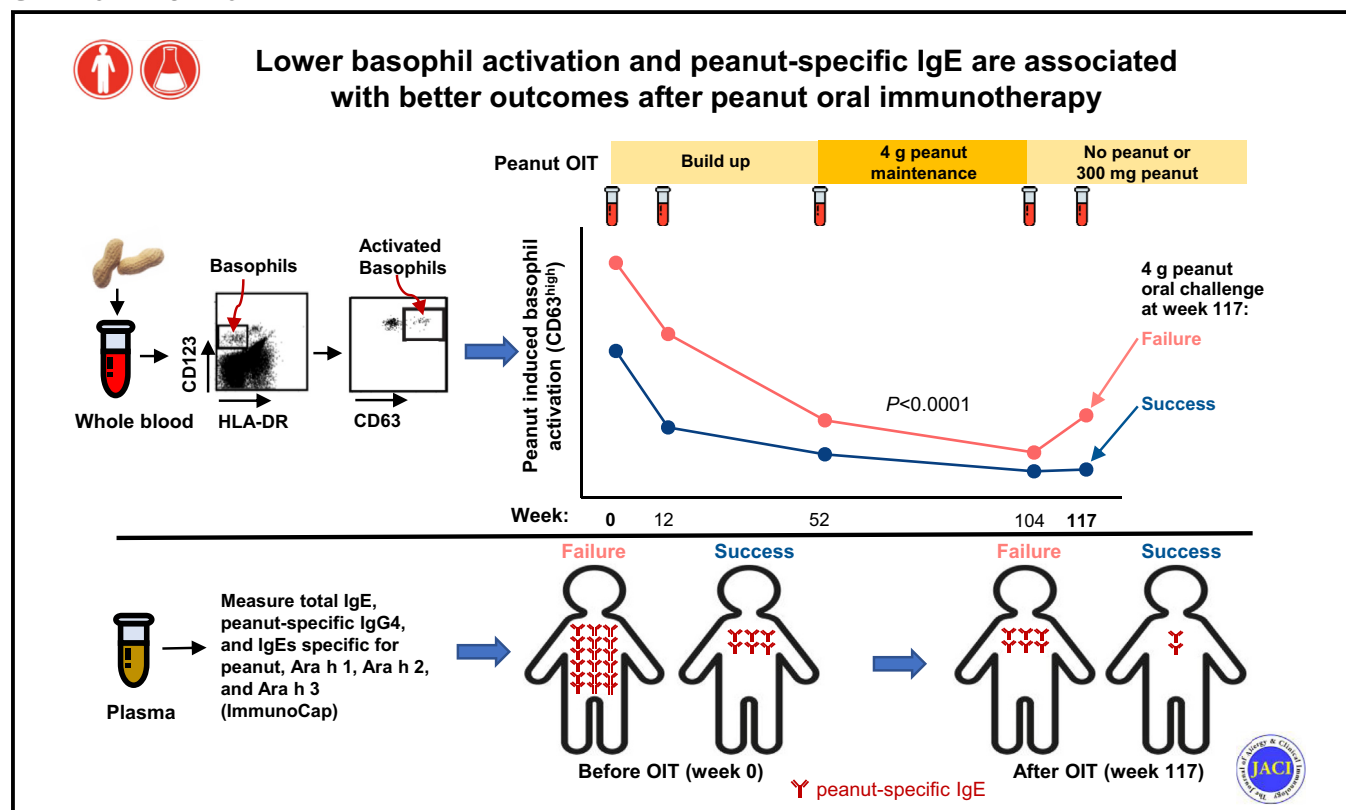


Sustained successful peanut oral immunotherapy associated with low basophil activation and peanut-specific IgE



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GRAPHICAL ABSTRACT



Background: Oral immunotherapy (OIT) can successfully desensitize many peanut-allergic subjects, but clinical tolerance diminishes over time on discontinuation, or low-dose maintenance, of peanut. Therefore, to improve the efficacy and sustainability of such therapy, we sought to identify biomarkers and clinical tools that can predict therapeutic outcomes and monitor treatment responses.

Objective: We evaluated whether basophil activation in whole blood, and plasma levels of peanut-specific immunoglobulins, are useful biomarkers for peanut OIT.

Methods: We longitudinally measured, before, during, and after OIT, basophil activation in whole blood *ex vivo* in response to peanut stimulation, and peanut-specific IgE (sIgE) and peanut-specific IgG₄ (sIgG₄), in a large, single-site, double-blind,

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randomized, placebo-controlled, phase 2 peanut OIT study. We compared basophil responsiveness and peanut-specific immunoglobulins between those who were clinically reactive and those who were tolerant to peanut oral challenges.

Results: Peanut OIT significantly decreased basophil activation, peanut sIgE, Ara h 1, Ara h 2, and Ara h 3 IgE levels, and sIgE/total IgE, but increased sIgG₄/sIgE. Participants who became reactive to 4 g of peanut 13 weeks off active OIT exhibited higher peanut-induced basophil activation *ex vivo* and higher peanut sIgE levels and sIgE/total IgE, but lower sIgG₄/sIgE. Notably, participants entering the study with low basophil responsiveness were more likely to achieve treatment success. Substantial suppression of basophil activation was required to maintain long-term clinical tolerance after peanut OIT.

Conclusions: Assessments of peanut-induced basophil activation and peanut-specific immunoglobulins can help to predict treatment outcomes, and to differentiate transient desensitization versus sustained unresponsiveness after OIT. (J Allergy Clin Immunol 2020;145:885-96.)

Key words: Basophil, basophil activation test, biomarkers, CD63, CD203c, oral immunotherapy, peanut allergy, peanut-specific IgE, peanut-specific IgG₄

There has been an increasing prevalence of food allergy, in both children and adults.^{1,2} However, there is currently no cure, and standard care, for example, dietary avoidance and symptomatic treatments, is suboptimal. Peanut allergy is particularly problematic. Only approximately 20% of peanut-allergic subjects are expected to outgrow this allergy.³ Moreover, it is associated with high rates of accidental exposure, which carries a dangerous risk of systematic anaphylaxis.⁴ Finally, diagnosing peanut allergy without oral peanut challenge is clinically challenging.⁵

Experimental therapies using oral immunotherapy (OIT), sublingual immunotherapy, or epicutaneous immunotherapy have evaluated the safety, efficacy, and immunologic changes associated with peanut immunotherapy.⁶ Such studies have demonstrated that immunotherapy can successfully desensitize patients to peanut.⁷⁻¹⁶ However, it is not clear whether long-term tolerance is sustainable without continuous peanut dosing after active therapy.

We recently completed a single-site, double-blind, randomized, placebo-controlled, phase 2 study (peanut oral immunotherapy study: safety, efficacy and discovery [POISED]) to evaluate the long-term effects of peanut OIT.¹⁷ POISED participants underwent peanut OIT build-up and maintenance followed by either peanut withdrawal or low-dose (300 mg) peanut daily dosing. More than 80% of participants tolerated up to 4 g of peanut protein after 2 years of active OIT therapy, but the tolerance declined substantially with or without low-dose daily peanut intake.¹⁷ Our findings in POISED,¹⁷ and others' studies,^{12,18-20} indicate that maintenance dosing and treatment regimens need to be improved further to achieve long-term protection after peanut immunotherapy.

Biomarkers for improving accuracy in diagnosis, and/or differentiating short-term desensitization versus long-term unresponsiveness or other outcomes, would be useful in improving treatment protocols that could ultimately be based on an individual's response to therapy. Although several immunologic parameters are being evaluated for such purposes, the basophil

Abbreviations used

AUC:	Area under the curve
BAT:	Basophil activation test
DBPCFC:	Double-blind, placebo-controlled, oral food challenge
HR:	Basophil high responder
IR:	Basophil intermediate responder
LR:	Basophil non/low responder
OIT:	Oral immunotherapy
POISED:	Peanut oral immunotherapy study: safety, efficacy and discovery
sIgE:	Specific IgE
sIgG ₄ :	Specific IgG ₄

activation test (BAT) has emerged as one of the most promising tools for food allergy testing.^{21,22} However, routine applications of BATs in clinical and research settings are not yet feasible because of the skills required to handle blood basophils, the lack of standardization of BAT protocols, and validation of BAT results with clinical outcomes.

To facilitate the utility of BATs, we recently developed a simple BAT protocol that produces consistent and reproducible results using whole blood samples collected in heparin, stored at 4°C, and processed within 24 hours after blood collection.²³ Using this protocol, basophil activation can be quantified in small volumes of whole blood by flow cytometric measurements of activation markers, such as CD63 and CD203c.²³ In the present study, we performed a comprehensive longitudinal analysis of basophil activation in the whole blood of participants enrolled in the POISED phase 2 peanut OIT study, and examined the association between basophil responsiveness and treatment outcomes after 2 years of peanut OIT followed by 13 weeks of peanut withdrawal (Peanut 0) or 300 mg low-dose peanut maintenance (Peanut 300). We reported recently that lower peanut-specific IgE levels and higher sIgG₄/sIgE at baseline are associated with treatment success in our POISED study.¹⁷ In this study, we examined whether OIT-induced changes in peanut-specific IgE (sIgE) and peanut-specific IgG₄ (sIgG₄) levels are associated with basophil activation and are predictive of sustained outcomes.

METHODS

Study design

Whole blood for BATs was collected from peanut-allergic participants (adults and children) enrolled in the POISED ([ClinicalTrials.gov](https://clinicaltrials.gov/NCT02103270) NCT02103270) at the Sean N. Parker Center for Allergy and Asthma Research at Stanford University. The clinical research protocol was approved by the Division of Allergy, Immunology, and Transplantation/National Institute of Allergy and Infectious Diseases Allergy and Asthma Data Safety Management Board, the Division of Allergy, Immunology, and Transplantation/National Institute of Allergy and Infectious Diseases Clinical Review Committee, the Stanford Institutional Review Board, and the Food and Drug Administration. For complete study design, baseline characteristics of the participants, and treatment outcomes, see Chinthrajah et al.¹⁷ Briefly, 120 participants who reacted to a cumulative ingested dose of less than or equal to 500 mg peanut by double-blind, placebo-controlled, oral food challenges (DBPCFCs) at enrollment were randomized into 3 arms: Peanut 0 (60 participants), Peanut 300 (35 participants), and Placebo (25 participants). Peanut 0 and Peanut 300 participants received OIT build up to, and maintenance of, 4 g of peanut protein until week 104, when participants in the Peanut 0 arm discontinued peanut and those in the Peanut 300 arm

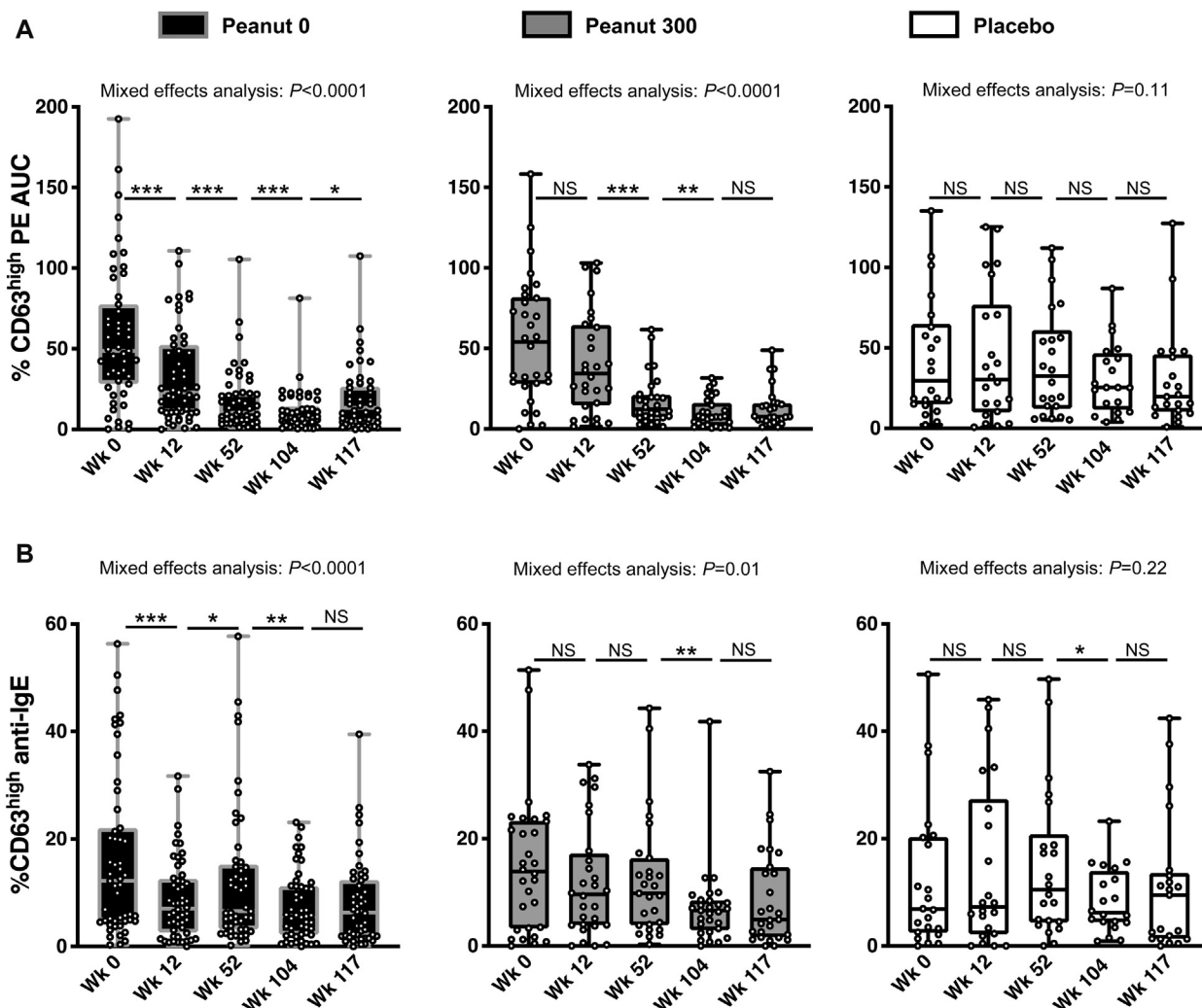


FIG 1. Peanut OIT reduces basophil responsiveness to peanut and anti-IgE over time. **A**, Peanut-induced %CD63^{high} basophils represented by AUC as a function of the PE protein dose (0–1000 ng/mL) in Peanut 0, Peanut 300, and Placebo arms at weeks 0, 12, 52, 104, and 117. **B**, Anti-IgE (1 μ g/mL)-induced %CD63^{high} basophils in Peanut 0, Peanut 300, and Placebo arms at weeks 0, 12, 52, 104, and 117. NS, Not significant. Data from dropout participants (ie, those who exited the study before week 104) were excluded. Whiskers represent min to max, boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. *P* values were determined by mixed-effects analysis with the Geisser-Greenhouse correction for longitudinal changes, or by Wilcoxon matched-pairs signed rank test for comparisons between adjacent columns. Source of week 0 data in Fig 1, A, was from Fig 4 of Chinthrajah et al,¹⁷ with permission from the Publisher (Elsevier).

maintained daily dosing of 300 mg peanut protein. Placebo received oat flour throughout the study. DBPCFCs were performed at baseline (week 0), week 104, week 117, and every 13 weeks for those who passed the previous DBPCFCs until the last DBPCFC at week 156. Intention-to-treat populations included all 120 participants randomized at enrollment. Treatment “success” is defined as passing DBPCFCs to 4 g of peanut protein. Participants exiting the study before week 104 were considered “dropouts.” There were 9 dropouts in Peanut 0, 5 dropouts in Peanut 300, and 3 dropouts in Placebo, including 2 Placebo participants who exited the clinical study before week 104 and another Placebo participant whose basophil data were not available at weeks 104 and 117. All dropouts were excluded from longitudinal analyses. One participant was randomized to Peanut 300, failed DBPCFC at week 104, and was not on 300 mg peanut from weeks 104 to 117, nor received food challenge at week 117. Data from this participant were included up to week 104.

Blood collection

Blood was collected from each participant before, during, and after OIT at weeks 0, 12, 52, 104, and 117.

Basophil activation test

BATs were performed using whole blood preserved in heparin as described in Mukai et al.²³ Briefly, basophil activation was assessed after stimulation for 30 minutes at 37°C with IL-3 (PeproTech, Rocky Hill, NJ, 2 ng/mL), polyclonal rabbit antihuman IgE (Bethyl Laboratories, Montgomery, Tex, 1 μ g/mL), or peanut protein extracted from the same peanut flour used in OIT at 0, 0.1, 1, 10, 100, and 1000 ng/mL. All stimuli were prepared in RPMI. Basophils were gated as CD123⁺HLA-DR⁺ cells and %CD63^{high} basophils and CD203c expression levels were quantified by flow cytometry. To assess changes in basophil reactivity and sensitivity in responses to OIT, upregulation

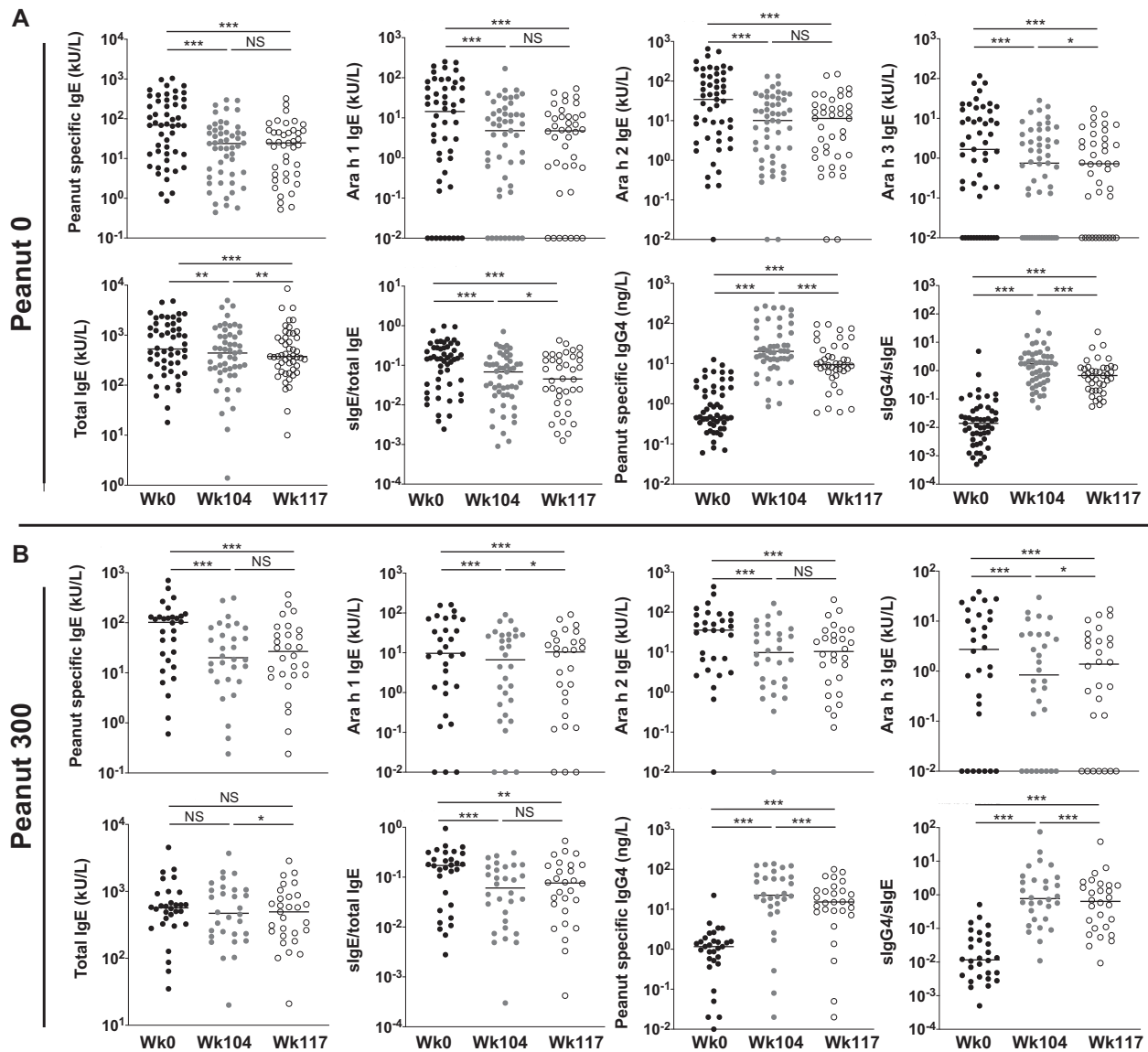


FIG 2. Changes in peanut-specific immunoglobulins associated with peanut OIT. Plasma immunoglobulins were quantified at weeks 0, 104, and 117. **A**, Peanut 0. **B**, Peanut 300. NS, Not significant. Values less than 0.1 in Ara h 1, Ara h 2, or Ara h 3 IgEs are displayed as 0.01. *P* values were determined by the Wilcoxon matched-pairs signed rank test. **P* < .05, ***P* < .01, ****P* < .001. NS *P* > .05. Horizontal bar = median. Data from dropout participants (ie, those who exited the study before week 104) were excluded.

of the basophil activation marker CD63 induced by peanut *ex vivo* stimulation was calculated as area under the curve (AUC) of %CD63^{high} basophils as a function of the peanut dose.²⁴ %CD63^{high} peanut extract (PE) AUC was calculated for each participant at each time point. Expression of CD203c was measured by mean fluorescent intensity. ΔCD203c is defined as CD203c mean fluorescent intensity after anti-IgE, IL-3, or peanut stimulation minus CD203c mean fluorescent intensity incubated with RPMI.

Immunoglobulin measurements

Total IgE, peanut sIgE and sIgG₄ levels, and component IgEs (Ara h 1, 2, 3) were measured at baseline, week 104, and week 117 using standardized methods in CLIA (Clinical Laboratory Improvement Amendments of 1988)-approved laboratories (Johns Hopkins University for peanut sIgE, peanut sIgG₄, and component IgE testing, and Stanford Clinical laboratories for total IgE measurements). Both laboratories (at Stanford and Johns

Hopkins) measured immunoglobulin levels using ImmunoCAP on the Phadia 250 Immunoassay Analyzers (Thermo Fisher Scientific, Waltham, Mass).

Statistical analysis

Statistical comparisons were performed using GraphPad Prism 8 or R version 3.6.0. Mixed-effects analysis with the Geisser-Greenhouse correction was used for the longitudinal analysis of basophil CD63 and CD203c. Mann-Whitney tests were performed for comparisons between different treatment arms and between success and failure outcomes. Wilcoxon matched-pairs signed rank test was used to assess changes in immunoglobulin levels in the same group. Spearman correlations were used to assess associations between basophil activation and immunoglobulins levels. Fisher exact test was used to test for differences in treatment outcomes between basophil responders.

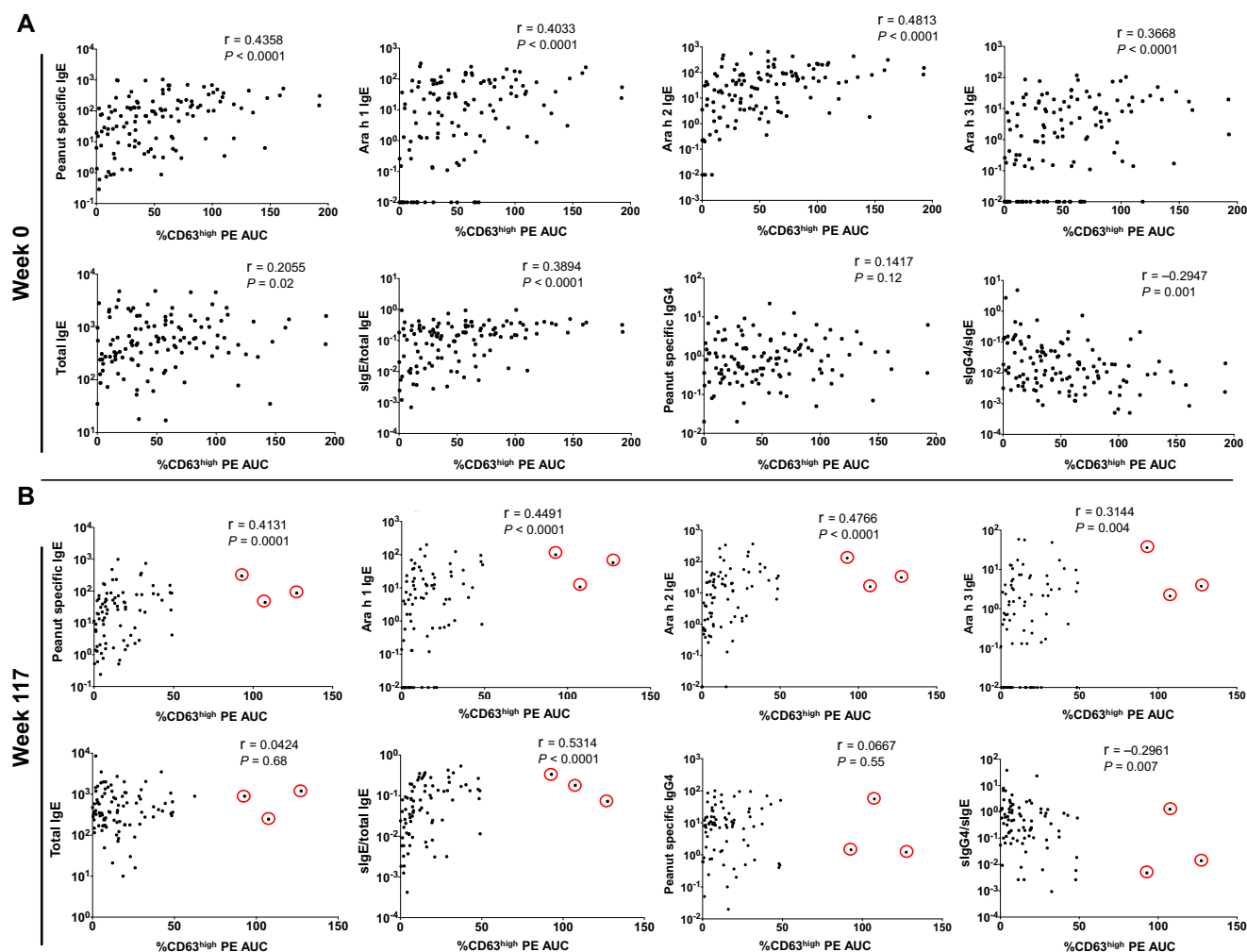


FIG 3. Correlations between basophil responses (%CD63^{high} PE AUC) and plasma immunoglobulins before and after OIT. **A**, Baseline at week 0. All participants in intention-to-treat population were included in the analysis. **B**, Thirteen weeks after active OIT at week 117. Data from dropout participants (ie, those who exited the study before week 104) were excluded. Values less than 0.1 in Ara h 1, Ara h 2, and Ara h 3 IgEs are displayed as 0.01. Spearman correlation coefficient (r) and P values are provided on each graph. In Fig 3, **B**, dots in red circles are data from 2 Placebo participants and 1 HR in the Peanut 0 arm who failed week 117 DBPCFC.

RESULTS

Peanut OIT suppresses basophil activation and reduces peanut sIgE

Before OIT, there were no differences in the induction of %CD63^{high} basophils by *ex vivo* stimulation with peanut or anti-IgE between Peanut 0, Peanut 300, and Placebo (see Fig E1, A and B, in this article's Online Repository at www.jacionline.org). Levels of CD203c expression in basophils after anti-IgE, IL-3, or peanut stimulation were also similar between the 3 treatment arms at baseline (Fig E1, C). Consistent with previous OIT studies of peanut allergy,^{7,18,25-27} our OIT protocol significantly decreased peanut-induced %CD63^{high} basophils as early as week 12 of the OIT build-up phase (Fig 1, A). Upregulation of CD63 in basophils was suppressed during the OIT maintenance phase from weeks 52 to 104. Basophils of Peanut 0 and Peanut 300 participants continued to exhibit reduced levels of peanut-induced activation until week 117 (Fig 1, A).

Peanut OIT also significantly reduced %CD63^{high} basophils in response to anti-IgE (1 μ g/mL) stimulation in Peanut 0 and Peanut 300 participants as early as week 12 (Fig 1, B). Interestingly, we detected a small but significant increase in peanut-induced, but not anti-IgE-induced, %CD63^{high} basophils in Peanut 0 participants between week 104 and week 117. This increase is likely due to peanut avoidance by the Peanut 0 participants during this period. However, there was no significant difference in %CD63^{high} PE AUC between weeks 104 and 117 in Peanut 300, during which these participants continued taking 300 mg peanut after active OIT (Fig 1).

Similarly, peanut OIT significantly inhibited basophil CD203c expression induced by anti-IgE, IL-3, and peanut stimulation over time (see Fig E2, A and B, in this article's Online Repository at www.jacionline.org). No significant change in CD63 and substantially lesser changes in CD203c were observed in basophils of Placebo participants (Fig 1 and Fig

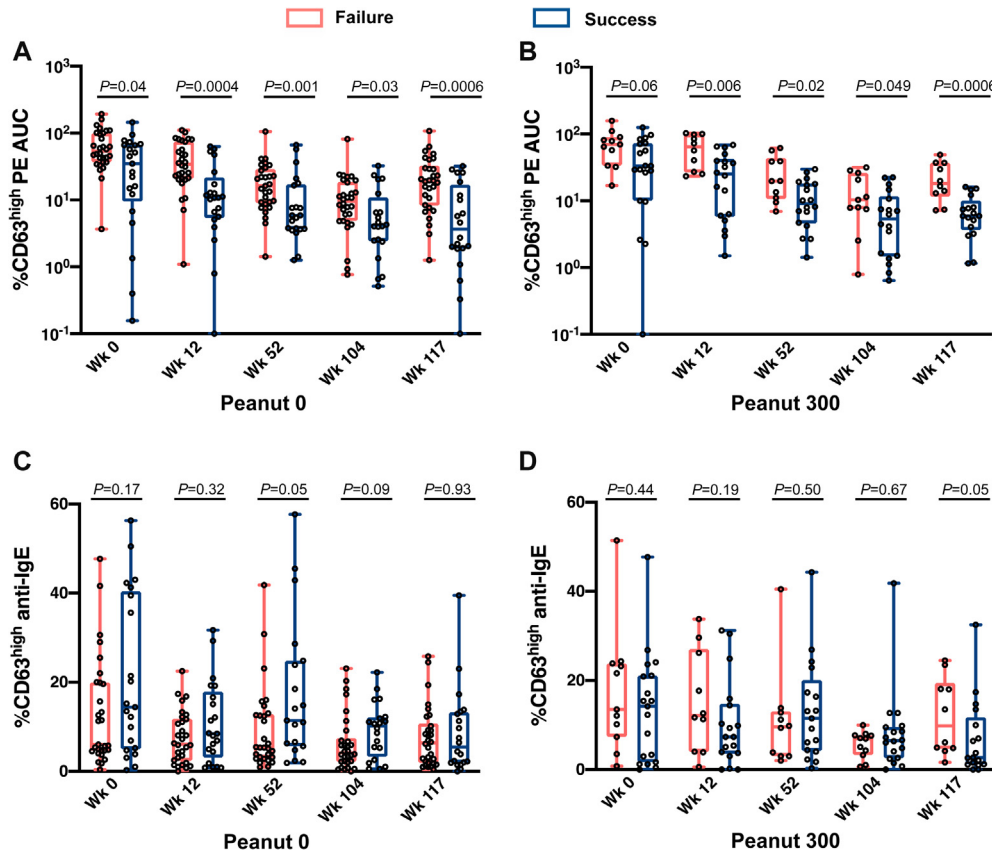


FIG 4. Peanut- and anti-IgE-induced %CD63^{high} basophils over time by treatment outcome at week 117. **A**, Peanut-induced %CD63^{high} basophils are represented by AUC as a function of the PE protein dose (0-1000 ng/mL) in Success vs Failure in Peanut 0. **B**, Peanut-induced %CD63^{high} basophils are represented by AUC as a function of the PE protein dose (0-1000 ng/mL) in Success vs Failure in Peanut 300. **C**, Anti-IgE (1 μ g/mL)-induced %CD63^{high} basophils in Success vs Failure in Peanut 0. **D**, Anti-IgE (1 μ g/mL)-induced %CD63^{high} basophils in Success vs Failure in Peanut 300. Whiskers represent min to max, boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. P values were determined by Mann-Whitney test. "Success" is tolerant to DBPCFCs to 4 g peanut protein at week 117. Source of week 0 data in Fig 4, A, B was from Fig 4 of Chinthrajah et al,¹⁷ with permission from the Publisher (Elsevier).

E2, C). Basophil responses were strongly suppressed by peanut OIT in Peanut 0 and Peanut 300 participants at weeks 104 and 117 compared with those in Placebo; however, there was no difference in peanut-induced basophil activation between Peanut 0 and Peanut 300 participants (see Fig E3 in this article's Online Repository at www.jacionline.org) at either time, suggesting that 300 mg peanut maintenance did not influence basophil responses at this time point.

Our peanut OIT also significantly decreased plasma levels of peanut-specific, Ara h 1, Ara h 2, and Ara h 3 IgEs, and the ratio of peanut sIgE versus total IgE (Fig 2). In contrast, peanut sIgG₄, and ratios of sIgG₄ versus sIgE, were elevated by peanut OIT (Fig 2). There were no changes in sIgE, Ara h 1, Ara h 2, or Ara h 3 IgE, sIgE/total IgE, or sIgG₄/sIgE between week 0 and 117 in Placebo participants (see Table E1 in this article's Online Repository at www.jacionline.org). However, we detected some small but significant reductions in IgEs specific for peanut, Ara h 1 and Ara h 2, and sIgE/total IgE, in Placebo participants at week 104. Importantly, IgEs specific for peanut, Ara h 1, Ara h 2, and Ara h 3, and sIgG₄, sIgE/total IgE, and sIgG₄/sIgE between Peanut 0 and Peanut 300 participants were not different at week 117,

indicating that 300 mg of peanut dosing for an additional 13 weeks after active OIT did not influence plasma levels of peanut-specific immunoglobulins.

Correlations of basophil activation and peanut-specific immunoglobulins

Induction of allergen-specific IgG₄ is a common outcome of immunotherapy and thought to be the primary mechanism that inhibits IgE-mediated effector cell activation.^{28,29} We recently reported that lower peanut-induced basophil activation and higher peanut sIgG₄/sIgE before treatment is associated with OIT-induced sustained effects (ie, passing DBPCFCs to 4 g peanut) after 13 weeks of peanut avoidance.¹⁷ Furthermore, lower Ara h 2 IgE and peanut sIgE are significantly associated with treatment success at the primary end point at week 117 in both Peanut 0 and Peanut 300 participants, and higher baseline sIgE/total IgE is associated with a higher % of dose-related adverse events.¹⁷

We examined whether basophil activation was correlated with the plasma levels of sIgE or sIgG₄ before and after active OIT treatment. At baseline before OIT, there were weak, but

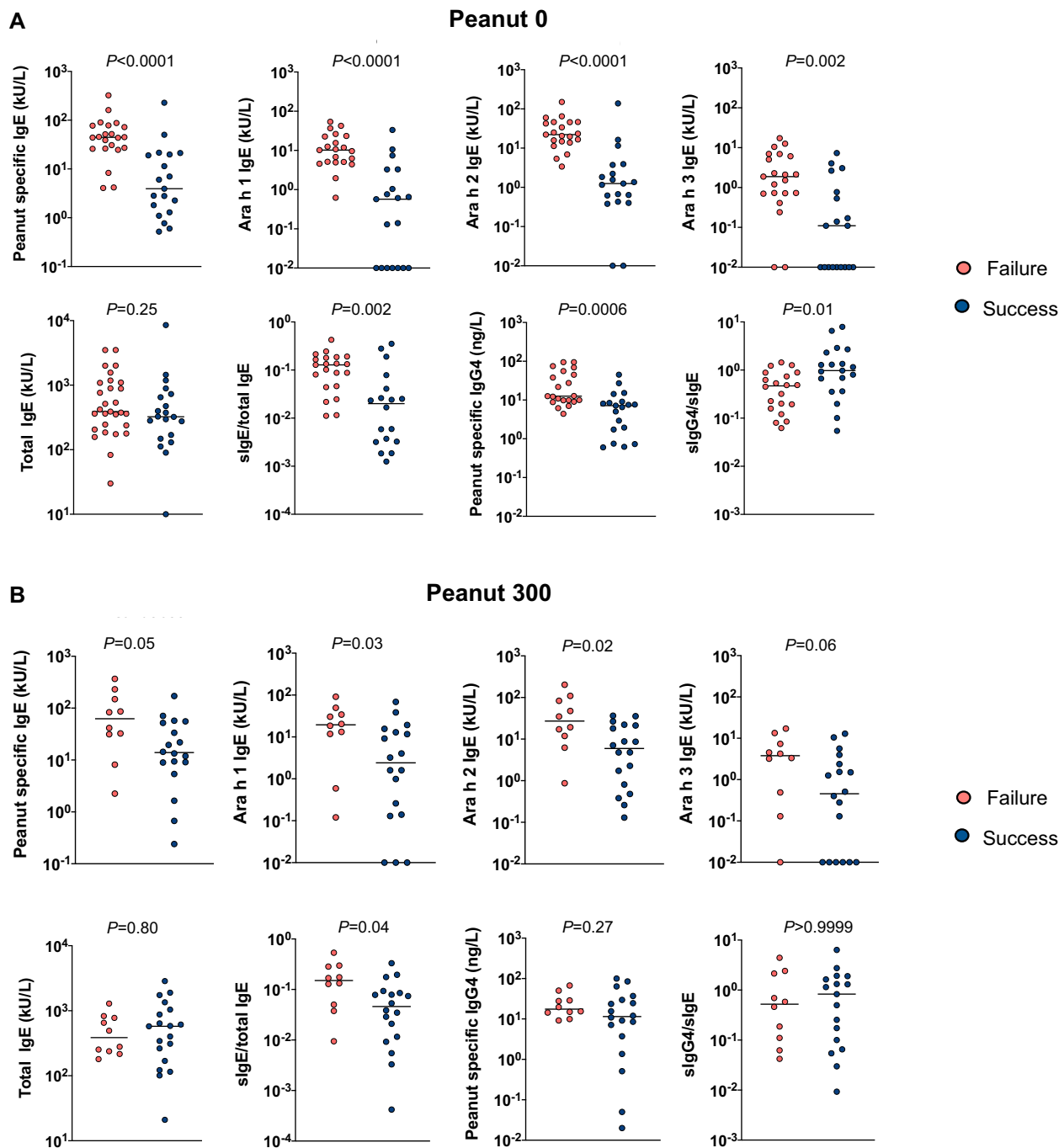


FIG 5. Plasma immunoglobulin levels by treatment outcome at week 117. Plasma immunoglobulins were quantified at week 117. **A**, Peanut 0. **B**, Peanut 300. Horizon bar = median. Values less than 0.1 in Ara h 1, Ara h 2, and Ara h 3 IgEs are displayed as 0.01. P values were determined by Mann-Whitney test. "Success" is tolerant to DBPCFCs to 4 g peanut protein at week 117. Source of the data in this figure is from Figs S5 and S6 of Chinthrajah et al,¹⁷ with permission from the Publisher (Elsevier).

significant correlations between peanut-induced basophil activation (%CD63^{high} PE AUC) and IgEs specific for peanut, Ara h 1, Ara h 2, or Ara h 3, or sIgE/total IgE, in 120 intention-to-treat participants (Fig 3, A). Although there was no correlation between basophil responsiveness and sIgG₄, we detected a weak, but significant, negative correlation between %CD63^{high} PE AUC and sIgG₄/sIgE at baseline (Fig 3, A).

We analyzed the primary end point at week 117 (ie, 13 weeks after active OIT treatment). We found significant weak to moderate correlations between peanut-induced basophil activation and IgEs specific for peanut, Ara h 1, Ara h 2, or Ara h 3, and sIgE/total IgE or sIgG₄/sIgE, but no correlation between sIgG₄ and peanut-induced basophil activation (Fig 3, B). These data suggest that peanut-induced basophil activation is regulated not just

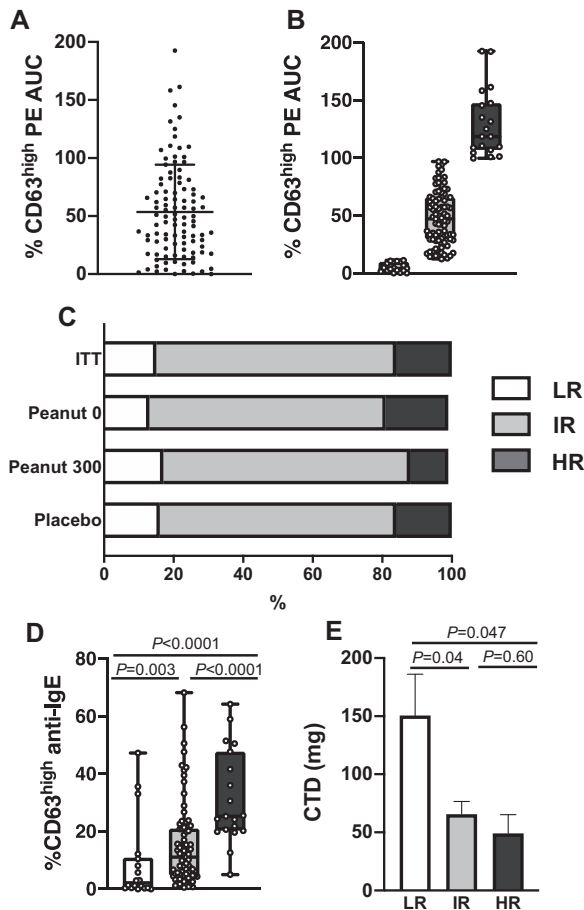


FIG 6. Three subgroups of peanut allergy participants based on basophil responses to peanut before OIT. **A**, Peanut-induced %CD63^{high} basophils (%CD63^{high} PE AUC) in the 120 participants at baseline. Scatter dot plot with mean $\pm$ SD. **B**, Peanut-induced %CD63^{high} basophils of LR (PE AUC < 12.09), IRs (PE AUC > 12.09 and < 97.37), and HRs (PE AUC > 97.37). Individual values are shown as circles. **C**, Distribution of 3 subgroups of basophil responders in the intention-to-treat (ITT) population, Peanut 0, Peanut 300, and Placebo. **D**, Anti-IgE (1 μ g/mL)-induced %CD63^{high} basophils in the 3 subgroups of basophil responders. Whiskers represent min to max, boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. **E**, Cumulative tolerated peanut challenge dose (CTD) at week 0 among the 3 subgroups of basophil responders. Data are mean $\pm$ SEM. P values were determined by Mann-Whitney test.

by sIgG₄ alone, but by the interactions of basophils with peanut sIgEs, peanut sIgG₄, and total IgE.

Peanut-induced basophil activation and sIgEs differentiate transient desensitization versus sustained unresponsiveness

The main goals of our peanut OIT study were to (1) assess sustained unresponsiveness to a cumulative dose of 4 g peanut protein after active peanut OIT treatment followed by 13 weeks of peanut avoidance, and (2) test the sustained effect of 300 mg daily dosing after active therapy. We compared longitudinally, between those passing and those failing food challenges at week 117, peanut-induced %CD63^{high} basophils at weeks 0, 12, 52, 104, and 117.

Peanut 0 and Peanut 300 participants who failed 4 g peanut DBPCFCs at week 117 exhibited higher %CD63^{high} basophils on

peanut stimulation at every time (Fig 4, A and B), whereas there was no significant difference in %CD63^{high} basophils to anti-IgE stimulation (1 μ g/mL) between those who passed and those who failed week 117 peanut challenges (Fig 4, C and D). Peanut 0 participants who failed week 117 oral food challenge also exhibited higher Δ CD203c in response to peanut stimulation, but not to anti-IgE or IL-3 (see Fig E4, A and B, in this article's Online Repository at www.jacionline.org). In Peanut 300, lower Δ CD203c in basophils of those who passed 4 g peanut DBPCFCs at week 117 were detected with high-dose peanut stimulation at week 117 (Fig E4, C and D). In addition, participants who failed week 117 DBPCFCs exhibited significantly higher IgEs specific for peanut, Ara h 1, Ara h 2, and Ara h 3, and sIgE/total IgE, at week 117 (Fig 5). Peanut 0 participants who failed week 117 DBPCFCs had (unexpectedly) significantly higher sIgG₄, but lower sIgG₄/sIgE, at week 117 (Fig 5, A). sIgG₄ and sIgG₄/sIgE were not different between Peanut 300 participants who failed and passed week 117 DBPCFCs (Fig 5, B).

We did not find any significant differences between Peanut 0 and Peanut 300 participants who failed or between Peanut 0 and Peanut 300 participants who passed week 117 DBPCFCs in %CD63^{high} PE AUC at weeks 0, 12, 52, 104, and 117, or in Δ CD203c and peanut sIgEs and sIgG₄ at week 0 and week 117.

Basophil non/low responders to peanut exhibit lower peanut sIgE and better treatment outcome

Basophils of the 120 participants enrolled in this study expressed a wide range of peanut-induced CD63 upregulation before treatment. On the basis of their sensitivity and extent of their basophil responses to *ex vivo* peanut stimulation at enrollment, participants could be classified as basophil "non/low," "intermediate," or "high" responders. We found that the induction of %CD63^{high} basophils (calculated by AUC as a function of the peanut dose-response curves [0-1000 ng/mL]) ranged from 0 to 192.6 with a mean of 54.73 (Fig 6, A). We defined the basophil "non/low responders" (LRs) as those with peanut-induced %CD63^{high} dose-response AUC of less than 12.09 (= mean (54.73) $- 1 \times$ SD (42.64) of %CD63^{high} PE AUC of 120 participants) and the basophil "high responders" (HRs) as those with peanut-induced %CD63^{high} dose-response AUC of more than 97.37 (= mean $+ 1 \times$ SD of %CD63^{high} PE AUC of 120 participants). The basophil "intermediate responders" (IRs) had peanut-induced %CD63^{high} dose-response AUC of more than 12.09 and less than 97.37 (Fig 6, B).

There were 18 (15%) LRs, 83 (69%) IRs, and 19 (16%) HRs in our POISED intention-to-treat population (Fig 6, C). Moreover, there was a similar distribution of LRs, IRs, and HRs among the 3 treatment arms (Fig 6, C). Participants were classified on the basis of their basophil responses to peanut, but we found that the basophil LRs to peanut also exhibited lower anti-IgE-induced CD63 upregulation and vice versa for the HRs (Fig 6, D). However, we found that basophil LRs tolerated 2 to 3 times more peanut protein at enrollment than the IRs and HRs (LR vs IR vs HR: 150.6 $\pm$ 35.51 mg vs 66.51 $\pm$ 10.92 mg vs 49.21 $\pm$ 15.96 mg) (Fig 6, E). Furthermore, before treatment, basophil LRs also had lower peanut sIgE, Ara h 1, Ara h 2, and Ara h 3 IgE, and sIgE/total IgE, compared with IRs and/or HRs (Fig 7, A). In contrast, basophil HRs had higher levels of peanut sIgE, Ara h 1, Ara h 2, and Ara h 3 IgE, and sIgE/total IgE than basophil LRs or IRs. Interestingly, no difference was detected in levels of

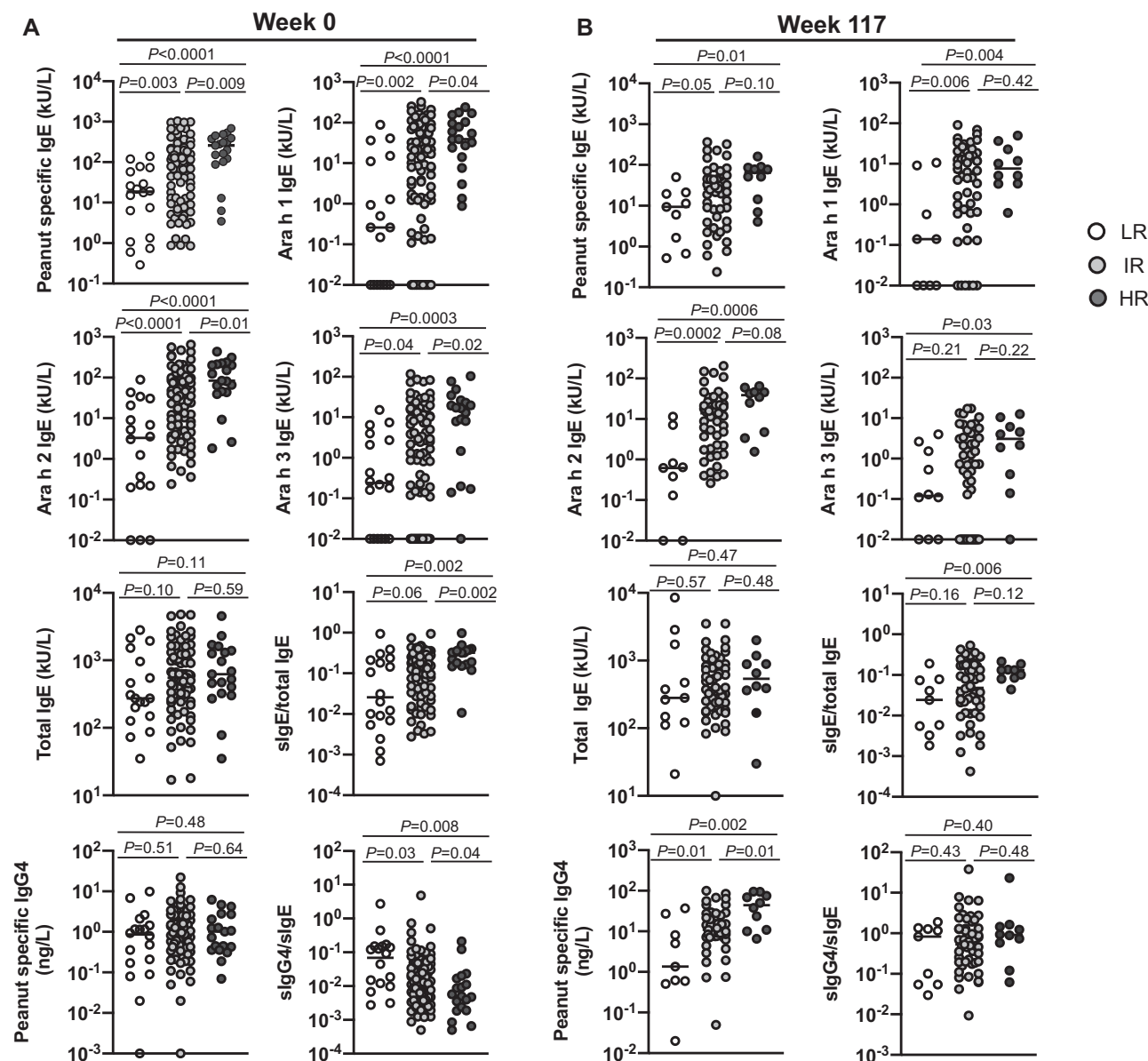


FIG 7. Plasma immunoglobulin levels in the 3 groups of basophil responders before and after OIT. **A**, Before OIT at week 0. Data from ITT (intention-to-treat) participants were included in the analysis. **B**, After OIT at week 117. Data from Peanut 0 and Peanut 300 participants were included; data from dropouts were excluded. Horizontal bar = median. Values less than 0.1 in Ara h 1, Ara h 2, and Ara h 3 IgEs were displayed as 0.01. *P* values were determined by Mann-Whitney test.

peanut sIgG₄ among LRs, IRs, or HRs, but the ratio of sIgG₄/sIgE was significantly higher in LRs compared with IRs or HRs (Fig 7, A).

We also compared plasma IgE and IgG₄ levels between the 3 groups of basophil responders at week 117 after active OIT treatment. LRs exhibited the lowest levels of IgE specific for peanut, Ara h 1, Ara h 2, and Ara h 3 compared with IRs and/or HRs, whereas the levels of these IgEs were not different between IRs and HRs (Fig 7, B). Notably, the levels of peanut sIgG₄ were also lower in LRs compared with either IRs or HRs after OIT, whereas peanut sIgG₄/sIgE was not significantly different between the 3 groups after OIT (Fig 7, B).

LRs not only exhibited lower basophil activation after peanut or anti-IgE stimulation, and lower peanut sIgEs, they also responded to OIT with better outcomes: 91% and 55% of LRs passed oral food challenge up to 4 g of peanut protein at week 117 and week 156, respectively (Table I). In contrast, only 17% of HRs passed oral food challenges at both weeks 117 and 156, and 48% and 22% of the IRs recorded treatment successes at weeks 117 and 156, respectively.

Notably, levels of peanut-induced %CD63^{high} basophils of IRs and HRs in the Peanut 0 arm who passed week 117 DBPCFCs were reduced to 20% ± 4% of their baseline levels by OIT, whereas those who failed still had 42% ± 7% of their baseline levels (*P* = .01). Similarly, peanut-induced %CD63^{high}

TABLE I. Low basophil activation at week 0 is associated with sustained effectiveness of peanut OIT

Basophil status (week 0)	Peanut 0 + Peanut 300						Peanut 0						Peanut 300					
	Week 117 DBPCFC			Week 156 DBPCFC*			Week 117 DBPCFC			Week 156 DBPCFC*			Week 117 DBPCFC			Week 156 DBPCFC*		
			P value			P value			P value			P value			P value			P value
	Pass	Fail		Pass	Fail		Pass	Fail		Pass	Fail		Pass	Fail		Pass	Fail	
LRs	10 (91%)	1 (9%)	.001	6 (55%)	5 (45%)	.08	5 (83%)	1 (27%)	.02	2 (33%)	4 (67%)	.48	5 (100%)	0 (0%)	.13	4 (80%)	1 (20%)	.23
IRs	28 (48%)	30 (52%)		13 (22%)	45 (78%)		15 (42%)	21 (58%)		5 (14%)	31 (86%)		13 (59%)	9 (41%)		8 (36%)	14 (64%)	
HRs	2 (17%)	10 (83%)		2 (17%)	10 (83%)		1 (11%)	8 (89%)		1 (11%)	8 (89%)		1 (33%)	2 (67%)		1 (33%)	2 (67%)	

Values are n (%).

P values were determined by Fisher exact test.

*Those who failed at week 117 were not retested (for DBPCFC) at week 156. However, in the table, these participants are included as “failures.”

basophils in IRs and HRs in the Peanut 300 arm who passed week 117 DBPCFCs were reduced to $11\% \pm 2\%$ of their baseline levels, compared with $45\% \pm 18\%$ in those who failed ($P < .0001$).

These data indicate that achieving sustained unresponsiveness 13 weeks off active OIT is observed primarily in 2 groups of subjects, those with lower basophil responses at study entrance and those who undergo a substantial reduction (80%-90%) of their peanut-induced basophil activation after OIT.

Basophil responsiveness in LR participants who reached treatment success remained low after OIT (%CD63^{high} PE AUC = 3.78 ± 1.21 & 5.67 ± 2.45 at week 0 & week 117, respectively; $P = .30$). Peanut OIT also did not alter sIgE in these LR participants (peanut sIgE [kU/L] = 25.70 ± 10.92 & 13.39 ± 5.26 at week 0 & week 117, respectively; $P = 0.13$), but increased sIgG₄ (ng/mL) levels from 0.82 ± 0.30 at week 0 to 8.93 ± 4.53 at week 117 ($P = .03$). In contrast, sustained tolerance in IR and HR subgroups was associated with lower %CD63^{high} PE AUC and lower IgEs specific for peanut, Ara h 1, Ara h 2, and Ara h 3, but not with higher sIgG₄, before and/or after OIT treatment (see Table E2 in this article's Online Repository at www.jacionline.org).

In summary, in our POISED study, assessment of basophil activation and measurement of peanut sIgEs are useful biomarkers in differentiating transient desensitization versus sustained tolerance.

DISCUSSION

Since its first application in food allergy 2 decades ago,³⁰ the BAT has been actively evaluated in this disorder.^{21,22} Enhanced spontaneous and food-induced basophil activation was shown to be associated with clinical reactivity to milk,^{31,32} peanut,^{33,34} egg,^{33,35} red meat,³⁶ and other foods.^{21,22} Some studies have suggested that BATs are more reliable than skin prick tests and sIgE levels in differentiating food allergy from tolerance in children.^{32,34} Although suppression of basophil activation by immunotherapy has been demonstrated in food allergy to egg,³⁷ milk,³⁸ and peanut,^{7,8,18,25-27,39,40} only a few studies have examined the association of basophil activation with treatment outcomes.^{18,26,27,37,40,41} Our analysis of BAT results obtained for more than 2 years in the POISED study shows that basophil activation remains suppressed even after 13 weeks off active OIT, and that sustained protection is associated with lower levels of basophil activation by peanut before, during, and after OIT.

In addition to inhibiting peanut-induced basophil activation, our peanut OIT decreased upregulation of CD63 and CD203c in responses to anti-IgE and/or IL-3. Antigen-nonspecific inhibition

of basophil activation also was observed in other peanut OIT studies.^{18,25} This “bystander effect” is likely due to reduction of FcεRI on basophils as a consequence of IgE reduction by OIT.^{42,43}

The mechanisms by which OIT suppresses basophil activation are still being investigated. We think that understanding the suppression of basophils by immunotherapy can help in efforts to devise better treatment strategies or preventive approaches for allergies to peanuts and other foods. Depletion of IgG, specifically IgG₄, has been shown to eliminate the inhibitory effect of OIT plasma in basophil activation.^{28,40,44} We did not find direct associations between peanut-induced %CD63^{high} basophils and levels of peanut sIgG₄ alone; however, we detected significant but weak correlations between peanut-induced %CD63^{high} basophils and sIgG₄/sIgE before and after OIT, as well as a moderate correlation between peanut-induced %CD63^{high} basophils and sIgE/total IgE after OIT. These findings suggest that basophil activation is regulated by the summation of activating (such as sIgE) and inhibitory (eg, IgG₁ and IgG₄) immunoglobulins. Indeed, our findings indicate that not only the levels of sensitization at study entrance but also immune responses shaped by OIT can have significant impacts on treatment outcome.

Furthermore, other immunoglobulin isotypes induced by OIT can potentially inhibit basophil activation.^{45,46} It is conceivable that peanut immunotherapy induces immunologic modifications (such as reduction in peanut sIgE and increase in peanut sIgG, sIgA, and sIgD), which in turn alter basophil phenotype (such as reduction of FcεR, Syk,⁴⁷ and other intracellular signaling machinery) and thus reduces basophil responsiveness to food allergens, and clinical reactivity. Therefore, we think that BATs ideally should be performed in whole blood, as we did in this study, to evaluate OIT-induced immune modifications external to basophils, as well as intrinsic changes in basophils.

We found that participants with or without 300 mg peanut daily dosing after OIT exhibited similar basophil responsiveness and peanut-specific immunoglobulins tested 13 weeks after active OIT. These findings are in line with clinical observations that Peanut 0 and Peanut 300 participants achieved similar treatment success rates 13 weeks after active OIT.¹⁷ However, Peanut 300 participants were 3 times more likely to tolerate 4 g peanut DBPCFCs than Peanut 0 participants 1 year after active OIT. One limitation of our study is that we were not able to associate BAT results with clinical outcomes beyond 3 months after stopping active OIT, because most of the participants who failed week 117 DBPCFCs did not return to the study and their basophils were not tested further.

In summary, we have demonstrated that BATs and blood biomarkers (including peanut and peanut component-specific IgEs, sIgG₄/sIgE, and sIgE/total IgE) measured before, during, and after immunotherapy can differentiate transient desensitization versus sustained tolerance after peanut OIT. Our analysis of basophil responsiveness to *ex vivo* peanut stimulation reveals phenotypic heterogeneity among basophils in peanut allergy, and identifies a peanut allergy subpopulation (ie, LRs) who are more amenable to OIT treatment. Our results support the hypothesis that BATs, in conjunction with other blood biomarkers, can identify allergic subjects who can most benefit from OIT. They also raise the possibility that prolonged administration of antigen, or antigen up dosing, may improve the efficacy and durability of OIT in difficult-to-treat populations.

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Key messages

- Peanut OIT suppresses basophil activation in responses to peanut and anti-IgE, reduces peanut sIgE, Ara h 1, Ara h 2, and Ara h 3 IgEs, and increases peanut sIgG₄.
- Measurements of basophil activation by peanut and peanut sIgEs can help to differentiate those who will achieve transient desensitization from those who will achieve sustained tolerance after peanut OIT.
- Basophil non/low responders exhibit higher treatment success to peanut OIT and, among other subjects, 80% to 90% suppression of peanut-specific basophil activation is required to maintain long-term clinical tolerance after peanut OIT.

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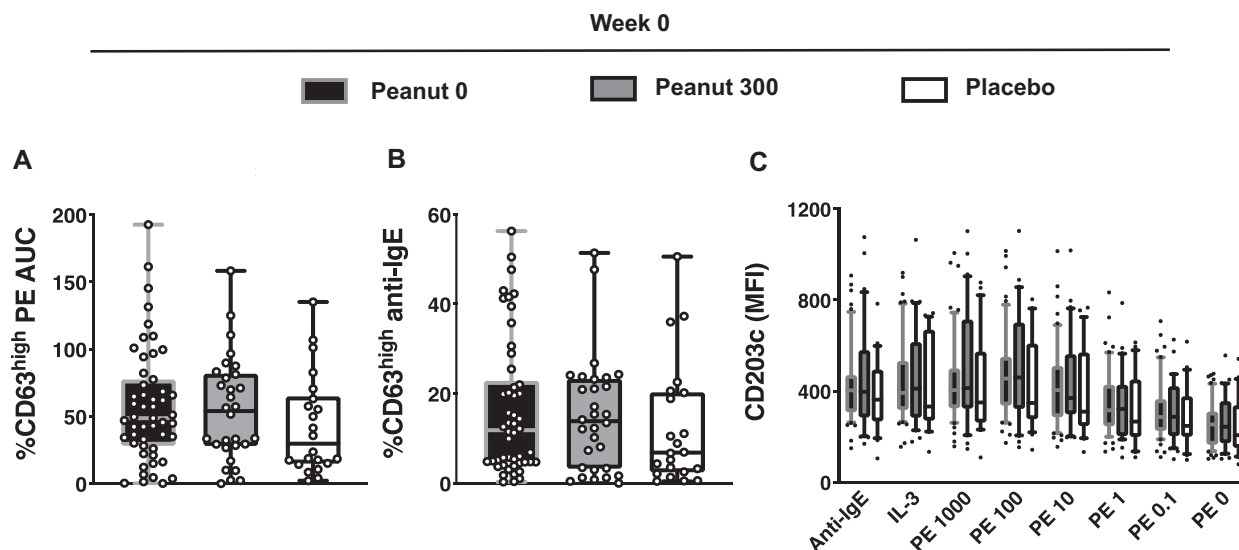


FIG E1. Baseline basophil responsiveness to peanut, anti-IgE, and IL-3 in Peanut 0, Peanut 300, and Placebo groups. **A**, Peanut-induced %CD63^{high} basophils represented by AUC as a function of the PE protein dose (0-1000 ng/mL) in Peanut 0, Peanut 300, and Placebo arms. **B**, Anti-IgE (1 μ g/mL)-induced %CD63^{high} basophils in Peanut 0, Peanut 300, and Placebo arms. **C**, Anti-IgE- (1 μ g/mL), IL-3- (2 ng/mL), and peanut-induced (0-1000 ng/mL) CD203c in Peanut 0, Peanut 300, and Placebo arms. Data from participants who exited the study before week 104 were excluded. In Fig E1, **A** and **B**, whiskers represent min to max, boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. In Fig E1, **C**, whiskers represent 10 to 90 percentile. Lower and higher 10% of all values are plotted individually. Boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. *MFI*, Mean fluorescence intensity.

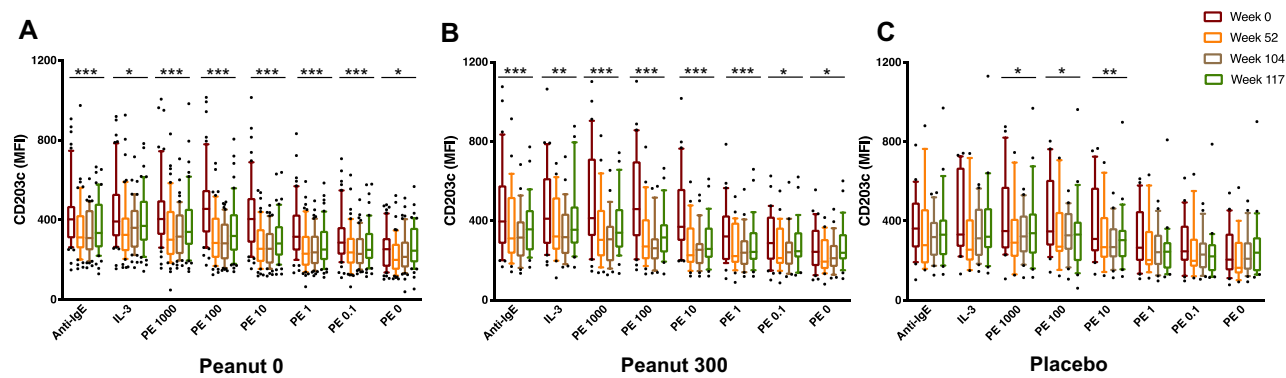


FIG E2. Peanut OIT reduces basophil CD203c expression in responses to anti-IgE, IL-3, and peanut over time. **A**, Peanut 0. **B**, Peanut 300. **C**, Placebo. Data from participants who exited the study before week 104 were excluded. *PE*, Peanut extract (0 to 1000 ng/mL). Whiskers represent 10 to 90 percentile. Lower and higher 10% of all values are plotted individually. Boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. *P* values were determined by mixed-effects analysis with the Geisser-Greenhouse correction. *MFI*, Mean fluorescence intensity. **P* < .05, ***P* < .01, ****P* < .001.

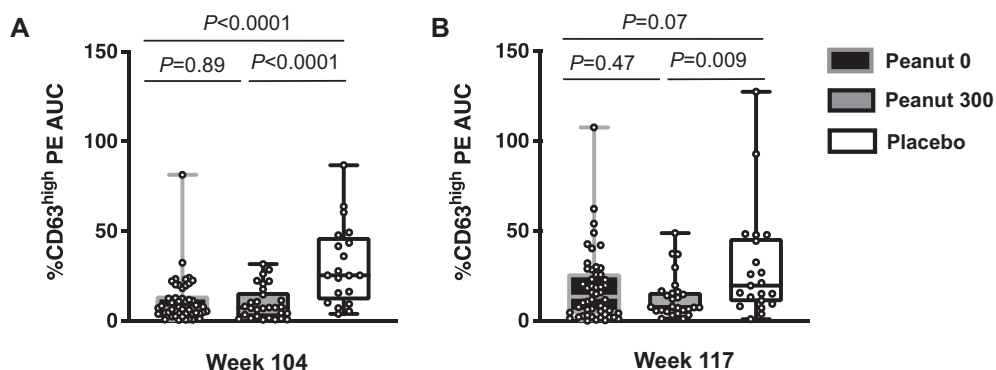


FIG E3. Comparisons of peanut-induced basophil activation between the 3 study arms at weeks 104 and 117. **A**, Peanut-induced %CD63^{high} PE AUC after desensitization at week 104. **B**, Peanut-induced %CD63^{high} PE AUC at week 117, which was 13 weeks after active peanut OIT followed by either peanut withdrawal (Peanut 0) or 300 mg peanut maintenance (Peanut 300). Whiskers represent min to max, boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. Individual values are shown as circles. *P* values were determined by Mann-Whitney test.

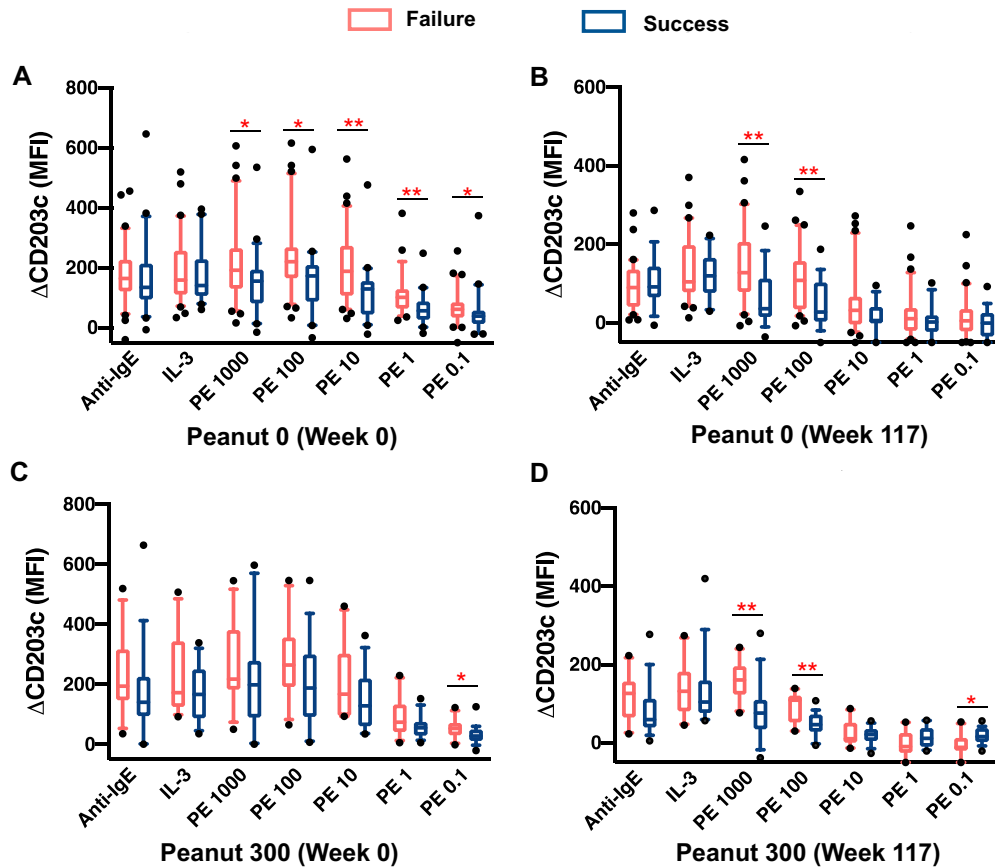


FIG E4. Anti-IgE-, IL-3-, and peanut-induced ΔCD203c in basophils by week 117 treatment outcome. **A**, Treatment failure vs success at week 0 in the Peanut 0 arm. **B**, Treatment failure vs success at week 117 in the Peanut 0 arm. **C**, Treatment failure vs success at week 0 in the Peanut 300 arm. **D**, Treatment failure vs success at week 117 in the Peanut 300 arm. Data from participants who exited the study before week 104 were excluded. *MFI*, Mean fluorescence intensity. Whiskers represent 10 to 90 percentile. Lower and higher 10% of all values are plotted individually. Boxes extend from the 25th to 75th percentiles. The lines in the middle of the boxes are medians. *P* values were determined by Mann-Whitney test. "Success" is tolerance in DBPCFCs to 4 g peanut protein at week 117. **P* < .05, ***P* < .01, ****P* < .001.

TABLE E1. Plasma immunoglobulin levels in the Placebo arm

Peanut sIgE (kU/L)		
Week 0	175.90 ± 63.61	<i>P</i> = .002 vs week 104 <i>P</i> = .84 vs week 117
Week 104	113.99 ± 37.02	<i>P</i> = .01 vs week 117
Week 117	182.26 ± 70.28	
Ara h 1 IgE (kU/L)		
Week 0	57.20 ± 18.46	<i>P</i> = .005 vs week 104 <i>P</i> = .85 vs week 117
Week 104	37.22 ± 9.64	<i>P</i> = .049 vs week 117
Week 117	50.00 ± 15.27	
Ara h 2 IgE (kU/L)		
Week 0	74.14 ± 27.93	<i>P</i> = .001 vs week 104 <i>P</i> = .84 vs week 117
Week 104	43.66 ± 13.91	<i>P</i> = .03 vs week 117
Week 117	76.45 ± 26.69	
Ara h 3 IgE (kU/L)		
Week 0	13.44 ± 5.34	<i>P</i> = .16 vs week 104 <i>P</i> = .56 vs week 117
Week 104	12.79 ± 5.26	<i>P</i> = .04 vs week 117
Week 117	12.97 ± 5.23	
Total IgE (kU/L)		
Week 0	884.32 ± 247.71	<i>P</i> = .35 vs week 104 <i>P</i> = .43 vs week 117
Week 104	723.73 ± 137.18	<i>P</i> = .59 vs week 117
Week 117	801.00 ± 168.98	
sIgE/total IgE		
Week 0	0.15 ± 0.03	<i>P</i> = .004 vs week 104 <i>P</i> = .15 vs week 117
Week 104	0.12 ± 0.02	<i>P</i> = .007 vs week 117
Week 117	0.14 ± 0.03	
Peanut sIgG ₄ (ng/mL)		
Week 0	1.41 ± 0.46	<i>P</i> = .0001 vs week 104 <i>P</i> = .04 vs week 117
Week 104	0.78 ± 0.28	<i>P</i> = .0002 vs week 117
Week 117	1.24 ± 0.47	
sIgG ₄ /sIgE		
Week 0	0.18 ± 0.12	<i>P</i> = .11 vs week 104 <i>P</i> = .46 vs week 117
Week 104	0.11 ± 0.05	<i>P</i> = .99 vs week 117
Week 117	0.14 ± 0.06	

P values were determined by Wilcoxon matched-pairs signed rank test. Data are mean ± SEM.

TABLE E2. Comparisons of %CD63^{high} PE AUC and peanut-specific immunoglobulins between treatment failure and success in IRs and HRs before (week 0) and after (week 117) OIT

Peanut 0 + Peanut 300			
%CD63 ^{high} PE AUC			
Week 0	Failure	69.98 ± 6.61	<i>P</i> = .18
	Success	56.52 ± 5.99	
Week 117	Failure	24.89 ± 3.26	* <i>P</i> < .0001
	Success	8.90 ± 1.61	
Week117/Week 0	Failure	0.40 ± 0.07	* <i>P</i> < .0001
	Success	0.16 ± 0.03	
Peanut sIgE			
Week 0	Failure	255.55 ± 41.58	* <i>P</i> < .0001
	Success	96.53 ± 38.16	
Week 117	Failure	74.78 ± 15.27	* <i>P</i> = .0003
	Success	30.71 ± 10.23	
Week117/Week 0	Failure	0.36 ± 0.05	<i>P</i> = .86
	Success	0.42 ± 0.07	
Ara h 1 IgE			
Week 0	Failure	66.20 ± 11.39	* <i>P</i> < .0001
	Success	19.86 ± 6.28	
Week 117	Failure	18.42 ± 3.87	† <i>P</i> = .001
	Success	8.36 ± 3.03	
Week117/Week 0	Failure	0.35 ± 0.07	† <i>P</i> = .009
	Success	0.63 ± 0.09	
Ara h 2 IgE			
Week 0	Failure	131.88 ± 23.47	* <i>P</i> < .0001
	Success	49.41 ± 23.01	
Week 117	Failure	39.38 ± 8.00	* <i>P</i> < .0001
	Success	13.04 ± 5.06	
Week117/Week 0	Failure	0.34 ± 0.04	<i>P</i> = .62
	Success	0.41 ± 0.05	
Ara h 3 IgE			
Week 0	Failure	16.55 ± 4.44	‡ <i>P</i> = .005
	Success	5.86 ± 1.67	
Week 117	Failure	4.32 ± 0.90	‡ <i>P</i> = .005
	Success	1.96 ± 0.64	
Week117/Week 0	Failure	0.51 ± 0.12	‡ <i>P</i> = .02
	Success	0.67 ± 0.07	
Peanut sIgG ₄			
Week 0	Failure	1.84 ± 0.31	<i>P</i> = .06
	Success	2.04 ± 0.83	
Week 117	Failure	29.53 ± 4.90	‡ <i>P</i> = .007
	Success	18.66 ± 4.76	
Week117/Week 0	Failure	25.64 ± 5.03	<i>P</i> = .42
	Success	25.94 ± 6.86	

"Success" is defined as tolerant to DBPCFCs to 4 g peanut protein at week 117. *P* values were determined by Mann-Whitney test. Data are mean ± SEM.

**P* < .001.

†*P* < .01.

‡*P* < .05.