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Eosinophilic gastrointestinal disease and peanut allergy are alternatively associated with IL-5$^+$ and IL-5$^-$ Th2 responses

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Abstract

Background—Both anaphylactic food allergy and eosinophil associated gastrointestinal disorders (EGIDs) are associated with Th2 responses and food specific IgE, yet have very different clinical presentations.

Objective—To determine if the clinical differences between anaphylactic food allergy and EGIDs are reflected in different Th2 responses to foods.

Methods—Peanut allergic (PA), allergic eosinophilic gastroenteritis (AEG) and nonatopic (NA) subjects were enrolled. Antigen specific IL-4, IL-5, IFN-γ and TNF T cell responses to peanut, soy and shrimp were measured using intracellular cytokine staining and polychromatic flow cytometry.

Results—Two distinct subpopulations of Th2 cells were found: IL-5$^+$ Th2 (IL-4$^+$, IL-5$^+$) and IL-5$^-$ Th2 (IL-4$^+$, IL-5$^-$) cells. Peanut specific IL-5$^+$ Th2 cells were present at a 20-fold greater frequency in AEG vs. PA (81 vs. 4 per 10^6 CD4 cells, p=0.05), whereas there were similar frequencies of IL-5$^-$ Th2 cells (67 vs. 41 per 10^6). For all foods, IL-5$^+$ Th2 cells accounted for a significantly greater fraction of the antigen specific cells in AEG relative to PA (29% vs. 4%, p<0.0001). In PA, but not AEG, IL-5$^-$ Th2 responses to peanut were highly correlated with peanut specific IgE (r= 0.87 vs. 0.55, respectively). All subject groups elicited similar very low magnitude Th1 responses to food Ags.

Conclusion—Th2 responses are composed of two subpopulations: IL-5$^+$ Th2 and IL-5$^-$ Th2 cells. IL-5$^+$ Th2 food allergen specific T cells are singularly associated with AEG, whereas PA is associated with a dominant IL-5$^-$ Th2 response. These results suggest heterogeneity within the Th2 cytokine response, with different Th2 responses alternatively favoring IgE mediated or eosinophil dominant immunopathology.

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Key Messages

- Two distinct subpopulations of Th2 cells were identified: IL-5$^+$ Th2 (IL-4$^+$, IL-5$^+$) and IL-5$^-$ Th2 (IL-4$^+$, IL-5$^-$) cells.
- Allergic eosinophilic gastroenteritis was associated with a significantly greater frequency of food allergen specific IL-5$^+$ Th2 cells than peanut allergy.
- Similar low magnitude food allergen specific Th1 responses were found in both the food allergic and non-atopic control groups.

Keywords

food allergy; peanut; T cell; Th2; eosinophil associated gastrointestinal disease; interleukin-4; interleukin-5; flow cytometry

Introduction

Food allergies are increasingly common and affect approximately 4% of the population[1, 2]. Eosinophil associated gastrointestinal disorders (EGIDs), including eosinophilic esophagitis (EoE) and eosinophilic gastroenteritis (EG), are a spectrum of increasingly recognized inflammatory diseases that are strongly associated with hypersensitivity to multiple foods [3-5]. Furthermore, EGIDs, in particular pediatric EoE, are responsive to an elemental diet[6], suggesting that food allergen driven immune responses contribute to disease pathogenesis.

Th2 cells are critical to both the IgE and eosinophilic responses that underlie allergic diseases[7, 8]. Both peanut allergy[9, 10] (PA) and EGIDs[11-14] are associated with abnormal cytokine (Th2-like) and IgE responses to food antigens (Ags). However, the two diseases have markedly dissimilar clinical presentations. The dominant feature of PA is immediate hypersensitivity and anaphylaxis, whereas EGIDs are associated with chronic eosinophilic gut inflammation, but typically not anaphylaxis[3]. Th2 responses play a dominant role in directing both IgE class switching (IL-4) and tissue eosinophila (in part IL-5-mediated). However, it is not clear how a uniform Th2 response could differentially direct the IgE-mediated or eosinophil dominant pathology characteristic of PA and EGIDs, respectively.

To understand how Th2 cells may differentially drive classic IgE-mediated food allergy vs. the eosinophilic gut inflammation, we used polychromatic flow cytometry to examine Ag specific T cell cytokine responses in EG, PA and healthy non-atopic (NA) control subjects. Notably, the IgE vs. eosinophil dominance of these diseases was reflected in the food allergen specific Th2 cytokine response. These results suggest heterogeneity within Th2 responses that may alternatively favor IgE production versus eosinophilic inflammation.

Methods

Subjects

Subjects were between 18 to 60 years. EG subjects had peak tissue eosinophil counts of ≥44 eos/hpf in gastric or duodenal biopsies (Table E1). The diagnosis of EG was based on typical gastrointestinal symptoms, the above tissue eosinophilia in stomach or duodenal biopsy specimens, and the exclusion of other causes of gut eosinophilia, including helminth infection. Crohn's disease was excluded by lack of typical pathologic findings (ulcerations, granulomata, or crypt architectural distortion) and clinical features (fistula, abdominal mass, and surgical obstructive disease). Subjects with immunodeficiency or a positive FIP1L1-PDGF-R fusion gene PCR were excluded. Subjects with EG were post-hoc grouped as either allergic EG (AEG) or non-allergic EG (NA-EG) as noted in Table 1. Subjects AEG1-4 and AEG6-9 are identical to EG1-4 and EG6-9 reported previously[15]; all samples were obtained prior to the study drug on that study. Four of the AEG subjects had concurrent EoE (Table E1). PA subjects had a history of systemic symptoms including at least one extracutaneous site (i.e. wheezing, gastrointestinal symptoms, laryngeal edema, angioedema, or hypotension) within 30 minutes of peanut ingestion, and a peanut specific IgE of ≥ 4 kIU/L. PA subjects did not undergo a peanut challenge as part of their work-up. Sixteen healthy non-atopic (NA) subjects had a negative history for allergy, asthma and food allergy, a negative Phadiatop® atopy screen (Phadia, Uppsala, Sweden), as well as undetectable levels (<0.35 kIU/L) of food allergen specific IgE to peanut, soy, shrimp, wheat, egg, and milk.

The NIAID Institutional Review Board approved the clinical protocols used for this study. All subjects signed informed consent.

Cell culture, staining and flow cytometry

T cell activation and intracellular cytokine staining were performed on cryopreserved samples according to previously published methods[16] that are detailed in the on-line repository text. The following 9-color panel was used: Violet LIVE/DEAD®, CD154 FITC (clone TRAP1), IL-4 PE (clone 25D2), CD4 PE/Cy5 (clone SK3), IFN-γ PE/Cy7 (clone B27), IL-5 APC (clone TRFK-5), TNF Alexa 700 (clone MAb11), all BD Biosciences, San Jose, CA; CD8 PE/TR (clone 3B5), CD3 APC/Alexa 750 (clone UCHT1), both Invitrogen.

Data analysis and statistics

Boolean analysis of cytokine coexpression was performed using SPICE software (M. Roederer, NIH, Bethesda, MD)[17] as detailed in the on-line repository text. Medians were used as the measure of central tendency. Proportional analyses represent a given subpopulation as a fraction of the total number of Ag specific cytokine producing cells. Statistics and linear regression were calculated with Prism software (GraphPad, La Jolla, CA) using Mann-Whitney U or Wilcoxon signed rank tests for comparisons, and the Spearman rank test for correlations. Box and whisker plots represent median, quartiles, minimum and maximum, respectively.

Results

Of the 17 EG subjects enrolled, 13 were designated allergic EG (AEG) and 4 non-allergic EG (NA-EG), based on the presence of multiple food hypersensitivities in the former (Table 1). PA subjects had significantly higher peanut specific IgE than did AEG subjects (16 vs. 1.2 kIU/L, p= 0.028).

After serial gating to identify viable CD4+ T cells (Fig 1A-D), we used the rapid upregulation of CD154 and cytokines to identify food Ag specific T cells[18, 19]. $CD154^+$, $cytokine^+$ cells were readily apparent in the peanut Ag activated samples (Fig 1E-F) from both PA and AEG subjects, but not in the unstimulated (media control) cultures (Fig 1G-H). CD8 T cytokine responses to food allergens were not detected (data not shown).

Although similar intracellular cytokine staining methods have been validated for pathogen associated Th1 immune responses[17, 20], they have not been previously used to analyze Th2 dominant allergen specific responses. To demonstrate that these food allergen specific responses are due to T cell recognition of MHC bound Ag, we used an anti-MHC class II mAb to block Ag presentation. Addition of anti-MHC class II decreased peanut Ag induced cytokine responses (Fig 1I-L; 80%, 91%, 94%, and 86% inhibition of IL-4, IL-5, IFN-γ, TNF, respectively). For all subsequent figures, the $CD4^+$, $CD154^+$, $cytokine^+$ gate was used to enumerate allergen specific cells.

For all cytokines and in all subject groups, the frequency of cytokine producing cells in the media control was exceeding low and was not significantly different among groups (Fig E1 and data not shown). Upon activation with peanut Ag, cytokine expression was highly induced in both the AEG and PA subjects (Fig E1).

Both EGIDs and PA are associated with Th2 responses and food allergen specific IgE, yet the two diseases have very different clinical presentations. To explore whether these differences are reflected in the T cell response, we measured the frequency of peanut specific CD4 T cells producing IL-4, IL-5, TNF or IFN-γ. Although the PA group had >10-fold higher peanut specific IgE than the AEG subjects, the frequency of peanut Ag specific

IL-4 producing CD4 T cells was not significantly different (Fig 2A, p=0.32). In contrast, AEG subjects had >10 times more IL-5 producing peanut specific T cells relative to the PA group (Fig 2B, p=0.038). IFN-γ and TNF expression was not significantly different between the two groups (Fig 2C, D).

None of the NA subjects had detectable peanut specific IL-5 responses and most had undetectable IL-4 expression (Fig 2A, B). In contrast, small but measurable IFN-γ and TNF responses were detected in about half of NA subjects.

Because previous studies have used ratios of Th1/Th2 frequencies to measure relative Th2 skewing[21], we next analyzed the ratio of peanut Ag specific cytokine producing cells. Both the AEG and PA responses were highly Th2 skewed, with IL-4:IFN ratios of 8.4 and 9.4, respectively (Fig E2A). The corresponding IL-5:IFN ratios were 4.2 and 0.67, respectively (Fig E2B). In summary, these results demonstrate that both PA and AEG are associated with increased peanut Ag specific IL-4 responses, whereas IL-5 responses are largely limited to AEG.

We next employed polychromatic flow cytometry to simultaneously analyze IL-4, IL-5, IFN, and TNF at the single cell level. Two major Th2 subpopulations were discernable: IL-5^+ Th2 (IL-4^+, IL-5^+) and IL-5^- Th2 (IL-4^+, IL-5^-) cells (Fig 3A, B: right and left upper quadrants, respectively). TNF was highly coexpressed with both IL-4 and IL-5 (Fig 3D and data not shown), but IFN-γ was not coexpressed with either of these cytokines (Fig 3C, E).

To evaluate the complexity of the T cell cytokine response more systematically, we used a Boolean gating analysis to examine the 15 cytokine coexpression patterns comprising every potential combination of the 4 individual cytokines (Fig 3F, bottom grid)[17]. The frequency of each individual cytokine combination was then assessed as a proportion of the total Ag specific cytokine response (Fig 3F).

Both AEG and PA were notable for a dominant Th2 response to peanut Ag. Th0 cells, defined as cells coexpressing IFN-γ and either Th2 cytokine, contributed minimally to the total cytokine expressing cells. TNF was highly coexpressed with both Th1 and Th2 cytokines.

Only 7 of the 15 possible cytokine combinations substantially contributed to the response. Because of the complexity of simultaneously analyzing 15 cytokine combinations, for further analysis we grouped these into 5 major cytokine subpopulations (IL-5^+ Th2, IL-5^- Th2, Th0, Th1, and TNF alone) as defined in Fig 3F.

To address the magnitude and quality of CD4 T cell responses, we next examined the frequency of these cytokine subpopulations responding to peanut, soy and shrimp Ag. Notably, peanut Ag specific IL-5^+ Th2 cells were 20-fold more frequent in AEG relative to PA (81 vs. 4 cells per 10^6 CD4 cells, p=0.05, Fig 4A), whereas IL-5^- Th2 cells were present in similar numbers (67 vs. 41 per 10^6, p= 0.89, Fig 4B). For all food Ags, AEG was associated with significantly greater frequencies of IL-5^+ Th2 cells than the PA population (p=0.05, 0.001, 0.01 for peanut, soy and shrimp, respectively).

Both allergic groups had significantly greater IL-5^+ and IL-5^- Th2 responses to foods than did the NA group (Fig 4A, B; $p<0.05$ for 11 of 12 comparisons). In contrast, food Ag specific Th1 responses were not significantly different between the groups (Fig 4D). The 4 NA-EG subjects had responses similar to the NA group (Figs 4, E1, and E3).

To examine the relative contribution of the 5 cytokine subpopulations, we next analyzed these food Ag specific cytokine responses by frequency (Fig 4E-G), or alternatively by

proportion (Fig E3). Notably, in AEG, Th2 responses and in particular IL-5$^+$ Th2 responses were greater in both magnitude and in proportion, relative to either the PA or NA groups. IL-5$^+$ Th2 cells were a significantly larger fraction of the food Ag response in AEG relative to PA (29% vs. 4%, $p<0.0001$). In contrast to the large differences in Th2 responses, all groups had similar magnitude Th1 responses (Fig 4E-G). When individual subject's Ag specific Th1 and Th2 responses were plotted against each other, we found no evidence for reciprocal correlation of these cytokine responses (data not shown). In sum, these findings indicate that AEG is singularly associated with an expansion of food allergen specific IL-5$^+$ Th2 cells.

To determine if the Th2 skewing found in AEG was limited to food Ags, SEB specific responses were examined (Fig 4H). Although IL-5$^-$ Th2 responses were similar among the groups, IL-5$^+$ Th2 cells were significantly greater in AEG (0.15% vs. 0.06% vs. 0.03% for AEG, PA and NA, respectively; $p<0.05$ for AEG vs. either group). Both allergic groups had lower frequencies of Th1 cells (2.3% vs. 2.7% vs. 5.0 % for AEG, PA and NA, respectively), although this was only significant for AEG vs. NA ($p=0.04$).

Because IL-5 has multiple pro-eosinophil actions we sought to determine if there was a relationship between IL-5$^+$ Th2 cells and eosinophilia. Accordingly, in AEG we found that the absolute eosinophil count correlated with the overall frequency of IL-5$^+$ Th2 cells ($r=0.6$, Fig 5A), but less so for IL-5$^-$ Th2 cells ($r=0.44$, data not shown). Similarly, food Ag specific IL-5+ Th2 responses for soy and peanut, but not shrimp, correlated with AEC (Fig E4 A-C). The frequency of IL-5$^+$ Th2 cells also correlated with tissue eosinophils in the gastric body but not those in the antrum, duodenum, or esophagus (Fig E4 D-G).

Because Th2 responses are required for IgE class switching, we next examined the relationship between Th2 responses and IgE. In PA but not in AEG, peanut specific IgE was highly correlated to the peanut specific Th2 response ($r=0.87$ vs. 0.55, respectively for IL-5$^-$ Th2; Fig 5B-C). In contrast, there was minimal correlation between the soy Th2 and IgE responses in either disease (Fig 5D).

Discussion

Both classic food allergy and EGIDs are associated with IgE and Th2 responses to food allergens, yet have distinct clinical manifestations of anaphylaxis and tissue eosinophilia, respectively. In this study we examined if these clinical differences are associated with different Th2 cytokine expression patterns. We thus investigated food allergen specific T cell cytokine responses at the single cell level and demonstrate that Th2 responses can be divided into two subpopulations based on IL-5 expression: IL-5$^+$ Th2 (IL-4$^+$, IL-5$^+$) and IL-5$^-$ Th2 (IL-4$^+$, IL-5$^-$) cells. We found that IL-5$^+$ Th2 responses are singularly associated with AEG, whereas PA is associated with a dominant IL-5$^-$ Th2 response (Figs 3 and 4). These results provide evidence for heterogeneity within the Th2 response, and demonstrate fundamental differences in the food allergen specific T cell responses found in AEG compared to classic anaphylactic food allergy. These results suggest that distinct Th2 subpopulations may alternatively contribute to IgE dominant or eosinophil dominant allergic disease.

It is unclear if the IL-5$^+$ and IL-5$^-$ Th2 subpopulations found in this study represent different lineages or Th2 differentiation states, or simply reflect differential expression of the IL-5 gene due to probabilistic[22], immunologic[23] or genetic factors[24]. Presumably, IL-5$^+$ Th2 cells serve as an abundant source of IL-5 to drive eosinophil differentiation, survival and activation[8]. Our finding that the frequency of IL-5$^+$ Th2 cells correlates with peripheral blood eosinophilia (Fig 5) supports this line of reasoning.

In agreement with the landmark findings of Turcanu and colleagues[21], we found that peanut Ag responses were highly Th2 skewed in PA subjects relative to the NA controls (Figs 4E, E2, E3). The relative Th1 skewing in NA subjects in this previous report led the authors to conclude that Th1-skewed responses underlie oral tolerance. Our results indicate that this apparent Th1 skewing of allergen specific responses in NA controls is an artifact of the experimental system used. The Turkanu study analyzed cytokine responses as ratios of Th1:Th2 cytokines. When our data was analyzed as ratios (Fig E2) or proportions (Fig E3) similar Th1 skewing was found. A deficiency of these ratio and proportional analyses is that they do not examine the magnitude of the T cell response. When our data are analyzed by magnitude by measuring the frequency of food Ag specific T cells, all subject groups had similar Th1 responses (Figs 2C, 4C, 4E-G). In the allergic groups this small Th1 response is dwarfed by the Th2 responses. In contrast, in NA subjects there is no Th2 response, resulting in a seemingly dominant Th1 response. Similar findings of Th1 dominated aeroallergen specific responses in NA subjects should be reassessed in this light.

In agreement with a recent report[9], we found that in PA peanut specific Th2 responses were highly correlated with peanut specific IgE (Fig 5). This relationship was not found in either the AEG group or with other food Ags. This tight association indicates that the peanut specific Th2 cells in PA may provide better help for IgE production than Th2 cells found in EGIDs.

Interestingly, EG subjects neatly dichotomized into those with multiple food hypersensitivities (AEG) or those with none, NA-EG (Table 1). Unlike the AEG group, the NA-EG subjects did not have Th2 responses to foods, but had responses similar to the NA group (Figs 4, E1 and E3). This suggests that the pathogenesis of NA-EG is distinct from AEG and may be T cell independent. Although there are no consensus diagnostic guidelines for EG, the AEG group demonstrated and relatively homogeneous clinical and immunological features, supporting the validity of the diagnosis.

In both allergic subject groups, the frequency of peanut Ag specific T cells was 100-200 per 10^6 CD4 T cells. In contrast, HIV and CMV specific responses are approximately 50-100-fold higher[20, 25]. This greater magnitude of viral specific T cell responses may be due to the greater pathogen associated molecular pattern “danger” signals, which more effectively prime virus specific immune responses relative to those associated with allergens. This low magnitude appears to be an intrinsic feature of allergen specific T cell responses[26], making their detection in translational research a technical challenge.

Notably, TNF was highly coexpressed with both IL-4 and IL-5 (Fig 3F). Although TNF is often considered a Th1 cytokine, Liu and colleagues have described *in vitro* differentiated “inflammatory Th2” cells that coexpress TNF and Th2 cytokines[27]. However, we have not examined the function of these TNF^+ cells to determine if they have inflammatory, or for that matter, regulatory function. To our knowledge, this work is the first demonstration of TNF coexpression by Th2 cells in an allergen specific manner and without extensive *in vitro* culture.

This work is also notable for several limitations. Although four of the AEG subjects had coexisting EoE, AEG differs from the most common EGIDs clinical entity of solitary EoE. As such, our findings of $IL\text{-}5^+$ Th2 cells may not be generalizable to EoE. IL-13 is a major effector cytokine in EGIDs, however, technical limitations did not allow us to concurrently examine IL-4, IL-5, and IL-13. Pilot experiments indicate that IL-4 and IL-13 are concordantly expressed (data not shown). A greater frequency of food Ag specific T cells was found in AEG relative to NA subjects. However, this may be due to the panel of cytokine antibodies we used that may not detect other subpopulations of T cells, such as

food allergen specific regulatory T cells, for which specific markers are less well defined. Although pediatric EoE responds to dietary management, similar studies have not been performed for adult AEG, making it difficult to gauge the pathogenic relevance of the food Ag specific Th2 cells in this study. Presumably, if IL-5^+ Th2 cells play a pathogenic role in EGIDs they home to the affected gut. A limitation of this work is that the observations of IL-5^+ Th2 cells are limited to the blood and have not been verified in the pathologic gut tissue itself.

In conclusion, we have shown that Th2 responses are composed of two subpopulations, IL-5^+ Th2 and IL-5^- Th2 cells, and that these Th2 subpopulations are respectively associated with eosinophilic inflammatory vs. IgE mediated food allergy. These findings suggest that different subpopulations of Th2 cells may alternatively contribute to the immediate hypersensitivity vs. the eosinophilic inflammatory components of allergic disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AEC Absolute eosinophil count

AEG	Allergic eosinophilic gastroenteritis
Ag	Antigen
EG	Eosinophilic gastroenteritis
EoE	Eosinophilic esophagitis
EGIDs	Eosinophil associated gastrointestinal disorders
NA	Nonatopic
NA-EG	Non-allergic EG
PA	Peanut allergic
PBMC	Peripheral blood mononuclear cells
SEB	Staphylococcal enterotoxin B

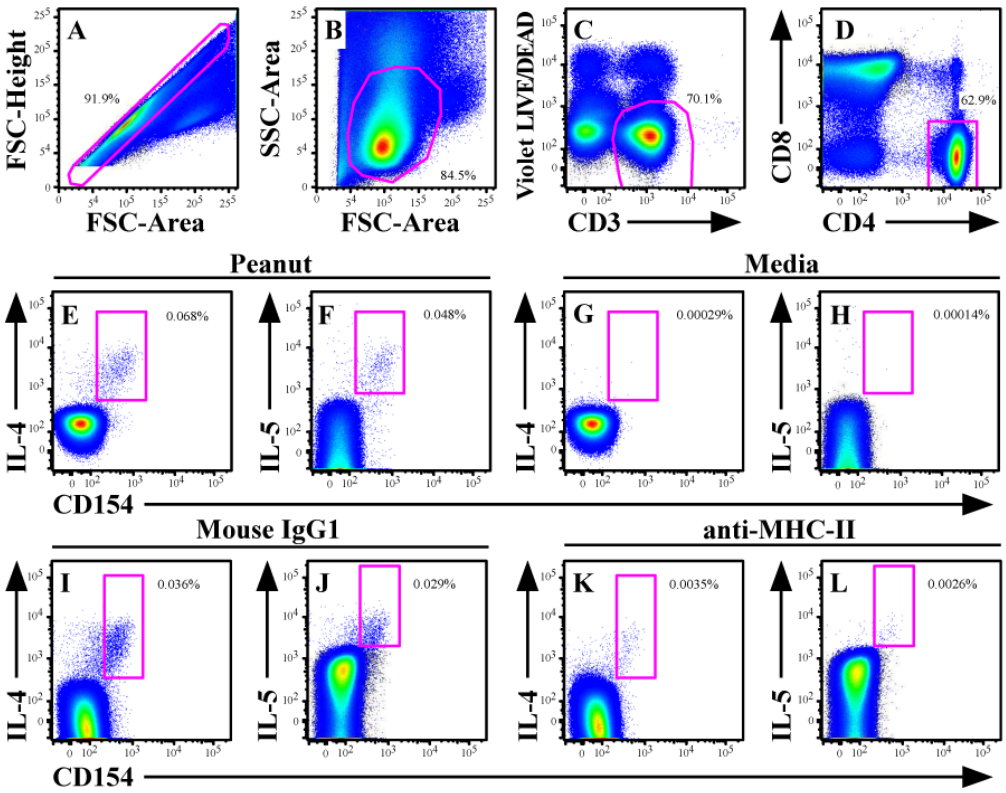

Fig 1.

Detection of food allergen specific T cell responses.

A-D: Gating. $CD4^+$ T cell expression of CD154 and either IL-4 (E, G) or IL-5 (F, H) after incubation with peanut Ag (E, F) or media (G, H). In a separate experiment, peanut Ag activated cultures were incubated with isotype control (I, J), or anti-MHC class II mAb (K, L), n=5.

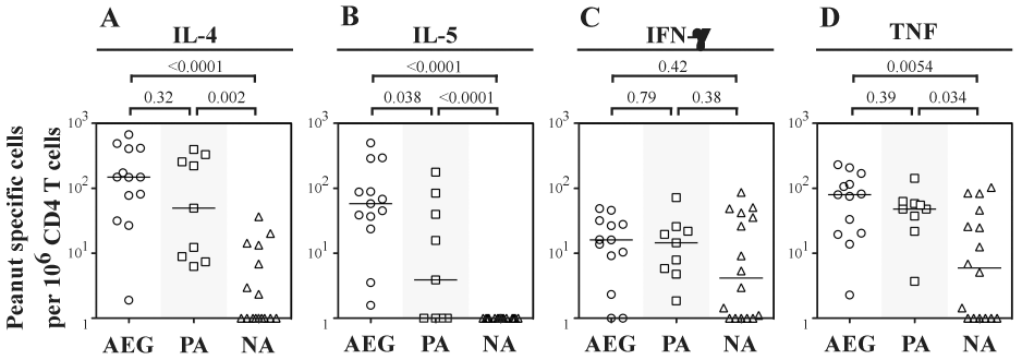

Fig 2.

AEG is associated with a greater frequency of peanut specific IL-5 producing T cells. The frequency of (A) IL-4, (B) IL-5, (C) IFN-γ and (D) TNF expressing peanut Ag specific CD4 cells was determined for each subject group. Each symbol represents an individual subject. The median value is denoted by a horizontal bar. Intergroup statistics are shown over the brackets.

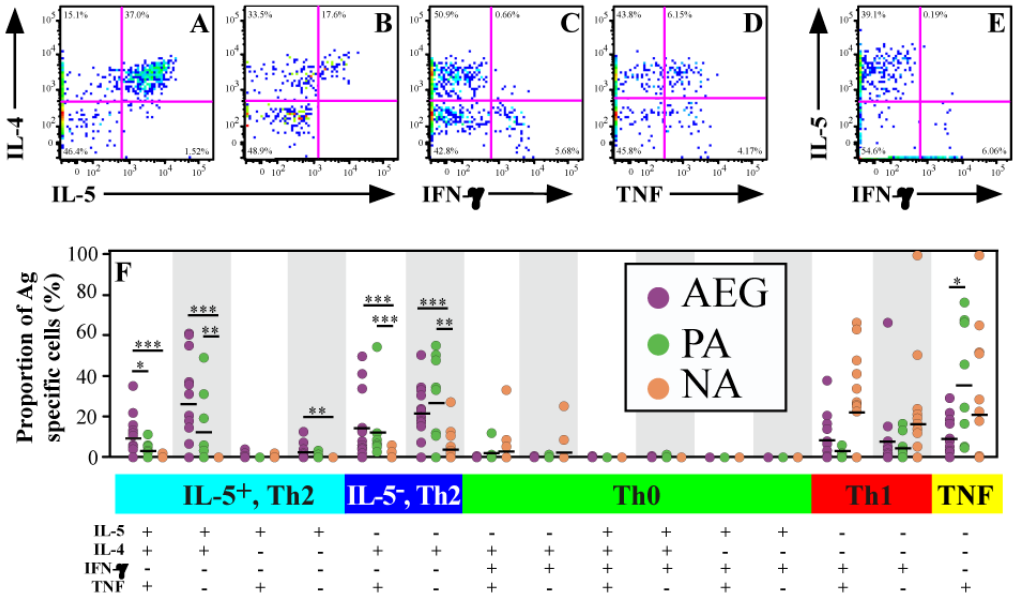

Fig 3.

Food specific T cells exhibit complex cytokine coexpression patterns.

Cytokine coexpression by peanut specific T cells from AEG (A, C-E) or PA (B) subjects. (F) Each of the 15 possible cytokine combinations are shown as a proportion of the total peanut response. Individual subjects and medians are denoted by dots and horizontal bars, respectively. All subjects from the 3 groups were studied. *, **, and *** represents p values of ≤0.05, ≤0.01, ≤0.001.

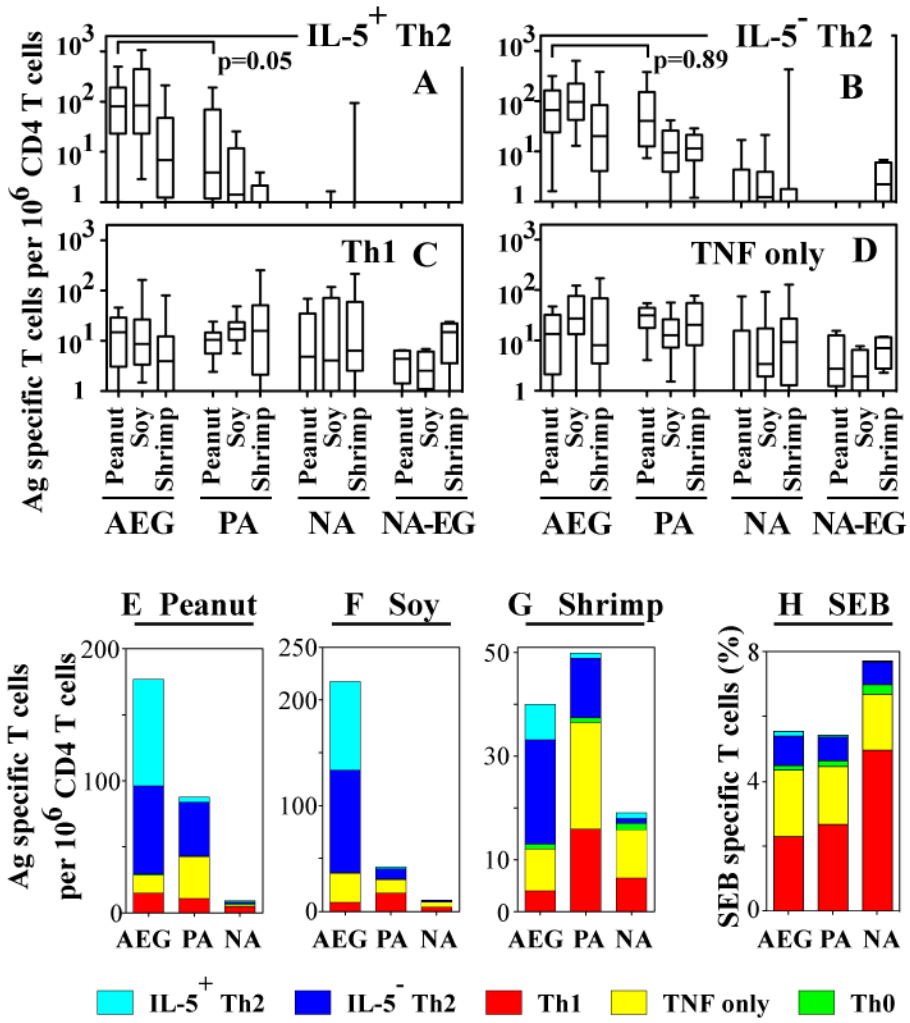

Fig 4.

AEG is singularly associated with food allergen specific IL-5^{+} Th2 cells.

A-D, the frequency of food Ag specific CD4 cells for each cytokine subpopulation and subject group. E-H, stack graphs depict the median values for each cytokine subpopulation summed to represent the total frequency of Ag specific cytokine producing cells.

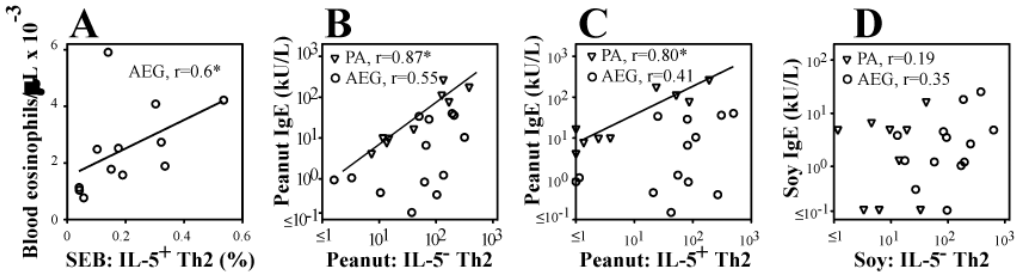

Fig 5.

Correlation of Th2 responses with IgE and eosinophilia.

A, correlation of IL-5$^+$ Th2 cells with eosinophil count. Correlation of peanut specific IgE with B, IL-5$^-$ Th2 and C, IL-5$^+$ Th2 cells. D, correlation of soy specific IgE with IL-5$^-$ Th2 cells. Linear regression curve fit is shown for AEG (A) and PA subjects (B, C), respectively.

Table 1

Subject characteristics

Subject	Age	Sex	Corticosteroid Therapy	AEC (cells/μL)	Presence of Dysphagia	Esophagus Peak eos >15/hpf	IgE (kIU/L)	Food allergen specific IgE (kIU/L)				
								Peanut	Soy	Shrimp	Wheat	Egg white
AEG1	37	M	None	**2732**	N	Y	583	**29**	4.5	2.9	15	12
AEG2	48	M	Bud 6	**1779**	N	N	780	**6.6**	3.5	0.8	56	10
AEG3	45	M	Bud 6	**1889**	Y	ND	555	**10**	4.9	2.1	20	15
AEG4	40	M	Pred 5	**2485**	Y	Y	42	**1.2**	1.0	ND	3.4	1.4
AEG5	25	M	None	**5910**	Y	Y	112	**0.41**	2.7	ND	9.0	0.53
AEG6	30	M	Pred 10	**4221**	N	Y	228	**0.84**	1.2	ND	3.6	1.1
AEG7	49	F	Bud 6	**768**	N	N	223	**1.1**	1.3	ND	12	13
AEG8	42	F	None	**1134**	N	Y	268	**34**	3.8	ND	3.8	14
AEG9	33	F	None	**1040**	N	Y	266	ND	ND	ND	12	1
AEG10	44	M	None	**2519**	Y	Y	28	**0.46**	0.37	ND	5.1	ND
AEG11	35	M	None	**4083**	N	Y	5530	**40**	25	25	32	144
AEG12	44	F	Bud 6	**822**	N	N	448	**0.94**	1.2	ND	0.98	0.39
AEG13	53	M	None	**1578**	N	Y	5840	**36**	18	0.38	52	>100
NA-EG1	60	M	None	**564**			370	ND	ND	ND	ND	ND
NA-EG2	56	M	None	**63**			60	ND	ND	ND	ND	ND
NA-EG3	52	F	None	**76**			18	ND	ND	ND	ND	ND
NA-EG4	41	F	None	**335**			4900	ND	ND	ND	ND	ND
PA1	26	F	None	**232**			428	**110**	1.3	ND	ND	ND
PA2	44	F	Pred 5	**247**			6850	**170**	4.7	1.7	65	14
PA3	21	M	None	**309**			330	**16**	ND	ND	0.49	ND
PA4	21	M	None	**890**			1470	**260**	16	2.0	3.2	3.2
PA5	22	F	None	**822**			736	**9.4**	4.7	1.3	0.46	ND
PA6	22	F	None	**268**			359	**74**	4.8	ND	ND	ND
PA7	18	F	None	**164**			474	**4.0**	6.5	ND	ND	ND
PA8	20	M	None	**89**			328	**9.6**	ND	ND	0.46	ND

Subject	Age	Sex	Corticosteroid Therapy	AEC (cells/μL)	Presence of Dysphagia	Esophagus Peak eos >15/hpf	IgE (kIU/L)	Food allergen specific IgE (kIU/L)				
								Peanut	Soy	Shrimp	Wheat	Egg white
PA9	37	M	None	**57**			175	**8.9**	ND	ND	ND	ND

Subject groups: AEG, allergic eosinophilic gastroenteritis; NA-EG, non-allergic eosinophilic gastroenteritis; PA, peanut allergic. ND: ≤0.35 kIU/L. Therapy: Bud, budesonide; Pred, prednisone; dose noted in mg/day