

Decreases in human dendritic cell–dependent T_H2-like responses after acute *in vivo* IgE neutralization

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Background: Dendritic cells (DCs) and other professional antigen-presenting cells express a variant of the high-affinity IgE receptor known as $\alpha\gamma_2$, which, on the basis of *in vitro* findings, has long been implicated to function in facilitating allergen uptake and presentation to T_H cells.

Objectives: To use omalizumab as an *in vivo* tool to neutralize IgE binding to circulating dendritic cells and to assess whether this results in altered DC-dependent T-cell responsiveness to allergen *ex vivo*.

Methods: Subjects with cat allergy were enrolled in a 3.5-month, double blind, randomized (3.5:1), placebo-controlled trial of omalizumab using standard dosing for allergic asthma. Blood plasmacytoid and myeloid DCs were assessed at baseline and posttreatment for expression of surface IgE, Fc ϵ RI α , and induction of CD4⁺T-cell proliferation and cytokine responses to cat allergen.

Results: IgE expression on plasmacytoid and myeloid DCs from omalizumab-treated subjects (n = 12) decreased by $\geq 95\%$ posttreatment ($P = .0005$), whereas Fc ϵ RI α expression decreased by 66% and 48%, respectively ($P = .0005$). Cat allergen–induced proliferation in DC/T-cell cocultures observed at baseline was suppressed $\sim 20\%$ to 40% postomalizumab treatment ($P = .001$). Multiplexing for cytokines in plasmacytoid DC/T-cell cocultures also showed decreases in IL-5, IL-13, and IL-10 ($P < .05$), whereas IL-2 and IFN- γ were unaltered or slightly increased. These changes were not evident in placebo-control subjects (n = 4).

Conclusion: IgE likely facilitates allergen presentation by dendritic cells *in vivo* and is also important in regulating

DC-dependent T-cell cytokines during effector phases of allergic disease. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Dendritic cells, IgE, antigen presentation, cytokine, receptor

Allergen-specific IgE plays an essential role in arming basophils and mast cells for the release of inflammatory mediators and proinflammatory cytokines that are pivotal to the pathogenesis of allergic disease.¹ It does so by binding both cell types through a uniquely expressed high-affinity receptor (Fc ϵ RI) consisting of an α chain (to which IgE binds), 1 β chain, and 2 γ chains that combine to form an $\alpha\beta\gamma_2$ tetramer. In human beings, IgE is also known to bind with high affinity to a trimeric variant of Fc ϵ RI, $\alpha\gamma_2$, which is predominantly expressed on antigen-presenting cells (APCs). Although the precise functional role of IgE binding to this variant remains poorly understood, *in vitro* studies have long suggested a role in facilitating (or “focusing”) allergen presentation to memory CD4⁺ T_H lymphocytes.² Whether this activity or any other might help drive T_H2 responses associated with allergic disease is not clear at this time.

Dendritic cells (DCs) are the most potent APCs, possessing a high capacity for capturing, processing, and presenting peripheral antigens to T cells. Two major subclasses have been described based on phenotypic differences: (1) CD123⁺CD11c⁺ plasmacytoid DCs (pDCs) and (2) CD123⁺CD11c⁺ myeloid DCs (mDCs), also known as conventional DCs, for which there are several subclasses.³ Both DC subtypes circulate in human blood as immature DCs and are reported to express the $\alpha\gamma_2$ variant of the IgE receptor.⁴⁻⁷

Omalizumab is a mAb immune modulator that is US Food and Drug Administration–approved to treat allergic asthma. It prevents IgE binding to Fc ϵ RI by targeting the same Fc region of the immunoglobulin that binds the receptor. A marked suppression in allergen-induced basophil responses is observed after *in vivo* administration of this drug. Moreover, basophils from subjects receiving omalizumab markedly lose surface-bound IgE within a few days, which is accompanied by a subsequent drop in Fc ϵ RI α expression by day 7.^{8,9} In contrast, skin tissue mast cells do not show reductions in Fc ϵ RI expression until ~ 70 days of omalizumab treatment.¹⁰

Prussin et al¹¹ initially reported that omalizumab administration reduces Fc ϵ RI α expression on human blood pDCs and mDCs, lending to the notion that IgE drives $\alpha\gamma_2$ expression on APCs much like it does for $\alpha\beta\gamma_2$ on basophils and mast cells. Whether this drop in IgE/Fc ϵ RI expression has any functional consequences has not yet been addressed. In this study, we investigate and uncover evidence that IgE is important not only for DC-dependent T-cell proliferation in response to allergen but also for the production of T_H2 cytokines.

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Abbreviations used

APC: Antigen-presenting cell
 BAU: Bioequivalent allergy unit
 BDC: Basophil-depleted cell
 BDCA: Blood dendritic cell antigen
 DC: Dendritic cell
 IQR: Interquartile range
 mDC: Myeloid dendritic cell
 nMFI: Net median fluorescence intensity
 pDC: Plasmacytoid dendritic cell

METHODS**Study subjects**

Subjects between 18 and 50 years of age with a history of cat allergy were recruited for a 15-week, double-blind, placebo-controlled trial at the Johns Hopkins Asthma and Allergy Center. Each gave informed consent to participate in a protocol approved by the Johns Hopkins Institutional Review Board and the National Institute of Allergy and Infectious Diseases' Data Safety Monitoring Boards (study no. NCT00604786). Subjects met a number of inclusion criteria for enrollment and are detailed by Eckman et al.¹² Eligible subjects were randomly assigned omalizumab or matched placebo in a 3.5:1 ratio. The dosage of omalizumab was equal to at least 0.016 mg/kg/IgE (IU/mL) per 4 weeks as approved for the treatment of allergic asthma. Venipuncture was performed at baseline (d0) and after approximately 3.5 months of treatment (~d105).

Cell preparation

Blood specimens were subjected to double-Percoll (GE Healthcare, Little Chalfont, UK) (1.075/1.081 g/mL) density centrifugation, producing both a basophil-depleted cell (BDC) interface and a basophil-enriched cell interface.⁶ Briefly, BDC interfaces were removed and washed to remove platelets. A portion was then removed, fixed in 4% buffered paraformaldehyde, washed, and frozen below -70°C for later analysis by using flow cytometry.¹³ The remaining BDCs underwent positive selection steps that sequentially enriched for pDCs, mDCs, and CD4⁺ lymphocytes by using immunomagnetic beads, LS columns, and a quadroMACs magnet (Miltenyi Biotec, Auburn, Calif). First, pDCs were isolated by using blood DC antigen (BDCA)-4 selection. Cells not bound in the column (the majority of the BDC suspension) were collected and portioned between 2 tubes with 90% of the cell suspension used for mDC isolation and 10% used for the isolation of CD4⁺ lymphocytes.⁶ For mDCs, this involved first removing CD19⁺ B cells followed by positive selection for BDCA-1⁺ mDCs.⁵

DC-dependent allergen-driven T-cell responses

Dendritic cell subtypes were individually cultured with CD4⁺ cells at a 1:5 ratio (DC:T-cell) in Iscove modified Dulbecco medium supplemented with 5% human AB serum, nonessential amino acids and 10 µg/mL gentamicin. Duplicate cultures (2 × 10⁴ DCs and 1 × 10⁵ CD4⁺ cells per 0.250 mL in 96-well round-bottom plates) were incubated in medium alone (control) and with cat allergen (Hollister-Stier lot number K76E7593 with 10,000 bioequivalent allergy units [BAU]/mL containing <0.03 EU/mL endotoxin units, dialyzed to remove the 50% glycerine). A comparison of dialyzed cat allergen with non-dialyzed stock showed no loss of activity as determined by basophil histamine release (data not shown).

Allergen-driven cytokine responses and proliferation were concurrently assessed in DC/T-cell cocultures. This was achieved by harvesting supernatants after 96 hours of incubation for analysis of cytokine content using multiplexing plates (Bioplex, BioRad). At this time, an equal volume of fresh medium and allergen (prewarmed to 37°C) was added to the culture wells for an additional 48 hours of incubation. ³H-thymidine (1 µCi/well) was added for the final 16 hours of incubation before harvesting onto glass fiber filters by using a cell harvester. Incorporated radioactivity was assessed by using liquid scintillation to obtain counts per minute (cpm).⁶

Flow cytometry to assess IgE and FcεRIα expression

Direct staining for pDCs in paraformaldehyde BDC suspensions was done by using anti-BDCA-2/allophycocyanin.¹³ Identification of mDCs was done by gating on BDCA-1⁺CD19⁻ cells (see this article's Fig E1 in the Online Repository at www.jacionline.org). Coexpression for cell surface IgE and FcεRIα was individually assessed by costaining with goat antihuman IgE/fluorescein isothiocyanate (KPL, Gaithersburg, Md) and anti-FcεRIα/phycoerythrin (AER-37; eBioscience, San Diego, Calif), respectively, and analyzed by using a FACSCalibur machine (BD Biosciences, San Jose, Calif). Readouts were net median fluorescence intensity (nMFI) after subtracting values obtained with isotype control antibodies.

Serum total, free, and cat-specific IgE

Total and cat-specific IgE were measured using the ImmunoCap-250 assay (Phadia, Portage, Mich). Free IgE (ie, IgE that is not complexed with omalizumab) was measured by using an in-house, label-free surface plasmon resonance-based technology (see this article's Methods in the Online Repository at www.jacionline.org for details).

Statistical analysis

Statistical analyses were performed with Prism4 (Graphpad Software Inc, San Diego, Calif) and involved regression analysis and the Wilcoxon test for matched pairs. *P* values ≤ .05 were considered significant.

RESULTS**IgE neutralization reduces surface IgE and FcεRIα expression on DC subtypes**

Two of the 14 enrolled subjects in the active treatment group relocated from the Baltimore area and could not complete the study. Of the 16 subjects completing the study, there were no detectable differences in baseline characteristics between the active and placebo groups.¹² Complete data sets for all 12 subjects on active treatment and the 4 on placebo were compiled for surface IgE and FcεRIα expression. Data for DC-dependent T-cell proliferation and cytokine secretion were not collected on the first subject entering the study who happened to receive active therapy.

Dendritic cell parameters were investigated at baseline (pre or d0) and posttreatment (~d105). Omalizumab administration did not significantly affect pDC or mDC frequencies in the BDC suspensions. The mean frequency of pDCs in the active group before treatment was 0.44 ± 0.05% versus 0.46 ± 0.06% posttreatment. Identical percentages of pDCs were also detected in the placebo group pre and posttreatment (0.44 ± 0.8% vs 0.44 ± 0.05%, respectively). The mean frequency of mDCs was slightly higher but was also unchanged after treatment in both the active (0.52 ± 0.06% pre vs 0.74 ± 0.26% post) and placebo (0.62 ± 0.19% pre vs 60 ± 0.30% post) groups.

This article's Fig E2 in the Online Repository at www.jacionline.org shows that both DC subtypes from all 16 subjects expressed surface FcεRIα before treatment, which correlated with total serum IgE levels (*r*² = 0.3609, *P* = .014 for pDCs and *r*² = 0.669, *P* = .0001 for mDCs). Surface-bound IgE was also detected and highly correlated with FcεRI expression on both pDCs (*r*² = 0.7506; *P* < .0001) and mDCs (*r*² = 0.955; *P* < .0001).

Changes in the expression of FcεRIα and surface IgE on pDCs and mDCs posttreatment (~d105) are represented in Fig 1 as fractional responses of baseline (d0) values. In this analysis, omalizumab decreased FcεRIα on pDCs and mDCs by 66% and 48%, respectively, whereas IgE expression was reduced by ≥95% on

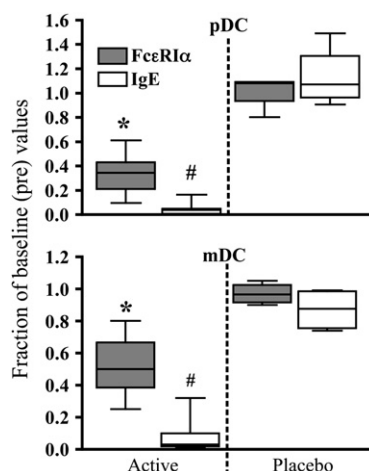


FIG 1. FcεRIα and surface IgE on human blood DC subtypes are down-regulated after omalizumab administration. BDCA2⁺ pDCs and BDCA1⁺CD19⁺ mDCs within PBMC suspensions were analyzed pre (baseline) and post (~d105) treatment with active (n = 12) drug or placebo (n = 4). Shown are the median, IQR, and lower/upper extreme fractional values posttreatment relative to baseline (see Figs E2 and E3 for specific nMFI values). *P = .0005; #P < .0001.

both DC subtypes. Individual changes in expression of FcεRIα and surface IgE are also provided as nMFI values in this article's Figs E3 (for pDCs) and E4 (for mDCs) in the Online Repository at www.jacionline.org.

Free IgE levels among the active group after ~d105 on omalizumab averaged just $4.5 \pm 1.2\%$ of the total IgE detected at this same time (Fig 1). Regression analyses in Fig 2 show that the percentage of free IgE levels at this time correlated with the fraction of FcεRIα remaining on pDCs (Fig 2, A) and mDCs (Fig 2, C). This relationship, although confounded by the capacity of omalizumab to increase total IgE levels *in vivo* by some 4-fold to 5-fold, remained intact when also reporting the latter as a percentage of the total IgE measured before treatment (Fig 2, B and D, respectively). This latter calculation indicated that free IgE levels at ~d105 of treatment averaged $20.6 \pm 4.9\%$ of the total IgE measured before treatment (d0), for a reduction of ~79.4%.

DC-dependent allergen-driven T-cell proliferation responses *in vitro* are reduced with IgE neutralization

With surface IgE and FcεRIα expression reduced on both DC subtypes, the next goal was to determine whether this reduction associated with functional changes in APC activity. To do this, we investigated allergen-induced proliferation in DC/CD4⁺ T-cell cocultures. As shown in Fig 3, there was an allergen-induced proliferation response that was dose-dependent, increasing with higher allergen concentration. Both pDCs and mDCs demonstrated similar levels of APC function. Using pDCs, the median cpm responses at baseline (to 100 and 500 BAU concentrations of the cat extract) among the placebo group were 2550 (interquartile range [IQR], 2310-2850) and 3690 (IQR, 3480-3870), respectively. These were unchanged posttreatment (cpms, 2850 and 3630). The median baseline values to the 100 and 500 doses of cat extract were comparable among the active group subjects (cpm, 2263, IQR, 1656-3414; and cpm, 5252, IQR, 4392-6846,

respectively). However, these significantly decreased by ~20% to 40% when assessed posttreatment (cpm, 1778, IQR, 1411-2513; and cpm, 3502, IQR, 3126-4296; P = .001). Similar findings were observed using mDCs. In this instance, the median baseline cpm responses to 100 and 500 BAU of cat extract among the placebo group were 3600 (IQR, 2700-3900) and 4680 (IQR, 3960-5250), respectively. These were unchanged posttreatment (cpm, 3660, IQR, 2490-4200; and cpm, 4590, IQR, 3570-5220). Comparable baseline responses were seen in the active group to both the 100 and 500 BAU doses of cat allergen (cpm, 1978, IQR, 1402-2758; and cpm, 3705, IQR, 2783-5249, respectively) but were reduced by ~20% to 40% posttreatment (cpm, 1624, IQR, 1115-2052; and cpm, 2541, IQR, 2223-3473; P = .001).

Baseline CD4⁺ T cells from several subjects (n = 4) were cultured with allergen (100 BAU) alone to determine whether the proliferation responses measured were indeed dependent on the presence of DCs. We found the mean cpms with T cells plus allergen were 906 ± 67 , only slightly above the 621 ± 63 cpms obtained in T-cell/DC cocultures activated with medium alone.

DC-dependent allergen-driven T_H2-like cytokine responses *in vitro* are reduced with IgE neutralization

Culture supernatants were assayed for a variety of cytokines as another measure of whether omalizumab administration affects DC-dependent T-cell responses. Fig 4, A, shows evidence that among those subjects receiving omalizumab, a statistically significant drop (~50%) in IL-5 production was seen posttreatment in pDC/T-cell cocultures stimulated with both the 100 and 500 BAU concentrations of cat extract (P < .05). Although the overall levels among the placebo subjects were lower at baseline, the posttreatment values were essentially unchanged. Similar decreases of approximately 50% were also observed for the IL-13 and IL-10 produced in response to the 100 BAU concentration of cat allergen (P < .05). Although trends for reduced IL-13 and IL-10 were also seen at the 500 BAU concentration of cat, these did not reach statistical significance. In contrast, these cytokines were unchanged posttreatment among the 4 placebo individuals.

Fig 4, B, shows that IL-2 and IFN-γ were among other cytokines detected in the supernatants from pDC/T-cell cocultures stimulated with cat allergen. However, unlike the IL-5, IL-13, and IL-10, the production of these cytokines did not significantly change posttreatment in either the active or placebo groups. In fact, a trend for increased IFN-γ secretion was observed among the active group in cultures stimulated with the 500 BAU concentration, but this did not reach statistical significance. There were no detectable levels of IL-4, IL-9, or IL-12, either pre or posttreatment (data not shown).

Cytokines were also analyzed in mDC/T-cell cocultures stimulated with cat allergen, both pre and postomalizumab administration. Although the same pattern of cytokines (IL-5/IL-13) was observed in a few subjects, levels in most mDC/T-cell cocultures fell below detection and thus did not allow an adequate analysis (data not shown).

Because DC-dependent T-cell responses to the cat allergen were significantly reduced among subjects receiving omalizumab, we next investigated whether these dysregulations in function were related to the reduction in FcεRIα expression posttreatment. With respect to allergen-induced proliferation, no

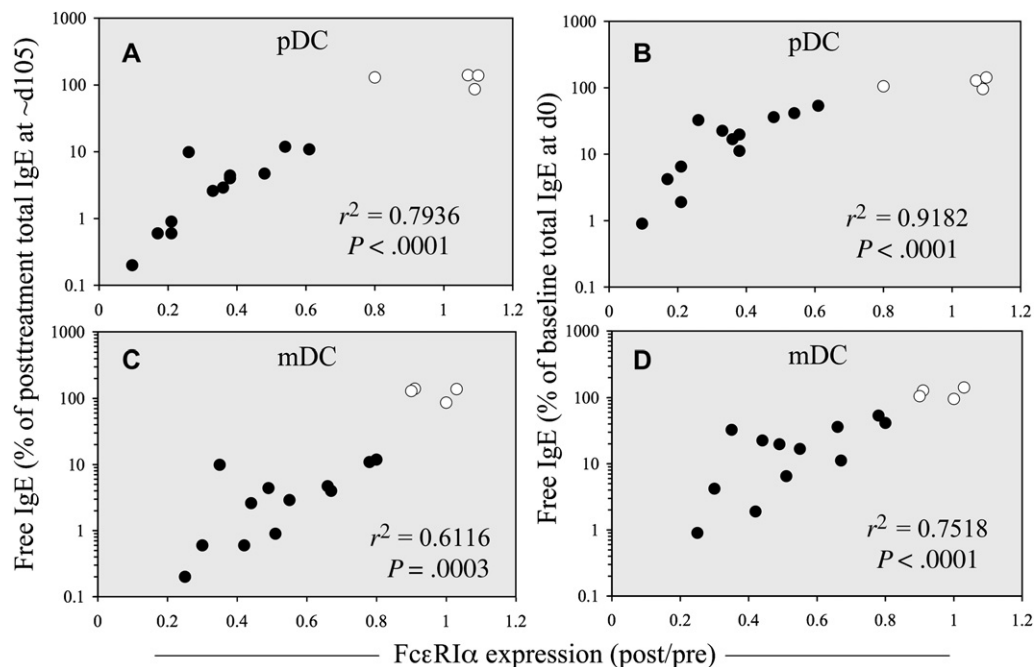


FIG 2. FcεRIα correlates with free IgE levels postomalizumab treatment. Free IgE serum levels were determined posttreatment (~d105). Shown are percentages of the total IgE at the same time point (**A and C**) and as the total IgE at baseline (**B and D**) vs fractional (post/pre) values for FcεRIα on both pDCs (**A and B**) and mDCs (**C and D**). Open circles denote placebo subjects.

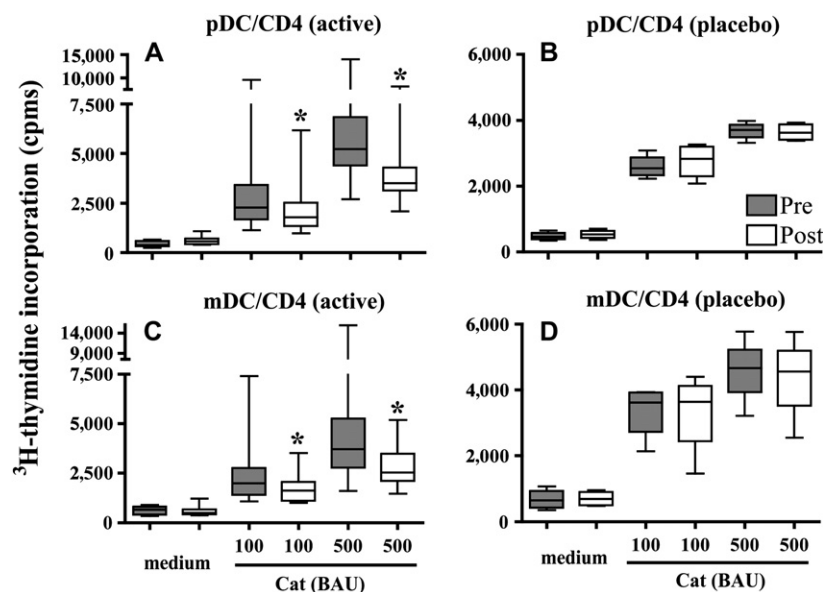


FIG 3. DC-dependent T-cell proliferation to allergen is inhibited with omalizumab administration. DC subtypes were cultured with CD4⁺ T cells as described in Methods. Shown are the median, IQR, and lower/upper extreme cpms for pDC/CD4 (**A and B**) and mDC/CD4 (**C and D**) cocultures using cells isolated from active (n = 11) and placebo (n = 4) subjects pre and posttreatment. * $P = .001$.

correlations were uncovered (data not shown). However, there was an indication that decreases in IL-5 and IL-13 protein tracked with reductions in FcεRI on pDCs, but these only became significant after excluding 1 apparent outlier in each analysis (see this article's Fig E5 in the Online Repository at www.jacionline.org). Doing this resulted in correlations for IL-5 ($r^2 = 0.70$; $P = .0026$) and IL-13 ($r^2 = 0.50$; $P = .0223$) but not for any of the other cytokines detected.

DISCUSSION

This study, to the best of our knowledge, is the first to report evidence of reduced APC-like activity by DCs after acute IgE neutralization in human beings receiving omalizumab. In doing so, it plays a significant part in substantiating a longstanding belief that the IgE receptor variant ($\alpha\gamma_2$) expressed on these cells is involved in allergen presentation or focusing *in vivo*. However, it is important to stress that these findings do not definitively show

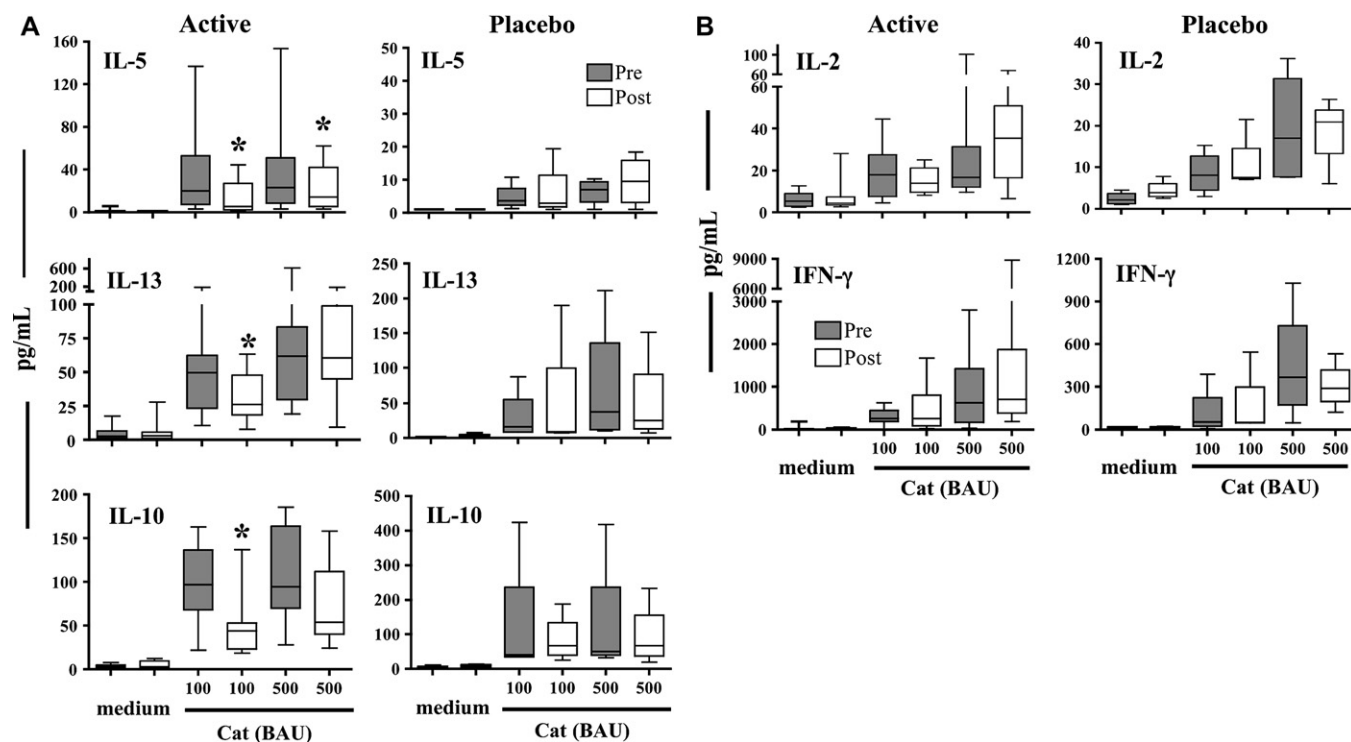


FIG 4. Cytokine secretion in pDC/T-cell cocultures after omalizumab administration. BDCA4⁺ DC and CD4⁺ T-cells were isolated pre and posttreatment and cocultured with 100 and 500 BAU/mL cat extract for 96 hours before assaying supernatants for common T_H2-like (A) and T_H1-like (B) cytokines by using multiplexing. Shown are the median, IQR, and lower/upper extreme pg/mL values for the indicated cytokines detected among the active (n = 11) and placebo (n = 4) groups. **P* ≤ .05.

that IgE mediates APC activity in DCs. It remains possible that omalizumab affects clonal expansion and/or T_H2-priming under mechanisms yet unknown.

Importantly, the data confirm an earlier report by Prussin et al¹¹ by showing that FcεRIα expression is significantly reduced on blood DCs from subjects receiving omalizumab. The current study demonstrates that decreased IgE/receptor expression on DC subtypes is associated with reductions in DC-dependent allergen-induced T-cell proliferation and T_H2 cytokine secretion in subjects receiving omalizumab. Thus, these new data suggest that acute reductions in IgE binding resulting from omalizumab administration not only dampen basophil (and mast cell) function but also reduce T_H2-cell responses by affecting DC FcεRI/IgE expression. Mechanistically, inhibition of DC functions may be an important effect of omalizumab in the treatment of allergic disease.

Most significantly, there were modest but consistent reductions (~20% to 40%) in DC-dependent T-cell proliferation to cat allergen among subjects receiving omalizumab, which were evident whether pDCs or mDCs were used as APCs (Fig 3). In contrast, responses among the placebo group were unchanged comparing pre versus posttreatment. With the assumption that surface IgE does play a role in APC activity yet was essentially undetectable on pDCs and mDCs after the ~3.5-month treatment period with omalizumab, it could be reasoned that proliferation responses would likewise be ablated. However, the observation that ~60% to 70% of baseline responses remained intact further supports the notion that IgE expression by DCs plays only a partial role in antigen uptake and presentation. Other pathways of endocytosis not requiring IgE for antigen uptake likely remain intact and can thus contribute to the APC activity that remains.

Alternatively, *in vitro* studies predict that allergen presentation is 100-fold to 1000-fold more effective if targeted to FcεRI.¹⁴ Thus it seems also possible that even an undetectable level of IgE facilitates antigen presentation. Still another possibility is that omalizumab affects coexpression of other accessory cell molecules (eg, CD80/86, MHC class II) and/or DC maturation. We did not explore these possibilities, but they are certainly potential mechanisms to explore in future studies.

We also considered the involvement of CD23, the low-affinity IgE receptor, and how neutralization of IgE by omalizumab might affect its expression and the capacity of DC subtypes to activate T cells in the presence of allergen. However, we have yet to detect CD23 expression on pDCs, even though staining for this molecule on monocytes is readily achieved (J.T.S., unpublished observations, July 2009). Moreover, we know of no reports indicating that CD23 is expressed by mDC or T cells and therefore conclude that it is not a factor in the results obtained here.

Although the reduction in T_H2 cytokines, namely IL-5 and IL-13, was not completely unexpected with a reduction in DC-associated IgE, the overall results are noteworthy. They further implicate that IgE plays a critical role in regulating T-cell cytokine responses during effector phases of allergic disease. Although the exact mechanism of how this is achieved remains unknown, it seems reasonable to suggest that IgE expression by DCs may be directly involved in activating memory T-cell responses. Indeed, allergen-driven T-cell proliferation and cytokine production are very much dependent on APC activity, and only the IgE-bearing DCs used in the coculture experiments should have been affected by omalizumab. To the best of our knowledge, there is no evidence that CD4⁺ T cells express IgE and therefore

should not be directly affected by omalizumab. Likewise, with the assumption that DC-dependent memory T_H2 cell responses are indeed suppressed by omalizumab, increases in T_H1 -like cytokines are also to be expected with this mode of therapy. We observed a trend for increased IFN- γ among the pDC/T-cell cocultures of the active group (Fig 4), which supports this belief. We also noted in a previous study that no changes in proliferation responses to tetanus toxoid followed when Fc ϵ RI decreased on pDCs.⁶ However, we did not test whether T-cell responses to non-allergens were similarly affected by omalizumab in the current study. Nonetheless, these findings raise the possibility that depleting IgE expression on DCs directly affects the T-cell cytokine milieu, and this in and of itself might ultimately play a role in dampening or suppressing effector cell functions, including those mediated by basophils, mast cells, and eosinophils.

It is important to note that the study herein was just 1 project in a multiproject protocol that overall had greater emphasis on basophil and mast cell clinical and mechanistic endpoints.¹² Thus, the protocol did not allow for an in-depth investigation of exactly when DC-dependent activity is suppressed on neutralization of IgE-binding *in vivo*. A previous study had shown maximal reductions in Fc ϵ RI α on pDCs (83%) and mDCs (53%) after 14 days and 28 days, respectively, during a ~45-day treatment schedule.¹¹ Remarkably, our findings after ~day 105 on omalizumab were comparable, with reductions in surface Fc ϵ RI α of 66% on pDCs compared with 48% on mDC (Fig 1), suggesting that IgE-dependent DC activity is indeed reduced much earlier—perhaps at a time comparable to when basophil function is likewise suppressed. This is an important observation because it raises the argument that reduced APC-like activity could have also contributed (along with reduced basophil responses) to the reduction in allergen-induced nasal symptoms observed among these same subjects.¹² The observation that nasal mast cell responses appeared intact at this same time point (~45d) was even more significant. Future studies are needed to test whether such clinical effects are a result of decreased APC function.

Finally, it is intriguing that, in the current study, pDCs supported T_H2 cytokine production to a greater extent than did mDCs, even though patterns were similar when cytokines were detected in mDC/T-cell cocultures (data not shown). Nonetheless, others have reported similar observations when conducting experiments on allergen-driven T-cell responses by using these DC subtypes.¹⁵ Although a variety of factors might account for this disparity, a lack of Fc ϵ RI α /IgE expression does not appear to be a contributing element. For example, the relative expression on mDCs indicated 5-fold greater levels on these cells compared with pDCs (Figs E3 and E4), and this is consistent with the original findings made by Foster et al,⁴ who reported 6.5-fold greater levels. These disparities are thought to reflect differences in receptor densities, even though actual numbers of Fc ϵ RI α on the 2 DC subtypes have yet to be determined. It is equally intriguing that pDCs, and even more so mDCs, do not downregulate receptor expression to the extent that basophils do. For example, our colleagues showed that basophil Fc ϵ RI α expression was down 84% after ~day 105 of treatment,^{12,15} whereas decreases in this receptor on pDCs and mDCs were only 66% and 48%, respectively. This observation is even more compelling given the fact that basophils appear to express 2-fold to 10-fold more Fc ϵ RI α than either mDCs or pDCs respectively. How well DCs downregulate Fc ϵ RI α is certainly related to free IgE

levels¹¹ (Fig 2), which adds to the support for this immunoglobulin regulating Fc ϵ RI α expression. However, surface IgE expression was down by more than 95% on all these cell types and is an unlikely factor in the retention of receptors that remained after treatment. Therefore, these observations raise the possibility that pDCs and mDCs regulate Fc ϵ RI expression differently than basophils (and mast cells). If so, this is another area of IgE-receptor biology requiring further investigation, because it will likely lead to a greater understanding of APC activity.

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Clinical implications: Reduced IgE binding to Fc ϵ RI α on DCs could alter the T-cell cytokine milieu in allergic disease and ultimately play a role in suppressing effector cell (basophil, mast cell, and eosinophil) functions.

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METHODS

Serum total, free, and cat-specific IgE

The levels of total serum IgE was quantified in kU/L using the Phadia ImmunoCAP-250, which has been shown to be minimally interfered with by the presence of omalizumab in the specimen.^{E1}

Free IgE quantification

The level of free (nonomalizumab-bound) IgE in human serum was quantified by using a surface plasmon resonance assay on the Biacore 3000 system (GE Healthcare, Little Chalfont, UK). In brief, omalizumab was insolubilized on carboxy-methyl dextran (CM5) coated chips using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinide chemistry. Remaining active sites were blocked with ethanolamine. All sera were mixed and then centrifuged just before analysis to remove microparticles and lipids that could interfere with the assay. Serum (90 μ L) diluted with 10 μ L buffer (PBS-1% BSA) or dialyzed omalizumab (at 50 mg/mL) were passed over the

omalizumab bound flowcell for 4 minutes at 10 μ L/min. The level of resonance units that was measured as the biomolecular interaction of IgE bound to insolubilized anti-IgE was recorded. This was followed by regeneration of the chip using 10 mmol/L acetate buffer (pH 1.5) for 30 seconds. The assay was calibrated by using reference specimens containing total serum IgE levels from 1 to 1000 kU/mL that were cross-calibrated to the World Health Organization IgE reference preparation. Internal controls were interspersed throughout the assay. The resonance unit levels measured in the unknown sera were interpolated from the calibration curve into kU/L levels of IgE. Finally, the soluble omalizumab inhibited IgE was taken as the nonspecific binding level for that specimen and subtracted from the uninhibited specimen to determine the level of free IgE in the specimen.

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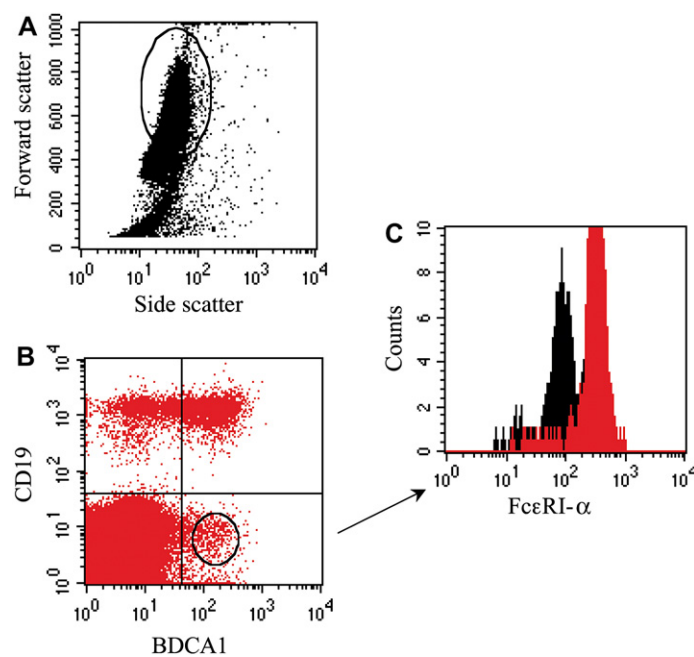


FIG E1. Gating parameters used in detecting mDC (BDCA1⁺CD19⁻ cells) for determination of FcεRIα and surface IgE expression. Representative data. **A**, Forward and side scatter analysis of a BDC suspension (ie, PBMCs). **B**, Staining parameters to identify BDCA1⁺CD19⁻ cells (ie, mDC). **C**, Staining for FcεRIα on mDCs. Shaded area represents staining with isotype control antibody.

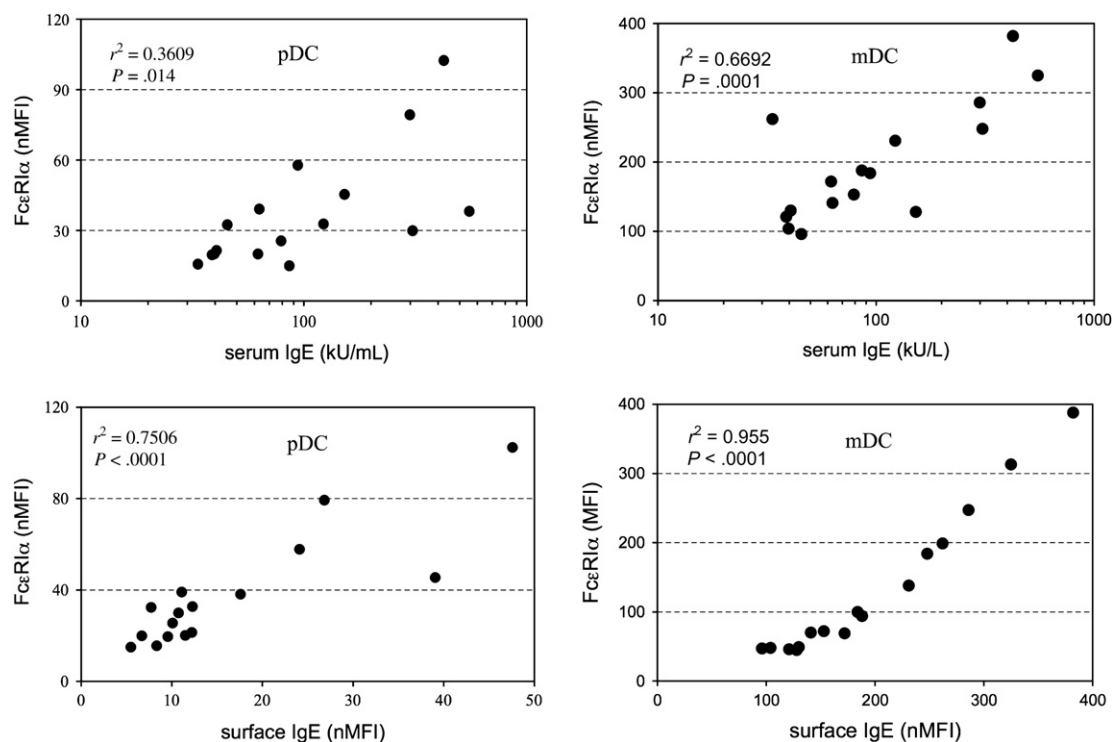


FIG E2. FcεRIα expression on pDCs and mDCs correlates with serum total IgE and surface IgE expression. BDCA2⁺ pDCs and BDCA1⁺CD19⁻ mDCs within PBMC suspensions were analyzed at baseline for FcεRIα expression and surface IgE staining by using flow cytometry. Total IgE levels were determined by the ImmunoCap-250 assay. Shown are regression analyses with r^2 and P values.

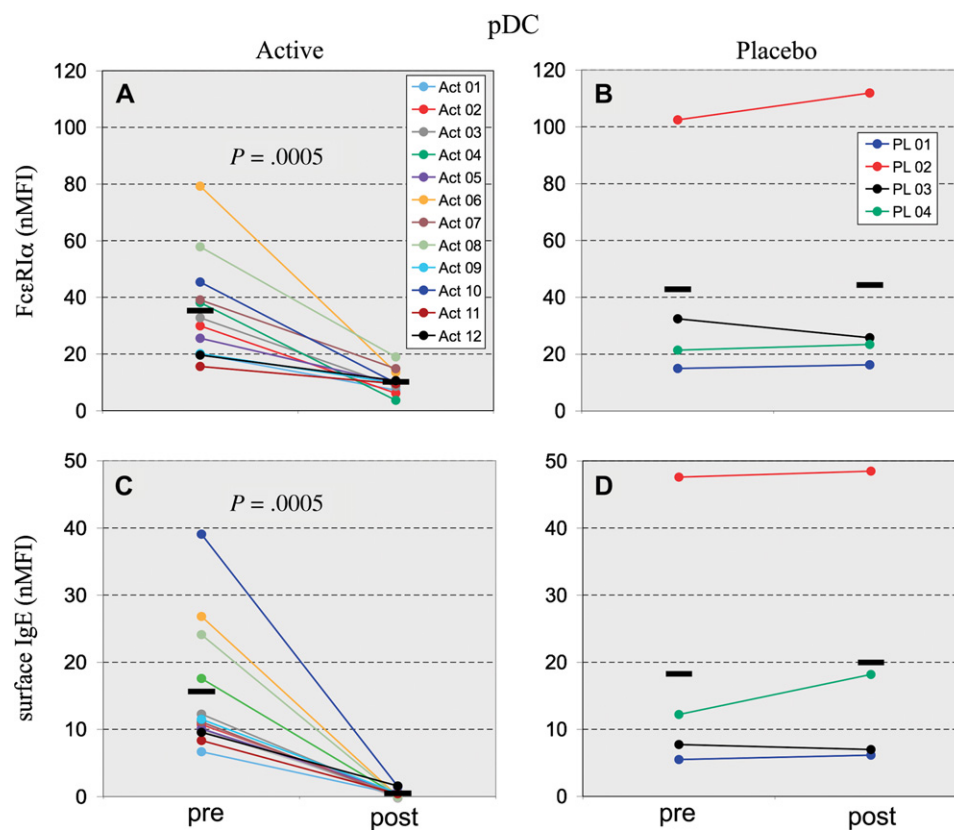


FIG E3. FcεRIα and surface IgE expression on pDCs are downregulated after omalizumab administration. PBMCs prepared pre (d0) and post (~d105) treatment from subjects receiving omalizumab (*Act*) or placebo (*PL*) were analyzed by flow cytometry for BDCA2⁺ cells (pDC) coexpressing FcεRIα (**A and B**) and surface IgE (**C and D**). Shown are results for individual subjects enrolled. Solid black bars denote mean values for the group indicated.

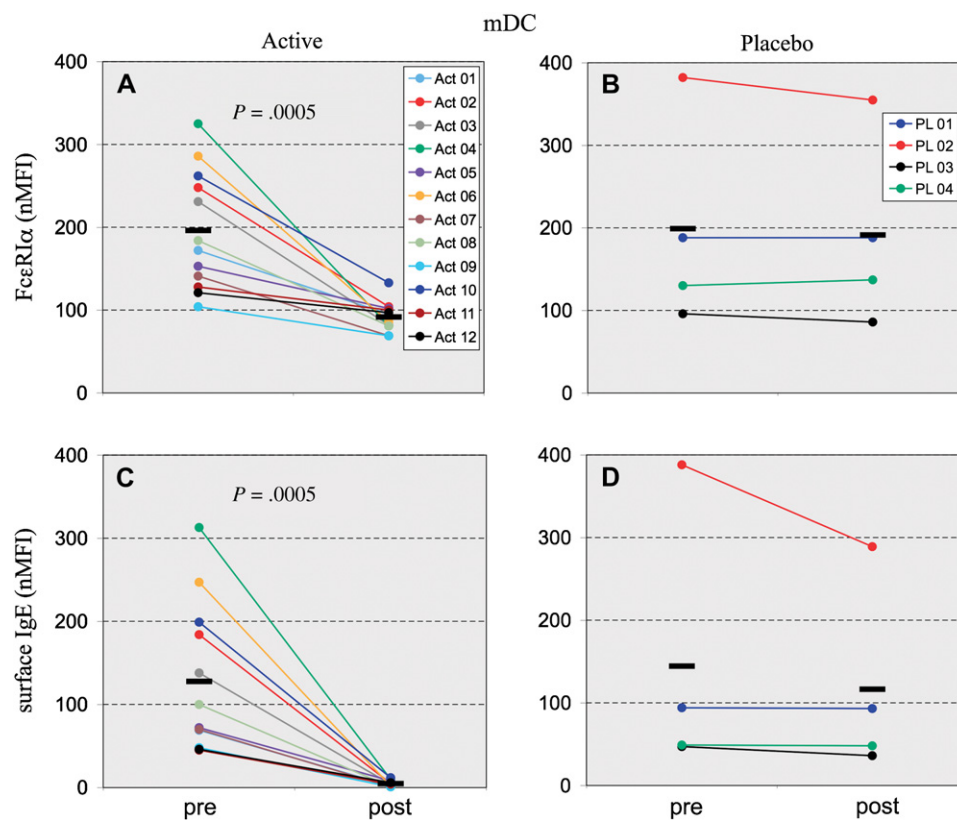


FIG E4. FcεRIα and surface IgE expression on mDCs are downregulated after omalizumab administration. PBMCs prepared pre (d0) and post (~d105) treatment from subjects receiving omalizumab (*Act*) or placebo (*PL*) were analyzed by flow cytometry for BDCA1⁺CD19⁻ cells (mDC) coexpressing FcεRIα (**A and B**) and surface IgE (**C and D**). Shown are results for individual subjects enrolled. *Solid black bars* denote mean values for the group indicated.

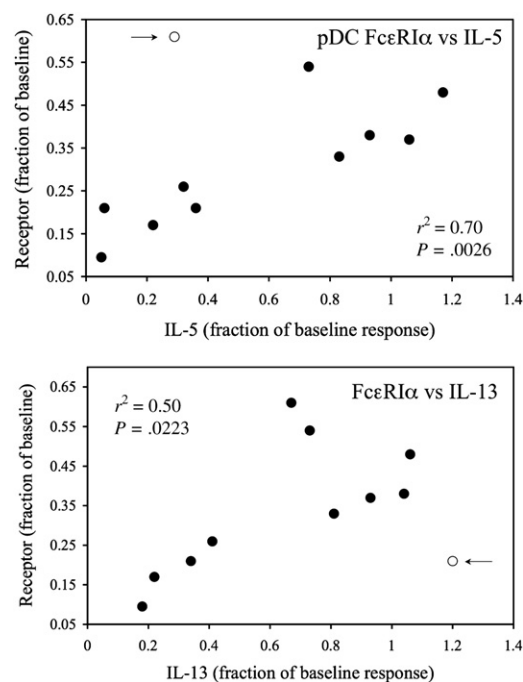


FIG E5. FcεRIα expression on pDC after omalizumab administration versus the secretion of IL-5 (**A**) and IL-13 (**B**) in pDC/CD4⁺ T-cell cocultures. Fractional responses posttreatment relative to baseline values are plotted for subjects receiving omalizumab (n = 11). Cytokine values are from those induced with the 100 BAU/mL concentration of cat extract. *Open circles with arrows* represent presumed outlier results, which were not included in regression analyses.