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Longitudinal study of the expression of FcεRI and IgE on basophils and dendritic cells in association with basophil function in two patients with severe allergic asthma treated with Omalizumab

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Key words

Asthma; basophils; dendritic cells; IgE; IgE receptor; omalizumab

Summary

Severe asthma is a challenging disease, and omalizumab has been an important tool to help clinicians address more efficiently this problem. Besides reduction of free and total serum IgE levels, there are a number of other immunologic effects of omalizumab that may be of relevance in its therapeutic action. We report two mite-allergic severe asthmatic patients successfully treated with omalizumab for one year.
Clinically, patients improved gradually, with no further need for systemic steroids or emergency department visits during that treatment period, and with Asthma Control Test (ACT) scores showing controlled disease, although pulmonary function didn't show any significant improvement.
Immunologically, we observed marked down-regulation of surface IgE and FcεRI on basophils, plasmacytoid and myeloid dendritic cells, as well as a reduction of basophil activation after specific allergen stimulation. These effects were clearly evident immediately after one month but were enhanced at 3, 6 and 12 months of omalizumab treatment, suggesting an advantage to continuing this therapy, and raising the hypothesis of some markers being useful to assess immunological responses to omalizumab, which could assist in the clinician's decision to stop or to restart this treatment.

Introduction

IgE play a central role in the pathogenesis of allergic asthma and chronic airway inflammation via high affinity (FcεRI) or low affinity (FcεRII-CD23) IgE receptors. FcεRI is predominantly expressed on mast cells, basophils and dendritic cells (DC). FcεRII-CD23 is expressed on a wide variety of inflammatory cells (1).
Omalizumab binds to circulating IgE antibodies, reducing serum IgE levels and preventing binding of IgE to high-affinity receptors (2,3). Accompanying the reduction of IgE levels, FcεRI expression is also decreased, leading to a reduction in allergen-mediated activation and degranulation of mast cells and basophils. Recent long-term clinical trials confirm that omalizumab reduces exacerbations and symptoms in adults and children with moderate-to-severe allergic asthma (2-4).
In this paper we describe the evolution of IgE and FcεRI expression on different cell types, and changes in basophil activation following allergen stimulation before and during successful

omalizumab treatment in two severe mite-allergic asthmatic patients.

Case reports

We present two non-related female patients, 25 and 26 years old, allergic to house-dust-mites. Both had long-standing moderate-to-severe asthma and rhinitis, receiving high-dose inhaled steroids, long-acting beta2-agonists, montelukast, nasal steroids and non-sedating H1-antihistamines. Both had uncontrolled disease with daily use of short-acting beta2-agonists and frequent emergency department visits and/or oral steroids (> 6/ year). Total IgE levels were 252 and 294 kU/L, respectively. Both patients had been treated with allergen immunotherapy, stopped due to frequent asthma exacerbations.

Pulmonary function showed reduced basal FEV1 values (50% and 78% of predicted, respectively) and mid-expiratory flow values (26% and 59%), with a 20-40% increase in all these values after 400 mcg salbutamol inhalation. Asthma control test (ACT) scores were 15 and 16, respectively.

Both patients started omalizumab 300 mg 2/2 weeks in January 2012, maintaining regularly this therapy. From a clinical perspective, both showed clinical improvements, without further need for systemic steroids or emergency department visits. ACT scores improved progressively to 23 and 24 at one-year. However, pulmonary function was only marginally improved.

For the immunologic evaluation blood samples were drawn before (T0) and at 1, 3, 6 and 12 months of omalizumab treatment. Flow cytometry (FACSCalibur, BD-Biosciences) was performed on 100 ul of whole blood using anti-IgE FITC; HLA-DR Per-CP, CD123 APC (eBiosciences), FcεRI PE (eBiosciences). IgE and FcεRI expression (mean fluorescence intensity) was evaluated on basophils and on dendritic cells, either myeloid (mDC) or plasmacytoid (pDC), gated according to phenotype (CD123+/ HLA-DR-; CD123-/HLA-DR+; CD123+/HLA-DR+, respectively). Additionally, basophil activation was determined according to CD63 expression, before and after allergen stimulation. Briefly, 100 ul of heparinised blood was incubated with a stimulation buffer (containing IL-3). Each sample was tested with negative control (PBS), positive control (N-formylmethionyl-leucil-phenylanine-FMLP) and allergen (*D. Pteronyssinus*). Analysis was performed using FlowJo software.

After the first two omalizumab injections (1 month) we observed significant reductions of surface IgE and FcεRI expression on basophils (93% and 89%, respectively), mDC (75% and 57%) and pDC (89% and 68%). These reductions were enhanced with continuation of therapy, albeit less pronounced (**figures 1a** and **1b**). This evolution was similar in both patients, despite different individual values (data not shown). Regarding basophil activation test (BAT) following mite stimulation, we observed a parallel trend with reductions in both patients in

the first month (70% in patient 1 and 45% in patient 2), with additional reductions of 16% and 21% in patients 1 and 2 respectively, between 1 and 12 months (**figure 1c**). We did not find any significant correlations between timings of the immunologic changes and clinical improvement.

Figure 1
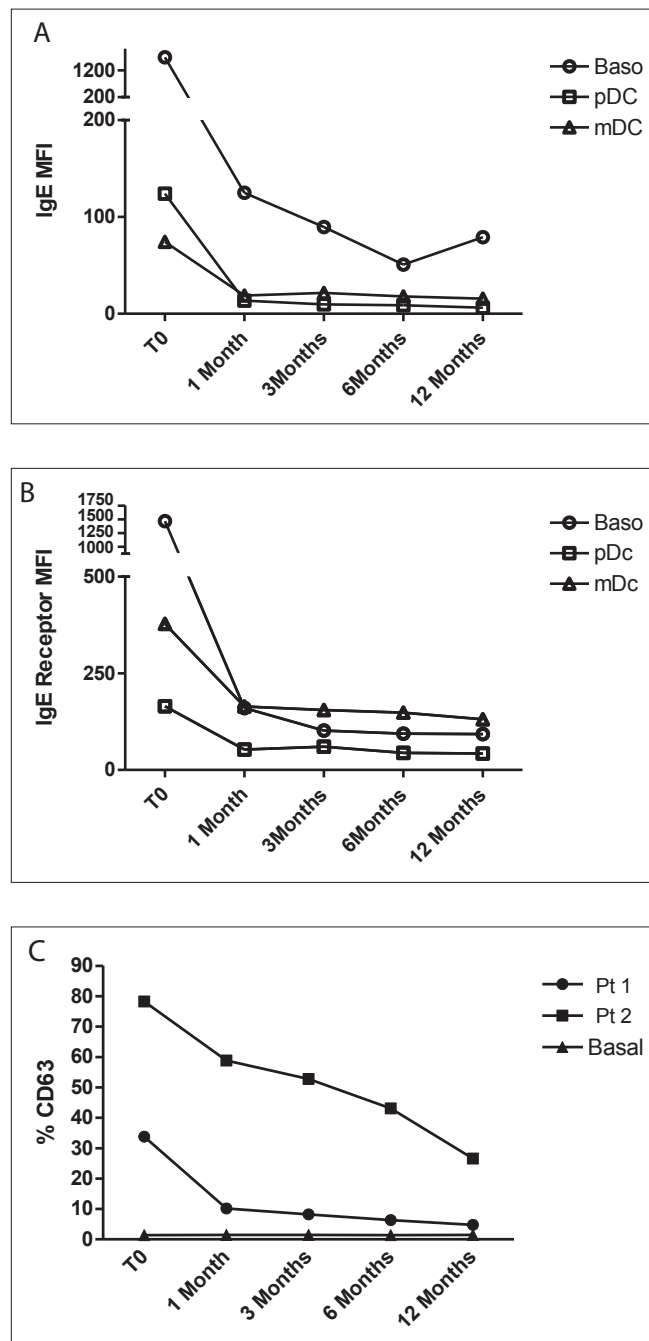
Discussion

During one year of omalizumab treatment surface IgE and FcεRI expression on basophils, mDC and pDC were consistently reduced in both asthmatic patients. Maximal reductions of FcεRI expression on basophils and DC were mostly achieved within one month of treatment, with further but smaller reductions during treatment. Substantial reductions of surface IgE and FcεRI expression on basophils and pDC have already been described after 6-52 weeks of omalizumab (2-5). Our study shows that omalizumab's immunologic effects are maintained and enhanced with continuation of therapy, a fact that parallels clinical evolution in most of our patients.

These case-reports highlight two less-studied effects of omalizumab: on mDC and on basophil activation following allergen stimulation. DC are antigen-presenting cells that play crucial roles in immune responses, whether innate or acquired. Some studies have shown in animal models of asthma that pDC play only a limited role in priming T cells in the OVA-allergic mouse model of asthma, while mDC are potent orchestrators of the asthmatic inflammatory response (6) Furthermore, in human lungs, mDC express CD80, CD86 and CD40 costimulatory molecules at higher levels than pDC, and stimulate more efficiently naïve T cell proliferation, suggesting a more important role of mDC in antigen presentation processes (7). Therefore, it is relevant to report that omalizumab reduces IgE and FcεRI expression on mDC, as already described for pDC.

Regarding omalizumab's effect on BAT following allergen stimulation, our study shows that both patients had very significant reductions; however in the patient with higher basal activation, reduction was not enough to render the test negative. This difference didn't correlate with any clinical data, both patients showing approximately the same degree of clinical improvement. Other authors have already described that amongst clinical responders to omalizumab, there are patients who achieve a negative BAT while others remain positive (8,9). In these cases, BAT intensity after omalizumab treatment seems to be more related to pre-treatment values than to the presence or absence of clinical improvement (8,10). Several studies have suggested that basophil response to allergen stimulation may reflect the underlying activity of allergic disease, being reduced after successful allergen immunotherapy (11). Our results suggest that this is also the case after successful omalizumab therapy.

In conclusion, these two case-reports of mite-allergic asthmatic patients show that omalizumab treatment induces reduction of different cellular activation mechanisms that can impact on effector mechanisms (basophil degranulation) but also on dendritic cell antigen-presentation mechanisms. These beneficial effects are evident immediately after the first two injections, and reinforced throughout the duration of therapy. These data raise the hypothesis that some laboratory cut-off values could be indicative of a complete, incomplete or non-response to omalizumab.

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