Eosinophil degranulation as an allergy activation marker
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Chapter 8

Effect of immunotherapy on eosinophil activation in pollen-sensitive children

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Effect of immunotherapy on eosinophil activation

Summary

Immunotherapy is a well-documented method for treatment of children with allergic airway diseases and allergic rhinitis. The aim of this investigation was to establish the effect of immunotherapy on the presence and activation state of eosinophils in pollen sensitive patients, measured by eosinophil counts and their degranulation product in serum, Eosinophil Cationic Protein, in comparison with the (specific) IgE concentration in the patients. Neither in patients treated with conventional anti-allergic therapy, nor in patients treated with immunotherapy, intra-individual variations in (specific) IgE concentrations, due to increase of pollen exposure, were observed. Blood eosinophil counts and serum ECP concentrations showed similar results before and after starting immunotherapy. Overall release of ECP per eosinophil remained also unaffected after the start of the immunotherapy. Results of ECP serum concentrations and eosinophil blood counts in the immunotherapy group did not show any deviation compared with the conventional treatment group. It is concluded that the laboratory parameters are not helpful in monitoring the efficacy of immunotherapy in patients with seasonal rhinitis.
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Introduction

Increased sensitivity to nonspecific allergen stimuli is a characteristic feature in case of individuals with allergic rhinitis (1). A wide range of different cells and mediators have been identified in the course of an allergic reaction. Pathophysiology in allergic diseases is characterised by ongoing tissue inflammation, according to type I immediate hypersensitivity reaction (2). Activation and recruitment of several effector cells will lead to hyperreactivity in target tissues, resulting in clinical symptoms (2, 3). In patients with allergic diseases, the concentration of eosinophils is increased in blood and tissues after contact with stimuli released from mast cells activated by IgE. Alterations of allergen exposure in the course of the pollen season may yield a suitable model to evaluate the dependency of eosinophils and IgE concentrations on allergen exposure in subjects with allergic diseases. Immunotherapy is a well-documented method for treatment of allergic airway diseases. Several studies with double-blind, placebo-controlled protocols have confirmed the efficacy of grass-pollen immunotherapy in patients with pollen allergy (4, 5, 6, 7). Knowledge of mechanisms underlying immunotherapy has increased in recent years. However, the exact mechanism of immunotherapy has not yet been clarified. It has been hypothesized that treatment with appropriate dosages of allergens induces a switch from Th2 to Th1 lymphocyte population (8, 9, 10, 11). Th2 cells, but not Th1 cells, have been found to produce cytokines responsible for differentiation, activation and viability of eosinophils (12). Thereby, Th2 cells, but not Th1 cells, have been found to produce cytokines responsible for production of IgE by B-lymphocytes.

The aim of this longitudinal investigation was to study the effect of immunotherapy on the presence and activation state of eosinophils measured by eosinophil blood count and the degranulation product ECP in serum, in comparison with the (specific) IgE concentration, in pollen sensitive patients.
Effect of immunotherapy on eosinophil activation

Materials and methods

Patients
Patients were selected according to the following criteria
1) history of summer hay fever
2) poor control of clinical symptoms in spring, despite regular anti-allergic treatment
   with addition of antihistamines, intra-nasal corticosteroids and sodium
cromoglycates
3) positive skin prick test with respect to tree pollen and/or grass pollen extract or a
   positive result for specific IgE against inhalation allergens.

Patients treated with immunotherapy (n = 12) and patients treated with conventional
anti-allergic therapy (n = 15) participated in the study. These children were aged
between 6 and 16 years.

Patients treated with conventional anti-allergic therapy received corticosteroids by
inhalation as well as β₂ mimetics or β₂ sympathicomimetics as clinically indicated.
Patients treated with immunotherapy received only β₂ mimetics or β₂
sympathicomimetics as clinically indicated.

Study design
Laboratory investigations were performed before, during and after the pollen season. In
1995 (period 1 to 4), patients were treated with a conventional anti-allergic medication.
In the beginning of 1996, immunotherapy was started; tree-pollen allergic children
started in January and grass-pollen children started in March 1996. Control patients
received the conventional anti-allergic therapy as usual. Effect of immunotherapy in
comparison to conventional anti-allergic medication was studied during 1996, 1997 and
1998.
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Immunotherapy scheme

Immunotherapy was performed by application of a modification according to the RUSH-protocol (13); within one day a dosage of 1000 Biological Units of purified pollen extracts was administered by injection, within 2 weeks 10,000 Biological Units and within 3 weeks 100,000 Biological Units. Immunotherapy was applied with purified extracts from ALK (Denmark).

Four patients received extracts with tree and grass pollen allergens and eight patients received extracts with only grass pollen allergens.

Testing of blood parameters

Blood collection was performed quarterly each year and immediately before injection with pollen extracts. The longitudinal study started in 1995 and ended in 1998:

<table>
<thead>
<tr>
<th>Year 1: Regular therapy</th>
<th>Period 1:</th>
<th>January - March 1995</th>
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<tr>
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<td>Period 2:</td>
<td>April - June 1995</td>
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<td>Period 3:</td>
<td>July - September 1995</td>
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<td>Period 4:</td>
<td>October - December 1995</td>
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<tr>
<td>Year 2: Start Immunotherapy</td>
<td>Period 5:</td>
<td>January - March 1996</td>
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<td>Period 6:</td>
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<td>Period 7:</td>
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<td>Period 15:</td>
<td>July - September 1998</td>
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<td></td>
<td>Period 16:</td>
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</table>
Blood samples were drawn for measurement of ECP, total IgE, specific IgE against inhalation allergens in serum, and leukocyte counts together with leukocyte differentiation in blood (Vacutainer®, ref. 367783 with addition of SST gel and clot activator and ref. 367652 with K$_3$EDTA as an anticoagulant, respectively, Becton Dickinson, Plymouth, UK). After clotting for 2 hours at 37°C, blood samples were centrifuged for 10 minutes at 1350 x g. Subsequently, the serum samples were stored at -20°C until analysis. ECP, and IgE (total and specific) concentrations were measured with commercially available immunoassays (Kabi Pharmacia, Uppsala, Sweden).

To establish the correlation between eosinophil blood count and serum ECP, regression analysis was applied to the results of 223 apparently healthy individuals, to calculate the 50% reference line \(y = -1524x + 3772 x^2 - 2960 x^3 + 315\).

Specific IgE concentrations are classified in four ranges; score 1 reflects 0.35-0.7 kIU/l, score 2 reflects 0.7-3.5 kIU/l score 3 reflects 3.5-17.5 kIU/l and score 4 reflects 17.5-50.0 kIU/l.

Leukocyte counts and leukocyte differentiation in blood samples were performed with a Sysmex NE-8000 haematology analyser (Charles Goffin Medical Systems BV, Tiel, The Netherlands).

**Seasonal registration of pollen counts**

The pollen count was continuously registered by application of a volumetric pollen trap (Burkard) placed on the top of a building, approximately 15 m. high, and microscopic evaluation. Measurements occurred from February until September by the Laboratory of Aerobiology, Leiden University Medical Centre.

**Statistical analysis**

The statistical package SPSS/PC, version +5.0 was used for statistical evaluation of results. The statistical significance of differences between groups was assessed by
application of ANOVA analysis of variance and a t-test when appropriate. A p-value less than 0.05 was considered to be statistically significant.

Results

Pollen counts with regard to trees and grasses demonstrated several peaks, viz. in the periods April-June 1995, April-June 1996, July-September 1997, February-March 1998 and April-June 1998 (figure 1). Laboratory parameters did not show any significant correlation with pollen counts. However, patients treated with immunotherapy and patients treated conventionally reported seasonal increases or onset of symptoms, e.g. wheezing and irritated mucous membranes, during pollen exposure.
Effect of immunotherapy on eosinophil activation

Patients treated with immunotherapy
Serum ECP levels in patients during treatment with immunotherapy revealed comparable results over the years; no significant decrease in serum ECP concentrations was established (figure 2A). Eosinophil blood counts also showed stability during the years of immunotherapy. However, after two years treatment with immunotherapy the eosinophil counts had returned within the reference range (figure 2B).

Figure 1: Registration of grass- and tree-pollen count per cubic meter during the period studied.
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Results for serum ECP per eosinophil in period 5 (January-March 1996) and period 13 (January-March 1998) were significantly higher compared with the first four periods, in which the patients were treated only with the regular medication (figure 2C). ECP release per eosinophil in relation to the eosinophil blood counts also increased during immunotherapy. At the start of the study, 67% of the patients had a serum ECP/eosinophil ratio below the 50% reference line established in 223 apparently healthy subjects (14). After 1, 2 and 3 years of immunotherapy 66%, 58% and 55% respectively, of the patients were situated below the 50% reference line (figure 2D).

Concentrations of total IgE were increased in 1995 (first year). After the start of immunotherapy in period 5, IgE values showed a tendency to decrease (figure 3A) but the values remained above the reference range (< 120 kIU/l). Scores for specific IgE concentrations showed variable results; however, the concentrations of specific IgE against grass pollen remained higher than phase 3 score (3.5-17.5 kIU/l; figure 3B) and specific IgE against tree pollen remained a phase 1 score (specific IgE between 0.35 and 0.70 kIU/l; figure 3C).

Control patient group

The control patients, who received conventional anti-allergic medication, showed results for eosinophil counts and serum ECP concentrations similar with patients treated with immunotherapy (figure 2A + 2B).

Serum ECP per eosinophil ratios in control patients showed similar sinusoidal variations when compared with patients treated with immunotherapy.

IgE concentrations measured in control patients remained above the reference range (< 120 kIU/l). No significant differences between the patient group and the control patients were established (figure 3A). Also IgE concentrations specific to grass- or tree-pollen showed comparable results (figure 3B + figure 3C).

In patients treated with immunotherapy as well as in control patients a significant correlation between ECP and eosinophil count was established (r = 0.78 and 0.75,
Effect of immunotherapy on eosinophil activation respectively). Other laboratory parameters did not show a significant relationship.

Figure 2A: Mean values (± SEM) of serum ECP concentrations for patients with immunotherapy (---) and control patients (--). Periodic results were calculated as an iterative average of results of the previous, present and future period. Reference range is marked with the horizontal lines (12-99 µg/l).
Figure 2B: Mean results (± SEM) of eosinophil blood counts for patients with immunotherapy (—) and control patients (- - -). Periodic results are calculated as an iterative average of results of the previous, present and future period. Reference range is indicated as the horizontal line (< 0.5 x 10^9/l).
Figure 2C: Mean results (± SEM) of serum ECP/eosinophil ratio for patients with immunotherapy (—) and control patients (---). Periodic results are calculated as an iterative average of results of the previous, present and future period. * means significantly higher in comparison to period 1 - 4. Reference range (61-367 μg/10⁶ cells) is marked with the horizontal line.
Figure 2D: Serum ECP per eosinophil release in relation to eosinophil blood count in a reference group \((n = 223; x)\) and the mean values of the serum ECP/eosinophil ratio in the patients treated with immunotherapy \((n = 12)\) in the first year \((\bigcirc)\), the second year \((\bullet)\), the third year \((\bigstar)\) and the fourth year \((\blacklozenge)\). Line represents the 50% reference line \(y = -1524x + 3772x^2 - 2960x^3 + 315\) (\(R^2 = 0.28, p<0.02\)).
Figure 3A: Mean concentration (± SEM) of total IgE for patients with immunotherapy (——) and control patients (- - -). Periodic results are calculated as an iterative average of results of the previous, present and future period. Reference range (< 120 kIU/l) is marked with the horizontal line.
Figure 3B: Mean score (± SEM) results of specific IgE against grass pollen for patients with immunotherapy (——) and control patients (----). Periodic results are calculated as an iterative average of results of the previous, present and future period.
Figure 3C: Mean score (± SEM) results of specific IgE against tree pollen for patients with immunotherapy (---) and control patients (----). Periodic results are calculated as an iterative average of results of the previous, present and future period.
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Discussion

Inflammation of airways is considered to be an important factor for aggravation of asthma symptoms. Laboratory parameters may yield additional information for monitoring disease activity. An allergic constitution can be deduced from the eosinophil counts in peripheral blood. Additionally, serum ECP concentration is accepted as an indicator reflecting eosinophil activity (15). Our results are not in agreement with results from previous studies, which demonstrated increased blood eosinophil counts and serum ECP concentrations during natural allergen exposure in sensitized patients (16, 17, 18, 19, 20, 21).

In our study, blood eosinophil counts were stable during the years of immunotherapy. A similar trend was established for ECP serum concentrations, in contrast to the results described by others (21, 22).

In individual persons, fluctuations of eosinophil blood counts coincided with fluctuations in serum ECP concentrations. However, when compared to patients receiving immunotherapy, the control patients showed a similar trend for these parameters. Serum ECP per eosinophil ratio, irrespective of the therapy, increased over the years. These results are in contrast with the hypothesis and the results described by Imai et al. (23), in which this serum ECP per eosinophil ratio decreased after discharge of the patients.

IgE concentrations and specific IgE scores remained above the reference range in both patient groups. In both groups no variation in (specific) IgE concentrations, due to increase of pollen exposure, was observed.

In conclusion, our results do not support evidence that serum ECP levels or blood eosinophil counts are sensitive markers reflecting the disease activity state in case of pollen exposure to asthmatic patients. Also, reaction to therapy cannot be followed by these parameters. The additional value of eosinophil activation expressed as ECP/eosinophil ratio during treatment with immunotherapy has not been proven.
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None of the laboratory parameters showed differences between both patient groups that could be helpful in monitoring of the effect of treatment.
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References


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