

Changes in Cytokine Production *In Vitro* during the Early Phase of Birch-Pollen Immunotherapy

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The kinetics of the immunological mechanisms during allergen-specific immunotherapy (IT) has not been thoroughly evaluated. In this investigation we study the changes in T-cell responses during the early phase of IT. Ten patients (IT group) with birch-pollen allergy were treated with conventional IT. Blood samples were collected at regular intervals for specific immunoglobulin (Ig)E measurements and preparation of peripheral blood mononuclear cells (PBMC). Seven allergic control patients (AC group) were included during the subsequent birch-pollen season. The PBMC were stimulated with birch-pollen extract or tetanus toxoid (TT) and mitogens. After a short decrease, probably owing to seasonal variation, the birch-pollen-specific proliferation and the interleukin (IL)-4, IL-5, and IL-10 production significantly increased when reaching the maintenance dose and during the subsequent pollen season. The increase in IL-4 correlated with a temporary increased serum level of birch-pollen-specific IgE. Interestingly, also the TT-specific response was affected by IT, resulting in weaker, but in time similar, changes in proliferation and cytokine production as in the birch-pollen-specific response. We speculate that the early phase of IT might lead to systemic changes in the capacity of Th2-like cytokine production, and that the early increase in allergen-specific IgE is a consequence of enhanced IL-4 production.

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INTRODUCTION

Allergen-specific immunotherapy (IT) has been used in treating pollen allergy for many decades. IT has been shown to be clinically effective in many double-blind placebo-controlled studies [1–3], but the mechanisms behind its effect remain to be fully elucidated. It has been difficult to correlate the clinical improvements after IT to the decrease in specific IgE or to the increased synthesis of specific IgG and IgG4 observed [3–5]. IT has been shown to reduce eosinophil activation and accumulation in the lung [6, 7] and to reduce the number of mast cells in the nasal mucosal epithelium [8]. In recent years focus has been on the different subgroups of Th cells, i.e. Th1-, Th2- and Th0-like cells [9]. It has been shown that successful IT is followed by a shift from allergen-specific Th2-like cells, producing high amounts of interleukin (IL)-4 and IL-5, to a dominating Th1-like immune response during allergen exposure, including elevated interferon (IFN)- γ production. However, these investigations have mostly concerned bee-venom IT performed with a rush or ultra-rush regime [10–12], or conventional pollen IT

performed for many months [13, 14]. Very few studies have been published regarding the kinetics of cytokine changes during the induction phase of conventional pollen IT [15, 16].

During the early phase of IT, total and specific IgE often increase [2, 4, 17]. It is well known that IL-4 is essential for IgE production [18]. In this study we investigated if a temporary increase in IgE production during the induction phase of IT was associated with an increased IL-4 production by T cells *in vitro*. We also measured the IL-5, IL-10 and IFN- γ production by the T cells and the proliferative response. During the subsequent birch-pollen season the IT-treated patients were compared with birch-pollen-allergic control subjects.

MATERIALS AND METHODS

Patients. This study included 17 patients with a history of birch-pollen allergy with rhinitis, conjunctivitis, and occasional broncho-obstructive symptoms during the birch-pollen season, but not fulfilling the American Thoracic Society's (ATS) criteria for the diagnosis of asthma [19]. They all had IgE antibodies to birch-pollen allergens as measured by

skin-prick test and/or the Pharmacia CAP System (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Besides sensitized to birch pollen, most patients were also sensitized to grass and/or weed pollen (82%). Other sensitizations were to animal dander (71%), and in a few cases to house dust mite (18%). The patients were fulfilling the criteria for IT according to Swedish practice. Ten patients, six men and four women, aged 25 to 50 years (median age 29 years), received treatment with birch-pollen IT (IT group) and were monitored during their early treatment phase and the subsequent birch-pollen season. Seven patients, four men and three women, aged 25 to 41 years (median age 33 years) served as allergic controls (AC group) during the birch-pollen season. The median levels (with range) of birch-pollen specific IgE for the IT group and AC group were 20.6 kU/l (0.35–114.6) and 17.1 kU/l (0.35–40.9), respectively. Two patients in the IT group were excluded from the study; one person after 2 weeks of IT owing to an unrelated disease, and the other shortly after reaching the maintenance dose because of his move from Uppsala. The initial values for the latter patient are included in the study.

Study design. The IT group was treated with Alutard SQ Birch (ALK Abello, Hørsholm, Denmark), starting in October and November with weekly subcutaneous injections of increasing doses until the maintenance dose of 100 000 SQ units was reached after about 4 months. The maintenance dose was then administered every 6 weeks for 3 years. Before each injection the patients were questioned about side-effects of the previous injection and about their general health. Peak Expiratory Flow (PEF) measurements were performed before and 30 min after each injection. The patients were using combinations of antihistamines, inhaled and nasal corticosteroids, sodium chromoglycate and β_2 agonists when needed. No patient was using oral or parenteral corticosteroids. Their use of medication differed in relation to their symptoms. Heparinized blood was drawn from each patient in the IT group before the start of IT (D0) and at different time points during IT; at 2 weeks (2 W) and at 1 month (1 M) after start of IT, when the maintenance dose (MD) was reached in February to March and during the subsequent birch-pollen season (PS) in May. All samples were collected at least one week after the previous allergen injection. Blood was collected from the AC group only during the pollen season. The birch-pollen season was followed by counting pollen grains sampled by a Burkard volumetric spore trap (Burkard Scientific, Uxbridge, UK). The blood collection was performed when the cumulative birch-pollen counts exceeded 2000 grains/m³.

Skin-prick testing. For skin-prick testing, commercial extracts of birch pollen, timothy pollen, mugwort pollen, cat dander, dog dander, horse dander, *Dermatophagoides pteronyssinus*, *Alternaria* and *Cladosporium* (Soluprick SQ, ALK Abello) were used following the manufacturer's instruction. Positive (histamine 10 mg/ml) and negative (buffer) controls were included. A reaction was considered positive if the mean diameter of the wheal was 3 mm or larger.

IgE measurements. The serum concentration of IgE to birch-pollen allergens and to tetanus toxoid (TT) was determined using the Pharmacia CAP System according to the protocol of the manufacturer (Pharmacia & Upjohn Diagnostics).

Clinical evaluation. The patients were retrospectively asked about their changed symptoms and their changed use of rescue medication during the birch- and grass-pollen seasons after as compared to before the start of IT. The questions were standardized and handled by the research nurse. For much worse symptoms, the improvement score was set to one (1), worse symptoms (2), no change (3), less symptoms (4), much less symptoms (5), and no symptoms (6). A similar system for scoring changed consumption of rescue medication was used. For much more medication the improvement score was set to one (1), more medication (2), no change (3), less medication (4), much less medication (5), and no use of rescue

medication (6). The scores for the three subsequent birch-pollen seasons after start of IT were analyzed for correlation with immunological parameters.

PBMC cultures. PBMC were isolated from heparinized blood by Ficoll-Paque density gradient centrifugation (Amersham-Pharmacia Biotech, Uppsala, Sweden). The cells were washed three times in phosphate-buffered saline (PBS), and were then 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, Kibbutz Beit Haemek, Israel). We used a culture method emanating from two previously published methods to study the Th1/Th2 profiles of T cells from allergic patients [20, 21]. The cells (1×10^6 cells/ml) were cultured for 7 days in 24-well, flat-bottom tissue culture plates (Costar, Cambridge, MA, USA) in a humidified chamber at 37 °C in 7% CO₂, in the presence of 2 µg/ml crude extract of birch pollen (BPE) (*Betula verrucosa*; Allergon, Ängelholm, Sweden) or 10 µg/ml TT (SBL Vaccin, Stockholm, Sweden). These concentrations had previously been shown to stimulate optimal proliferation in PBMC cultures (data not shown). The cells were then harvested, washed, and restimulated (5×10^5 cells/ml) with 10 µg/ml phytohemagglutinin (PHA; Amersham-Pharmacia Biotech) and 10 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO, USA). Twenty-four hours later, the culture supernatants were harvested by centrifugation and frozen at –20 °C until analysis. During these culture conditions, the major cytokine producing cells are the antigen-activated T-cell blasts that are enriched during the antigen-specific prestimulation step. We have previously shown that this method can be used to study Th2-like cytokine responses by T cells to allergens in vitro [22]. In order to determine the T-cell proliferative response, PBMC (2×10^6 cells/ml) were cultured in the presence or absence of BPE or TT at optimal concentrations in 200 µl CM in 96-well, flat-bottom cell culture plates (Costar) in four-replicates for 5 days. The cells were pulsed with 1 µCi ³H-labelled thymidine (Amersham-Pharmacia Biotech) for the last 4 h of culture and the incorporated radioactivity was measured in a beta-counter (Beckman, Fullerton, CA, USA). The stimulation index (SI) was calculated as counts per minute (cpm) in antigen stimulated cultures divided by cpm in the nonstimulated cultures.

Cytokine assays. The concentrations of IFN- γ , IL-4, IL-5, and IL-10 in the cell culture supernatants were measured by commercial enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN, USA) according to the supplier's recommendations. The detection limits of the assays were 7 pg/ml for IFN- γ , 10 pg/ml for IL-4, 3 pg/ml for IL-5 and 3.9 pg/ml for IL-10.

Statistical analysis. The Mann–Whitney U-test (two tailed *P*-value) was used to identify significant differences between the IT group and AC group during pollen season, and Wilcoxon-signed ranks test to identify changes within the IT group. Correlation was evaluated by Spearman's rank-correlation test. Only *P*-values less than 0.05 were considered significant.

The study was approved by the local Ethics Committee of the Medical Faculty of Uppsala University, Sweden, and the patients gave their informed consent.

RESULTS

Clinical efficacy

The clinical efficacy of the IT was evaluated for the patients that continued their therapy through the first pollen season. The

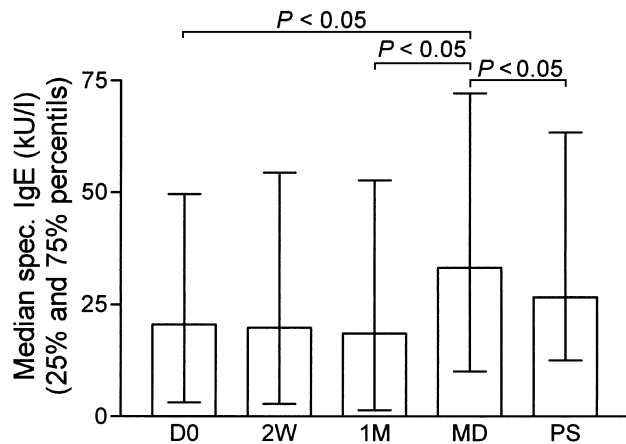


Fig. 1. The median levels of specific serum immunoglobulin (IgE) to birch pollen during the early phase of immunotherapy (IT).

outcome of the IT was shown to be successful. All IT-treated patients reported improvements in symptoms (improvement score 4.1 ± 0.8 [mean $\pm$ SD]) and/or reduction in their medication (improvement score 4.4 ± 1.0) already during the first birch-pollen season. The improvements were maintained or even enhanced during the two subsequent birch-pollen seasons (second year: 4.2 ± 0.5 [symptoms], 4.6 ± 1.1 [medication], third year: 5.0 ± 0.8 [symptoms], 5.2 ± 0.7 [medication]).

Specific serum IgE

No significant changes in the concentration of birch-pollen-specific serum IgE were seen during the first month of IT, although it decreased in some of the patients (data not shown). Later, the IgE temporarily increased in all but one patient. The increase was from a median level of 20.6 kU/l before IT to 33.1 kU/l at reaching the maintenance dose (Fig. 1). After the maintenance dose was reached, the specific IgE significantly decreased to 26.6 kU/l at the following birch-pollen season. No TT-specific IgE antibodies were detected at any time (data not shown).

Antigen-specific proliferation

The mean proliferative response to BPE stimulation slightly decreased (nonsignificantly) during the first month of IT. Then the proliferative response increased significantly when reaching the maintenance dose, and it was still elevated during the pollen season (Fig. 2). The response to TT before IT was in the same magnitude as to BPE (Fig. 2). Interestingly, the TT-specific proliferation was significantly increased from the maintenance dose and onwards, compared to 1 month of IT.

Cytokine profile of the immune response to birch pollen and TT

The cytokine profile of the cellular immune response to birch-pollen allergens and to TT were studied in cultures of PBMC isolated (at D0) from the patients to be treated with IT. After restimulation with PHA/PMA, the BPE-stimulated PBMC

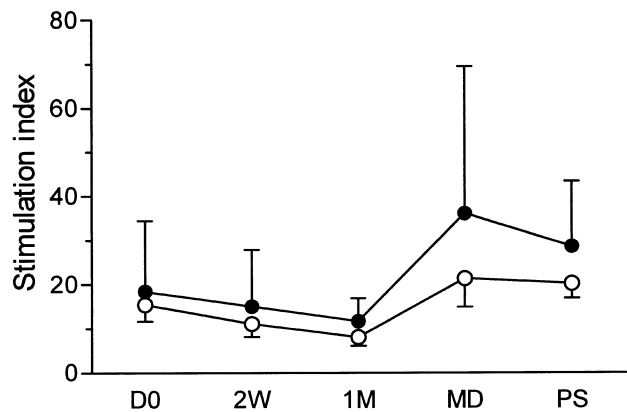


Fig. 2. Proliferative responses of peripheral blood mononuclear cells (PBMC) during the early phase of IT. PBMC were isolated at points indicated in the figure and stimulated in four-replicate cultures with BPE (closed circles) or TT (open circles). ^3H -TdR incorporation was measured after 5 days of culture. Results are shown as stimulation index (SI) (mean $\pm$ SD). The BPE-specific SI increased significantly from 2 weeks of IT (2W) to maintenance dose (MD) ($P < 0.05$) and pollen season (PS) ($P < 0.01$), and from 1 month of IT (1M) to MD and PS (both $P < 0.05$). The TT-specific SI increased significantly from 1M to MD and PS (both $P < 0.05$).

produced significantly more Th2-type cytokines than the TT-prestimulated cells (IL-4: 41.5 ± 32.9 pg/ml [mean $\pm$ SD] versus 6.5 ± 8.3 pg/ml, $P < 0.01$; and IL-5: 2228 ± 1573 pg/ml versus 319 ± 246 pg/ml, $P < 0.05$). There was also a trend towards less IFN- γ production in the BPE-stimulated compared to the TT-stimulated cultures; 1266 ± 1418 pg/ml versus 3481 ± 2663 pg/ml ($P = 0.074$).

Changes in IL-4, IL-5 and IFN- γ production during IT

The BPE-specific IL-4 and IL-5 production significantly decreased during the first month of IT (Fig. 3A, C). After this decrease, the Th2 cytokine levels increased when reaching the maintenance dose. The mean IL-4 concentration at the maintenance dose was higher than before the IT started, although not reaching the level of significance ($P = 0.055$). The concentrations of these Th2-type cytokines, at the time for the maintenance dose, were equal to, and in some patients even higher than, those observed during the following pollen season. We also noticed a small but significant increase in IFN- γ from 2 weeks of IT to the time for the maintenance dose (Fig. 3E). In the IT group, the IL-5 level was significantly higher during the pollen season compared to the AC group, and there was also a clear trend of elevated IL-4 production in the former group (Table 1). However, IFN- γ did not differ between the IT and AC groups.

There were no significant changes in TT-specific production of IL-4 or IL-5 during the first month of IT (Fig. 3B, D). Interestingly, the TT + PHA/PMA-induced IL-4 and IL-5 production increased when reaching the maintenance dose and during the birch-pollen season, although never to the same levels as in BPE + PHA/PMA-stimulated cultures. The increase was

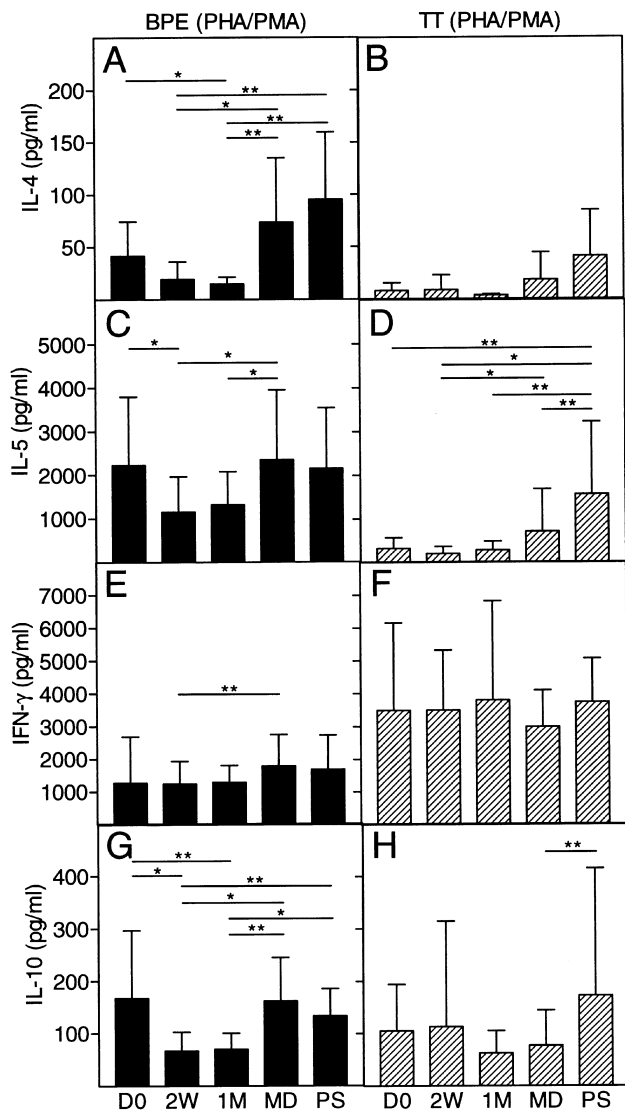


Fig. 3. Cytokine production (mean $\pm$ SD) in cultures of PBMC during the early phase of IT. The cells were isolated at points indicated in the figure and stimulated for 7 days with BPE (closed bars) or TT (hatched bars) and then restimulated with PHA/PMA to induce (A and B) interleukin (IL)-4, (C and D) IL-5, (E and F) interferon (IFN)- γ , or (G and H) IL-10 production. * $P < 0.05$, ** $P < 0.01$.

significant only for IL-5. No change in TT + PHA/PMA-induced IFN- γ production was observed during the entire study period (Fig. 3F). No significant differences in TT-specific cytokine production could be found between the IT and AC groups during pollen season (Table 1), although a trend towards increased IL-5 production could be identified in the IT group.

Changes in IL-10 production during IT

To investigate the immuno-regulatory role of IL-10 during IT we measured IL-10 in the supernatants. IL-10 has a number of effects on the immune system, among others it induces T-cell unresponsiveness (anergy). BPE + PHA/PMA stimulation resulted in

Table 1. Cytokine production (pg/ml) by PBMC isolated from the IT group and the AC group during the pollen season

	IT group	AC group	<i>P</i> -value
BPE + PHA/PMA			
IL-4	95.1 $\pm$ 64.4	33.6 $\pm$ 29.6	0.054
IL-5	2148 $\pm$ 1394	800.5 $\pm$ 709.6	0.020*
IFN- γ	1667 $\pm$ 1054	1098 $\pm$ 502.1	0.46
IL-10	133.6 $\pm$ 52.2	102.9 $\pm$ 86.8	0.23
TT + PHA/PMA			
IL-4	40.7 $\pm$ 43.8	4.0 $\pm$ 2.1	0.15
IL-5	1559 $\pm$ 1665	156 $\pm$ 92.8	0.072
IFN- γ	3731 $\pm$ 1326	3586 $\pm$ 957.7	0.87
IL-10	172.0 $\pm$ 242.7	47.6 $\pm$ 61.9	0.094

Data is shown as mean concentration $\pm$ SD. * Significant ($P < 0.05$).

significantly higher IL-10 production than TT + PHA/PMA stimulation before start of treatment (D0) (167 $\pm$ 129 pg/ml versus 105 $\pm$ 89.2 pg/ml, $P < 0.01$). The BPE-specific IL-10 production initially decreased (Fig. 3G), but it had recovered at reaching the maintenance dose and remained elevated during the pollen season, similar to the IL-4 and IL-5 production. The changes observed during TT + PHA/PMA stimulation were smaller but we noticed a significant increase during the pollen season (Fig. 3H). No significant difference was found between the IT and AC group concerning the IL-10 production during the pollen season (Table 1).

We also analyzed the IL-10 production in PBMC cultures stimulated with BPE for 7 days without any restimulation with PHA/PMA to study if the increased allergen-specific proliferation observed during the induction phase was owing to less IL-10 production. The obtained results (shown as mean concentration $\pm$ standard deviation) were 62.6 $\pm$ 48.5 pg/ml (D0, $n = 9$), 49.2 $\pm$ 34.0 pg/ml* (1M, $n = 9$), 91.6 $\pm$ 51.8 pg/ml* (MD, $n = 9$), and 61.8 $\pm$ 31.3 pg/ml (PS, $n = 8$). After the small decrease during the first month of IT (not significant), the production of IL-10 was significantly enhanced when reaching the maintenance dose (* $P < 0.01$), i.e. a negative correlation to the proliferative response was not observed.

Four of the IT-treated patients had a TT-specific IL-10 production below the detection limit for the assay when the restimulation step was excluded. For all of the patients, from which we obtained measurable levels, the TT-induced IL-10 production increased when reaching the maintenance dose (from 11.0 $\pm$ 6.5 pg/ml [D0] to 34.4 $\pm$ 32.8 pg/ml [MD], mean $\pm$ SD, $n = 4$) and the pollen season (from 11.5 $\pm$ 6.3 pg/ml [D0] to 40.9 $\pm$ 17.1 pg/ml [PS], mean $\pm$ SD, $n = 4$). The few samples rendered reliable estimations of significance impossible.

Correlation between immunological parameters and clinical improvements

Calculations based on Spearman's rank-correlation test revealed

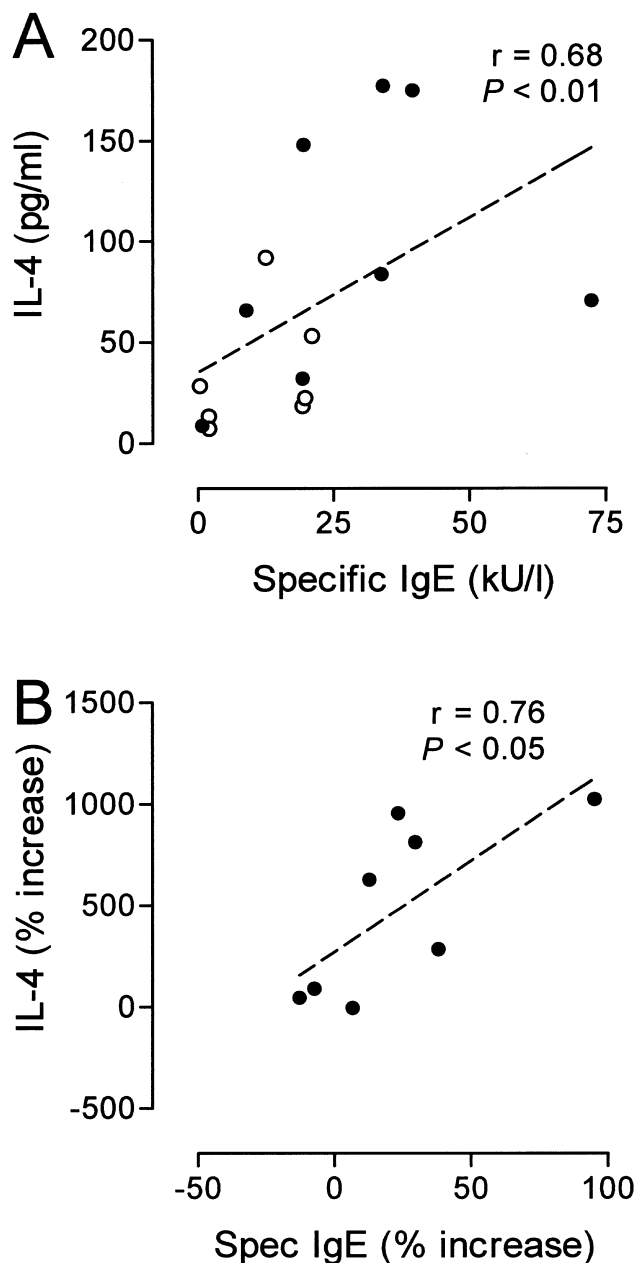


Fig. 4. Correlation analyses of the association between BPE + PHA/PMA-stimulated IL-4 production *in vitro* and birch-pollen-specific serum immunoglobulin (IgE). (A) Correlation between the levels of IL-4 and IgE during the birch-pollen season in IT-treated patients (closed circles) and allergic controls (AC) patients (open circles). (B) Correlation between the relative changes in the IL-4 and IgE levels from 2 weeks of IT to the maintenance dose (one IT patient with specific IgE below the detection limit of the assay (<0.35 kU/l) was excluded before evaluation).

a significant correlation during the pollen season between the IL-4 secretion in the BPE + PHA/PMA-stimulated PBMC cultures and the *in vivo* concentration of birch-pollen-specific IgE (Fig. 4A). It was also a significant correlation between the relative increase in IL-4 and the relative increase in specific

serum IgE from the second week of IT (2 W) to the maintenance dose (Fig. 4B).

Low IL-5 production during the first birch-pollen season after start of IT correlated with a high rate of symptom improvements ($r = -0.88$, $P < 0.01$). No correlation with other cytokines was found.

DISCUSSION

In the present investigation we studied the changes in the immune response in birch-pollen-allergic patients with severe rhinoconjunctivitis during the induction phase of conventional IT and during the following birch-pollen season. The start of IT was spread out over approximately 4 weeks, reducing the risk of possible experimental variation between points of time to influence the outcome of the study. The IT was successful, because all treated patients reported clinical improvements.

The proliferative response of BPE-stimulated PBMC tended to decrease during the first month of IT (not significant). The proliferative response then increased significantly when reaching the maintenance dose and was retained at a high level during the following pollen season.

We observed a typical Th2-type cytokine profile when stimulating the PBMC from the allergic patients with BPE compared to the bacterial antigen TT. Initially after start of IT, the production of Th2-like cytokines decreased. This early decrease seen already after 2 weeks of treatment might either be a consequence of migration of peripheral T cells to the site of local inflammation owing to the allergen injections, or perhaps more likely, owing to a natural decrease in Th2-type cytokines from the last pollen season [23, 24]. According to our experiences, the IL-4 and IL-5 levels were still relatively high when the IT started in the autumn. In concordance, the specific serum IgE decreased during the first month of IT in some patients (data not shown). However, when reaching the maintenance dose, the BPE + PHA/PMA-induced IL-4 and IL-5 production by PBMC *in vitro* increased significantly in the allergic patients undergoing IT treatment, confirming the results in our previous pilot study, in which we found increased IL-4 and IL-5 production during the induction phase of semirush birch-pollen IT [15]. The increases coincided with the enhanced proliferative response. Also a small increase in IFN- γ was observed. The significant increase in IL-4 secretion correlated with an elevation of specific IgE *in vivo*, which indicates a role for IL-4 in the temporary increase of specific IgE often observed during the early phase of IT [2, 4, 17].

During the subsequent pollen season, the Th2-type cytokine levels were elevated in the IT group compared to the AC group. A shift towards a Th1/Th0-like phenotype as a result of the IT treatment could not be observed during this first birch-pollen season. On the other hand, we did not analyze the patients' cytokine profiles during the pollen seasons before the start of IT. Interestingly, however, low IL-5 production during this first pollen season correlated significantly with symptom improvement, which is in line with the findings of an important role for

IL-5 in the late phase inflammatory reaction [25]. None of the other cytokines analyzed correlated with clinical improvements.

The fact that the IL-4 production in the IT group was still enhanced during the pollen season while the birch-pollen-specific IgE decreased might be the result of inhibited expression of CD40 on the B cells during IT [26]. However, during the pollen season, the birch-specific IL-4 production *in vitro* was significantly correlated with the level of birch-pollen-specific serum IgE.

Interestingly, the TT + PHA/PMA-induced cytokine production also changed during the early phase of IT. The TT-specific production of IL-4 and IL-5 increased at the maintenance dose (significant only for IL-5) and continued to increase during the birch-pollen season. It is noteworthy that the increase in the Th2-like cytokine production coincided with an enhanced TT-specific proliferation of the PBMC. The small elevation of IL-4 observed was not associated with any measurable production of TT-specific IgE *in vivo*.

Our results differ from the results of other groups showing either a Th2 to Th1/Th0 shift in the cytokine profile [13, 14] and/or a reduced proliferative T-cell response [27] during pollen-specific IT. However, these groups did not study the kinetics of the early immunological changes during IT as we did. The early symptom improvements of IT observed in this and many other studies might be the consequence of immunological changes in the shock organ rather than in the circulation.

In a study by Akdis *et al.* [12], the immuno-regulatory cytokine IL-10 was reported to increase during ultra-rush bee-venom IT, and it was speculated that IL-10 induced a state of T-cell anergy. When analyzing the IL-10 secretion in the supernatants of BPE- or TT-stimulated PBMC cultures both before and after PHA/PMA restimulation we revealed that the changes in IL-10 production during the induction phase of IT were very similar to the changes observed for IL-4 and IL-5. When comparing the IL-10 production with the proliferative response during the induction phase of IT, no IL-10-induced T-cell anergy could be observed. The discrepancy between the studies might be explained by the different IT regimes used and the different forms of allergic diseases, i.e. bee-venom allergy versus birch-pollen allergy. Methodological differences regarding the *in-vitro* stimulation might also have influenced the results.

The results in this study point out a systemic effect as a result of the allergen injections during the early phase of IT leading to a strengthening of the Th2-like phenotype. Recently, another IT study was published by Benjaponpitak *et al.* [16] that supports our data. The mechanism is not known, but as indicated in a study by Hamid *et al.* [28], IL-12 production by antigen-presenting cells (APC) can be influenced by IT. IL-12 is believed to be an important regulator of the Th1/Th2 balance of immune responses (reviewed in [29]). There are also indications that allergen exposure during pollen season has a nonspecific priming effect on the Th2-type cytokine production by PBMC *in vitro* [23, 24]. Other studies have shown that low allergen doses lead to an immune response of Th2 type, while high doses results in a Th1-type response [30, 31]. It is plausible that the low allergen

doses injected during the induction phase of conventional IT are causing a strengthening of the Th2-like phenotype, with increased production of Th2-type cytokines and IgE antibodies. Later, when a number of maintenance doses have been administered, the IgE level decreases [3–5] accompanied with a Th2 to Th1/Th0 shift [13, 14]. Furthermore, aluminum hydroxide, used as a depot adjuvant in most allergen preparations for conventional IT (e.g. Alutard), has been shown to be a potent Th2 inducer in mice, and to stimulate IgE production [32, 33].

In a recently published study by Hedlin *et al.* [34], polysensitized children, allergic to cat dander as well as to grass- and birch-pollen allergens, were treated with mixed pollen extracts only. Interestingly, the children improved not only in their pollen allergy but also in their cat allergy, indicating that also a nonspecific effect of so-called allergen-specific IT indeed might exist.

More research will be needed to understand the regulation of the Th1/Th2 balance of the immune system during the development of allergic diseases as well as during the treatment with allergen-specific IT.

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