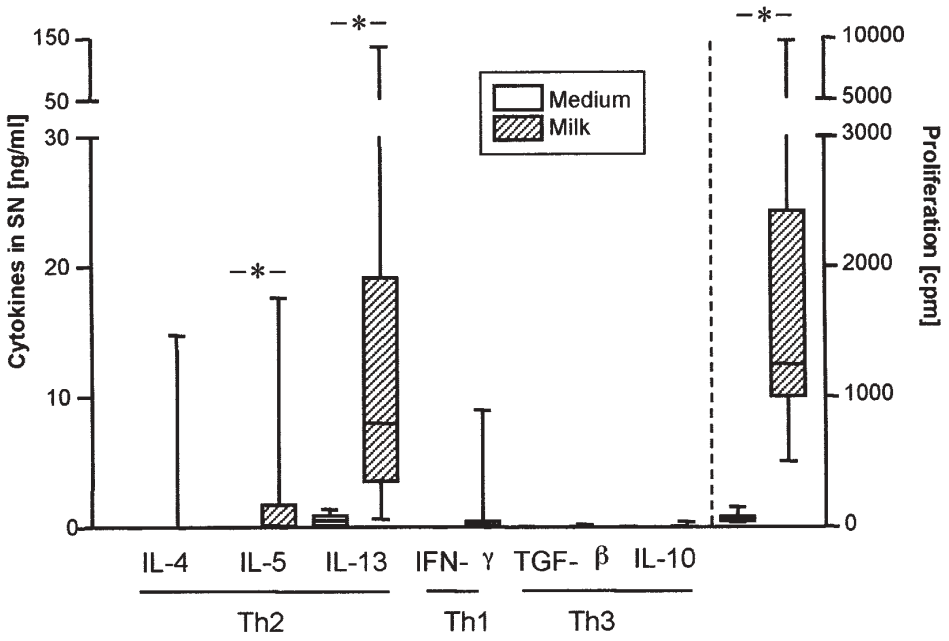

FIG 3. Whisker plot of T_H2, T_H1, and T_H3 cytokine release and proliferation after restimultion of milk-specific mucosal T cells with milk. Shown are the median, 25th-75th percentile, and range in the 6 patients with positive responses to milk-specific stimulation. *$P < .05$.

TABLE II. Growth of T cells in culture

	Patients with milk-induced GI disorders	Control subjects
Nonspecific expansion	90% (9/10)	75% (9/12)
Milk-specific expansion	60% (6/10)	0% (0/12)

Shown is the percentage of cultures that show cell growth under the given culture conditions. In addition, total numbers are given in brackets. If no cell growth was observed within 4 weeks, biopsy material was discarded.
GI, Gastrointestinal.

with atopic dermatitis with and without cow's milk allergy are able to proliferate after milk-specific stimulation, only lymphocytes from patients with cow's milk allergy display a T_H2 cytokine profile, whereas patients without milk allergy display a T_H1 phenotype.[17]

In contrast to the large amount of T_H2 cytokines, almost no TGF-β or IL-10 was released from the milk-specific mucosal T cells in the present study. Both TGF-β and IL-10 contribute to tolerance development in the mucosa. A unique subset of $CD4^+$ T cells secrete TGF-β, and these cells have been designated as T_H3 cells. TGF-β is a multifunctional cytokine with multiple roles in the immune system. TGF-β acts as a switch factor for B cells, increasing IgA production, and is a powerful immunosuppressant depressing the proliferation of T cells.[18,19] TGF-β also acts indirectly on T cells by regulating the function of antigen-presenting cells. It is able to inhibit in vitro activation and maturation of dendritic cells (DCs); critical T-cell costimulatory molecules on the surface of DCs become inhibited, and the antigen-presenting capacity of the DCs is reduced.[20] TGF-β knockout mice present with generalized inflammatory infiltrates and uncontrolled B-cell responses, such as a lack of anti-inflammatory IgA in the gastrointestinal tract and elevated IgE levels.[21] It was also shown that TGF-β in colostrum seems to prevent the development of atopic disease during exclusive breast-feeding and promotes specific IgA production in human subjects.[22]

Similar to TGF-β IL-10 is known to suppress T-cell proliferation either by acting directly on T lymphocytes or by interfering with T-cell–antigen-presenting cell interactions.[23,24] IL-10 initiates peripheral tolerance by blocking the CD28 costimulatory signal in T cells. Coprecipitation experiments revealed that on stimulation, CD28 and IL-10 receptor are physically associated in T cells.[25] Blocking of IL-10 enhances IL-4 and IL-13 secretion. In contrast to peripheral blood lymphocytes, activated T cells of the human intestinal lamina propria are high producers of IL-10.[26] It has been shown that chronic enterocolitis develops in IL-10 knockout mice.[27]

In the present study we were able to show that milk-specific mucosal T cells in children with milk-induced gastrointestinal diseases display a cytokine profile that promotes an allergic response but lacks the generation of immunosuppressive cytokines, such as IL-10 and TGF-β. These findings might explain the inflammatory changes seen in milk-induced gastrointestinal disorders.

We thank Scott Sicherer, MD, and Margret Magid, MD, for helpful discussions.

REFERENCES

1. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Allergy 1990;45:587-96.
2. Sampson HA, Sicherer SH, Birnbaum AH. AGA technical review on the evaluation of food allergy in gastrointestinal disorders. American Gastroenterological Association. Gastroenterology 2001;120:1026-40.
3. Sampson HA. Food allergy. Part 1: Immunopathogenesis and clinical disorders. J Allergy Clin Immunol 1999;103:717-28.
4. Kelly KJ, Lazenby AJ, Rowe PC, et al. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino-acid based formula. Gastroenterology 1995;109:1503-12.
5. Lee C, Changchieen C, Chen P, et al. Eosinophilic gastroenteritis: 10 years experience. Am J Gastroenterol 1993;88:70-4.
6. Prussin C. Cytokine flow cytometry: understanding cytokine biology at the single-cell level. J Clin Immunol 1997;17:195-204.
7. Jaffe J, James S, Mullins G, et al. Evidence for an abnormal profile of interleukin-4 (IL-4), IL-5, and gamma interferon in peripheral blood T cells from patients with allergic eosinophilic gastroenteritis. J Clin Immunol 1994;14:299-309.
8. Hogan SP, Mishra A, Brandt EB, et al. A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. Nat Immunol 2001;2:353-60.
9. Vandezande LM, Wallaert B, Desreumaux P, et al. Interleukin-5 immunoreactivity and mRNA expression in gut mucosa from patients with food allergy. Clin Exp Allergy 1999;29:652-9.
10. Desreumax P, Bloget F, Seguy D, et al. Interleukin 3, granulocyte-macrophage colony-stimulating factor, and interleukin 5 in eosinophilic gastroenteritis. Gastroenterology 1996;110:768-74.
11. Hamelmann E, Gelfand EW. Role of IL-5 in the development of allergen-induced airway hyperresponsiveness. Int Arch Allergy Immunol 1999; 120:8-16.
12. Wardlaw AJ. Molecular basis for selective eosinophil trafficking in asthma: a multistep paradigm. J Allergy Clin Immunol 1999;104:917-26.
13. De Vries JE. The role of IL-13 and its receptor in allergy and inflammatory response. J Allergy Immunol 1998;102:165-9.
14. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol 2000;105:399-408.
15. Hamid QA, Minshall EM. Molecular pathology of allergic disease I: lower airway disease. J Allergy Clin Immunol 2000;105:20-36.
16. Nagata S, McKenzie C, Pender SLF, et al. Human Peyer's patch T cells are sensitized to dietary antigen and display a Th cell type 1 cytokine profile. J Immunol 2000;165:5315-21.
17. Schade RP, Van Ieperen-Van Dijk AG, Van Reijsen FC, et al. Differences in antigen-specific T cell responses between infants with atopic dermatitis with and without cow's milk allergy: relevance of Th2 cytokines. J Allergy Clin Immunol 2000;106:1155-62.
18. Cerwenka A, Swain SL. TGF-beta1: immunosuppressant and viability factor for T lymphocytes. Microbes Infect 1999;1:1291-6.
19. Ludviksson BR, Seegers D, Resnick AS, Strober W. The effect of TGF-beta1 on immune responses of naïve versus memory CD4+ Th1/Th2 T cells. Eur J Immunol 2000;30:2101-11.
20. Strobl H, Knapp W. TGF-beta1 regulation of dentritic cells. Microbes Infect 1999;1:1283-90.
21. Van Ginkel FW, Wahl SM, Kearney JF, et al. Partial IgA-deficiency with increased Th2-type cytokines in TGF-beta 1 knockout mice. J Immunol 1999;163:1951-7.
22. Kalliomaki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clin Immunol 1999;104:1251-7.
23. De Waal Malefyt R, Haanen JBAG, Spits H, et al. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T-cell proliferation by diminishing the antigen-presenting capacity for monocytes via down-regulation of class II major histocompatibility complex expression. J Exp Med 1991;174:915-24.
24. De Waal Malefyt R, Yssel H, De Vries JE. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific inhibition of IL-2 production and proliferation. J Immunol 1993;150:4754-65.
25. Akdis CA, Blaser K. Mechanisms of Interleukin-10-mediated immune suppression. Immunology 2001;13:131-6.
26. Braunstein J, Qiao L, Autschbach F, Schurman G, Meuer S. T cells of the human intestinal lamina propria are high producers of interleukin-10. Gut 1997;41:215-20.
27. Kuehn, Loehler J, Rennick D, Rajewski K, Mueller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263-74.