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Subcutaneous Allergen Immunotherapy Restores Human Dendritic Cell Innate Immune Function

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Summary

Background—We recently reported that human blood dendritic cells from allergic subjects have impaired IFN- α production following TLR9-dependent innate immune stimulation. It is not known how subcutaneous allergen immunotherapy (SCIT) affects dendritic cell immune responses.

Objective—The aim of this study is to determine how SCIT affects human dendritic cell function.

Methods—PBMC and plasmacytoid dendritic cells (pDCs) were isolated from the blood of 7 dust mite allergic subjects at baseline and upon reaching a standard SCIT maintenance dose that included dust mite and other aeroallergens. Cells were stimulated with various adaptive and innate immune receptor stimuli, or media alone for 20hrs with secreted cytokine levels determined by ELISA. A portion of the cells were used to measure intracellular signaling proteins by flow cytometry. Humoral immune responses were measured from plasma.

Results—SCIT resulted in a 3-fold increase in PBMC production of IFN- α in response to CpG at 100 nM ($P = 0.015$) and at 500 nM ($P = 0.015$), $n = 7$. The predominant cell type known to produce IFN- α in response to CpG (CpG ODN-2216) and other TLR9 agonists is the pDC. As expected, a robust innate immune response from isolated pDCs was re-established among allergic subjects undergoing SCIT resulting in a 5-fold increase in IFN- α production in response to CpG at 500 nM ($P = 0.046$), $n = 7$. In contrast, IL-6 production was unaffected by SCIT ($P = 0.468$). Consistent with published reports, IgG4 blocking antibody increased 10-fold with SCIT ($P = 0.031$), $n = 7$. There was no significant increase in the frequency of pDCs or the expression of TLR9 that would account for the rise in IFN- α production.

Conclusions—Allergen immunotherapy increases dendritic cell TLR9 mediated innate immune function, which has previously been shown to be impaired at baseline in allergic subjects.

Keywords

allergy; immunotherapy mechanisms; SCIT; dendritic cell; pDC; IFN- α ; TLR9; cytokines

INTRODUCTION

Dendritic cells are known to express innate immune receptors (TLRs) and receptors for immunoglobulin such as Fc ϵ RI (the high affinity receptor for IgE). Previous studies have

examined the function of TLRs in detail, however the effect of direct activation of the IgE receptor on dendritic cells is not completely understood. While crosslinking of the IgE receptor on the surface of mast cells and basophils by allergen is considered to be the primary trigger for allergic disease responses, it is not clear if the IgE receptor on dendritic cells plays a role in the manifestation of allergic disease.

Mounting evidence links IgE receptor function with TLR function in DCs. Our group has previously shown that TLR9 and FcεRI on immature plasmacytoid dendritic cells (pDCs) are cross-regulated [1]. More specifically, activation of pDCs with a well-known TLR9 receptor agonist, CpG, induces a reduction in expression and function of the IgE receptor. Conversely, activation of the IgE receptor leads to a reduction of TLR9 expression and function.

We have also recently reported a clinical correlate to these *in vitro* findings. Dendritic cells from allergic subjects expressed higher levels of the IgE receptor while their ability to produce IFN-α in response to a TLR9 agonist was significantly impaired [2]. Consistent with other reports, these findings suggest that counter-regulatory mechanisms exist between innate (TLR) and adaptive (FcεRI) immune receptors on dendritic cells such that one modulates the other [1,3–5]. It is not known whether or how allergen immunotherapy affects this dendritic cell immune axis.

The precise mechanism(s) underlying the clinical efficacy of subcutaneous allergen immunotherapy (SCIT) remains unknown. Established immunological markers associated with SCIT include both serological and immune cell changes [6–9]. In particular, there is often an initial rise then a decline in allergen specific IgE followed by a rise in IgG antibody, especially of subclasses 1 and 4. More recently, SCIT has been shown to increase the frequency of T regulatory cells [6,8,10]. These findings suggest that SCIT may function in part by shifting the Th2 dominated immune response to allergens towards a more balanced or Th1 type response. However, few have examined this opposing innate arm of the immune system as it relates to allergic responses and SCIT mechanisms. For example, does SCIT help restore impaired dendritic cell TLR function that has been shown to be dysfunctional in allergic subjects? Given the fact that SCIT helps attenuate the adaptive immune response, would SCIT induced restoration of innate immune function provide further evidence of a shift from the Th2 to the Th1 axis?

In the present study, we address these questions by analysing human plasmacytoid dendritic cell (CD123⁺, CD11c[−]) innate and adaptive immune receptor function in a cohort of subjects with allergic rhinitis before and after the initiation of conventional subcutaneous allergen immunotherapy. As anticipated, SCIT resulted in an initial rise in allergen specific IgE as well as a 10-fold increase in allergen specific IgG4. Compared to non-allergic subjects (in prior studies), dendritic cell production of IFN-α via TLR9 receptor stimulation was greatly impaired at baseline in our allergic group. IFN-α production increased several fold upon reaching maintenance immunotherapy. There was no significant change in dendritic cell frequency or production of IL-6 in response to FcεRI stimulation.

Our findings further illuminate some of the primary immunological changes associated with allergen immunotherapy. By tracking and dissecting these interactions, more rationale and targeted therapies may emerge to treat allergic diseases. As sublingual and oral immunotherapy gain popularity and TLR9/CpG-based therapies improve, it is essential that we better understand these fundamental mechanisms.

METHODS

Subjects

Seven volunteer subjects with moderate to severe allergic rhinitis aged 22 to 56 consented to participate in this study. Skin test sensitivity to dust mite (*D. farinae*) and at least two other aeroallergens and a desire to undergo allergen immunotherapy was required for inclusion in the study. Individuals with severe concurrent disease, those taking immunosuppressive agents, oral steroids or undergoing prior immunotherapy were excluded from the study. An IRB approved protocol for venipuncture was performed at baseline and upon reaching maintenance immunotherapy. Subject use of antihistamines and/or topical nasal steroids was recorded pre and post-SCIT. As a secondary measure, subjects were asked, “yes or no” in an unblinded fashion if they noticed a decrease in allergic symptoms associated with SCIT.

Immunotherapy Protocol

Subcutaneous allergen immunotherapy was performed as per routine standard of care at the Johns Hopkins Asthma and Allergy Center using allergens obtained from Hollister-Stier (Spokane, WA) or Greer Laboratories (Lenoir, NC). Each subject received an individualized formula that included *D. farinae*, *D. pteronyssinus* and other allergens such as cat, dog, tree, weed and grass which are common to the Northeastern United States. The build-up phase began at 1:1,000 of the final concentration and increased at weekly to bi-weekly intervals as tolerated. Maintenance immunotherapy was reached when a full dose of 1:1 final concentration containing at least 12 µg of each relevant protein or combined cross-reactive species was administered. Maintenance immunotherapy was reached at approximately 6 months on average.

Special Reagents and Buffers

The following reagents were purchased: crystallized human serum albumin (Calbiochem-Behring Corp, La Jolla, CA); PIPES, fetal calf serum (FCS), and crystallized BSA (Sigma Chemical Co, St Louis, MO); gentamicin, IMDM and nonessential amino acids (Life Technologies, Inc, Grand Island, NY); Percoll (Pharmacia Biotech, Inc, Piscataway, NJ); CpG ODN-2216 (TriLink, San Diego, CA); *D. farinae* (Greer laboratories, Lenoir, NC). Phycoerythrin (PE)-labeled antibodies to human TLR9 (eBioscience, San Diego, CA), allophycocyanin (APC) or fluorescein isothiocyanate (FITC) labeled anti-BDCA-2 (Miltenyi Corp, Auburn, CA), PE labeled anti-Syk (splenic tyrosine kinase) (Santa Cruz Biotechnology, Santa Cruz, CA) and isotype (IgG2a) conjugated with Alexa Fluor 647 (Invitrogen, Carlsbad, CA), anti-human FcεRIα monoclonal antibody (eBioscience) conjugated with APC or PE and relevant rat IgG₁ isotype controls were used for flow cytometry. Use of polyclonal goat anti-human IgE for stimulation and preparation of PIPES-albumin-glucose (PAG) and isotonic percoll has been described elsewhere [1]. Conditioned medium (C-IMDM) consisted of IMDM supplemented with 5% heat-inactivated (56°C for 30 minutes) FCS, 1x nonessential amino acids, and 10 µg/mL gentamicin.

Cell preparation

Blood was processed as previously described [2,11,12]. Briefly, whole blood in 10mM EDTA underwent centrifugation at 600xg to isolate a buffy coat. Buffy coats were diluted 1:1 (vol/vol) with PAG containing 4 mM EDTA (PAG-EDTA) and then subjected to double-Percoll (1.075/1.081 g/mL) density centrifugation. This produced a basophil-depleted peripheral blood mononuclear cell (PBMC) suspension. Platelets were removed from the PBMC suspension using four low-speed (100–150xg) centrifugations. pDCs were purified from PBMC using blood dendritic cell antigen (BDCA)-4 positive selection

(Miltenyi Corp, Auburn, CA). An initial analysis of pDC suspensions prepared in this manner indicated >80% purity, as determined by BDCA-2 staining.

Culture conditions

PBMC were cultured at 5×10^6 /ml in C-IMDM. Specifically, 1.25×10^6 PBMC were added to 96-well plates (0.125 mL per well). pDCs were cultured simultaneously in separate wells at 8×10^4 /ml (2×10^4 per well). After incubating for 15 minutes to equilibrate to 37°C and 5% CO₂, an equal volume of stimulus or C-IMDM was added to duplicate cultures. The following stimuli were used for separate culture conditions in each subject with optimal concentrations having been pre-determined: goat polyclonal anti-human IgE (3 µg/mL), *D. farinae* (100 or 500 AU/ml) or CpG (20, 100 or 500 nM). After a 20-hour incubation period, supernatants were harvested and frozen at -70°C and ultimately assayed for cytokines by ELISA.

Cytokine measurement

IL-6 protein levels in culture supernatants were determined using a commercial ELISA kit (eBioscience, San, Diego, CA). IFN-α measurements were performed with an in-house assay [1], using mouse monoclonal anti-human IFN-α (clone MMHA-2 at 2.5 µg/mL) as the capture antibody and rabbit polyclonal anti-human IFN-α (5000 neutralization units/mL) as the detection antibody (PBL Biomedical Laboratories, Piscataway, NJ). Detection of the rabbit anti-IFN-α was achieved using goat anti-rabbit/HRP-conjugate at a 1:1000 dilution (R&D, Minneapolis, MN). Human recombinant IFN-α (Biosource) was used for assay standardization.

Flow cytometric analysis

A portion of each PBMC suspension (9×10^6 cells) was fixed in 4% buffered paraformaldehyde and stored in multiple aliquots at -70°C in 10% DMSO for flow cytometry. pDCs staining positive for BDCA-2⁺ (CD303) were further analysed for FcεRIα, Syk and/or intracellular TLR9 (CD289) with APC-, PE-, FITC- or Alexa Fluor 647-conjugated antibodies and appropriate isotype controls using methods previously described [1,2,12]. Flow cytometry was performed using a FACSCalibur instrument.

Humoral Immune Response Measurements

Plasma was collected from blood obtained by venipuncture using EDTA and stored at -20°C until analysed. Total serum IgE and *D. farinae* specific IgE and IgG4 were measured by the Johns Hopkins Dermatology Allergy and Clinical Immunology Reference Laboratory using the ImmunoCAP 250 (Phadia, Kalamazoo, MI) which is a fluorescent-based enzyme immunoassay (FEIA).

Statistics

Data are presented as mean ±SEM unless otherwise indicated. Statistical analysis was performed with InStat software (GraphPad, San Diego, CA) by a two-tailed nonparametric Mann-Whitney test unless otherwise stated. A linear regression was used to calculate correlations as indicated. *P* values ≤ 0.05 were considered significant.

RESULTS

Participant characteristics

Seven subjects (mean age 35) underwent subcutaneous allergen immunotherapy that was initiated during various seasons throughout the year (2007–2008). As shown in Table 1, there was no significant difference in the use of medications pre and post-SCIT. All subjects

were skin-test positive to *D. farinae*. The mean duration of SCIT was 25.1 weeks. There was a subjective reduction in symptoms of allergic rhinitis associated with immunotherapy (5 out of 7 subjects, $P = 0.031$) but this outcome was not the primary focus of this study.

Serum factors associated with allergen immunotherapy

As shown in Table 1, there was a modest increase in mean total serum IgE from 290.7 to 330.6 kUA/L that was not statistically significant. However, there was a significant rise in allergen specific IgE (IgE to *D. farinae* present in the SCIT formula) from 1.07 to 6.17 kUA/L ($P = 0.015$). Based on prior reports, specific and total IgE initially rises and then falls during continued allergen immunotherapy. However, this was not systematically evaluated further by us [6,8,10]. As seen in Table 1 and Figure 1, IgG4 to *D. farinae* increased ten-fold (0.1 to 1.02 mgA/L) with SCIT ($P = 0.031$). A rise in IgG4 (also referred to as blocking antibody) associated with immunotherapy has been a consistent observation in published reports [6–9,13]. However, the biological relevance of this rise has not been fully investigated.

FcεRIα expression on dendritic cells correlates with total serum IgE

Consistent with previously published reports, IgE receptor (FcεRI) expression on dendritic cells correlated with total serum IgE levels [2,14,15]. As shown in Figure 2, plasmacytoid dendritic cell (BDCA-2⁺ pDC) expression of FcεRIα as measured by flow cytometry rose in proportion with increased levels of serum IgE ($P < 0.0001$). The modest increase in total serum IgE (post-SCIT) was associated with a subsequent rise in dendritic cell FcεRIα expression (MFI 48.5 to 59.5) but was not statistically significant. However, as a consequence of this trend, all but one of the subjects had higher dendritic cell expression of FcεRIα after receiving allergen immunotherapy.

Allergen immunotherapy restores dendritic cell TLR9 mediated IFN-α production

In all seven allergic subjects analysed in this study, pre-SCIT PBMC and pDC production of IFN-α was expectedly low compared to IFN-α levels that we have previously reported in non-allergic cohorts with similar demographics (see open circles Fig. 3) [2]. As seen in Figure 3a, baseline pre-SCIT PBMC production of IFN-α in response to 100 nM and 500 nM CpG-2216 was 1248 and 1483 pg/10⁶ cells respectively.

The predominant cell type producing IFN-α in response to the type A CpG used in this study is the pDC. Therefore, PBMC derived IFN-α levels would be expected to correlate with levels of IFN-α produced by isolated pDCs. Nonetheless, as a confirmation of our findings we isolated and directly stimulated pDCs in addition to PBMC suspensions. Baseline pDC production of IFN-α in response to 100 nM and 500 nM CpG was 1717 and 6773 pg/10⁶ cells respectively (Figure 3b). IFN-α levels were undetectable in cultures containing 20 nM CpG or medium alone.

In order to test the hypothesis that allergen immunotherapy would restore dendritic cell innate immune function, we isolated and stimulated dendritic cells with CpG upon reaching maintenance immunotherapy in a manner identical to what was done at baseline. As shown in Figure 3a (closed circles), allergen immunotherapy resulted in a 3-fold increase in PBMC production of IFN-α ($P = 0.015$). More specifically, stimulation of PBMC with 100 and 500 nM CpG following SCIT resulted in an increase in IFN-α to 4326 and 4569 pg/10⁶ cells, respectively. As depicted in Figure 3b (closed circle), stimulation of isolated pDCs with 500 nM CpG post-SCIT resulted in a 5-fold increase in IFN-α production to 34,947 pg/10⁶ cells ($P = 0.046$). This value approached levels that we have previously reported for non-allergic subjects [2]. IFN-α levels increased about 2-fold post-SCIT (from 1717 to 3663 pg/10⁶ cells) in pDCs stimulated with 100 nM CpG. However, this increase did not reach statistical

significance in part because post-SCIT IFN- α levels remained below the level of detection in 3 of the 7 allergic subjects.

IgE receptor mediated production of IL-6 is unaltered by SCIT

To confirm the viability of dendritic cells pre and post-SCIT we examined cellular responses to other (non-TLR) mediated stimuli. In contrast to TLR9 mediated stimulation of DCs, there was no increase in IgE receptor mediated cytokine production associated with immunotherapy. PBMC (Fig. 4a) and isolated pDCs (Fig. 4b) were stimulated with *D. farinae*, anti-IgE, or media alone pre and post-SCIT. As shown, PBMC and pDC IgE receptor mediated production of IL-6 was unaltered by allergen immunotherapy. Stimulation of DCs with *D. farinae* or anti-IgE did not produce IFN- α above baseline (data not shown).

Dendritic cell frequency and TLR9 expression are unchanged by SCIT

There was no significant increase in the frequency of pDCs post-SCIT that would account for the rise in IFN- α production associated with immunotherapy. pDC frequency in PBMCs was $0.45 \pm 0.1\%$ at baseline and $0.62 \pm 0.1\%$ post-SCIT ($P = 0.578$) as determined by flow cytometry (see Figure 5a). Likewise, there were no appreciable variations in the expression of intracellular signaling elements TLR9 and Syk that would account for the observed differences in cytokine production. As shown in Figure 5b, the mean fluorescence intensity of TLR9 was 46.0 ± 1.5 at baseline and 45.8 ± 1.5 at maintenance immunotherapy ($P = 0.999$). pDC expression of total Syk was also remarkably stable pre and post-SCIT, as shown in Figure 5c (MFI 2268 ± 49.6 and 2385 ± 41.2 respectively, $P = 0.578$). Syk present in mast cells and basophils is well known to be an essential component of Fc ϵ RI mediated activation [16,17]. Despite the high levels of Syk found in pDCs there was no difference in total Syk expression associated with immunotherapy. This suggests that other downstream signaling components or transiently phosphorylated elements account for the differential production of IFN- α associated with allergen immunotherapy.

DISCUSSION

Dendritic cells are a critical component of an immune axis poised to simultaneously receive and interpret molecular triggers that activate both the innate and the adaptive immune systems. Evidence suggests that skewing of the immune system towards an adaptive (Th2) immune response leads to allergic disease while a bias towards an innate receptor mediated (Th1) response may lead to autoimmune disorders [18–20]. In the present study we demonstrate that subcutaneous allergen immunotherapy may help re-establish a more balanced immune response in part by restoring the dendritic cell capacity to respond to innate immune receptor stimuli. Allergen immunotherapy resulted in a 3–5 fold increase in dendritic cell production of IFN- α in response to a TLR9 agonist, CpG. This increase in IFN- α associated with SCIT approached levels that are seen in non-allergic subjects [2].

Consistent with the work of others, we recently reported an impairment of CpG/TLR9 mediated IFN- α production from dendritic cells of allergic subjects [2,21,22]. While the mechanism by which this impairment occurs is not clear, it helped support the notion that elements of the innate immune system may be dysregulated in allergic individuals. By definition, allergic individuals have a more robust IgE mediated adaptive immune response compared to non-allergic individuals. It is, therefore, becoming increasingly clear that the innate and the adaptive immune system must be examined in tandem to fully comprehend the mechanisms that are involved in allergic disease processes and its treatment.

We have previously demonstrated a counter-regulatory apparatus between TLR9 and IgE receptor function on dendritic cells *in vitro* [1]. Others have reported similar findings

suggesting that innate and adaptive immune receptor function on dendritic cells can be antagonistic [3–5]. In the present study, we take this concept one step further by examining dendritic cell TLR function in subjects receiving traditional IgE mediated allergen immunotherapy. Attenuation of the IgE mediated adaptive immune response with immunotherapy has long been appreciated but few have examined the effects of immunotherapy on the innate immune system.

Several questions have emerged. When IgE receptors are activated in experimental (*in vitro*) conditions, TLR9 receptor level and function are down-regulated [1]. However, when intracellular TLR9 levels were measured in an effort to explain the impairment of IFN- α production in allergic subjects, there was no difference [2]. Similarly, TLR9 levels remained constant in allergic subjects pre and post-SCIT despite a restoration in IFN- α production. Recently, we have reported that autocrine TNF- α is responsible for IgE receptor mediated TLR9 downregulation in pDCs, however IFN- α levels were shown to be suppressed before this feedback mechanism was involved [23]. The cellular mechanisms that result in the increase in IFN- α production post-SCIT are likely more complex than simple variations in the expression of TLR9.

Honda et al. have shown that IRF-7 (interferon regulatory factor 7) may be a master switch for type I IFN production in pDCs [24]. Interruption of this essential component may result in a deficiency in TLR9 mediated IFN- α production and, therefore, needs to be investigated in future studies that address the mechanisms of allergen immunotherapy. Other TLR associated signaling molecules, including high mobility box 1 protein (HMGB1) and Receptor for Advanced Glycation End Products (RAGE), may also need to be investigated [25]. In the current study, levels of TLR9 and pDC frequency did not explain the variable production of IFN- α pre and post-SCIT. Expression of spleen tyrosine kinase (Syk), an early signaling element shown to be important in IgE receptor mediated function in basophils and mast cells, was likewise unaltered by SCIT. Its expression was investigated even though it has yet to be definitively linked to Fc ϵ RI-dependent signaling in DCs.

Activation of dendritic cells with monoclonal anti-IgE antibody resulted in the release of pro-inflammatory cytokines including IL-6. This antibody attaches to IgE that is bound to Fc ϵ RI on the surface of DCs. IL-6 production remained constant in allergic subjects pre and post allergen immunotherapy. This finding supports the observation that dendritic cell frequency and viability remains unchanged with immunotherapy. However, given our previous report that anti-IgE causes a down-regulation in TLR9 and an up-regulation in Fc ϵ RI, one might expect IL-6 production to increase and IFN- α to decrease as DCs are exposed to increasing quantities of allergen in immunotherapy. This was not the case. IFN- α levels increased and IL-6 levels remained constant with immunotherapy. One plausible explanation for this is that the transient down-regulation of TLR9 that occurs with *in vitro* anti-IgE stimulation does not mimic the long-term effects of repeated subcutaneous allergen immunotherapy that occur over months to years.

In mouse models, bone marrow derived dendritic cells have been shown to respond to dust mite by producing prodigious amounts of IL-6 that in turn promotes a Th2/Th17 biased pathway [26]. The receptor by which dust mite allergen binds and activates DCs in the mouse to produce IL-6 is presumed to be Fc ϵ RI, but this is not known. Cholera toxin, which binds the innate immune receptor ganglioside GM1, was also shown to induce IL-6 production from mouse DCs. In our study, IgE receptor targeted stimuli (*D. farinae*) activated human DCs to produce large amounts of IL-6 while innate immune receptor stimuli (CpG) produced minimal amounts of IL-6. *D. farinae* did not induce detectable amounts of IFN- α , suggesting that certain allergens such as dust mite preferentially favor IgE receptor interactions over TLR9. However, dust mite extract is known to contain TLR4

receptor agonists (LPS). LPS causes the mDC to produce IL-10, a cytokine that is thought to enhance the effects of immunotherapy [6–8,12,27]. Future studies should further examine mDC production of IL-10 pre and post-SCIT.

We found a 10-fold increase in dust mite specific IgG4 associated with allergen immunotherapy. This magnitude of increase in IgG4 has been a consistent finding in several other studies [7,9,13]. Dendritic cells express receptors for IgG that are thought to modulate both innate and adaptive immune receptor mediated responses [28–32]. It is not clear what effect, if any, the IgG4 subclass has on DC innate immune receptor function, but this too needs to be investigated in future studies.

For nearly 100 years, clinicians have utilized subcutaneous allergen immunotherapy. Yet many of the principles that govern its mechanisms of action remain elusive. For reasons that are unclear, some individuals respond well to SCIT whereas others do not [6,8,33]. One of the main goals of allergen immunotherapy has been to attenuate IgE mediated responses. Our data show that while mitigation of the effects of IgE may well be important, there may be a simultaneous revival of the complementary innate arm of the immune system that demands attention. As sublingual techniques, food-containing oral immunotherapy and CpG-based technologies emerge, a better understanding of the dendritic cell innate and adaptive immune axis is required in order to fully exploit these new forms of therapy.

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Fig. 1.

Immunoglobulin subtype G4 (IgG4) specific to *D. farinae* (*Df*) was measured in the serum of seven dust mite allergic subjects before and after a standard subcutaneous allergen immunotherapy protocol (SCIT) and expressed in mgA/L. Serum IgG4 levels pre-SCIT (open circles) increased approximately 10-fold ($P = 0.031$) upon reaching maintenance immunotherapy (closed circles). SCIT, subcutaneous allergen immunotherapy; *Df*, *Dermatophagoides farinae*.

Fig. 2. Serum IgE levels expressed in kU/ml prior to (open circles) and post (closed circles) subcutaneous allergen immunotherapy correlate with median fluorescence intensity (MFI) of FcεRIα. A linear regression (solid line) calculation has a correlation coefficient (r) of 0.866 and is highly statistically significant ($P < 0.0001$). FcεRIα, high-affinity IgE receptor α .

Fig. 3.

(a) IFN- α cytokine levels in PBMC cultures from seven dust mite allergic subjects pre-SCIT (open circles) and upon reaching maintenance allergen immunotherapy (closed circles) as measured by ELISA (reported as pg/10⁶ cells). Average IFN- α levels ($\pm$ SEM) following immunotherapy are 3-fold higher ($P = 0.015$) in PBMC cultures stimulated with 100nM or 500nM CpG. (b) IFN- α levels in pDC cultures stimulated with 100 or 500nM CpG-2216 increased 2-fold ($P = 0.687$) and 5-fold ($P = 0.046$) respectively following immunotherapy ($\pm$ SEM). CpG, CpG-DNA oligonucleotide type 2216.

Fig. 4.

(a) Bar graph showing IL-6 cytokine levels in PBMC cultures from seven dust mite allergic subjects pre-SCIT (open bars) and upon reaching maintenance allergen immunotherapy (closed bars) as measured by ELISA (reported as pg/10⁶ cells). Average IL-6 levels ($\pm$ SEM) pre and post-SCIT are not significantly different in PBMC cultures stimulated with media ($P = 0.937$), 100 AU/ml *Df* ($P = 0.437$), 500 AU/ml *Df* ($P = 0.937$), or 3 μ g/ml anti-IgE ($P = 0.964$). (b) IL-6 levels ($\pm$ SEM) pre and post-SCIT are not significantly different in pDC cultures stimulated with media ($P = 0.250$), 100 AU/ml *Df* ($P = 0.625$), 500 AU/ml *Df* ($P = 0.296$), or 3 μ g/ml anti-IgE ($P = 0.812$).

Fig. 5.

(a) Bar graph showing the frequency of BDCA-2⁺ cells (pDC) in seven dust mite allergic subjects pre-SCIT (open bars) and upon reaching maintenance immunotherapy (closed bars) as measured by flow cytometry $\pm$ SEM. Average pDC frequency pre and post-SCIT is not significantly different ($P = 0.578$). (b) There is no significant difference in pDC average expression of intracellular TLR9 before and after immunotherapy reported as net MFI $\pm$ SEM ($P = 0.999$). (c) Intracellular Syk expression in pDCs is unchanged with immunotherapy and reported as net MFI $\pm$ SEM ($P = 0.578$). TLR9, toll-like receptor 9; MFI, median fluorescence intensity; Syk, spleen tyrosine kinase.

Table 1

Participant characteristics at baseline and on allergen immunotherapy (SCIT)

Characteristic	Baseline (n=7)	Maintenance SCIT (n=7)	P value
Medication Use	6/7	5/7	0.999
Duration of SCIT (weeks)	0	25.1 (± 0.8)	0.007 ^{*†}
Total Serum IgE (kU/L)	290.7 (± 55)	330.6 (± 52)	0.109
<i>D. farinae</i> IgE(kUA/L)	1.07 ($\pm .19$)	6.17 ($\pm .79$)	0.015 [*]
<i>D. farinae</i> IgG4 (mgA/L)	0.1 ($\pm .01$)	1.02 ($\pm .11$)	0.031 [*]
Symptom Improvement	0/7	5/7	0.031 ^{*†}

* significant

† one-tailed non-parametric test

SCIT, subcutaneous allergen immunotherapy