N-FPIES 初期の疾患概念構築、2 論文

1） Nomura I, Morita H et al. Four distinct subtypes of non-IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms. *J Allergy Clin Immunol*, 2011; 127(3): 685-8.e1-8.

2） Nomura I, Morita H et al. Non-IgE-mediated gastrointestinal food allergies: distinct differences in clinical phenotype between Western countries and Japan. *Curr Allergy Asthma Rep.* 2012 Aug;12(4):297-303.

EGE 初期の疾患概念構築

Kinoshita Y, Furuta K et al.. Clinical characteristics of Japanese patients with eosinophilic esophagitisand eosinophilic gastroenteritis. *J Gastroenterol.* 2013;48:333-9.

TABLE I. Sensitivity and specificity* for Ara h 2 and whole peanut extract

Test	Cutoff point (kU_A/L)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
Ara h 2	0.30	100.00	90.20	93.75
	0.32	100.00	94.12	95.00
	0.35	100.00	96.08	97.50
	0.38	96.55	96.08	96.25
	0.40	93.10	98.04	96.25
	0.55	93.10	100.00	97.50
	0.87	89.66	100.00	96.25
Whole extract	0.35	96.55	26.92	51.85
	3.91	79.31	84.62	82.72
	5.00	75.86	90.38	85.19
	5.30	75.86	94.23	87.65
	5.96	72.41	94.23	86.42
	7.81	72.41	96.15	87.65
	15.00	55.17	96.15	81.48
	43.86	34.85	98.08	75.31

Analysis included all children with available data (81 for sIgE to whole peanut extract and 80 for sIgE to Ara h 2).
*Sensitivity refers to the proportion of subjects who have peanut allergy and give positive test results. Specificity refers to the proportion of subjects without the target condition and a negative test result for peanut allergy.

peanut allergy and 50 are peanut-tolerant. By using sIgE to component Ara h 2 with a cutoff point of 0.35 kU_A/L, all children with peanut allergy would be correctly classified. The specificity of this test is given as 96.1% (Table I). In this example we expect 2 children who are not allergic to peanuts to be misclassified as having peanut allergy and the other 48 children to have a negative result. By using this cutoff point, 97.5% of the population is correctly classified. A similar proportion of children would be correctly classified by using a cutoff point of 0.55 kU_A/L; however, in this case 3 children with peanut allergy would be misclassified as tolerant. This cutoff point corresponds to a gain in specificity (100%) but a loss in sensitivity (93.1%). Given the importance of not misdiagnosing children with peanut allergy as being tolerant, we propose that the optimal cutoff point in our population is 0.35 kU_A/L.

The cutoff for whole peanut sIgE of 5.30 kU_A/L provides the maximum proportion of correctly classified subjects (87.6%), with a sensitivity of 75.9% and a specificity of 94.2%. However, approximately 24% of children with peanut allergy would be inappropriately classified as peanut-tolerant. The cutoff of 15 kU_A/L has excellent specificity, with 96.2% of children at greater than this level being correctly classified as allergic; however, this decision point has relatively poor sensitivity, with almost half of the subjects with peanut allergy being classified as tolerant. These data suggest that in our population the quantification of whole peanut sIgE has lower accuracy in discriminating peanut allergy from tolerance compared with quantification of sIgE to Ara h 2.

In conclusion, having identified sIgE to Ara h 2 as an important predictor of clinical reactivity to peanut using microarray technology,[5] we have now demonstrated the value of its quantification using a routinely available laboratory test. Among school-aged children in the United Kingdom, a cutoff of 0.35 kU_A/L Ara h 2 IgE confers 100% sensitivity and 96.1% specificity. By using this cutoff point, 97.5% of the subjects in our study population were correctly classified, with all children with peanut allergy given the correct classification. The importance of Ara h 2 has been suggested in studies from other Central and Northern European countries[7,8]; however, in other populations and geographic areas, IgE to other components might be relevant (eg, Ara h 9 in the Mediterranean[9]). Our findings need to be replicated in other populations and age groups before general application.

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Four distinct subtypes of non–IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms

To the Editor:

Although most food allergies are IgE-mediated, there are a number of non–IgE-mediated gastrointestinal food allergies that affect mainly infants and young children.[1,2] Because most such

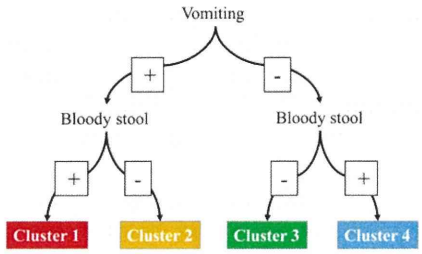

FIG 1. Tree analysis using 2 variables (vomiting and bloody stool at initial presentation) enables assignment of patients into 4 clusters.

patients develop the allergy more than 2 hours after ingestion of the offending food and show negative skin prick tests and/or absence of serum specific IgE against the offending food, these allergies are thought to be cell-mediated. However, the precise pathogenetic mechanisms of these disorders remain poorly understood. Several investigators have described different subtypes of non–IgE-mediated gastrointestinal food allergies: food protein-induced enterocolitis syndrome (FPIES),[3] food protein-induced proctocolitis syndrome (hereafter referred to as "proctocolitis"),[4] food protein-induced enteropathy syndrome (hereafter referred to as "enteropathy"),[5] celiac disease, and allergic eosinophilic gastroenteropathies.

Presumably because the main target organ of these syndromes is the gastrointestinal tract, patients with non–IgE-mediated gastrointestinal food allergies often exhibit similar symptoms, such as vomiting and diarrhea. However, it remains unclear whether these syndromes have the same pathogenesis and merely differ in severity, or whether the pathogenesis of each is distinct, meaning that they should be classified as separate clinical entities.

We applied cluster analysis to the clinical and laboratory findings to characterize these non–IgE-mediated food allergies and determine whether they are made up of distinct clinical entities. A total of 176 patients with detailed clinical records who had been registered in the database of the Japanese Research Group for Neonatal, Infantile Allergic Disorders from 2007 to 2010 were enrolled. Among them, 136 patients fulfilled 3 of the Powell[6] criteria: (1) a switch to therapeutic milk led to resolution of symptoms, (2) differential diagnosis from other disorders was possible, and (3) there was verified body weight gain. Definitive diagnosis was possible for 46 patients by oral food challenge tests that were performed after complete resolution of the initial symptoms (see this article's Fig E1 in the Online Repository at www.jacionline.org). These 46 patients were subjected to further analysis. Details of food challenge test are available in this article's Food challenge test, method section in the Online Repository at www.jacionline.org. Our total cohort included 15 patients who developed the most severe reactions, including ileus, shock, and developmental retardation. The clinical characteristics of those patients are summarized in this article's Table E1 in the Online Repository at www.jacionline.org. Because of the medical and ethical justification, even though these patients fulfilled 3 elements of the Powell[6] criteria, oral challenge tests were not performed. Thus, these patients were excluded from this cluster analysis of 46 patients. This study was approved by the Ethics Committee of the National Center for Child Health and Development.

We omitted clinical and laboratory findings found only in a few patients and finally selected 5 variables: birth weight, age at first presentation (days after birth), severity of vomiting (ranked as 0, none; 1, 1-2 times a day; 2, 3-5 times a day; and 3, more than 5 times a day or bilious vomiting) and severity of bloody stool (0, none; 1, spotty; 2, intermediate; and 3, massive) at first presentation, and milk-specific IgE antibody titer (class 0-6). Unsupervised cluster analysis and discriminant analysis were performed by using SPSS version 18 software (SPSS, Inc, Chicago, Ill). The Wald minimum-variance hierarchic clustering method was performed by using an agglomerative (bottom-up) approach and Ward's linkage. The squared Euclidean distance was used as a proximity measure. Values were transformed by a maximum magnitude of 1. ANOVA, the Tukey-Kramer test, and the χ^2 test were used for parametric continuous, nonparametric continuous, and categoric variables. As a result, the 46 definitively diagnosed patients were classified into 4 distinct clusters, and a dendrogram was generated (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Stepwise discriminate analysis identified the 2 strongest discriminatory variables for cluster assignment: vomiting and bloody stool (Fig 1). Cluster 1 was the patient group with vomiting and bloody stool at initial presentation. Cluster 2 had vomiting but not bloody stool. Cluster 3 had neither vomiting nor bloody stool. Cluster 4 had bloody stool but not vomiting. One patient initially assigned to cluster 3 in fact had clear bloody stool, and was thus reassigned to cluster 4 in accordance with Fig 1. As a result, clusters 1 through 4 consisted of 14, 16, 5, and 11 patients, respectively.

Table I presents the demographic data for each cluster. Cluster 3 showed a significantly lower birth weight and later onset of disease. Clusters 1 and 4 both had bloody stool, but they had normal birth weight and a somewhat earlier onset (median of 7 days after birth).

The laboratory data generated within the initial several days after onset showed that the peripheral blood eosinophil ratio was high in all clusters, with no significant differences among them. In contrast, eosinophils were found in the stool mainly of patients in clusters 1 and 4, in which all patients, by definition (Fig 1), had bloody stool. The presence of eosinophilia suggests that patients with non–IgE-mediated gastrointestinal food allergies tend to have a T_H2-prone immune deviation at baseline, but some additional factors such as overproduction of eosinophil-attracting chemokines are probably necessary to induce immune responses involving eosinophils in the gut (see this article's Fig E3 in the Online Repository at www.jacionline.org).

A positive milk-specific IgE antibody titer was observed in 37% of the patients, with no statistically significant differences among any of the clusters. In addition, almost all symptoms at initial presentation as well as in oral food challenge tests began to manifest at more than 2 hours after ingestion of the offending food, whereas no patients developed typical IgE-mediated symptoms such as urticaria or wheeze. These results strongly suggest that the presence of milk-specific IgE antibody neither causes the gastrointestinal symptoms nor rules out a diagnosis of non–IgE-mediated gastrointestinal food allergy.

One of the most notable findings of this study was the remarkably high reproducibility of symptoms provoked in the oral food challenge tests and those found at the initial presentation in all 4 clusters, even though the oral challenge tests were performed several months after the initial presentation (Table I). This observation suggests that the upper or lower gastrointestinal tract–specific hypersensitivity and perhaps the responsible

TABLE I. Demographic data of the patients (total = 46) whose diagnosis was confirmed by oral food challenge tests

Clinical characteristics	Cluster 1 (n = 14)		Cluster 2 (n = 16)		Cluster 3 (n= 5)		Cluster 4 (n = 11)		*P* value
Birth weight (g)		2642 (2410-3030)		2745 (2223-3079)		1008 (907-2491)		2678 (2512-3170)	.03*
Male/female (n)		6/8		9/7		2/3		5/6	.95
Initial presentation									
Day of onset		7.5 (3-23)		16.5 (9.5-33.5)		37 (8.5-132)		7 (2-56)	.17
Vomiting (%)		100		100		0		0	.000*
Bloody stool (%)		100		0		0		100	.000*
Fever (%)		7.1		18.8		20.0		0	.45
(Laboratory data)†	n		n		n		n		
Blood eosinophil ratio (%)‡	13	15 (3.0-23)	14	7 (3.9-19.3)	5	27 (3.2-39.3)	11	14 (4.5-25)	.63
WBC ($\times 10^3$/mL)§	13	18.4 (13.7-22.7)	14	15.7 (11.4-21.9)	5	21.8 (11.0-27.7)	11	13.1 (8.2-18.3)	.64
Total IgE (IU/mL)‖	14	5.2 (4.8-28.3)	16	11.4 (5.0-80.8)	5	7.4 (5.5-653.5)	10	5.0 (2.0-5.8)	.36
Positive for milk-specific IgE (class ≥1) (%)	14	57	16	37.5	5	40	11	9	.28
C-reactive protein (% positive, ≥0.5)	13	46	14	50	5	40	10	30	.47
Stool eosinophil (% positive)	8	50	6	33	3	0	7	100	.01*
Diet (reaction to each milk, %)									
Cow's milk	14	100	16	100	5	100	10	100	1.00
Breast milk	8	38	7	0	2	50	7	27	.40
Hydrolyzed formula	9	0	10	20	2	0	8	63	.02*
Oral food challenge test									
Onset of reaction (h)		6 (1.8-12)		10 (2-24)		48 (24-60)		24 (24-48)	.17
Vomiting (%)		85.7		81.3		0		9.1	.000*
Bloody stool (%)		28.6		6.3		0		72.7	.001*
Diarrhea (%)		21.4		31.3		60.0		18.2	.33

WBC, White blood cell count.
Data are shown as the median and the interquartile range.
*$P < .05$.
†*n*, Number with medical records.
‡Normal range of blood eosinophils is 0% to 4%. However, it is known to rise to some degree in the neonatal period, especially in low-birth-weight infants.[10]
§Normal range of WBC in neonatal period is 7.0 to 25.0 $\times$ $10^3/\mu$L.
‖Normal range of total IgE in infantile period is less than 20 IU/mL.

immune cells remain in the same part of the gastrointestinal tract even after several months' remission.

Because the patients in clusters 1 and 2 had vomiting that was provoked at relatively early time points, they are likely to be diagnosed as having FPIES, although the bloody stool and eosinophilia seen mainly in cluster 1 patients were not emphasized in earlier reports.[7,8] The nearly simultaneous manifestation of vomiting and bloody stool suggests that FPIES may affect both the upper and lower gastrointestinal tracts.

The main symptoms of the patients in cluster 3 were poor weight gain and diarrhea and were similar to those found in patients with enteropathy. The significantly lower birth weight and marked eosinophilia characteristically found in cluster 3 patients imply the involvement of immature gastrointestinal function in the pathogenesis of this syndrome.

Bloody stool was the main symptom of the patients in cluster 4. Some patients in this cluster had no systemic manifestation other than bloody stool, whereas others also had diarrhea and/or poor weight gain. Therefore, these patients may be diagnosed as having proctocolitis or early onset of allergic eosinophilic gastroenteropathies, respectively. However, the pathogenetic similarity and/or disparity of proctocolitis and allergic eosinophilic gastroenteropathies need to be studied further.

In our cohort, 3 children with exclusive breast-feeding have developed FPIES. This information is available in this article's Breast-feeding and FPIED section in the Online Repository at www.jacionline.org.

Elevated serum C-reactive protein levels were found in 30% to 50% of patients with non–IgE-mediated gastrointestinal food allergies. In addition, some patients developed a fever during oral food challenge tests, suggesting that TNF-α and other proinflammatory cytokines may be involved in the pathogenesis of these syndromes.[9]

To confirm the results of cluster analysis, we performed the same analysis for the aforementioned 136 patients who fulfilled 3 of the Powell[6] criteria (consisting of the 46 patients definitively diagnosed by oral food challenge and 90 patients not subjected to oral food challenge; Fig E1). We obtained exactly the same results: the patients were assigned to 4 clusters in accordance with the tree analysis shown in Fig 1. The patients' demographics (see this article's Table E2 in the Online Repository at www.jacionline.org), birth weight (see this article's Fig E4 in the Online Repository at www.jacionline.org) and peripheral blood eosinophils (see this article's Fig E5 in the Online Repository at www.jacionline.org) confirmed the earlier cluster analysis findings.

In our ongoing cohort, 52% of the patients acquired tolerance to the offending food by 1 year of age, 88% by 2 years, and 94% by 3 years. Therefore, assuming that identification and elimination of the offending food had been done properly, it can be assumed that most patients outgrew their allergy by the age of 2 to 3 years. On the other hand, just like patients with severe IgE-mediated food allergy, patients with non–IgE-mediated gastrointestinal food allergies may develop severe reactions

(Table E1). Thus, early diagnosis is very important, and refinement of the diagnostic method is truly necessary.

Our findings clearly demonstrated that patients with these non–IgE-mediated gastrointestinal food allergies showed similar T_h2-prone laboratory data (eosinophilia and presence of specific IgE antibody), but the disease entities of each cluster had distinct clinical features and may have different pathogenetic mechanisms.

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FOOD CHALLENGE TEST, METHOD

Generally, oral challenge tests were performed at 4 to 6 months of age. First, 4 mL milk/kg body weight was administered. If no reaction occurred, the dose of milk was increased daily until symptoms manifested. If the reaction had been evoked by a very small volume of milk in the initial presentation, the test was started using a lesser volume to avoid a serious reaction. Because of the medical and ethical justification for oral food challenge tests, patients with the most severe reactions were excluded from the initial cluster analysis. Their clinical characteristics are summarized in Table E1.

BREAST-FEEDING AND FPIES

Six of the 46 patients were exclusively breast-fed. Three of them were included in cluster 1 and can be diagnosed as FPIES. Those 3 patients showed a positive reaction to cow's milk as well as breast milk even after their mothers stopped consuming milk products. These patients also developed symptoms when orally challenged with rice and/or soy. Therefore, these findings indicate that not only proctocolitis but also FPIES can develop even in children who are exclusively breast-fed. A recent case report supports our findings.[E1]

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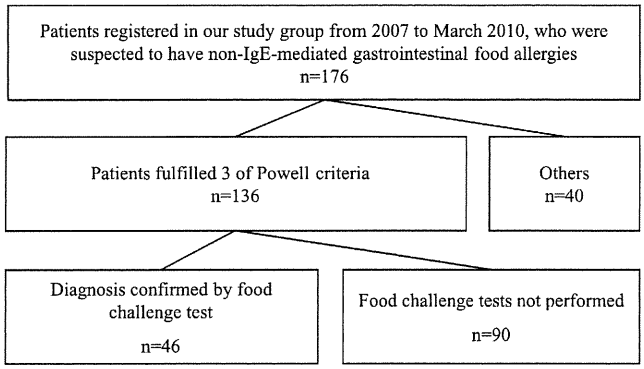

FIG E1. A total of 176 patients with gastrointestinal symptoms who were suspected of having non–IgE-mediated allergy from 1999 to 2009 were registered by doctors of the Japanese Research Group for Neonatal, Infantile Allergic Disorders. Of them, 136 patients fulfilled elements 1 through 3 of the Powell criteria. Forty-six patients underwent food challenge tests and had a positive result, whereas the remaining 90 patients were not tested. Seventeen patients showed no reaction in the oral challenge tests. However, it was unclear whether this was because the patients had outgrown their allergy or because of misdiagnosis. Those 17 patients were excluded from further analysis in this study.

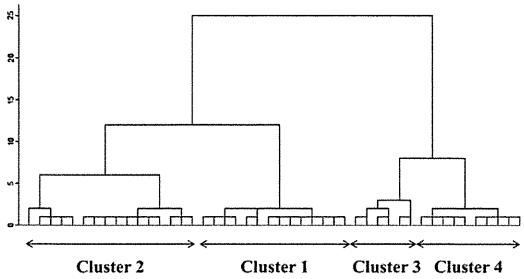

FIG E2. The 46 patients definitively diagnosed with non–IgE-mediated food allergies were analyzed for 5 variables by using an agglomerative (bottom-up) approach and Ward's linkage, and a dendrogram was generated.

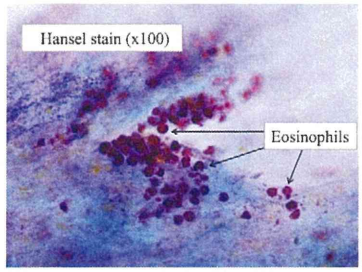

FIG E3. Detection of accumulations of eosinophils in the stool mucus. The mucous part of the stool was thinly smeared on a glass slide and stained by using Hansel stain. The stool sample was taken from a patient in cluster 2 after a positive food challenge test. Representative images were found in a total of 13 patients (Table I).

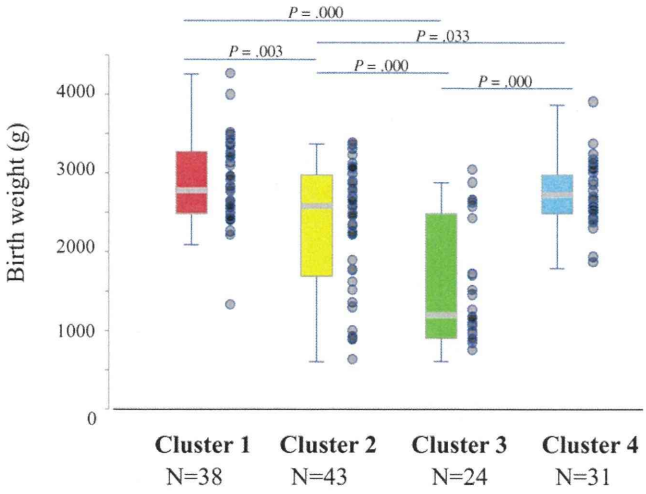

FIG E4. The birth weights in each cluster of the 136 patients who fulfilled 3 elements of the Powell criteria for a non–IgE-mediated allergy are shown.[E2] The birth weights in cluster 3 were significantly lower than in the other clusters. Moreover, 2 subgroups seem to be identified in cluster 3: a lower birth weight group and a normal birth weight group.

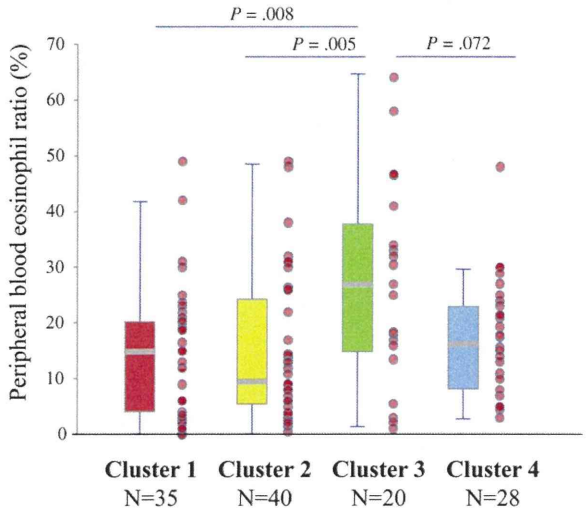

FIG E5. The peripheral blood eosinophil ratios in each cluster of the 136 patients who fulfilled 3 elements of the Powell criteria are shown.[E2] Although eosinophilia was found in all 4 clusters, the eosinophil ratios of the patients in cluster 3 were significantly higher than those of the patients in clusters 1 and 2.

TABLE E1. Clinical features of most severe cases of non–IgE-mediated gastrointestinal food allergies*

Patient no.	Sex	Cluster	Complication	Day of onset	Diet right before the onset of complications	Remarks
1	F	1	Ileus	8	Cow's milk 7 d	
2	M	1	Ileus	5	Cow's milk 3 d, breast milk 6 d	Relieved by surgical operation
3	F	1	Ileus	8	Breast milk 9 d	Relieved by surgical operation
4	F	1	Shock	2	Cow's milk 2-3 times	Massive bloody stool, blood infusion required
5	F	1	Shock	21	Breast milk 18 d	Massive bloody stool, disseminated intravascular coagulation
6	F	2	Ileus	14	Breast milk 2 d	
7	F	2	Shock	36	Breast milk 30 d	Apnea, vomiting
8	M	2	Shock	30	Cow's milk 50 mL by chance	Vomiting
9	M	2	Shock	241	Soy food 2-3 times	Vomiting and diarrhea, ICU admission
10	M	3	Ileus	61	Breast milk 45 d	Cholestasis
11	F	3	Shock	22	Cow's milk 21 d, breast milk 21 d	ICU admission
12	F	3	Severe weight loss	12	Breast milk several months	Developmental retardation
13	M	3	Severe weight loss	46	Cow's milk 30 d, breast milk 30 d	Developmental retardation
14	F	4	Ileus	2	Cow's milk 6 d, breast milk 3 d	Stenosis of sigmoid colon
15	F	4	Ileus	7	Cow's milk 10 d	

F, Female; *ICU,* intensive care unit; *M,* male.
*These patients fulfilled 3 elements of the Powell criteria,[E2] but oral challenge tests were not performed.

TABLE E2. Demographics of the 136 patients who fulfilled 3 elements of the Powell criteria[E2]

Clinical characteristics	Cluster 1 (n = 38)		Cluster 2 (n = 43)		Cluster 3 (n = 24)		Cluster 4 (n = 31)		*P* value
Birth weight (g)	2823 (2501-3267)		2581 (1779-3016)		1363 (1023-2611)		2778 (2512-3100)		.000 *
Male/female	19/19		28/15		13/11		12/19		.16
Initial presentation									
Day of onset	6 (4-8)		29 (7.5-52)		16.5 (9.5-37.5)		7 (2-35)		.01*
Vomiting (%)	100		100		0		0		.000*
Bloody stool (%)	100		0		0		100		.000*
(Laboratory data)†	**n**		**n**		**n**		**n**		
Blood eosinophil ratio (%)	35	15 (3.5-21.0)	40	9 (5.3-25.0)	20	26 (14.1-39.3)	28	17 (8.5-23.8)	.005*
WBC ($\times 10^3$/mL)	32	18.7 (14.5-23.5)	40	13.8 (10.4-22.1)	23	15.9 (13.9-24.4)	27	13.9 (11.4-19.5)	.16
Total IgE (IU/mL)	32	5.2 (4.1-23.1)	40	5.8 (4.0-17.8)	22	13.2 (5.5-122.9)	28	5.0 (3.3-6.0)	.001*
Positive for milk-specific IgE (class ≥1) (%)	31	41.9	38	23.7	20	50	27	19	.24
C-reactive protein (% positive, ≥0.5)	36	61	40	45	20	70	27	33	.69

WBC, White blood cell count.
Data are shown as the median and the interquartile range.
*$P < .05$.
†*n*, Number with medical records.

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FOOD ALLERGY (D ATKINS, SECTION EDITOR)

Non–IgE-Mediated Gastrointestinal Food Allergies: Distinct Differences in Clinical Phenotype Between Western Countries and Japan

Ichiro Nomura · Hideaki Morita · Yukihiro Ohya · Hirohisa Saito · Kenji Matsumoto

Abstract Non-IgE-mediated gastrointestinal food allergies, including food-protein-induced enterocolitis, enteropathy, proctocolitis and allergic eosinophilic gastroenteritis, seem to be increasing in several regions in the world. However, unlike the case of IgE-mediated food allergy, development of diagnostic laboratory tests and our understanding of the immunological mechanisms involved in non-IgE-mediated gastrointestinal food allergies lag. Although the clinical entities in Western countries have been well established, the clinical phenotypes might differ somewhat among the human races and geographical regions. In Japan, non-IgE-mediated gastrointestinal food allergies have increased sharply since the late 1990s, and clinicians have sometimes experienced confusion because of differences in the clinical phenotypes from those seen in Western countries. Aiming to solve this problem, we performed clinical research and determined a useful method for dividing patients into four clusters with distinctive clinical symptoms. We are confident this method will help in diagnosing and treating these patients. We also tried to clarify the differences between these patients in Japan and Western countries.

Keywords Non–IgE-mediated gastrointestinal food allergy · Gastrointestinal allergy · GI allergy) · Food protein–induced enterocolitis syndrome (FPIES) · Food protein–induced enteropathy syndrome (enteropathy) · Food protein–induced proctocolitis syndrome (proctocolitis) · Eosinophilic esophagitis (EoE) · Allergic eosinophilic gastroenteritis (AEG) · Food challenge test · Food allergy · Eosinophil · Lymphocyte proliferation test · Cluster analysis · Phenotype · Japan

Two Japanese Patients with Non-IgE-Mediated Gastrointestinal Food Allergy

Patient 1: A Baby Girl with Vomiting and Bloody Stool

A baby girl was born at full term and normal birth weight. She was happy and drinking dairy-based formula until the 8th day after birth. Then she started vomiting once a day. On the next day she became less energetic. On the 11th day, she had 20 bloody stools. On the 12th day, she developed apnea and went into shock. She was transferred to the emergency department of a children's hospital. On arrival, no arterial pulse could be detected, and cyanosis was apparent. Life support was started, and she gradually recovered. Before 2000, we would never have considered gastrointestinal allergy (GI allergy) as the diagnosis for such a seriously ill patient. But now the order of differential diagnosis has changed. Open abdominal surgery was performed but found no abnormality. An increased peripheral eosinophil count (22 %) and cow's milk-specific IgE (3+) were detected, and

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GI allergy (food-protein-induced enterocolitis syndrome; FPIES) was suspected (30 % of patients with FPIES in Japan test positive for IgE to the offending food). She was placed on an elemental diet and recovered.

Patient 2: A Growth-Retarded One-Year-Old Boy with No Apparent GI Symptoms

A one-year-old boy was transferred from a university hospital to National Research Center for Child Health and Development. He was born with a normal birth weight, but weight gain had been slower since 4 months of age. Vomiting, bloody stools and diarrhea were absent. He had been breast fed, but gradually lost his appetite. The cause of weight loss was not identified, in spite of various examinations at the university hospital. At one year and nine months of age, he was transferred to our hospital. His weight was -3SD, with prominent emaciation and brain atrophy, and he only sat in his baby stroller. Gastrointestinal endoscopy revealed prominent eosinophilic infiltration extending from the duodenum to the large intestine, and duodenal villi were torn off. A diagnosis of allergic eosinophilic gastroenteritis (AEG) was made. Chronic tolerance tests revealed rice, soy and cow's milk to be the causes of AEG. After elimination of the offending foods, his weight began to increase quickly. Five months later, he was able to stand at the top of a jungle gym!

Introduction

Non-IgE-mediated gastrointestinal food allergies (GI allergy) appear to have increased around the world in recent decades. Eosinophilic esophagitis (EoE)—which is recognized as a mixed IgE- and cell-mediated disorder—has been the most extensively studied food allergy, and it has increased dramatically [1]. In neonates and infants, there are well-established clinical entities such as food-protein-induced enterocolitis syndrome (FPIES) [2, 3, 4•]. However, these entities are not sufficiently recognized by general pediatricians, and many patients experience rapid worsening of their clinical course. As a result, serious complications can develop, including intestinal obstruction, intestinal perforation, shock and developmental retardation. There also seem to be some differences in the clinical features and laboratory findings among the human races and geographical regions. If clinicians in non-Western countries tried to make a diagnosis based only on the reports and textbooks from Western countries, they might have serious difficulty. The outlines of two severe Japanese cases are presented above to highlight the importance of GI allergy. Next, the differences between IgE-mediated reactions and GI allergy will be discussed, followed by a description of the known clinical entities. Finally, the clinical features of Japanese patients will be reviewed and a simple method for classifying and comparing them with the known entities will be described.

Differences Between IgE-Mediated Food Allergy and GI Allergy

When we think about the time courses of food allergy, there are two types of reactions; namely, immediate reactions (IgE-mediated) and non-immediate type reactions (non-IgE-mediated) [5, 6]. Mast cells and IgE antibodies play key roles in immediate reactions. Allergenic proteins are bound by two molecules of specific IgE antibodies on the surface of mast cells, and high-affinity IgE receptors activate signal transduction pathways leading to the release of histamine, leukotrienes and prostaglandins. Urticaria, wheezing and anaphylactic shock may be induced. On the other hand, since GI allergies are non-immediate type reactions, the central player may be T cells. Antigen-presenting cells, several subsets of T cells, epithelial cells, mast cells and eosinophils may be involved in the reactions, but the precise mechanisms and true effector cells remain elusive.

Since food-specific IgE antibodies do not play a key role in the immunological reaction of GI allergy, we have no effective laboratory tests for identifying offending foods in daily practice. A lymphocyte proliferative response to an offending food protein might have great potential as an in vitro diagnostic test. The mechanism of lymphocyte proliferation is that allergens are endocytosed by APCs and then digested in lysosomes. Next, allergen fragments of around ten amino acids in length are bound by MHC class II molecules and expressed on the surface of the APCs. T cells bearing specific T-cell receptors bind to the MHC class II-peptide complexes. Then the T cells are activated and start cell division. It should be noted that the reaction is initiated by tiny, 10-amino-acid peptides, not large molecules. Hydrolyzed milk contains peptides of around molecular weight 1000, which is close to ten amino acids. This might explain why hydrolyzed milk, which is effective for treatment of IgE-mediated allergy, is not effective in a significant percentage of patients with GI allergy [7•].

The central mechanism of chronic inflammation of the GI mucosa was elucidated by studies on EoE [8, 9]. Offending food proteins contact the surface of the esophageal mucosa and are incorporated by APCs, after which food allergen-specific T cells recognize them and are activated. Lymphocytes begin to produce IL-13 and other cytokines. IL-13 directs epithelial cells of the GI mucosa to produce eotaxin-3. Eotaxin-3 attracts eosinophils into the stratified squamous epithelial layer. However, eosinophils may not be the main effector cells, because depletion of eosinophils by anti-IL-5 treatment does not improve the symptoms of

patients with EoE [10]. We need to identify the main effector cell: is it a certain subset of T-lymphocytes, mast cells or innate immune cells?

GI Allergies Already Established in Western Countries

The following five clinical entities have been well studied and characterized. [5, 6]

In neonates and infants (classified as non-IgE-mediated gastrointestinal food allergies)

1. Food protein-induced enterocolitis syndrome (FPIES)
2. Food protein-induced enteropathy syndrome (enteropathy)
3. Food protein-induced proctocolitis (proctocolitis)

In children to adults (classified as mixed IgE- and cell-mediated gastrointestinal food allergies)

4. Eosinophilic esophagitis (EoE)
5. Allergic eosinophilic gastroenteritis (AEG)

Food Protein-Induced Enterocolitis Syndrome (FPIES)

The main symptoms of FPIES are vomiting, diarrhea and shock. Offending foods are cow's milk, wheat, rice, soy, breast milk, etc. [11]. Onset is usually before 3 months of age, but ranges from the day of birth to 1 year old. Laboratory data show no specific IgE and no eosinophilia [4•]. Since making the correct diagnosis at first glance is almost impossible, medical doctors have to use a "therapeutic diagnosis" to save patients. Five steps of diagnostic and treatment procedures—proposed by Powell in the 1970s [2, 3]—are useful for diagnosing FPIES and may be useful for other subsets of GI allergies. Those steps are: 1) suspect FPIES from the initial symptoms, 2) rule out other disorders in the differential diagnosis, 3) a switch to therapeutic milk leads to resolution of symptoms (therapeutic diagnosis), 4) verify weight gain every month and 5) confirm the diagnosis by oral food challenge tests performed after complete resolution of the initial symptoms. Patients with FPIES show prompt responses to both therapeutic diagnosis and oral food-challenge tests. Oral challenge tests show a specific pattern of response different from IgE-dependent food allergy. If the allergen dose exceeds the threshold of the patient, symptoms (vomiting, diarrhea and sometimes fever) will be provoked within 1 to 24 hours after ingestion of the offending food. Peripheral blood neutrophils will increase more than 3500/microL from the baseline [2]. C-reactive protein may become positive on the next day. Care must be taken not to cause serious damage when conducting food-challenge tests. It is essential to start with a very small amount of the food in severe cases, or not to challenge and wait for 2–3 years with elimination of the offending food(s). To prove remission of FPIES for a food allergen, a chronic tolerance test lasting 2–3 weeks is recommended to exclude possible delayed onset. If the treatment is appropriate, the prognosis is good. Ninety percent of patients enter remission by the age of 3 years. A few reports have investigated the pathophysiology of FPIES. TNF-alpha might be a key cytokine. Chung et al. reported [12] that TNF-alpha is expressed in epithelial cells and mononuclear cells in the lamina propria of the intestinal mucosa. At the same time, TGF-beta, an important cytokine for protecting the GI mucosa from excessive immune system reactions, is decreased in the GI mucosa. However, the precise molecular mechanism of FPIES is unclear. The mechanism of allergen-specific responses not involving IgE antibody and starting a few hours after ingestion of an offending food is a big puzzle in the field of immunology.

Food Protein-Induced Enteropathy Syndrome (Enteropathy)

Food protein-induced enteropathy syndrome (enteropathy) affects infants and children aged 0–2 years. The main symptoms are weight loss and sometimes diarrhea. Offending foods can be cow's milk, soy, wheat, eggs, etc. The small intestine is the most affected organ of the GI tract. Laboratory data show no specific IgE or eosinophilia, but hypoproteinemia and malabsorption syndrome occur. Pathological studies show patchy villous atrophy, a prominent mononuclear cell infiltrate and few eosinophils. The five steps of diagnostic and treatment procedures described above may not be effective for enteropathy, and pathological examination is required for diagnosis [13]. Also, we are unable to distinguish enteropathy from allergic eosinophilic gastroenteritis (AEG) without observing the pathology of the intestinal mucosa. Scientific studies, such as microarray analysis of the GI mucosa, clinical research, etc., are needed to elucidate the differences.

Food-Protein-Induced Proctocolitis (Proctocolitis)

Food-protein-induced proctocolitis (proctocolitis) affects babies aged 0–6 months [14–16]. The main symptom is bloody stool. There is no weight loss. If a patient shows weight loss, a diagnosis of AEG might be more appropriate. Offending foods are breast milk, cow's milk, soy, etc. Laboratory data show no specific IgE, and eosinophilia is occasionally seen. Lesions are confined to the distal large bowel and consist of mucosal edema with infiltration of eosinophils. Although patients show a rather slow response to therapeutic diagnosis and the food-challenge test, the above-mentioned five steps of diagnostic and treatment procedures might be useful. A chronic tolerance test, lasting

up to 2 weeks, is needed for diagnosis of proctocolitis. The prognosis is good if treatment is appropriate.

Eosinophilic Esophagitis (EoE)

Eosinophilic esophagitis (EoE) is now increasing in Western countries. EoE affects a wide age range, from 1-year-old children to adults. Symptoms are gastroesophageal reflux, excessive spitting-up or emesis, dysphagia, etc. Offending foods are cow's milk, wheat, eggs, etc. Some patients have multiple food allergens, which can be difficult to determine, especially in older children. Pathological examination is required to establish diagnosis [17].

Allergic Eosinophilic Gastroenteritis (AEG)

AEG affects infants to adults. Symptoms vary among patients due to differences in age and the affected GI organs. Recurrent abdominal pain, irritability, easy satiety, vomiting and weight loss may occur. The offending foods are sometimes difficult to determine, especially in older children. Food-specific IgE and a positive prick-test are seen in 50 %. Eosinophilia is also seen in 50 % of patients. Pathological examination is required to establish diagnosis, and eosinophil infiltration of the mucosa is observed [18].

Incidence of These Syndromes in Western Countries

In Israel, the prevalence was investigated in a large scale, population-based study. The cumulative incidence of FPIES was 0.34 %. Bloody diarrhea was seen in only 4.5 % of patients [19•].

Spergel et al. reported the prevalence of EoE and AEG in the USA as 52 and 28/100,000, respectively [20]. Chang et al. reviewed retrospective data for the USA and found that, unlike EoE, AEG is a rare disease [21]. Based on those results, EoE is much more prevalent in the USA compared to AEG.

GI Allergy in Japanese Neonates and Infants

GI Allergy Is Increasing in Japan

GI allergy in neonates and infants has been increasing in Japan since the late 1990s. Today, its incidence is 0.21 % [22]. There are three major differences in the clinical features of GI allergy between Western countries and Japan. (1) In FPIES, bloody stool is rare in Western countries, but frequent in Japan (47 % of patients). (2) Food-specific IgE antibody is negative in Western countries, but positive in 32 % of Japanese patients. (3) Peripheral blood eosinophils are normal in Western countries but often increased in Japan (Table 1). Since GI allergies are poorly described in Japanese textbooks and literature, doctors have to rely on accounts from Western countries. But if the clinical features of the patients differ in regard to the above three points, reaching a correct diagnosis can be difficult. Confusion and delayed diagnosis and treatment might occur. Approximately 10 % of patients develop severe complications such as mechanical ileus, perforation of the GI wall, shock and

Table 1 *Differences in clinical features of FPIES between Western countries and Japan*

		FPIES	
		Western countries	Japan
Symptoms and signs	Onset	First day-1 year	First day-1 year; mostly neonatal period
	Offending foods	Cow's milk, soy, etc.	Cow's milk, breast milk, rice, soy
	Vomiting	Frequent	Frequent
	Diarrhea	Frequent	Frequent
	Bloody stool	Rare	Frequent (47 %)
	Shock	10-15 %	10-15 %
	Weight loss	Present	Present
Laboratory data	Prick test	Negative	Negative
	Food-specific IgE antibodies	Negative	Positive in 30 %
	Peripheral blood eosinophils	Normal range	Often elevated maximum 70 % of white blood cells
	Stool mucous eosinophils	Sometimes positive	Often positive, especially with bloody stool
Food challenge test		Vomiting (3–4 hr) Diarrhea (5–8 hr)	Vomiting (3–4 hr), diarrhea (5–8 hr), bloody stool (next day)
Prognosis		Good	Good; remission by 3 years old

developmental retardation due to delayed start of treatment. Also, enteropathy and allergic eosinophilic gastroenteritis (AEG) require histological examination—which is not an easy technique for many pediatricians—for diagnosis.

Four Clusters Were Identified for GI Allergies in Neonates and Infants

We worried about this situation and tried to establish a method for classifying patients based only on the initial symptoms and clear-cut, simple clinical data [7•]. The goal would be prompt, proper diagnosis and treatment of affected babies. First, we included all the patients with suspicion of non-IgE-mediated gastrointestinal food allergies into one term, "GI allergy." We constructed a nation-wide database by using an internet online system. To date, clinical data for 450 babies have been collected from all over Japan (130 were treated in our department). We performed cluster analysis among patients whose confirmatory diagnosis was established by food challenge test, using five clinical parameters: birth weight, day of onset, severity of vomiting, severity of bloody stool and milk-specific IgE antibody titers. Four clusters were identified, and the discriminatory variables for cluster assignment were found to be vomiting and bloody stool (Fig. 1). Cluster 1 showed both vomiting and bloody stool. Since vomiting is the representative symptom of damage of upper GI tract and bloody stool is that of lower GI tract, inflammation of the GI mucosa can be imagined to spread to the entire GI tract. Cluster 2 showed vomiting but not bloody stool. So inflammation is imagined as prominent at the upper GI tract but not at lower GI tract. Cluster 3 showed neither vomiting nor bloody stool, but there was weight loss. Since weight loss seems to result from disturbed absorption of nutritional elements, the small intestine might be site of the main lesion in Cluster 3. Cluster 4 had bloody stool but no vomiting. Because many patients had red-colored fresh bloody stool, the bleeding sites may be located in the large intestine. At first, we did not know whether or not these four clusters had any important biological meaning, so we compared the clinical and laboratory data among the clusters. The day of onset was earlier in Clusters 1 and 4 (median 7th day of life) compared to Clusters 2 (median 16th day) and 3 (median 37th day). The birth weight was significantly less in Cluster 3 patients. The blood eosinophil percentages were significantly higher in Cluster 3 (median 26 %), although other clusters also showed abnormally high percentages. We can thus conclude that the 4 clusters have distinct biological differences. The greatest surprise was seen in the results of the food-challenge tests: in most patients, even after several months' remission, food-challenge generated the same symptoms as had been seen at initial onset of the disease. That means that the responsible immune cells remained in the same part of the GI tract.

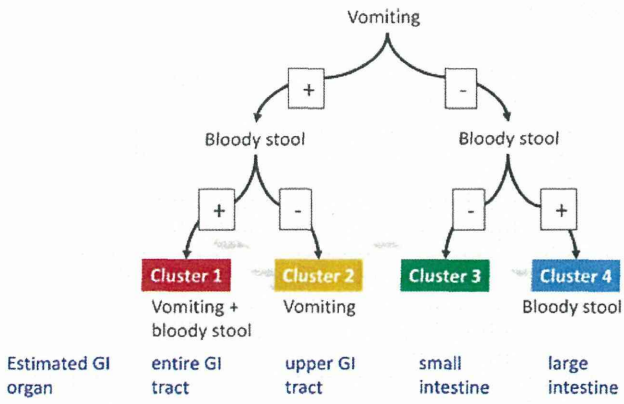

Fig. 1 Four clusters of GI allergies in neonates and infants. This classification can be used in any patients with suspected GI allergy. Estimation of affected organs in GI tract and prediction of the outcome in the food-challenge test are possible. When patients have both vomiting and bloody stool at initial presentation, they are classified as Cluster 1. One can estimate the affected organs of Cluster 1 as the entire GI tract. If patients have neither vomiting nor bloody stool but show weight loss, they can be classified as Cluster 3. The most affected organ might be the small intestine. (*Adapted with permission from* Nomura I, Morita H, Hosokawa S, et al.: Four distinct subtypes of non-IgE-mediated gastrointestinal food allergies in neonates and infants, distinguished by their initial symptoms, J Allergy Clin Immunol, volume 127(3):685–688.e8. Copyright 2011; Mosby, Inc.)

Cluster 1 showed vomiting and bloody stool at the same time. Bloody stool in FPIES has not often been reported in Western countries. However, Cluster 1 should be included in FPIES because the response in the food-challenge test is similar to FPIES. Cluster 2 is compatible with FPIES, but eosinophilia was seen in many patients. Although the blood of 30 % of Cluster 1 and 2 patients was positive for milk-specific IgE, these clusters could be diagnosed as FPIES. That is because they did not show any IgE-mediated reactions like urticaria or wheezing even in the food-challenge test at 5–8 months old, prick tests were all negative, reactions were confined to the GI organs, most symptoms started more than 2 hours after ingestion, and the food-challenge test showed reactions typical of FPIES. Cluster 3 resembles enteropathy or AEG. In Cluster 4, the diagnosis should be proctocolitis when weight gain is normal, but AEG when it is poor.

This classification is useful because it is easy to apply, using only the initial clinical data, and it will increase the likelihood of achieving a correct diagnosis. The involved portion of the GI tract can be deduced, and the outcome of the food-challenge test can be predicted.

A limitation of this cluster method is that it can be used only for babies under 6 months old. For older patients, another analytical method is needed. The symptoms during the first month after onset of the disease should be used, because a longer duration of chronic inflammation may lead to many other GI symptoms.

Remaining Problems in GI Allergies in Neonates and Infants

Two important problems remain to be solved. One is the difficulty of differential diagnosis at the initial presentation of the disease because of an absence of diagnostic tests. The other is a lack of in vitro tests to determine which food is responsible for the disease. Actually, this second issue is easier to circumvent in babies—who do not refuse an elimination diet aimed at identifying the offending food—than it is in teenagers suffering from AEG or EoE. In infants, when developing a diagnostic test, it is very important not to overlook GI allergy.

The lymphocyte proliferation test for GI allergy has been largely neglected by scientists in recent years. There was controversy regarding its diagnostic value for FPIES [23], but now it seems likely to become a reliable diagnostic method [24].

Pathological examination using advanced molecular techniques would be a very powerful diagnostic tool [8] for distinguishing the inflammation patterns of each clinical entity. Ohtsuka et al. performed microarray analysis of mucosal samples in infant with proctocolitis and reported expression of CCL-1 and CXCL-13 [25].

Since many patients begin to manifest symptoms within 2 weeks after birth, intrauterine sensitization is suspected. It would be of interest to investigate the immune mechanism of intrauterine sensitization. More importantly, risk factors for the development of GI allergy should be identified, enabling prevention of these diseases.

Older Children and Adults with EoE and AEG in Japan

EoE (eosinophilic esophagitis) and AEG (allergic eosinophilic gastroenteritis) are the two main GI allergy diseases in older children and adults. EoE is increasing in Western countries, and is now more prevalent than AEG. AEG seems to have only 10-50 % of EoE's prevalence [19•, 20]. In contrast, Japanese reports suggest that prevalence of pure EoE might be much lower than that of AEG's [26, 27]. For these reasons, systematic review of GI allergy from childhood to adults is needed to clarify the differences in the clinical features, laboratory data and histological distribution among human races, regions and age groups.

Conclusions

Much work remains to be done regarding non-IgE-mediated GI allergies. Clinical studies to elucidate the precise natures of these diseases are the most important. The frequencies of each offending food, prognosis and complications should be investigated. Systematic review of clinical data from all over the world is needed to compare racial and regional differences. Because prenatal exposure to risk factors may cause GI allergy in neonates, those factors must be investigated and identified. Immunological research is needed to develop reliable diagnostic tests for determination of offending foods and to understand the disease mechanisms. International cooperation to save babies living far from medical resources is important. Combination of these efforts will decrease the incidence of diseased babies and save lives.

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