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Relationship of IgE to basophil phenotypes in peanut-sensitized adults

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To the Editor

Peanut allergy affects approximately 0.5 to 2% of the US population, and this prevalence appears to be rising.¹ Unlike other food allergies, which are typically outgrown by teenage years,² only 20% of children will outgrow this condition.³ While the prevalence of peanut allergy among adults is estimated at 0.6%,¹ 8.7% of adults have evidence of sensitization to peanut based on the presence of specific IgE.⁴ Despite the increasing numbers of adults that will be affected by peanut allergy, few studies have examined mechanisms of food allergy in this population.

Children with food allergy have an altered basophil phenotype, manifested by elevated spontaneous histamine release (SHR),⁵ elevated baseline CD203c expression,⁶ and increased sensitivity to peanut allergen as measured by CD63.⁷ While we recently demonstrated that 50% of adults with double-blind placebo controlled food challenge (DBPCFC)-confirmed peanut allergy have elevated basophil SHR,⁸ it is unknown whether other measures of basophil activation also persist into adulthood, if they are also present in asymptomatic sensitized adults, and how they change with the use of IgE-depleting therapy.

Two groups of adult subjects with peanut sensitization were recruited for this study. **Group A** included adults with a history of childhood-onset peanut allergy who failed a DBPCFC to 1000 mg peanut and were recruited for a AADCRC-NIAID clinical trial involving the use of omalizumab. **Group B** included adults who either did not react to a challenge with 4000 mg of peanut protein or ate peanuts regularly without difficulty. All individuals provided written informed consent, which was approved by the Johns Hopkins or Western IRB.

Blood samples were collected and serum was used for total IgE and peanut-allergen (PA) specific measurements. Basophils were isolated from blood and analyzed for SHR, PA-stimulated basophil histamine release (BHR), baseline basophil activation markers, and PA-stimulated basophil CD203c expression. Serum IgE transfer experiments were then performed before and after 3 weeks of *in vivo* treatment with omalizumab. The paired student t test, Wilcoxon matched-pairs signed-rank test, and Fisher's exact test were used for statistical analysis, and p values < 0.05 were considered statistically significant. Further details regarding these methods can be found in the Online Supplement.

A total of 24 individuals were included in this study (Group A = 15; Group B = 9), as is described in the Online Supplement. There were no significant differences in demographics or allergic disease between the two groups (Table I). Total IgE trended higher in Group B, whereas peanut-specific IgE, the ratio of peanut-specific to total IgE, and IgE to Ara h 1, 2, 3, and 8 by ImmunoCAP were all significantly higher in Group A. Using the ISAC assay, more subjects in Group B had IgE to pathogenesis-related 10 (PR-10) allergens, allergens cross-reactive with pollens. Peptide arrays performed in a subset of individuals demonstrated that those in Group A tended to have greater recognition of IgE binding to sequential epitopes of Ara h 1, 2, and 3 than individuals in Group B (Supplementary Figure E1).

Baseline basophil SHR was significantly higher in Group A than in Group B (median 6.7; range 2.4–42.1%; versus 3.6; range 2.6–5.9%; p = 0.03; Figure 1A). Group A subjects also

had higher net maximum BHR to PA-stimulation than those in Group B (Supplementary Figure E2). The PA concentrations for threshold BHR (>10%) and maximum BHR were significantly lower in the peanut allergic group. However, BHR stimulation with optimal anti-IgE did not differ between the groups, indicating the observed difference in PA-triggered release may be due to differences in peanut-specific IgE profiles rather than the overall IgE-receptor basophil responsiveness between groups. Patients with peanut allergy also had significantly higher baseline basophil expression of CD63 (Figure 1B) (median 94.0; range 9.4–1828 net mean fluorescence intensity (MFI); versus 20.33; range 4.1–51.7 net MFI; $p=0.004$) than those with asymptomatic sensitization, but no difference in baseline CD69 or CD203c expression was seen. Similar to PA-induced BHR, Group A subjects had a lower threshold concentration for PA-induced basophil CD203c expression than those in Group B (Supplementary Figure E3). No difference in anti-IgE mediated CD203c expression was found between the groups.

To further examine the contribution of IgE in this basophil phenotype in peanut allergic adults, baseline basophil measures were repeated after *in vivo* treatment with the monoclonal anti-IgE antibody, omalizumab, as part of an AADCRC-NIAID clinical trial⁸ (Supplementary Figure E4). As previously reported, baseline SHR significantly decreased after treatment with omalizumab for ~3 weeks. Likewise, baseline CD63 levels significantly decreased with omalizumab treatment. Average baseline CD203c expression did not decrease, but there was a significant decrease in the subset of six subjects with elevated CD203c at baseline (median 127.8; range 66.8–300.6 net MFI; versus 34.8; range 9.1–69.9 net MFI; $n=6$; $p=0.009$). Passive serum transfer experiments using serum before and after *in vivo* treatment with omalizumab showed impairment in the transfer of this activated basophil phenotype with omalizumab (see the Online Supplement and Supplementary Figure E5 for details).

While children with food allergy are known to have an altered basophil phenotype,^{5–7} this study is the first to characterize both the IgE profiles and basophil functional measures in adults with peanut allergy. Compared to asymptotically PA sensitized adults, adults with confirmed peanut allergy had higher levels of peanut-specific IgE, a higher ratio of peanut-specific to total IgE, and a trend toward broader epitope expression of specific IgE to peanut Ara h 1, 2, and 3. Similar to children, about one-half of adults with peanut allergy have an altered basophil phenotype, characterized by elevated SHR, elevated markers of baseline basophil activation (CD63), and heightened sensitivity to PA, as measured by both BHR and CD203c expression. This basophil phenotype is distinct from adults with asymptomatic peanut sensitization and adults with other atopic conditions, such as allergic rhinitis.⁹

When treated with *in vivo* IgE-reducing therapy (omalizumab), basophils from adults with peanut allergy had a significant decrease in basophil SHR and baseline CD63 expression and a decrease in baseline CD203c in those subjects with elevated CD203c prior to therapy. When transferring peanut allergic serum to IgE-stripped basophils *in vitro*, before and after treatment with omalizumab, we found that omalizumab presence in serum impaired the transfer of this phenotype. While the mechanism underlying this altered basophil phenotype is currently unknown, our *in vivo* and *in vitro* results lead us to suspect that IgE, and possibly differences in FcεRI signaling, may be implicated in the baseline expression of

elevated CD203c and SHR on basophils of peanut allergic adults, but further studies are needed.

This study is limited by the small sample size of peanut allergic and asymptotically sensitized individuals. However, significant differences in serum IgE and functional basophil measures were still noted between the two groups. Furthermore, it is possible that the crude peanut extract used for the basophil activation studies may have contained larger quantities of Ara h 1–3 than Ara h 8, which may partly explain the differences in stimulation assay results noted between Groups A and B. Finally, the serum transfer assays have substantial variation in the percentage of surface IgE removed and the amount of serum transferred due to donor serum and recipient basophil characteristics. Despite these limitations, however, a trend towards decreased SHR and decreased CD203c expression was noted after transfer of donor serum IgE with omalizumab.

In conclusion, this study is the first to demonstrate that approximately one-half of adults with peanut allergy were found to have an altered basophil phenotype, characterized by elevated SHR, elevated markers of baseline basophil activation (CD63), and heightened sensitivity to peanut allergen, as measured by both basophil histamine release and CD203c expression. Removal of IgE both *in vivo* and *in vitro* diminished these markers, which may implicate a role for IgE or altered FcεRI signaling in this altered basophil phenotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AADCRC	Asthma and Allergic Diseases Cooperative Research Centers
DBPCFC	Double blind placebo controlled food challenge
PA	peanut allergen
HR	histamine release
SHR	spontaneous histamine release
BHR	basophil histamine release

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Figure 1. Comparison of baseline basophil measures between peanut allergic and asymptotically sensitized adults

A. Comparison of spontaneous histamine release (% histamine) between those with peanut allergy (blue) and those with asymptomatic sensitization to peanut (red). **B.** Comparison of baseline CD63 expression (net MFI). **C.** Comparison of baseline CD69 expression (net MFI) **D.** Comparison of baseline CD203c expression.

Table I

Patients' demographic, clinical, and serologic characteristics*

Characteristic	Peanut Allergy (n=15)	Peanut Sensitized (n=8 ^{**})	P Value
Age (yr)	26.53 ± 2.45	28.44 ± 2.48	0.62
Male Sex	4 (27)	3 (38)	0.44
Allergic rhinitis	12 (86)	7 (88)	0.90
Atopic dermatitis	1 (7)	2 (25)	0.24
Asthma	7 (50)	4 (50)	1.00
Total IgE (kU/L) [†]	260 ± 50	908 ± 387	0.14
Peanut-specific IgE (kU/L)	28.39 ± 7.5	0.92 ± 0.23	< 0.001
Peanut-specific/Total IgE (%)	16.81 ± 4.57	0.18 ± 0.05	< 0.0001
ImmunoCAP Ara h 1 (kU/L)	11.73 ± 5.50	0.06 ± 0.01	0.005
ImmunoCAP Ara h 2 (kU/L)	14.92 ± 5.07	0.08 ± 0.02	< 0.001
ImmunoCAP Ara h 3 (kU/L)	3.62 ± 1.75	0.07 ± 0.02	0.04
ImmunoCAP Ara h 8 (kU/L)	2.58 ± 2.30	1.37 ± 0.58	0.04
ImmunoCAP Ara h 9 (kU/L)	2.17 ± 2.08	0.11 ± 0.04	0.66
ImmunoCAP Σ Ara h 1,2,3,8,9	34.58 ± 11.11	1.67 ± 0.59	<0.001
ISAC Σ Ara h 1,2,3,8 (ISU) [‡]	28.27 ± 11.41	1.35 ± 0.52	0.005
Presence of Ara h 1, 2, or 3	14 (93)	4 (50)	0.03
Cross-reactivity to PR-10 allergens	3 (21)	6 (75)	0.01

* Values are expressed as mean ± SE or as number (%)

^{**} n=8 for all analyses, except for age, gender, total IgE, peanut-specific IgE, and peanut-specific/Total IgE, where n=9[†] As the cut-off value for ImmunoCAP is 0.10 kU/L, a value of 0.05 kU/L was used for all values < 0.10.[‡] As the cut-off value for ISAC is 0.30 ISAC standard units (ISU), a value of 0.15 ISU was used for all values < 0.30.