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Allergen immunotherapy in intermittent allergic rhinitis reduces the intracellular expression of IL-4 by $CD8^+$ T cells

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Summary
Background: T helper subset dysregulation is evident in allergic disorders. The role of T cytotoxic subsets is less understood. We investigated whether allergen immunotherapy in intermittent allergic rhinitis influences the intracellular expression of IL-4 and IFN-gamma by $CD3^+CD8^-$ and $CD3^+CD8^+$ cells.
Methods: Nineteen adult patients with intermittent allergic rhinitis were evaluated before the pollen season, and then after one preseasonal course of subcutaneous allergen immunotherapy. Twelve healthy nonatopic patients matched for age and sex served as controls. Intracellular expression of IFN-gamma and IL-4 by $CD3^+CD8^-$ (Th1 and Th2, respectively) and $CD3^+CD8^+$ (Tc1 and Tc2, respectively) was estimated by flow cytometry in peripheral blood cells after stimulation with PMA and ionomycin.
Results: Before immunotherapy the percentages of Th1, Th2, Tc1 and Tc2 did not significantly differ between the patients and the controls. After immunotherapy the percentage of Tc2 was lower in the rhinitic patients than in the controls (0.38% vs. 0.45%, $p = 0.04$). The percentage of Tc2 cells decreased significantly after immunotherapy in the intermittent allergic rhinitis group (0.64% vs. 0.38%, $p = 0.02$) with tendency to decrease in ratios of Tc2/Tc1 ($p = 0.059$) and with no changes in ratios of Th2/Th1. The percentages of Th1, Th2 and Tc1 were comparable before and after immunotherapy within the rhinitic patient group.
Conclusions: The preseasonal allergen subcutaneous immunotherapy applied to intermittent allergic rhinitis patients suppressed the percentage of IL-4 producing $CD3^+CD8^+$ cells. Decreased number of $CD3^+CD8^+IL\text{-}4^+$ cells may participate in the regulatory mechanisms of immunotherapy.

Introduction

After the discovery of two functionally distinct subsets of Th cells in mice by Mossmann et al. in 1986 [1], Th1/Th2

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imbalance has been proved to play a key role in allergic inflammation. Recently it has been shown that naïve CD8 T cytotoxic/suppressor cells similarly to CD4 cells secrete not only proinflammatory cytokines such as IFN-gamma or TNF-beta, but they may also produce Th2-type cytokines, e.g. IL-4, IL-5 and IL-10 [2,3]. Thus T cytotoxic/suppressor cells may differentiate into two subsets of effector cells accordingly to the profile of secreted cytokines: ''Tc1'' and ''Tc2''. The role of Tc1 and Tc2 cells in pathomechanism of allergic reactions, including anaphylactic and delayed hypersensitivity, has not been definitely established, and data are equivocal. IL-4 producing Tc2 subset has been assumed to play a role in the regulation of allergic response because of its potential to stimulate IgE class switching [4]. Moreover, it has been reported that IL-4 promotes the development of a CD8 T-lymphocyte phenotype with low perforin and granzyme expression, so of lower cytotoxic properties [5]. On the other hand inhibition of IgE responses by both Tc1 and Tc2 cells has been found [6]. There are also some data coming from animal [7,8] and human studies [9] suggesting the role of T cytotoxic cells and Tc2 cells in allergic asthma.

Intermittent allergic rhinitis (IAR) belongs to a group of atopic diseases and is characterized by enhanced IgE synthesis and the involvement of eosinophils, mast cells and T cells in allergic inflammation in the nasal mucosa. The most effective method of treatment of IAR is an avoidance of culprit allergen but sometimes it is not possible. The alternative method of disease modifying treatment may be allergen-specific immunotherapy that has a profound potential to change the natural history of the respiratory allergy [10]. This method has been recommended by EAACI and WHO as an important part of allergy treatment, particularly in cases of severe disease [11,12].

Allergen immunotherapy is a therapeutic modality applied to allergic patients for more than a century [13]. Despite its apparent efficacy, in most properly managed patients its mechanism of action remains not completely understood. Recently a thesis of decrease in production of IL-4, IL-5 and IL-13 by $CD4^+$ T cells has been proposed [14]. Neutralisation of cytokine activity is parallel with the induction of activity of $CD4^+CD25^+$ T cells and T cell tolerance. There are some data that percentage of CD4 and CD8 type 2 T cells decreased in asthmatic children treated with immunotherapy [15]. However the data about the role of $CD8^+$ T cell subsets in immunotherapy of intermittent allergic rhinitis is unclear. So we decided to assess the effect of preseasonal immunotherapy on the secretion of IL-4 and IFN-gamma by T $CD4^+$ and T $CD8^+$ cells on a single cell level in patients with intermittent allergic rhinitis.

Materials and methods

Nineteen patients with intermittent allergic rhinitis (IAR group, $n = 19$, 11 females, mean age 26 ± 7.53 years) sensitive to tree pollen (3 patients) or grass and rye pollen (16 patients) were included into the study. The patients were recruited from the District Outpatient Clinic of Allergology, Zabrze. Diagnosis of intermittent allergic rhinitis was based on clinical history and skin prick test results. Rhinitis was stated as intermittent according ARIA criteria. In some cases serum level of allergen-specific IgE against the culprit allergens was estimated to confirm the diagnosis. None of the patients had asthma diagnosed. All patients were examined outside the pollen season, in autumn or early winter before starting the immunotherapy. Then 13 subjects from this group were estimated once again after a course of preseasonal immunotherapy. The other patients who were seen earlier were not followed-up because of: one case of pregnancy, one case of cessation of immunotherapy due to a large local adverse reaction, in one case because of moving to another part of the country and the other three subjects refused the second blood sampling.

Twelve healthy nonatopic subjects (eight females, mean age 24 ± 4.7 years) formed a control group (CON). Nonatopic state of the control subjects was confirmed by anamnesis and skin prick test results. None of the participants was taking antihistamines or corticosteroids, nor any other medications before blood sampling.

The study was approved by the Local Ethics Committee at the Medical University of Silesia, Katowice and written informed consent was obtained.

Immunotherapy

Immunotherapy was applied according to a preseasonal schedule comprising the up-building dose and the maintenance dose phases. Preparations containing modified allergen were used (Allergovit®; Allergopharma, Reinbeck, Germany). Patients were given injections of Allergovit® according to a standard protocol, beginning at a dose 0.1 mL of 1000 TE/mL extract (bottle A) and increasing at weekly intervals for 7 weeks until a maintenance dose of 0/6 mL of 10,000 TE/mL extract (bottle B) was reached and then continued monthly until the pollen season started.

Antibodies and reagents

CD3 PerCP, mouse IgG, CD69 PE, anti-IL-4 and anti-IFN-gamma conjugated with PE, CD8 FITC and FACS lysing solution and FACS permeabilizing solution were purchased from Becton Dickinson, phorbol-12-myristate acetate (PMA) and ionomycin from Sigma, RPMI from BioWhittaker.

Intracellular cytokine expression

Flow cytometric evaluation of intracellular expression of IL-4 and IFN-gamma was performed according to the manufacturer's instruction (Becton Dickinson, Application Note: Detecting Intracellular Cytokines in Activated Lymphocytes). Briefly, whole blood was collected into tubes containing sodium heparin and diluted 1:1 in RPMI 1640 and was assayed within 6 h of collection. All activation procedures were performed in capped polystyrene test tubes. The cells were activated with PMA (10 ng/mL) and ionomycin (1 mcgL/mL) in the presence of monensine and then incubated for 4 h at 37°C in a water bath. Surface staining with 20 mcL of CD3 PerCP (BD Biosciences) and 20 mcL of CD8 FITC was then performed. After mixing and 15 min incubation at room temperature in the dark 2 mL of FACS lysing solution was added followed by 10 min incubation. Then the sample was centrifugated for 5 min and the supernatant was