

Short communication

Study of the Th1/Th2 balance, including IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients

Background: Excessive production of interleukin (IL)-4, IL-5, IL-10, and IL-13 is thought to be important in the development of allergy and asthma. The objective of this investigation was to study Th1/Th2-like cytokine profiles *in vitro* in seven patients allergic to birch pollen and six nonallergic controls during the birch-pollen season.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated and cultured with birch-pollen extract (BPE) or tetanus toxoid (TT) for 7 days, harvested, and restimulated with the mitogens phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) for 24 h. Cytokine production was determined by ELISA, and logarithmic cytokine ratios were compared between the two groups and between the antigens.

Results: In the allergic group, the cultures prestimulated with BPE had a more Th2-like cytokine response than the TT-prestimulated cultures; i.e., lower IFN- γ and higher IL-10 production ($P < 0.05$), as well as higher IL-5/IFN- γ and IL-13/IFN- γ ratios ($P < 0.05$). There were also significantly higher IL-4/IFN- γ ($P < 0.005$) and IL-5/IFN- γ ($P < 0.05$) ratios in BPE-stimulated cultures in the allergic group than in the control group. The IL-4 and IL-13 production *in vitro* correlated with the specific serum IgE levels.

Conclusions: BPE stimulation induces a Th2-like cytokine response by PBMC isolated during the pollen season from birch-pollen-allergic patients, indicating a Th2-type immune response to birch pollen *in vivo*.

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T helper (Th) cells are thought to be important in the development of allergy. While Th cells of the Th1 type predominantly produce interferon (IFN)- γ and are involved in cell-mediated immune responses, Th cells of the Th2 type produce large quantities of interleukin (IL)-4, IL-5, and IL-13, which promote the development of allergy and asthma (1–4). The Th1/Th2 balance is regulated by different cytokines. IL-4 promotes differentiation of naive Th cells toward a Th2-like profile *in vitro*, while IFN- γ , on the other hand, has been reported to downregulate the development of Th2 cells (5). IL-12 is produced by antigen-presenting cells (APC) and promotes Th1-type development. The immune-regulatory cytokine IL-10 can be produced in high amounts by Th2 cells, but also by other immune cells (6, 7). IL-10 acts by downregulating IL-12 production by APC and thereby promoting the differentiation of naive Th cells toward a Th2 profile although the Th2-type response in some situations can also be downregulated (reviewed in ref. 7).

Different types of allergies can be distinguished by their characteristics, such as allergen dose, time of exposure, induced immune responses, and clinical features. To our knowledge, only a few studies have focused on the Th1/Th2 balance in birch-pollen-allergic patients, although birch pollen is the major cause of seasonal rhinitis and conjunctivitis in northern Europe because of its high pollen counts during a few weeks in spring (8). The aim of this work was to study the balance between Th2-type cytokines (IL-4, IL-5, and IL-13) and a Th1-type cytokine (IFN- γ) during the birch-pollen season in allergic patients with rhinoconjunctivitis and in nonallergic individuals by short-term stimulation of their peripheral blood mononuclear cells (PBMC). The production of IL-10 was also measured. Furthermore, correlations between the cytokine concentrations *in vitro* and the serum levels of birch-pollen-specific IgE were evaluated.

Material and methods

Thirteen subjects were included in the study, which was approved by the ethics committee of the Faculty of Medicine of Uppsala University (Sweden). Seven patients (two men and five women, age range 24–38 years) had a clinical history of birch-pollen allergy (rhinoconjunctivitis and in one case also atopic eczema) and specific serum IgE to birch pollen and/or positive skin test to birch pollen (allergy group). The six control individuals (one man and five women, age range 26–47 years) were all RAST-negative to birch pollen (<0.35 kU/l) and lacked all symptoms of allergic disease (control group). All blood samples were collected during three successive days in the birch-pollen season immediately following the birch-pollen peak for the study year. This peak was 1668 pollen grains per cubic meter, which is a pollen peak above average. The specific IgE was determined with the Pharmacia CAP System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

PBMC were separated from heparinized blood by centrifugation through Ficoll-Paque (Amersham-Pharmacia Biotech, Uppsala, Sweden). After three washes in PBS, the cells were suspended in conditioned medium (CM) composed of F-DMEM (National Veterinary Institute, Uppsala, Sweden) supplemented with 4 mM l-glutamine (Life Technologies, Paisley, UK), 10% heat-inactivated human AB+ sera, and 50 µg/ml gentamycin sulfate (Biological Industries, Israel).

The PBMC were cultured at 37°C in humidified, 7% CO₂ atmosphere in 24-well, flat-bottom cell-culture plates (Costar, Cambridge, MA, USA) at 1×10^6 /ml in the presence of 2.0 µg/ml BPE, a crude extract of birch pollen (*Betula verrucosa*; Allergon, Angelholm, Sweden), or 10–20 µg/ml tetanus toxoid (TT; SBL Vaccin AB, Stockholm, Sweden). After 7 days, the cells were harvested and washed. Aliquots of $5\text{--}10 \times 10^4$ cells were stained with anti-CD3-PE, anti-CD4-FITC, anti-CD8-FITC, and appropriate control antibodies according to the supplier's recommendation (Becton-Dickinson, San Jose, CA, USA) and immediately analyzed in a Coulter EPICS XL flow cytometer (Beckman-Coulter, Miami, FL, USA). The rest of the cells were seeded in new 24-well, flat-bottom cell-culture plates at 5×10^5 /ml and restimulated with 10 µg/ml phytohemagglutinin (PHA; Amersham-Pharmacia Biotech) and 10 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma, St Louis, MO, USA) for 24 h to induce high cytokine production. Finally, the cells were harvested by centrifugation, and the supernatants were frozen (-20°C) until the cytokine analysis was performed.

The antigen-driven proliferation was tested by culturing the PBMC at 2×10^6 /ml in CM in the presence or absence of BPE or TT at optimal concentrations in 96-well, flat-bottom cell-culture plates (Costar). The cells were seeded in four replicates and cultured for 5 days at

37°C. For the final 4 h of culture, the cells were incubated with 1 µCi ³H-labeled thymidine (Amersham-Pharmacia Biotech). The cultures were then harvested on glass fiber disks, and the incorporated radioactivity was measured in a beta-counter (Beckman, Fullerton, CA, USA). The stimulation index was calculated as counts per minute (cpm) in antigen-stimulated cultures divided by cpm in the nonstimulated cultures.

Concentrations of cytokines in the cell-culture supernatants were measured by commercial ELISA for IFN-γ, IL-4, IL-5, IL-10, and IL-13 (R&D Systems, Minneapolis, MN, USA).

The Mann-Whitney U-test (two-tailed *P* value) was used to identify significant differences between the two patient groups, and the Wilcoxon signed ranks test to identify changes within the groups. The cytokine ratios were log-transformed prior to statistical analysis. Correlation analyses were performed by Spearman's rank method. Only *P* values of <0.05 were considered significant.

Results

There was a significantly higher cell proliferation in response to BPE in the allergy group than the control group, confirming the higher number of birch-pollen-specific T cells in the peripheral blood of allergics than in nonallergic subjects (Table 1). When TT was used for stimulation, no significant difference was seen between the two groups. Analysis by flow cytometry of the T-cell blast formation in the BPE-stimulated cultures indicated that most of the activated T cells belonged to the CD3 + CD4 + T helper cell population (data not shown).

In the allergy group, the IFN-γ production by PBMC stimulated with BPE + PHA/PMA was significantly lower than with stimulation with TT + PHA/PMA (2586 ± 1458 pg/ml; mean $\pm$ SD, and 5011 ± 3066 , respectively; $P < 0.05$). Higher concentrations of IL-4, IL-5, and IL-13 were found in the BPE + PHA/PMA-stimulated cultures in the allergy group than in the TT + PHA/PMA-stimulated cultures, but the differences never reached significance (IL-4: 25.4 ± 16.2 pg/ml [BPE] mean $\pm$ SD and 14.3 ± 16.7 pg/ml [TT]; IL-5: 1378 ± 1260 pg/ml and 281.7 ± 275.5 pg/ml; IL-13: 874.6 ± 701.6 pg/ml and 186.1 ± 311.5 pg/ml). No significant differences were observed in the control group. The BPE + PHA/PMA-induced cytokine production in the allergy group was lower (IFN-γ) and, respectively,

Table 1. Stimulation index for PBMC activated with BPE or TT for 5 days. Data are shown as median stimulation index with range

	BPE stimulation	TT stimulation
Allergy group	12.4 (5.4–110.6)*	16.8 (1.7–99.3)
Control group	2.4 (1.3–8.9)*	20.8 (6.6–81.6)

* $P < 0.05$.

higher (IL-4, IL-5, and IL-13) than in the control group (not significant, data not shown).

BPE+PHA/PMA stimulation resulted in significantly higher IL-4/IFN- γ and IL-5/IFN- γ ratios in the allergy group than the control group (Fig. 1A and B). No statistical differences between the two subject groups were observed when PBMC were prestimulated with TT. When BPE and TT prestimulation in the allergy group was compared, the former antigen induced significantly higher IL-5/IFN- γ and IL-13/IFN- γ ratios (Fig. 1B and C). In the control group, there were no significant differences in the cytokine ratios between the BPE- and TT-prestimulated cultures.

In the allergy group, significantly higher production of IL-10 was induced by BPE+PHA/PMA stimulation than TT+PHA/PMA stimulation (Fig. 2). No significant difference in IL-10 production between the two antigens was seen in the control group. When the two study groups were compared, the higher concentrations of IL-10 after BPE+PHA/PMA stimulation in the allergy group never reached significance.

In the PBMC cultures, the IL-4, IL-5, and IL-13 concentrations correlated very well with each other ($r=0.86$ – 0.89 , $P<0.0001$) and also with IL-10 ($r=0.41$ – 0.46 , $P<0.05$). None of these four cytokines correlated with the production of IFN- γ (data not shown).

In the allergic patients, the mean level of birch-pollen-specific IgE was 19.1 kU/l (range: <0.35 – 76.2 kU/l). There was a significant correlation between the specific serum IgE levels and the BPE+PHA/PMA-induced IL-4 production *in vitro* ($r=0.82$, $P<0.05$). There was also a significant correlation between specific IgE and the IL-13 production ($r=0.93$, $P<0.01$), but not between specific IgE and IL-5, IL-10, or IFN- γ .

Discussion

In the present study, we analyzed the cytokine production in short-term cultures of PBMC isolated during the birch-pollen season from both birch-pollen-allergic patients suffering from rhinoconjunctivitis and nonallergic control subjects. The response to birch-pollen allergens was compared between the two study groups, as well as the response to TT, a bacterial antigen that seems to induce a Th0-like immune response in man (3, 9). Analysis of the proliferative response to BPE in PBMC cultures revealed allergen-specific T cells in both study groups, although there was a lower proliferative response in the control group than the allergy group. Our observation is similar to those obtained in previously published studies (10–12). We observed no difference in TT-specific proliferation between the two study groups, indicating that the

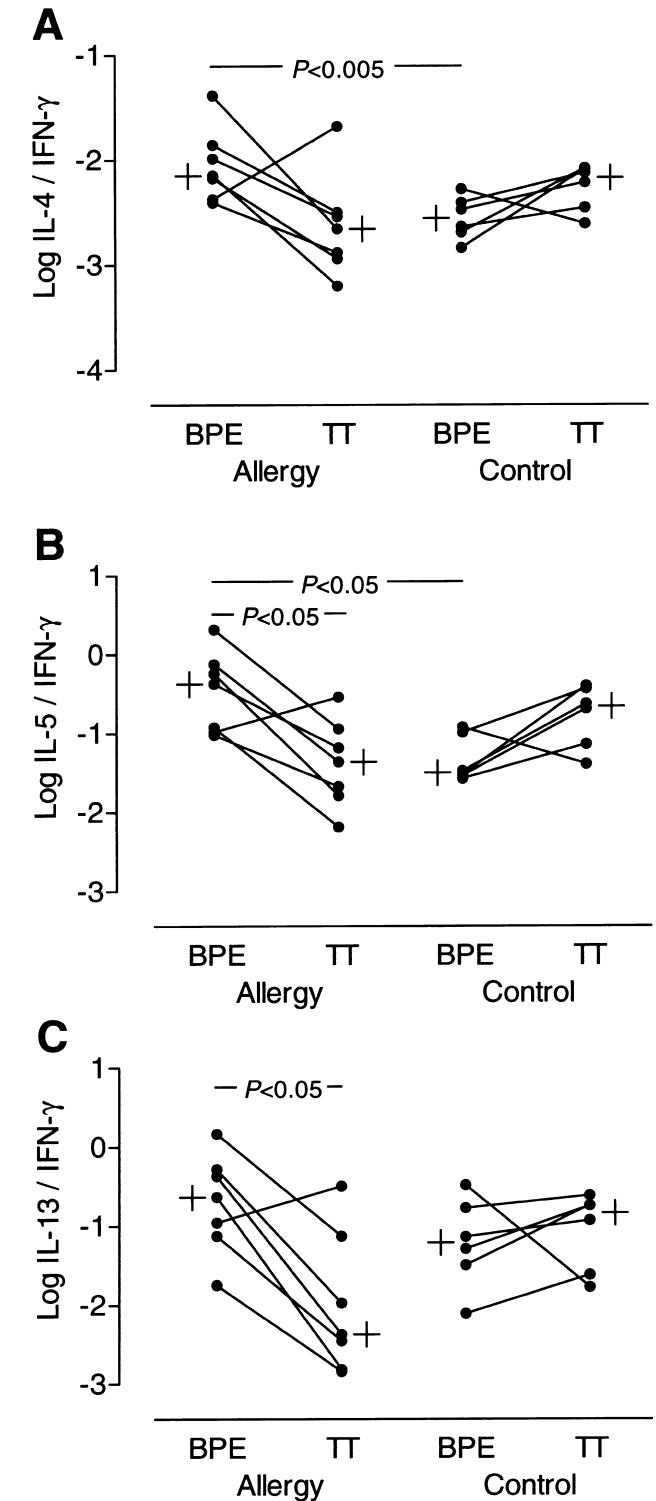


Figure 1. Logarithmic (A) IL-4/IFN- γ , (B) IL-5/IFN- γ , and (C) IL-13/IFN- γ ratios in BPE- or TT-stimulated PBMC cultures restimulated with PHA and PMA. Median ratios for allergy group (Allergy) and nonallergic control group (Control) are indicated (+).

antigen-presenting capacity by the APC was similar. The flow cytometry analysis showed that most of the activated T cells were of the helper phenotype. It is

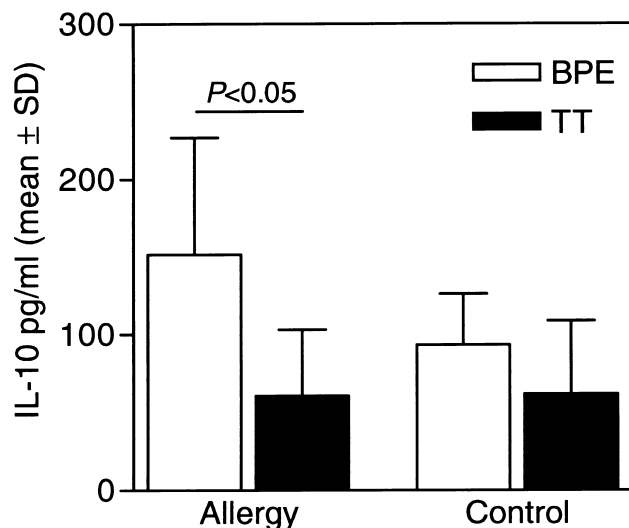


Figure 2. Concentration of IL-10 in PBMC cultures stimulated with BPE or TT for 7 days and restimulated with PHA and PMA. probably these activated Th cells that produce most of the cytokines studied.

In the allergy group, there was a significantly lower production of the Th1 cytokine IFN- γ in cultures prestimulated with BPE than with TT. On the contrary, the mean concentrations of the allergy-promoting cytokines IL-4, IL-5, and IL-13 were higher after BPE than TT prestimulation, although the differences never reached significance. By comparing the IL-4/IFN- γ , IL-5/IFN- γ , and IL-13/IFN- γ cytokine ratios, we could show that the Th1/Th2 balance in the birch-pollen-specific Th-cell response in the allergic patients was significantly skewed toward a Th2-like response. In the allergy group, the cytokine ratios were significantly higher after BPE stimulation than TT stimulation (IL-5/IFN- γ , IL-13/IFN- γ) or compared to the control group (IL-4/IFN- γ , IL-5/IFN- γ). Furthermore, significantly higher IL-10 concentrations were found in the BPE-prestimulated PBMC cultures than in the TT-prestimulated cultures. This finding is supported by the fact that Th2-like cells are a major source of IL-10 (6). In the control group, no significant differences between the two antigens were observed. The concentrations of IL-4, IL-5, and IL-13 correlated very well with each other and also with IL-10, but not with the IFN- γ levels. That indicates that different cells in the PBMC cultures might be producing the Th1- and Th2-type cytokines. New

data have also shown that the transcription of the genes coding for the Th2 cytokines is regulated differently from the transcription of the IFN- γ gene (13, 14).

Our results confirm earlier studies of cytokine profiles in birch-pollen-allergic patients (15–19). In these studies, Ebner et al. used T-cell cloning techniques (15, 16), Gabrielsson et al. used ELISPOT assays (17, 18), and Lagging et al. stimulated PBMC in bulk cultures (19). The studies performed by the last two groups used asthmatic patients. Our study included patients with rhinoconjunctivitis as the main symptom, and, to our knowledge, it is the first one to report elevated IL-10 production in bulk cultures of PBMC from birch-pollen-allergic patients during the pollen season.

IL-4 has an important role in the B-cell switch from IgG to IgE production (20). IL-13 has also been shown to induce IgE switching (21). Interestingly, we observed a correlation between the concentration of birch-pollen-specific serum IgE and the BPE-specific IL-4 and IL-13 production *in vitro*, but not with IL-5, IL-10, or IFN- γ production. Similar correlations have recently been reported by others (18, 22). These results point to the relevance of measuring IL-4 and IL-13 secretion by PBMC *in vitro* in studying IgE-mediated diseases.

Taken together, these results strongly indicate the existence of a Th2-like response to birch allergens in birch-pollen-allergic individuals during the pollen season. It includes elevated IL-10 production leading to an impaired Th1-like response and favoring the development of Th2-like cells. The Th2-type cytokines IL-4, IL-5, and IL-13 promote typical allergic reactions to inhaled birch-pollen allergens, manifested by increased production of allergen-specific IgE and elevated numbers of activated eosinophils at the site of inflammation, such as the bronchial mucosa (23). In nonallergic individuals, the response to birch pollen is of the Th0 or Th1 type.

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