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Abstract

Background: The pathogenesis of eosinophilic esophagitis involves immunoglobulin G4 (IgG4) deposition. However, the relationship between IgG4 and eosinophilic gastroenteritis (EGE) is unclear.

Aims: To investigate gastrointestinal deposition of IgG4 in EGE.

Methods: Biopsies of the esophagus, stomach, and small intestine were evaluated in patients with and without EGE. Immunohistochemical staining for IgG4 was performed, and the proportions of the stained areas were compared. Sera from patients with EGE were assayed for food-specific IgG4, including egg white, wheat, rice, soy, and cow milk.

Results: Seventeen patients were included in this study (EGE group, n = 10; control group, n = 7). Compared with the control group, the proportion of IgG4-stained area in the EGE group was approximately three-fold higher (40.2% [32.3–49.5]) vs. 12.1% [4.0–21.9], p = 0.014) in the esophagus, five-fold higher in the stomach (17.3% [11.1–26.2] vs. 3.7% [1.5–5.2], p = 0.014), and six-fold higher in the small intestine (28.0% [15.0–33.2] vs. 4.5% [2.6–9.8], p = 0.019). There was no significant association between the proportion of IgG4-stained area and the number of infiltrating eosinophils. Serum egg white-specific IgG4 levels were correlated with the proportion of IgG4-stained areas in the small intestine (R = 0.7, p = 0.035).

Conclusions: IgG4 accumulated within the gastrointestinal mucosa in EGE. The positive correlation between serum egg white-specific IgG4 levels and the proportion of IgG4-stained

areas in the small intestine suggests a role for IgG4 in the disease pathophysiology.

Keywords: Eosinophils; immunohistochemistry; immunoglobulin G; allergy and immunology

Introduction

Eosinophilic gastrointestinal disorders (EGIDs) are defined as chronic allergic disorders with eosinophilic inflammation along the gastrointestinal (GI) tract. EGIDs are divided into eosinophilic esophagitis (EoE) and eosinophilic gastroenteritis (EGE) on the basis of involved organs. The pathophysiology of EGIDs is thought to be a chronic T helper (Th) type 2-mediated allergic reaction, which is caused mainly by food allergens [1–7]. Generally, food allergies are classified as immunoglobulin E (IgE)-mediated, cell-mediated, and mixed immunity based on the mechanism(s) [8]. Elevated total IgE levels and IgE sensitization to food and aeroallergens have been found in EGIDs [2,9,10]; however, the causal role of IgE in the pathogenesis of EGIDs has not yet been elucidated [2,11,12]. Recent studies have suggested a possible association between total and food-specific immunoglobulin G4 (IgG4) in the pathogenesis of EoE [13,14]. Both esophageal deposition of IgG4 and IgG4 sensitization to food has been observed in EoE, suggesting that EoE is an IgG4-associated disease [13]. The possible causal mechanisms of IgG4 included formation of immune complex [11] and direct blocking of endogenous adhesion molecules [15]. In contrast to EoE, the number of studies on EGE is limited, and the association between IgG4 and EGE has not been investigated. Therefore, the purpose of this study was to determine the relationship between EGE and IgG4.

Methods

Patients and disease definition

This was a single-center cross-sectional study that included patients with EGE and patients without pathological eosinophil infiltration in the GI tract as a control group. Pathological eosinophil infiltration was defined by at least 15 eosinophils/high-power field (HPF) (× 400) for the esophagus and 20 eosinophils/HPF for other parts of the GI tract [16,17]. The diagnosis of EGE required pathological eosinophil infiltration in the stomach, small intestine, or colon, as confirmed by biopsies, as well as related symptoms, such as nausea, dysphagia, abdominal pain, and diarrhea [18,19]. We defined the control group as patients who underwent oral and anal double-balloon enteroscopy and whose biopsies from the esophagus, stomach, upper and lower small intestine, and colon showed no pathological eosinophil infiltration. Finally, the patients in the control group were diagnosed as follows; Meckel's diverticulum, nonsteroidal anti-inflammatory drug-induced intestinal ulcer, irritable bowel syndrome, centrally mediated abdominal pain syndrome, diarrhea caused by medications, anemia without any GI tract disease, and neurofibromatosis type 1. We confirmed that all controls had no malignancy and the results of biopsies were non-specific inflammation or normal mucosa.

We enrolled patients with EGE who were evaluated for serum food-specific IgG4 and control patients who visited Osaka City University Hospital between January 2008 and July 2020. Ten patients with EGE were prospectively recruited and seven patients in the control group were retrospectively identified. We had no subjects under systemic glucocorticoid therapy in both

EGE group and controls. Serum food-specific IgG4 was prospectively evaluated in ten patients with EGE who provided written consent before glucocorticoid therapy for EGE. The exclusion criteria were as follows: history of severe respiratory, cardiovascular, hepatic, hematological, and/or renal disease, previous GI surgery, age < 20 years, and insufficient tissue biopsies. Patients with other diseases that cause GI eosinophil infiltration such as autoimmune disorders including eosinophilic granulomatosis with poly angiitis, parasitic infection, viral infection, fungal infection, inflammatory bowel diseases, hypereosinophilic syndrome, and drug hypersensitivity reaction were also excluded. We collected data including age, sex, current alcohol drinking (presence or absence), current cigarette smoking (presence or absence), and concomitant allergic diseases from the patients' medical records.

The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (protocol numbers: 2020-151 and 4144) and was performed in accordance with the principles of the Helsinki Declaration. The need for informed consent to collect retrospective data was waived by the Ethics Committee of the Osaka City University Graduate School of Medicine. We disclosed the study information on our website, and the patients had the opportunity to opt out.

Immunoassays

Using ImmunoCAP specific IgG4 (Thermo Fisher Scientific, Uppsala, Sweden), sera of ten

patients with EGE were assayed for food-specific IgG4 for egg white, wheat, rice, soy, and cow milk. Whole allergens were tested for egg white, wheat, rice, and soy, and purified allergens were tested for cow milk (nBos d 8). The detection range was 0.0700–30.0 mg_A/L, and the measured values above and below the detection range were presumed to be the upper and lower limits of the range, respectively.

The levels of food-specific IgE for soy, rice, wheat, cow milk, and egg white were also examined in five patients with EGE. Serum food-specific IgG4 measurements were categorized into sIgE (−) or sIgE (+) groups according to whether serum IgE to the same food was below or greater than the cutoff value (0.35 U_A/mL).

Immunohistochemical staining and image analysis

Mucosal biopsy specimens were obtained endoscopically from the esophagus, stomach, and small intestine. Sections of formalin-fixed, paraffin-embedded tissue were subjected to EDTA-based antigen retrieval using Histofine (Nichirei Bioscience, Tokyo, Japan). Immunoperoxidase staining for IgG4 was performed using rabbit monoclonal anti-IgG4 (ab109493; Abcam, Cambridge, UK) at a 1/5000 dilution as the primary antibody; EnVision+ System-HRP-labeled polymer anti-rabbit (Dako, Agilent, Santa Clara, CA, USA) as the secondary antibody; and Liquid DAB + Substrate Chromogen System (Dako) for visualization.

Hematoxylin and eosin-stained slides were evaluated for the presence and distribution of

eosinophils. The peak numbers of eosinophils per HPF were recorded for the esophagus, stomach, and small intestine. The eosinophil counts were measured by pathologists in our institute. IHC was performed by a researcher in the department of gastroenterology (S.K.).

Digital images of the diaminobenzidine-stained tissue were obtained at ×40 magnification. To minimize observation bias, the fractions of stained areas were measured using thresholding with ImageJ version 1.53e (National Institutes of Health, Bethesda, Maryland, USA).

Evaluation of endoscopic findings

Endoscopic images were reviewed and assessed for mucosal inflammation. We evaluated the presence of typical endoscopic findings of EoE (mucosal edema, esophageal ring, exudate, furrow, and stricture) and reflux esophagitis. In the stomach, we evaluated the presence of gastric ulcer, erosion, erythema, nodularity, discoloration, and crack, which we reported as characteristic endoscopic findings for EGE [20]. We evaluated the presence of erythema and erosion of the small intestine.

Statistical analysis

Data are presented as medians with interquartile ranges for continuous variables and numbers with proportions for categorical variables. Statistical analyses between two groups were performed using Fisher's exact test for categorical data and the Mann–Whitney *U* test for

continuous data. Spearman's rank correlation coefficient was used to estimate correlations. Statistical analysis was performed to evaluate the difference in the proportion of IgG4-stained area between patients with and without each endoscopic finding if there were more than two patients with each finding. The overall significance level was set at a *p*-value of 0.05. All statistical analyses were performed using R ver. 3.6.2 (The R Foundation for Statistical Computing).

Results

Demographics of participants

Ten patients with EGE and seven patients in the control group were identified (Table 1). Among patients with EGE, pathological eosinophilic infiltration was found in three patients for the esophagus, in five patients for the stomach, and in seven patients for the small intestine. Patients with EGE were younger (33.5 [27.0–63.0] vs. 73.0 [44.0–80.0] years, $p = 0.031$), and females were predominant in both the EGE (80%) and control groups (57%). None of the patients in the control group had concomitant allergic diseases, including bronchial asthma (BA), atopic dermatitis (AD), hay fever (HF), and self-reported food allergy (FA), whereas the prevalence was 40% for BA and FA and 20% for AD and HF in the EGE group. A complete blood count test revealed significantly higher absolute eosinophil counts in the EGE group (683 [492–985] vs. 43 [17–96], $p < 0.001$), which is a laboratory characteristic of eosinophilic

gastroenteritis.

IgG4 deposition was significantly increased in the esophagus, stomach, and small intestine of patients with EGE compared with controls

Immunohistochemistry of biopsy specimens from the esophagus, stomach, and small intestine revealed remarkable IgG4 deposition in the EGE group compared with in the control group (Figure 1A–F). The proportion of stained area in the EGE group was approximately three-fold higher (40.2% [32.3–49.5]) vs. 12.1% [4.0–21.9], $p = 0.014$) in the esophagus, five-fold higher in the stomach (17.3% [11.1–26.2] vs. 3.7% [1.5–5.2], $p = 0.014$), and six-fold higher in the small intestine (28.0% [15.0–33.2] vs. 4.5% [2.6–9.8], $p = 0.019$) than those in the control group (Figure 1G). The location of reactivity was the intercellular space between keratinocytes in the esophagus (Figure 2A), within the lamina propria both in the stomach (Figure 2B) and small intestine (Figure 2C), and in the intercellular space between the epithelium in the small intestine (Figure 2D).

IgG4 deposition was independent of local eosinophil infiltration

IgG4 deposition was found to be independent of pathological eosinophil infiltration in the EGE group (Figure 3A). The numbers of patients with and without pathological eosinophil infiltration were three and seven for the esophagus, five and five for the stomach, and seven

and three for the small intestine, respectively. The tissue eosinophil count did not correlate with the IgG4-stained area in the esophagus ($R = -0.4$, $p = 0.252$) (Figure 3B), stomach ($R = -0.1$, $p = 0.713$) (Figure 3C), and small intestine ($R = 0.03$, $p = 0.946$) (Figure 3D).

Proportion of the IgG4-stained area was unrelated to endoscopic findings

EGD revealed various endoscopic findings, including ulcers, erosions, erythema, nodularity, discoloration, cracks, and atrophy in the stomach of patients with EGE, whereas erythema was the only finding in the small intestine (Online Resource 1). The prevalence of each endoscopic finding was low and not more than 30%, except for gastric erythema (40%) and erosions (50%); however, the prevalence of patients with at least one endoscopic finding was high in the esophagus (70%) and stomach (80%). The proportion of IgG4-stained areas was not significantly different between patients with and without endoscopic findings.

Relationship among serum food-specific IgG4, the IgG4-stained area, and serum food-specific IgE

The serum levels of food-specific IgG4 for soy, rice, wheat, cow milk, and egg white in the EGE group are shown in Figure 4A. The levels of soy- and rice-specific IgG4 tended to be lower than those of the other allergens (Figure 4A). The levels of egg white-specific IgG4 had a moderate positive correlation with the proportion of IgG4-stained area in the small intestine

(R = 0.7, p = 0.035) (Figure 4B). Furthermore, the levels of cow milk-specific IgG4 and wheat-specific IgG4 tended to correlate with the proportion of IgG4-stained area in the small intestine (R = 0.6, p = 0.060; R = 0.6, p = 0.069) (Figures 4C and D). Other food-specific IgG4s had no significant relationship with the IgG4-stained area in the small intestine (Figures 4E and F). Egg white-specific IgG4 levels did not correlate with the IgG4-stained area in the esophagus or stomach (data not shown).

Food-specific IgE levels for soy, rice, wheat, cow milk, and egg white were measured in five patients (Online Resource 2). Serum food-specific IgE levels correlated with serum food-specific IgG4 levels (R = 0.7, p < 0.001) (Figure 5A). There were 17 food-specific IgG4 measurements in the sIgE (−) group and eight in the sIgE (+) group. The levels of serum food-specific IgG4 were significantly higher in the sIgE (+) group than in the sIgE (−) group (18.0 [14.7−26.6] vs. 1.5 [0.1−6.9], p = 0.002) (Figure 5B).

Discussion

To the best of our knowledge, this study is the first to show IgG4 deposition in the GI tract of patients with EGE. We confirmed that the proportion of the IgG4-stained area in the esophagus, stomach, and small intestine was significantly increased in the EGE group compared with the control group; however, the presence of IgG4 deposition was not specific to EGE. These results imply that local IgG4 is partly associated with the pathophysiology of EGE. In contrast to EoE,

research on EGE is limited due to its low prevalence and disease heterogeneity. The pathogenesis of both EGE and EoE is unclear; however, these diseases are thought to have a similar pathophysiology based on their biochemical and clinical similarities [19]. As a consequence of the Th2-mediated inflammatory response, esophageal IgG4 deposition in patients with EoE has been reported in several studies [11,21–26].

We observed esophageal intrasquamous IgG4 deposition in EGE, which was similar to that observed in EoE. Two previous studies reported that esophageal intrasquamous IgG4 deposits were present in EoE and were absent in GERD; the authors concluded that the presence of esophageal intrasquamous IgG4 deposits was a useful marker to distinguish EoE from GERD with high sensitivity and specificity [21,24]. We confirmed that intraepithelial IgG4 deposition was also observed in the stomach and small intestine; however, IgG4 was mostly deposited within the layer of the lamina propria in the stomach and small intestine. The localization of IgG4 was consistent with the location of eosinophilic infiltration, and electron microscopy revealed that the esophageal deposits in patients with EoE were immune complexes [11]. In addition, transcriptional analyses of cytokine levels revealed that the production of IgG4 is promoted in the esophageal mucosa of EoE patients [27]. The role of IgG4 in EoE remains controversial, and it may have a causal or protective role. If IgG4 plays a causal role in EoE, the formation of an immune complex in the esophageal mucosa may be one of pathogenesis [28,29]. Indeed, other studies have also provided evidence supporting the pathogenic role of

IgG4 in EoE. In pediatric EoE, a cow milk elimination diet was found to reduce serum IgG4 to cow milk [14]. Another study showed that esophageal IgG4 levels and food-specific IgG4 levels were decreased following an empirical food elimination diet [13]. Serum IgG4 levels and the number of esophageal IgG4-positive cells decreased after budesonide therapy in adult patients with EoE [25]. Esophageal IgG4 levels were also correlated with histopathological disease activity in EoE [27,30]. Another possible pathogenic mechanism of IgG4 is that IgG4 binds to molecules that maintain tissue structure in the GI mucosa and lead to impaired mucosal integrity and barrier function. Autoantibodies of IgG4 have been found in various autoimmune diseases, such as pemphigus vulgaris, which leads to barrier impairment of the skin [15]. Anti-desmoglain-1 and 3 antibodies have been found to abolish the binding of desmoglein, which is a cell-to-cell adhesion molecule. In EGE, IgG4 may bind to adhesion molecules between the epithelium given the intraepithelial IgG4 deposition in the esophagus and small intestine. Accordingly, we consider that there is a possibility that IgG4 is associated with the pathogenesis of EGE, similar to EoE, by the modification of intestinal permeability. However, another possibility is that the increased GI IgG4 accumulation in EGE is simply a consequence of increased passing of allergens through the GI mucosa due to impairment of the mucosal barrier induced by inflammation.

Interestingly, we found no significant relationship between the proportion of IgG4-stained areas and the number of infiltrating eosinophils in EGE cases. This was surprising because we

assumed that IgG4 and eosinophils were also involved in a Th2-mediated inflammatory response and that IgG4 deposition was secondary to mucosal eosinophilic infiltration. Our results imply that local IgG4 does not reflect disease activity, as demonstrated by the eosinophil counts. These results were different from previous findings in EoE, which may be a characteristic of EGE. Furthermore, if IgG4 has a causal role, evaluating eosinophil counts may underestimate disease activity because some patients showed a high proportion of IgG4-stained area, even if tissue eosinophil infiltration was low. In contrast, if IgG4 has a protective role, increased IgG4 levels may be a consequence of the production of anti-inflammatory cytokines such as IL-10. IL-4 and IL-10 are both Th2-mediated cytokines; IL-4 induces B-cell class switching to produce IgE and IgG4, and IL-10 enhances IgG4 production [31]. IgG4 antibodies are thought to inhibit the binding of allergens to IgE and attenuate mast cell degranulation, followed by inhibition of eosinophil infiltration. Therefore, it is acceptable that the discrepancy between IgG4 levels and eosinophil counts was observed in some patients as a consequence of the anti-inflammatory response. Another possible reason for this discrepancy is a tissue sampling bias. The tissue peak eosinophil count can vary among biopsy sites in a single organ, which may lead to a discrepancy between eosinophil counts and IgG4-stained areas. In addition, various endoscopic findings were observed in EGE, although the prevalence of each finding was low. In our study, endoscopic findings were not significantly associated with the proportion of IgG4-stained areas. These results imply that endoscopic findings do not reflect disease

activity as evaluated by local IgG4 staining; therefore, it may be difficult to evaluate disease activity using only endoscopic findings.

Concerning the relationship between IgG4 and IgE, we found that serum food-specific IgG4 levels were increased when the same food-specific IgE levels were increased in patients with EGE. These similar behaviors of IgE and IgG4 are probably due to exposure to the same allergens and the effect of common B-cell switching factors such as IL-4. The production of IgE is a consequence of an immediate allergic reaction to the exposure to allergens, and IgE production often occurs prior to substantial IgG4 production. Increased IgG4 levels are associated with delayed allergic reactions due to frequent and/or chronic antigen exposure [28]. Therefore, it is possible that these results may be different if the timing of sample collection or the duration of exposure is different.

The level of serum food-specific IgG4 varied among patients with EGE. Elevated levels of serum food-specific IgG4 have been observed in patients with GI diseases, including EoE, Crohn's disease, irritable bowel syndrome (IBS), celiac disease, and chronic gastritis, [13,32–35] as well as in those with food allergies. However, we did not evaluate the levels of serum food-specific IgG4 in the control group, and there is no consensus on the cutoff value for pathologic food-specific IgG4 levels; therefore, this study lacked the power to address the question of whether serum food-specific IgG4 levels were elevated in patients with EGE. However, we found a correlation between serum egg white-specific IgG4 levels and the

proportion of IgG4-stained areas in the small intestine. Similar results have been reported for patients with EoE. Indeed, Wright *et al.* found accumulation of food-specific IgG4s in esophageal biopsy specimens from EoE patients and an association between the levels of serum food-specific IgG4 and esophageal food-specific IgG4 [13]. Therefore, local IgG4 deposition might be mainly composed of food-specific IgG4, which also circulates in the blood stream. Our finding of a positive correlation between the level of serum egg white-specific IgG4 and IgG4-stained area in the small intestine supports this theory. We also observed that serum levels of IgG4 in cow milk and wheat tended to correlate with the IgG4-stained area in the small intestine. These correlations might indicate that the production of IgG4 in response to food is triggered in the small intestine. The relationship between serum and tissue IgG4 to food may indicate that food-specific IgG4 measurement is useful for the identification of allergens. An elimination diet is an effective therapy for EGE; however, it is difficult to identify food allergens with conservative tests such as examination of serum food-specific IgE, skin patch tests, and prick tests. An IgG4-guided elimination diet has been reported to be effective for Crohn's disease and IBS [36,37]. Although an IgG4-guided elimination diet may be a therapeutic option for EGE, this study lacked data on IgG4-guided elimination diet and clinical response. Thus, further studies are required to address this issue.

A limitation of our study is that we did not quantify the amount of tissue IgG4 directly because biopsy specimens were not collected for protein analysis. There was a possibility to

overestimate IgG4-stained area because we considered that both intracellular and extracellular-stained area were positive, which might include non-specific staining. Another limitation is that we did not evaluate the change in tissue IgG4 deposition after treatment for EGE. It is also a limitation that patients in the control group were not tested for serological immunoassays. Accordingly, future investigation will be needed to evaluate our findings more properly. Because we could not match age between the EGE and the control group, there was a possibility that our results might partially depend on age. Other limitations include the small population and its setting in a single institution. Further studies are needed to evaluate the correlation between serum food-specific IgG4 and IgE in bigger study populations. Moreover, in patients with IgE-mediated food allergies, it is needed to identify whether the correlation holds true and whether increased IgG4 is indeed a distinguishing feature of EGE or a consequence of IgE production.

In conclusion, diffuse IgG4 deposition in the esophagus, stomach, and small intestine is a novel characteristic of EGE, which may be associated with its pathogenesis. Our findings are consistent with those of a previous study on EoE, supporting the hypothesis that EoE and EGE are partly based on common pathophysiology. However, further studies are required to determine the exact role of IgG4 in the pathophysiology of EGE.

Compliance with Ethical Standards

Funding: None

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (protocol numbers: 2020-151 and 4144). For this type of study formal consent is not required.

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Table 1. Demographics of the study groups

	Control (n = 7)	EGE (n = 10)	p-value
Age, years	73.0 (44.0–80.0)	33.5 (27.0–63.0)	0.031
Female, n (%)	4 (57)	8 (80)	0.593
BMI, kg/m^2	24.3 (20.2–25.8)	23.3 (20.3–26.0)	0.962
Current alcohol drinking, n (%)	2 (30)	5 (50)	0.622
Current smoking, n (%)	3 (43)	1 (10)	0.250
Concomitant allergic disease, n (%)	0 (0)	9 (90)	<0.001
Bronchial asthma	0 (0)	4 (40)	0.103
Atopic dermatitis	0 (0)	2 (20)	0.485
Hay fever	0 (0)	2 (20)	0.485
Food allergy	0 (0)	4 (40)	0.103
WBC count, ×10^9/L	5.8 (5.4–6.6)	5.8 (5.3–8.1)	0.982
Eosinophil ratio, %	0.7 (0.3–1.5)	12.1 (6.0–16.9)	<0.001
Absolute eosinophil count, /L	43 (17–96)	683 (492–985)	<0.001
Total IgE, IU/mL	n/a	235.0 (66.0–543.8)	
Tissue eosinophil count, /HPF			
Esophagus	0.0 (0.0–0.0)	2.0 (0.0–36.5)	0.029
Stomach	3.0 (1.0–3.0)	24.5 (4.0–72.8)	0.019
Small intestine	5.0 (4.0–7.0)	25.0 (17.0–46.5)	0.029

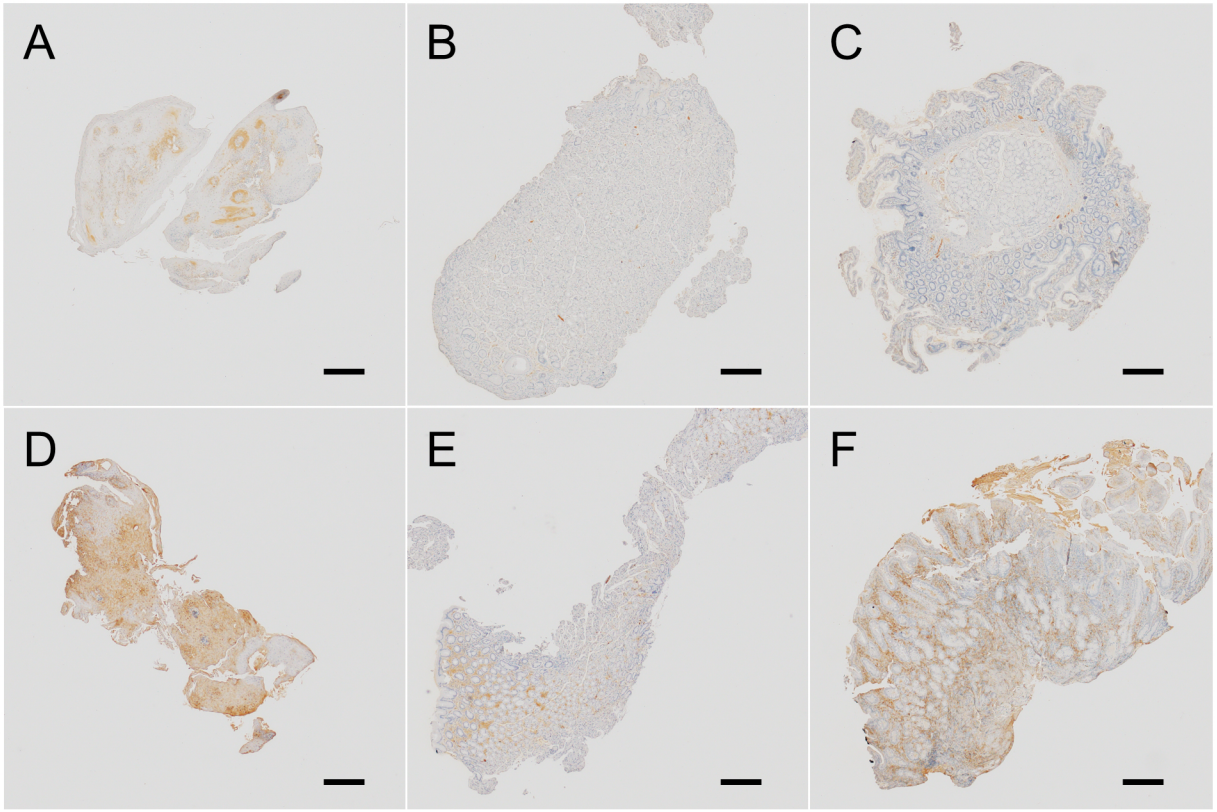
Continuous variables are presented as medians and interquartile ranges (IQR; 25th, 75th percentile).

BMI: Body mass index, EGE: Eosinophilic gastroenteritis, WBC: white blood cell.

Figure 1. Comparison of IgG4-stained area between the control and EGE group

A–F, Representative photomicrographs of IgG4 immunohistochemistry (×40). The proportion of IgG4-stained area was small in the control group (A: esophagus, B: stomach, C: small intestine), but considerably larger in the EGE group (D: esophagus, E: stomach, F: small intestine). Each bar in A–F is 200 μm. G, Comparison of IgG4-stained areas between the control and EGE groups. Statistical significance ($p < 0.05$) between the EGE and control groups is indicated by an asterisk. EGE, eosinophilic gastroenteritis; IgG4, immunoglobulin G4.

Fig. 1


A
B
C
D
E
F

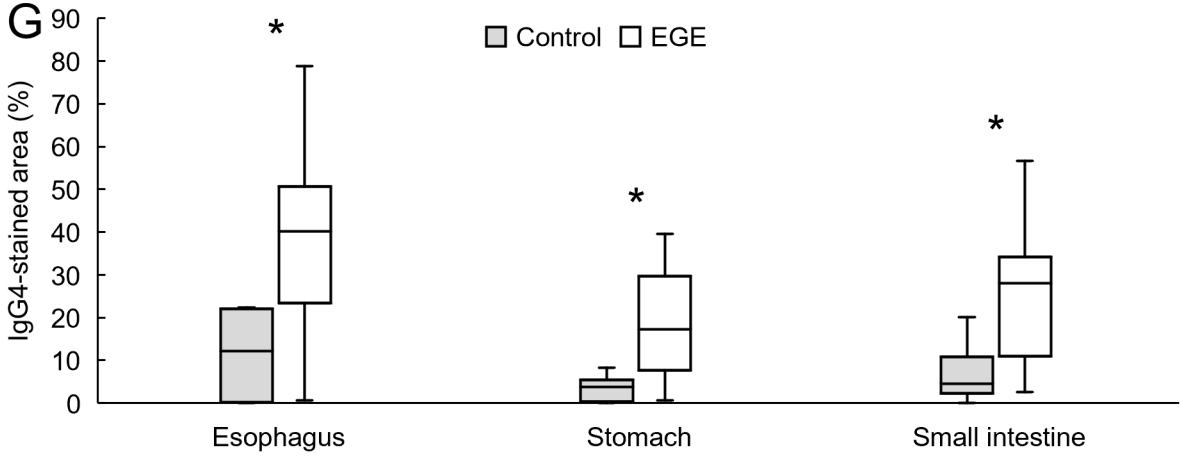

G
IgG4-stained area (%)
90
80
70
60
50
40
30
20
10
0
Control
EGE
*
*
*
Esophagus
Stomach
Small intestine

Figure 2. Localization of IgG4 staining in the EGE group

A–D, Representative photomicrographs of IgG4 immunohistochemistry (×400). Strong reactivity was observed in the intercellular space between keratinocytes in the esophagus (A) and interstitial tissues surrounding the glands in the mucosa of the stomach (B) and small intestine (C). Note the intercellular deposition between the epithelium of the small intestine (arrowhead) (D). Each bar in A–D corresponds to 40 μm. EGE, eosinophilic gastroenteritis; IgG4, immunoglobulin G4.

Fig. 2

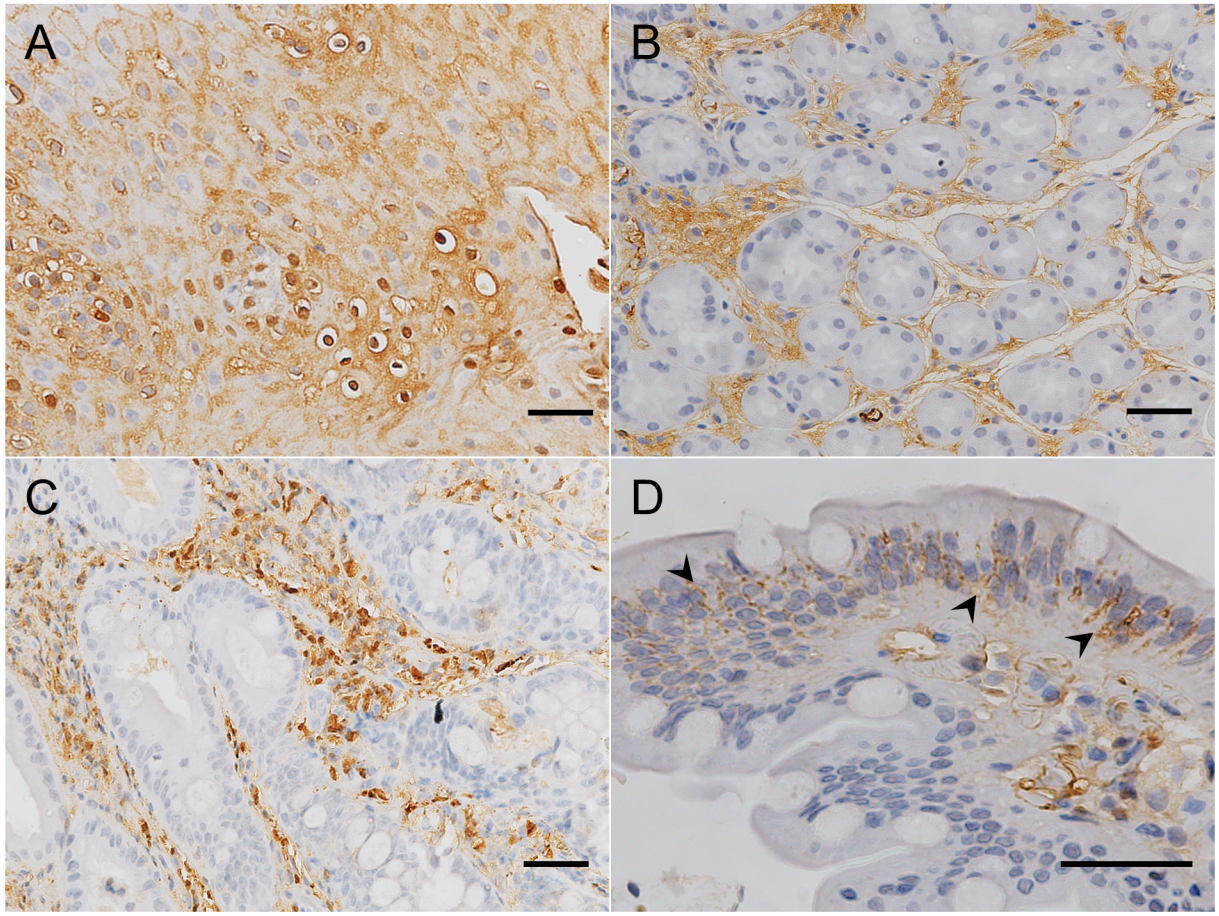


Figure 3. Local proportion of IgG4-stained area and eosinophil infiltration

A, Comparison of the proportion of IgG4-stained areas in patients with or without abnormal eosinophil infiltration. The cutoff value of abnormality was 15 eosinophils/HPF for the esophagus and 20 eosinophils/HPF for the stomach and small intestine. The proportion of IgG4-stained area was not significantly different between the groups in the esophagus, stomach, and small intestine. Correlation between the proportion of IgG4-stained area and the peak eosinophil counts in the esophagus (B), stomach (C), and small intestine (D). EGE, eosinophilic gastroenteritis; HPF, high-power field; IgG4, immunoglobulin G4.

Fig. 3

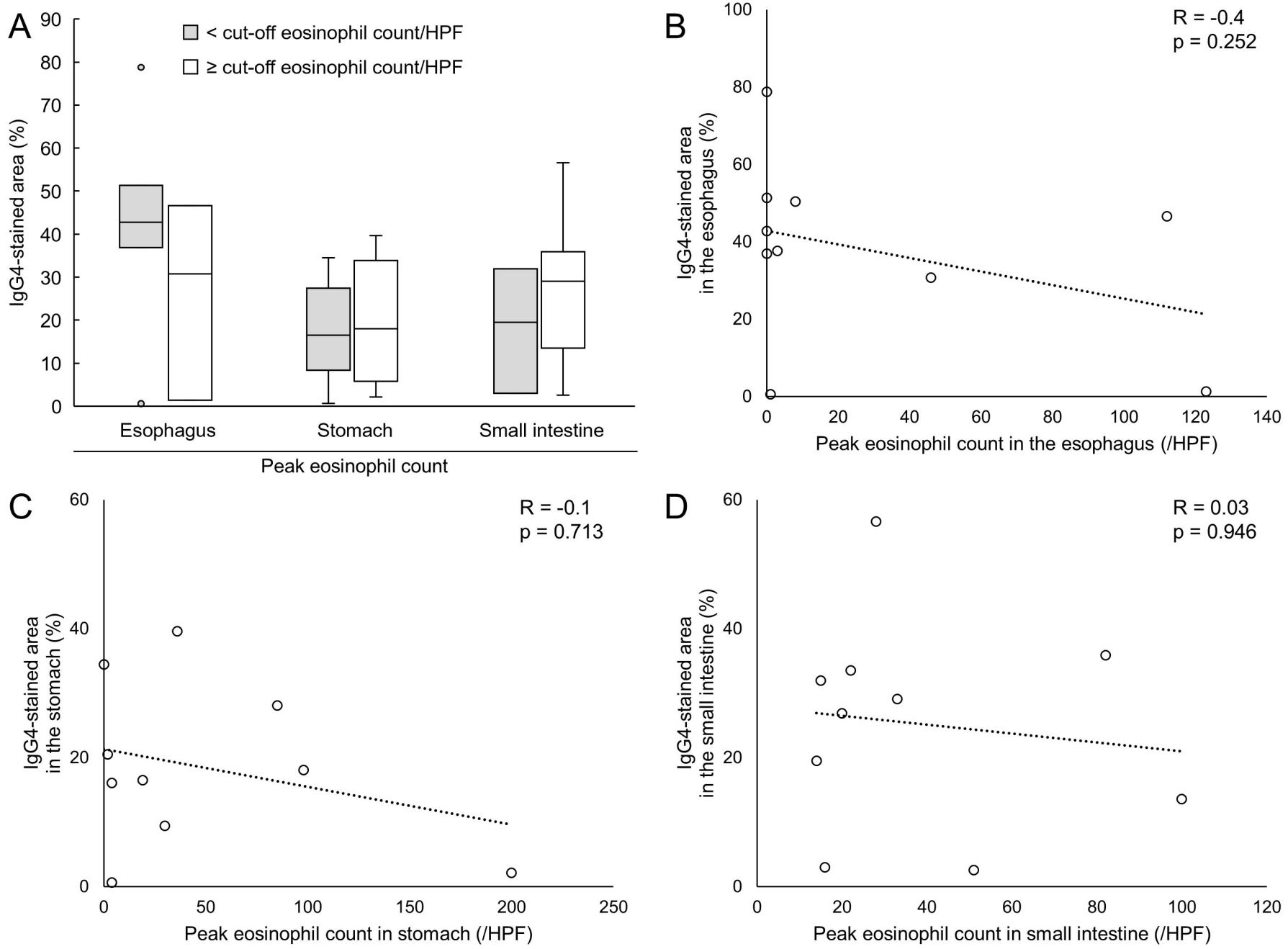

A
< cut-off eosinophil count/HPF
≥ cut-off eosinophil count/HPF
IgG4-stained area (%)
Esophagus
Stomach
Small intestine
Peak eosinophil count
B
R = -0.4
p = 0.252
IgG4-stained area in the esophagus (%)
Peak eosinophil count in the esophagus (/HPF)
C
R = -0.1
p = 0.713
IgG4-stained area in the stomach (%)
Peak eosinophil count in stomach (/HPF)
D
R = 0.03
p = 0.946
IgG4-stained area in the small intestine (%)
Peak eosinophil count in small intestine (/HPF)

Figure 4. Correlation between the levels of serum food-specific IgG4 and the local proportion of IgG4-stained area in the small intestine of patients with EGE

The levels of serum soy- and rice-specific IgG4 tended to be lower than those of other allergens (A). B–F, Relationship between the proportion of IgG4-stained area in the small intestine and serum IgG4 level, which is reactive to egg white, cow milk, wheat, soy, and rice. Serum egg-white-specific IgG4 levels were positively correlated with the IgG4-stained area (B). The levels of serum cow milk- and wheat-specific IgG4 tended to correlate with the stained area (C, D). EGE, eosinophilic gastroenteritis; IgG4, immunoglobulin G4.

Fig. 4

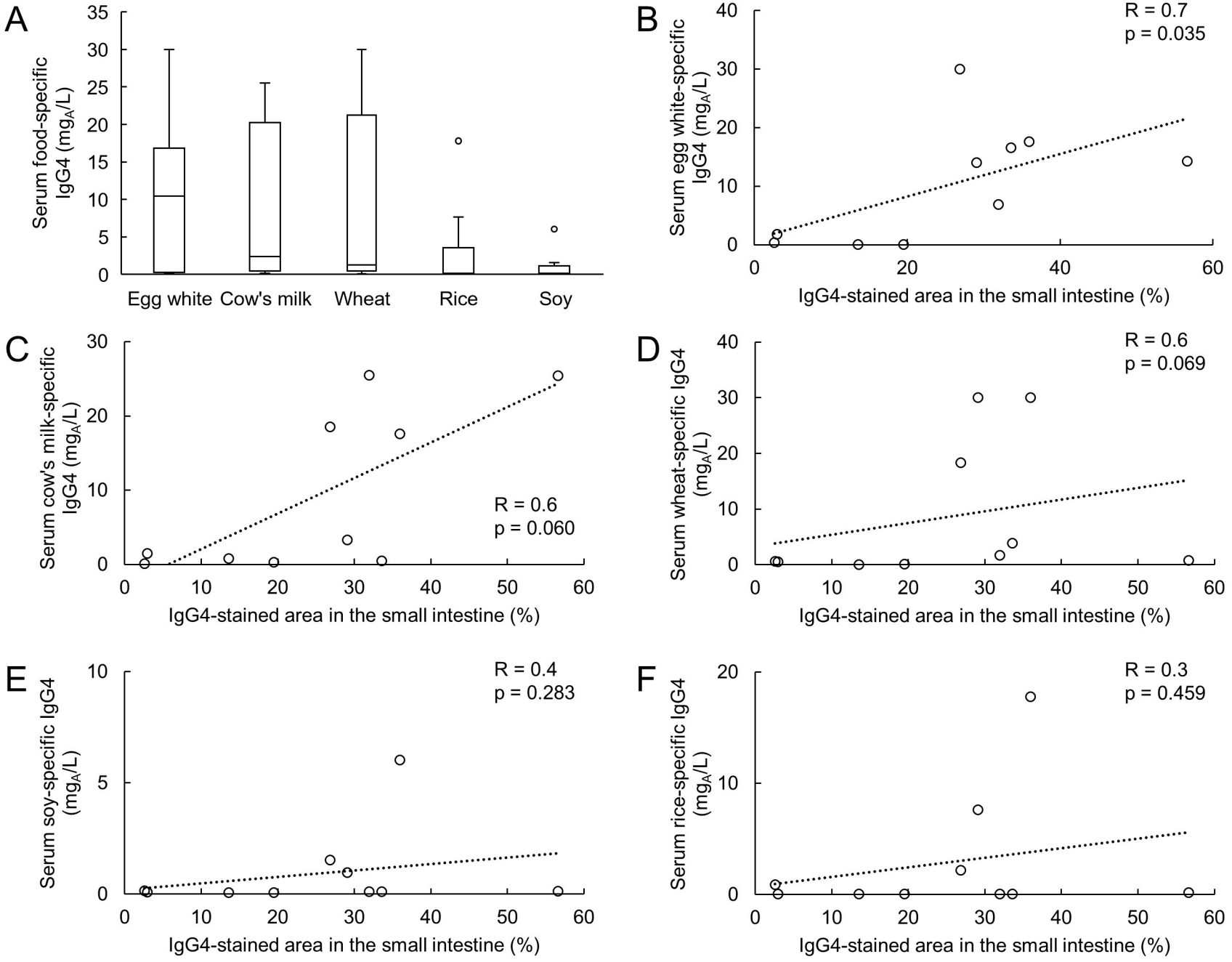
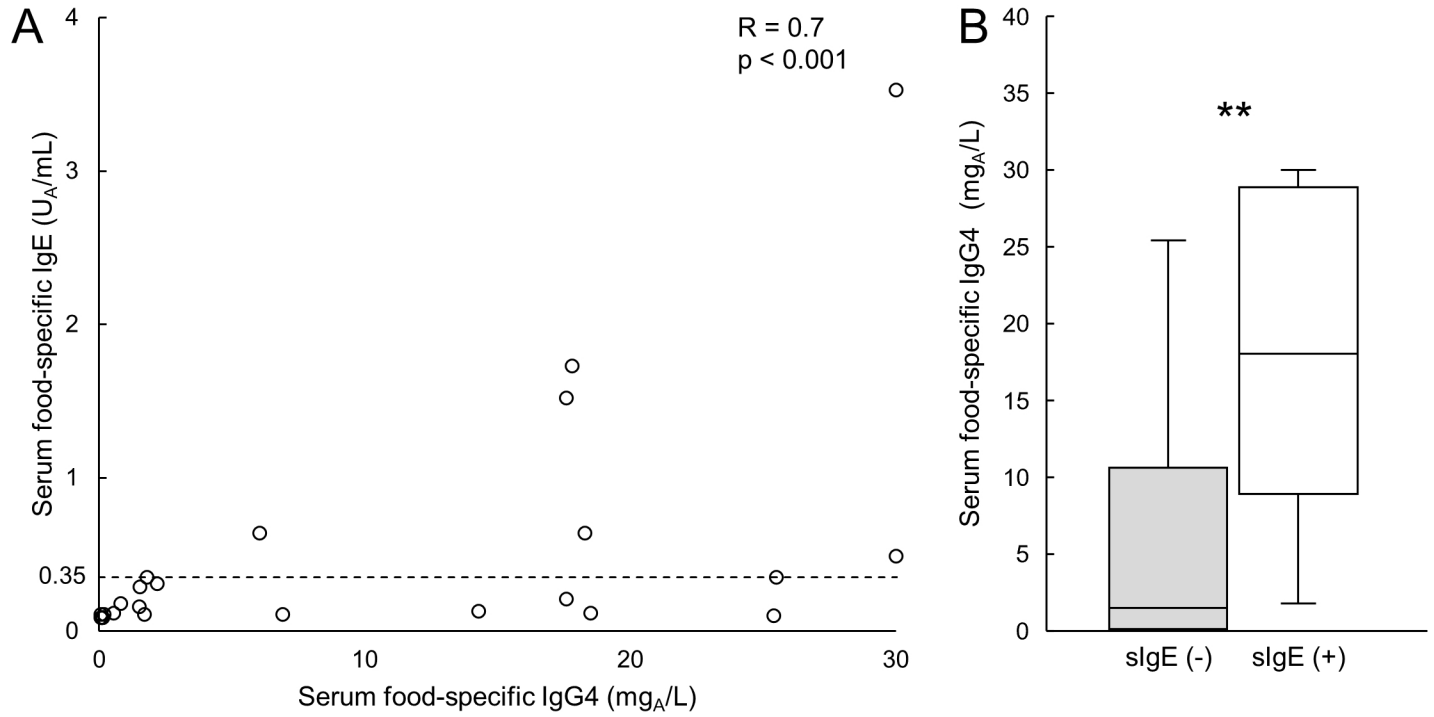

A
Serum food-specific IgG4 (mgA/L)
35
30
25
20
15
10
5
0
Egg white
Cow's milk
Wheat
Rice
Soy
B
Serum egg white-specific IgG4 (mgA/L)
R = 0.7
p = 0.035
IgG4-stained area in the small intestine (%)
C
Serum cow's milk-specific IgG4 (mgA/L)
R = 0.6
p = 0.060
IgG4-stained area in the small intestine (%)
D
Serum wheat-specific IgG4 (mgA/L)
R = 0.6
p = 0.069
IgG4-stained area in the small intestine (%)
E
Serum soy-specific IgG4 (mgA/L)
R = 0.4
p = 0.283
IgG4-stained area in the small intestine (%)
F
Serum rice-specific IgG4 (mgA/L)
R = 0.3
p = 0.459
IgG4-stained area in the small intestine (%)

Figure 5. Correlation between the levels of serum food-specific IgE and IgG4 in the EGE group.

A, Correlation between the levels of serum food-specific IgG4 and IgE in patients with EGE. The levels of serum food-specific IgG4 were positively correlated with the levels of serum food-specific IgE. The horizontal dashed line represents the cutoff value (0.35 U_A/mL) for serum food-specific IgE levels. B, Serum food-specific IgG4 measurements were categorized into sIgE (−) or sIgE (+) groups according to whether the serum IgE to the same food was below or greater than 0.35 U_A/mL. Comparison of serum food-specific IgG4 levels between the sIgE (−) and sIgE (+) groups. The levels of IgG4 were significantly higher in the sIgE (+) group than in the sIgE (−) group. Statistical significance ($p < 0.01$) is indicated by two asterisks. EGE, eosinophilic gastroenteritis; IgE, immunoglobulin E IgG4, immunoglobulin G4.

Fig. 5


A
R = 0.7
p < 0.001
Serum food-specific IgE (UA/mL)
Serum food-specific IgG4 (mgA/L)
0
0.35
1
2
3
4
0
10
20
30
B
**
Serum food-specific IgG4 (mgA/L)
0
5
10
15
20
25
30
35
40
sIgE (-)
sIgE (+)

Supplemental Table 1. Endoscopic findings and IgG4–stained area

Endoscopic findings	Present		Absent		
	n	IgG4-stained area (%)	n	IgG4-stained area (%)	p-value
Esophagus					
Edema	2	1.4, 46.6	8	40.2 (35.4–50.7)	n/a
Ring	3	30.8, 42.8, 46.6	7	37.6 (19.1–50.9)	1
Exudate	2	1.4, 46.6	8	40.2 (35.4–50.7)	n/a
Furrow	3	1.4, 30.8, 46.6	7	42.8 (37.3–50.9)	0.383
Stricture	0	n/a	0	n/a	n/a
Reflux esophagitis	3	0.6, 37.6, 50.5	7	42.8 (33.8–49.0)	0.667
Any lesions	7	37.6 (16.1–44.7)	3	36.9, 51.3, 78.8	0.183
Stomach					
Ulcer	1	9.4	9	18.1 (16.1–28.1)	n/a
Erosions	5	18.1 (16.1–20.5)	5	16.5 (9.4–28.1)	1
Erythema	4	22.1 (12.6–31.0)	6	17.3 (11.2–19.9)	0.762
Nodularity	1	18.1	9	16.5 (9.4–28.1)	n/a
Discoloration	2	2.2, 39.6	8	17.3 (14.4–22.4)	n/a
Cracks	1	2.2	9	18.1 (16.1–28.1)	n/a
Atrophy	1	28.1	9	16.5 (9.4–20.5)	n/a
Any lesions	8	19.3 (14.4–29.7)	2	0.6, 16.5	0.914
Small intestine					
Erythema	2	13.6, 56.6	8	28.0 (15.4–32.3)	n/a

Continuous variables are presented as medians and interquartile ranges (IQR; 25th, 75th percentile) or individual value(s) if $n \leq 3$.

Supplemental Table 2. Food-specific IgG4s and IgEs

	Serum food-specific IgG4 (mg_A/L)					Serum food-specific IgE (U_A/mL)				
Patient No.	**Soy**	**Rice**	**Wheat**	**Cow milk**	**Egg white**	**Soy**	**Rice**	**Wheat**	**Cow milk**	**Egg white**
EGE4	0.081	<0.07	0.533	1.5	1.79	<0.10	<0.10	0.12	0.16	0.35
EGE6	6.03	17.8	>30.0	17.6	17.6	0.64	1.73	3.53	0.21	1.52
EGE7	1.54	2.18	18.3	18.5	>30.0	0.29	0.31	0.64	0.12	0.49
EGE8	0.129	0.189	0.81	25.4	14.3	<0.10	0.11	0.18	0.1	0.13
EGE10	0.111	<0.07	1.69	25.5	6.91	<0.10	0.11	0.11	0.35	0.11