

Comparison between the *in vitro* cytokine production of mononuclear cells of young asthmatics with and without immunotherapy (IT)

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Summary

Background The underlying mechanisms of immunotherapy (IT) are still unknown but may be related to modifications of cytokine production of T lymphocytes.

Objective In this study we determined the *in vitro* allergen-induced production of IL-2, IL-4, IL-5, IL-12 and IFN γ of peripheral blood mononuclear cells (PBMC) of eight young asthmatics, aged 15 ± 2 years, receiving IT (IT group) and of eight comparable asthmatics, aged 13 ± 3.5 years, who never received IT (non-IT group).

Methods All patients suffered from perennial asthma and were allergic to house dust mite (HDM). They were selected if they showed a positive stimulation index (SI) of PBMC after *in vitro* incubation with HDM (i.e. $SI > 2$). Cells were incubated with and without HDM ($10 \mu\text{g/mL}$) during 24 h, 48 h and 7 days. Cytokines were determined in the supernatant at the three time points and are expressed as median values in pg/mL.

Results In the IT group the secretion of IL-2 was lower compared with the non-IT group after 7 days incubation of PBMC with HDM (0 vs 33.2, $P = 0.008$). In both groups maximal secretion of IL-2 was observed after 48 h. In the non-IT group a high value of IL-2 persisted after 7 days, whereas in the IT group a significant decline of IL-2 occurred after 7 days. Although IL-4 secretion was low in all subjects, more patients of the non-IT group showed detectable IL-4 in the HDM cultures after 24 h and 48 h, although the difference was not statistically significant ($P = 0.08$ and $P = 0.28$, respectively). Furthermore, IL-4 secretion was lower in the HDM cultures after 24 h in the IT group (1.75 vs 4.1, $P = 0.011$) and 48 h (2.2 vs 4.1, $P = 0.035$). IL-5 secretion was lower in the HDM cultures after 24 h (12.4 vs 47.6, $P = 0.035$) and 48 h (26.8 vs 135, $P = 0.046$) in the IT group than in the non-IT group. After 7 days of incubation with HDM there was no difference between the groups. There was no difference between both groups in secretion of IFN γ and IL-12.

Conclusions These results show a difference *in vitro* cytokine secretion of PBMC of asthmatics receiving IT compared with asthmatics who never received IT. PBMC of patients receiving IT secrete less IL-2 and IL-5 after *in vitro* incubation with HDM and show a tendency to secrete less IL-4. The efficacy of IT may be attributed to a modified cytokine secretion of PBMC.

Keywords: asthma, immunotherapy, cytokines, IL-2, IL-4, IL-5, IL-12, IFN γ

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Introduction

Although its underlying mechanisms are still largely unknown, evidence suggests that immunotherapy (IT)

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preferentially affects T lymphocytes [1]. A number of lymphocytic changes, such as the induction of allergen-specific CD8⁺ cells [2], a decreased antigen-specific proliferative response [1,3], and an altered interleukin-2 (IL-2) production and responsiveness [3] have been described in patients receiving IT, indicating a modified immune response of T lymphocytes to antigens. A characteristic clinical feature of successful IT is the inhibition of the allergen-induced late responses [4–6]. In different studies, using skin and nasal biopsies, it was demonstrated that the inhibition of late responses is accompanied by a decreased infiltration of CD4⁺ cells and eosinophils [7–10]. Furthermore, in patients receiving IT a significant increase in cells expressing mRNA for IL-12 and interferon-gamma (IFN γ) was observed [10,11]. In another study it was found that IT reduced the production of IL-4 in peripheral blood [12] and in a recent study by Hsieh *et al.* an increased production of IL-8 was demonstrated in asthmatic children who received IT successfully [13]. The observed modifications induced by IT suggest a switch from a TH₂-type lymphocyte to a TH₁-type lymphocyte [10]. To further elucidate the mechanisms of IT we studied the *in vitro* allergen-induced cytokine production of peripheral blood mononuclear cells (PBMC) of young asthmatics with and without IT.

Methods

Patients

Sixteen young asthmatics (children and adolescents) suffering from perennial asthma were selected for in this study. Diagnoses were based on criteria listed in an international consensus report on treatment of asthma [14]. All subjects were monoallergic to house dust mite (HDM) (*Dermatophagoides pteronyssinus*) as shown by positive skin-prick tests and by the presence of specific IgE to HDM. Patients were divided into two groups, those who had been receiving IT at the time of this study ($n=8$, IT group) and those who had never received IT ($n=8$, non-IT group). Severity of asthma was determined for each patient [14]. The two groups were comparable for clinical evolution of asthma (asthma severity), allergy testing, lung function testing and usage of antiasthmatic drugs. At the time of the study all patients were symptom free for at least 1 month and all had normal lung function tests. In the IT group five patients were on daily inhaled corticosteroids (beclomethasone dipropionate) and in the non-IT group six were on daily inhaled corticosteroids. All patients took β -agonists as required. Before blood sampling inhaled corticosteroids were stopped for 48 h and β -agonists were stopped for 12 h. Details of the patients are given in Table 1.

Culture of mononuclear cells

In all patients blood sampling was performed between 08.00 and 10.00h. PBMCs were isolated out of 30 mL peripheral blood using Nycoprep (Nycomed, Oslo, Norway) density gradient centrifugation (density 1.077 g/mL). One hundred thousand lymphocytes in 150 μ L serum free AIM-V medium (Life Technologies, Ghent, Belgium) were cultured in 96-well round bottomed microtitre plates (Costar, Cambridge, MA, USA) and stimulated with 10 μ g/mL HDM-extract (*Dermatophagoides pteronyssinus*, ALK, Copenhagen, Denmark) (i.e. HDM cultures) or 20 μ L medium as a negative control (i.e. control cultures). The plates were incubated at 37°C in a humidified 5% CO₂ incubator (Jouan, Saint Herblain, France) during 3 or 7 days. Cells were pulsed for the final 24 h with 20 μ L of {methyl-³H} thymidine (20 μ Ci/well; specific activity 25 Ci/mmol, Amersham Life Sciences, Amersham, UK). Afterwards cells were harvested on glass fibre paper (GF/C-Unifilter, Canberra Packard Instruments) using an automatic cell harvester (Filtermate 186, Canberra Packard Instruments, Meriden, CT, USA). The incorporated radioactivity was measured with a liquid scintillation counter (Topcount, Canberra Packard Instruments). Results were expressed as SI, i.e. counts per minute (cpm) of the HDM-stimulated lymphocytes divided by cpm of the control culture with AIM-V medium alone. Patients were selected for the study if they showed a SI > 2.

Cytokine determination

PBMCs, separated as described above and concentrated to 2.10⁶ cells/mL in AIM-V medium, were stimulated with 10 μ g/mL HDM during 24 h, 48 h and 7 days at 37°C in a humidified 5% CO₂ incubator in 24 well plates (Costar). At the respective time points, plates were centrifuged during 10 min at 200 g. Supernatant was collected and frozen at –20°C until further analysis. Sandwich ELISA techniques were used to determine IFN γ , IL-2, IL-4, IL-5 (R&D Systems, Oxon, UK) and IL-12 (Medgenix – BioSource, Fleurus, Belgium). Tests were performed exactly as described by the manufacturer. Results are expressed as median and range in pg/mL. The lower detection limits of the cytokines are 3 (IFN γ), 7 (IL-2), 4.1 (IL-4), 3 (IL-5) and 1.5 pg/mL (IL-12).

Immunotherapy protocol

IT was administered according to a standardized protocol previously described [15]. In all patients IT was started using a semirush procedure during which five injections were given at 30 min intervals with increasing amounts (0.1, 0.3, 0.5, 0.8 and 1.0 mL) of an aqueous extract of HDM

Table 1. Clinical data, FEV₁, skin tests with HDM, total IgE and HDM-specific IgE of the 16 patients studied

	IT group	Non-IT group	P-value
Number of patients	8	8	
Mean age (months) (mean $\pm$ SD)	184 ($\pm$ 24.3)	153 ($\pm$ 41.9)	0.085
Gender (M:F)	7:1	6:2	
Mean FEV ₁ (%) (mean $\pm$ SD)	94 ($\pm$ 10.4)	87 ($\pm$ 5.7)	0.111
Total IgE (kU/L) (median and range)	440 (24–2000)	360 (43–2000)	0.916
HDM-specific IgE (kU/L) (median and range)	73 (3–100)	72 (29–100)	0.752
Skin test HDM (mm) (median and range)	5 (3–12)	4.5 (3–5)	0.318
Severity of asthma and antiasthmatic medication*	moderate 5 (IC + IB) mild 3 (IB)	moderate 6 (IC + IB) mild 2 (IB)	
Mean duration of IT (months)	53 $\pm$ 4.4	–	

*antiasthmatic medication: IC, inhaled corticosteroids (daily); IB, inhaled β -agonists (as required).

(*Dermatophagoides pteronyssinus*) of 50 Allergy Units per millilitre (50 AU/mL) (Allerset, HAL, Haarlem, The Netherlands). One week later the same amounts were injected with a tenfold higher concentration (500 AU/mL). Subsequently twice weekly injections were given with increasing amounts (0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.9 and 1 mL) of the allergen extract dosed 5000 AU/mL. Treatment was continued by injecting this dose, once after one week, then once after 2 weeks, then once after 3 weeks and finally every 4 weeks. At the time of the study all patients received IT for at least 4 years (mean: 53 $\pm$ 4.4 months).

Statistical analyses

Normally distributed data are expressed as mean and standard deviation (SD) and were analysed using the Student *t*-test. Not normally distributed data are expressed as median and range and were analysed using non-parametric tests (Mann–Whitney test for unpaired data and Wilcoxon matched pairs test for paired data). Differences between frequencies were evaluated by χ^2 analysis with Yates' correction. Probability (*P*) values of less than 0.05 were regarded as significant.

Results

Both groups were comparable for age, asthma severity, lung

function parameters, total IgE, HDM-specific IgE, skin-prick test with HDM and usage of antiasthmatic medication (Table 1).

Stimulation index

Patients were selected for this study if they showed a SI > 2. There was no difference between the two groups in median SI: in the IT group the median SI was 7.3 (range 3–13.7) and in the non-IT group the median SI was 4.8 (range 3–20.5) (*P* = 0.71).

IFN γ

IFN γ was only detectable in a minority of subjects in control cultures and in HDM cultures after 24 h and 48 h. After 7 days of incubation with HDM, IFN γ was detectable in 5/8 supernatants of the non-IT group and in 6/8 of the IT group. At that time point, the values were not different between both groups; the median value in the IT group was 6.65 (range 0–13.6) and in the non-IT group it was 6.10 (range 0–16.6).

IL-2

In the control cultures IL-2 was detectable only in a few

Table 2. Values of IL-2 (pg/mL) in the two groups (median and range)

IL-2 in:	IT group	Non-IT group	P-value
HDM culture after 24 h	2.2* (0–23.7)	11.1 (0–229.0)	0.090
HDM culture after 48 h	20.8 (0–82.2)	43.9 (0–1358.1)	0.160
HDM culture after 7 days	0* (0–8.4)	33.2 (0–2677.1)	0.008

* below detection limit.

cases. However, in the HDM cultures after 24 h IL-2 was found in 6/8 cases of the non-IT group and in 4/8 of the IT group. In the IT group the median value was 2.2 (range 0–23.7) (below detection limit), while in the non-IT group it was 11.1 (range 0–229) ($P = 0.09$). After 48 h IL-2 was found in 7/8 supernatants of HDM of the non-IT group, and in 5/8 of the IT group. The median value in the IT group was 20.8 (range 0–82.2) and in the non-IT group the median value was 43.9 (range 0–1358.1) ($P = 0.16$). After 7 days IL-2 was above the lower detectable limit in only 1/8 cases in the IT group and in 7/8 cases of the non-IT group. The median value in the IT group was 0 (range 0–8.4) and the median value in the non-IT group was 33.2 (range 0–2677.1) ($P = 0.008$). Peak secretion of IL-2 was reached after 48 h in the IT group and values were significantly lower at 7 days ($P = 0.017$). In the non-IT group the peak secretion time was also after 48 h, but the values remained high after 7 days ($P = 0.41$) (Table 2).

IL-4

In all control cultures of both groups IL-4 was below the detection limit. This was also the case in the IT group in all HDM cultures at the three time points, except in one. In contrast, in the non-IT group IL-4 could be detected and was found in 4/8 of HDM cultures both after 24 h ($P = 0.08$) and 48 h ($P = 0.28$). After 7 days IL-4 was also below the lower limit in all HDM cultures in the non-IT group.

By extrapolation on the standard curve (values were below the detection limit) a significant difference in

median IL-4-value was found between the two groups in the HDM cultures both after 24 h and 48 h; children of the non-IT group showed higher values of IL-4 than those of the IT group (Table 3).

IL-5

In the control cultures, IL-5 was below the detection limit in most subjects. However, IL-5 was largely above the detection limit in all supernatants of the HDM cultures except in 1 (at 24 h, IL-5 could not be detected in a child from the non-IT group) (Table 4). IL-5 secretion was higher in the non-IT group than in the IT group at time points 24 h ($P = 0.035$) and 48 h ($P = 0.046$). After 7 days incubation with HDM there was no difference in IL-5 between the two groups ($P = 0.833$).

IL-12

In the control cultures, IL-12 was only detectable in half of the subjects. In the HDM cultures IL-12 was found in most supernatants (73%). The median values and range of IL-12 are shown in Table 5. There was no difference found at any time point between IL-12 secretion of children with and without IT. In each group there was also no difference between IL-12 of control cultures and of HDM cultures.

Discussion

In a number of recent studies it was demonstrated that T

Table 3. Values of IL-4 (pg/mL) in the two groups (median and range)

IL-4 in:	IT group	Non-IT group	P-value
HDM culture after 24 h	1.75* (0.7–2.6)	4.10 (1.8–9.8)	0.011
HDM culture after 48 h	2.20* (0.7–4.3)	4.10 (1.8–15.3)	0.035
HDM culture after 7 days	1.45* (1.0–3.2)	1.90* (1.4–2.5)	0.490

* below detection limit.

Table 4. Values of IL-5 (pg/mL) in the two groups (median and range)

IL-5 in:	IT group	Non-IT group	P-value
HDM culture after 24 h	12.4 (3–31)	47.6 (0–248)	0.035
HDM culture after 48 h	26.8 (13–110)	135.0 (5–799)	0.046
HDM culture after 7 days	1054.0 (29–1805)	993.7 (380–2586)	0.833

lymphocytes may play a central role in asthma [16,17]. Furthermore, it has been shown that IT preferentially affects T lymphocytes, suggesting that IT may have a place in the treatment of asthmatic patients [1–3]. A possible mechanism of IT may be the induction of a different cytokine production of T lymphocytes. In the present study it is demonstrated that PBMC of young asthmatics receiving IT have a different *in vitro* cytokine profile from PBMC of young asthmatics who never received IT. Both groups showed the same degree of *in vitro* proliferation of PBMCs after incubation with HDM (i.e. the same SI after incubation of PBMC with HDM). Therefore, the observed differences in cytokine production between the two groups can not be attributed to a different proliferative response of PBMCs and should be regarded as qualitative cellular modifications induced by IT. Furthermore, the results of the present study are in agreement with results of a recent study demonstrating a modified cytokine production of PBMC, including an increased production of IL-8, in asthmatic children receiving IT [13].

We were unable to detect any difference in IFN γ production between the two groups. In a study by Durham *et al.* however, an increased mRNA expression of IFN γ was shown in the nasal mucosa of rhinitis receiving IT [10]. The main difference between the results of the present study and those from the study by Durham *et al.* is that we looked at secretion of IFN γ after *in vitro* stimulation of PBMC, while in Durham's study IFN γ expression was determined in the target organ (the nose). However, in another study on bee venom immunotherapy by Jutel *et al.* an increase of IFN γ secretion in specific allergen-stimulated T cell cultures was found [18]. On the other hand, in a recent study by

O'Brein *et al.* a reduction in expression of IFN γ in circulating lymphocytes was found in patients receiving IT [19]. Although the reason for these contradictory findings is unclear, it might be that the different experimental protocols employed are responsible for it.

A decreased secretion of IL-2 was observed in patients receiving IT. This finding seems contradictory to the general assumption that IT induces a switch from TH₂ to TH₁. However, the findings of the present study are on PBMC and we speculate that these findings are due to an IT-induced IL-2-depletion of PBMC *in vivo*, leading to a TH₁ effect in the target organs (i.e. nose, bronchi), as an upregulation of mRNA expression of IL-2 was found in patients receiving IT [9]. Furthermore, in different studies decreased levels of soluble IL-2 receptor in asthmatic children receiving IT were found, suggesting decreased activity of peripheral T lymphocytes [20–22]. In a study by Hsieh, a decreased production of IL-2 was found in antigen-stimulated lymphocytes of asthmatic children receiving IT, while their lymphocytes showed increased responsiveness to IL-2 [3].

Although IL-4 was not detectable in most cultures, more children of the non-IT group showed detectable IL-4, although statistically not different. Furthermore, a small, but significant difference was found in IL-4 secretion between both groups; the secretion of IL-4 was reduced in the IT group after 24 h and 48 h in the HDM cultures compared with the non-IT group. These results are in agreement with those from recent studies by Secrist *et al.* and by O'Brien *et al.* showing a decreased production and expression of IL-4 by peripheral lymphocytes after IT [12,19]. Therefore, it can be assumed that IT is able to

Table 5. Values of IL-12 (pg/mL) in the two groups

IL-12 in:	IT group	Non-IT group	P-value
HDM culture after 24 h	2.00 (0–8.3)	1.90 (0–7.9)	0.915
HDM culture after 48 h	1.90 (0–13.4)	6.40 (0–49.2)	0.206
HDM culture after 7 days	6.30 (0.4–29.5)	8.95 (0.4–272.5)	0.562

induce a switch from TH₂ to TH₁, leading to less stimulation of B cells to produce HDM-specific IgE. However, in the present study HDM-specific IgE and skin test with HDM did not differ between the two groups. Therefore, it seems that the effect of IT is too small, at least in our patients, to result in a decrease of HDM-specific IgE or that other non-IL-4 regulated mechanisms are involved in maintaining the formation of HDM-specific IgE.

A decreased secretion of IL-5 was found in the HDM-stimulated PBMC of subjects receiving IT. This finding suggests decreased ability to stimulate eosinophils by IL-5 which is in concordance with studies demonstrating decreased activity of eosinophils, including decreased levels of eosinophil cationic protein (ECP), in patients receiving IT [7,23]. The consequence of this decreased stimulation of eosinophils is the inhibition of bronchial inflammation, as shown by a decreased ability to develop late asthmatic reactions after allergen provocation in patients receiving IT [4,6,15].

In an animal model it has been shown that IL-12 is able to suppress the antigen-induced airway eosinophilic infiltration and bronchial hyperresponsiveness [24]. Furthermore, it was suggested that the effectiveness of IT might be due to an IT-induced switch from a TH₂ to TH₁ lymphocytic profile, due to an increased secretion of IL-12 by resident macrophages [11]. In the present study, however, we could not find any difference in IL-12 secretion between subjects receiving IT and subjects who never received IT. We speculate that this is due to the applied technique, being the *in vitro* secretion of IL-12 by PBMC, while it has been assumed that resident macrophages are responsible for the main secretion of IL-12 [11,25].

In conclusion, these results show a different *in vitro* cytokine secretion of PBMC of asthmatics receiving IT compared with asthmatics who never received IT. PBMC of patients receiving IT secrete less IL-2, IL-4 and IL-5 after *in vitro* incubation with HDM. An important immunological effect of IT may be a modification of cytokine secretion of PBMC. However, further longitudinal studies on larger groups of patients are needed to confirm these results.

References

- 1 Van Bever HP, Bridts CH, Moens MM et al. Lymphocyte transformation test with house dust mite (*Dermatophagoides pteronyssinus*) in normal children, asthmatic children and asthmatic children receiving hyposensitisation. *Clin Exp Allergy* 1993; 23:661–8.
- 2 Bonno M, Fujisawa T, Iguchi K et al. Mite-specific induction of interleukin-2 receptor on T lymphocytes from children with mite-sensitive asthma: modified immune response with immunotherapy. *J Allergy Clin Immunol* 1996; 97:680–8.
- 3 Hsieh KH. Altered interleukin-2 (IL-2) production and responsiveness after hyposensitization to house dust. *J Allergy Clin Immunol* 1985; 76:188–94.
- 4 Warner JO, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitisation to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978; 2:912–5.
- 5 Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 1985; 76:729–34.
- 6 Van Bever HP, Bosmans J, De Clerck LS, Stevens WJ. Modification of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*) or grass pollen. *Allergy* 1988; 43:378–85.
- 7 Furin MJ, Norman PS, Creticos PS et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991; 88:27–32.
- 8 Iliopoulos O, Proud D, Adkinson NF et al. Effects of immunotherapy on the early, late and challenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; 87:855–66.
- 9 Varney VA, Hamid QA, Gaga M et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; 92:644–51.
- 10 Durham SR, Ying S, Varney VA et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon- γ . *J Allergy Clin Immunol* 1996; 97:1356–65.
- 11 Durham SR, Schotman E, Jacobson M, Walker S, Hamid Q. Grass pollen immunotherapy (IT): inhibition of allergen-induced late skin responses (LPR) is associated with an increase in IL-12 messenger RNA+ cells {abstract}. *J Allergy Clin Immunol* 1995; 95:304.
- 12 Secrist H, Chelen CJ, Wen Yan Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993; 178:2123–30.
- 13 Hsieh KH, Chou CC, Chiang BL. Immunotherapy suppresses the production of monocyte chemotactic and activating factor and augments the production of IL-8 in children with asthma. *J Allergy Clin Immunol* 1996; 98:580–7.
- 14 Sheffer AL. Guidelines for the diagnosis and management of asthma. I. Definition and diagnosis. *J Allergy Clin Immunol* 1991; 88:427–38.
- 15 Van Bever HP, Stevens WJ. Effect of hyposensitisation upon the immediate and late asthmatic reaction and upon histamine reactivity in patients allergic to house dust mite (*Dermatophagoides pteronyssinus*). *Eur Resp J* 1992; 5:318–22.
- 16 Kay AB, Durham SR. T lymphocytes, allergy and asthma. *Clin Exp Allergy* 1991; 21 (Suppl. 1):17–21.
- 17 Chang JH, Chan H, Quirce S et al. In vitro T-lymphocyte response and house dust mite-induced bronchoconstriction. *J Allergy Clin Immunol* 1996; 98:922–31.
- 18 Jutel M, Pichler WJ, Skrbic D et al. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ in specific allergen-stimulated T cell cultures. *J Immunol* 1995; 154:4187–94.

- 19 O'Brein RM, Byron KA, Varigos GA, Thomas WR. House dust mite immunotherapy results in a decrease in Der p2-specific IFN- γ and IL-4 expression by circulating T lymphocytes. *Clin Exp Allergy* 1997; 27:46–51.
- 20 Hsieh KH. Decreased production of interleukin-2 receptors after immunotherapy to house dust. *J Clin Immunol* 1988; 8:171–7.
- 21 Tsai LC, Tang RB, Hung MW, Chen HM, Tsai SJ. Expression of serum IL-, 2 IL-, 2R, CD8 levels during hyposensitisation in house-dust sensitive asthmatics. *J Asthma* 1990; 27:307–13.
- 22 Moens MM, Van Bever HP, Stevens WJ et al. Influence of hyposensitization on soluble interleukin-2 receptor, eosinophil cationic protein, in vitro lymphocyte proliferation, in vitro lymphocyte adhesion and lymphocyte membrane markers in childhood asthma. *Allergy* 1994; 49:653–8.
- 23 Rak S, Björnson A, Hakanson L, Sörenson S, Venge P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 1991; 88:878–88.
- 24 Kips JC, Brusselle GJ, Joos GF et al. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1996; 153:535–9.
- 25 Fan X, Sibalic V, Niederer E, Wuthrich RP. The proinflammatory cytokine interleukin-12 occurs as a cell membrane-bound form on macrophages. *Biochem Biophys Res Commun* 1996; 225:1063–7.