J Gastroenterol
DOI 10.1007/s00535-012-0640-x

ORIGINAL ARTICLE—ALIMENTARY TRACT

Clinical characteristics of Japanese patients with eosinophilic esophagitis and eosinophilic gastroenteritis

Yoshikazu Kinoshita · Kenji Furuta · Norihisa Ishimaura · Shunji Ishihara · Shuichi Sato · Riruke Maruyama · Shuichi Ohara · Takayuki Matsumoto · Choitsu Sakamoto · Toshiyuki Matsui · Satoshi Ishikawa · Tsutomu Chiba

Received: 30 March 2012 / Accepted: 4 July 2012

Abstract
Background The clinical characteristics of Japanese patients with eosinophilic esophagitis (EoE) and eosinophilic gastroenteritis (EGE) have not been fully clarified. For understanding the pathogenesis as well as providing support for accurate diagnosis, precise information regarding clinical characteristics of these diseases is important.
Methods A questionnaire-based survey of EoE and EGE was conducted in 1,078 teaching hospitals. Clinical data of patients with confirmed EoE or EGE diagnosed from 2004 to 2009 were collected.
Result Clinical data from 26 patients with EoE and 144 patients with EGE were collected. The mean ages of patients in both groups were in the 40s. Those with EoE frequently complained of dysphagia and heartburn, and had characteristic endoscopic features such as longitudinal furrows and multiple concentric rings in the esophagus, while only 34 % had peripheral eosinophilia. Patients with EGE frequently complained of abdominal pain and diarrhea, and approximately 80 % of them have peripheral eosinophilia. They did not have characteristic endoscopic features helpful for diagnosis. Computed tomography (CT) findings and the presence of peripheral eosinophilia were diagnostic for EGE. EGE patients with a small intestinal involvement showed the highest peripheral eosinophil counts. Glucocorticoid administration was the most widely used treatment for these diseases and its effect was favorable for at least induction of remission.
Conclusion EGE is more prevalent than EoE in Japan. Patients with EGE have abdominal pain and diarrhea, high peripheral eosinophil counts, and gastrointestinal wall thickening identifiable by CT findings, while EoE is characterized by dysphagia and characteristic endoscopic features.

Electronic supplementary material The online version of this article (doi:10.1007/s00535-012-0640-x) contains supplementary material, which is available to authorized users.

Y. Kinoshita (✉) · K. Furuta · N. Ishimaura · S. Ishihara · S. Sato
Department of Gastroenterology and Hepatology,
Shimane University School of Medicine,
89-1, Enya, Izumo, Shimane 693-8501, Japan
e-mail: kinosita@med.shimane-u.ac.jp

R. Maruyama
Department of Pathology,
Shimane University School of Medicine, Izumo, Japan

S. Ohara
Department of Gastroenterology,
Tohoku Rosai Hospital, Sendai, Japan

T. Matsumoto
Department of Medicine and Clinical Science,
Graduate School of Medical Sciences,
Kyusyu University, Fukuoka, Japan

C. Sakamoto
Division of Gastroenterology,
Department of Internal Medicine,
Nippon Medical School, Tokyo, Japan

T. Matsui · S. Ishikawa
Department of Gastroenterology,
Fukuoka University Chikushi Hospital,
Fukuoka, Japan

T. Chiba
Department of Gastroenterology and Hepatology,
Graduate School of Medicine, Kyoto University,
Kyoto, Japan

Published online: 31 July 2012

Keywords Eosinophile · Inflammation · Dysphagia · Heartburn · Diarrhea · Computed tomography

Introduction

Eosinophilic esophagitis (EoE) and eosinophilic gastroenteritis (EGE), which are included in eosinophilic gastrointestinal disorders (EGIDs), are rare pathological conditions characterized by dense infiltration of eosinophils in esophago-gastro-intestinal mucosa [1, 2]. Motor, digestive, and sensory functions of the involved alimentary tract are severely damaged by chronic inflammation, mainly caused by eosinophils. When eosinophil infiltration is demonstrated only in the esophageal epithelial layer, the pathological condition is called EoE. On the other hand, when it is found in gastric and/or intestinal/colonic mucosa irrespective of esophageal involvement, it is called EGE. Common pathological conditions including exaggerated Th2 response to environmental and food allergens are considered to have important roles in these conditions [3–5]. Partly because of changing environmental factors and food antigens, and partly because of lower exposure to microorganisms that change from Th2 dominant individuals to Th1 dominant ones, the prevalence of EoE is rapidly increasing, especially in western countries [6–9]. The clinical characteristics of EoE patients in western countries have been extensively investigated [5, 10, 11], whereas those of EGE have not been fully clarified. In Japan, the prevalence and clinical characteristics of EoE and EGE have yet to be investigated in a large cohort, though we previously reported the prevalence of EoE in a smaller study [12]. A nationwide survey of EoE and EGE is important not only for understanding prevalence rates, characteristics, and commonly used therapies, but also for revealing possible clinical differences between Japanese and western patients with EoE and EGE.

We conducted a nationwide survey of EoE and EGE, and analyzed their clinical characteristics. We found similarities and differences for these two diseases, which may be important not only for the diagnosis but also for understanding their pathogeneses.

Materials and methods

We sent questionnaires to 1,078 teaching hospitals who were rated as high quality by the Japanese Society of Gastroenterology in 2010. The questionnaire contained queries concerning the clinical characteristics of patients with EoE and EGE diagnosed in each hospital from 2004 to 2009. The items surveyed in the questionnaire included age, sex, symptoms, laboratory data, radiologic/ultrasonographic examination findings, endoscopic examination findings, and treatments (Supplemental Figure).

Responses to the questionnaires were analyzed and evaluated by an independent analysis team (KF, NI, SI, SS). Cases with clinical characteristics that were appropriately described and had an appropriate diagnosis confirmed by histopathological findings were subjected to analysis. The diagnostic criteria employed were the same as those previously reported in the literatures [10, 13]. Proton pump inhibitor (PPI) responsive symptomatic patients with esophageal eosinophilic infiltration over 20 eosinophils/HPF (high poewr field) were also collected as EoE in this survey. The study protocol was approved by the ethics committee of Shimane University School of Medicine.

Statistical analyses were done using Stat View software. Chi-square tests and unpaired t tests were used as appropriate. When the data were not normally distributed, non-parametric tests were used. Values shown represent the mean ± SE or the median and ranges. $p < 0.05$ was considered to be statistically significant.

Results

Basic clinical characteristics

Twenty-six patients with EoE and 144 with EGE were identified in the questionnaire-based survey. Thus, we concluded that the prevalence of EGE is approximately 5.5 times higher than that of EoE in Japan. The male/female ratio for EGE was 1.2:1, while that for EoE was 3.3:1 with male preponderance. Both diseases were frequently observed in middle aged persons, with younger patients more frequently found to have EGE (Table 1; Fig. 1).

In both EoE and EGE cases, approximately half of the patients had a history of allergic diseases. Bronchial asthma was the most frequently observed allergic condition and was found in 23.1 and 27.8 % of the patients with EoE and EGE, respectively. Atopic dermatitis, food allergy, and allergic rhinitis were also frequently observed (Table 1).

In 26 EoE cases, 4 patients showed symptomatic improvement after PPI administration. There was no difference in clinical characteristics between PPI responsive and non-responsive cases.

Symptoms

Patients with EoE complained of dysphagia, heartburn, and other esophageal symptoms such as chest discomfort, throat discomfort, and regurgitation. Dysphagia was the most frequently observed symptom in 46 % of the patients. In contrast to findings in western countries [10, 14, 15], none of the present patients had a history of food

Table 1 Clinical characteristics of surveyed patients with EoE and EGE

	EoE	EGE
N	26 (including 4 PPI responsive cases)	144
M/F	20/6	78/66[a]
Age (years)	49 ± 3	46 ± 2
Presence of allergic disease (duplicate count)	50 %	46 %
Bronchial asthma	23.1 %	27.8 %
Atopic dermatitis	7.7 %	6.3 %
Food allergy	11.5 %	6.3 %
Allergic rhinitis	15.4 %	10.4 %
Symptoms (duplicate count)		
Dysphagia	46 %	–
Heartburn	8 %	–
Chest pain	–	15 %
Abdominal pain	–	53 %
Diarrhea	–	54 %
Others	50 %	42 %
GI wall thickening by CT	53 % (9 cases/17 investigated cases)	75 % (83 cases/111 investigated cases)
Endoscopic features (duplicates counted)		
Longitudinal furrows	35 %	–
White plaque	23 %	–
Concentric rings	19 %	–
Erythema	–	38 %
Edema	–	42 %
Erosion	–	43 %
Others	–	3 %
Normal	42 %	11 %
Involved GI tract (duplicates counted)		
Esophagus	100 %	9 %
Stomach	0 %	31 %
Small intestine	0 %	72 %
Colon	0 %	42 %
Ascites	0 %	56 % (48/85 investigated cases)

PPI proton pump inhibitor, *EoE* eosinophilic esophagitis, *EGE* eosinophilic gastroenteritis

[a] Significantly different from EoE ($p < 0.05$)

impaction. All patients with EoE had some symptoms that suggested the presence of esophageal disease.

Patients with EGE frequently complained of abdominal pain, diarrhea, and chest pain. Abdominal colic pain with diarrhea was the most frequent complaint, and over 40 % of the EGE patients had this type of symptom complex. There were no age-related or sex-related differences in regard to the reported symptoms.

Laboratory findings

Peripheral leukocyte counts, peripheral eosinophil counts, and CRP were routinely measured when a diagnosis of EoE or EGE was made (Table 2). Among the patients with EoE, 23.1 % had an elevated leukocyte count greater than 9,000/μl (Fig. 2), while the median and range for peripheral leukocyte count were 6,830 (4,400–21,400)/μl. Eosinophilia was defined as a peripheral eosinophil count over 600/μl according to the previous publication [16]. An elevated peripheral eosinophil count greater than 600/μl was found in 34.6 % of the patients with EoE, and the median and range for eosinophil count were 446 (162–8,774)/μl. The median CRP level in patients with EoE was 0.1 mg/dl.

In patients with EGE, peripheral leukocytes and eosinophils counts and plasma CRP level were all higher as compared to EoE, with 49.3 % of the EGE patients showing an elevated leukocyte count greater than 9,000/μl [median and range for all EGE cases, 8,970 (3,100–97,800)/μl]. Peripheral eosinophil count was elevated in 80.6 % of patients with EGE. The CRP level was also elevated over 2.0 mg/dl in 17.4 % of the patients with EGE.

In patients with EGE, involved lesions were most frequently found in the small intestine followed by the colon. Nine percentages of patients with EGE had esophageal lesions as well. The median peripheral eosinophil count in patients with esophago-gastric, small intestinal and colonic lesions was 1,462/μl, 2,656/μl, and 616/μl, respectively. Patients with small intestinal involvement had a significantly higher peripheral eosinophil count (Table 3; Fig. 3).

Radiological examination findings

Seventeen of 26 patients with EoE were examined using CT, and over 50 % of those demonstrated thickened esophageal walls. CT examinations were also performed in 77 % of the patients with EGE, and thickened gut walls were seen in 75 % of those cases. In addition, ascites were detected in approximately 56 % of the investigated patients with EGE. Except thickened esophago-gastrointestinal walls, no specific morphological feature that could be helpful for the diagnosis was found in the description of the radiological findings on the questionnaires.

Endoscopic findings

Endoscopic examinations were performed for all of the reported cases. Esophageal mucosal longitudinal furrows (35 %), white plaque (23 %), and multiple concentric rings (19 %) were frequently observed characteristic endoscopic features of EoE, as has also been reported in western countries [17, 18] (Table 1).

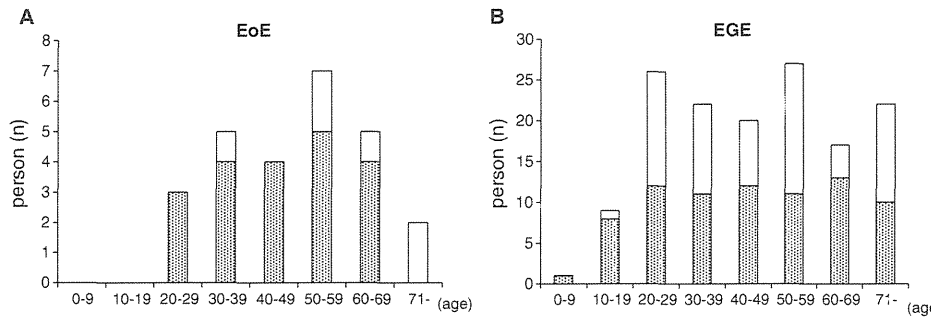

Fig. 1 Age distribution of surveyed patients with EoE (**a**) and EGE (**b**). *Open columns* represent female patients and *hatched columns* represent male patients. In addition to the middle-aged patients, younger patients with EGE were frequently found

Table 2 Laboratory data from surveyed cases

	EoE	EGE
CRP	0.1 (0.03–2.9) mg/dl	0.29 (0.0–18.0) mg/dl[a]
WBC	6,830 (4,400–21,400)/μl	8,970 (3,100–97,800)/μl[b]
Eosinophils	446 (162–8,774)/μl	2,130 (3–58,860)/μl[b]

Medians and ranges in parenthesis

EoE eosinophilic esophagitis, *EGE* eosinophilic gastroenteritis

[a] Significantly different from EoE ($p < 0.05$)

[b] Significantly different from EoE ($p < 0.01$)

Over 95 % of the patients with EGE had endoscopic abnormalities found on the mucosal surface of involved alimentary tract. The most frequently observed mucosal lesions were edema, erosion, and erythema, though no specific characteristic endoscopic findings for EGE was found.

Treatments and response

Oral glucocorticoid administration was employed in two thirds of the EoE patients, and all except one responded favorably. Oral glucocorticoid therapy was also used for almost all of the patients with EGE, and the responses were favorable, at least in short-term observations. A variety of anti-allergic drugs such as montelukast, epinastine, and suplatast tosilate was given with and without combined administration of glucocorticoids, although their therapeutic effects were not confirmed to be effective.

Discussion

In this report, the largest number of EGEs in the world literature and the largest number of EoEs in the Japanese literature were analyzed. This survey clarified the clinical characteristics of Japanese patients with EoE and EGE. Although they were similar to those reported in western countries [12, 19, 20], a remarkable difference was that the prevalence of EoE in Japan was lower than that of EGE.

The lower prevalence of EoE in Japan may be explained in part by the Japanese general practitioners' limited knowledge about EoE and also because of the difficulty in diagnosis, in addition to a true lower incidence of EoE in Japan [21]. Elevated CRP levels and peripheral leukocyte counts were found in fewer than 35 % of the analyzed cases of EoE. The finding of thickened esophageal walls detected by CT was similar to that reported in EoE patients from western countries [22–24]. Endoscopic findings in the present cases were also similar to western populations, with longitudinal furrows, white plaque, and multiple concentric rings frequently observed endoscopically as mucosal changes [18, 25, 26]. Importantly and similar to the western reports, 40 % of the surveyed patients had no esophageal mucosal lesions identified on endoscopic examinations [18]. The difference we found was older age of the Japanese patients with EoE as compared to western patients [18, 27, 28]. The similar characteristics of EoE between the present Japanese cases and those in western regions suggest

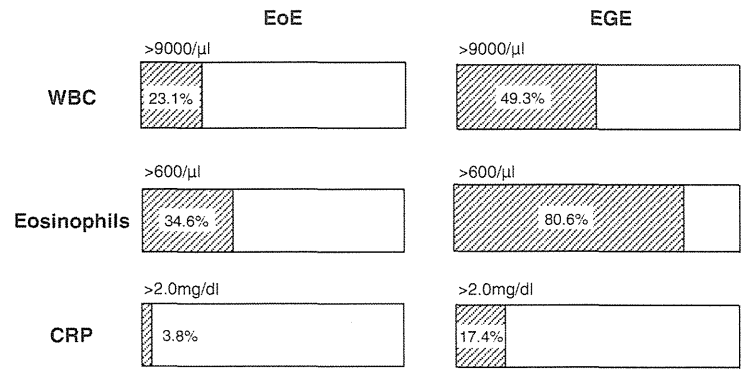

Fig. 2 Percentage of patients with EoE or EGE who showed abnormally elevated peripheral leukocyte, and eosinophil count or CRP level. *Hatched columns* represent the patients with abnormal elevation of peripheral leukocytes, eosinophils, or CRP level

Table 3 Clinical and laboratory data from EGE cases classified by involved organs

	EGE		
	Esophagus/stomach	Small intestine	Large intestine
N	19	104	23
M/F	10/9	59/45	9/14
Age	48 ± 4	47 ± 2	45 ± 4
Presence of allergic disease	47 %	45 %	44 %
Symptoms (duplicates counted)			
Chest pain	26 %	14 %	4 %
Abdominal pain	21 %	56 %[a]	65 %
Diarrhea	16 %	59 %[a]	61 %
Others	58 %	41 %	30 %
CRP (mg/dl) median (range)	0.30 (0.0–9.2)	0.29 (0.0–13.5)	0.10 (0.0–18.0)
WBC (/μl) median (range)	7,740 (3,100–21,780)	10,415 (4,200–97,800)[a, b]	6,900 (3,620–19,700)
Eosinophils (/μl) median (range)	1462 (117–15,202)	2,656 (3–58,860)[a, b]	616 (90–13,790)

Esophagus/stomach group includes cases with esophagus and/or stomach lesions but without small intestinal lesions

Small intestine group includes cases with small intestinal lesions

Large intestine group includes cases with large intestinal lesions but without small intestinal lesions

Two cases are included in both the esophagus/stomach and large intestine groups, as they had gastric and large intestinal lesions, but no small intestinal lesions

EGE eosinophilic gastroenteritis

[a] Significantly different from esophagus/stomach group ($p < 0.05$)

[b] Significantly different from large intestine group ($p < 0.05$)

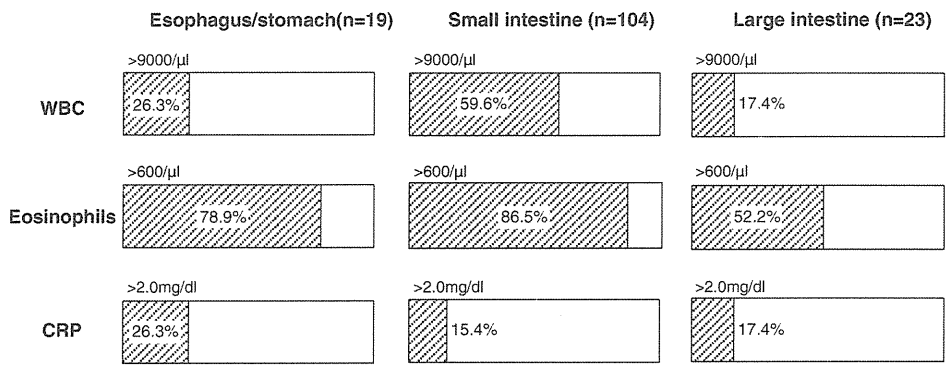

Fig. 3 Percentage of EGE patients with different involved site of gastrointestinal tracts who showed abnormally elevated peripheral leukocyte and eosinophil counts or CRP level. *Hatched columns* represent the patients with abnormal elevation of peripheral leukocytes, eosinophils, or CRP level

a common pathogenesis of EoE among ethnically different populations.

In this survey, we analyzed 144 cases of EGE, making this the largest of its kind reported worldwide. The characteristics of the Japanese patients with EGE were similar to those reported from other Asian countries [29]. Patients with EGE had several clinical characteristics similar to those with EoE, although there were more abnormalities in the laboratory data than in EoE patients. The reason why the abnormalities of peripheral eosinophil count and CRP level were more prominent in EGE than in EoE cases is not clear. One possible reason is the larger volume of the involved gastrointestinal tract in patients with EGE as compared to EoE. In addition, the specific involved lesion may be important for determining the peripheral eosinophil count and CRP level. In the present survey, the small intestine was the most frequently involved organ in patients with EGE, as also reported in western countries [13, 30]. The small intestine, which has abundant lymphatic tissues, is the most active alimentary tract participating in immune reactions [31]. Therefore, patients with small intestinal lesions may show stronger immunologic and inflammatory responses. Indeed, in patients without small intestinal involvement, the mean peripheral eosinophil count was less

than 9,000/μl, while in those with small intestinal lesions it was 14,209/μl (Table 3).

Approximately half of the surveyed patients with EoE had dysphagia, which may be caused by esophageal motor dysfunction or esophageal stenosis related to edema or fibrosis. Patients with EoE have been reported to have esophageal motor dysfunctions and decreased esophageal distensibility [32–35]. As for surveyed patients with EGE, more than 50 % complained of abdominal pain and/or diarrhea, as also noted in other surveys [16, 29, 36]. In patients with small intestinal and/or colonic involvement, abdominal pain and diarrhea were frequently observed, while in those with only esophago-gastric involvement, a minority reported abdominal pain or diarrhea.

For diagnosis of EoE, an endoscopic examination with a biopsy is considered to be useful, since affected patients frequently have characteristic endoscopic features such as longitudinal furrows and multiple concentric rings in the esophagus, which was clarified in the present survey and also reported in western populations [18]. Approximately 40 % of the present patients with EoE, however, had no characteristic endoscopic finding. Therefore, a biopsy is recommended for the evaluation of patients with PPI-resistant esophageal symptoms, even if they have no endoscopic abnormalities. Miller et al. [37] reported the good cost-effectiveness of this type of approach in the US. Since both the prevalence of EoE and cost of an endoscopic biopsy examination are lower in Japan, the cost-effectiveness of the diagnostic strategy with an endoscopic biopsy examination for PPI resistant patients should be re-examined in Japan.

In patients with EGE, an endoscopic examination can only detect non-specific mucosal lesions such as edema, erosion, and erythema. Therefore, the diagnostic value of an endoscopic examination is lower in EGE if an endoscopic biopsy is not performed. Unlike EoE, patients with EGE frequently have ascites and increased peripheral eosinophils. Therefore, for the diagnosis of EGE, a combination of laboratory tests and endoscopic/radiological image analysis is considered to be important. Of note, CT is especially useful, as over 70 % of the involved lesions were detected by such an examination in this survey.

For treatment of both EoE and EGE, systemic or topical glucocorticoid administration was employed as first-line treatment in nearly all the surveyed cases, as noted in the consensus recommendations [11]. The long-term effects of such treatment, especially after the initial induction treatment, have not been fully clarified, while the short-term remission induction effect was found to be adequate in our survey. Recently, the natural history of EGE was reported in a study of 43 patients, of whom 40 % experienced spontaneous disease resolution, while the clinical course was more complex and disease relapse was common despite the repeated glucocorticoid treatments in other cases [38]. The long-term clinical courses of patients with EoE and EGE were not surveyed; thus, a future nationwide cohort study will be necessary to clarify the long-term clinical course in Japanese patients.

This study has several limitations especially in the study design. The questionnaire we sent has several descriptive questions in it. Therefore, the quality and quantity of the information collected strongly depend on the attending gastroenterologists. In addition, for the diagnosis of EoE and EGE, other diseases that may cause esophago-gastrointestinal eosinophil infiltration need to be ruled out. This diagnostic process also depends on the attending gastroenterologists. These uncertainties and the possible inhomogeneity of the diagnostic process may limit the value of this survey. Diagnosing EGE may have an additional difficulty. Because of the resident eosinophils present in normal gastrointestinal mucosa, the histological diagnosis of EGE is not easy without the defined cutoff value of infiltrating eosinophils. Therefore, based on the results of this survey, the establishment of diagnostic criteria containing the cutoff value of the number of tissue eosinophils especially for EGE is considered important. The third limitation of this survey is the lack of a maximum eosinophil infiltration number in different parts of the gastrointestinal tract of the patients with EGE. When the survey was conducted, the necessary maximum number of eosinophils infiltrating the gastrointestinal tract was not described in the diagnostic criteria. Therefore, in future studies, we need to investigate the number of infiltrating eosinophils and to compare it to the patients' clinical characteristics.

In summary, we surveyed the clinical characteristics of Japanese patients with EoE or EGE. EGE was more prevalent in Japan. EGE patients were typified by abdominal pain and diarrhea symptoms, high peripheral eosinophil counts, and gastrointestinal wall thickening, identifiable in CT findings. EoE was characterized by dysphasic symptoms and characteristic endoscopic features.

Acknowledgments The authors thank the Japanese gastroenterologists who sent clinical and laboratory data of their patients with EoE and EGE. This study was supported by a grant from the Ministry of Health, Labour, and Welfare of Japan.

Conflict of interest We have no COI with any commercial groups concerning the manuscript. We, the authors of this manuscript, indicate that we have no financial relationship to any commercial groups. We also state that we have full control of all primary data and that we agree to allow the journal to review our data if requested.

References

1. Straumann A. Idiopathic eosinophilic gastrointestinal diseases in adults. Best Pract Res Clin Gastroenterol. 2008;22:481–96.

2. Spergel JM, Book WM, Mays E, et al. Variation in prevalence, diagnostic criteria, and initial management options for eosinophilic gastrointestinal diseases in the United States. J Pediatr Gastroenterol Nutr. 2011;52:300–6.
3. Yan BM, Shaffer EA. Primary eosinophilic disorders of the gastrointestinal tract. Gut. 2009;58:721–32.
4. Prussin C, Lee J, Foster B. Eosinophilic gastrointestinal disease and peanut allergy are alternatively associated with IL-5+ and IL-5(−) T(H)2 responses. J Allergy Clin Immunol. 2009;124:1326–32.
5. Rothenberg ME. Biology and treatment of eosinophilic esophagitis. Gastroenterology. 2009;137:1238–49.
6. Dellon ES, Peery AF, Shaheen NJ, et al. Inverse association of esophageal eosinophilia with Helicobacter pylori based on analysis of a US pathology database. Gastroenterology. 2011;141: 1586–92.
7. Dellon ES, Gibbs WB, Fritchie KJ, et al. Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. Clin Gastroenterol Hepatol. 2009;7:1305–13.
8. Liacouras CA, Spergel JM, Ruchelli E, et al. Eosinophilic esophagitis: a 10-year experience in 381 children. Clin Gastroenterol Hepatol. 2005;3:1198–206.
9. Prasad GA, Alexander JA, Schleck CD, et al. Epidemiology of eosinophilic esophagitis over three decades in Olmsted County. Minnesota. Clin Gastroenterol Hepatol. 2009;7:1055–61.
10. Furuta GT, Liacouras CA, Collins MH, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133:1342–63.
11. Liacouras CA, Furuta GT, Hirano I, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol. 2011;128:3–20.
12. Fujishiro H, Amano Y, Kushiyama Y, et al. Eosinophilic esophagitis investigated by upper gastrointestinal endoscopy in Japanese patients. J Gastroenterol. 2011;46:1142–4.
13. Talley NJ, Shorter RG, Phillips SF, et al. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. Gut. 1990;31:54–8.
14. Sperry SL, Crockett SD, Miller CB, et al. Esophageal foreign-body impactions: epidemiology, time trends, and the impact of the increasing prevalence of eosinophilic esophagitis. Gastrointest Endosc. 2011;74:985–91.
15. Desai TK, Stecevic V, Chang CH, et al. Association of eosinophilic inflammation with esophageal food impaction in adults. Gastrointest Endosc. 2005;61:795–801.
16. Méndez-Sánchez N, Chávez-Tapia NC, Vazquez-Elizondo G, et al. Eosinophilic gastroenteritis: a review. Dig Dis Sci. 2007;52: 2904–11.
17. Veerappan GR, Perry JL, Duncan TJ, et al. Prevalence of eosinophilic esophagitis in an adult population undergoing upper endoscopy: a prospective study. Clin Gastroenterol Hepatol. 2009;7:420–6.
18. Müller S, Pühl S, Vieth M, et al. Analysis of symptoms and endoscopic findings in 117 patients with histological diagnoses of eosinophilic esophagitis. Endoscopy. 2007;39:339–44.
19. Abe Y, Iijima K, Ohara S, et al. A Japanese case series of 12 patients with esophageal eosinophilia. J Gastroenterol. 2011;46: 25–30.
20. Furuta K, Adachi K, Kowari K, et al. A Japanese case of eosinophilic esophagitis. J Gastroenterol. 2006;41:706–10.
21. Straumann A, Spichtin HP, Bernoulli R, et al. Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings. Schweiz Med Wochenschr. 1994;124:1419–29.
22. Straumann A. Eosinophilic esophagitis; a rapidly emerging disease. Esophagus. 2008;5:177–83.
23. Straumann A, Conus S, Degen L, et al. Long-term budesonide maintenance treatment is partially effective for patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2011;9: 400–9.
24. Fox VL, Nurko S, Teitelbaum JE, et al. High-resolution EUS in children with eosinophilic "allergic" esophagitis. Gastrointest Endosc. 2003;57:30–6.
25. Collins MH, Blanchard C, Abonia JP, et al. Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. Clin Gastroenterol Hepatol. 2008;6:621–9.
26. Dellon ES, Gibbs WB, Rubinas TC, et al. Esophageal dilation in eosinophilic esophagitis: safety and predictors of clinical response and complications. Gastrointest Endosc. 2010;71:706–12.
27. Sgouros SN, Bergele C, Mantides A. Eosinophilic esophagitis in adults: what is the clinical significance? Endoscopy. 2006;38: 515–20.
28. García-Compeán D, González González JA, Marrufo García CA, et al. Prevalence of eosinophilic esophagitis in patients with refractory gastroesophageal reflux disease symptoms: a prospective study. Dig Liver Dis. 2011;43:204–8.
29. Zhang L, Duan L, Ding S, et al. Eosinophilic gastroenteritis: clinical manifestations and morphological characteristics, a retrospective study of 42 patients. Scand J Gastroenterol. 2011;46: 1074–80.
30. Khan S. Eosinophilic gastroenteritis. Best Pract Res Clin Gastroenterol. 2005;19:177–98.
31. Rothenberg ME, Mishra A, Brandt EB, et al. Gastrointestinal eosinophils. Immunol Rev. 2001;179:139–55.
32. Savarino E, Gemignani L, Zentilin P, et al. Achalasia with dense eosinophilic infiltrate responds to steroid therapy. Clin Gastroenterol Hepatol. 2011;9:1104–6.
33. Aceves SS, Chen D, Newbury RO, et al. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF-β1, and increase esophageal smooth muscle contraction. J Allergy Clin Immunol. 2010;126:1198–204.
34. Korsapati H, Babaei A, Bhargava V, et al. Dysfunction of the longitudinal muscles of the oesophagus in eosinophilic oesophagitis. Gut. 2009;58:1056–62.
35. Kwiatek MA, Hirano I, Kahrilas PJ, et al. Mechanical properties of the esophagus in eosinophilic esophagitis. Gastroenterology. 2011;140:82–90.
36. Oh HE, Chetty R. Eosinophilic gastroenteritis: a review. J Gastroenterol. 2008;43:741–50.
37. Miller SM, Goldstein JL, Gerson LB. Cost-effectiveness model of endoscopic biopsy for eosinophilic esophagitis in patients with refractory GERD. Am J Gastroenterol. 2011;106:1439–45.
38. de Chambrun GP, Gonzalez F, Canva JY, et al. Natural history of eosinophilic gastroenteritis. Clin Gastroenterol Hepatol. 2011;9: 950–6.

2. Liu CH, Wu KH, Lin TY, Wei CC, Lin CY, Chen XX, et al. Wiskott-Aldrich syndrome with IgA nephropathy: a case report and literature review. Int Urol Nephrol 2012 [Epub ahead of print].
3. Matsukura H, Kanegane H, Miya K, Ohtsubo K, Higuchi A, Tanizawa T, et al. IgA nephropathy associated with X-linked thrombocytopenia. Am J Kidney Dis 2004; 43:e7-12.
4. Tomana M, Novak J, Julian BA, Matousovic K, Konecny K, Mestecky J. Circulating immune complexes in IgAN consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. J Clin Invest 1999;104:73-81.
5. Shimizu M, Nikolov NP, Ueno K, Ohta K, Siegel RM, Yachie A, et al. Development of IgA nephropathy-like glomerulonephritis associated with Wiskott–Aldrich syndrome protein deficiency. Clin Immunol 2012;142:160-6.
6. Lasseur C, Allen AC, Deminière C, Aparicio M, Feehally J, Combe C. Henoch-Schöenlein purpura with immunoglobulin A nephropathy and abnormal IgA in Wiskott-Aldrich syndrome carrier. Am J Kidney Dis 1997;29:285-7.
7. Moldoveanu Z, Wyatt RJ, Lee JY, Tomana M, Julian BA, Mestecky J, et al. Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. Kidney Int 2007;71:1148-54.
8. Higgins EA, Siminovitch KA, Zhuang DL, Brockhausen I, Dennis JW. Aberrant O-linked oligosaccharide biosynthesis in lymphocytes and platelets from patients with the Wiskott-Aldrich syndrome. J Biol Chem 1991;266:6280-90.
9. Nguyen DD, Maillard MH, Cotta-de-Almeida V, Mizoguchiv E, Klein C, Fuss I, et al. Lymphocyte-dependent and Th2 cytokine-associated colitis in mice deficient in Wiskott-Aldrich syndrome protein. Gastroenterology 2007;133:1188-97.

Available online October 31, 2012.
http://dx.doi.org/10.1016/j.jaci.2012.08.040

Antigen-specific T-cell responses in patients with non–IgE-mediated gastrointestinal food allergy are predominantly skewed to T_H2

To the Editor:

IgE-mediated allergy is triggered by cross-linking of antigen-specific IgE antibodies on the cell surfaces of mast cells and basophils, followed by local accumulation and activation of inflammatory cells, including eosinophils and T_H2 cells. T_H2 cells produce such cytokines as IL-4, IL-5, and IL-13, which promote IgE production and eosinophilopoiesis and play central roles in the development of chronic allergic inflammation. On the other hand, non–IgE-mediated allergies, such as hypersensitivity pneumonitis, are considered mediated by cellular immunity, which has not been thought to involve antigen-specific T_H2 cells because IgE antibody would be detected if T_H2 cells were activated. Non–IgE-mediated gastrointestinal food allergies include food protein–induced enterocolitis syndrome (FPIES), food protein–induced proctocolitis, and food protein–induced enteropathy. The precise underlying mechanisms are almost unknown, except for a fundamental role of TNF-α,[1] presumably because this disease entity is relatively rare in incidence and is encountered during infancy in human subjects but not seen in experimental animals. Here, for the first time, we were able to detect antigen-specific T_H2 cell responses in infants with non–IgE-mediated gastrointestinal food allergies by analyzing 89 blood samples collected from all over Japan.

The antigen-specific lymphocyte stimulation test is a classic method for investigating antigen-specific T-cell proliferation and theoretically should be applicable to the study of gastrointestinal food allergies. However, a couple of previous studies demonstrated that the antigen-specific lymphocyte stimulation test was useful, whereas another study found no such usefulness.[2] We hypothesized that this controversy was due to contamination of the antigen preparations with LPS and tested this hypothesis. The limulus amebocyte lysate assay detected high concentrations of LPS in commercially available milk protein preparations, as previously reported (see Table E1 in this article's Online Repository at www.jacionline.org).[3] In addition, significant lymphoproliferative responses were found in the presence of as little as 10 pg/mL LPS (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org), and PBMCs from younger children showed more pronounced lymphoproliferation in response to LPS (see Fig E1, *B*). Therefore we attempted to remove contaminating LPS from milk protein preparations by passing them through a prepacked endotoxin affinity column. However, a high LPS concentration was detected even after that treatment (see Table E1), and therefore we obtained a special β-lactoglobulin preparation with very low contaminating LPS levels (kindly provided by Bean Stalk Snow, Tokyo, Japan). Further studies were performed by using these milk protein preparations, which contained LPS at a final concentration of less than 5 pg/mL.

Next, to elucidate what types of antigen-specific immune responses are induced in patients with gastrointestinal food allergies, we cultured PBMCs from patients and control subjects in the presence and absence of LPS-depleted milk component proteins. The study enrolled 65 patients with gastrointestinal food allergies, 12 patients with IgE-mediated cow's milk allergy (CMA) who showed only nongastrointestinal symptoms on ingestion of milk, and 12 control subjects who showed absolutely no symptoms on ingestion of milk. Table I[4] summarizes the clinical symptoms, clinical diagnosis, and demographic data for the 2 patient groups. None of the patients with gastrointestinal food allergies had detectable levels of IgE against milk proteins in sera. We were unable to recruit infants with IgE-mediated CMA who were age matched with the infants with non–IgE-mediated

TABLE I. Demographic characteristics of the patients

	IgE-mediated CMA		Gastrointestinal food allergies	
	No.		No.	
Age (mo)	12	38.0 (26.5-60.0)	65	2.0 (1.0-4.0)
Male/female sex	12	7/5	65	40/25
Day of onset	12	—	65	32.5 (7.0-115.5)
Symptoms at onset				
Vomiting	12	0% (0/12)	65	53.8% (35/65)
Bloody stool	12	0% (0/12)	65	47.7% (31/65)
Diarrhea	12	0% (0/12)	65	47.7% (31/65)
Failure to thrive	12	0% (0/12)	65	38.4% (22/65)
Lethargy	12	0% (0/12)	65	38.4% (22/65)
Fever	12	0% (0/12)	65	18.5% (12/65)
Eczema	12	100% (12/12)	65	7.7% (5/65)
Wheeze	12	33.3% (3/12)	65	0% (0/65)
Laboratory data				
Milk-specific IgE (IU/mL)	12	56.95 (11.74-90.8)	65	<0.34 (<0.34)
Peripheral blood eosinophils (%)		Not examined	53	7.7 (3.6-13.5)

Data are expressed as medians (interquartile ranges). The inclusion criteria were as follows: (1) gastrointestinal symptoms were present more than 2 hours after ingestion of milk and (2) 3 of Powell's criteria were fulfilled,[4] including (a) switch to therapeutic milk leading to resolution of symptoms, (b) differential diagnosis from other disorders, and (c) verified body weight gain. A definitive diagnosis based on the results of oral food challenge tests that were performed after complete resolution of the initial symptoms was achieved in 19 patients. Patients with gastrointestinal symptoms within 2 hours after ingestion of milk were excluded. On the basis of such symptoms as vomiting, diarrhea, and failure to thrive, the patient group (n = 65) consists of 34 patients with FPIES, 4 patients with food protein–induced enteropathy syndrome (enteropathy), and 27 patients with food protein–induced proctocolitis syndrome (proctocolitis). A definitive diagnosis based on the results of oral food challenge tests was achieved in 13 and 6 patients with FPIES and proctocolitis, respectively. None of the patients underwent endoscopic biopsy.

TABLE II. Antigen-specific lymphoproliferation and cytokine production profiles in patients with gastrointestinal food allergies, patients with IgE-mediated allergy, and control subjects

	Control subjects		IgE-mediated CMA		Gastrointestinal food allergies			
	No.		No.		No.		*P* value†	*P* value‡
Proliferation (SI)*	20	1.290 (0.830-1.738)	9	3.077 (2.484-3.492)	65	2.894 (2.004-7.147)	<.01	<.001
Cytokine (pg/mL)								
TNF-α	12	74.69 (58.44-144.8)	10	77.78 (58.04-141.4)	65	241.0 (89.21-729.6)	NS	<.05
IL-6	12	79.24 (36.36-193.8)	10	337.9 (57.43-1021)	65	1151 (157.0-4802)	NS	<.01
IL-1β	11	26.02 (6.880-46.47)	10	27.49 (6.548-65.04)	64	48.75 (11.7-136.1)	NS	NS
IL-2	12	4.15 (0.0-10.04)	10	12.31 (7.23-17.58)	58	16.32 (7.760-39.49)	NS	<.01
IL-3	12	0.0 (0.0-0.38)	10	0.40 (0.0-3.61)	62	4.22 (0.0-29.49)	NS	<.05
IL-4§	12	5.365 (2.895-6.358)	10	3.795 (2.033-7.788)	65	5.670 (2.775-12.06)	NS	NS
IL-5	12	2.080 (0.0-19.56)	10	46.59 (4.663-173.5)	65	63.66 (7.360-310.4)	NS	<.01
IL-10	12	9.285 (3.075-15.71)	10	56.17 (18.74-76.91)	65	57.92 (12.61-198.8)	NS	<.05
IL-13	12	21.61 (0.270-65.04)	10	82.56 (16.28-555.3)	65	291.7 (22.10-1417)	NS	<.01
IFN-γ	11	3.910 (0.0-67.06)	10	31.91 (3.635-102.0)	65	71.86 (5.49-303.4)	NS	NS
IL-17	12	0.0 (0.0-2.350)	10	7.635 (1.710-39.63)	65	7.150 (0.0-17.83)	NS	NS

PBMCs from each patient were stimulated separately with each of 5 different milk protein preparations, and the data show the highest concentration of each cytokine detected in response to the 5 different stimuli. Data are expressed as medians (interquartile ranges).

*The stimulation index *(SI)* was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).

†Nonparametric test to compare control subjects and patients with IgE-mediated CMA.

‡Nonparametric test to compare control subjects and patients with gastrointestinal food allergies.

§According to the standard curve, the minimal detection limit was 5.88 pg/mL.

gastrointestinal food allergies. This study was approved by regional ethics committees, and written informed consent was obtained from the guardians of all patients and control subjects.

The details of the lymphoproliferation test and cytokine production assay are described in the Methods section in this article's Online Repository at www.jacionline.org. In brief, PBMCs from heparinized peripheral blood were suspended at a cell density of 1×10^6/mL in AIM-V medium (Gibco, Grand Island, NY) without serum. Lymphoproliferation was measured by using tritiated thymidine uptake during a 16-hour period after a 5-day stimulation with 100 μg/mL of each LPS-depleted milk protein preparation (α-lactalbumin, β-lactoglobulin, and α-, β- and κ-caseins). PBMCs were suspended at 1×10^6/mL in RPMI 1640 medium supplemented with 5% autologous plasma to investigate the antigen-specific cytokine production profiles. Culture supernatants were harvested at day 6 after stimulation with 100 μg/mL of each LPS-depleted milk protein preparation, and the cytokine production profiles were investigated by using the Luminex multiplex cytokine analysis kits (Millipore, Bedford, Mass) and ELISA (R&D Systems, Minneapolis, Minn).

In the first series of experiments, we investigated milk protein–specific lymphoproliferation in the control subjects, patients with IgE-mediated CMA, and patients with gastrointestinal food allergies. The lymphoproliferation level was similar in the patients with IgE-mediated CMA and those with gastrointestinal food allergies. Unlike in previous studies, however, the control subjects showed almost no proliferation (Table II). We presume that this was due to the extensive depletion of LPS contaminating the antigen preparations and the use of serum-free medium.

In the next experiments we investigated the cytokine production profiles in these subjects. TNF-α concentrations in the culture supernatants of milk protein–stimulated PBMCs from patients with gastrointestinal food allergies were significantly greater than those seen in patients with IgE-mediated CMA or control subjects. However, TNF-α levels in supernatants from patients with IgE-mediated CMA and control subjects were similar (Table II). Significantly higher concentrations of another proinflammatory cytokine, IL-6, were also seen only in the patients with gastrointestinal food allergies.

The concentrations of 3 T_H2 cytokines, IL-3, IL-5, and IL-13, in the supernatants of milk protein–stimulated PBMCs from patients with IgE-mediated CMA tended to be higher than those in the control subjects, but the differences did not reach statistical significance. In contrast, statistically significant and much higher concentrations of these T_H2 cytokines were found for the patients with gastrointestinal food allergies. Another T_H2 cytokine, IL-4, was undetectable in almost all subjects, and there were no differences among the 3 groups.

Concentrations of the T_H1 cytokine IFN-γ and the T_H17 cytokine IL-17 did not show statistically significant differences between any 2 groups.

The milk component that caused the most prominent tritiated thymidine uptake or the most prominent IL-2 or TNF-α production varied among the patients (see Fig E3 in this article's Online Repository at www.jacionline.org), suggesting that the lymphoproliferation and cytokine production observed in these assays were indeed antigen specific. In addition, the IL-5 concentration in the culture supernatant of cow's milk protein–stimulated PBMCs from patients with gastrointestinal food allergies correlated significantly with the peripheral blood eosinophil ratio at disease onset (see Fig E4 in this article's Online Repository at www.jacionline.org), suggesting that our *in vitro* assay reflects the *in vivo* conditions in these patients.

Collectively, T_H2 cytokines, including IL-3, IL-5, and IL-13, but not the T_H1 cytokine IFN-γ or the T_H17 cytokine IL-17 were significantly produced *in vitro* by milk protein–stimulated PBMCs from patients with gastrointestinal food allergies. The findings that tritiated thymidine uptake correlated significantly with IL-13 production (data not shown) along with the absence of milk-specific IgE antibody strongly suggest that the IL-13 detected in our assay was not produced by basophils in the PBMC fraction. IL-13 is a well-established mediator of intestinal

epithelial cell damage in patients with injuries and inflammatory diseases through activation of the tumor necrosis factor-like weak inducer of apoptosis-fibroblast growth factor-inducible molecule 14 (TWEAK-Fn14) axis.[5] Thus in addition to the previously known TNF-α, IL-13 might play a crucial role in the pathogenesis of gastrointestinal food allergies.

In conclusion, antigen-specific T-cell responses in patients with non–IgE-mediated gastrointestinal food allergy are predominantly skewed to T_H2. It remains unclear why antigen-specific IgE antibodies were not detected in these patients. Possible explanations are that neonatal B cells scarcely express IL-4/IL-13 receptors[6] or that production of IgE antibodies had just started but was still undetectable. This question warrants further study.

We express our sincere gratitude to all the members of the Japanese Research Group for Neonatal Infantile Allergic Disorders. We also thank all the doctors, nurses, and technicians, especially Ms Nao Aida from the Division of Allergy, Gastroenterology, Pathology, Surgery, Interdisciplinary Medicine and Neonatology of the National Center for Child Health and Development, for their hard work and valuable comments. We also thank Professor Mitsuaki Kimura (Department of Allergy and Clinical Immunology, Shizuoka Children's Hospital) for his valuable suggestions.

Hideaki Morita, MD, PhD[a,b]
Ichiro Nomura, MD, PhD[a,c]
Kanami Orihara, PhD[a]
Koichi Yoshida, MD[c,d]
Akira Akasawa, MD, PhD[c,d]
Hiroshi Tachimoto, MD, PhD[e]
Yoshikazu Ohtsuka, MD, PhD[f]
Yoshiyuki Namai, MD[g]
Masaki Futamura, MD, PhD[c]
Tetsuo Shoda, MD[a,c]
Akio Matsuda, PhD[a]
Norio Kamemura, MSc[h]
Hiroshi Kido, MD, PhD[h]
Takao Takahashi, MD, PhD[b]
Yukihiro Ohya, MD, PhD[c]
Hirohisa Saito, MD, PhD[a]
Kenji Matsumoto, MD, PhD[a]

From [a]the Department of Allergy and Immunology, National Research Institute for Child Health and Development, Tokyo, Japan; [b]the Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; [c]the Division of Allergy, National Center for Child Health and Development, Tokyo, Japan; [d]the Department of Allergy, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan; [e]the Department of Pediatrics, Jikei University School of Medicine, Tokyo, Japan; [f]the Department of Pediatrics and Adolescence Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan; [g]the Department of Pediatrics, Ohta Nishinouchi Hospital, Koriyama, Japan; and [h]the Division of Enzyme Chemistry, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan. E-mail: nomura-i@ncchd.go.jp, matsumoto-k@ncchd.go.jp.

Supported in part by Health and Labour Sciences Research Grants, Research on Intractable Diseases from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid for Clinical Research from the National Hospital Organization of Japan.

Disclosure of potential conflict of interest: I. Nomura has received support from the Ministry of Health, Labor, and Welfare. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Chung HL, Hwang JB, Park JJ, Kim SG. Expression of transforming growth factor beta1, transforming growth factor type I and II receptors, and TNF-alpha in the mucosa of the small intestine in infants with food protein-induced enterocolitis syndrome. J Allergy Clin Immunol 2002;109:150-4.
2. Giavi S, Megremis S, Papadopoulos NG. Lymphocyte stimulation test for the diagnosis of non-IgE-mediated cow's milk allergy: a step closer to a noninvasive diagnostic tool? Int Arch Allergy Immunol 2012;157:1-2.
3. Brix S, Bovetto L, Fritsché R, Barkholt V, Frøkiaer H. Immunostimulatory potential of beta-lactoglobulin preparations: effects caused by endotoxin contamination. J Allergy Clin Immunol 2003;112:1216-22.
4. Powell GK. Food protein-induced enterocolitis of infancy: differential diagnosis and management. Compr Ther 1986;12:28-37.
5. Kawashima R, Kawamura YI, Oshio T, Son A, Yamazaki M, Hagiwara T, et al. Interleukin-13 damages intestinal mucosa via TWEAK and Fn14 in mice—a pathway associated with ulcerative colitis. Gastroenterology 2011;141:2119-29.
6. Tian C, Kron GK, Dischert KM, Higginbotham JN, Crowe JE Jr. Low expression of the interleukin (IL)-4 receptor alpha chain and reduced signalling via the IL-4 receptor complex in human neonatal B cells. Immunology 2006;119:54-62.

Available online October 16, 2012.
http://dx.doi.org/10.1016/j.jaci.2012.09.005

Forkhead box protein 3 (*FOXP3*) hypermethylation is associated with diesel exhaust exposure and risk for childhood asthma

To the Editor:

Traffic-related air pollutants, such as diesel exhaust particles (DEP), significantly contribute to the pathogenesis of wheezing and asthma in early childhood.[1] These illnesses are characterized by chronic airway inflammation caused by a dysregulated immune system.[2] Attention has recently been directed toward regulatory T (Treg) cells because they are important in suppressing immune responses against nonspecific stimuli,[3] such as DEP. The suppressive phenotype of Treg cells is conferred by stable expression of forkhead box protein 3 (*FOXP3*).[3] Transcriptional silencing of *FOXP3* through hypermethylation of CpG islands in the promoter and intronic regions has been identified as a hallmark of committed Treg cells and human diseases, including asthma.[3,4] As such, Nadeau et al[4] reported increased *FOXP3* hypermethylation in blood DNA to be associated with diminished Treg cell function and increased asthma severity in children exposed to polycyclic aromatic hydrocarbons, a component of DEP. In this study we test the novel hypothesis that early (birth) and consistent exposure to high levels of traffic pollution alters *FOXP3* methylation status in DNA from saliva in a manner that correlates with DEP exposure, predicts wheezing/asthma in later life, or both. The oral cavity provides an important first line of defense against DEP exposure for children because mouth breathing is a common path of exposure.[5] Furthermore, other aerodigestive track tissues, such as buccal cells, have been successful in characterizing DNA methylation with respect to air pollutants and airway inflammation.[6,7] The ancillary goal is to establish a noninvasive, high-throughput, and quantitative assay for measuring risk of asthma linked to traffic-related air pollution.

TABLE I. Distribution of *FOXP3* percentage methylation in the sample population stratified by respiratory outcomes

Description	Mean	Minimum	Maximum	SD
Study sample	21.30	0.00	62.40	17.40
Wheezing phenotype				
Nonwheezers	17.00	0.00	55.10	16.20
Persistent wheezers*	35.84	18.10	58.35	15.20
Early transient wheezers*	24.16	0.32	61.30	17.90
Asthma status				
Nonasthmatic	19.50	0.00	62.40	16.90
Asthmatic†	32.70	1.10	58.40	17.90

*Significant difference between persistent wheezers ($P < .01$) and early transient wheezers ($P < .05$) compared with nonwheezers.
†Significant difference between asthmatic and nonasthmatic children ($P < .05$).

METHODS

Heparinized blood samples were stored at room temperature and transferred to the National Research Institute for Child Health and Development in Tokyo. The following procedures were performed no later than 24 hours after phlebotomy. PBMCs were obtained from peripheral blood by using Ficoll-Hypaque gradient sedimentation (Lymphocyte Separation Medium; ICN Biochemicals, Aurora, Ohio). The viability determined by using trypan blue dye exclusion (Sigma, St Louis, Mo) always exceeded 95%. PBMCs were suspended at a cell density of 1×10^6/mL in AIM-V medium (Gibco) without serum for lymphoproliferation, and in RPMI 1640 medium (GIBCO/Life Technologies, Gaithersburg, Md) in the presence of 5% autologous plasma for cytokine production assays.

Lymphoproliferation was measured based on tritiated thymidine (Amersham, Tokyo, Japan) uptake during a 16-hour period after 5 days of stimulation with 100 μg/mL of each LPS-depleted milk protein preparation (α-lactalbumin, Sigma; β-lactoglobulin, Bean Stalk Snow; and α-, β-, and κ-caseins, Sigma) at 37°C in a humidified 5% CO_2 atmosphere. Incorporated tritiated thymidine was counted with a liquid scintillation counter (TopCount NXT; PerkinElmer Life Sciences, Boston, Mass). The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).

Culture supernatants were harvested at day 6, and the cytokine production profiles were investigated by using Luminex multiplex cytokine analysis kits (Millipore) and ELISA (R&D Systems).

The lymphoproliferation assays and cytokine production assays were performed in duplicates and triplicates, respectively.

There was a significant positive correlation between the IL-2 concentration in the PBMC culture supernatant and lymphoproliferation (stimulation index) after stimulation with κ-casein ($r = 0.269$, $P = .025$; see Fig E2). Similar tendencies were also found when PBMCs were stimulated with other milk protein preparations (data not shown).

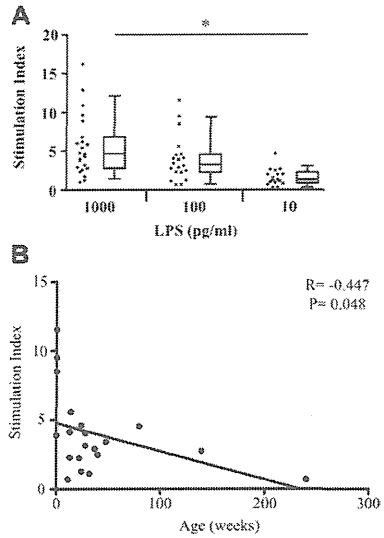

FIG E1. A, LPS at as little as 10 pg/mL can induce lymphoproliferation. PBMCs from young children (n = 60, 0-60 months of age) were stimulated with various concentrations of LPS (Sigma) for 5 days. Lymphoproliferation was measured by using tritiated thymidine uptake. The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm). *$P < .05$. **B,** LPS-induced lymphoproliferation was inversely associated with age. PBMCs from young children (n = 21, 0-240 weeks of age) were stimulated with 100 pg/mL LPS (Sigma) for 5 days. Lymphoproliferation was measured by using tritiated thymidine uptake. The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm).

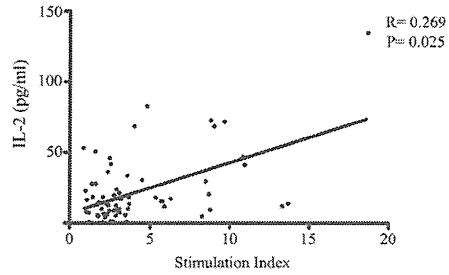

FIG E2. IL-2 concentrations in culture supernatant of cow's milk protein–stimulated PBMCs correlated significantly with antigen-specific lymphoproliferation. PBMCs from children with gastrointestinal food allergies were stimulated separately with 100 μg/mL of each of 5 LPS-depleted milk protein preparations in the absence of serum for the antigen-specific lymphoproliferation assay and in the presence of 5% autologous plasma for the IL-2 production assay. The stimulation index was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm), and the highest stimulation index shown among the 5 tested protein preparations was used as that patient's data in the plot. Even under slightly different culture conditions, antigen-specific lymphoproliferation and antigen-specific IL-2 production were significantly correlated ($r = 0.269$, $P = .025$).

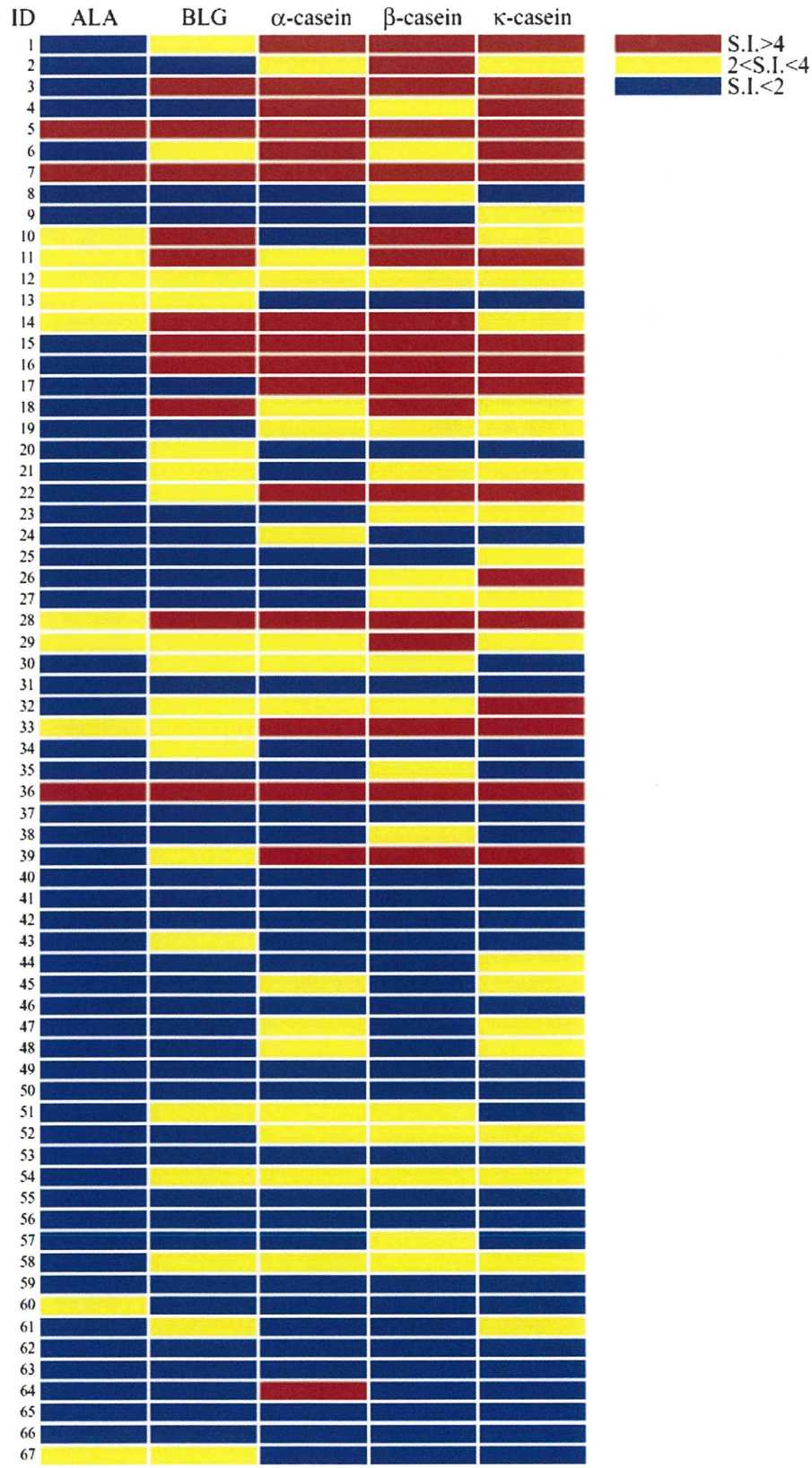

FIG E3. The milk protein component causing the most prominent tritiated thymidine uptake varied among the patients. PBMCs from children with gastrointestinal food allergies were stimulated separately with 100 μg/mL of each of 5 LPS-depleted milk protein preparations in the absence of serum. Lymphoproliferation was measured based on tritiated thymidine uptake. The stimulation index *(S.I.)* was calculated as milk protein–specific tritiated thymidine uptake (cpm)/vehicle-induced tritiated thymidine uptake (cpm). For

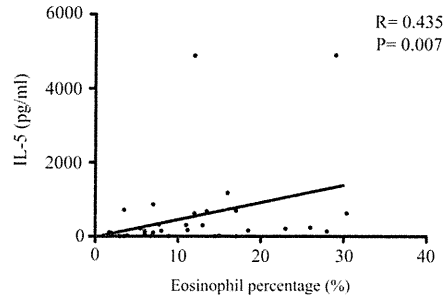

FIG E4. IL-5 concentration in the culture supernatant of cow's milk protein–stimulated PBMCs correlated significantly with the peripheral blood eosinophil percentage. PBMCs from children with gastrointestinal food allergies were stimulated separately with 100 μg/mL of each of 5 LPS-depleted cow's milk protein preparations in the presence of 5% autologous plasma for 6 days. Antigen-specific IL-5 production correlated significantly with the peripheral blood eosinophil percentage at disease onset (r = 0.435, P = .007).

each patient, the SI is shown for the PBMCs' response to each of the 5 milk protein preparations. Each *row* represents a single patient, and each *column* represents one of the 5 milk proteins. *ALA*, α-Lactalbumin; *BLG*, β-lactoglobulin; *blue*, SI < 2; *yellow*, 2.0 < SI < 4.0; *red*, SI > 4.

TABLE E1. Concentrations of LPS in commercially available milk protein preparations before and after treatment with a prepacked endotoxin affinity column

Cow's milk protein preparation	Before treatment (pg/mg)	After treatment (pg/mg)
α-Lactalbumin (Sigma L-6010)	184,200	14
β-Lactoglobulin (Sigma L-3908)	206,700	1,880
α-Casein (Sigma C-6780)	540	23
β-Casein (Sigma C-6905)	500	34
κ-Casein (Sigma C-0406)	400	41
LPS-depleted β-lactoglobulin (Bean Stalk Snow)	29	—

The indicated milk protein preparations were treated with a prepacked endotoxin affinity column (Detoxi-Gel; Pierce Chemical, Rockford, Ill) in accordance with the manufacturer's instructions. LPS concentrations were measured by using the limulus amebocyte lysate assay.

Novel Insights from Clinical Practice

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2012;158(suppl 1):83–86
DOI: 10.1159/000337797

Published online: May 15, 2012

Eosinophilic Gastrointestinal Disorder in an Infant with Feeding Dysfunction

Yoshiyuki Yamada[a] Masahiko Kato[a] Fumiaki Toki[b] Mio Watanabe[c]
Akira Nishi[b] Ikue Matsushita[d] Junko Hirato[f] Yasuhide Hayashi[e]

Divisions of [a]Allergy and Immunology, [b]Surgery, [c]Neurology, [d]Rehabilitation and [e]Hematology and Oncology, Gunma Children's Medical Center, Shibukawa, and [f]Department of Pathology, Gunma University Hospital, Maebashi, Japan

Established Facts

- A few recent studies have shown that feeding dysfunction, including maladaptive learned behaviors and physical difficulties in eating mechanics, is a prevalent symptom complex in young children with eosinophilic gastrointestinal disorders.
- Feeding dysfunction that adversely affects development, feeding and nutrition in affected children does not respond well to the medical treatments that improve eosinophilic inflammation.

Novel Insights

- Not only eosinophilic esophagitis, but also other eosinophilic gastrointestinal disorders, should be considered in the differential diagnosis of an infant with feeding dysfunction.

Key Words
Eosinophilic gastrointestinal disorders · Feeding dysfunction · Elemental diet

Abstract
Feeding dysfunction (FD) has recently been considered to comprise a prevalent set of symptoms in eosinophilic gastrointestinal disorders (EGIDs) in young children. We report the case of an 8-month-old girl with an EGID who visited our hospital due to vomiting, poor weight gain and feeding difficulties; her condition was discovered during the examination of the symptoms including FD. Tracheal aspiration and reduced esophageal clearance showed up in a barium swallow test and upper gastrointestinal contrast radiography, respectively. Delayed clearance from the stomach was also detected on gastrointestinal scintigraphy. Gastrointestinal endoscopy and biopsies revealed esophagitis with some eosinophils and duodenitis with eosinophilic inflammation. She was not a likely candidate for eosinophilic esophagitis. On administration of an elemental diet, the patient gained weight. Esophageal and stomach clearance subsequently improved, although the vomiting and FD persisted to some extent. We conclude that it is important to consider other EGIDs as well as eosinophilic esophagitis in the differential diagnosis of FD.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/12/1585–0083$38.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Yoshiyuki Yamada
Division of Allergy and Immunology, Gunma Children's Medical Center
779 Shimohakoda Hokkitsu
Shibukawa, Gunma 377-8577 (Japan)
Tel. +81 279 52 3551, E-Mail yamaday@gcmc.pref.gunma.jp

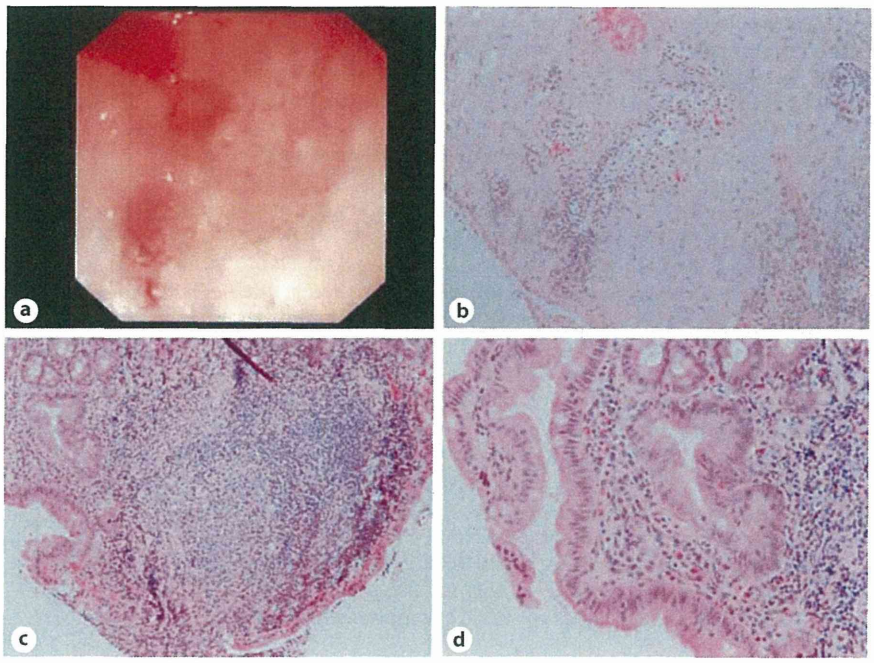

Fig. 1. Endoscopic and histological findings. **a** Endoscopic findings of the duodenum. Histopathologic analyses of the esophagus (**b**) and duodenum (**c**, **d**) were performed using hematoxylin and eosin staining. ×100 (**b**, **c**). ×200 (**d**).

Introduction

Eosinophilic gastrointestinal diseases (EGIDs) are characterized by primary eosinophilic infiltration of the gastrointestinal (GI) tract accompanied by GI symptoms. Clinical studies on EGIDs have demonstrated an obvious association between these conditions and food allergies. Over the past decade, the incidence of primary EGIDs, especially eosinophilic esophagitis (EoE), has increased along with the increase in the incidence of atopic diseases [1]. This may be due to an actual increase in the number of affected patients as well as better disease awareness [2]. EGIDs are classified into 4 groups (EoE, eosinophilic gastritis, eosinophilic gastroenteritis and eosinophilic colitis) on the basis of the primarily affected GI sites. The symptoms of EGIDs vary by anatomic location and include dysphagia, abdominal pain, cramping, bloating, nausea, vomiting, diarrhea, ascites and obstruction. In addition to these symptoms, feeding dysfunction (FD), a symptom complex including maladaptive learned behaviors, developmental differences, immature diet selection, dysphagia, oral sensory skill deficits and oral motor skill deficits, has recently been found to be prevalent in pediatric patients with EGIDs [3]. Significant FD was observed in 16.5% of patients, and patients with EGIDs other than EoE were also included in the study. Besides unexplained oral aversion, feeding refusal or difficulty, vomiting and poor weight gain in patients less than 4 years of age are symptoms of EGID, EoE [4]. The authors present a case of pediatric EGID (unlikely to be EoE) discovered by the examination of FD including food refusal, exaggerated responses to touch in the mouth or around the face and poor weight gain.

Case Report

An 8-month-old girl presented at our hospital with vomiting, poor weight gain and feeding difficulties. She was born with a low birth weight of 1,936 g at 35 weeks of gestation. She had been admitted to another hospital because of poor weight gain at 2 months of age. Her weight gain improved after her mother was educated on how to feed her milk. However, after the initial recovery, the amount of feeding decreased gradually, and she began vomiting frequently, again resulting in poor weight gain. Finally, the infant started refusing oral intake and tube feeding was started; she was referred to our hospital at the age of 8 months. At the first visit, her body weight was 5,360 g [–3 standard deviation (SD)] and her height was 59.8 cm (–3.8 SD). She had severe food refusal and exaggerated responses to touch in the mouth or around the face. A pediatric neurologist found that her motor development was slightly delayed. Laboratory data showed undetectable C-reactive protein levels and marginally elevated levels of total and milk-specific immunoglobulin E (28.7 IU/ml and 0.41 UA/ml, respectively). The eosinophil count was 456 cells/μl, and no anemia, hypoproteinemia or electrolyte imbalance was detected. A barium swallow test showed tracheal aspiration. Tube feeding was continued with rehabilitations, because she had gained a little weight. However, the vomiting and food refusal did not really im-

prove. At 11 months of age, she was admitted to our hospital for further examination. Reduced esophageal clearance was observed in upper GI contrast radiography and GI scintigraphy showed delayed clearance from the stomach. Upper GI endoscopy showed lymphoid hyperplasia (LH) and mucosal erythema in the duodenum (fig. 1a). Esophageal biopsy showed basal zone hyperplasia and papillary elongation with a few eosinophils (fig. 1b). LH (fig. 1c) with eosinophil infiltration [26 eosinophils per high-power field (HPF), fig. 1d] was observed in duodenal biopsy. EGID was suspected on the basis of the findings. The patient was started on an elemental diet; this is sometimes considered as a therapeutic approach for EGID when any causative foods are not found [1]. Rapid weight gain occurred (fig. 2) and the amount of vomiting each time was reduced (but not the frequency). The food refusal and exaggerated responses to touch in the mouth or around the face also became milder. At 16 months of age, a reevaluation was performed using the barium swallow test, upper GI contrast radiography and upper GI endoscopy. Tracheal aspiration and esophageal clearance had improved, shown by both the barium swallow test and upper GI contrast radiography. However, LH and mucosal erythema in the duodenum were still detected on endoscopy. The eosinophil infiltration in the duodenum also persisted. The elemental diet was continued, as the child's symptoms had been improving.

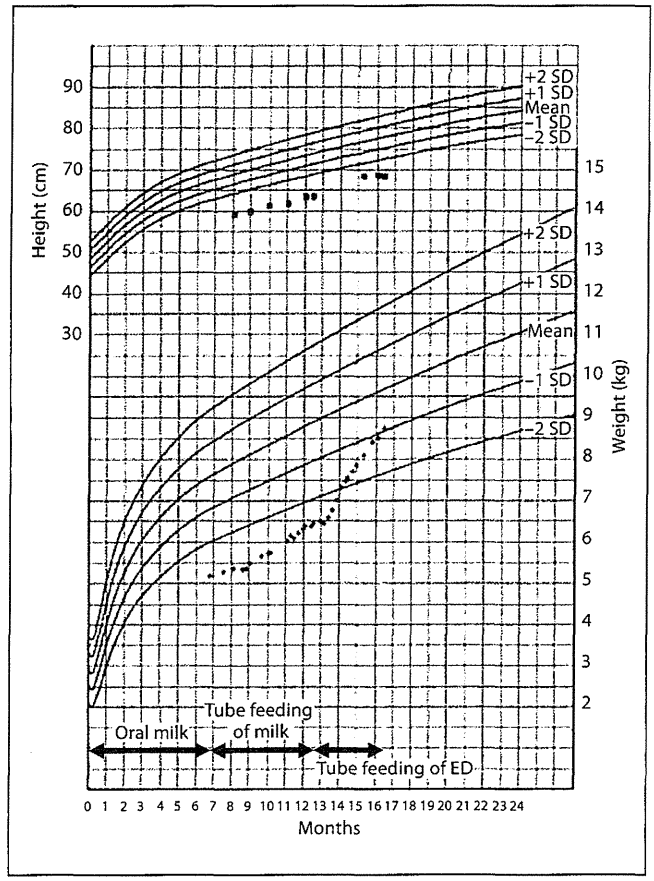

Fig. 2. Growth charts of the patient plotted on the Japanese sex-matched standard growth curves (0–24 months). The dots represent the actual height and weight. From the top, the lines indicate +2 SD, +1 SD, the mean, −1 SD and −2 SD. The periods for which each mode of nourishment was implemented are indicated. ED = Elemental diet.

Discussion

Widely accepted diagnostic criteria have not been established for EGIDs, but an increase in the number of eosinophils in biopsy specimens from the GI tract is an indicator [5]. A study that evaluated 14 biopsies showed that the mean number of eosinophils in the lamina propria of the duodenum was 9.6 ± 5.3 per HPF [6]. In addition, a previous review article defined duodenal eosinophilia as >10 eosinophils per HPF in 2 or more locations of the duodenum [5]. In this case, therefore, the observation of 26 eosinophils per HPF was considered to indicate significant eosinophilia. The endoscopic findings for the patient demonstrated duodenal LH with mucosal erythema indicative of duodenitis as well as eosinophilia. LH is mostly a benign condition in children and usually recedes spontaneously, but it is significantly associated with food allergy when seen in the duodenal bulb [7]. On the other hand, although obvious inflammation was observed histologically in this case, only a few eosinophils were found in the esophagus. Thus, the etiology of the esophagitis could not be determined. Collectively, the findings led us to suspect that the patient had an EGID that was most likely not EoE.

EGIDs are commonly associated with allergies [1, 5]. However, it is sometimes difficult to identify EGID-specific allergens because EGIDs have properties that fall between IgE-mediated and cellular-mediated allergy [1]. In fact, an association of food allergy in this patient was not clear. In addition, the symptoms of FD made it more difficult to perform further examinations e.g. challenge tests. Once disease remission had been observed by means of an elemental diet, the specific food groups would be slowly reintroduced. This food reintroduction process could be helpful for identifying the causative foods.

The symptoms of FD are generally associated with neurological diseases, developmental delays and gastroesophageal reflux disease (GERD). The painful swallowing and heartburn observed in GERD cause FD [8]. Recently, EGIDs have been considered as a differential diagnosis of FD. Several distinguishable FD characteristics of GERD are observed in young children with EGIDs [3]. As the symptoms of EGIDs (particularly EoE) and GERD

are sometimes similar, GERD is the most important differential diagnosis of EGID [9]. Therefore, differences in FD characteristics may be useful to distinguish EGIDs from GERD.

A recent report showed the detailed characteristics of FD and defined it in depth [3]. The characteristics include learned maladaptive behaviors, developmental differences, dysphagia, oral sensory skill deficits and oral motor skill deficits. The patient in our study had all 6 characteristics. Notably, learned maladaptive behaviors such as food refusal were more marked than other characteristics.

Interestingly, in a previous study, almost half the EGID patients with active FD showed GI eosinophilia that fulfilled the required criteria. FD can persist, however, even after the eosinophilic inflammation has subsided [3]. Dysphagia is exclusively correlated with the histological score [10], suggesting that it is difficult to estimate the severity of eosinophilic infiltration in the tissues on the basis of changes in symptoms other than dysphagia, including FD. Indeed, endoscopic reevaluation after confirmation of weight gain showed poor improvements in the eosinophil inflammations in our patient. However, there may have been an unnoted time lag between the reduction of symptoms and histological improvement, because subsequently, continuing with an ED, the patient's symptoms improved, despite the presence of duodenal eosinophilia at the reevaluation.

In summary, the EGID in this patient was discovered on the basis of FD symptoms. An elemental diet was effective for weight gain as well as improving esophageal clearance and symptoms. It is important to consider not only EoE but also other EGIDs in the differential diagnosis of infants with FD.

Acknowledgements

This project was supported by Research on Intractable Diseases, Health and Labour Sciences research grants from the Ministry of Health, Labour and Welfare of Japan (H22-Nanchi-Ippan-070 for Y.Y. and Y.H. and H22-Nanchi-Ippan-066 for Y.Y.).

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of this article.

References

1 Rothenberg ME: Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol 2004;113:11–28;quiz 29.

2 Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS: Eosinophilic esophagitis: updated consensus recommendations for children and adults. J Allergy Clin Immunol 2011;128:3–20 e26;quiz 21–22.

3 Mukkada VA, Haas A, Maune NC, Capocelli KE, Henry M, Gilman N, Petersburg S, Moore W, Lovell MA, Fleischer DM, Furuta GT, Atkins D: Feeding dysfunction in children with eosinophilic gastrointestinal diseases. Pediatrics 2010;126:e672–677.

4 Pentiuk SP, Miller CK, Kaul A: Eosinophilic esophagitis in infants and toddlers. Dysphagia 2007;22:44–48.

5 Liacouras CA: Nutrition issues in gastroenterology: eosinophilic gastrointestinal disorders. Pract Gastroenterol 2007;48:53–67.

6 DeBrosse CW, Case JW, Putnam PE, Collins MH, Rothenberg ME: Quantity and distribution of eosinophils in the gastrointestinal tract of children. Pediatr Dev Pathol 2006;9:210–218.

7 Kokkonen J, Karttunen TJ: Lymphonodular hyperplasia on the mucosa of the lower gastrointestinal tract in children: an indication of enhanced immune response? J Pediatr Gastroenterol Nutr 2002;34:42–46.

8 Hyman PE: Gastroesophageal reflux: one reason why baby won't eat. J Pediatr 1994;125:S103–109.

9 Dellon ES, Gibbs WB, Fritchie KJ, Rubinas TC, Wilson LA, Woosley JT, Shaheen NJ: Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. Clin Gastroenterol Hepatol 2009;7:1305–1313;quiz 1261.

10 Aceves SS, Newbury RO, Dohil MA, Bastian JF, Dohil R: A symptom scoring tool for identifying pediatric patients with eosinophilic esophagitis and correlating symptoms with inflammation. Ann Allergy Asthma Immunol 2009;103:401–406.