Original Article

Neutrophilia and hyperamylasemia in patients with immediate food allergy

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Abstract   **Background**: Presently, little is known about the laboratory data several hours after oral food challenge (OFC) in patients with immunoglobulin (Ig)E-mediated immediate food allergy (FA).
Methods: One hundred and twelve subjects who underwent OFC at the present institute between 1 June 2016 and 31 March 2018, were enrolled in this study. Changes in laboratory data several hours after OFC were examined.
Results: OFC was positive in 76 patients and negative in 36. Increase in absolute neutrophil count (ANC) was significantly higher in OFC-positive than in OFC-negative subjects (median, 2,306/μL vs 637/μL; $P < 0.00001$). On multivariate regression analysis, a significant correlation was seen between neutrophilia and the development of gastrointestinal symptoms ($t = 3.63$; $P < 0.001$). Serum interleukin-6 increased in 43.8% of the patients with marked neutrophilia and had a significant positive correlation with ANC ($r = 0.64$; $P < 0.001$). Serum amylase increased in 33.3% of the OFC-positive patients and was >100 U/L (median, 642 U/L) in five patients in whom serum lipase also increased markedly (1,197 U/L). There was a significant negative correlation between increase in serum amylase and decrease in absolute eosinophil count ($r = -0.36$, $P < 0.01$).
Conclusions: Marked neutrophilia was seen after OFC in patients with immediate FA presenting gastrointestinal symptoms, which may provide an insight into the relationship between symptoms and laboratory data. A considerable increase in serum amylase after OFC was also seen in patients with immediate FA, suggesting that the pancreas is a target organ for immediate FA.

Key words   amylase, eosinophil, food protein-induced enterocolitis syndrome, IgE-mediated food allergy, neutrophil.

Immunoglobulin (Ig)E-mediated food allergy (FA), or immediate FA, is the predominant form of FA in which histamine, released from mast cells after cross-linking the cell surface IgE antibody, plays a critical role in the induction of various clinical manifestations in the early phase reactions (EPR).[1,2] Cutaneous, respiratory, and gastrointestinal (GI) symptoms are well-known major manifestations in EPR of immediate FA. So far, however, little is known about the laboratory findings in patients with immediate FA, except for histamine.

In contrast to immediate FA, food protein-induced enterocolitis syndrome (FPIES) is a representative non-IgE-mediated FA characterized by GI symptoms such as vomiting, diarrhea, and bloody stool.[3,4] Although immediate FA is an entirely different disease from FPIES by definition, the actual distinction between them may not be so simple. For example, although patients with typical FPIES lack food-specific IgE antibody (sIgE), some patients have increased serum food-sIgE and are classified as

having atypical FPIES.[5–7] Further studies are needed to understand the relationship between immediate FA and FPIES.

Acute pancreatitis due to immediate FA has been sporadically reported.[8] In some cases, pancreatitis developed after oral food challenge (OFC).[9] Although harmful effects of OFC on the pancreas have not yet been recognized, clinicians should pay attention to laboratory data in patients with immediate FA to prevent serious complications.

In this study we therefore analyzed changes in laboratory data several hours after OFC in patients with immediate FA, in order to (i) investigate the increase in ANC after OFC, a well-known laboratory features of FPIES,[3,7,10] to facilitate understanding of the relationship between immediate FA and FPIES; and (ii) elucidate the changes in serum pancreatic enzymes, to determine the possible effect of OFC on the pancreas.

Methods

Subjects

Subjects who underwent OFC between 1 June 2016, and 31 March 2018, for the diagnosis of immediate FA or the

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estimation of tolerance acquisition, were enrolled in this study. Patients with suspected FPIES were not included in this study. Subjects who had clear cutaneous, respiratory, or GI symptoms after OFC were classified into the OFC-positive immediate FA group, while those who did not show any symptoms were allocated to the OFC-negative control group.

Patients in the OFC-positive group were usually treated as needed with anti-histamines, inhalation of β-2 adrenergic agonist, and/or i.v. fluid infusion. Three patients given adrenalin and/or corticosteroids for the treatment of symptoms were not included in this study because those drugs could cause neutrophilia.

Clinical subject data were collected from medical records.

OFC protocol

Oral food challenge was performed according to institutional protocol in a ward of the present hospital, in which infectious disorders are critically controlled. Health condition, as well as prior contact with sick persons, was also checked before admission. The initial dose of food varied from 0.1 g to 3 g (for cow's milk [CM], 0.1–3 mL), which was determined according to sIgE level, subject age, or the severity of previous episodes. If subjects showed no symptoms during a 20 min observation period, the quantity of food was increased three times in the next challenge. This process was repeated three times if the subjects had no reaction. If subjects developed any symptoms, the test was discontinued instantly, and medical intervention was applied as soon as required. All subjects were instructed to remain in the institute for 6 h after OFC for observation of symptoms.

Before OFC, venous access was routinely gained in preparation for severe allergic reactions. Blood samples for laboratory examinations were obtained twice via this venous access before, and 5–6 h after OFC.

Laboratory tests

Absolute neutrophil count (ANC), absolute eosinophil count (AEC), serum amylase, and plasma cortisol were measured simultaneously with other routine blood examinations before and 5–6 h after OFC. In some cases, amylase isozyme and the serum lipase were also measured.

Changes in laboratory data were estimated using the difference in value after OFC. Exceptionally, changes in plasma cortisol were estimated using the ratio of that after OFC to that before OFC (i.e. fold change) to reduce the influence of individual variation before OFC.

An increase in ANC ≥3,500/μL after OFC was defined as a marked increase. The upper limit of the change in the serum amylase was set at 4 U/L, which corresponds to the 99th percentile in the OFC-negative group. Similarly, the upper limit of the ratio of plasma cortisol was set to 1.81.

Total IgE and food-sIgE were measured using the Immuno-CAP system (Thermo Fisher Scientific, Tokyo, Japan). Serum levels of 10 cytokines (interleukin [IL]-1β, −2, −4, −5, −6, −8, −10, −12p70, interferon [IFN]-γ, and tumor necrosis factor [TNF]-α) were measured using an AimPlex Immunoassay kit (YSL Bioprocess Development, Pasadena, CA, USA). Change in each serum cytokine was estimated using the ratio of that after OFC to that before OFC (i.e. fold change), similar to the method for plasma cortisol. The upper limit of the change in each cytokine was set to the 99th percentile in the OFC-negative group.

Ethics

This study was approved by the ethics committee of Shizuoka Children's Hospital, Japan (2015-78). The parents provided written informed consent before participating in this study, and patient anonymity was preserved using methods approved by the ethics committee.

Statistical analysis

Mann–Whitney *U*-test was used to estimate the significance of the differences. The significance of the difference in frequency was estimated using Fisher's exact test, and the significance of the correlation was calculated using Spearman's rank correlation coefficient. The significance of the association of a laboratory parameter with various factors was estimated on multivariate regression analysis. All analyses were performed using STATA version 13 (Light Stone, Tokyo, Japan).

Results

Subject profile

One hundred and twelve subjects were analyzed in this study (Table 1). Seventy-six subjects had a positive OFC (35 to hen's egg white [EW]; 21 to CM; 10 to wheat; and 10 to nuts: peanut, $n = 6$; walnut, $n = 3$; cashew nut, $n = 1$]) and were classified into the OFC-positive immediate FA group. In contrast, 36 subjects were negative at OFC (17 to EW; six to CM; six to wheat; and seven to nuts: peanut, $n = 5$; walnut, $n = 1$; almond, $n = 1$), and were assigned to the OFC-negative control group. There were no significant differences in age, sex ratio, frequency of complications, quantity of challenged food, or serum total IgE between these two groups. Although median serum sIgE was higher in the OFC-positive group than in the OFC-negative group, the difference was not significant.

Increase in ANC

Change in ANC after OFC was significantly larger in the OFC-positive group than in the OFC-negative group (median, 2,306 vs 637/μL; $P < 0.00001$; Table 2). Marked increase in ANC (≥3,500/μL) after OFC occurred in 38.2% of patients in the OFC-positive group, which was significantly higher than in the OFC-negative group (0%; $P < 0.00001$; Fig. 1a). The prevalence of marked increase in ANC varied according to food: it was highest for nuts (90.0%; 9/10), lowest for CM

Table 1 Subject profile

Food	OFC	n	Age (years) Median (range)	Sex (M/F)	Complication AD % (n)	Complication BA % (n)	Quantity of food ingested at OFC (g) or (mL)† Median (range)	Time between ingestion of food and symptoms (min) Median (range)	Total IgE (IU/mL) Median (range)	sIgE (kU/L) Median (range)
Total	Total	112	5 (1–12)	81/31	82.1 (92/112)	20.5 (23/112)			440 (5–3,900)	
	Positive	76	5 (1–12)	57/19	82.9 (63/76)	23.7 (18/76)			370 (20–3,900)	
	Negative	36	4 (1–11)	24/12	80.6 (31/36)	13.8 (5/36)			756 (5–3,544)	
EW	Positive	35	5 (1–12)	26/9	82.9 (29/35)	17.1 (6/35)	3 (0.1–30)	20 (5–180)	320 (17–3,900)	27.9 (1.92–>100)
	Negative	17	2 (1–8)	10/7	82.4 (14/17)	5.9 (1/17)	3 (1–30)		370 (17–3,000)	15.5 (0.77–>100)
CM	Positive	21	4 (1–12)	17/4	90.5 (19/21)	33.3 (7/21)	3 (0.1–30)†	20 (3–180)	320 (83–2,400)	12.6 (2.89–>100)
	Negative	6	4 (1–11)	6/0	66.7 (4/6)	23.8 (1/6)	30 (1–100)†		700 (5–2,355)	3.88 (0.13–50)
Wheat	Positive	10	2.5 (1–12)	7/3	80.0 (8/10)	30.0 (3/10)	10 (3–100)	23 (5–120)	775 (83–2,800)	27.8 (1.2–>100)
	Negative	6	4.5 (1–7)	4/2	83.3 (5/6)	0 (0/6)	10 (1–30)		520 (21–3,544)	4.57 (0.21–>100)
Nuts	Positive	10	6.5 (5–11)	7/3	70.0 (7/10)	20.0 (2/10)	3 (0.3–10)	31 (5–74)	420 (72–2,700)	‡
	Negative	7	6 (3–7)	4/3	85.7 (6/7)	42.9 (3/7)	10 (3–10)		1,247 (48–3,197)	§

†Volume of cow's milk is expressed as mL; ‡Peanut, n = 6; median, 13.1 kU/L; range, 0.68–36.6 kU/L; walnut, n = 3; median, 6.57 kU/L; range, 2.52–8.22 kU/L; cashew nut, n = 1; median, 5.26 kU/L. §Peanut, n = 5; median, 0.54 kU/L; range, 0.21–>100 kU/L; walnut, n = 1; median, 3.02 kU/L; almond, n = 1; median, 4.4 kU/L. AD, atopic dermatitis; BA, bronchial asthma; CM, cow's milk; EW, hen's egg white; IgE, immunoglobulin E; OFC, oral food challenge; sIgE, allergen-specific IgE antibody.

(4.8%; 1/21), and intermediate for EW (45.7%; 16/35) and wheat (30%; 3/10; Fig. 1b).

Factors associated with neutrophilia

On multivariate regression analysis, change in ANC was significantly correlated with the development of GI symptoms ($t = 3.63$; $P < 0.001$) and skin symptoms ($t = 2.75$; $P < 0.01$) but not with respiratory symptoms (Table 3). In fact, the prevalence of marked increase in ANC was significantly higher in patients with GI symptoms than in those without GI symptoms (58.1%, 25/43 vs 12.1%, 4/33; $P < 0.001$; Fig. 2a).

With regard to skin symptoms, an extreme increase in ANC (>7,000/μL) was seen only in patients with skin symptoms, although the prevalence was not significantly different from that in patients without skin symptoms (19.6%, 11/56 vs 0%, 0/20; $P = 0.058$).

Increase in ANC did not correlate significantly with age, sex, or total IgE antibody.

Change in the number of other peripheral blood cells

Decrease in AEC after OFC was significantly larger in OFC-positive than in OFC-negative subjects (median, −188 vs −78/μL; $P < 0.01$; Table 2). In OFC-positive patients, change in AEC was significantly negatively correlated with serum total IgE ($r = -0.32$; $P < 0.01$) and change in ANC ($r = -0.52$; $P < 0.0001$; data not shown).

There was no significant difference in change of absolute lymphocyte or of monocyte count between the OFC-positive and -negative groups.

Fold change in serum cytokines

Fold change in serum cytokines was examined in 16 OFC-positive patients with marked neutrophilia (EW, $n = 9$; nuts, $n = 6$; wheat, $n = 1$) and in nine OFC-negative patients (EW, $n = 4$; nuts, $n = 5$). Serum IL-6 was increased in 43.8% of subjects (7/16) with marked neutrophilia (Fig. 3a). There was a significant positive correlation between increase in ANC and fold change in serum IL-6 ($r = 0.64$; $P < 0.001$; Fig. 3b).

Two patients and one patient with marked neutrophilia had a considerable increase in serum IL-8 and in IL-10, respectively (Fig. 3a). The fold change in those cytokines, however, did not correlate with an increase in ANC. No remarkable increases were seen in the other seven cytokines: IL-1β, IL-2, IL-4, IL-5, IL-12p70, IFN-γ, or TNF-α (Fig. 3c).

Increase in serum amylase

Change in serum amylase was not significantly different between the OFC-positive and -negative groups (Table 2). The prevalence of a distinct increase (≥4 U/L) was significantly higher in OFC-positive patients than in OFC-negative subjects (33.3%, 21/63 vs 3.0%, 1/33; $P < 0.001$; Fig. 4a). An increase in serum amylase >100 U/L occurred in five OFC-positive

Table 2 Change in data after oral food challenge

	OFC		*P*-value
	Positive Median (range) (*n*)	Negative Median (range) (*n*)	
Neutrophils (/μL)	2,306 (−1,298 to 17 325) (76)	637 (−2,130 to 2,918) (36)	<0.00001
Eosinophils (/μL)	−188 (−980 to 294.6) (76)	−78 (−639 to 99.1) (36)	<0.01
Lymphocyte (/μL)	−353 (−3,692 to 976) (76)	−259 (−6,492 to 1,387) (36)	NS
Monocyte (/μL)	16 (−1,094 to 568) (76)	2 (−1,104 to 218) (36)	NS
Amylase (U/L)	−3 (−34 to 1,117) (63)	−3 (−21 to 4) (33)	NS
Cortisol (μg/dL)[†]	0.86 (0.19 to 5.02) (63)	0.91 (0.39 to 1.82) (24)	NS
BUN (mg/dL)	1 (−7 to 7) (76)	1 (−4 to 6) (36)	NS
CRE (mg/dL)	0.02 (−0.12 to 0.20) (76)	0.02 (−0.12 to 0.24) (36)	NS
T.Bil (mg/dL)	0.0 (−0.2 to 0.2) (75)	0.0 (−0.2 to 0.3) (35)	NS
AST (IU/L)	−1 (−24 to 23) (72)	−1 (−7 to 7) (36)	NS
ALT (IU/L)	−1 (−3 to 9) (72)	0 (−4 to 3) (35)	NS
LDH (IU/L)	−10 (−117 to 203) (71)	−9 (−80 to 84) (35)	NS
ALP (IU/L)	−31 (−308 to 89) (51)	−26 (−144 to 45) (23)	NS
γ-GTP (IU/L)	−1 (−8 to 2) (63)	−1 (−3 to 1) (34)	NS
CK (IU/L)	−11 (−33 to 41) (65)	0.5 (−28 to 64) (32)	<0.0001
TP (g/dL)	−0.3 (−1.0 to 0.3) (76)	−0.2 (−0.9 to 0.2) (36)	NS
Alb (g/dL)	−0.1 (−0.6 to 0.3) (76)	−0.1 (−0.6 to 0.2) (36)	NS
Na (mmol/L)	0 (−4 to 3) (71)	0 (−3 to 2) (34)	NS
K (mmol/L)	−0.2 (−0.9 to 0.6) (71)	−0.2 (−0.9 to 1.0) (34)	NS
Cl (mmol/L)	0 (−5 to 4) (71)	0 (−2 to 3) (34)	NS

[†]Fold change. Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; Cl, chlorine; CRE, creatinine; γ-GTP, γ-glutamyl transpeptidase; K, potassium; LDH, lactate dehydrogenase; Na, sodium; OFC, oral food challenge; T.Bil, total bilirubin; TP, total protein.
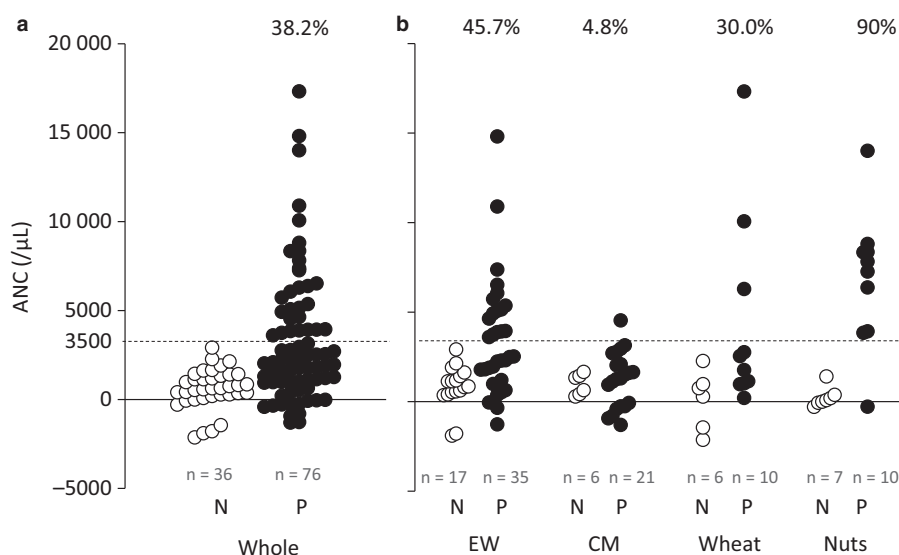

Fig. 1 Change in absolute neutrophil count (ANC) after oral food challenge (OFC) (a) in all patients and (b) according to food. CM, cow's milk; EW, hen's egg white; N, negative OFC; P, positive OFC. (%), percentage of patients with marked increase in ANC (≥3,500/μL).

patients (Table 4). In those patients, pancreatic amylase comprised the majority of the serum amylase, and a marked increase in serum lipase was also observed. There was a significant positive correlation between serum amylase and lipase (*r* = 0.97; *P* < 0.0001; data not shown).

On multivariate regression analysis, increase in serum amylase after OFC was significantly negatively correlated with

change in AEC (*t* = −2.74; *P* < 0.01; Table 3). There was a significant negative correlation between the change in serum amylase and the change in AEC (*r* = −0.36; *P* < 0.01; Fig. 4b).

In contrast, an increase in serum amylase did not correlate significantly with a change in ANC (Fig. 4c). Age, sex, symptoms, and total IgE were not correlated with an increase in serum amylase.

Table 3 Factors correlated with neutrophilia and hyperamy-lasemia

	t-value	P-value
Neutrophilia		
Age	1.16	NS
Sex	0.90	NS
Total IgE	0.15	NS
Symptom		
GI	3.63	<0.001
Skin	2.75	<0.01
Respiratory	0.17	NS
Hyperamylasemia		
Age	1.26	NS
Sex	−0.06	NS
Total IgE	−1.88	NS
ANC	1.09	NS
AEC	−2.74	<0.01
Symptom		
GI	−0.32	NS
Skin	−0.05	NS
Respiratory	1.81	NS

ANC, absolute neutrophil count; AEC, absolute eosinophil count; GI, gastrointestinal; IgE, immunoglobulin E.

Fold change in plasma cortisol

The fold change in plasma cortisol was not significantly different between the OFC-positive and -negative groups (Table 2). The prevalence of distinct increase in the ratio (≥1.81) was also not different between the two groups (15.9 vs 4.2%). There was no significant correlation between the fold change in plasma cortisol and that in IL-6.

Changes in other laboratory data

Change in serum creatine kinase was significantly larger in the OFC-positive group than in the OFC-negative group (median, −11 vs 0.5 IU/L; P < 0.0001, Table 2), but this was not significantly correlated with any symptoms or laboratory data.

There were no significant differences in change in other routine laboratory parameters between the OFC-positive and -negative groups (Table 2). Serum C-reactive protein was <0.1 mg/dL in all subjects before OFC and did not increase after OFC in any subjects (data not shown).

Discussion

In this study, to elucidate further the relationship between immediate FA and FPIES, we analyzed changes in laboratory data several hours after OFC in patients with immediate FA. As a result, a marked increase in ANC was found in one-third of patients with immediate FA. Moreover, there was a significant positive correlation between marked neutrophilia and development of GI symptoms. Although a marked increase in ANC has been considered to be a feature of FPIES, it is now suggested to be shared by some patients with immediate FA. This observation may also provide a useful clue about the association between symptoms and laboratory data in patients with immediate FA.

With regard to cytokine production, serum IL-6 was increased in approximately half of the patients with marked neutrophilia. IL-6 is a well-known pro-inflammatory cytokine that induces neutrophilia.[11] Indeed, a significant positive correlation was seen between the increase in ANC and the fold change in serum IL-6. Therefore, IL-6 appears to play a critical role in the induction of marked neutrophilia in patients with immediate FA.

There was a significant positive correlation between marked neutrophilia and the development of GI symptoms in patients with immediate FA. A weak but significant correlation was also seen between skin symptoms and marked
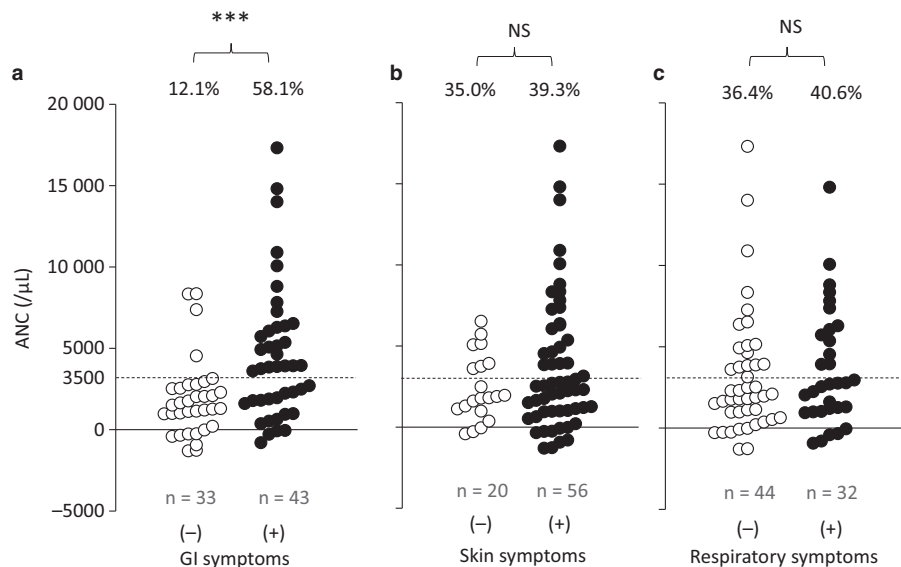

Fig. 2 Change in absolute neutrophil count (ANC) according to presence of (a) gastrointestinal (GI) symptoms, (b) skin symptoms and (c) respiratory symptoms. (%), percentage of patients with marked increase in ANC. ***P < 0.001; NS, not significant.
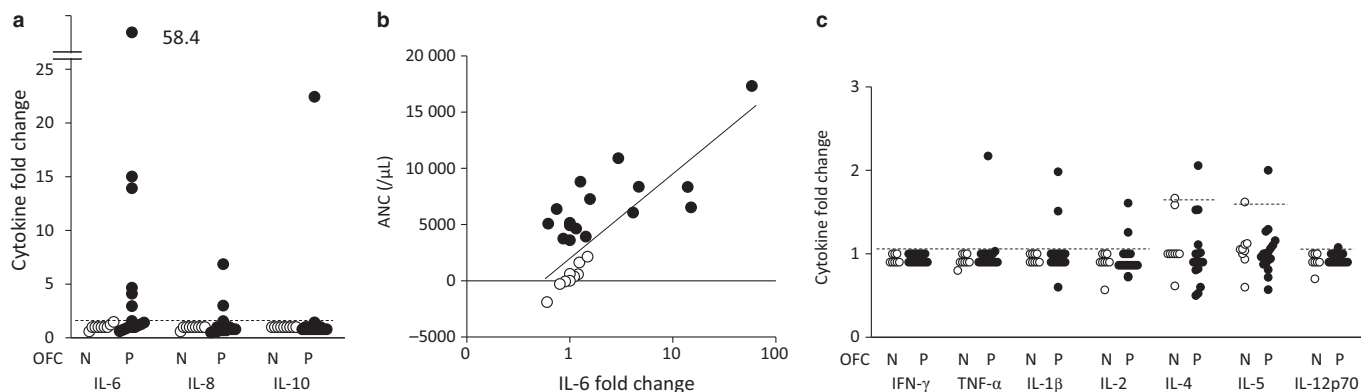
Fig. 3 Fold change in (a,c) serum cytokines vs result of oral food challenge (OFC; N, negative OFC; P, positive OFC) in 16 OFC-positive and nine OFC-negative subjects; and (b) change in absolute neutrophil count (ANC) vs fold change in interleukin (IL)-6 (●, OFC positive with marked neutrophilia; ○, OFC negative; $r = 0.64$; $P < 0.001$). IFN, interferon; TNF, tumor necrosis factor. (- - -), 99th percentile of OFC-negative subjects.
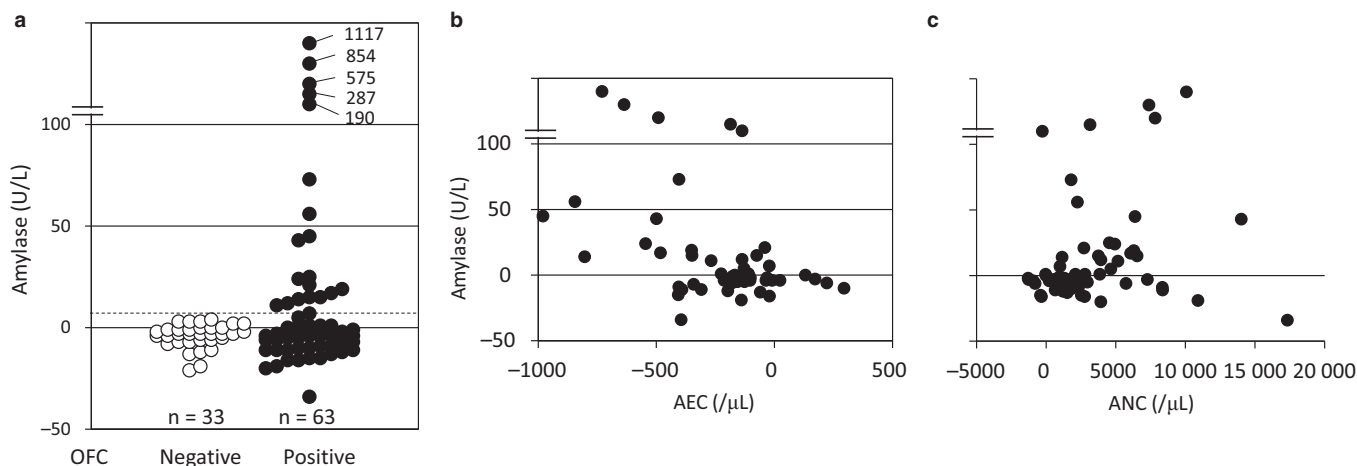

Fig. 4 Change in serum amylase vs (a) result of oral food challenge (OFC); (b) change in absolute eosinophil count (AEC; $r = -0.36$; $P < 0.01$; $n = 63$) and (c) change in absolute neutrophil count (ANC; $r = -0.23$; $P = $ n.s.; $n = 63$) in (●) OFC-positive and (○) OFC-negative patients. (a) - - -, upper limit of the normal range in change in amylase (4 U/L), which was set to the 99th percentile of the OFC-negative group.

Table 4   Characteristics of subjects with remarkable hyperamylasemia

Patient ID no.	Age (years)	Sex	Food	Symptoms	Change in		Amylase (U/L)		Pancreatic amylase (%)		Lipase (U/L)	
					ANC (/μL)	AEC (/μL)	Before OFC	After OFC	Before OFC	After OFC	Before OFC	After OFC
1	3	M	EW	S,R	7,380	−637	130	984	36.5	88.4	22	2,329
2	9	M	Wheat	S,R,GI(P)	10,072	−730	38	1,155	40.5	97.3	20	3,620
3	5	M	Walnut	GI(Vo)	−279	−137	68	258	34.8	81.3	25	403
4	6	F	Walnut	S,R,GI (Vo)	7,825	−491	67	642	36.5	94.7	22	1,197
5	3	M	CM	S	3,148	−186	78	365			19	771

ANC, absolute neutrophil count; AEC, absolute eosinophil count; CM, cow's milk; EW, hen's egg white; GI, gastrointestinal symptoms; OFC, oral food challenge; P, abdominal pain; R, respiratory symptoms; S, skin symptoms; Vo, vomiting.

neutrophilia. In contrast, respiratory symptoms were not associated with marked neutrophilia. The cause of such a difference in the association between symptoms and marked neutrophilia remains to be elucidated.

Interestingly, some investigators have reported that, although serum IL-6 correlates with the severity of anaphylaxis, it does not correlate with the severity of respiratory symptoms in patients with acute allergic reactions.[12,13]

Moreover, in contrast to IL-5, serum IL-6 was not increased in children with acute asthma attack.[14] This suggests that IL-6 is not a major cytokine in the pathophysiology of bronchial lesion induced by aeroallergens.[15] Similarly, IL-6 may not be a critical factor in food-induced respiratory symptoms.

The frequency of marked neutrophilia after OFC was also influenced by the type of food. It is surprising that almost all patients with an immediate nut allergy had a marked increase in ANC, while patients with an immediate CM allergy seldom had marked neutrophilia. Such a difference cannot be explained by the level of serum food-sIgE because it is not significantly different between foods. This suggests that some aspects of EPR are regulated in a food-specific manner. Further study is needed to clarify the relationship between food species and the frequency of marked neutrophilia.

Interleukin-8 is another representative chemokine that induces neutrophilia.[16] In patients with FPIES, IL-8 has been shown to increase after OFC,[17,18] and the fold change in serum IL-8 is closely correlated with increase in ANC.[19] In contrast, in the present study, increase in serum IL-8 was seldom seen in patients with immediate FA. This may correspond with the observation that serum IL-8 does not increase in patients with anaphylaxis.[12] Thus, although marked neutrophilia was seen in both patients with FPIES and in those with immediate FA, the underlying mechanism of each is suggested to be different.

Another important finding was that of hyperamylasemia in patients with immediate FA. Thus far, harmful effects on the pancreas have not yet been recognized as a major manifestation of immediate FA. Acute pancreatitis in patients with immediate FA, however, has occasionally been reported.[8,9] This study has shown that pancreatic dysfunction is not a rare complication of immediate FA because an increase in serum amylase was observed in one-third of OFC-positive patients after OFC.

Although an increase in serum amylase is usually mild, a remarkable increase (>100 U/L) was seen in 7.9% (5/63) of OFC-positive patients. Given that serum lipase was simultaneously increased, hyperamylasemia after OFC may derive from damage to the pancreas. The association between such a remarkable increase in pancreatic enzymes and the development of acute pancreatitis, however, is still unclear because abdominal symptoms were not serious and subsided in several hours. Moreover, two of five subjects did not have GI symptoms. Because OFC is now performed frequently by many pediatric allergists, it is important to accurately estimate the potential risk of OFC-related damage to the pancreas.

In conclusion, marked neutrophilia was noted after OFC in patients with immediate FA and GI symptoms. This may provide a new insight into not only the association between symptoms and laboratory data, but also the pathophysiological association between immediate FA and FPIES. A considerable increase in serum amylase after OFC was also noted in patients with immediate FA. Further studies are needed to clarify the mechanism of pancreatic dysfunction and its

association with acute pancreatitis, a rare complication of immediate FA.

Disclosure

The authors declare no conflict of interest.

Author contributions

M.K. designed the study and wrote the manuscript; Y.I. and Y.A. measured cytokines; M.S., K.Y., C.N. and T.M. collected data. All authors read and approved the final manuscript.

References

1 Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J. Allergy Clin. Immunol.* 2014; **133**: 291–307.
2 Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.* 2010; **125**: S73–80.
3 Powell GK. Food protein-induced enterocolitis of infancy: Differential diagnosis and management. *Compr. Ther.* 1986; **12** (2): 28–37.
4 Nowak-Węgrzyn A, Szajewska H, Lack G. Food allergy and the gut. *Nat. Rev.* 2017; **14**: 241–57.
5 Sicherer SH, Eigenmann PA, Sampson HA. Clinical features of food protein-induced enterocolitis syndrome. *J. Pediatr.* 1998; **133**: 214–9.
6 Kimura M, Oh S, Narabayashi S, Taguchi T. Usefulness of lymphocyte stimulation test for the diagnosis of intestinal cow's milk allergy in infants. *Int. Arch. Allergy Immunol.* 2012; **157**: 58–64.
7 Nowak-Węgrzyn A, Chehade M, Groetch ME *et al.* International consensus guideline for the diagnosis and management of food protein-induced enterocolitis syndrome: Executive summary – Workshop Report of the Adverse Reactions to Foods Committee, American Academy of Allergy, Asthma and Immunology. *J. Allergy Clin. Immunol.* 2017; **139**: 1111–26.
8 Manohar M, Verma AK, Venkateshaiah SU, Goyal H, Mishra A. Food-induced acute pancreatitis. *Dig. Dis. Sci.* 2017; **62**: 3287–97.
9 Ogura K, Iikura K, Yanagida N, Sato S, Ebisawa M. Two patients with acute pancreatitis after undergoing oral food challenges. *J. Allergy Clin. Immunol. Pract.* 2016; **4**: 984–6.
10 Powell GK. Milk- and soy-induced enterocolitis of infancy. Clinical features and standardization of challenge. *J. Pediatr.* 1978; **93**: 553–60.
11 Hashizume M, Higuchi Y, Uchiyama Y, Mihara M. IL-6 plays an essential role in neutrophilia under inflammation. *Cytokine* 2011; **54**: 92–9.
12 Stone SF, Cotterell C, Isbister G, Holgate A, Brown SGA. Elevated serum cytokines during human anaphylaxis: Identification of potential mediators of acute allergic reactions. *J. Allergy Clin. Immunol.* 2009; **124**: 786–92.
13 Lin RY, Trivino MR, Curry A *et al.* Interleukin 6 and C-reactive protein levels in patients with acute allergic reactions:

An emergency department-based study. *Ann. Allergy Asthma Immunol.* 2001; **87**: 412–6.

14 Kato M, Suzuki K, Yamada Y, Maruyama K, Hayashi Y, Mochizuki H. Virus detection and cytokine profile in relation to age among acute exacerbations of childhood asthma. *Allergol. Int.* 2015; **64**: S64–70.

15 Fireman P. Understanding asthma pathophysiology. *Allergy Asthma Proc.* 2003; **24**: 79–83.

16 Terashima T, English D, Hogg JC, van Eden SF. Release of polymorphonuclear leukocytes from the bone marrow by interleukin-8. *Blood* 1998; **92**: 1062–9.

17 Kimura M, Ito Y, Shimomura M *et al.* Cytokine profile after oral food challenge in infants with food protein-induced enterocolitis syndrome. *Allergol. Int.* 2017; **66**: 452–7.

18 Caubet JC, Bencharitiwong R, Ross A, Sampson HA, Berin MC, Nowak-Węgrzyn 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.

19 Shimomura M, Ito Y, Tanaka H, Meguro T, Kimura M. Increased serum cortisol on oral food challenge in infants with food protein-induced enterocolitis syndrome. *Pediatr. Int.* 2018; **60**: 13–8.