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Omalizumab therapy is associated with reduced circulating basophil populations in asthmatic children

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Abstract

Basophils have been implicated in promoting the early development of T_H2 cell responses in some murine models of T_H2 cytokine-associated inflammation. However, the specific role of basophils in allergic asthma remains an active area of research. Recent studies in animal models and human subjects suggest that IgE may regulate the homeostasis of human basophil populations. Here, we examine basophil populations in children with severe asthma before and during therapy with the IgE directed monoclonal antibody omalizumab. Omalizumab therapy was associated with a significant reduction in circulating basophil numbers, a finding that was concurrent with improved clinical outcomes. The observation that circulating basophils are reduced following omalizumab therapy supports a mechanistic link between IgE levels and circulating basophil populations and may provide new insights into one mechanism by which omalizumab improves asthma symptoms.

Keywords

Asthma; Basophil; Omalizumab; IgE; Allergy

Introduction

The incidence of asthma continues to increase and represents a significant source of morbidity, mortality and healthcare cost (1). Allergic asthma is characterized by production of interleukin (IL)-4, IL-5, IL-9 and IL-13 by CD4⁺ T helper type 2 (T_H2) cells, immunoglobulin E (IgE) production by B cells, and the recruitment of innate effector cell populations including eosinophils, mast cells and basophils to inflamed tissues. In addition to their role as late phase effector cells that migrate into inflamed tissues after the

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Author contributions

D.A.H, M.S., D.A. and J.M.S designed the research; D.A.H., M.S., K.R., and E.D.T.W performed the research; D.A.H, M.S., D.A. and J.M.S analyzed the data; and D.A.H, M.S., D.A. and J.M.S wrote the manuscript.

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inflammatory response is established, basophils have been implicated in promoting the early development of T_H2 cell responses (2). While the influence of basophils on the initiation and progression of allergic inflammation suggests that they may represent a viable therapeutic target, the specific role of basophils in allergic asthma remains an active area of research (3).

In addition to the well-established role of IgE antibodies in mediating the release of effector molecules from granulocyte populations, IgE molecules can influence other aspects of granulocyte homeostasis (4). For example, IgE promotes the population expansion of basophils from bone marrow-resident progenitor populations in murine models of allergic disease and helminth infection (5). Furthermore, elevated serum IgE levels correlate with increased frequencies of circulating basophils in patients, suggesting that IgE may regulate the homeostasis of human basophil populations (5). However, the effect of reducing IgE levels on the percentage and number of circulating basophils in the context of allergic disease remains unknown.

Omalizumab is a monoclonal antibody directed against IgE and an FDA-approved treatment for allergic asthma (6). Omalizumab blocks the interaction between IgE and the high-affinity IgE receptor (FcεRI) expressed on the surface of basophils and mast cells (6). Omalizumab therapy correlates with reduced IgE levels in serum (6, 7), reduced FcεRI expression on basophils (7) and altered IgE-mediated basophil activation including reduced numbers of FcεRI required for activation via IgE-crosslinking and reduced allergen-mediated histamine release (8–11). However, the quantitative effects of omalizumab therapy on circulating basophil populations are not well understood. Here, we show that circulating basophils are reduced following omalizumab therapy, a finding that may provide a better understanding of the pathophysiology of asthma as well as one mechanism through which omalizumab improves asthma symptoms.

Materials and methods

Study Organization

This study was approved by the medical ethics committee of the Children's Hospital of Philadelphia. Participants and guardians signed informed consent. Inclusion criteria: age 5–18 years, severe asthma, body weight and IgE level compatible with omalizumab administration chart. Exclusion criteria: immunotherapy in the past year, history of malignancy, immunodeficiency, autoimmune condition, anaphylaxis, or β-blocker use. Dose and frequency of omalizumab administration was determined by the dosing administration chart as provided by Genentech/Novartis. 7 subjects were dosed every two weeks, 2 subjects were dosed monthly. Asthma symptom assessments were administered.

Flow Cytometry

Blood samples were obtained before and during therapy. Peripheral blood mononuclear cells were isolated by Ficoll (GE) gradient, stained with anti-human fluorochrome-conjugated monoclonal antibodies against 2D7, CD11c, CD19, CD56, CD117, CD123, FcεRIα, IgE or TCRαβ (BD Bioscience, eBioscience), fixed with 4% PFA, and acquired on an LSR II using DiVa software (BD Bioscience) and analyzed with FlowJo software (Tree Star).

Statistical Analysis

12 subjects were enrolled in the study, 3 were lost to follow-up and 1 outlier was deemed significant using the extreme studentized deviate method (coefficient of variation with outlier = 672.31%, coefficient of variation with outlier = 132.94%) and excluded from the analysis. Significance of the remaining 8 data-points was determined using the Wilcoxon

Signed Rank Test. Statistical analyses were performed using GraphPad software (GraphPad Software, Inc.).

Results and Discussion

Clinical characteristics of the study subjects are presented in Table 1. Twelve subjects, aged 7–16 years, with severe persistent asthma were enrolled in the study. Three patients were lost to follow-up. The mean IgE level of the remaining subjects was 969 IU/mL (range 137–2510 IU/mL). Omalizumab dose and administration frequency was determined by the drug manufacturers dosing administration chart. There was no correlation between monthly or bimonthly dosing schedule and measured outcomes.

To determine the influence of omalizumab therapy on asthma control, a survey of asthma symptoms was utilized. Consistent with previous reports (6), omalizumab therapy was associated with an average ten point improvement in asthma control (Figure 1a). To test whether omalizumab therapy was associated with alterations in circulating basophil populations, we examined basophil frequencies and total numbers in the blood of subjects prior to and during therapy. Basophils were identified by flow cytometric analysis as being negative for surface markers associated with B cells, NK cells, T cells and dendritic cells, and positive for the basophil-associated surface markers CD123 and FcεRI. In addition, basophil identification was confirmed by intracellular expression of the basophil-specific granular protein 2D7 (12) (Figure 1b).

Consistent with a previous report (7), we observed a 70–80% reduction in the mean fluorescence intensity (MFI) of FcεRI on circulating basophil populations following the initiation of omalizumab therapy (Figure 1c). Despite a 70–80% reduction in the MFI of FcεRI expression, human basophil populations exhibited a 10 fold higher MFI of FcεRI expression compared to FcεRI negative cell populations (Figure 1c) and were readily identifiable based on their expression of FcεRI (7) and CD123 (Figure 1d). Compared to samples collected prior to therapy, the percentage of blood basophils was reduced after the initiation of omalizumab (Figure 1d). While total white blood cell numbers did not change over the course of therapy (data not shown), there was an average reduction in the total number of circulating basophils (basophils/mL) after initiating omalizumab therapy with variability in the magnitude and timing of the reduction when comparing individual subjects (Figure 1e). Based on our previous studies in animal models (5), we hypothesized that significant reductions in basophil numbers would occur after four to eight weeks of therapy. Consistent with this hypothesis, we observed a significant reduction in the total number of circulating basophils after 2 omalizumab injections or an mean duration of therapy of 8 weeks (range 4–21 weeks) (Figure 1f). Together, these findings indicate that circulating basophil frequencies and numbers are reduced following omalizumab therapy.

We report that on average circulating basophil frequencies and numbers in asthmatic children are reduced with omalizumab therapy and that reductions in circulating basophils were concurrent with improved asthma control. However, two subjects in our study did not have a reduction in circulating basophils at the time point measured. As such, a decline in circulating basophil responses may not always parallel omalizumab therapy or individual clinical improvement. Further investigation is warranted to determine whether these subjects who do not display reductions in circulating basophils represent a clinically relevant sub-population with implications for future treatment failure. A study by Lin et al. reported that omalizumab therapy does not alter circulating basophil numbers (7). Our observations may differ from the Lin et al. study as a result of differences in adult versus pediatric patient populations, study design or new 9 color flow cytometric techniques we employed that may identify human basophil populations with higher resolution than previously possible with 4

color flow cytometric techniques (Lin et. al). Though our study is limited by the absence of placebo group, the observation that circulating basophil frequencies and numbers are reduced following omalizumab therapy is consistent with previous findings in allergic patients and animal models, and supports a mechanistic link between reduced systemic IgE levels and lower circulating basophil populations. This finding may contribute to a better understanding of the pathophysiology of asthma as well as one mechanism through which omalizumab may improve asthma symptoms.

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Figure 1.

Omalizumab therapy results in improved asthma control and reduced frequencies and numbers of circulating basophils in asthmatic children. (a) Change in asthma symptom/quality of life score with omalizumab therapy (n=5). (b) Identification of blood basophils from asthmatic subjects by flow cytometric analysis as CD11c⁻, TCRαβ⁻, CD19⁻, CD56⁻, CD123⁺, FcεRIα⁺, 2D7⁺ cells. 2D7 staining of basophils (red) was compared to FcεRIα⁺, CD123⁻ cell populations (black). (c) Representative flow cytometric analysis of blood basophils from asthmatic subjects before and during therapy illustrating the MFI (italics) of FcεRIα expression of basophils pre-treatment (red, 5338) and post-treatment (green, 1318) compared to FcεRIα-negative cells (black, 112). (d) Representative percentage (black) and MFI (red) of blood basophils from asthmatic subjects before (baseline) and after (omalizumab) therapy. (e) Number of basophils in blood from asthmatic subjects over the course of therapy. (f) Basophil numbers before (baseline) and after (omalizumab) an average of eight weeks of therapy (mean = 7.8 weeks, range = 4–21 weeks) (*, P = 0.05; n=8).

Table 1

Characteristics of the study subjects.

Characteristic	Omalizumab (n=9)
Age (y), mean (range)	12 (9–17)
Male sex (%)	56
Concurrent allergic rhinitis (%)	100
Concurrent atopic dermatitis (%)	44
Therapy with LABA/ICS [#] (%)	100
Days of asthma symptoms per week [*] , mean (±SEM)	3 (±1)
Asthma symptom-related physician visits [*] , mean (±SEM)	2 (±1)
Asthma symptom-related ED visits [*] , mean (±SEM)	2 (±1)
Days of albuterol use per week [*] , mean (±SEM)	3 (±1)
Prednisone courses [*] , mean (±SEM)	3 (±1)
Total IgE (IU/mL), mean (±SEM)	969 (±251)
Omalizumab dose (mg), mean (±SEM)	333 (±13)

[#] Long acting beta agonist/Inhaled corticosteroid^{*} 12 months preceding the start of Omalizumab treatment