

The effect of treatment with montelukast on *in vitro* interleukin-10 production of mononuclear cells of children with asthma

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Summary

Background The effects of leukotriene modifiers on IL-10 production have not been studied in children with asthma.

Objective The primary objective of this study was to determine the changes in IL-10 concentrations, clinical efficacy and peripheral blood eosinophil counts after treatment with montelukast.

Methods The study was conducted on 27 patients: 13 patients monoallergic to grass pollen during the pollen season (GPs group) and out of the pollen season (GPos group), and on 14 patients monoallergic to house dust mite (HDM) from May to September (HDM group). Main outcome measures were changes in concentrations of IL-10 in the supernatant after a 4-week treatment with montelukast. Measurements of asthma severity score, forced expiratory volume in 1 s (FEV₁) and peripheral blood eosinophil counts were the secondary end-points.

Results Montelukast resulted in a within-group significant increase in IL-10 concentration in the supernatant in the GPs (54.0 vs. 125.5 pg/mL) and in the HDM (51.2 vs. 77.1 pg/mL) group. Montelukast had no effect on changes of IL-10 concentration in the supernatant from peripheral blood mononuclear cell (PBMC) culture after non-sensitizing allergen stimulation. Montelukast significantly improved asthma control and FEV₁, and significantly decreased eosinophil blood count in the GPs and in the HDM group after a 4-week treatment. Montelukast did not lead to changes of all measured parameters within the GPos group.

Conclusion Montelukast increased IL-10 concentration in supernatants from sensitizing allergen-stimulated PBMC culture obtained from children with asthma monoallergic to grass pollen during the pollen season, and from children with asthma monoallergic to HDM.

Keywords childhood asthma, IL-10, montelukast

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Introduction

The normal respiratory tract is characterized by elevated concentrations of IL-10 [1]. IL-10 levels are reduced in asthmatic airways; a regulatory mechanism involving IL-4-induced allergen-specific IL-10 production might be defective in allergic subjects, potentially contributing to more intense inflammation [2]. Subsequent studies have demonstrated IL-10 production by human T-helper types 1 and 2 (Th1 and Th2) lymphocytes, by cytotoxic T cells, mast cells, monocytes, B lymphocytes, keratinocytes, eosinophils and various tumour cells [3]. Monocytes and B lymphocytes are major sources for human IL-10 [3, 4].

IL-10 inhibits proliferation of T cells activated by anti-CD3 antibodies in the absence of antigen-presenting cells. This occurs through an inhibition of IL-2 production by T cells at

the transcriptional level [3]. Some studies indicate that IL-10 does not affect the proliferative responses of T cells that were stimulated by anti-CD3. IL-10 can suppress cytokine synthesis in T cells by inhibiting accessory CD28–B7.1 receptor interaction [5]. It has been shown that IL-10 inhibits the proliferative T cell response in peripheral blood mononuclear cell (PBMC) to various antigens, and the superantigen staphylococcal enterotoxin B [5]. Lymphocyte-derived TNF- α production is also inhibited by IL-10; however, IL-4, INF- γ and granulocyte macrophage-colony stimulating factor (GM-CSF) are not affected significantly. In addition to its effects on Th1 and Th2 cytokines, IL-10 also inhibits pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, IL-12 and TNF- α), growth factors (GM-CSF and granulocyte colony-stimulating factor), and chemokine (macrophage-inflammatory protein-1 α) production by mononuclear phagocytes, as well as IFN- γ and TNF- α by natural killer cells. IL-10 has inhibitory effects on mast cells and basophils [6]. Several studies have established the ability of IL-10 to inhibit the release of the eosinophilopoietin and eosinophil-activating cytokine IL-5 by human T cells [7]. IL-10 also suppresses the lipopolysaccharide-induced generation of GM-CSF and

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TNF- α by eosinophils and consequently accelerates eosinophil death [3, 7, 8].

The inhibitory effects of IL-10 are in contrast to its effect on B lymphocytes in which it functions as an activating factor that stimulates cell proliferation, IgG secretion and the γ -isotype switch. Other pro-inflammatory activities of IL-10 include the abilities to enhance IL-2- and IL-3-dependent T cell proliferation and the local accumulation of T cells *in vivo*. It has been shown that children with atopic asthma had lower Bla g2-induced IL-10 [9]. However, some studies demonstrate that elevated IL-10 levels are not associated with less allergic disease [10]. IL-10 may also promote airway hyper-responsiveness [11] and even eosinophilia [12] in allergic models.

Despite the pro-inflammatory role of IL-10, the observation that IL-10 is also produced by Th1 lymphocytes and that it inhibits cytokine production by Th2 lymphocytes has led to the concept that IL-10 might be beneficial in mitigating allergic inflammation. A noteworthy additional finding from one analysis was the inverse relationship between weal diameter and house dust mite (HDM)-specific IL-10 response [13]. Also, recent findings indicate that IL-10 is crucial in suppression of the immune response to aeroallergens and controls allergic inflammation due to mucosal allergen in specific immunotherapy [6, 14, 15]. Grass pollen immunotherapy results in a population of circulating T cells that express the IL-10⁺CD4⁺CD25⁺ phenotype in response to allergen restimulation [15] and in an increase in allergen-specific IL-10 mRNA expression in PBMC [6].

As IL-10 inhibits cytokines associated with cellular immunity and allergic inflammation, we have decided to investigate the effect of anti-asthma therapy on IL-10 in children. In our previous study, we demonstrated that treatment with the leukotriene (LT) antagonist – montelukast sodium – significantly increased the serum level of IL-10 in children with moderate asthma [16]. Those data suggested that one possible way by which anti-LTs contribute to inhibition of inflammation is by increasing IL-10 levels. It encouraged us to conduct the present study. We hypothesized that treatment with montelukast sodium increases the production of IL-10 by PBMC-stimulated *in vitro* with allergen. We measured the production of IL-10 by PBMC of children with mild-to-moderate atopic asthma, before and after 4 weeks of treatment with montelukast, while monitoring lung function, clinical severity score and eosinophil blood count.

Materials and methods

Patients

Inclusion criteria Male and female outpatients, aged 6–18 years living in the same geographical location, with a clinical diagnosis of bronchial asthma with a duration of at least 6 months before the first visit were enrolled. To become eligible for the active treatment period, patients and their parents were required to demonstrate an understanding of the diary and patients were required to be able to perform reproducible spirometry.

Exclusion criteria Study exclusions included active upper respiratory tract infection within 3 weeks and acute sinus

disease requiring antibiotic treatment within 1 month before the first visit. Additional criteria included other major clinically significant pulmonary, haematologic, hepatic, gastrointestinal, renal, endocrine, neurologic, cardiovascular and/or psychiatric diseases or malignancy that either put the patient at risk when participating in the study, or may influence the results of the study, or the patient's ability to participate in the study as judged by the investigator. Excluded medications were β -blockers (eye drops included), astemizole within 3 months or oral corticosteroids within 1 month before the first visit. Patients who were receiving immunotherapy were excluded.

Participants in the 8-week study comprised 27 children with a typical history of mild-to-moderate asthma, who were sensitive only to HDM ($n = 14$) or sensitive only to grass pollen ($n = 13$) as shown by positive skin-prick tests and by the presence of specific IgE to *Dermatophagoides pteronyssinus* (Der p) or *Phleum pratense* (Phl p). The diagnosis of asthma was established by symptoms of asthma and by improvement in the prebronchodilator forced expiratory volume (FEV₁) $\geq 12\%$ after administration of salbutamol (200 μ g). We defined mild and moderate asthma according to established guidelines [17].

For the purposes of this study, skin prick tests (Nexter-Allergopharma, Reinbek, Germany) to standard allergen extracts were performed; a mean weal diameter of ≥ 3 mm at 15 min was defined as a positive response. Serum-specific IgE levels were measured by using a Pharmacia CAP kit (Uppsala, Sweden); > 0.7 kU/L were defined as positive. Subjects were recruited from our Clinic Center in Zgierz, Poland.

Study design

The study was conducted on 13 patients who were mono-allergic to grass pollen (*P. pratense*) from June to July (during the pollens season) (GPs group), from November to January (out of the pollens season) (GPos group) and from May to September on 14 patients who were monoallergic to HDM (*D. pteronyssinus*) (HDM group). All subjects from the first group were treated at the same time of the season. The study design is presented in Fig. 1.

At the time of asthma diagnosis, anti-allergen measures were recommended for all patients and all children remained in the same environment during the study. For patients sensitive to HDM, anti-dust measures including removal of carpets, pillows and quilts with feathers; covering of mattresses with plastic; frequent cleaning with a wet mop; and keeping pets (if present) out of the bedroom environment, to reduce allergen exposure were recommended. For patients sensitive to grass pollen, during the pollen season, anti-allergen measures including avoiding the outdoors between 05:00 and –10:00 hours; keeping windows in the home and car closed; and drying clothes in an automatic dryer rather than hanging them outside were recommended.

This study assesses the effect of montelukast sodium (Singulair, MSD, Whitehouse Station, NJ, USA) on production of IL-10, by PBMC stimulated *in vitro* with sensitizing/non-sensitizing allergen. Children 6–14 years old received 5 mg and children > 14 years old received 10 mg oral tablet once daily at bedtime. The main outcome measures were changes in supernatant concentrations of IL-10; asthma

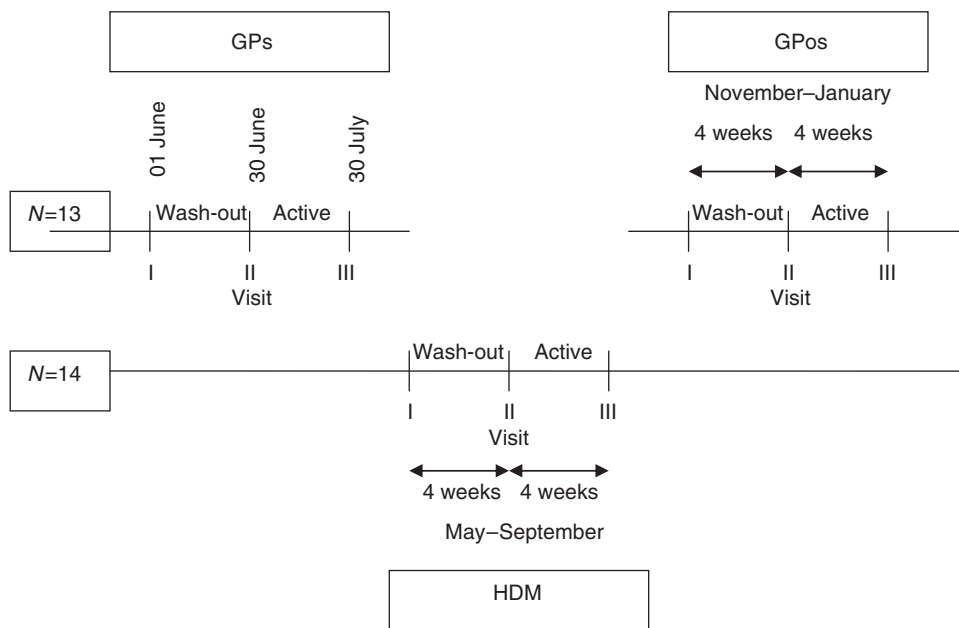


Fig. 1. Study design: The study was conducted on 13 patients monoallergic to grass pollen: during the pollen season (June–July) (GPs group) and out of the pollen season (November–January) (GPos group) and on 14 patients monoallergic to house dust mite (May–September) (HDM group). In all three groups, a 4-week wash-out period was followed by 4-week active period.

severity score, FEV₁ and peripheral blood eosinophil counts were the secondary end-points.

There were three study visits. At the first visit, previous asthma medications were withheld (inhaled steroids, long-acting β_2 -agonists, nedocromil sodium), and patients were administered β_2 -agonist (Ventolin, Glaxo Wellcome, Greenford, UK) 'as needed' for symptomatic relief. In addition, the patients were informed of the purpose of the study and were instructed in scoring of asthma symptoms at home on a daily diary card. At the second visit (4 weeks after the first visit), the subjects received montelukast. The third visit was after 4 weeks of treatment with montelukast. At visits 2 and 3, baseline spirometry was performed, and a blood sample was obtained for PBMC culture and measurement of eosinophil levels. Visits were scheduled so that blood sampling and lung function testing in individual patients always took place on the same day of the week and at the same time. Before lung function testing, short-acting β -agonists were withheld for 12 h. During the entire study, patients filled daily diary cards to assess symptoms score and every morning and evening they assigned themselves a score that expressed their asthmatic well-being; diary cards were evaluated at visits two and three.

Diary card

The daily diary card included daytime symptoms (completed at bedtime) and nocturnal awakening (recorded in the morning upon awakening) scales previously shown to have acceptable evaluative measurement properties [18]. In addition, the amount of as-needed β -agonist were recorded daily as the number of puffs. Daytime asthma symptoms and nocturnal awakenings were scored subjectively as follows: 0, no symptoms during the day/night; 1, symptoms, but they do not affect any activities during the day or night sleep; 2, symptoms that affect at least one daily activity or disturb night sleep and 3, symptoms that affect two or more daily activities or disturb sleep all night or most of the night. Use of β -agonists was scored as 0, none; 1, once a day; 2, two to

three times a day; 3, more than three times a day. The minimum score for each day was 0 (no symptoms during the day, no symptoms at night and no use of β -agonists) and the maximum score was 9 (severe symptoms during the day and at night, and more than three administrations of β -agonists). The mean values from the 4-week (visits two and three) period were taken.

Lung function measurements

FEV₁ was measured by a spirometer Lungtest 1000 (MES, Krakow, Poland); the highest of three successive measurements was taken. Reference values were computed according to the recommendations of the American Thoracic Society Standards of Acceptability and Reproducibility [19].

Number of eosinophils measurement

Blood samples (2 mL) were obtained from each patient at both visits two and three. Blood was collected into serum separator tubes (S-Monovette, Sarstedt, Germany), which were allowed to stand at room temperature for 1 h, and then centrifuged at 3000 *g* at 4 °C for 10 min. Aliquots of serum were stored for analysis. The number of eosinophils was measured by an automated haematology analyser K-4500 (Sysmex, Kobe, Japan) and expressed as the number of cells per mm³.

Cultures of mononuclear cells and cytokine determination

In all patients, blood sampling was performed between 08:00 and 00:10 hours. PBMCs were separated from 9 mL heparinized blood (obtained from each patient at both visits two and three) by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. The PBMC layer was washed three times with sterile phosphate-buffered saline. PBMC (10⁶/mL) were stimulated with 10 μ g of Der p2/mL or 10 μ g of Phl p5/mL (kindly provided by Allergopharma) in serum-free RPMI 1640 medium (Sigma-Aldrich Co., Steinheim,

Germany) at 37 °C in a humidified atmosphere with 5% CO₂. For GPs and Gpos groups, the sensitizing allergen was Phl p5 and the non-sensitizing allergen was Der p2; for the HDM group, the sensitizing allergen was Der p2 and the non-sensitizing one was Phl p5. Kinetics performed on the pilot group showed that IL-10 peaks at 48 h. After 48 h incubation, cell cultures were centrifuged and cell-free supernatants were performed (pooled and frozen at –20 °C until assayed for cytokine). IL-10 were assayed by sandwich ELISA (R&D Systems, Minneapolis, MN, USA). The sensitivity of the IL-10 ELISA was 3.9 pg/mL.

Statistical methods

The results were analysed according to well-known statistical methods using StatSoft Statistica for Windows, release 6.0 (StatSoft, Inc., Tulsa, OK, USA). Data were presented as mean with standard error or standard deviation (for demographic data). Before analysis, IL-10 concentrations were transformed to the normal distribution.

To determine differences within groups, Student's *t*-test was used as well. To compare changes with treatment between groups, all parameters were analysed using ANOVA for repeated measures. Also, ANOVA was used to compare changes in IL-10 concentration in supernatant from PBMC culture obtained from children during the pollen season and out of the pollen season before treatment with changes in IL-10 concentration with 4-week treatment with montelukast during the pollen season. To determine correlation between IL-10 concentration and clinical parameters, Pearson's correlation analysis was conducted. *P*-values <0.05 were considered to be significant.

Ethics

The study was approved by the Medical Ethical Committee of the Medical University and all patients' parents or guardians gave their written consent for participation in this study.

Result

All 27 subjects completed the study. The characteristics of the patients who completed the study are given in Table 1.

Primary and secondary outcome measurements before and after treatment with montelukast are presented in Table 2.

Montelukast significantly improved asthma control (based on asthma symptom score), and FEV₁, and significantly decreased eosinophil blood count after a 4-week treatment in the GPs and in the HDM group. The effect of montelukast on all parameters of clinical manifestation of asthma and on eosinophil blood count was greater in the GPs group than in the HDM group. Montelukast did not significantly result in improvement in clinical parameters: clinical score, FEV₁ and in decrease in eosinophil blood count within the Gpos group.

In the GPs group of children (sensitive to grass pollen, observed during the pollen season), montelukast significantly increased the concentration of IL-10 in supernatant from PBMC culture after sensitizing allergen stimulation (Fig. 3). To assess the effect of natural grass pollen allergen exposure on IL-10 production during the pollen season, we compared

Table 1. Patient baseline characteristics

	GPs/GPos group	HDM group
Number of patients	13	14
Men gender (%)	38.5	46.1
Age (years)	12 ± 5.5	14 ± 4.1
Duration of asthma (years)	3.5 ± 1.1	2.5 ± 1.2

Data are presented as mean with standard deviation (± SD).

GPs/GPos group, patients monoallergic to grass pollen during/out of the pollen season; HDM group, patients monoallergic to house dust mite.

changes in IL-10 concentration in supernatant from PBMC culture obtained from children during the pollen season and out of the pollen season before treatment with changes in IL-10 concentration with 4-week treatment with montelukast during the pollen season. There was a significant difference between natural grass pollen allergen exposure and the effect of montelukast treatment in IL-10 concentration in supernatant (Fig. 2). IL-10 concentration in supernatant increased after natural grass pollen allergen exposure; however, it did not reach a level of significance.

Also, in the HDM group, montelukast significantly increased the concentration of IL-10 in supernatant from PBMC culture after sensitizing allergen stimulation (Fig. 3).

In the GPos group, montelukast had no significant effect on IL-10 concentration (Fig. 3). PBMC response to sensitizing allergen stimulation measured by IL-10 concentration in supernatant was greater in the GPs group than in the HDM group after a 4-week treatment with montelukast. Montelukast had no effect on changes of IL-10 concentration in supernatant from PBMC culture after non-sensitizing allergen stimulation.

Three replicate cultures were used for each subject (Table 2, Fig. 4). Before and after treatment with montelukast, IL-10 concentration in supernatant from sensitizing allergen stimulation PBMC culture was significantly higher than the supernatant concentration of IL-10 from non-sensitizing allergen-stimulated PBMC and unstimulated PBMC culture. There were no significant differences in IL-10 concentration in the supernatant from non-sensitizing allergen-stimulated and unstimulated PBMC culture (Fig. 4).

Correlations

We found a significant correlation between IL-10 concentration and clinical score ($r = -0.38$; $P = 0.016$) and FEV₁ ($r = 0.46$; $P = 0.003$) before treatment with montelukast. We also found a significant correlation between changes with treatment of IL-10 concentration and changes with treatment of FEV₁ ($r = 0.35$; $P = 0.028$) and eosinophil blood count (-0.59 ; $P < 0.001$).

Discussion

This study is the first to assess the effect of montelukast on IL-10 production by PBMC. Our data revealed that montelukast increased the concentration of IL-10 in supernatants from PBMC culture obtained from children with asthma monosensitive to HDM and from children monosensitive to grass pollen during the pollen season. Montelukast

Table 2. Effect of treatment with montelukast sodium on asthma symptoms score, FEV₁, eosinophil count and IL-10 concentration in supernatant from PBMC culture after sensitizing and non-sensitizing allergen stimulation

	Before treatment		After treatment		P-level		
	Mean	SEM	Mean	SEM	Within group (t-test)	vs. GPos group (ANOVA)	vs. HDM group (ANOVA)
GPs group							
Asthma symptoms* (score)	7.0	0.4	3.8	0.5	<0.0001	0.0016	0.0249
FEV ₁ (% pred.)	76.4	2.0	96.1	3.0	0.0016	0.0089	0.0313
Eosinophil count (cells/ μ L)	403.8	26.3	297.7	17.4	0.0088	0.0043	0.0270
IL-10 [†] (pg/mL)	54.0	12.6	125.5	11.0	0.0003	0.0001	0.0046
IL-10 [‡] (pg/mL)	24.5	5.2	21.7	4.4	0.1594	0.1507	0.2812
IL-10 [§] (pg/mL)	23.6	5.2	22.9	5.0	0.99	0.60	0.269
HDM group							
Asthma symptoms* (score)	6.2	0.5	3.9	0.4	<0.0001	0.0280	—
FEV ₁ (% pred.)	80.5	1.7	89.2	4.1	0.0003	0.0115	—
Eosinophil count (cells/ μ L)	203.3	29.0	154.3	21.0	0.0071	0.0065	—
IL-10 [†] (pg/mL)	51.2	10.5	77.1	11.6	0.0151	0.0256	—
IL-10 [‡] (pg/mL)	28.3	8.7	23.7	8.4	0.3174	0.6512	—
IL-10 [§] (pg/mL)	25.8	6.9	23.8	7.1	0.558	0.479	—
GPos group							
Asthma symptoms* (score)	2.5	0.2	2.0	0.2	0.1006	—	—
FEV ₁ (% pred.)	82.5	0.8	87.5	2.6	0.0506	—	—
Eosinophil count (cells/ μ L)	242.6	27.1	218.5	26.0	0.0946	—	—
IL-10 [†] (pg/mL)	27.7	7.6	28.4	7.3	0.0758	—	—
IL-10 [‡] (pg/mL)	10.7	1.9	11.1	2.2	0.7229	—	—
IL-10 [§] (pg/mL)	10.1	1.8	9.6	1.8	0.981	—	—

Values are expressed as means with standard error mean (SEM).

GPs group: patients who were monoallergic to grass pollen (Phl p) observed during the pollen season; HDM group: patients who were monoallergic to house dust mite (Der p); GPos group: patients who were monoallergic to grass pollen (Phl p) observed out of the pollen season.

Pred., predicted; FEV₁, forced expiratory volume in 1 s; PBMC, peripheral blood mononuclear cell.

*Daytime, nocturnal asthma symptoms and use of β -agonists were scored as 0 (best) to 3 (worst) each.

[†]IL-10 concentration in supernatant from PBMC culture after sensitizing allergen stimulation.

[‡]IL-10 concentration in supernatant from PBMC culture after non-sensitizing allergen stimulation.

[§]IL-10 concentration in supernatant from PBMC unstimulated culture.

Statistically significant results are indicated in bold.

also significantly improved asthma control, lung function and decreased eosinophil blood counts in these children.

Although a randomized placebo-controlled trial would have been the best design, there was a clear reason that our observational study was not verified by placebo. Our study involved children with moderate persistent asthma who were left without asthma controller, suggested by guidelines, for 4 weeks. This could lead to a deterioration of symptoms and lung function particularly among children sensitive to grass pollen during the pollen season, and this could not be acceptable from an ethical point of view. Therefore, the comparison with the effect of allergen/winter season alone on IL-10 production to compare the effect of montelukast with, was of absolute necessity.

There are considerable data showing a significant improvement in lung function and quality of life in patients treated with anti-LTs [20, 21]. However, the evidence concerning the anti-inflammatory properties of these agents is still controversial. The effects of anti-LTs on cytokine synthesis have been not thoroughly studied. One *in vitro* study showed that a high concentration of pranlukast suppresses the production of IL-4, IL-5 and GM-CSF by PBMCs from mite-sensitive asthmatic patients [22]. These data suggest that endogenous cysteinyl LTs (cys-LTs) released by allergen-specific blood mononuclear cells can stimulate the synthesis of cytokines

implicated in eosinophilia and IgE synthesis, and that this can be inhibited by a specific cys-LT receptor antagonist. Lin et al. [23] demonstrated that zafirlukast is effective in decreasing T cell-related cytokine mRNA expression in ovalbumin-sensitized rats. In our previous studies, we showed the effect of montelukast on serum concentrations of IL-4, soluble intercellular adhesion molecule (sICAM)-1 [24] and on serum concentration of IL-10 [16]. In the present study, a lower level of IL-10 in the supernatant was observed among children with higher clinical manifestation of asthma measured by clinical score and FEV₁. Additionally, we found a significant correlation between changes with treatment of IL-10 concentration and changes with treatment of FEV₁. Collectively, these findings are consistent with the hypothesis that a low expression of IL-10 can induce severe allergic inflammation in asthmatics [1, 25].

One recent study demonstrated slightly elevated numbers of IL-10⁺ lymphocytes before and during the birch pollen season; the number remained unchanged during the season, but decreased significantly after the pollen season [26]. In our study, there was no significant difference in IL-10 concentrations in the supernatant between out of pollen seasons and during the pollen season.

The specific mechanisms by which cys-LTs promote eosinophilia are unknown, but may involve direct chemotaxis

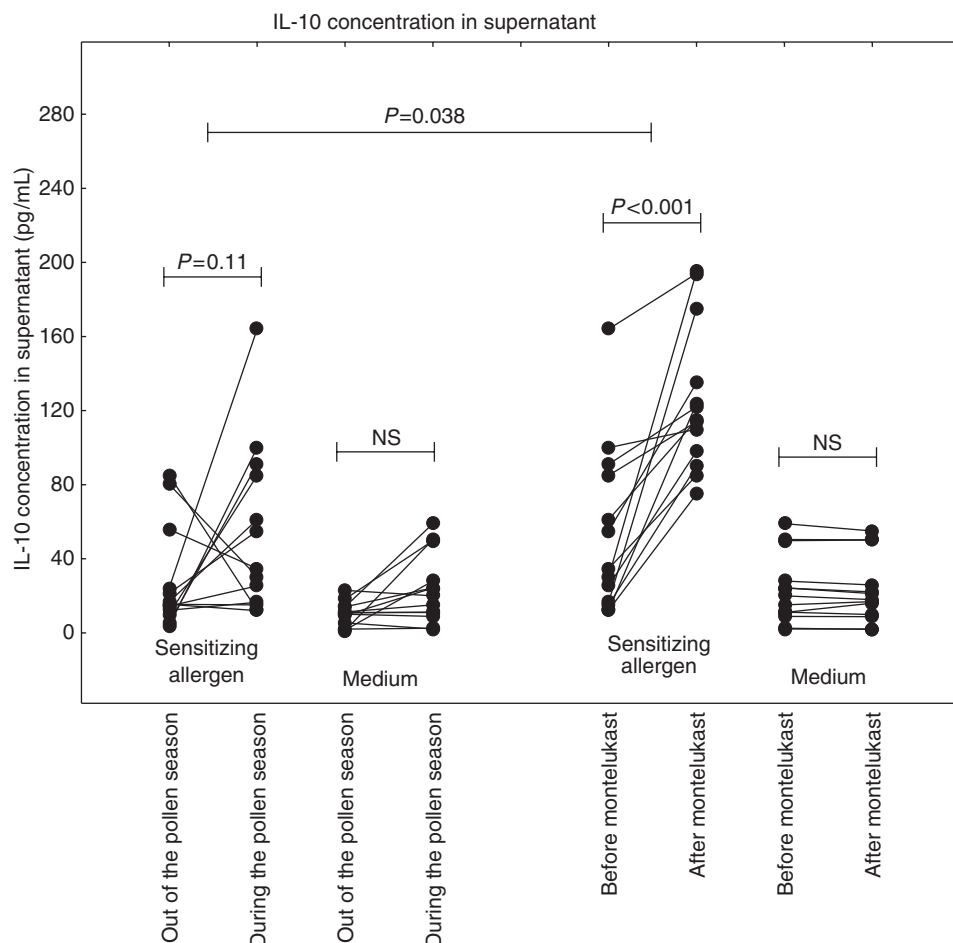


Fig. 2. Concentration of IL-10 pg/mL in supernatants out of the pollen season (GPos group before treatment with montelukast), during the pollen season (GPs group before treatment with montelukast) and before and after treatment with montelukast (GPs group). Supernatants were obtained from 48 h culture of peripheral blood mononuclear cell (PBMC) stimulated *in vitro* with 10 μ g of Phl p5/mL (sensitizing allergen) and from 48 h unstimulated PBMC culture (medium).

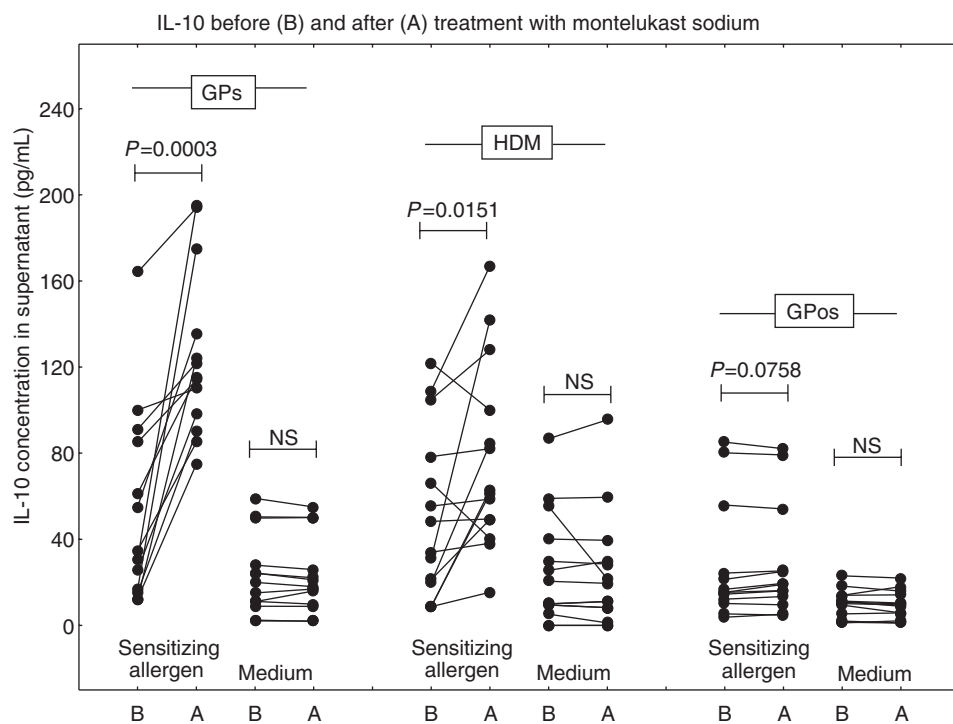


Fig. 3. Concentration of IL-10 in supernatants pg/mL before and after treatment with montelukast in the patients monoallergic to grass pollen during the pollen season (GPs group) (left panel), in the patients monoallergic to house dust mite (HDM group) (central panel) and in the patients monoallergic to grass pollen out of the pollen season (GPos group) (right panel). Supernatants were obtained from 48 h unstimulated peripheral blood mononuclear cell (PBMC) culture (medium) and from 48 h culture of PBMC stimulated *in vitro* with sensitizing allergen: 10 μ g of Phl p5/mL (GPs and GPos group) and 10 μ g of Der p2/mL (HDM group).

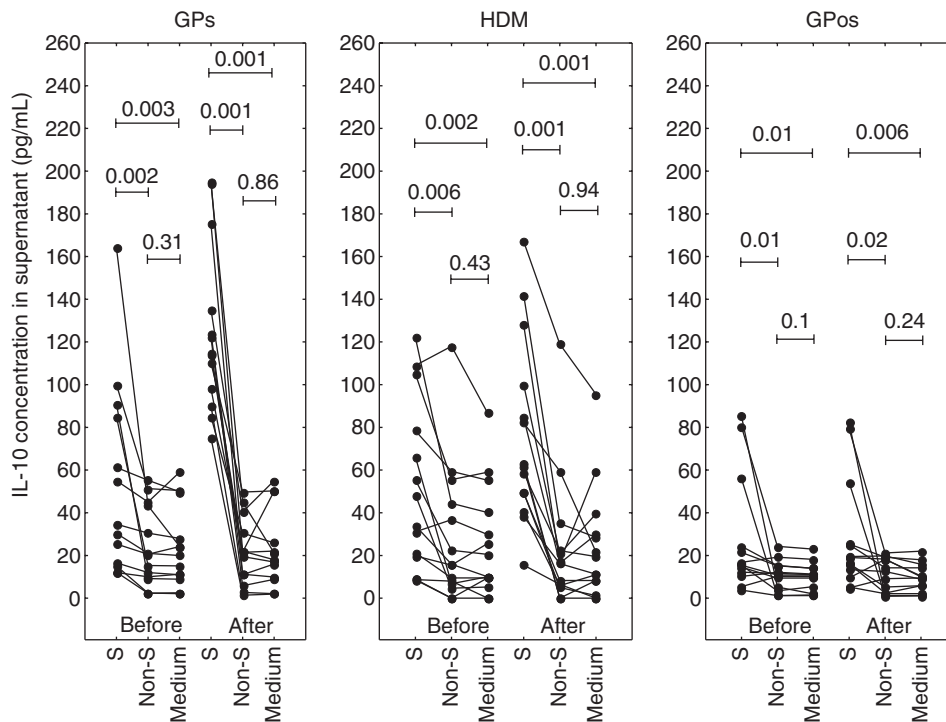


Fig. 4. Concentration of IL-10 (pg/mL) in supernatants from sensitizing allergen stimulation peripheral blood mononuclear cell (PBMC) culture (S), from non-sensitizing allergen stimulation PBMC (non-S) culture and unstimulated PBMC culture (medium). Supernatants were obtained from 48 h culture of PBMC unstimulated (serum-free RPMI 1640 medium) and stimulated *in vitro* with sensitizing allergen: 10 µg of Phl p5/mL (GPs/GPos), 10 µg of Der p2/mL (HDM) and non-sensitizing allergen: 10 µg of Der p2/mL (GPs/GPos), 10 µg of Phl p5/mL (HDM). PBMCs were obtained from children before (left panel) and after (right panel) treatment with montelukast.

of eosinophils in inflamed tissues, up-regulation of adhesion molecules on eosinophils and on the vascular endothelium, effects on eosinophilopoiesis and the release of eosinophil precursors from the bone marrow and enhanced eosinophil survival.

Clear evidence of an effect of cys-LT1 receptor antagonist on eosinophilia has been obtained [27, 28]. Although the mechanism by which this effect is accomplished is obscure, we showed significant reduction in blood eosinophils after 4 weeks of treatment in our previous [24] and in the present study. Additionally, the present study demonstrates a significant correlation between changes with treatment of IL-10 concentration and changes with treatment of eosinophil blood count. However, on the basis of these results we cannot conclude the mechanisms of cytokine and eosinophil changes.

Our study demonstrated that PBMC response to sensitizing allergen stimulation measured by IL-10 concentration in supernatant was greater in children monosensitive to grass pollen observed during the pollen season than that in children monosensitive to HDM after a 4-week treatment with montelukast. Additionally, we showed that montelukast had no effect on IL-10 concentration in supernatant of children without current clinical manifestation of asthma who were monoallergic to grass pollen, observed out of the pollen season. Different cytokine response to different aeroallergens was previously described [29, 30]. Rudin et al. showed that after stimulation with HDM allergen, PBMCs from children sensitized only to HDM expressed increased mRNA levels of the Th2 cytokines, whereas ryegrass stimulation did not result in increased cytokine expression. PBMC from children sensitized to HDM and ryegrass expressed increased Th2 cytokines after stimulation with either of the two allergens [30]. They concluded that expression of cytokines after *in vitro* stimulation of PBMC from atopic children is specific to the sensitizing allergen and suggested that T cells specific to

seasonal allergens circulate in the blood out of season only if the child is concomitantly sensitized to a perennial allergen.

As mentioned, our previous study showed the effect of treatment with montelukast on serum IL-10 in asthmatic children [16]; the present study provides some valid new information. In this study, montelukast had no effect on IL-10 concentration in supernatant from PBMC culture after non-sensitizing allergen stimulation; these data suggest that montelukast modifies only allergen-specific PBMC response. Additionally, we demonstrate that changes in supernatant concentration of IL-10 were greater in children monosensitive to grass pollen observed during the pollen season than that in the children monosensitive to HDM after a 4-week treatment with montelukast. This phenomenon could explain the heterogeneity of responsiveness to LT antagonists. However, our data do not explain a possible way by which anti-LTs increase IL-10 but this was not the aim of the present study; therefore, we did not discuss the cellular source of IL-10, nor directly the effect of montelukast. Therefore, further clinical trials, in a larger group of asthmatics, will increase our understanding of anti-LT' role in anti-asthma therapy.

In conclusion, this trial demonstrates that montelukast administered once daily in the evening over a 4-week treatment period significantly increases the concentration of IL-10 in supernatants from PBMC culture obtained from children with mild-to-moderate persistent asthma and provides clinical benefit to these children.

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