

Original article

Sublingual immunotherapy in children modulates allergen-induced *in vitro* expression of cytokine mRNA in PBMC

Background: During subcutaneous immunotherapy (SCIT), there is a local mucosal shift from Th2 to Th1 type cytokine predominance and downregulation of interleukin (IL)-5 and eosinophilia. According to recent studies IL-10- and transforming growth factor (TGF)- β -induced tolerance is another key phenomenon in SCIT. Few data to date is available on mechanisms and roles of these cytokines in sublingual immunotherapy (SLIT).

Scope: This study was undertaken to analyse the allergen-induced *in vitro* mRNA expression of IL-4, IL-5, IL-10, TGF- β and interferon (IFN)- γ during SLIT in peripheral blood mononuclear cells (PBMC) of children with allergic rhinitis (AR).

Methods: Ten patients with AR undergoing pollen SLIT with a weekly dose of 200 000 SQ-U, 10 with a weekly dose of 24 000 SQ-U of glycerinated mixture of *Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa* and 10 with placebo were included in the study. Peripheral blood mononuclear cell samples were collected and stimulated with pollen allergen extract prior to the treatment, after 1 and 2 years of the treatment. The cytokine mRNA expression was assessed using kinetic real time reverse transcription polymerase chain reaction (RT-PCR; TaqMan[®]).

Results: The *in vitro* allergen-induced mRNA expression of IL-5 by PBMC in the placebo group at 1 ($P = 0.0065$) and 2 ($P = 0.013$) years of therapy were increased in comparison with the highest dose. The expression of IL-10 mRNA was increased in the highest dose group ($P = 0.0016$) and the lower dose group ($P = 0.034$) at 2 years of therapy when compared with placebo. The change in the expression of allergen-induced TGF- β had an inversed correlation with the change of IL-5 ($r = -0.38$, $P = 0.036$) and positive correlation with the change of IL-10 ($r = 0.58$, $P = 0.0019$).

Conclusions: Sublingual immunotherapy induced a dose-dependent systemic allergen-specific immunological response in children with AR. During high-dose SLIT, there was activation of regulatory cytokine IL-10 and an inhibitory effect on IL-5 expression increase that was associated with TGF- β .

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Key words: allergic rhinitis; specific immunotherapy; Th1 cells; Th2 cells; regulatory T cell.

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Accepted for publication 16 June 2006

Allergic rhinitis (AR) is a common disorder with usually seasonal mucosal inflammation characterized by mast cell and eosinophil activation. The inflammation displays a predominant interleukin (IL)-4 expression (Th2 cells) in CD4⁺ T cells over interferon (IFN)- γ expression (Th1 cells). The clinical effect of sublingual immunotherapy (SLIT) has been shown in several studies. Of the 22 double blind placebo controlled (DBPC) SLIT trials published in peer-reviewed journals, all but three have confirmed statistically the clinical efficacy of SLIT with the most common allergens: grass, mites, birch and parietaria (1, 2).

Interleukin-10 is an anti-inflammatory cytokine, produced by T cells and dendritic cells that downregulates both Th1 and Th2 type responses (3–5). In addition, IL-10 has direct effects on T cells, including induction of

non-responsive anergic state (6). Furthermore, IL-10 has inhibitory effects on mast cells and basophils (7, 8). Transforming growth factor (TGF)- β is another regulatory, pleiotropic cytokine that affects the growth, differentiation and function of a variety of cells, including T cells (9). Transforming growth factor- β antagonizes both Th1 and Th2 type inflammatory responses and is an essential cytokine in the immunosuppression induced by oral tolerance (9–11). CD4⁺ T cells secreting IL-10 and TGF- β have been designated regulatory T cells (Treg) and further divided into two subsets: Tr1 cells with predominance of IL-10 production and Th3 cells with predominance of TGF- β production (11–13). Interleukin-5 is a crucial cytokine in maturation, growth and activation of eosinophils (14).

In patients with AR treated with subcutaneous immunotherapy (SCIT) there is a local mucosal shift from Th2 to Th1 type cytokine predominance (15). Expression of IL-5 mRNA in the nasal mucosa is increased in AR and decreased after SCIT (16, 17). It has been firmly established that IL-10-induced tolerance is a key phenomenon in venom immunotherapy (VIT) and recently it has been shown that IL-10, TGF- β and CD4+CD25+ Treg are involved also in SCIT (18–22). Although their important role in SCIT is well established, there are to date no reports on the expression of these cytokines during SLIT.

This study was undertaken to investigate the allergen-induced *in vitro* mRNA expression of Th1 type effector cytokine IFN- γ , Th2 type cytokines IL-4 and IL-5 and regulatory cytokines IL-10 and TGF- β during SLIT in allergic children suffering from rhinoconjunctivitis caused by allergy to tree pollen.

Material and methods

Patients

Thirty children aged 5–15 years with a clinical history of tree pollen-induced allergic rhinoconjunctivitis with/without seasonal asthma for at least 2 years were randomly selected from a single centre, randomized, double-blind, placebo-controlled dose–response phase II trial including tree pollen allergic children [positive skin prick test (SPT) ≥ 3 mm to tree mix and either one of *Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa*; Soluprick® SQ 10 HEP, (ALK-Abelló, Hørsholm, Denmark) or positive specific immunoglobulin (Ig)E to tree mix and either one of *B. verrucosa*, *C. avellana* and *A. glutinosa*; Magic Lite® SQ, $\geq$ class 2; 23]. The patient characteristics are presented in Table 1. The study was performed with the approval of the local ethics committee and with the parents' written informed consent.

Sublingual immunotherapy

The allergen extract used for SLIT was a tree pollen extract of SQ-standardized *B. verrucosa* (birch), *C. avellana* (hazel) and *A. glutinosa* (alder). The children were randomized into three groups receiving SLIT up to 18 months. Ten children were included in each

group. Distributed on 5 days each week, the patients received the following maintenance doses.

Dose group 1. Maintenance phase using a concentration of 12 000 SQ-U/ml. The accumulated weekly dose corresponded to 24 000 SQ-U or 3.6 μ g major allergen Bet v 1/Aln g 1/Cor a 1.

Dose group 2. Maintenance using a concentration of 100 000 SQ-U/ml. The accumulated weekly dose corresponded to 200 000 SQ-U or 30 μ g major allergen Bet v 1/Aln g 1/Cor a 1.

Placebo. The placebo medication was for the up-dosing and maintenance phase a diluent containing 50% glycerol and 50% saline buffer.

The detailed description of the SLIT treatment is described elsewhere (23). The blood samples of the 30 patients were collected, during pollen-free season, before SLIT was commenced and after 1 and 2 years from the start of the treatment.

Clinical evaluation: patient diary

Symptom and medication data were collected using a patient diary as described elsewhere (23). Briefly, the patient diary was filled in during the tree pollen season for 10 weeks and included the following parameters registered daily: nose symptoms (runny nose, sneezing, blocked nose), eye symptoms (streaming and swelling, redness and itching) and lung symptoms (breathlessness, cough, wheeze and chest tightness).

A telephonic survey of the study patients' parents assessing asthmatic symptoms and use of asthma medication was conducted 5 years after the commencement of the study. The survey questionnaire listed the possible use of any asthma medication during the past 12 months, including the latest prescribed medication and dosage, use of β -agonists during the last 2 weeks and the number of months on inhaled corticosteroids. In addition daytime, nocturnal and exercise-induced cough, dyspnoea and wheezing during the last 12 months was registered.

Late phase skin reaction

Late phase skin reaction (LPSR) was performed with an allergen solution (0.05 ml corresponding to 50 SQ-U Tree Mix; Aquagen SQ®; ALK-Abelló) injected intracutaneously. The early skin reaction was measured after 15 min and recorded. The LPSR was measured in a similar way after 24 h. A control intradermal injection using albumin diluent was performed for evaluation of unspecific reactions. The early and LPSR sizes were determined by using a computerized scanner. Only the LPSRs were analysed.

Lymphocyte cultures

The peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by Ficoll (Ficoll-Hypaque; Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. After washing, the mononuclear cells were suspended in RPMI (Gibco Life Technologies, Paisley, UK), supplemented with 5% autologous serum, 2 mM L-glutamine (Gibco Life Technologies), 100 U/ml penicillin and 100 mg/ml gentamicin (Nordvacc Media Skärholmen, Sweden). The cultures were incubated at +37°C, in a humidified atmosphere with 5% CO₂. The cells rested for 3 days. The rested cells were applied on the wells, 2×10^5 cells per well in duplicate. For allergen stimulation there were thus 2×10^5 cells in 400 μ l/well on 48-well plates. The allergen concentration of the whole extract of birch

Table 1. Study patients: baseline demographic data

	Weekly dose (SQ-U)		
	Dose group 2 (200 000)	Dose group 1 (24 000)	Placebo
n	10	10	10
Gender (M/F)	5/5	4/6	5/5
Mean age (SD; years)	10.2 (2.3)	7.8 (2.2)	11.1 (3.4)
Mean symptoms* after SLIT year 2000 (SD)	1.9 (1.6)	3.2 (2.7)	3.6 (3.4)
Mean LPSR (SD) 1998	3164 (2270)	2733 (1475)	3761 (1877)
1999	1274 (1088)†	2664 (1599)	3176 (1656)
2000	895 (505)	1324 (1080)	1916 (1577)

LPSR, late phase skin response; SLIT, sublingual immunotherapy.

*Symptom score index.

†200 000 vs placebo ($P = 0.012$).

(*B. verrucosa*), kindly provided by ALK-Abelló (Hørsholm, Denmark), in the final volume of culture medium was 50 µg/ml, based on assessment in preliminary experiments. As controls, the cells were cultured in the presence of purified protein derivative (PPD) (10 µg/ml; Statens Seruminstitut, Copenhagen, Denmark) and medium alone. The cultures were incubated for 3 days. After centrifugation the culture supernatants were stored at -70°C and the cell pellets were collected, lysed and frozen at -70°C in 500 µl of Trizol (Gibco Life Technologies) solution for RNA extraction.

Cytokine mRNA detection by RT-PCR (TaqMan®)

The RNA isolation and the TaqMan® (Applied Biosystems, Foster City, CA, USA) reverse transcription polymerase chain reaction (RT-PCR) were performed as previously described (24). Briefly, RNA was isolated according to Trizol instructions and the extracted RNA was stored in 75% ethanol at -20°C. The RT reaction was performed with First-Strand cDNA Synthesis Kit (Pharmacia Biotech) using oligo (dT) primers, and cDNA was stored at -70°C. The amplification of β-actin, IL-4, IL-5, IL-10, IFN-γ and TGF-β cDNA was performed in MicroAmp® optical 96-well reaction plate (Applied Biosystems, Foster City, CA, USA). A negative PCR control without template and a positive PCR control with a template of known amplification were included in each assay. The reaction was performed in ABI PRISM 7700 Sequence Detection System (Applied Biosystems). For analysis of IL-5, IL-10 and TGF-β predeveloped assay reagents (PDAR), kits were purchased from Applied Biosystems. The primer and probe sequences of β-actin, IL-4 and IFN-γ were designed to be cDNA-specific and to work under equivalent reaction conditions using PRIMER EXPRESS software (Applied Biosystems) and were labelled with FAM (6-carboxyfluorescein) at the 5'-end and with TAMRA (6-carboxytetramethylrhodamine) at the 3'-end as previously described (24). During PCR, the Ct values (the cycle number at which the detected fluorescence exceeds the threshold) for each amplification product were determined using a threshold value of 0.03. The cytokine-specific signals were normalized by constitutively expressed β-actin signals using the formula $2^{-\Delta Ct} = 2^{-(Ct, \beta\text{-actin} - Ct, \text{Cytokine})}$. The cytokine stimulation indices were thereafter calculated by the formula $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct \text{ for stimulated culture} - \Delta Ct \text{ for unstimulated culture})}$ as described in ABI PRISM 7700 SDS relative quantitation of gene expression protocol by PE Applied Biosystems.

Statistics

The Wilcoxon signed rank test was used for paired comparisons within the individuals and the Spearman rank order correlation test for paired comparison of groups. The Mann-Whitney *U*-test was used for unpaired comparison of groups.

Results

The *in vitro* allergen-induced mRNA expression of IL-5 and ratio of IL-4/IFN-γ mRNA expression by PBMC at three time points in the three study groups are illustrated in Fig. 1. There is a significant difference between the placebo group and the dose group 2 at 1 ($P = 0.0065$) and 2 ($P = 0.013$) years in IL-5 mRNA expression. Low levels of IL-5 are maintained in the dose group 2 throughout the study. There are no significant differences

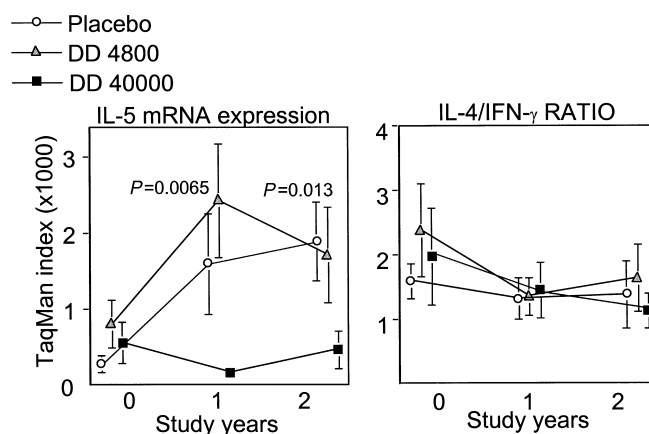


Figure 1. *In vitro* allergen-induced interleukin (IL)-5 mRNA expression and the ratio of *in vitro* allergen-induced mRNA expression of IL-4 and interferon (IFN)-γ by peripheral blood mononuclear cell (PBMC) cultures of study patients assessed with real time kinetic reverse transcription polymerase chain reaction (RT-PCR; TaqMan®). The expression is shown as TaqMan index (IL-5), indicating fold increase in comparison with no stimulus, or ratio of TaqMan indices (IL-4/IFN-γ). The mean and SEM are shown separately for patients treated in dose group 2 (black square), dose group 1 (grey triangle) and placebo (white circle) before the treatment and after 1 and 2 years of therapy. The *P*-values refer to non-parametric unpaired comparison of dose group 2 to placebo group at each time point (Mann-Whitney *U*-test).

in IL-4/IFN-γ ratio between the treatment groups at any time point. No significant differences are either seen when IL-4 and IFN-γ are studied separately.

The *in vitro* allergen-induced mRNA expression of regulatory cytokines IL-10 and TGF-β by PBMC are illustrated in Fig. 2. There is a significant difference between the placebo group and the dose group 2 ($P = 0.016$) and between the placebo group and the dose group 1 ($P = 0.034$) at 2 years in IL-5 mRNA expression. The expression of TGF-β mRNA remains low. However, when changes in the expression of allergen-induced TGF-β from commencement to 1 year is compared with the changes in mRNA expression of IL-5 and IL-10, it appears that TGF-β has an inversed correlation with IL-5 ($r = -0.38$, $P = 0.036$) and positive correlation with IL-10 ($r = 0.66$, $P < 0.0001$; Fig. 3). The best correlation between changes in TGF-β and IL-10 is seen in the highest dose group (dose group 2; $r = 0.79$, $P = 0.0045$). A good correlation is also seen between the TGF-β and IL-10 mRNA expression after 1 year of therapy ($r = 0.61$, $P = 0.0002$). No correlations were observed between IL-5 and IL-10. No differences between time points or treatment groups were seen in PPD-induced cytokine mRNA expressions (results not shown). PPD stimulation resulted in slightly decreased IL-10 mRNA expression when compared with unstimulated in all groups at all time points.

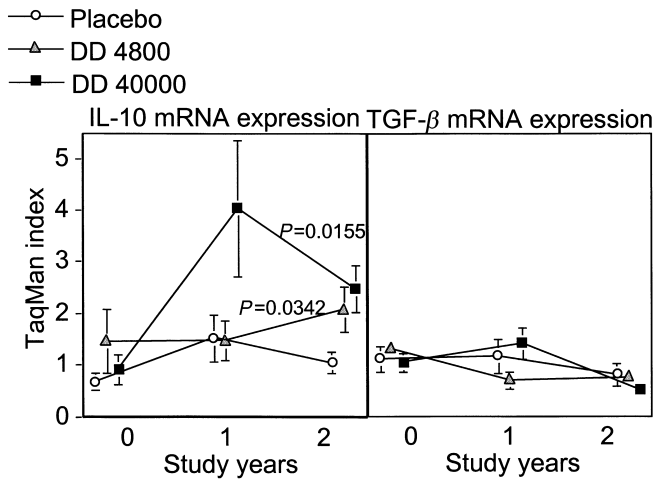


Figure 2. *In vitro* allergen-induced interleukin (IL)-10 and transforming growth factor (TGF)- β mRNA expression in peripheral blood mononuclear cell (PBMC) cultures of the study patients assessed with real time kinetic reverse transcription polymerase chain reaction (RT-PCR; TaqMan®). The expression is shown as TaqMan index, indicating fold increase in comparison with no stimulus. The mean and SEM are shown separately for patients treated in dose group 2 (black square), dose group 1 (grey triangle) and placebo (white circle) before the treatment and after 1 and 2 years of therapy. The *P*-values refer to non-parametric unpaired comparison with placebo group at each time point (Mann-Whitney *U*-test).

○ Placebo
△ DD 4800
■ DD 40000

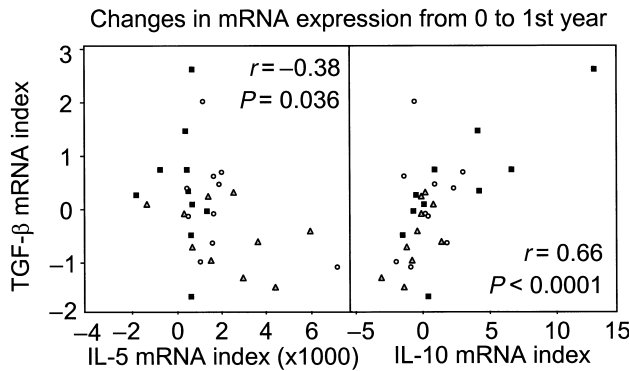


Figure 3. The correlations of the observed change in cytokine expression from baseline to 1 year. Change of transforming growth factor (TGF)- β mRNA expression is compared with that of interleukin (IL)-5 and IL-10 mRNA expression. The expression is shown as TaqMan index, indicating fold increase in comparison with no stimulus. The plot symbols are shown separately for patients treated in dose group 2 (black square), dose group 1 (grey triangle) and placebo (white circle).

The comparison of the IL-5 mRNA expression and the symptom score at 2 years is illustrated in Fig. 4. A good correlation is seen only in the dose group 2 ($r = 0.69$,

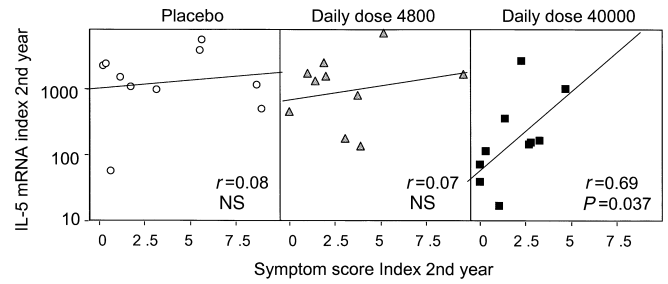


Figure 4. The correlations of the symptom score index and interleukin (IL)-5 after second study year. IL-5 mRNA expression is expressed as TaqMan index, indicating fold increase in comparison with no stimulus. The correlations are shown separately for patients treated in dose group 2 (black square), dose group 1 (grey triangle) and placebo (white circle).

○ Placebo
△ DD 4800
■ DD 40000

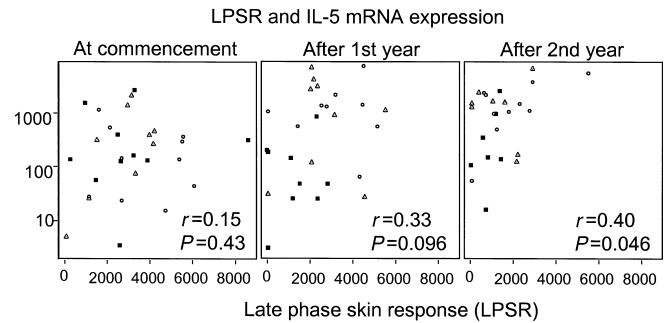


Figure 5. The correlations of the late phase skin response and interleukin (IL)-5 at commencement, after the first study year and after the second study year. IL-5 mRNA expression is expressed as TaqMan index, indicating fold increase in comparison with no stimulus. The correlations are shown separately for patients treated in dose group 2 (black square), dose group 1 (grey triangle) and placebo (white circle).

$P = 0.037$). Least symptoms and lowest IL-5 levels are seen in the actively treated group. The correlations between IL-5 mRNA expression and LPSR at different time points are illustrated in Fig. 5. A good correlation is established at 2 years ($r = 0.40$, $P = 0.045$) mainly because both LPSR and IL-5 are low in the actively treated groups while they are high in the placebo group.

Based on the telephonic survey 5 years after the 30 study subjects could be divided into two groups, one group with no signs of bronchial asthma ($n = 16$) and another group being on inhaled steroids and/or having had asthmatic wheezing during the past 12 months. Some patients on inhaled steroids were asymptomatic. The IL-4/IFN- γ mRNA expression ratio, IL-5 mRNA expression and the LPSR at the three time points, the symptoms and medication at 2 years and the number of patients in each treatment group are shown in Table 2. It appears that a significant decrease of IL-4/IFN- γ ratio ($P = 0.03$)

Table 2. Comparison of study parameters according to later (2003) reported bronchial asthma

	Year	No asthma	WSR	Asthma	WSR	MWU
IL-4/IFN- γ *	1998	2.56 (0.60)		1.31 (0.25)		0.20
	1999	1.51 (0.32)	0.23	1.20 (0.22)	0.98	0.59
	2000	1.26 (0.29)	0.03	1.52 (0.44)	0.78	0.84
IL-5†	1998	546 (186)		528 (234)		0.71
	1999	1201 (489)	0.33	1613 (570)	0.05	0.18
	2000	934 (230)	0.12	1828 (564)	0.04	0.23
LPSR	1998	3120 (551)		3220 (425)		0.63
	1999	1671 (384)	0.01	3246 (407)	0.94	0.02
	2000	785 (192)	0.002	2151 (381)	0.02	0.003
Symptoms‡	2000	1.66 (0.33)		4.29 (0.84)		0.02
Medication§	2000	2.17 (0.96)		5.69 (1.49)		0.02
Group						
Dose group 2						
200 000¶ (n = 10)		8		2		
Dose group 1						
24 000¶ (n = 10)		4		6		
Placebo (n = 10)		4		6		

WSR, Wilcoxon signed rank test (paired comparison with year 1998); MWU, Mann-Whitney U-test (unpaired comparison between the groups); LPSR, late phase skin response; IL, interleukin; IFN, interferon. Statistically significant values in bold.

*mRNA expression ratio.

†IL-5 mRNA TaqMan index indicating fold increase.

‡Symptom score index.

§Medication score index.

¶Weekly dose (SQ-U).

is seen in the non-asthma group at 2 years and an increase in IL-5 mRNA expression is seen in the asthma group at 1 ($P = 0.048$) and 2 ($P = 0.036$) years. No differences were seen in TGF- β and IL-10 mRNA expression. LPSR decreased in both groups but less in the asthma group so that a significant difference between the groups is seen at 1 ($P = 0.02$) and 2 ($P = 0.003$) years. Both symptom ($P = 0.02$) and medication ($P = 0.02$) scores at 2 years are higher in the asthma group than in the non-asthma group. The majority of the subjects in the dose group 2 (the highest dose group, eight of 10) are in the non-asthma group while the majority of the subjects in the low dose and placebo groups (12 of 20) are in the asthma group ($P = 0.038$, chi-square test).

Discussion

Our study demonstrated that SLIT had systemic effects demonstrated in PBMC. In addition, a clear dose-response effect was demonstrated. The important role of regulatory cytokine IL-10 in the highest dose SLIT and in addition the inhibition of upregulation of IL-5 was demonstrated, but no changes were seen in the actual IL-4/IFN- γ ratio.

The importance of the regulatory cytokine IL-10 in VIT and SCIT for pollen allergy has been demonstrated in several studies (18–22). For the first time, we have demonstrated that IL-10 has a role in SLIT of pollen

allergic children. Recently an increase in PHA-induced IL-10 production was demonstrated in house dust mite SLIT in young adults (25). Data taken together with earlier studies suggest that in SLIT for respiratory allergy, as in VIT and subcutaneous immunotherapy, upregulation of regulatory cytokines, especially IL-10 is essential, and the regulatory elements in peripheral blood may be similar in different form for immunotherapy (15, 18–29). Interleukin-10 is a T cell-derived cytokine that downregulates both Th1 and Th2 type responses and appears to suppress both IgE-mediated inflammation and delayed type hypersensitivity (DTH)-type inflammation (3–5). It can also, together with IFN- γ , decrease the release of histamine and other mediators from mast cells and basophils (7, 8). Interleukin-10 has been regarded as the main factor in the peripheral tolerance observed in VIT (18, 19). Also this peripheral tolerance is an important phenomenon in subcutaneous injections allowing the repeated injections of high allergen doses (20, 21). During SCIT, an early IL-10 response has been associated with good therapeutic outcome, whereas in SLIT no systemic changes could be seen in cytokine responses during dose build-up phase or early maintenance period (30). The timing of the regulatory response might thus differ between the two routes of immunotherapy.

From several studies in animal models, it is well known that the oral route is 'tolerogenic' and can redirect the Th1/Th2 differentiation (10, 31, 32). By using radiolabelled purified allergens, it has been shown that the contact of the allergen with the oral mucosa is critical and that the allergen is not directly absorbed in the mouth but is retained for up to 40 h in the mouth. On the contrary, the chemically modified allergen (monomeric allergoid) was detected unmodified in traces in plasma, whereas in the gut the allergen seems to be absorbed as peptides, not as native protein (33, 34).

Transforming growth factor- β is an essential cytokine in the immunosuppression induced by oral tolerance and it antagonizes both Th1 and Th2 type inflammatory responses (9–11). There were no consistent significant changes in the TGF- β mRNA expression during the study. Yet, it has been recently suggested that, in addition to IL-10, increased TGF- β is associated with the tolerance regulation in SLIT and the cytokines co-operate in the regulation (20). It has been shown that IL-10 enhances TGF- β receptor II expression and consequently T-cell responsiveness to TGF- β (35). The regulation of these two cytokines are thus connected together, with IL-10 having a regulatory role over TGF- β responses. We demonstrated a significant correlation between the changes in IL-10 and TGF- β mRNA expression, mainly because in the lower dose and placebo groups, the IL-10 and TGF- β mRNA levels decreased or showed little increase. In the highest dose group there were clear increases of IL-10 and TGF- β mRNA expressions that furthermore showed good correlation with each other. TGF- β is a cytokine that is secreted by monocytes,

macrophages and lymphocytes as a latent isoform that forms a complex with latent TGF- β -binding protein. Bioactive TGF- β is generated by release of TGF- β from the complex. Macrophages and plasmin and urokinase are essential in this activation process (36). The secretion and extracellular activation of the latent isoform of TGF- β is essential for its function. Therefore, the slight changes observed in the expression of TGF- β mRNA may be significant.

There is no prospective data available on allergen-induced IL-5 or TGF- β mRNA expression as a function of age in children with AR. Our data suggest that AR could imply gradually increasing levels of allergen-induced IL-5 mRNA expression. The increased IL-5 expression was clearly inhibited by high-dose SLIT while only a trend but no statistically significant decrease was seen in IL-4/IFN- γ ratio. A relatively small sample size and lower levels of allergen-induced IL-4 mRNA expression when compared with IL-5 may explain the results partly. Decreased serum levels of IL-13, another Th2 type cytokine, have been observed during SLIT (37). Interleukin-5 is a key cytokine in eosinophil differentiation and activation and subsequent development of mucosal inflammation in AR and asthma (14, 16, 38). It is known that SCIT performed at early age prevents later development of bronchial asthma (39). It is possible that the mechanism behind this preventive effect is the inhibition of a 'natural' increase of IL-5 levels and consequent eosinophil maturation and accumulation on bronchial mucosa. Previously the anti-inflammatory effects of SLIT have been shown by observation that SLIT decreases the mucosal expression of the intracellular adhesion molecule (ICAM-1) and, as a consequence, the inflammatory

infiltration (40). There was a negative correlation between the observed change of IL-5 and TGF- β mRNA expression mainly because of increased IL-5 and decreased TGF- β mRNA observed in the dose group 2 and decrease or no change in TGF- β and increase or no change in IL-5 in the dose group 1 and placebo group. Transforming growth factor- β mRNA appeared to decrease by age and the highest dose SLIT inhibited this decrease. Even though the function of bioactive TGF- β can only be distantly associated with mRNA expression, the observed minimal decrease of TGF- β mRNA expression and associated increase of IL-5 at a certain age may be phenomena in the development of bronchial asthma in children with AR. It seems that inhibition of IL-5 mRNA expression and thereby inhibition of eosinophil maturation and activation in the bronchial mucosa may be behind the prevention of bronchial asthma observed in our patients. The results from our non-validated telephonic survey of the asthma symptoms and medication use 5 years after showed that signs of asthma were seen in patients who, at the time of the study, had most symptoms and medication and who remained with elevated levels of LPSR, IL-5 mRNA expression and IL-4/IFN- γ ratio.

During SLIT using a high concentration of SQ-standardized allergen there is increased expression of regulatory cytokine IL-10 mRNA and in addition an inhibitory effect on IL-5 mRNA expression increase that is associated with TGF- β mRNA expression. These cytokine responses may contribute to downregulation of the allergic inflammation decreasing symptoms and LPSR by treatment. It remains to be investigated whether these phenomena eventually decrease the risk for later outcome of bronchial asthma.

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