Altered T Helper 17 Responses in Children with Food Allergy

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Key Words

Anaphylaxis · Food allergy · Interleukin 17 · Peanut allergy · T helper 17

Abstract

Background: While a central role for the T helper (Th) 1/Th2 axis in food allergy has been established, the Th17 response in food-allergic humans has not been addressed. **Methods:** Th17 responses in 18 peanut-allergic children, who were also allergic to at least one additional food allergen, were assessed relative to 15 age-matched healthy controls. To account for the atopy background in the allergic children, 7 atopic, but not food-allergic, individuals and their age-matched controls were included in this study. PBMCs were analyzed by flow cytometry ex vivo or were stimulated in vitro with peanut allergens, gliadin, or tetanus toxoid followed by analysis of proliferation and cytokine production in antigen-responsive cells. **Results:** We observed a significantly lower interleukin (IL) 17 production in CD4+ T cells of food-allergic individuals ex vivo ($p < 0.02$). In vitro, we found that IL-17 production in CD4+ T cells in response to all antigens tested was significantly impaired in food-allergic subjects compared to healthy controls (Ara: $p < 0.005$; gliadin: $p < 0.004$; TT: $p < 0.03$). No significant differences were observed between atopic and nonatopic individuals with no

food allergy. **Conclusion:** Our results thus reveal a systemic, non-allergen-specific defect in Th17 responses to antigen stimulation in food allergic individuals, suggesting a role for Th17 cells in the control of food allergy and implicating IL-17 as a potential biomarker for tolerance to food antigens.

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Introduction

Food-related anaphylaxis rates are reported to have increased by as much as 350% in the last few decades, and peanut allergy accounts for the majority of severe allergic reactions in English-speaking countries [1]. A number of studies show that allergen-specific T helper (Th) 2-mediated responses characterize patients with food allergies, and that resolution of food allergy coincides with a shift to a Th1 response [2, 3]. Recently, this Th1/Th2 dichotomy in allergy has been expanded to other T cell effector subsets including Th17 cells. Th17 cells are characterized by their hallmark production of interleukin (IL) 17. While Th17 cells have been implicated in a number of autoimmune

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Table 1. Characteristics of the subjects

	Allergic (n = 18)	Nonallergic (n = 20)	Atopic (n = 8)	Nonatopic (n = 7)
Age, years	6.58 (3.93–9.01)	6.6 (5.42–12.68)	35.27 (34.75–37.44)	34.60 (30.75–41.08)
Males	55.55 (31.35–77.60)	70 (45.66–87.16)	25 (4.45–64.42)	14.28 (0.75–57.99)
White ethnicity	70.58 (44.04–88.62)	78.95 (53.90–93.02)	87.50 (46.68–99.34)	57.14 (20.24–88.19)
Asthma	66.66 (41.15–85.64)	5.26 (0.28–28.11)	25 (4.45–64.42)	0
Eczema	72.22 (46.41–89.29)	5.26 (0.28–28.11)	25 (4.45–64.42)	0
Hay fever	50 (29.03–70.97)	5 (0.26–26.94)	75 (35.57–95.54)	0
Drug allergy	0	0	25 (4.45–64.42)	0
Other diseases	0	0	0	14.28 (0.75–57.99) ^a
Regular ICS	16.66 (4.41–42.26)	0	12.5 (0.66–53.32)	0

With the exception of median age (IQR), values represent percentages (95% CI).

^a One subject had hemochromatosis.

diseases [4], their role in the regulation of IgE-mediated allergic reactions in several models remains unclear. In atopic dermatitis, both decreased [5] and increased levels of IL-17+ cells have been found in the peripheral blood of patients [6]. Moreover, increased IL-17 levels have been detected in the sputum and bronchoalveolar lavage fluids of asthmatic patients [7]. Interestingly, a trend towards a negative correlation between whole blood IL-17A levels and allergic sensitization to some food allergens was suggested, although specific Th17 responses were not assessed [8]. Further, there are no studies investigating the antigen specificity of Th17 responses to human food allergy. In order to gain insight into the involvement of the Th17 subset in allergic responses to food antigens, we investigated the ex vivo Th17 profile and in vitro antigen-specific Th17 responses in children with multiple food allergies.

Materials and Methods

Subjects

Eighteen children with allergies to peanut, and to at least one additional food allergen, were recruited from the Montreal Children's Hospital Allergy Clinic (table 1 and online suppl. table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000354028). Fifteen age-matched, nonallergic, nonatopic healthy control subjects were also recruited. In addition, a control group of 8 atopic, but not food-allergic, adults (parents of subjects in our food allergy cohort) was included in the study along with their age-matched healthy controls (table 1). In our study, atopy was defined as a positive allergen-specific serum IgE test or skin prick test to any common food or inhalant allergens [9]. As previously described [10], a diagnosis of food allergy was established only if one of the following conditions was fulfilled: (1) the child had both a convincing clinical history of an allergic reaction and a positive skin prick test, or food-specific IgE ≥ 0.35 kU/l, and (2) the child had never or rarely ingested the food or had an uncertain

clinical history of an allergic reaction to the food but had (a) a specific IgE greater than or equal to the cutoff of 15 kU/l for milk, peanut, and tree nut and 20 kU/l for fish, or (b) a positive food challenge as previously described [10].

Flow Cytometry

PBMCs from patients and controls were isolated by Ficoll-Paque (GE Healthcare) and cryopreserved. For cytokine analysis, PBMCs were incubated with PMA (25 ng/ml), ionomycin (1 μ g/ml) (Sigma-Aldrich), and GolgiStop (BD Biosciences) for 5 h followed by staining with antibodies against CD4, CD8, IL-17, IFN- γ , TNF- α , IL-4, and IL-13 (eBioscience) and analyzed on a FACSCanto II analyzer (BD Biosciences).

Cell Culture

PBMCs were plated in Xvivo-15 serum-free medium (Lonza) supplemented with 5 U/ml rhIL-2 in 96-well round bottom plates at 2×10^5 cells/well. PBMCs were either cultured with IL-2 alone or stimulated with a 1:1 mixture of native peanut allergens Ara h 1 + Ara h 2 (20 μ g/ml), gliadin (10 μ g/ml, G3375; Sigma-Aldrich), or tetanus toxoid (TT; 10 μ g/ml; Statens Serum Institute, Copenhagen, Denmark) for 9 days, followed by flow cytometric analysis of cytokine production in antigen-responsive cells identified by the expression of Ki-67 (BD Biosciences).

Statistical Analysis

All statistical analyses on graphs were performed using the two-tailed Student t test.

Results

Ex vivo flow cytometric analysis of CD4+ T cells revealed a significantly reduced proportion of IL-17-producing CD4+ T cells in children with food allergy compared to healthy controls [median (IQR) 0.40 (0.35–0.64) vs. 0.61 (0.47–2.70); $p < 0.02$; fig. 1a]. We also observed a significantly lower frequency of CD4+FOXP3+ cells in children with food allergy [5.70 (4.70–6.20) vs. 8.10 (6.94–

Fig. 1. Altered Th17/Treg axis in food-allergic individuals. **a** Ex vivo percentage of IL-17-producing cells within the CD4+ subset measured by flow cytometry. **b, c** Ex vivo flow cytometric analysis of the CD4+FOXP3+ cell compartment. Shown are the percentages of FOXP3+ cells within the CD4+ subset (**b**), and the FOXP3 mean fluorescence intensity (MFI) of CD4+FOXP3+ cells (**c**). **d–f** In vitro cytokine profiles of antigen-responsive CD4+ T cells

identified by Ki-67 expression at the end of the antigen stimulation. Shown are representative FACS plots gated on CD4+ T cells (**d**), the percentage of IL-17+ cells within CD4+Ki-67+ cells (**e**), and the percentage of cytokine-expressing cells within CD4+Ki-67+ cells of Ara-stimulated PBMCs (**f**). All results are shown for allergic ($n = 18$) and nonallergic ($n = 15$) patients; all p values are based on Student's t test. n.s. = Not significant.

8.95); $p < 0.00006$; fig. 1b] coupled with a reduced expression of the FOXP3 protein in these cells as measured by the mean fluorescence intensity [47.0 (37.0–65.0) vs. 76.4 (66.8–91.5); $p < 0.00002$; fig. 1c]. This suggests a reduced Treg compartment in children with food allergy, and that the lower IL-17 production in allergic subjects is unlikely to be due to enhanced regulation by the Treg cells.

We next sought to examine the Th17 responses following in vitro stimulation with the peanut allergens and control antigens. PBMCs were stimulated with the dominant peanut allergens Ara h 1 and Ara h 2 (Ara) [11], the wheat allergen gliadin that was tolerated by all subjects, or the recall antigen TT for 9 days, after which the cytokine expression of antigen-activated cells (as determined by the

expression of the Ki-67+ mitotic/proliferative marker) was assessed by flow cytometry as previously described [12, 13]. Whereas all antigens triggered IL-17 production in the antigen-responding CD4+ T cells of healthy controls, the IL-17 response was significantly impaired in CD4+ T cells of allergic individuals regardless of the stimulating antigen used [Ara: 2.76 (1.20–5.40) vs. 11.0 (3.61–24.8); $p < 0.005$; gliadin: 3.70 (1.01–6.04) vs. 8.03 (5.04–19.1); $p < 0.004$; TT: 2.23 (1.46–4.77) vs. 7.18 (2.54–12.4); $p < 0.03$; fig. 1d, e]. Notably, the cytokine defect we observed was restricted to IL-17 as in vitro analysis of other cytokines revealed normal IFN- γ and TNF- α levels in response to all antigens tested, and enhanced IL-4 [16.5 (13.7–22.3) vs. 12.5 (7.8–14.7); $p < 0.04$] and IL-13 [18.8 (15.2–26.1) vs. 14.6 (5.96–18.3); $p < 0.007$] responses to Ara but not to gliadin or TT, in the allergic group compared to healthy controls (fig. 1f). Given that all of the allergic subjects had other atopic conditions, we sought to investigate whether atopy, rather than food allergy, contributed to the altered IL-17 responses observed. To this end, we analyzed Th17 responses to antigen stimulation, as described above, in a cohort of atopic, but not food-allergic, individuals relative to nonatopic healthy controls. Our results show similar levels of IL-17 produced ex vivo (online suppl. fig. S1A) and in vitro in response to all of the antigens tested in atopic versus nonatopic subjects who had no food allergies (online suppl. fig. S1D). Moreover, we observed no differences in IFN- γ , IL-4, or IL-13 responses between atopic and nonatopic adults in response to any of the tested antigens (online suppl. fig. S1E). The frequencies of FOXP3+ cells and FOXP3 MFI were also comparable in atopic and nonatopic subjects (online suppl. fig. S1B, C). Finally, multivariate regression analyses, adjusted for the presence of atopy, age, and sex in the allergic and nonallergic subjects, indicated that the presence of food allergy is the only significant predictor of reduced Th17 responses. These results suggest that the defect in Th17 production may be a unique aspect of the mucosal response to foods that predisposes to food allergy.

Discussion

We report here the novel finding that Th17 responses to antigen stimulation are impaired in food-allergic children; this is an impairment that is unique to the food allergy patients tested, but not atopic patients without food allergy. In addition, it appears to not be allergen specific but rather dominates in response to antigen stimulation in general.

The systemic defect in Th17 responses observed here may suggest impairment in the differentiation of this subset in food-allergic individuals. Interestingly, our data and those of others also suggest potential defects in the FOXP3+ Treg compartment of food-allergic individuals [14]. Given the shared TGF- β -dependent differentiation pathway between peripherally induced Treg and Th17 cells [4], upstream defects in the generation of both subsets may account for their reduced frequency in food-allergic individuals and lead to dysregulated mucosal immune responses to allergens. Moreover, IL-17 has been shown to repress Th2 cytokines and Th2-recruiting chemokine production [15], suggesting that the low IL-17 production in T cells of allergic patients could allow aberrant Th2 responses and ultimately lead to the development of the allergic condition. Finally, Th17 cells could be enriched in the target organs, such as is seen in asthma [16], and thus the peripheral IL-17 responses may reflect a trafficking and compartmentalization of the response.

In summary, we provide the first evidence of a systemic impairment in Th17 responses in children with food allergy. Our results provide a novel insight into the potential role played by the Th17 subset in the control of food allergy in humans, and implicate IL-17 as a potential biomarker for tolerance to food antigens.

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