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ORIGINAL ARTICLE

The role of active B cells in allergen immunotherapy

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Abstract
Background: The purpose of this study is to examine the changes in B lymphocyte subsets in patients receiving allergen immunotherapy.
Methods: B lymphocyte subsets of patients before immunotherapy and one year after immunotherapy began were examined using the flow cytometric method. Age-matched healthy children served as the control group.
Results: Twenty-two patients with asthma and/or allergic rhinitis and 14 healthy, age-matched controls were included in the study. The median age of the patients was 13 years old (range: 6–20 years), and eleven (50.0%) were male. The median age of the healthy controls was also 13 years old (range: 7–17), and seven (50.0%) were male. In the age group from 11 to 15 years; the patients' relative and absolute counts of active and mature sensitive B cells were higher than those of the healthy children ($p = 0.027–0.012$ and $p = 0.032–0.010$, respectively) before immunotherapy. The relative and absolute counts of active B cells before immunotherapy were also significantly higher than those of after immunotherapy ($p = 0.001–0.001$, $p = 0.025–0.037$, and $p = 0.029–0.035$, respectively). Before immunotherapy, the relative and absolute counts of mature sensitive B cells were significantly higher than those obtained after immunotherapy ($p = 0.024–0.006$) in the 11–15-year-old age group.
Conclusions: Allergen immunotherapy directly influences B cell differentiation and causes a decrease in the count of active B cells. This finding is relevant because the B cell count can be used as a guide in the assessment of an individual patient's treatment response and also when determining whether to continue the immunotherapy.

Introduction

During sensitisation to an allergen, the priming of allergen-specific T helper 2 (Th2) cells results in the production of Th2 cytokines (interleukin-4 (IL-4) and IL-13)), which

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are responsible for class switching to the ε immunoglobulin heavy chain and allow IgE production by B cells. IgE sensitises mast cells and basophils by binding with the high-affinity receptor for IgE (FcεRI), which is expressed on the surface of these cells. When the allergen crosslinks with the IgE-FcεRI complexes, mast cells and basophils degranulate, releasing vaso-active amines (mainly histamine), lipid mediators, chemokines, and other cytokines, all of which characterise the immediate phase of the allergic reaction. IgE also binds with FcεRI on the surface of dendritic cells and monocytes, as well as to the low-affinity receptor for IgE, FcεRII (also known as CD23), on the surface of B cells. This process increases the uptake of the allergen by these antigen-presenting cells and the subsequent presentation of allergen-derived peptides to specific CD4+ T cells, which drive the late phase of the allergic reaction. Allergen immunotherapy (AIT) is associated with improved tolerance to allergen challenges as well as a decrease in immediate-phase and late-phase allergic inflammation.[1]

The possible mechanisms of AIT include a reduction in mast cell reactivity, decreases in basophil responses, a drop in the specific immunoglobulin IgE, increases in IgG4, and induction of regulatory T cells.[2] Recently, a new subset of B cells has been identified as regulatory (Bregs) due to its capacity to secrete interleukin-10 (IL-10); these B cells likely play a role in the mechanism of AIT.[3] CD23 is located on B cells and has been proposed to participate in both positive and negative feedback mechanisms in the regulation of IgE synthesis.[4] However, there is a lack of information about the role of B cell subsets and CD23 (FcεRII) in AIT mechanisms.

The purpose of this study is to examine the changes in B lymphocyte subsets in patients receiving AIT.

Materials and methods

Study population

This study included 22 patients and 14 age-matched, healthy control children. The patients and the healthy children were divided into three groups as follows: 6–10 years, 11–15 years, and children >15 years of age. B lymphocyte subsets of the age-matched healthy children and the patients were assessed both before and one year after a monthly AIT treatment. The patients were also assessed using an asthma symptom score (cough, wheezing, dyspnoea, chest pain) and an allergic rhinitis symptom score (sneezing, itchy nose, runny nose, and nasal blockage) both before and after AIT. The association between the symptoms and sleep and daily activities was also examined. In addition, patients with allergic rhinitis were assessed using a visual analogue score.[5–7]

Asthma symptom scores (cough, wheezing, dyspnoea, chest pain)

0 p: No complaints during either the night or the day

1 p: Complaints do not affect daily work or sleep

2 p: Complaints have a light effect on daily work and sleep

3 p: Complaints affect daily work and sleep on more than two days per week

Note: Chest pain was not evaluated because it is rare in children.

Allergic rhinitis symptom score (sneezing, itchy nose, runny nose, and nasal blockage)

0 p: No complaints during either the night or the day

1 p: Complaints are present but are not disturbing

2 p: Complaints are disturbing but do not affect daily work or sleep

3 p: Complaints affect both daily work and sleep

Laboratory studies

Venous blood samples 3 ml in volume were obtained from each participant using tubes containing ethylenediamine tetraacetic acid (EDTA). Immunophenotyping was performed with the following monoclonal antibodies: IgD PE, CD19 APC, and CD27 FITC (BD Biosciences, Pharmingen, Germany). The relatives of the B lymphocyte subsets were analysed using flow cytometry (BD FACS Calibur; BD Biosciences, San Jose, CA, USA). The peripheral $CD19^+$ B cell subsets were defined as follows: active ($CD19^+CD38^-CD21^-$), memory ($CD19^+CD27^+$), naive ($CD19^+IgD^+CD27^-$), mature ($CD19^+IgD^-CD27^+$), mature and sensitive ($CD19^+IgD^-CD23^+$), mature and insensitive ($CD19^+IgD^-CD23^-$), naive and sensitive ($CD19^+IgD^+CD23^+$), naive and insensitive ($CD19^+IgD^+CD23^-$), non-switched ($CD19^+$ IgD^+ $CD27^+$), and class-switched memory B cells as $CD19^+IgD^-CD27^+$.[8,9] CD23-positive B cells were considered sensitive, while CD23-negative B cells were labelled insensitive.

For the atopy assessment, a skin prick test was performed. Histamine (10 mg/ml) was used as a positive control in the skin prick test, and physiological serum was used as the negative control. The size of the wheal was measured 15–20 min after the allergen had been applied to the skin. According to the physiological serum response, a wheal of 3 mm or greater was considered significant.[10] The following allergens were used in the skin prick test: tree mix (*Castanea vulgaris, Quercus robur, Fagus sylvativ ACE*), grass mix (*Anthoxant odoratum, Dactylis glomerata, Lolium pere me, Phleum protein France, Poa pratensis*), weeds (*Chenopodium album, Amaranthus retrolexus*), *Dermatophagoides farinea, Dermatophagoides pteronyssinus* (Stallergenes SA, 92160 Antony, France).

Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 15). All data were expressed as the median or relatives that were caused by distributions that were not considered normal. A Mann–Whitney *U*-test was used, and values of $p < 0.05$ were accepted as statistically significant.

Ethical disclosure

Ethical approval was granted in decision no. OMU KAEK 2013/347 by Ondokuz Mayis University's Ethics Committee of Medical Research.

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