## Food-specific $IgG_4$ is associated with eosinophilic esophagitis

*To the Editor:*

Our understanding of eosinophilic esophagitis (EoE) pathogenesis is incomplete, but repetitive antigen exposure plays a role in most patients. Skin prick testing, serum IgE levels, and atopy patch testing in some centers have been used to guide dietary elimination, but there is insufficient data to suggest that they are superior to empiric elimination of common food triggers.[1] Recent data suggest a possible association between EoE and elevated levels of total $IgG_4$ (T-$IgG_4$), and that $IgG_4$ may be implicated in disease pathogenesis.[2]

The role of $IgG_4$ in EoE is unclear. Clayton et al[2] showed that esophageal $IgG_4$ deposits in EoE derive from dense plasma cell infiltrates in the lamina propria. Moreover, data from one study noted that intrasquamous deposits of $IgG_4$ may distinguish patients with gastroesophageal reflux disease versus EoE.[3] These observations suggest potential associations between EoE and $IgG_4$-related diseases. Nontheless, food-specific $IgG_4$ (FS-$IgG_4$) has also been shown to inhibit basophil and mast cell responses[4]; indeed, FS-$IgG_4$:IgE ratios have been identified as markers of sustained unresponsiveness[5] and oral tolerance.[6] We speculate that $IgG_4$ may be a marker of epithelial disruption produced initially to attenuate IgE-mediated disease but that, ultimately, results in a proinflammatory process in susceptible atopic hosts. Evidence for this concept may be supported by the development of EoE in some subjects undergoing oral immunotherapy.[7]

Because common food allergens are primary triggers of EoE, the aim of this study was to examine the role of FS-$IgG_4$ in the pathogenesis of EoE. We hypothesized that FS-$IgG_4$ levels would be elevated in subjects with active EoE compared with controls, decrease with successful dietary elimination, and correlate with known food triggers.

We performed a case control study of 20 adult subjects with EoE and 10 controls without EoE using prospectively banked specimens from the University of North Carolina EoE Registry and Biobank. Details regarding recruitment, specimens, and data collection have been published previously.[8] Diagnosis of active EoE was defined by consensus guidelines.[9] Non-EoE controls were subjects with dysphagia or gastroesophageal reflux disease who did not meet histologic critieria for EoE. Subjects with proton pump inhibitor-responsive esophageal eosinophilia were excluded. Subjects diagnosed with EoE were treated with a six food elimination diet where dairy, egg, wheat, soy, peanut/tree nuts, and seafood were eliminated in addition to suspected dietary triggers on the basis of clinical history. Eleven subjects demonstrated histologic resolution of esophageal eosinphilia (<15 eos/hpf) after 6 to 8 weeks of dietary elimination and were classified as diet responders. The 9 remaining subjects were classified as nonresponders. Clinical food triggers were identified by serial reintroduction of individual food groups followed by repeat endoscopy and biopsy at approximately 6-week intervals. Fig E1 in this article's Online Repository at www.jacionline.org illustrates the study design. We measured T-$IgG_4$ and FS-$IgG_4$ levels for peanut, soy, egg white, casein, and wheat by ELISA in paired plasma and esophageal homogenates at baseline and following dietary elimination. Details regarding the study population, methods used for $IgG_4$ measurement, and statistical analysis are detailed in this article's Online Repository at www.jacionline.org.

Table E1 in this article's Online Repository at www.jacionline.org details the demographic and clinical characteristics of the study population. At baseline, subjects with EoE demonstrated significantly higher levels of T-$IgG_4$ and FS-$IgG_4$ in the esophagus in comparison to non-EoE controls (Table I). Similar results were seen in the plasma, although plasma T-$IgG_4$ did not differ significantly in subjects with EoE. Significant differences were not observed for baseline T-$IgG_4$ or FS-$IgG_4$ levels when nonresponders were compared with diet responders. Linear regression analysis of T-$IgG_4$ and FS-$IgG_4$ measurements in all subjects revealed positive correlations between esophageal FS-$IgG_4$ levels and those seen in the plasma (see Fig E2 in this article's Online Repository at www.jacionline.org). Following dietary elimination, esophageal T-$IgG_4$ declined significantly among responders ($P = .04$) (Fig 1, *A*), but not among nonresponders. In addition, postelimination T-$IgG_4$ levels were significantly lower in the responder group than in the nonresponder group ($P = .03$). In diet responders with identified triggers, we observed significant declines in esophageal, but not serum, FS-$IgG_4$ levels for all trigger foods ($P = .02$) (Fig 1, *B*). Interestingly, the only subject who demonstrated a slight increase in egg white-specific $IgG_4$ level was consuming baked egg at the time of follow-up biopsy.

Among the 11 diet responders, baseline esophageal and plasma FS-$IgG_4$:T-$IgG_4$ ratios were elevated for most trigger foods (Fig 1, *C* and *D*). Some subjects with marked elevations in FS-$IgG_4$ levels did not have corresponding clinical triggers. Of note, the 1 subject with soy as a trigger was the only subject with elevated esophageal and plasma levels of soy-specific $IgG_4$. We were not able to derive predictive thresholds of FS-$IgG_4$ for individual food triggers given the small size of our cohort and large variations in $IgG_4$ levels among subjects. Interestingly, some subjects with EoE had persistently elevated T-$IgG_4$ levels despite dietary elimination. Although this may reflect nonadherence, it may also be due to the presence of $IgG_4$ to food allergens that have not been successfully identified, aeroallergens, or other environmental triggers of EoE.

While interpreting our results, we acknowledge certain limitations. This is a single-center, exploratory study of adult subjects; therefore, the results cannot be generalized to children. The sample size is also relatively small and insufficiently powered to make definitive comparisons between responders and nonresponders or draw conclusions regarding FS-$IgG_4$ as a marker of specific food triggers. Because most esophageal $IgG_4$ levels were measured only from a single biopsy specimen, it is possible that more focal deposits of $IgG_4$ were not detected. Moreover, we did not assess food-specific IgE responses. However, these limitations are balanced by a number of strengths including the study's prospective design, utilization of banked samples collected, stored, and processed in a uniform fashion for all subjects, detailed clinical and dietary elimination information, and blinded analysis of matched tissue and plasma samples.

In summary, we observed in this small sample of adult subjects with EoE that T-$IgG_4$ and FS-$IgG_4$ levels are elevated in the esophagus compared with non-EoE controls. We found that when food

**TABLE I.** Baseline absolute $IgG_4$ levels in subjects with EoE and controls*

| Food-specific $IgG_4$ levels | Controls (n = 10) | EoE cases (n = 20) | *P* value† |
|---|---|---|---|
| Esophageal biopsies, median (IQR) | | | |
| Peanut | 0.01 (0.01-0.50) | 4.05 (0.39-14.5) | .003 |
| Soy | 0.01 (0.01-0.01) | 2.12 (0.12-4.82) | <.001 |
| Egg white | 2.88 (0.01-12.7) | 61.4 (21.1-147.3) | <.001 |
| Casein | 0.67 (0.01-3.78) | 55.8 (5.87-271.5) | <.001 |
| Wheat | 1.10 (0.01-3.82) | 19.9 (5.13-184.6) | <.001 |
| Total | 469 (210-848) | 1,847 (551-3,487) | .008 |
| Plasma, median (IQR) | | | |
| Peanut | 609.0 (153-1731) | 2,324 (703.9-18,709) | .04 |
| Soy | 54.8 (0.01-1204) | 1,141 (436.9-3,549) | .02 |
| Egg white | 16,400 (1,683-78,833) | 84,147 (30,720-151,476) | .01 |
| Casein | 2928 (384-7232) | 22,316 (5,113-123,456) | .01 |
| Wheat | 4,984 (298-10,388) | 14,773 (4,486-119,263) | .04 |
| Total | 276,839 (101,755-725,261) | 441,092 (146,954-747,240) | .65 |

*IQR*, Interquartile range.
*Total and antigen-specific $IgG_4$ measurements are reported in ng/mL.
†Comparisons of medians by Wilcoxon rank-sum test.

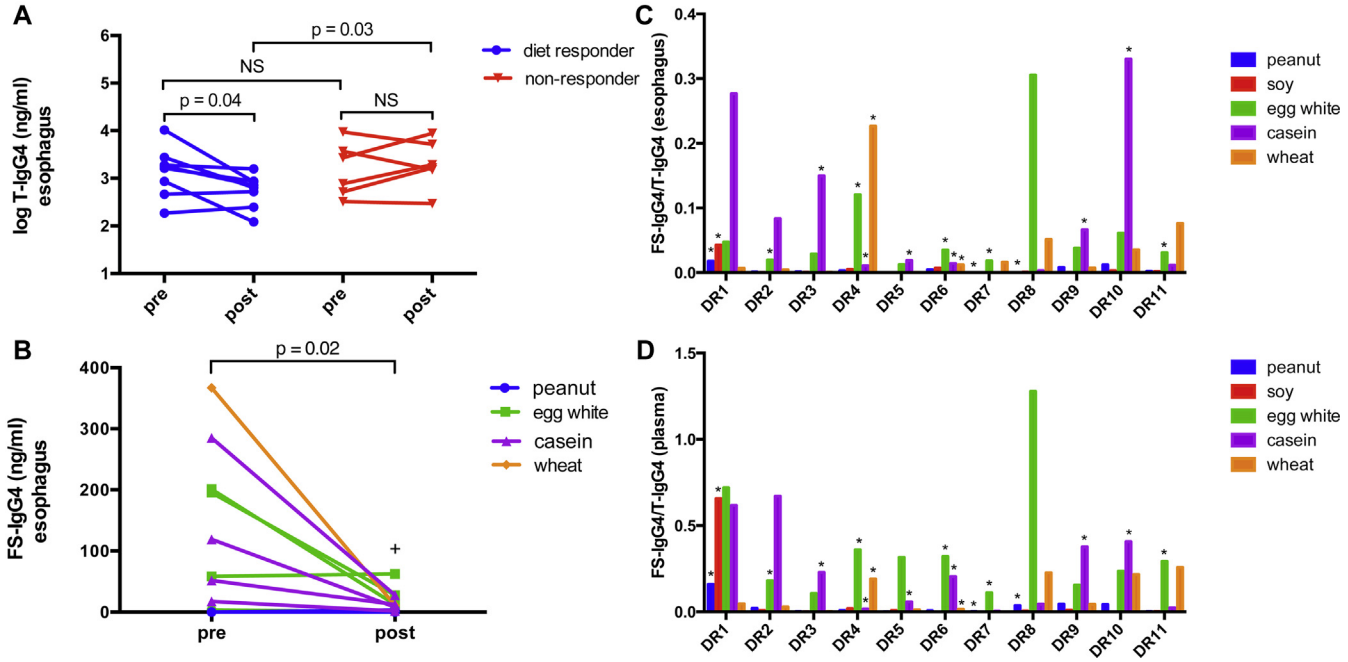


**FIG 1.** Esophageal $IgG_4$ level is elevated in subjects with EoE and decreases in response to dietary elimination. **A,** Esophageal T-$IgG_4$ levels decrease after dietary elimination in diet responders but not in nonresponders. **B,** Esophageal FS-$IgG_4$ levels to known triggers decrease significantly following dietary elimination. Baseline esophageal **(C)** and plasma **(D)** FS-$IgG_4$/T-$IgG_4$ ratios for diet responders with identified triggers. +Denotes a subject who continued to consume baked egg (*B*). *Denotes confirmed food triggers (*C* and *D*).

triggers are eliminated, esophageal T-$IgG_4$ and FS-$IgG_4$ levels decrease in diet responders although these trends are not clearly seen in plasma. The presence of FS-$IgG_4$ in the esophagus of subjects with EoE suggests that chronic antigen exposure in an atopic individual appears to be associated with an antigen-specific $IgG_4$ response that may contribute to the pathogenesis of EoE.

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## Regulatory B cells and T follicular helper cells are reduced in allergic rhinitis

*To the Editor:*

Although the role of human regulatory B (Breg) cells in autoimmunity has been extensively studied, there are limited studies exploring the potential importance of Breg cells in allergy.[1-7] A subset of peripheral blood Breg cells that produces IL-10 (ie, $CD19^+CD73^-CD25^+CD71^+$ Breg cells) has recently been demonstrated to inhibit $T_H2$ responses.[1-3] Breg cells express the cell surface markers CD19 (a B-cell marker), CD25 (the alpha chain of the IL-2 receptor also expressed by regulatory T [Treg] cells), and CD71 (a known activation marker of B cells), but express low amounts of CD73 (an ectonucleotidase thought to play a role in T-cell suppression).[1,2] Murine *in vivo* studies have demonstrated that antigen specificity is required to develop Breg cells capable of secreting IL-10.[8] Human Breg cells also exhibit allergen specificity as demonstrated in studies of bee venom-allergic subjects in whom lower circulating phospholipase $A_2$-specific Breg cells were found in venom-allergic patients compared with venom-tolerant beekeepers and venom immunotherapy patients.[1] Importantly, T follicular helper ($T_{FH}$) cell production of IL-21 is critical for the maturation of Breg cells and their production of IL-10.[8]

In this study, we investigated whether subjects with allergic rhinitis had reduced numbers of Breg cells, as well as reduced numbers of Breg cells expressing $CD25^{hi}$, as previous studies of Treg cells have identified the $CD25^{hi}$ population as the Treg-cell subset most effective in inhibiting T-cell responses.[9] In addition to examining levels of Breg cells and $T_{FH}$ cells in peripheral blood, we ascertained whether Breg cells and $T_{FH}$ cells could be detected in human lymph nodes, a potential site of interaction between Breg cells, $T_{FH}$ cells, and $T_H2$ cells not previously studied in humans.

To compare circulating levels of Breg cells and $T_{FH}$ cells, PBMCs were isolated from 10 individuals with allergic rhinitis (mean age, 33.8 years; sex, 3 males and 7 females) and 7 nonallergic individuals (mean age, 37.9 years; sex, 2 males and 5 females) in a protocol approved by the University of California San Diego Human Subjects Protection Committee. Allergy status was confirmed by ImmunoCAP specific IgE levels or skin prick testing to cat, dog, cockroach, dust mite, grasses, trees, weeds, and molds (see Table E1 in this article's Online Repository at www.jacionline.org). Using a previously described gating strategy,[1] Breg cells ($CD19^+CD73^-CD25^+CD71^+$) were identified in isolated PBMCs by fluorescence-activated cell sorting (FACS) using fluorescently labeled CD19, CD73, CD25, and CD71 antibodies as well as their corresponding isotype controls (eBioscience, San Diego, Calif) (Fig 1, *A*). We also examined the percentage of Breg cells expressing $CD25^{hi}$, as previous studies of Treg cells have identified the $CD25^{hi}$ population as the Treg-cell subset most effective in inhibiting T-cell responses (Fig 1, *B*).[9] $T_{FH}$-like cells (previously defined in peripheral blood as $CD4^+PD\text{-}1^+CXCR5^+$ T cells)[E1,E2] were also detected in PBMCs by FACS using fluorescently labeled CD4, CXCR5, PD-1 antibodies, and their corresponding isotype controls (eBioscience) (Fig 1, *A*). Human lung lymph nodes were obtained from human lungs postmortem as previously described at the Arkansas Children's Hospital Research Institute in an institutional review board–exempted protocol.[E3] A single-cell suspension was prepared by mechanical disruption of lymph nodes for FACS analysis to identify Breg cells and $T_{FH}$ cells (as described above for PBMCs). Flow cytometry was performed using the BD Accuri C6 (BD Biosciences, San Jose, Calif) and Novocyte Flow Cytometer (ACEA Biosciences, Inc, San Diego, Calif) and was analyzed using FlowJo software (version 10.0.7; Tree Star, Inc, Ashland, Ore). Statistical analyses were performed using 1-tailed *t* tests and results reported as mean ± SEM.

We found that percentages of $CD25^+$ Breg cells (2.45 ± 0.41 vs 5.05 ± 1.42; $P < .05$) and $CD25^{hi}$ Breg cells (0.25 ± 0.05 vs 0.49 ± 0.06; $P < .01$) were lower in individuals with allergic rhinitis than in nonallergic controls (Fig 2, *A*). The lower levels of Breg cells in subjects with allergic rhinitis tended to cluster in a similar range, while nonallergic individuals exhibited a wider distribution. Our studies are consistent with reports showing that Breg cells are reduced in allergic individuals compared with controls.[1,2,4,5,7]

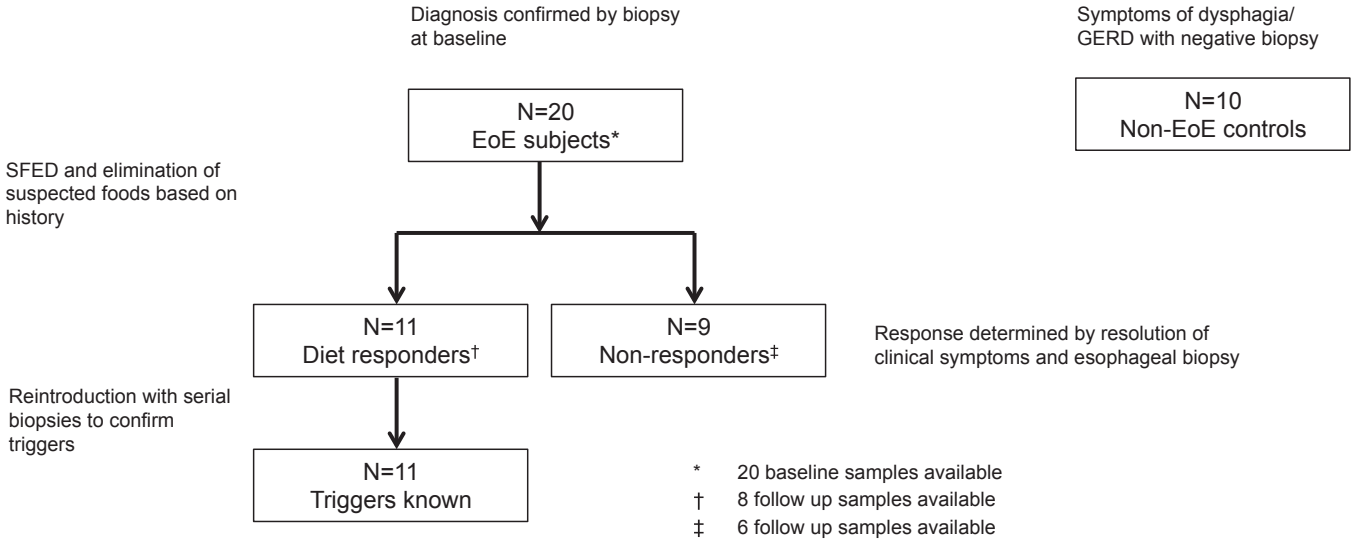


**FIG E1.** Prospectively collected and banked esophageal and plasma specimens were obtained at baseline from subjects with EoE (n = 20) and non-EoE controls (n = 10). After 6 to 8 weeks of dietary elimination, responder status was determined by follow-up endoscopy. Dietary triggers were elucidated in diet responders (n = 11) by serial reintroduction of foods and repeat esophageal biopsy.

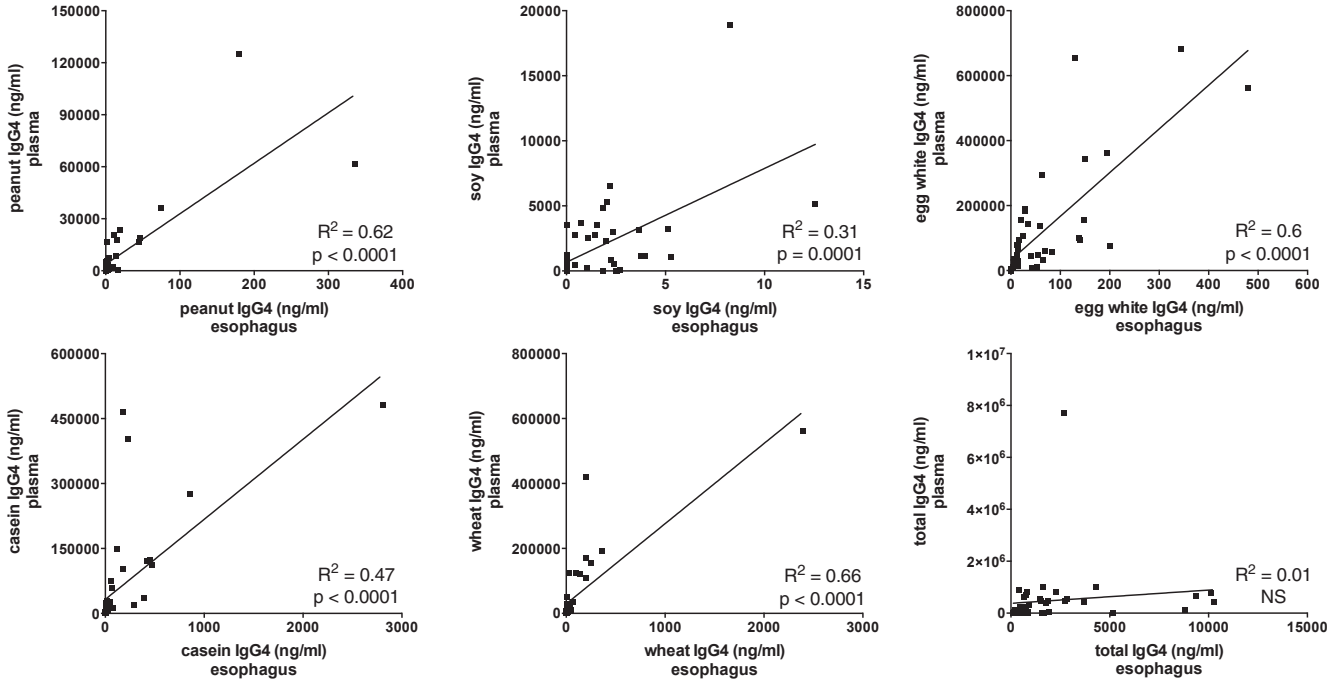


**FIG E2.** Esophageal FS-IgG$_4$ correlates with plasma FS-IgG$_4$. Linear regression plots of absolute T-IgG$_4$ and FS-IgG$_4$ (peanut, soy, egg white, casein, and wheat) from plasma (y-axis) and esophageal homogenates (x-axis). $R^2$ values reported for each food antigen.

**TABLE E1.** Baseline characteristics of the study population

| Characteristic | Controls (n = 10) | EoE cases (n = 20) |
|---|---|---|
| Age (y), mean ± SD | 43.6 ± 13.2 | 35.0 ± 8.4 |
| Sex: male, n (%) | 2 (20) | 8 (40) |
| White, n (%) | 7 (70) | 18 (90) |
| Atopic conditions, n (%) | | |
| Asthma | 4 (40) | 5 (25) |
| Eczema | 0 (0) | 1 (5) |
| Seasonal allergies | 5 (50) | 16 (80) |
| Food allergies | 1 (10) | 10 (50) |
| Diagnoses | | |
| EoE | — | 20 (100) |
| GERD | 8 (80) | — |
| Esophageal dysmotility | 1 (10) | — |
| Functional | 1 (10) | — |
| Peak eosinophil counts, mean eos/hpf ± SD | | |
| Baseline | 0.2 ± 0.4 | 69.2 ± 65.6 |
| Postdietary elimination (overall) | — | 42.9 ± 56.5 |
| Postelimination (responders; n = 11) | — | 4.5 ± 3.8 |
| Postelimination (nonresponders; n = 10) | — | 89.8 ± 55.5 |

*GERD*, Gastroesophageal reflux disease.