Letter to the Editor

Food protein–induced enterocolitis syndromes with and without bloody stool have distinct clinicopathologic features

To the Editor:

The vast majority of IgE-mediated food allergy patients develop cutaneous symptoms, whereas non-IgE-mediated food allergy patients most frequently develop gastrointestinal symptoms. We have set up a nationwide online database of patients with non-IgE-mediated gastrointestinal food allergy and medical records of >600 patients have been registered. After hierarchical clustering analysis was applied to the clinical and laboratory findings of 136 patients who fulfilled ≥3 of the Powell criteria, the patients were classified into 4 distinct clusters according to the presence or absence of vomiting and bloody stool.[1] Among our findings, we were particularly intrigued by the fact that patients exhibiting severe vomiting—a characteristic feature of food protein–induced enterocolitis syndrome (FPIES)—were divided into 2 clinically different clusters according to the presence or absence of macroscopic bloody stool. Unlike reports from Western countries in which only 4% to 10% of patients with FPIES exhibited bloody stool,[2] our earlier study found that approximately 47% of patients with FPIES in Japan exhibit macroscopic bloody stool.[1,3] Because our earlier study also found that patients with FPIES with macroscopic bloody stool (FPIESwBS) (see Fig E1 in this article's Online Repository at www.jacionline.org) and patients with FPIES without macroscopic bloody stool (FPIESnoBS) had very similar clinical and laboratory findings (other than bloody stool), these results raised the questions of whether FPIESwBS is simply a more severe and/or chronic form of FPIESnoBS, and whether they are based on similar antigen-specific immune responses.

To solve these questions, this study newly enrolled 115 patients with FPIES to cow's milk who fulfilled ≥4 of the diagnostic criteria for FPIES described by Miceli Sopo et al[4]: (1) age <9 months at initial presentation; (2) exposure to the incriminated food elicited repetitive vomiting and/or diarrhea within 4 hours without any other cause for the symptoms; (3) symptoms limited to the gastrointestinal tract; (4) avoidance of the offending protein from the diet resulted in resolution of symptoms; and (5) a standardized food challenge or isolated reexposure elicited the typical symptoms. The demographic data of the patients with FPIESwBS and FPIESnoBS are shown in Tables E1 and E2 (in the Online Repository at www.jacionline.org). Antigen-specific lymphoproliferation tests and antigen-specific cytokine production assays were performed for 59 patients using each of 5 different milk proteins (α-lactalbumin, β-lactoglobulin, and α-casein, β-casein and κ-casein after lipopolysaccharide-depletion), as previously described (see details in the Methods in the Online Repository at www.jacionline.org).[5] Culture supernatants were harvested on day 6, and cytokine production profiles were measured by multiplex assay or ELISA. Because each patient responded to different milk proteins (see Fig E2 in the Online Repository at www.jacionline.org), we selected the 1 specific milk protein among 5 tested milk proteins that induced the maximal cytokine production or proliferation in that patient and then used those data for further analysis and the figures.

As previously described,[6] the levels of such proinflammatory cytokines as TNF and IL-6, as well as T_H1 and T_H2 cytokines, in the culture supernatants of milk protein-stimulated PBMCs from the patients were significantly increased compared with those in the culture supernatants of vehicle control-stimulated PBMCs (Fig 1, *B-D*). Although stimulation index of the antigen-specific lymphoproliferation tests and the level of IL-2 in the culture supernatants were slightly higher in patients with FPIESwBS than with FPIESnoBS (Fig 1, *A*), the levels of such proinflammatory cytokines as TNF-α, IL-6, and IL-1β were comparable in the 2 patient groups (Fig 1, *B*). In addition, the concentrations of a T_H1 cytokine (IFN-γ) and a T_H17 cytokine (IL-17) were also comparable in the 2 groups (Fig 1, *D*). In sharp contrast, however, the concentrations of T_H2 cytokines—that is, IL-4, IL-5 and IL-13—were significantly higher in patients with FPIESwBS than in patients with FPIESnoBS (Fig 1, *C*), as was the concentration of a regulatory cytokine, IL-10 (Fig 1, *D*). These results clearly showed that, despite the similarity in proinflammatory properties, patients with FPIESwBS and FPIESnoBS have clearly different antigen-specific T-helper cytokine production profiles. The significant correlations of the IL-13 concentration with both the IL-5 and IL-10 concentrations strongly suggested that the T_H2-prone cytokine profiles in FPIESwBS were due to "classic" antigen-specific T_H2 cells (see Fig E3 in this article's Online Repository at www.jacionline.org).

In addition to the antigen-specific T-cell responses, 2 key features differed significantly between FPIESwBS and FPIESnoBS despite the similarity in most of their clinical features (other than bloody stool). First, the day of onset in patients with FPIESwBS (day 7 [IQR, 8-10]) was significantly earlier ($P < .0001$) than that in patients with FPIESnoBS (day 26 [IQR, 8.5-100], which is in agreement with findings for a Western country[7,8]) (Table E1). Importantly, this early onset feature in patients with FPIESwBS was also observed in our earlier report.[1] Such early onset implies involvement of prenatal or perinatal events, but none of gestational age, birth weight, the mode of delivery, or feeding differed between FPIESwBS and FPIESnoBS (Table E1). Possible environmental factors associated with FPIES warrant further investigation.

Second, by following 37 patients for up to 38 months, we found that patients with FPIESwBS (median follow-up, 7 months) developed tolerance significantly earlier than those with FPIESnoBS (median, 23 months) (Fig 2), and also earlier than patients with FPIES in previous reports.[7-9]

Although such T_H2 cytokines as IL-4, IL-5, and IL-13 were significantly produced by PBMCs from patients with FPIESwBS compared with patients with FPIESnoBS, the peripheral neutrophilia, eosinophilia, total IgE levels, and specific-IgE to milk, soy, wheat, and rice at onset and during follow-up were comparable in these 2 groups (Table E2), suggesting that cytokine production was enhanced only in the inflamed tissue sites. That notion is supported by reappearance of bloody stool in oral food challenge tests even several months after the initial symptoms, which strongly suggests involvement of antigen-specific immune cells remaining in the gastrointestinal tract.[1] Therefore, we believe that not only inflammatory cytokines but also T_H2 cytokines may play some roles in the pathogenesis of bloody stool.

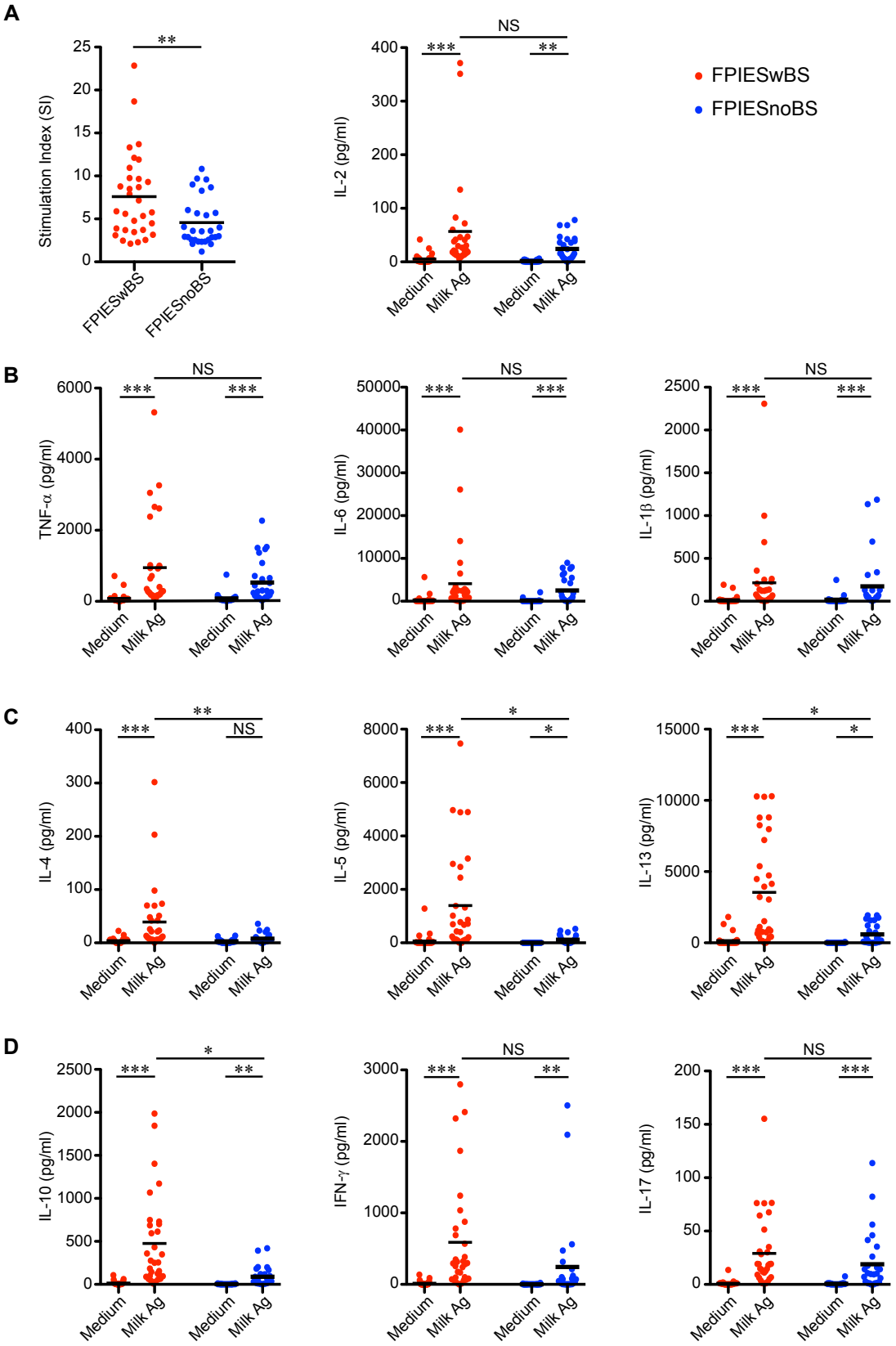

FIG 1. Milk protein-specific lymphocyte proliferation and cytokine production in patients with FPIESwBS and FPIESnoBS. PBMCs from each patient were stimulated separately with each of 5 different milk proteins (α-lactalbumin, β-lactoglobulin, α-casein, β-casein, and κ-casein). The data show the highest concentration of each cytokine or the highest proliferation detected in response to each stimulus. The stimulation index was calculated as milk protein-induced ^{3}H-thymidine uptake (cpm)/vehicle-induced ^{3}H-thymidine uptake (cpm). **A,** Proliferation and IL-2 levels. **B,** TNF-α, IL-6, and IL-1β levels. **C,** IL-4, IL-5, and IL-13 levels. **D,** IL-10, IFN-γ, and IL-17 levels. Each dot represents data from 1 patient, and medians are shown. *P < .05, **P < .01, and ***P < .001.

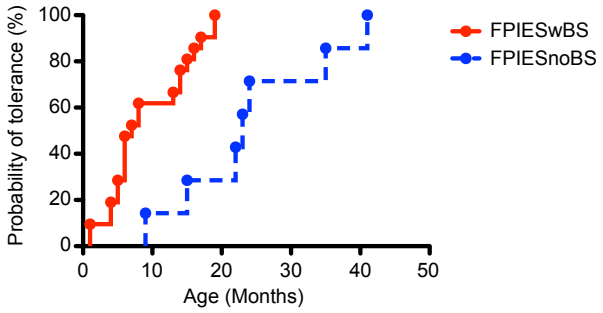

FIG 2. Kaplan-Meier plot for cumulative probability of cow's milk tolerance in patients with FPIES. FPIESwBS *(solid line)* and FPIESnoBS *(dotted line)*. Twenty-nine patients with FPIES (FPIESwBS, n = 21; FPIESnoBS, n = 8) were followed for ≤38 months until they showed tolerance to milk ingestion.

Regulatory cytokines such as IL-10 were also significantly produced by PBMCs from patients with FPIESwBS compared with patients with FPIESnoBS. Because IL-10 is known to be a suppressive cytokine of T-cell proliferation and is essential for peripheral tolerance to allergens, increased production of IL-10 in patients with FPIESwBS may contribute to the better prognosis of this manifestation of the disease.

In conclusion, patients with FPIESwBS and FPIESnoBS share several clinical features and proinflammatory properties in their antigen-specific immune responses. However, they have obviously different cytokine production profiles and several different key clinical features such as the day of onset and the prognosis, suggesting that FPIESwBS is not simply a more severe form of FPIESnoBS.

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Hideaki Morita, MD, PhD[a]
Hiroko Suzuki, MD, PhD[a]
Kanami Orihara, PhD[a,b]
Kenichiro Motomura, MD[a]
Akio Matsuda, PhD[a]
Yukihiro Ohya, MD, PhD[c]
Hirohisa Saito, MD, PhD[a]
Ichiro Nomura, MD, PhD[a,c]
Kenji Matsumoto, MD, PhD[a]

From [a]the Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, Japan; [b]the Waseda Institute for Advanced Study, Waseda University, Tokyo, Japan; [c]the Division of Allergy, National Center for Child Health and Development, Tokyo, Japan. E-mail: morita-hi@ncchd.go.jp. Or: matsumoto-k@ncchd.go.jp.

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REFERENCES

1. Nomura I, Morita H, Hosokawa S, Hoshina H, Fukuie T, Watanabe M, 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;127:685-8, e1-8.
2. Mane SK, Bahna SL. Clinical manifestations of food protein-induced enterocolitis syndrome. Curr Opin Allergy Clin Immunol 2014;14:217-21.
3. Nomura I, Morita H, Ohya Y, Saito H, Matsumoto K. Non-IgE-mediated gastrointestinal food allergies: distinct differences in clinical phenotype between Western countries and Japan. Curr Allergy Asthma Rep 2012;12:297-303.
4. Miceli Sopo S, Greco M, Monaco S, Tripodi S, Calvani M. Food protein-induced enterocolitis syndrome, from practice to theory. Expert Rev Clin Immunol 2013;9:707-15.
5. Morita H, Nomura I, Orihara K, Yoshida K, Akasawa A, Tachimoto H, et al. Antigen-specific T-cell responses in patients with non-IgE-mediated gastrointestinal food allergy are predominantly skewed to T(H)2. J Allergy Clin Immunol 2013;131:590-2, e1-6.
6. Caubet JC, Bencharitiwong R, Ross A, Sampson HA, Berin MC, Nowak-Wegrzyn A. Humoral and cellular responses to casein in patients with food protein-induced enterocolitis to cow's milk. J Allergy Clin Immunol 2017;139:572-83.
7. Nowak-Wegrzyn A, Katz Y, Mehr SS, Koletzko S. Non-IgE-mediated gastrointestinal food allergy. J Allergy Clin Immunol 2015;135:1114-24.
8. Katz Y, Goldberg MR, Rajuan N, Cohen A, Leshno M. The prevalence and natural course of food protein-induced enterocolitis syndrome to cow's milk: a large-scale, prospective population-based study. J Allergy Clin Immunol 2011;127:647-53, e1-3.
9. Caubet JC, Ford LS, Sickles L, Jarvinen KM, Sicherer SH, Sampson HA, et al. Clinical features and resolution of food protein-induced enterocolitis syndrome: 10-year experience. J Allergy Clin Immunol 2014;134:382-9.

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METHODS

Subjects and ethics

This study enrolled 115 patients who visited the outpatient clinics of the Japanese Research Group for Neonatal, Infantile Allergic Disorders during the period from December 2009 through October 2014 and who fulfilled ≥4 of diagnostic criteria for FPIES described by Miceli Sopo et al.[E1] This study was approved by the regional ethics committees and the Ethics Committee of the National Center for Child Health and Development, and written informed consent was obtained from the guardians of all patients.

Antigen-specific lymphoproliferation tests and antigen-specific cytokine production assays

Heparinized peripheral blood samples were stored at room temperature and transferred to the National Research Institute for Child Health and Development in Tokyo. PBMCs were obtained by Ficoll-Hypaque gradient sedimentation (Lymphocyte Separation Medium; ICN Biochemicals, Aurora, Ohio). For the lymphoproliferation tests, the PBMCs were suspended at a cell density of 1×10^6/mL in AIM V medium (Gibco, Grand Island, NY) without serum, and for cytokine production assays they were suspended in RPMI1640 medium (GIBCO/Life Technologies, Gaithersburg, Md) containing 5% autologous plasma. Lymphoproliferation was measured by ^{3}H-thymidine (Amersham, Tokyo, Japan) uptake during a 16-hour period following 5-day stimulation with 100 μg/mL of each of 5 LPS-depleted milk-protein preparations (α-lactalbumin, Sigma, St. Louis, Mo; β-lactoglobulin, Bean Stalk Snow, Shinjuku-Ku, Japan; α-, β- and κ-caseins, Sigma) at 37°C in a humidified 5% CO_2 atmosphere. Incorporated ^{3}H-thymidine was counted with a liquid scintillation counter (TopCount NXT; PerkinElmer Life Sciences, Boston, Mass). The stimulation index was calculated as milk protein-specific ^{3}H-thymidine uptake (counts per minute: cpm)/vehicle-induced ^{3}H-thymidine uptake (cpm). Culture supernatants were harvested on day 6, and the cytokine production profiles were investigated using Luminex multiplex cytokine analysis kits (Millipore, Bedford, Mass) and ELISA (R&D Systems, Minneapolis, Minn).

Statistical analysis

Differences between groups were analyzed using the Kruskal-Wallis test or Mann-Whitney *U* test.

REFERENCE

E1. Miceli Sopo S, Greco M, Monaco S, Tripodi S, Calvani M. Food protein-induced enterocolitis syndrome, from practice to theory. Expert Rev Clin Immunol 2013; 9:707-15.

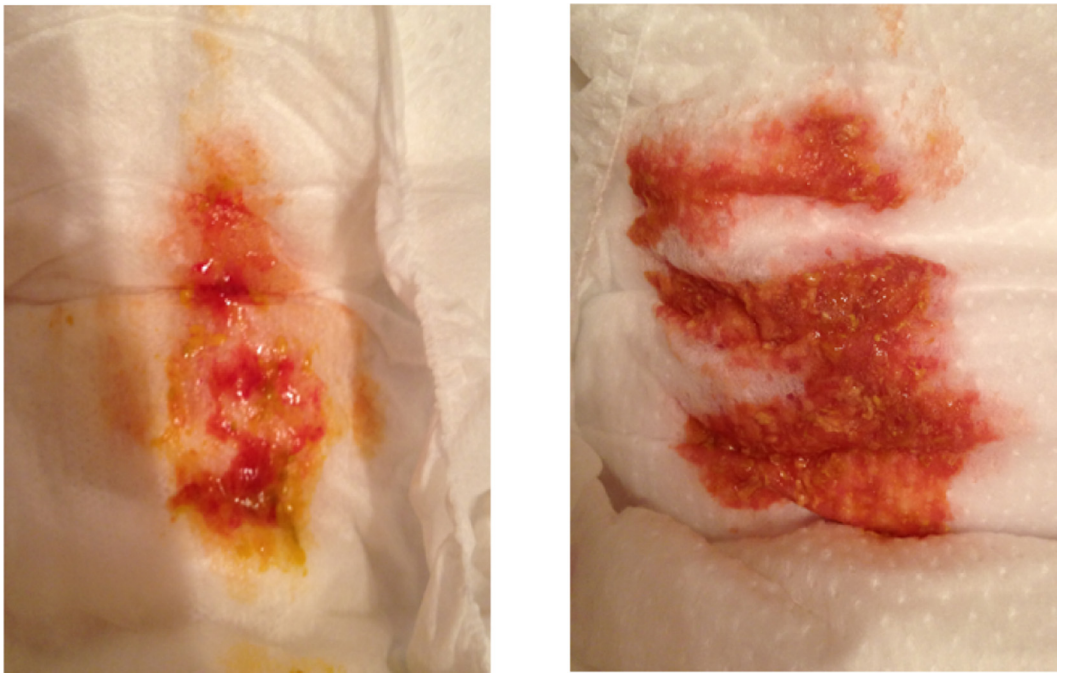

FIG E1. Microscopic bloody stools seen in patients with FPIESwBS.

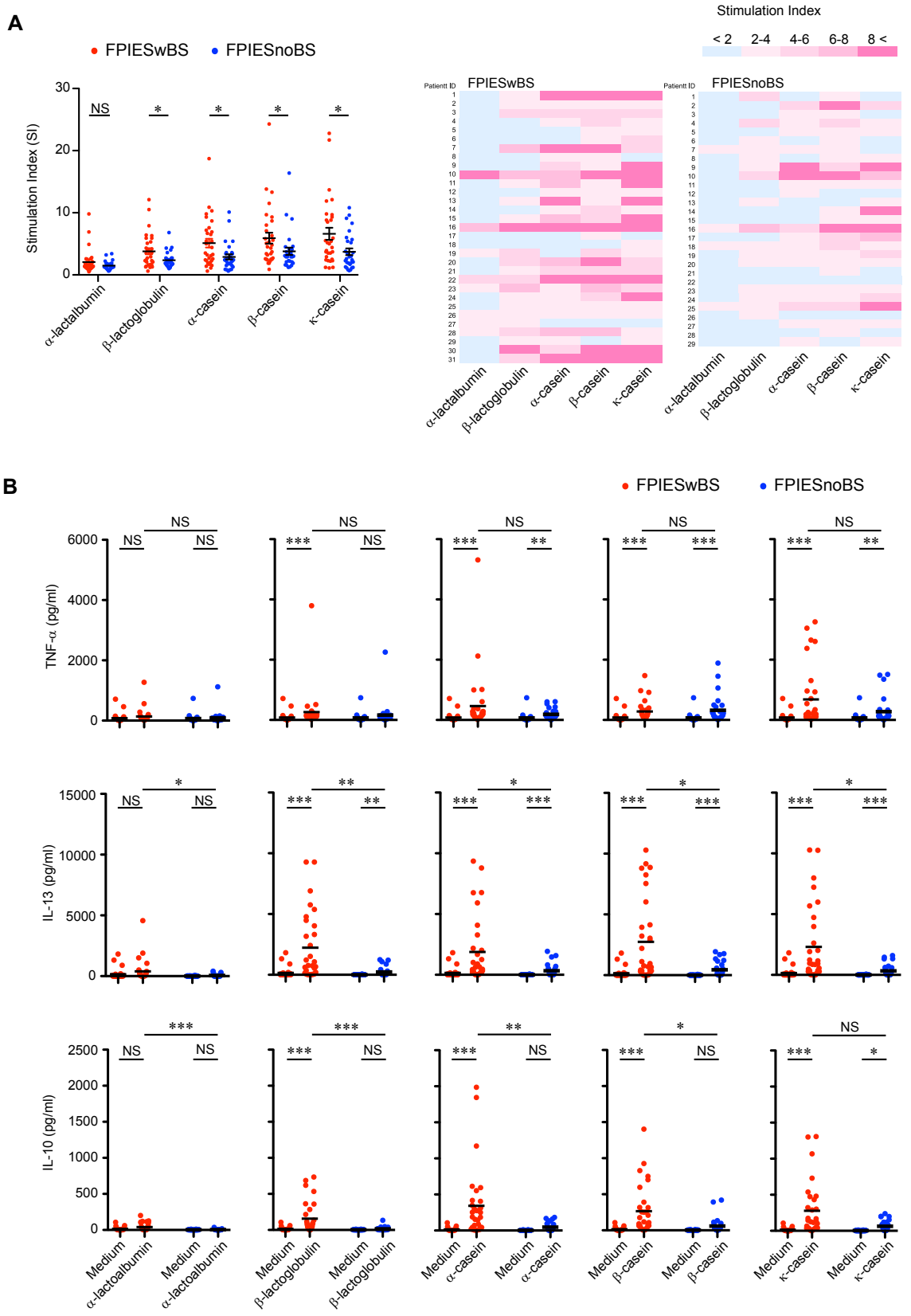

FIG E2. Responses to each milk protein component. PBMCs from children with FPIES were stimulated separately with 100 μg/mL of each of 5 LPS-depleted cow's milk protein preparations. **A,** The stimulation index was calculated as milk protein-induced ^{3}H-thymidine uptake (cpm)/vehicle-induced ^{3}H-thymidine uptake (cpm). **B,** The cytokine production profiles were measured by multiplex assay or ELISA. Each dot represents data from 1 patient, and medians are shown. *$P < .05$, **$P < .01$, and ***$P < .001$.

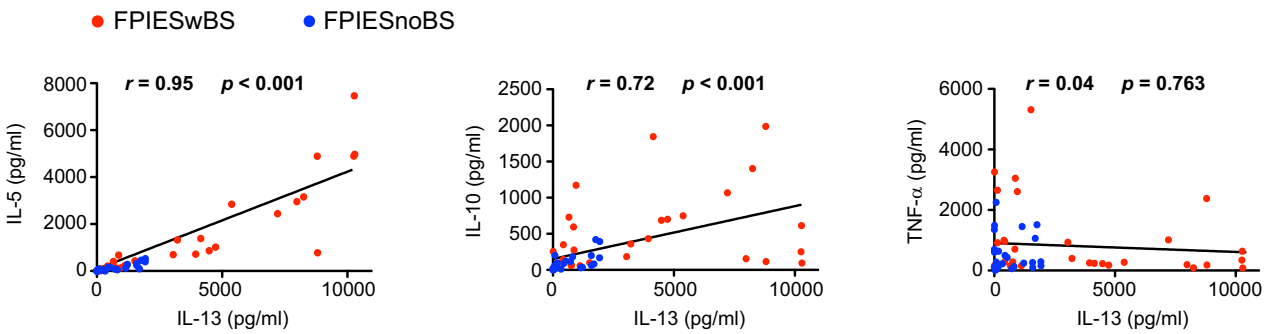

FIG E3. Correlation among cytokines in culture supernatants. PBMCs from children with FPIES were stimulated separately with 100 μg/mL of each of 5 LPS-depleted cow's milk protein preparations in the presence of 5% autologous plasma for 6 days. The concentration of IL-13 in the culture supernatants correlated significantly with the concentrations of IL-5 and IL-10, but not with the concentration of TNF-α.

TABLE E1. Demographic characteristics of patients

	n	FPIESwBS (n = 65)	n	FPIESnoBS (n = 50)	*P* value
Female:male		33:32		25:25	
Onset (d)	65	7.0 (6.0, 8.0)	50	26.0 (8.5, 100.0)	<.0001*
Delivery					
Vaginal	64	67.1 (43/64)	46	80.4 (37/46)	.092
Caesarean section	64	32.8 (21/64)	46	19.6 (9/46)	.136
Vacuum extraction	64	4.7 (3/64)	46	2.2 (1/46)	.639
Forceps delivery	64	1.6 (1/64)	46	0 (0/46)	1
Gestational age (wks)	50	38 (36, 39)	47	39 (36, 40)	.461
Birth weight (g)	65	2825 (2428, 3523)	48	2820 (2351, 3120)	.276
Nutrition					
Bottle-feeding		52.3 (34/65)		52.0 (26/50)	1
Mixed-feeding		46.2 (30/65)		40.0 (20/50)	.572
Exclusive breast-feeding		1.5 (1/65)		8.0 (4/50)	.165
Symptoms					
Vomiting	65	100 (65/65)	50	100 (50/50)	—
Bloody stool	65	100 (65/65)	50	0 (0/50)	—
Diarrhea	65	36.9 (24/65)	50	40.0 (20/50)	.847
Failure to thrive	65	41.5 (27/65)	50	38.0 (19/50)	.848
Lethargy	65	43.1 (28/65)	50	40.0 (20/50)	.850
Fever	65	15.4 (10/65)	50	22.0 (11/50)	.466
Eczema	65	3.1 (2/65)	50	14.0 (7/50)	.040*
Ileus	65	13.8 (9/65)	50	8.0 (4/50)	.386
Hypotension	65	3.1 (2/65)	50	6.0 (3/50)	.651
Acidosis	65	4.6 (3/65)	50	2.0 (1/50)	.631
Amount of milk to induce symptoms in oral food challenge test (mL)	10	37 (27, 80)	13	50 (22, 100)	.663

Data are expressed as the medians (interquartile ranges) or percentages (n of n). *P* values were calculated using the chi-square test or Fisher exact test.
*$P < .05$.

TABLE E2. Laboratory findings of patients

	n	FPIESwBS (n = 65)	n	FPIESnoBS (n = 50)	*P* value
WBCs at onset (/μL)	62	13,205 (9,885, 20,325)	43	12,400 (9,690, 18,820)	.614
WBC max (/μL)	54	18,500 (13,975, 24,325)	33	19,300 (13,640, 24,800)	1
Neutrophils at onset (/μL)	56	5,522 (3,640, 8,411)	31	4,604 (2,844, 11,266)	.509
Neutrophil max (/μL)	45	8,143 (6,721, 13,692)	26	9,779 (6,608, 14,945)	.799
Eosinophil at onset (/μL)	57	493 (294, 818)	33	530 (264, 1353)	.461
Eosinophils max (/μL)	49	1,981 (833, 3,647)	26	2,145 (853, 3,708)	.879
CRP at onset (mg/dL)	62	0.29 (0.10, 0.70)	40	0.31 (0.04, 0.96)	.724
CRP max (mg/dL)	49	0.73 (0.30, 2.00)	30	0.98 (0.37, 6.53)	.231
Total IgE at onset (IU/mL)	53	5.0 (2.0, 8.5)	39	5.0 (3.0, 15.3)	.107
Specific IgE at onset (IU/mL)					
Milk	50	0.0 (0.0, 0.38)	37	0.0 (0.0, 0.0)	.492
Casein	38	0.0 (0.0, 0.0)	29	0.0 (0.0, 0.0)	.781
α-lactalbumin	37	0.0 (0.0, 0.0)	26	0.0 (0.0, 0.0)	.587
β-lactoglobulin	37	0.0 (0.0, 0.35)	29	0.0 (0.0, 0.0)	.073
OVA	15	0.0 (0.0, 0.0)	21	0.0 (0.0, 0.0)	.141
Soy	15	0.0 (0.0, 0.0)	19	0.0 (0.0, 0.0)	—
Wheat	10	0.0 (0.0, 0.0)	15	0.0 (0.0, 0.0)	—
Rice	7	0.0 (0.0, 0.0)	11	0.0 (0.0, 0.0)	—
Milk specific IgE positivity at 6-mo follow-up	14	21.4 (3/14)	8	12.5 (1/8)	1

Data are expressed as medians (interquartile ranges) or percentages (n of n). *P* values were calculated by chi-square test or Fisher exact test.
CRP, C-reactive protein; *OVA*, ovalbumin; *WBC*, white blood cells.