Suppression of the immunologic response to peanut during immunotherapy is often transient

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Background: Studies suggest that oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) for food allergy hold promise; however, the immunologic mechanisms underlying these therapies are not well understood.
Objective: We sought to generate insights into the mechanisms and duration of suppression of immune responses to peanut during immunotherapy.
Methods: Blood was obtained from subjects at baseline and at multiple time points during a placebo-controlled trial of peanut OIT and SLIT. Immunologic outcomes included measurement of spontaneous and stimulated basophil activity by using automated fluorometry (histamine) and flow cytometry (activation markers and IL-4), measurement of allergen-induced cytokine expression in dendritic cell (DC)–T-cell cocultures by using multiplexing technology, and measurement of MHC II and costimulatory molecule expression on DCs by using flow cytometry.
Results: Spontaneous and allergen-induced basophil reactivity (histamine release, CD63 expression, and IL-4 production) were suppressed during dose escalation and after 6 months of maintenance dosing. Peanut- and dust mite–induced expression of T_H2 cytokines was reduced in DC–T-cell cocultures during immunotherapy. This was associated with decreased levels of CD40, HLA-DR, and CD86 expression on DCs and increased expression of CD80. These effects were most striking in myeloid DC–T-cell cocultures from subjects receiving OIT. Many markers of immunologic suppression reversed after withdrawal from immunotherapy and in some cases during ongoing maintenance therapy.
Conclusion: OIT and SLIT for peanut allergy induce rapid suppression of basophil effector functions, DC activation, and T_H2 cytokine responses during the initial phases of immunotherapy in an antigen-nonspecific manner. Although there was some interindividual variation, in many patients suppression appeared to be temporary. (J Allergy Clin Immunol 2015;135:1283-92.)

Key words: *Peanut allergy, oral immunotherapy, sublingual immunotherapy, sustained unresponsiveness, basophil activation, dendritic cells, food allergy*

Peanut allergy, a public health concern with substantial morbidity, affects 1% of the Western world.[1,2] Current clinical management focuses on avoidance and treatment of reactions after accidental exposures.[3] However, we and others have recently demonstrated[4] that oral immunotherapy (OIT) and sublingual immunotherapy (SLIT) might allow subjects to tolerate increased amounts of peanut compared with baseline values, although clinical reactivity often returns once subjects discontinue treatment, suggesting these therapies more likely induce transient desensitization rather than longer-term tolerance.[5]

The immunologic mechanisms underlying the clinical effects of immunotherapy continue to be elucidated. Initial studies in peanut OIT and SLIT demonstrated decreases in peanut-specific IgE levels with concomitant increases in IgG_4 levels, as well as reduced T_H2 cytokine responses to peanut and upregulation of regulatory T cells, especially in OIT.[6-10] Hypomethylation of the forkhead box protein 3 *(FOXP3)* locus as a result of peanut OIT and subsequent remethylation with regained sensitivity to peanut has also been proposed to be associated with clinical desensitization.[10] Changes in basophil reactivity during food immunotherapy have been another area of interest because basophils express the high-affinity receptor for IgE and are critical effector cells in allergic reactions through their release of histamine, cytokines, and leukotrienes on stimulation.[11,12] Decreased peanut-induced expression of the basophil activation markers CD63 and CD203c has been demonstrated during peanut OIT.[6,13] One study also suggested that immunotherapy can induce basophil hyporesponsiveness in an antigen-nonspecific manner.[14] Studies in

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Abbreviations used
DBPCFC: Double-blind, placebo-controlled food challenge
DC: Dendritic cell
HR: Histamine release
mDC: Myeloid dendritic cell
OFC: Open food challenge
OIT: Oral immunotherapy
pDC: Plasmacytoid dendritic cell
SHR: Spontaneous histamine release
SLIT: Sublingual immunotherapy

milk and egg immunotherapy have demonstrated similar findings, although in some cases with less robust basophil suppression.[14,15]

Dendritic cells (DCs) are professional antigen-presenting cells that direct T-cell responses to food and other antigens and therefore likely drive the changes in T-cell responses during immunotherapy. Two major classes of DCs have been identified in human peripheral blood: plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs).[16-18] Both subtypes regulate food allergen–driven T_H2 cytokine release by $CD4^+$ T cells and have been shown to exhibit phenotypic changes during the course of venom immunotherapy.[19,20]

In this pilot study we sought to evaluate the systemic effects of peanut OIT and SLIT[4] on the function of immune cells critical in allergic responses and tolerance, including basophils and DCs, to gain insight into the immunologic changes exerted by these therapies and to explore whether cellular immune responses qualitatively correlate with the clinical effects of OIT and SLIT.

METHODS

See the Methods section in this article's Online Repository at www.jacionline.org for a full description of the methods used in this study. Peripheral blood was collected from subjects during a clinical trial comparing peanut OIT and SLIT. A summary of the study design and clinical outcomes is contained in the Methods section in this article's Online Repository; Fig E1 in this article's Online Repository at www.jacionline.org summarizes the clinical study protocol, and Fig E2 in this article's Online Repository at www.jacionline.org is a CONSORT flow diagram detailing outcomes of subjects. The accompanying article by Narisety et al[4] also provides further details of the design and outcome of the study. Blood was collected at the following time points: T1, baseline double-blind, placebo-controlled food challenge (DBPCFC); T3, end of blinded escalation; T4, DBPCFC after 6 months of maintenance; T5, DBPCFC after 12 months of maintenance; T6, open food challenge (OFC) after 6 months of continued or add-on treatment (see Narisety et al[4] for details); and T7, OFC after 4 to 6 weeks off treatment. Cell preparation procedures have been described previously[21] and are described further in the Methods section in this article's Online Repository. Basophil-enriched suspensions were cultured in media alone or with crude peanut extract, dust mite extract *(Dermatophagoides pteronyssinus)*, anti-IgE, or ionomycin. Measures of stimulated and spontaneous basophil histamine release (HR; by using automated flourometry) and CD63 and CD203c expression (by using flow cytometry) were performed. Whole-blood samples were treated in an analogous fashion and used to assess basophil IL-4 expression by using an intracellular flow cytometric assay (J. T. Schroeder, unpublished data). $CD4^+$ T cells were cultured alone or with mDCs and pDCs and incubated with medium alone, peanut, or dust mite for 5 days. IL-13, IL-5, IFN-γ, IL-10, TNF-α, and IL-17 levels were measured in culture supernatants by using the multiplex bead immunoassay, and measures of HLA-DR and costimulatory molecule expression on DCs were assessed by using flow cytometry.

Statistical analyses are described in the Methods section in this article's Online Repository. This study was approved by the Johns Hopkins Institutional Review Board.

RESULTS

Spontaneous and peanut-induced basophil HR and CD63 expression

To investigate changes in basophil reactivity during OIT and SLIT, we measured spontaneous histamine release (SHR) and constitutive expression of the basophil activation markers CD63 and CD203c in basophil-enriched suspensions (Fig 1 and see Fig E3 in this article's Online Repository at www.jacionline.org). We also examined the same parameters in response to 3 different doses of peanut (Fig 2 and see Fig E4 in this article's Online Repository at www.jacionline.org). At the end of dose escalation (T3) and after 6 (T4) and 12 (T5) months of maintenance immunotherapy, spontaneous CD63 expression and SHR were reduced markedly in the OIT group ($P < .01$) compared with baseline values (Fig 1, *A* and *B*). A qualitatively similar decrease was seen in the SLIT group at T3 and T4 but did not reach significance (Fig 1, *A* and *B*). Peanut-induced HR and CD63 expression were also suppressed versus baseline by the end of dose escalation in the OIT group (T3) and in both the OIT and SLIT groups after 6 months of maintenance therapy (T4), especially at higher peanut doses (Fig 2).

After this initial suppression, constitutive CD63 expression increased in subjects receiving OIT despite continued maintenance dosing such that CD63 expression was no longer significantly decreased compared with baseline values by the end of the maintenance period (T6; Fig 1, *B*). A qualitatively similar pattern was evident for SHR (Fig 1, *A*) and for both parameters in subjects receiving SLIT (Fig 1, *A* and *B*). Peanut-induced HR and CD63 expression also reverted in subjects receiving OIT while they continued maintenance therapy (Fig 2). These parameters remained suppressed in the SLIT cohort (Fig 2). Of note, because of a crossover study design, all subjects in the SLIT group had OIT added between T5 and T6. This did not appear to inhibit SHR or constitutive CD63 expression, whereas peanut-induced HR and CD63 expression remained suppressed (Figs 1, *A* and *B*, and Fig 2). Additionally, 3 of 7 patients receiving OIT were augmented with SLIT between T5 and T6. This addition of SLIT resulted in qualitatively greater suppression of both constitutive and stimulated basophil reactivity in 2 of 3 subjects compared with 2 of 4 of those subjects who continued on OIT alone (data not shown).

Increases in both constitutive and peanut-induced CD63 expression and HR were also evident after a short period off therapy (T7), especially when compared with the point of maximal suppression (Figs 1, *A* and *B*, and 2). Constitutive CD203c expression was not suppressed at any time point and actually increased at T6 versus baseline in both the OIT and SLIT groups ($P = .043$ and $P = .018$, respectively) and after therapy withdrawal at T7 for the SLIT group ($P = .011$; Fig 1, *C*). CD203c upregulation in response to peanut was generally small and did not show significant changes during OIT or SLIT (see Fig E4). Finally, CD63 expression and HR were strongly correlated ($P < .001$, $r = 0.92$), although this relationship was significant but weaker for HR and CD203c expression ($P < .01$, $r = 0.11$; see Fig E5 in this article's Online Repository at www.jacionline.org).

Despite some qualitative differences in spontaneous basophil activity during OIT and SLIT, direct comparisons between these 2 arms showed no significant differences overall. However, OIT

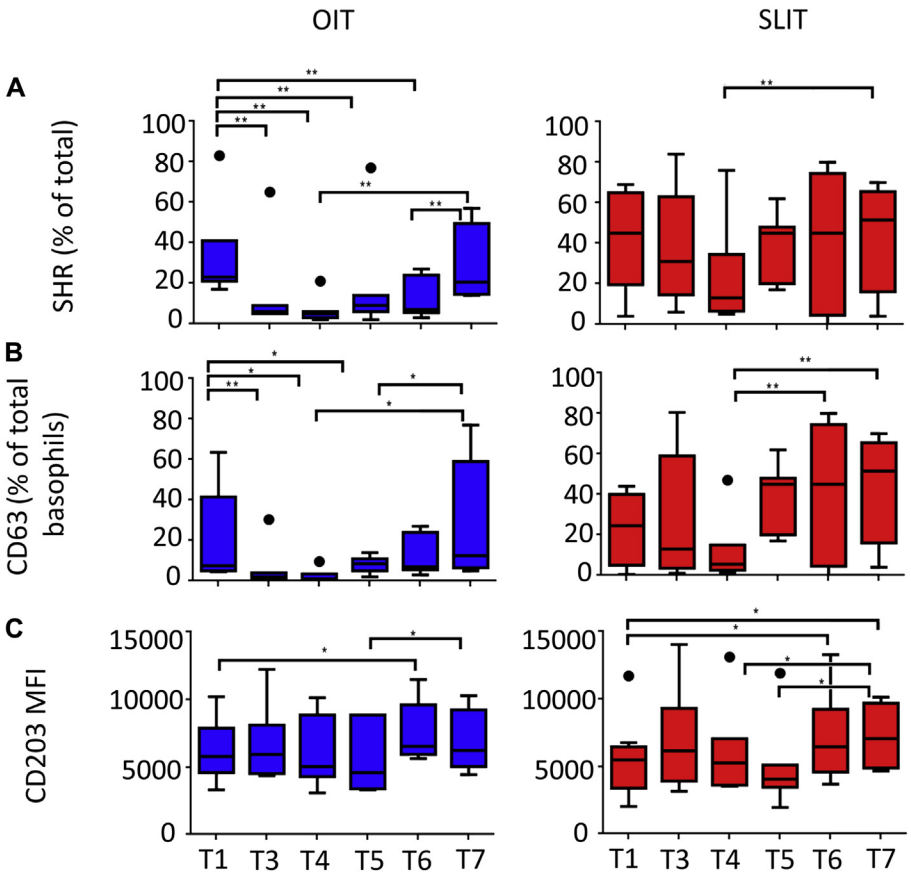

FIG 1. SHR and constitutive expression of basophil activation markers during immunotherapy. SHR **(A)**, constitutive CD63 expression **(B)**, and CD203c mean fluorescence intensity (*MFI*; **C**) were measured in basophil-enriched suspensions from subjects undergoing OIT *(left panels)* and SLIT *(right panels)* for peanut. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. **P* < .05 and ***P* < .01.

resulted in more rapid suppression of basophil responses to peanut compared with SLIT (eg, for rate of decrease in HR between T1 and T3: $P = .001$ and $P = .038$ for 0.1 and 1 ng/mL peanut, respectively).

Peanut-induced basophil IL-4 expression

To determine whether changes in basophil IL-4 expression might play a role in desensitization, we incubated whole-blood samples with peanut, anti-IgE, dust mite, and ionomycin. Peanut-stimulated basophil IL-4 expression was significantly reduced from the end of dose escalation (T3) through maintenance (T4-T6) compared with baseline (T1) values in both the OIT (T1 vs T3-T6, $P < .05$ at 0.1 ng/mL and $P < .001$ at 1 and 10 ng/mL of peanut) and SLIT (T1 vs T5, $P < .05$ at 0.1 ng/mL and $P < .001$ for T1 vs T3-T6 at 1 and 10 ng/mL) groups, but unlike CD63 expression and HR, IL-4 expression did not revert to higher levels during maintenance therapy (Fig 3, *A*). However, IL-4 expression did increase once subjects were taken off therapy (T7 vs T6) in both cohorts (Fig 3, *A*). Direct comparisons between the SLIT and OIT cohorts revealed no significant differences in basophil IL-4 expression.

Specificity of basophil suppression during peanut OIT and SLIT

To determine whether the changes we observed in basophil reactivity were specific for peanut, we evaluated responses from basophil-enriched suspensions (CD63, CD203c, and HR) and whole-blood samples (IL-4) incubated with a polyclonal anti-human IgE cross-linking antibody, 2 doses of dust mite, or ionomycin, an inducer of FcεRI-independent basophil degranulation. Suppression of basophil IL-4 expression during OIT and SLIT was not peanut specific because it was evident after stimulation with both dust mite and anti-IgE (Fig 3, *B-D*). In both the OIT and SLIT groups, IL-4 responses to anti-IgE and dust mite increased after the therapy withdrawal period (T7), although these changes did not always reach significance (Fig 3, *B-D*). Basophil CD63 expression and HR to anti-IgE and dust mite were low and did not change significantly with therapy in either the OIT or SLIT groups (see Fig E6 in this article's Online Repository at www.jacionline.org). No marker of basophil reactivity (IL-4, CD63, or HR) to the IgE-independent stimulus ionomycin changed during the course of OIT or SLIT (Fig 3 and see Fig E6). Finally, CD203c expression did not change significantly after stimulation with any of these stimuli in either cohort (data not shown).

DC-driven T-cell cytokine responses

Because DCs play a central role in dictating T-cell responses to allergens, we explored how SLIT and OIT affected cytokine responses by $CD4^+$ T cells cocultured with either pDCs or mDCs and stimulated with peanut or dust mite (an allergen for which subjects have not received immunotherapy). As seen in

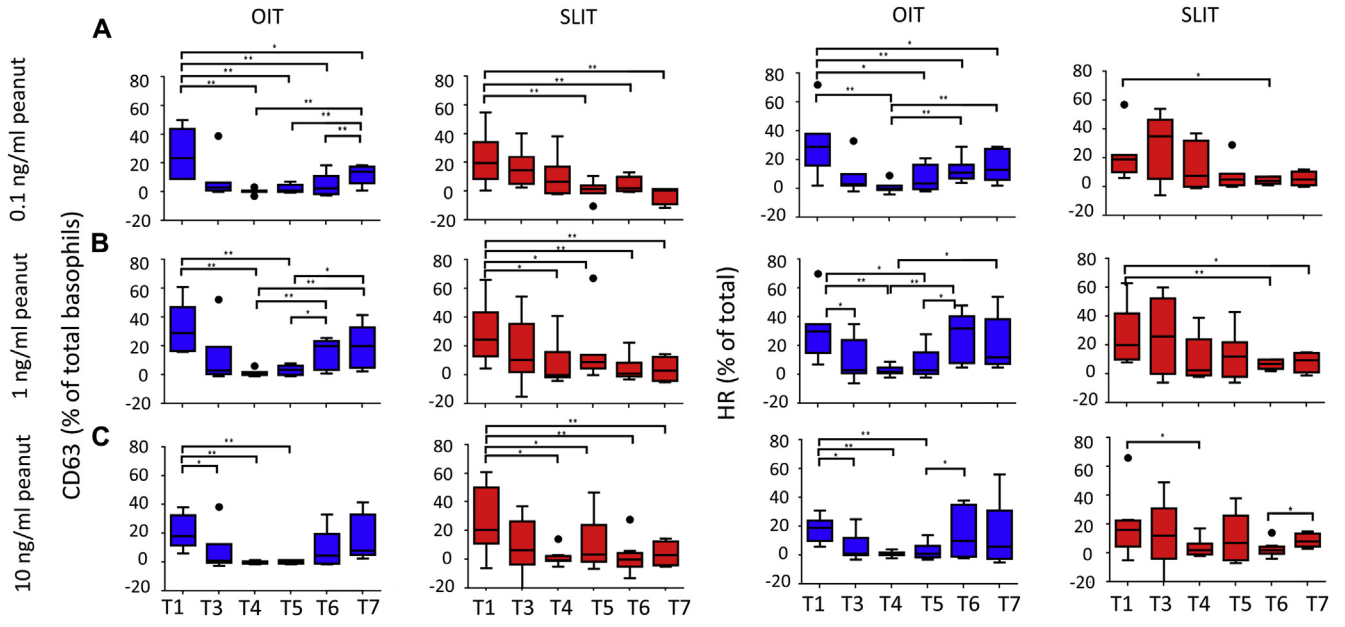

FIG 2. Effect of OIT and SLIT on basophil CD63 expression and HR in response to peanut. Basophil-enriched suspensions were stimulated with crude peanut extract at 0.1 ng/mL **(A)**, 1 ng/mL **(B)**, and 10 ng/mL **(C)**. SHR and CD63 expression in medium alone were subtracted to obtain stimulated values. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. **P* < .05 and ***P* < .01.

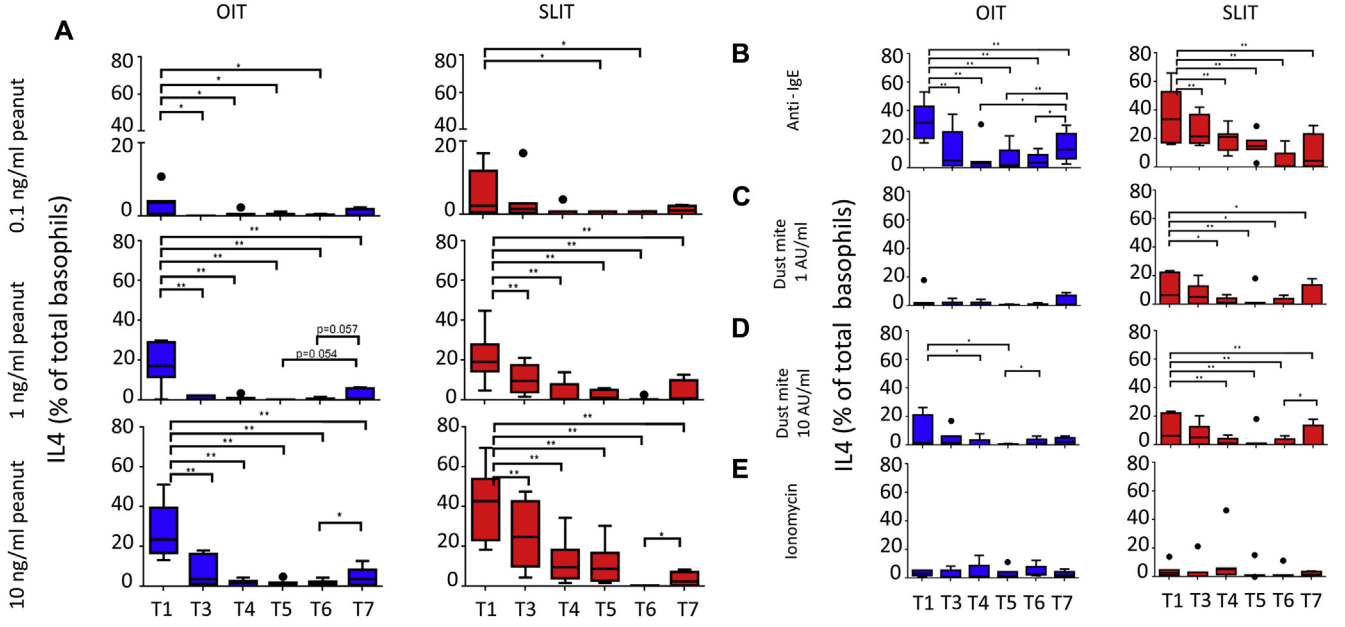

FIG 3. Changes in basophil IL-4 expression during peanut OIT and SLIT. Basophil expression of IL-4 was measured by using intracellular flow cytometry after incubation of whole-blood samples with peanut **(A)**, anti-IgE **(B)**, dust mite at 1 AU/mL **(C)** or 10 AU/mL **(D)**, and ionomycin **(E)**. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. **P* < .05 and ***P* < .01.

Fig 4, T_H2 cytokine responses (IL-13 and IL-5) to peanut were robust at baseline (T1), with significantly higher expression in mDC–T-cell than pDC–T-cell cocultures (IL-13, $P = .003$; IL-5, $P = .007$). After 12 months of maintenance therapy (T5), T_H2 cytokine release to peanut, as well as dust mite, was suppressed in both pDC–T-cell and mDC–T-cell cocultures from subjects receiving OIT and subjects receiving SLIT compared with baseline values (T1; Fig 4). However, T_H2 cytokine expression subsequently increased despite continued treatment in the OIT cohort such that levels at T6 were no longer significantly decreased compared with baseline (T1) values (Fig 4). This reversion was less evident in the SLIT group, perhaps because OIT had been

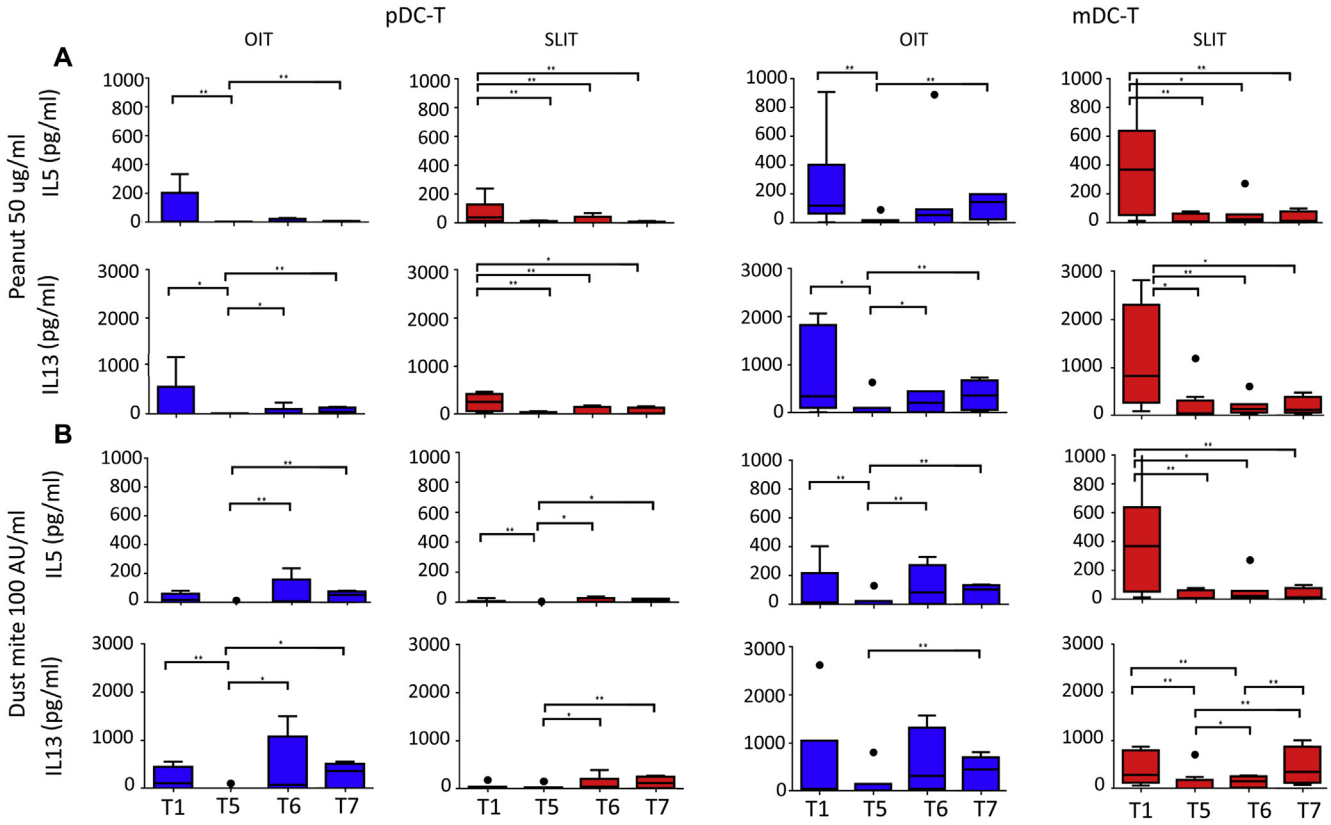

FIG 4. Effect of peanut OIT and SLIT on T_H2 cytokine responses in DC–T-cell cocultures. IL-5 and IL-13 were measured in supernatants from cocultures of pDCs and mDCs with autologous $CD4^+$ T cells stimulated with 50 μg/mL crude peanut extract **(A)** or 100 AU/mL dust mite **(B)**. Spontaneous cytokine secretion, as measured in medium alone, was subtracted to obtain allergen-induced values. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. *$P < .05$ and **$P < .01$.

added to their treatment regimen between T5 and T6 (Fig 4). Although levels of T_H2 cytokines produced in response to peanut remained lower at T7 (after subjects discontinued treatment) compared with baseline values in the SLIT cohort, neither peanut-induced IL-5 nor IL-13 levels were significantly different between T7 and T1 in the OIT group (Fig 4, *A*). OIT and SLIT also significantly altered peanut-induced expression of IFN-γ, IL-10, and TNF-α in a manner similar to the changes in T_H2 cytokine responses, with suppression followed by increased expression (see Fig E7 in this article's Online Repository at www.jacionline.org), whereas IL-17 expression was generally unchanged (data not shown). A similar pattern was also seen in cultures stimulated with dust mite (data not shown). For all cocultures, medium-only conditions resulted in low to non-detectable cytokine production (data not shown). Direct comparisons did not show any significant differences in cytokine responses between the OIT and SLIT cohorts.

Expression of HLA-DR and costimulatory molecules

To evaluate whether changes in DC-driven release of T_H2 cytokines were associated with changes in expression of costimulatory molecules and HLA-DR by these cells over the course of immunotherapy, we stained the cocultures described above after incubation with medium alone, peanut, or dust mite for CD40, CD80, CD86, and HLA-DR. As shown in Fig 5, significant changes were seen in a number of costimulatory molecules over the course of OIT and SLIT. CD40 and CD86 were significantly suppressed on mDCs and pDCs from the OIT cohort after peanut and dust mite stimulation after 12 months of maintenance dosing (T5), and a similar trend was seen for HLA-DR (Fig 5). This suppression was less evident in the SLIT cohort, particularly on pDCs (Fig 5). DCs cultured in medium alone showed qualitatively similar trends to cultures treated with allergen, although levels of expression of costimulatory molecules were lower than those observed after antigen stimulation (Fig 5 and see Fig E8 in this article's Online Repository at www.jacionline.org).

The decrease in expression of CD40, CD86, and HLA-DR on DCs during immunotherapy appeared to only be temporary in many subjects. After the initial decrease after 12 months of maintenance therapy (T5), expression of these markers on mDCs increased while maintenance dosing continued (T6) and after withdrawal of therapy (T7) in the OIT group (Fig 5 and see Fig E8). A similar pattern was seen in the SLIT group (Fig 5 and see Fig E8). Although these changes were most robust with mDCs, the pattern was often visible in pDC cultures as well but did not always reach significance (Fig 5 and see Fig E8). Direct comparisons between OIT and SLIT did not show any significant difference in HLA-DR or costimulatory molecule expression.

Expression of CD80 showed a very different pattern than HLA-DR and the other costimulatory molecules (Fig 6). Although essentially not detectable on mDCs at baseline (T1), CD80

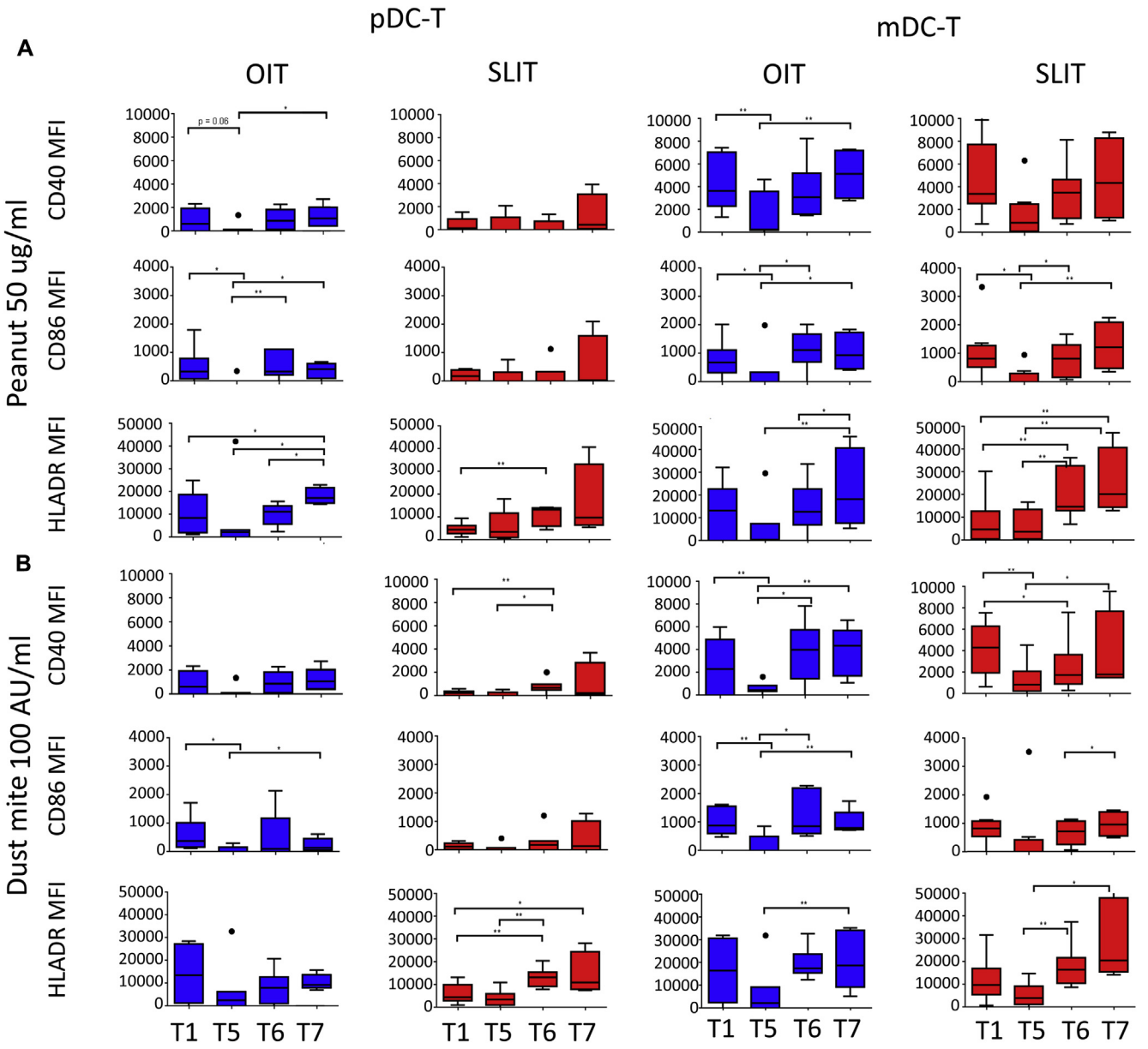

FIG 5. Effect of peanut OIT and SLIT on DC expression of costimulatory molecules and HLA-DR. Mean fluorescence intensity *(MFI)* of CD40, CD86, and HLA-DR on pDCs *(left 2 columns)* or mDCs *(right 2 columns)* was measured after coculture with $CD4^+$ T cells stimulated with 50 μg/mL peanut **(A)** or 100 AU/mL dust mite **(B)**. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. **P* < .05 and ***P* < .01.

expression was significantly increased after 12 months of maintenance dosing in subjects receiving OIT and, to a lesser extent, in subjects receiving SLIT (T1 vs T5, Fig 6). However, after 18 months of maintenance therapy (T6), expression was nearly completely lost and remained absent after withdrawal of treatment (T7; Fig 6). Expression of CD80 on pDCs was extremely low and did not change significantly with therapy (data not shown).

Correlation between mechanistic and clinical outcomes

Several biomarkers were significantly correlated with certain clinical outcomes (Table I). A negative correlation was found between achievement of sustained unresponsiveness and baseline basophil CD63 expression, HR, and IL-4 production observed at the low dose (0.1 ng/mL) of peanut. Four of 5 patients with baseline basophil CD63 expression of 10% or less (when incubated with 0.1 ng/mL peanut) achieved sustained unresponsiveness, whereas all patients with baseline basophil CD63 expression of greater than 10% did not ($P = .002$, Fisher exact test). Average HR and IL-4 production at 0.1 ng/mL peanut was lower at baseline in patients who achieved sustained unresponsiveness (28.3% vs 15.3% of total for HR and 5.3% vs 1.7% of total basophils for IL-4), but these differences were not significant. Basophil IL-4 expression in response to all 3 doses of peanut correlated positively with peanut-specific IgE levels.

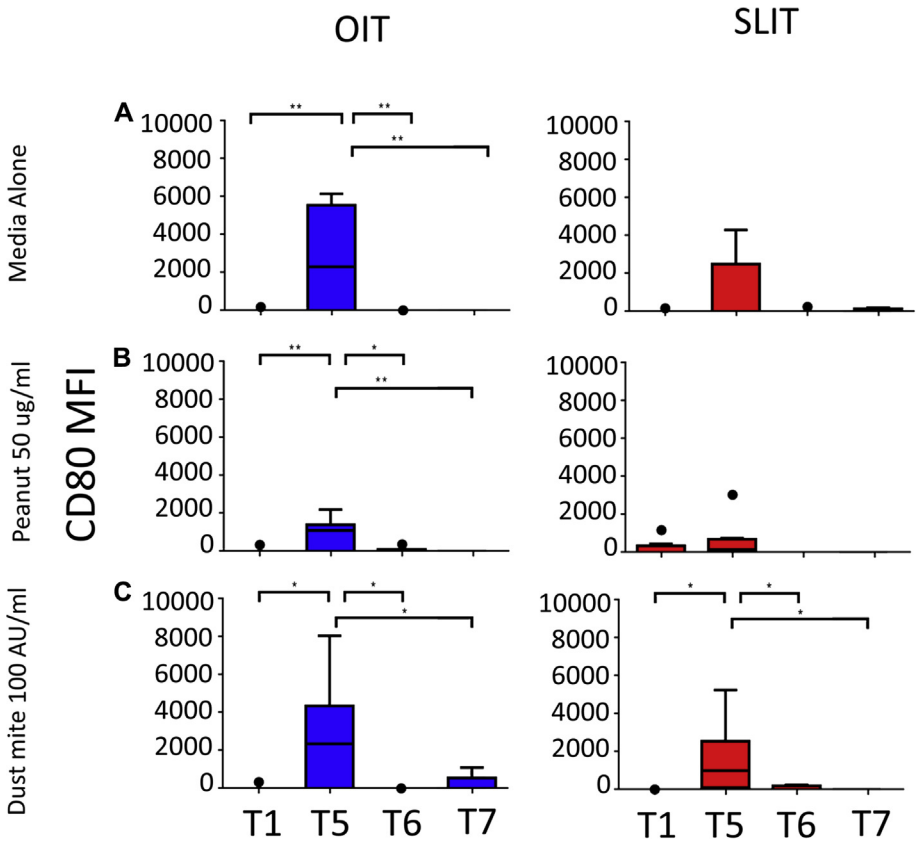

FIG 6. Effect of peanut OIT and SLIT on expression of CD80 on mDCs. CD80 mean fluorescence intensity *(MFI)* was measured on mDCs after coculture with $CD4^+$ T cells stimulated with media alone **(A)**, 50 μg/mL peanut **(B)**, or 100 AU/mL dust mite **(C)**. T1 to T7 correspond to time points at which blood was collected. OIT: n = 7 at T1 to T5, n = 5 at T6, and n = 4 at T7. SLIT: n = 8 at T1 to T4, n = 7 at T5, n = 8 at T6, and n = 4 at T7. **P* < .05 and ***P* < .01.

Additionally, a consistent positive correlation was noted between T_H2 cytokine production in mDC–T-cell cocultures, which showed the most robust changes during immunotherapy, and peanut-specific IgG_4 levels, whereas peanut-specific IgE/IgG_4 levels correlated negatively with this outcome (Table I). Finally, correlation of the specific number of reactions and the severity of these at the time of DBPCFCs and OFCs was evaluated based on expression of certain markers, such as CD63. No significant correlations were seen.

Despite the several correlations found between clinical and mechanistic data, a significant amount of interindividual variation was seen, with some patients maintaining clinical desensitization while levels of immunologic markers increased. For example, subjects who were able to maintain sustained unresponsiveness had generally lower peanut-induced CD63 expression at baseline but not while therapy continued, and in fact, some successful patients had a greater increase in CD63 expression after initial suppression than nonsuccessful patients (see Fig E9 in this article's Online Repository at www.jacionline.org). A different pattern was evident with IL-4, with subjects achieving sustained unresponsiveness generally demonstrating lower IL-4 expression to higher doses of peanut as therapy continued (see Fig E9). Significant interindividual variation was also seen in other outcomes, including expression of T_H2 cytokines and costimulatory molecules on DCs (see Fig E10 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Immunotherapy trials have generated great excitement that a treatment for food allergy is on the horizon; however, this enthusiasm has been tempered by the knowledge that side effects are common and only a minority of subjects achieve sustained unresponsiveness. How these clinical observations correlate with the degree and nature of immunologic suppression is not well understood. Here we demonstrate that OIT (and, to a lesser degree, SLIT) for peanut allergy effectively suppresses basophil effector cell function and DC-driven T_H2 cytokine responses to peanut. However, these parameters reversed in many subjects once they withdrew from therapy and, in some cases, while they continued maintenance dosing. To our knowledge, this is the first study to demonstrate that systemic immunologic suppression mediated by immunotherapy for peanut allergy might not be long lasting.

Stimulated expression of CD63 and CD203c on basophils has been studied as a potential biomarker of IgE-mediated food-induced allergic responses.[22] SLIT and OIT potently suppressed peanut-induced expression of CD63 and HR from basophils but not CD203c. HR strongly correlated with CD63 and more weakly with CD203c, which is consistent with reports that CD63 is the best indicator of anaphylactic degranulation.[22] Expression of CD203c can be induced by IL-3, exhibits different kinetics than CD63, and might reflect piecemeal rather than anaphylactic degranulation.[23] Although some previous studies have shown suppression of CD203c expression over the course of peanut OIT,[13]

TABLE I. Correlation of selected biomarkers with clinical outcomes

Biomarker	Pass or fail	Total dose	SPT	Log IgE	Log IgG_4	Log IgE/IgG_4
Spontaneous CD63 media	.476	.757	.272	.393	**.018** (−)	.434
CD63, peanut, 0.1 ng/mL	**<.001** (−)	.673	.411	.959	.058	.226
CD63, peanut, 1 ng/mL	.089	.727	.064	.318	.174	.836
CD63, peanut, 10 ng/mL	.949	.638	.092	.576	.082	.927
CD63, anti-IgE	NS	NS	NS	NS	NS	NS
CD63, ionomycin	NS	NS	NS	NS	NS	NS
CD63, dust mite, 1 AU/mL	NS	NS	**.041** (+)	NS	NS	NS
CD63, dust mite, 10 AU/mL	**.017** (−)	.305	.075	NS	.872	NS
IL-4, peanut, 0.1 ng/mL	**.013** (−)	.851	.591	**.047** (+)	.742	.152
IL-4, peanut, 1 ng/mL	.053	.536	.079	**.001** (+)	.524	.057
IL-4, peanut, 10 ng/mL	.980	.051	.147	**.011** (+)	.510	.099
IL-4, anti-IgE	**.018** (−)	.076	.936	.116	.849	.879
IL-4, ionomycin	NS	NS	NS	NS	NS	NS
IL-4, dust mite, 1 AU/mL	.552	.935	.791	.191	.501	.924
IL-4, dust mite, 10 AU/mL	.485	.125	.703	**.038** (+)	.605	.650
Histamine, medium (SHR)	.332	.548	.749	.280	.915	.344
Histamine, peanut, 0.1 ng/mL	**.047** (−)	.075	.995	.103	**.012** (−)	.104
Histamine, peanut, 1 ng/mL	.233	.556	.262	.330	.083	.739
Histamine, peanut, 10 ng/mL	.892	.930	.248	.332	.090	NS
Histamine, anti-IgE	NS	NS	NS	.748	NS	NS
Histamine, ionomycin	NS	.388	NS	.406	NS	NS
Histamine, dust mite, 1 AU/mL	NS	.116	**.018** (+)	NS	NS	NS
Histamine, dust mite, 10 AU/mL	NS	NS	NS	.042	NS	NS
IL-13 pDC, peanut, 50 μg/mL	.412	NS	.221	.581	.978	.739
IL-13 mDC, peanut, 50 μg/mL	.492	.745	.667	.209	**.049** (+)	**.029** (−)
IL-13 pDC, dust mite, 100 AU/mL	.382	**.044** (−)	**.048** (+)	.923	.920	.896
IL-13 mDC, dust mite, 100 AU/mL	.845	.872	.885	.345	**.007** (+)	**.016** (−)
IL-5 pDC, peanut, 50 μg/mL	.409	.660	.458	.225	.861	.377
IL-5 mDC, peanut, 50 μg/mL	.486	.974	.771	.450	**.008** (+)	**.033** (−)
IL-5 pDC, dust mite, 100 AU/mL	.757	.171	**.013** (+)	.823	.635	.646
IL-5 mDC, dust mite, 100 AU/mL	.701	.779	.943	.982	**.021** (+)	.139

P values describing significant correlations are shown in boldface. A (+) sign adjacent to the correlation indicates positive correlation, while a (−) sign adjacent to the correlation indicates negative correlation. Pass or fail outcomes were correlated to baseline biomarkers only, whereas all other outcomes were correlated over the entire course of the clinical trial.

Log IgE, Log of peanut-specific IgE (in kilounits of antigen per liter); *Log IgE/IgG$_4$*, ratio of the logs of peanut-specific IgE and IgG_4; *Log IgG$_4$*, log of peanut-specific IgG_4 (in milligram of antigen per liter); *NS*, $P > .05$, Wald test; *Pass or fail*, success or failure in achieving sustained hyporesponsiveness; *SPT*, peanut skin prick test wheal size in millimeters; *Total dose*, milligrams of peanut protein ingested during each challenge without symptoms.

these studies were performed in whole blood with IL-3 stimulation, as opposed to the washed basophil suspensions without IL-3 in our study. Basophils additionally support T_H2 immune responses by producing IL-4 after activation through FcεRI. This function of basophils was also attenuated during immunotherapy, suggesting a novel mechanism by which these cells might contribute to immunologic suppression during immunotherapy.

Basophils from the majority of children with food-induced allergy have been shown to spontaneously release histamine.[24] Although the clinical relevance and mechanisms responsible for this phenomenon are not well understood, high SHR appears to be IgE dependent and might indicate more severe clinical reactivity to cow's milk.[25,26] Consistent with our previous findings in a trial of milk OIT,[14] peanut OIT significantly reduced both SHR and constitutive CD63 expression by the end of the dose-escalation period. Collectively, these data support an overall decrease in IgE-dependent pathway activation in basophils early in the course of OIT.

Induction of T-cell tolerance, anergy, or both is purported to be central to the mechanisms of immunotherapy. Both pDCs and mDCs direct memory responses by $CD4^+$ T cells to food allergens, but in our study mDCs appeared to promote greater T_H2 cytokine responses to peanut than pDCs. Interestingly, Ara h 1, a major peanut allergen, directly binds to DC-specific ICAM-grabbing nonintegrin (DC-SIGN) on mDCs and acts as a T_H2 adjuvant to activate T cells.[27] Peanut-induced levels of T_H2 cytokines decreased in DC–T-cell cocultures after 12 months of maintenance dosing and were associated with reduced expression of the costimulatory molecules CD86 and CD40, as well as HLA-DR, on DCs. On the other hand, expression of CD80 increased on mDCs at the same time point. Although the mechanisms of T-cell costimulation are highly complex,[28] some studies suggest that CD80 is the preferential ligand for the inhibitory T-cell molecule cytotoxic T lymphocyte–associated antigen 4,[29] suggesting the increase in CD80 on mDCs during immunotherapy might serve to dampen T-cell responses. Nearly all of the changes in DC phenotype and function we observed during peanut immunotherapy were more prominent with mDCs than pDCs. Consistent with other immunotherapy trials for food allergy,[9] we did not find a switch from T_H2 to T_H1 cytokine responses during immunotherapy but rather a generalized suppression of effector cytokine expression. It was recently demonstrated that the increase in IFN-γ levels during SLIT for grass pollen allergy is not mediated by T cells[30]; therefore we cannot exclude the possibility that other cell types led to increased T_H1 and IL-10 responses in our study that we never detected because we only evaluated the T-cell arm.

Although peanut immunotherapy effectively suppressed basophil, DC, and T-cell reactivity by the end of dose escalation and after the first 6 months of maintenance dosing, remarkably, almost all of the changes were only temporary in a majority of subjects. Peanut-induced and spontaneous HR and CD63 expression by basophils increased despite continued maintenance therapy compared with earlier time points, and IL-4 responses also reverted once subjects withdrew from therapy. IL-4 might have been more persistently suppressed than HR/CD63 because our IL-4 assay was performed in whole blood rather than washed cells, and therefore basophils in this assay had continued exposure to inhibitory serum factors. Even constitutive expression of CD203c, which had not changed during the early stages of immunotherapy, significantly increased after 1 year of maintenance dosing. This might have clinical relevance because patients with lower levels of milk tolerance have been demonstrated to have increased constitutive CD203c expression at baseline.[25] Likewise, the reduction in DC-driven T_H2 cytokine responses to peanut, as well as expression of activation markers and HLA-DR on DCs, was often only transient.

The immunologic effects of immunotherapy were largely not antigen specific. DC and T-cell responses to dust mite were also reduced, and this suppression appeared to be transient as well in many subjects. This is consistent with previous findings suggesting immunotherapy promotes a pathway-specific, antigen-nonspecific basophil anergy.[13] Although basophil HR and CD63 expression in response to anti-IgE and dust mite did not significantly change during treatment, neither of these stimuli evoked potent responses, perhaps because the doses used were more optimal for IL-4 expression than HR. Basophil expression of IL-4 to these stimuli was significantly attenuated, whereas no change in any measure of basophil reactivity to ionomycin, a non–IgE-dependent stimulant, was observed.

Our study had several important limitations. First, we did not have a placebo group that received no intervention, although the study was placebo controlled during the double-blind treatment phase.

Second, there was a high dropout rate because of adverse effects, and mechanistic analyses were not performed on subjects who discontinued study participation, which might have excluded subjects with less robust immunologic suppression and skewed toward those better able to tolerate immunotherapy. An exception to the lack of mechanistic data on dropouts was data from HR. This was obtained at baseline and at 1 or 2 subsequent visits for all patients, including patients who later dropped out. The addition of these data improves the significance obtained for the predictive value of baseline HR for achieving sustained unresponsiveness at 0.1 ng/mL from .047 to .008 (Table I) but otherwise does not alter the data. Detailed clinical information on the patients who dropped out can be found in Narisety et al.[4]

Third, the crossover design,[4] in which all patients receiving SLIT were augmented with OIT and some patients receiving OIT were augmented with SLIT, created added complexity in analyzing the data but remarkably revealed that add-on treatments generally did not prevent the increase in immunologic reactivity. For instance, we found that OIT augmentation on SLIT seemed to be associated with continued suppression of stimulated CD63 expression, but most other immunologic markers showed reversion despite OIT augmentation.

Finally, only subjects who completed the treatment and had no reaction at their T6 oral food challenge proceeded to T7, which resulted in a total of only 9 subjects at the T7 time point.

The degree of immunologic suppression achieved was qualitatively more pronounced in subjects receiving OIT than in subjects receiving SLIT, which is consistent with our clinical observations that subjects receiving OIT generally experienced greater clinical improvement than those receiving SLIT.[4] Subjects receiving OIT might have a more pronounced response because of the overall larger dose of allergen received. Subjects receiving SLIT were more likely in some cases to exhibit persistent immunologic suppression, perhaps because they were augmented with OIT late in the maintenance phase because of the crossover design of the study. The high frequency of reactions and failure of most subjects to achieve sustained unresponsiveness to peanut is consistent with the transient nature of immunologic suppression to peanut that we observed, although it is interesting to note that many subjects remained able to ingest peanut despite an apparent loss of immunologic suppression, as mediated by both basophils and DCs. This might be due to the small sample size and the considerable interindividual variation within the sample or might suggest that other unknown mechanisms could play a role in induction and maintenance of desensitization. Although the study was not powered to identify predictors of clinical outcome, baseline basophil CD63 expression, HR, and IL-4 production at low doses of peanut correlated significantly with achievement of sustained unresponsiveness, suggesting that those patients with the lowest baseline basophil responsiveness to peanut might have a better clinical outcome. The consistent correlation between T_H2 cytokine production in mDC–T-cell cocultures, which showed the most robust changes during immunotherapy, and peanut-specific IgG_4 levels suggests that further investigation into the role of IgG_4 in modulating T-cell responses during immunotherapy is warranted. A caveat in the interpretation of these data is the inherent risk of increased type 2 error when making multiple comparisons simultaneously. Although larger clinical studies are needed to verify our findings and further inform their clinical significance, this pilot study raises the important possibility that current forms of immunotherapy for food allergy do not elicit persistent immunologic suppression.

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Key messages

- **OIT and SLIT for peanut allergy suppress basophil and DC-driven T-cell effector functions, although this inhibition is often transient.**
- **Although there was significant interindividual variation and mechanistic outcomes did not always correlate with clinical outcomes, these findings might offer a mechanistic basis for the relatively low rates of sustained unresponsiveness seen in immunotherapy trials for food allergy.**

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