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High and Low Dose Oral Immunotherapy Similarly Suppress Pro-Allergic Cytokines and Basophil Activation in Young Children

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Abstract

Background: Mechanisms underlying oral immunotherapy (OIT) are unclear and the effects on immune cells at varying maintenance doses are unknown.

Objective: We aimed to determine the immunologic changes caused by peanut OIT in preschool aged children and determine the effect on these immune responses in groups ingesting low or high dose peanut OIT (300 mg or 3000 mg, respectively) as maintenance therapy.

Methods: Blood was drawn at several time points throughout the OIT protocol and PBMCs isolated and cultured with peanut antigens. Secreted cytokines were quantified via multiplex assay, whereas Treg and peanut-responsive CD4 T cells were studied with flow cytometry. Basophil activation assays were also conducted.

Results: Th2-, Th1-, Th9-, and Tr1-type cytokines decreased over the course of OIT in groups on high and low dose OIT. There were no significant differences detected in cytokine changes between the high and low dose groups. The initial increase in both the number of peanut-responsive CD4 T cells and the number of Tregs was transient and no significant differences were found between groups. Basophil activation following peanut stimulation was decreased over the course of OIT and associated with increased peanut-IgG4/IgE ratios. No differences were found between high and low dose groups in basophil activation at the time of desensitization or sustained unresponsiveness oral food challenges.

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M. Kulis, A.W. Burks, and B.P. Vickery designed and oversaw the research project, including contributing to data collection, analysis, and manuscript preparation. X. Yue, R. Guo, H. Zhang, K. Orgel, P. Ye, and E. Kim all helped to collect data reported in the manuscript. Q. Li and Y. Liu performed statistical analysis of the data reported. All authors contributed to the preparation and editing of the initial version of the manuscript. All authors approved submission of the final version of the manuscript.

Conflicts of Interest:

The authors declare no conflicts of interest.

Conclusions and Clinical Relevance: Peanut OIT leads to decreases in pro-allergic cytokines, including IL-5, IL-13, and IL-9, and decreased basophil activation. No differences in T cell or basophil responses were found between subjects on low or high dose maintenance OIT, which has implications for clinical dosing strategies.

Keywords

Basophil; Food Allergy; Oral Immunotherapy; Peanut Allergy; Regulatory T Cell

Introduction

Oral immunotherapy (OIT) has been used in research settings for the past decade and is now being evaluated in a Phase III clinical trial in subjects with peanut allergy. Subjects with peanut, milk, and egg allergies have been successfully desensitized with OIT to the offending allergen in several landmark studies.^{1–6} Importantly, a large range of OIT doses have been used as maintenance therapy.⁷ In the case of peanut OIT, one study demonstrated that as little as 300 mg was effective for desensitization,⁸ whereas others have used 800 mg,¹ 1800 mg,³ 2000 mg,⁹ and 4000 mg⁵ maintenance doses. While OIT protocols were initially being developed and implemented, the consensus was that higher maintenance doses would cause robust desensitization and may lead to long-term sustained unresponsiveness (SU) after therapy was stopped. However, SU is not readily induced in the majority of subjects on peanut OIT,⁶ or those on egg and milk OIT,^{2, 10} even after several years on maintenance therapy at doses into the hundreds or thousands of milligrams of protein per day.

The underlying mechanisms of OIT have not been fully elucidated, although some immunologic changes have been shown across trials. Effector cell suppression has been demonstrated in OIT studies for peanut, milk, and egg OIT.^{2–3, 5, 11} Within 6–12 months of initiating OIT, mast cell reactivity as measured by skin prick testing is decreased and basophils become hyporesponsive to *ex vivo* antigen stimulation.^{3, 12} Peanut-specific IgE levels in serum often increase during the build-up phase of OIT then later decrease below baseline levels, typically after greater than 12 months of therapy.^{5–6} Peanut-specific IgG and IgG4 increase within the first 3 months of therapy and stay elevated with ongoing daily exposure to antigen.^{3, 5–6} Changes in T cells have also been shown during OIT, with increases in Tregs and decreases in Th2-type cytokine secreting T cells.^{3, 5, 13–14} There are suggestions that the pathogenic peanut-specific Th2 cells become anergic or are depleted during OIT,¹⁵ although residual Th2 effector cells have been found following OIT,¹⁴ and it remains unclear whether pathogenic Th2 cells re-emerge once therapy is stopped. One study demonstrated that cellular changes induced by peanut OIT are often transient,¹⁶ which seem to reflect the return of clinical symptoms during oral food challenge (OFC) after OIT has been stopped.¹⁷ While it is clear that OIT modulates the immune response to peanut allergens, there are no studies showing how these changes are impacted by the amount of antigen consumed as daily maintenance therapy.

The first trial of peanut OIT conducted exclusively in preschool age children aged 9–36 months with new-onset peanut allergy was recently reported by our group.⁸ We demonstrated the highest rate of SU to-date at 78%. This trial was highly innovative in that it

captured young children, presumably with a more malleable immune response to peanut that could be altered with OIT. Importantly, this is the first trial to directly compare two doses of peanut OIT, with a low dose of 300 mg and a high dose of 3000 mg. In addition to the clinical outcomes reported,⁸ we investigated the immune responses during OIT from sequential blood draws throughout the trial. The aims of the immunologic studies were to determine how OIT modulates the immune response to peanut and to determine whether there were differences between groups of subjects receiving high or low dose maintenance therapy. Here, we report our findings on changes in the peanut-induced cytokine production, Treg numbers, peanut-responsive CD4+ T cells, and basophil reactivity and compare these outcomes between groups.

Methods

Clinical Trial

The trial was approved by the University of North Carolina at Chapel Hill's Institutional Review Board (#11–2307), registered with clinical [trials.gov](https://clinicaltrials.gov) (#NCT00932828), and performed under an IND with the FDA. For details of the clinical trial please refer to Vickery, et al.⁸ The salient points of interest for the immunologic studies are as follows: Dosing was double-blinded; Subjects entered the trial and began peanut OIT at a dose of 0.1 mg, then underwent build-up to 300 mg of peanut protein daily; Upon reaching 300 mg peanut daily (at approximately 11–12 months on the OIT protocol), subjects randomized to high dose continued escalating to 3000 mg of peanut protein, while subjects randomized to low dose continued to receive more flour product, with the active peanut content held steady at 300 mg and eventually the placebo filler (oat flour) reached 2700 mg. A total of 49 subjects were consented and enrolled in the trial.

Blood samples

Subjects underwent blood draws at baseline and approximately 4, 8, and 12 months, then annually until the time of final OFCs. Blood was collected in serum separator tubes for IgE and IgG4 quantification and sodium-heparin tubes for cellular assays. For T cell assays, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll separation as previously reported.⁵

Cytokine quantification

PBMCs were suspended in RPMI-1640 media supplemented with 10% autologous plasma within 24 hours of blood collection, as previously described.⁵ Wells were seeded with 0.5 million PBMCs and antigens added as follows: 200 µg/mL peanut protein extract; 200 µg/mL egg white protein extract; 40 µg/mL ConA; or no antigen added as a negative control. Cells were cultured at 37°C in a humidified 5% CO₂ incubator for 72 hours and the cell supernatants were collected. Supernatants were immediately frozen and kept at –80°C until analysis. All cytokine quantifications were conducted by batch analysis following the completion of the clinical trial, thus minimizing any assay drift or subtle changes that may influence assay read-outs. Cytokine concentrations were assayed in multiplex fashion using the MesoScaleDiscovery platform. We quantified IL-13, IL-5, IFN-γ, TNF-α, IL-17, IL-10, IL-12p70, and IL-4. An ELISA kit (Biolegend) was used to quantify human IL-9.

CFSE assay

PBMCs were stained with CFSE using the CellTrace kit (Thermo Fisher) for 10 minutes in a 37°C water bath. Cells were then cultured as in the cytokine assay with peanut, egg, ConA, or no antigen. Cells were cultured for 7 days in a 37°C CO₂ incubator, then surface stained with anti-human CD4 (PE-Cy5; BD Biosciences). Flow cytometry data was acquired on a CyAn ADP (Beckman Coulter).

Treg assay

PBMCs were cultured as in the cytokine assay using the same stimulants. Cells were cultured for 7 days in a 37°C CO₂ incubator, then surface stained with anti-human CD4 (PE-Cy5, BD Biosciences), anti-human CD25 (APC; BD Biosciences), and intracellularly stained for FoxP3 (FITC; eBioscience) using BD permeabilization buffer. Flow cytometry data was acquired on a CyAn ADP. Tregs are reported here as % CD4+ CD25+ FoxP3+ within the entire CD4+ T cell population.

Whole blood basophil activation assay

At the time of clinical OFCs, blood was assayed for basophil activation responses to peanut antigens. Whole blood was stimulated with peanut protein extract, anti-human IgE (Bethyl Labs), or left unstimulated. All stimulations were conducted in the presence of 2 ng/mL human IL-3. Upon antigen addition to the whole blood, tubes were placed in a 37°C CO₂ incubator for 30 minutes, then the reaction was stopped with 20 mM EDTA. Cells were stained with basophil identification and activation markers, anti-human CD123 (PE-Cy5; BD Biosciences), anti-human CD203c (PE; Beckman Coulter), and anti-human CD63 (FITC; BD Biosciences). Red blood cells were lysed with BD Fix/perm solution for 15 minutes. Flow data was collected on a CyAn ADP. Basophils were gated from the lymphocyte population and identified as CD123+ CD203c+ (i.e. double-positive) cells. At least 300 basophils were acquired for each assay. Activated basophils were identified as CD63+ and reported as a percentage of total basophils.

IgE-stripped basophil assay

To assess the ability of plasma from subjects at various time points throughout the OIT protocol to activate basophils we used an IgE-stripping assay.¹⁸ Healthy donor blood was used as a source of PBMCs, then stripped of IgE with 10 mM lactic acid, 0.14 M NaCl, 5 mM KCl at pH 3.9 for 10 minutes on ice. Cells were washed, 1 million PBMCs were resuspended in 200 µL of RPMI (per test condition), and then primed with 200 µL of plasma from OIT subjects at 0, 12, 24, and 36 months in a 37°C CO₂ incubator for 1 hour. Stimulations were conducted with 0.1 µg/mL peanut protein extract for 30 minutes in a 37°C CO₂ incubator. Basophil activation was assessed as described above. Unstimulated activation was subtracted from the peanut-stimulated values and are reported here.

Peanut-specific IgG4/IgE ratio

Peanut-specific IgE (in kU/L) and IgG4 (in mg/L) were quantified on an ImmunoCAP100 instrument as previously reported.⁵ Ratios were calculated by first converting both IgG4 and

IgE into $\mu\text{g/L}$ then dividing peanut-specific IgG4 quantities by peanut-specific IgE quantities. A conversion factor of $2.42 \mu\text{g/L} = 1 \text{ kU/L}$ was used for IgE.

Statistical Analysis

For the longitudinal analysis of immune responses, the immune response variables were log-transformed (represented as natural logarithm, $\ln$) and regressed against time (in months) using Generalized Additive Mixed Model (GAMM).¹⁹ B-splines functions were used for fitting the GAMM. The analysis was performed for each outcome and each dosage group separately using R (www.r-project.org) version 3.1.2 with the mgcv package. Plots show the fitted mean temporal trajectories together with the 95% confidence band in each dosage group for each outcome. The likelihood ratio test²⁰ was performed to assess if the outcome significantly increases or decreases over the whole study span. In addition, another likelihood ratio test was performed to assess if the temporal changes differ significantly between the two dosage groups. All hypothesis tests were two-sided, with a p-value of less than .05 considered significant.

Results

Peanut-allergic subjects' PBMC responses to peanut are skewed towards Th2 and Th17 compared to egg responses that favor production of Tr1 and Th1 cytokines

Baseline levels of PBMC cytokine production following peanut and egg stimulation were assessed for subjects at enrollment. No subjects were allergic to egg and all were freely consuming egg in their diet. There are large differences in peanut and egg-induced cytokines, with peanut responses dominated by IL-13, IL-5, and IL-17 (**Figure 1**), whereas egg responsive cells produced higher levels of IL-10, a well-established regulatory cytokine and TNF- α , a Th1-type cytokine. There were no differences in IFN- γ secretion between peanut- and egg-responsive cells. These cytokine profiles establish the Th2-skewed immune response to peanut in peanut-allergic individuals and shows specificity since egg-induced cytokine profiles are skewed towards a Tr1 and Th1 phenotype, typical of an individual that has developed oral tolerance to a particular food antigen.

Peanut OIT suppresses Th2-, Th9-, Th1-, and Tr1-type cytokines without differences between high and low dose OIT groups

Longitudinal analysis of PBMC cytokine production in response to peanut stimulation was monitored for subjects throughout the course of OIT. The pro-allergic, Th2-type cytokines IL-13 and IL-5 were both significantly decreased from baseline throughout the OIT protocol (**Figure 2 and Supplemental Figure S1**), but there were no differences between subjects receiving 300 mg or 3000 mg of peanut OIT. IL-9, a mast cell growth factor and cytokine that enhances IgE production from B cells was also significantly decreased throughout the course of OIT. As with the Th2-type cytokines, no differences between high or low dose OIT groups were detected for IL-9 secretion. These findings indicate that OIT modulates the pro-allergic cytokine production from T cells and there is no apparent difference whether the subject ingested high or low dose peanut protein OIT as maintenance. Of note, IL-4 and IL-12p70 were not detectable and are thus not shown or discussed further.

Additional cytokines were quantified to determine effects of OIT on potential skewing of the immune response (**Figure 3 and Supplemental Figure S1**). Somewhat surprisingly, we measured significant decreases in IL-10, TNF- α , and IFN- γ in both the high and low dose groups. As with the Th2 cytokines, there were no differences detected between the groups. IL-17 secretion was decreased in the low dose OIT group but not the high dose group, although no significant difference was found when comparing the two groups. These data indicate an absence of immunologic skewing caused by peanut OIT and instead show a decrease in important Th1 and regulatory cytokines.

Cytokines secreted from PBMCs stimulated with egg antigen are modestly modulated during peanut OIT

Egg stimulation of PBMCs was carried out at the same time points described above for peanut stimulation. Interestingly, some of the cytokines induced by egg stimulation were reduced during peanut OIT (**Figure 4**). For example, IL-13 was decreased ($p<0.05$) in both high and low dose groups, although the slope was only approximately half that with peanut stimulation. TNF- α production resulting from egg stimulation was also decreased in both groups. Other cytokines were either decreased during OIT in only one of the groups or were not significantly altered. There were no differences in cytokine production between the high and low dose groups.

Peanut-responsive CD4 T cells and peanut-induced Tregs increase early but are not significantly altered over the course of OIT

Peanut-specific CD4 T cells were assessed by CFSE assay to determine whether the number of cells changed during OIT. CD4 T cells stained with CFSE become “CFSE low” as the cells proliferate in response to peanut.²¹ A significant increase in peanut-responsive CD4 T cells was noted during the first 4 and 8 months on OIT compared to baseline while all subjects were in the build-up phase and on the same dose of OIT (**Figure 5A**). **Figure 5B** shows the high and low dose groups over the entire course of OIT. Notably, the initial increase in CD4+ CFSE-low T cells is not sustained beyond 8 months of OIT. There is no difference between the high and low dose groups over the course of therapy and no significant change in the percentage of CD4+ CFSE-low cells.

Peanut-induced Tregs were also assessed over the course of OIT. We found a small, but statistically significant increase from baseline at 4 months on OIT while all subjects were in the build-up phase and on the same dose of OIT (**Figure 5C**), which was not sustained over the course of therapy (**Figure 5D**). **Figure 5D** shows Treg responses for high and low dose groups, neither demonstrate a significant change from baseline over the course of the OIT protocol. Importantly, there is no difference between the groups in terms of Treg numbers induced by peanut stimulation during OIT.

Basophil suppression in response to peanut allergens are not different between high and low dose OIT groups

Basophil reactivity in response to peanut antigen stimulation was measured longitudinally by stripping IgE from healthy donor basophils and priming with plasma from subjects at annual visits. Limited plasma volumes restricted testing in this assay to n=6 high dose and

n=9 low dose OIT subjects. Nevertheless, significant suppression was found at 12, 24, and 36 months compared to baseline reactivity and there was no difference between high and low dose OIT groups (**Figure 6A**). These findings indicate that subjects' peanut-IgE following OIT was not cross-linked as efficiently as at baseline. This could be due to a shift in the balance of peanut IgG4/IgE ratio, which significantly increased in both groups (**Figure 6B**).

Whole blood basophil activation assays were conducted at the time of desensitization and SU oral food challenges (**Figure 7**). A total of 23 subjects (n=13 high dose and n=10 low dose) had blood collected at both oral food challenges and were included in the analysis. These assays indicate highly suppressed basophil reactivity to peanut as all four doses used in the assay had medians < 5% CD63+ basophils. There were no increases in either group in terms of reactivity during the four weeks off of OIT (i.e. between DS and SU OFC). Importantly, there was no difference in terms of basophil reactivity at the DS or SU challenge at any of the four doses when comparing the high and low dose OIT groups. These findings indicate that OIT suppressed basophil reactivity, which did not return during a four week period without peanut dosing and that high and low dose maintenance had equivalent effects on suppression of the basophil response to peanut.

Discussion

OIT is a promising approach to desensitize food allergic individuals and has demonstrated efficacy for several major food allergies, including peanut, milk, and egg. However, key knowledge gaps remain, including optimal dose selection, immunologic mechanisms underlying OIT, and changes in immune responses leading to sustained unresponsiveness. The peanut OIT trial reported by Vickery *et al* had two unique elements in the study design: (1) Enrollment of young children <3 years of age and (2) the comparison of two maintenance doses, 300 and 3000 mg of peanut.⁸ This trial demonstrated the highest rate of SU to-date at 78% and there was no difference in clinical outcomes between the high and low dose OIT groups. Here, we report, for the first time, the changes in cellular immunologic effects of OIT on young children receiving either high or low dose OIT with the surprising finding that no differences in immunologic responses were found with a 10-fold difference in maintenance OIT dose.

Substantial changes in T cell-derived cytokines were observed over the course of OIT. Th2 cytokines, IL-13 and IL-5, known to contribute to peanut allergy were sharply decreased and remained suppressed during the course of OIT. The quantities of IL-9 in response to peanut were also decreased by OIT. The reduction in IL-9 during OIT has not been demonstrated before and may be especially important as two reports have found that IL-9 differentiates peanut-sensitized and peanut-allergic individuals.^{22–23} Thus, decreasing IL-9 with OIT could possibly restore a state of tolerance. We did not find any evidence of increased Th1-type or Tr1-type cytokines that would suggest a skewing away from Th2-driven responses to peanut. Instead, we found decreases in IL-10, TNF- α , and IFN- γ . Looking at the immune cytokine profiles of egg responses, there is a clear Th1 and Tr1 favored profile compared to peanut in these subjects (**Figure 1**). We postulate that OIT thus does not restore a state of “natural” tolerance like is seen for egg in these subjects, but instead OIT leads to an

“induced” state of tolerance characterized by low cytokine production in response to peanut stimulation.

It is unclear what mechanisms are involved in the T cell compartment that ultimately lead to SU. Several mechanisms, such as anergy, deletion, and/or exhaustion could cause decreased antigen-responsive cell numbers leading to decreased cytokine production.²⁴ The initial increase in Tregs during build-up in this study coincides with suppression of Th2 cytokines and suggests a role for Tregs in OIT-induced immunologic changes. Of note, we observe what may be bystander suppression of egg-induced T cell cytokines seemingly caused by peanut OIT. However, these findings remain speculative because without a placebo group it is not possible to rule out other explanations such as immune changes caused by aging in these young children. Bystander suppression has been demonstrated clinically with peanut OIT¹⁷ and in mouse model studies,²⁵ which may be caused by Treg induction that have antigen non-specific effects. One of the limitations of our study is that we did not perform assays for Treg subsets, FoxP3 methylation, or Treg function that may be more closely tied to tolerance.¹³ Additionally, the early increase of peanut-responsive cells during build-up may be important for driving desensitization and tolerance, however, we do not have a clear picture of the phenotype of these peanut-specific cells. One report showed the emergence of an anergic T cell population during peanut OIT and this will be interesting to investigate further in larger studies.¹⁵ Regardless of which cellular mechanisms lead to tolerance, our study demonstrates that even with a 10-fold difference in maintenance dose, similar changes in the T cell cytokine profiles emerge, dramatically suppressing cytokine production in response to peanut antigens.

OIT clearly leads to suppression of basophil responses to allergens while continuing to take doses.^{3, 5, 12, 17} Here, we demonstrated that plasma from subjects on OIT leads to lower basophil reactivity and is likely linked to the balance between peanut-IgG4 and IgE. Additionally, in whole blood assays we found low median levels of basophil reactivity at time of desensitization OFC while subjects were taking vastly different maintenance doses. While it is still unclear if decreased IgE combined with increased IgG4, or some other mechanism is causing these suppressive effects, we have shown that a high and low dose maintenance therapy yields similar outcomes in terms of suppressing basophil reactivity. Importantly, the basophil reactivity in this cohort of preschool age children did not return after four weeks avoidance following the OIT protocol at the SU OFC, unlike the finding in another OIT study, which enrolled older children.¹⁷ The findings here of low basophil reactivity and a high rate of SU appears to indicate that basophil reactivity may reflect clinical outcomes of SU.

Clinically, the low dose OIT maintenance therapy in this preschool age cohort was sufficient in most subjects to prevent allergic symptoms during a 5000 mg peanut protein OFC at the end of treatment and again one month after stopping treatment.⁸ Findings in the present work indicate that low dose OIT may serve as a method to drive down Th2 cytokines, peanut-specific IgE, and basophil reactivity, while increasing IgG4 and Tregs leading to long-term “correction” of the aberrant allergic response to peanut. Key drivers of SU may be reduction in Th2 and IL-9 responses and increased IgG4/IgE ratio. While these have been demonstrated in other trials with older children, it did not always result in SU, hinting that

the younger age group may be more amenable to treatment. Interestingly, in the LEAP trial, which examined early exposure to peanut as a prevention approach, the elevated peanut-specific IgG4 response, but not a decrease in peanut-specific IgE, was found to associate with prevention of peanut allergy.²⁶ It appears that OIT may work by boosting the peanut-IgG4 as happens with regular exposure in oral tolerance induction in LEAP, while also driving down the peanut-IgE response, which was not seen in LEAP. This restoration of a balance between IgG4 and IgE following OIT may ultimately lead to SU in young children.

It is important to note the limitations of our findings, primarily due to the clinical trial design and available cellular assays when this study was implemented in 2009. For example, a placebo control group was not included due to the young age of the participants, the unestablished nature of OIT, and safety and regulatory concerns. Immunologic outcomes from a placebo group would help strengthen our findings and more conclusively demonstrate the cellular changes found here were induced by OIT. Furthermore, more sophisticated assays, such as intracellular cytokine staining and antigen-specific T cell assays (e.g. CD154+ T cells or tetramer-stained T cells), would give additional insight into how OIT changed the peanut-specific T cell phenotypes in these young children. Missing baseline samples from the whole blood basophil activation assay also present a limitation. While we acknowledge the limitations of our study, we have laid important groundwork to be addressed in future clinical and mechanistic studies that will lead to better understanding of how OIT modulates the immune response to peanut.

Future directions based on these data would be to conduct dose finding trials. Since a 10-fold difference in maintenance OIT doses produce the same results clinically and immunologically, one must wonder what the lowest threshold would be, assuming therapy with less than 300 mg would be clinically effective. Another critical question to address is how long these cellular changes, and clinical benefit persist after OIT. An ongoing trial (clinical trials # NCT01867671) will address this question as subjects will undergo OIT then stop dosing for six months. It is possible that in these young children with newly diagnosed peanut allergy, we may have induced a very long-lived change in immunologic parameters along with protection from ingestion of peanut allergens. Ultimately, it will be important to relate the changes in immunologic responses to clinical outcomes in future studies with larger numbers of subjects.

In conclusion, we have demonstrated that OIT modulates key pro-allergic immune compartments, namely T cells and basophils, along with changes in peanut-IgG4 and IgE. The most striking finding from this study is that high and low dose OIT lead to similar changes in these compartments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Secreted cytokine profiles from cultured PMBCs at enrollment. Peanut and egg antigens were used to stimulate PBMCs for 72 hours and cytokines were quantified with a multiplex assay. Individual subject data are shown with median values indicated by a red line.

Figure 2.

Peanut-induced IL-13, IL-5, and IL-9 changes over the course of OIT in high and low dose OIT groups. Individual data were log-transformed (ln) and are shown with a mean temporal trajectory curve and 95% CI. Statistics for each cytokine indicate the slope of the curve with a corresponding p-value indicating change over time.

Figure 3.

Peanut-induced IL-10, TNF- α , IFN- γ , and IL-17A changes over the course of OIT in high and low dose OIT groups. Individual data were log-transformed (ln) and are shown with a mean temporal trajectory curve and 95% CI. Statistics for each cytokine indicate the slope of the curve with a corresponding p-value indicating change over time.

Figure 4. Egg-induced cytokine changes in high and low dose peanut OIT groups. Individual data were log-transformed (ln) and are shown with a mean temporal trajectory curve and 95% CI.

Figure 5.

CD4 T cell changes during the course of peanut OIT. (A) Peanut-responsive (CFSE-low) CD4 T cells for all subjects during build-up at 0, 4, and 8 months and (B) in high and low dose groups over the course of OIT. (C) CD4+CD25+FoxP3+ T cells during build-up and (D) in high and low dose groups over the course of OIT. Individual data are shown; red lines indicate median values; mean temporal trajectory curves are shown with 95% CI.

Figure 6.

Basophil activation and peanut-specific IgG4/IgE ratios over the course of OIT. (A) Basophil activation of lactic acid-stripped basophils primed with plasma from 0, 12, 24, and 36 months in groups on high and low dose OIT. (B) Peanut-specific IgG4/IgE ratios over the course of OIT for high and low dose groups. Individual data are shown with mean temporal trajectory curves and 95% CI.

Figure 7.

Whole blood basophil activation assays at desensitization (DS) and SU OFCs. High and low dose groups were stimulated with 4 log-fold doses of peanut antigens, anti-IgE control, or left unstimulated (media alone). Individual subject data are shown with medians indicated by red lines.