

A randomized, double-blind trial of the effect of glucocorticoid, antileukotriene and β -agonist treatment on IL-10 serum levels in children with asthma

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Summary

Background Levels of an immunoregulatory and anti-inflammatory cytokine IL-10 are reduced in asthmatic airways, potentially contributing to more intense inflammation. Triamcinolone has anti-inflammatory properties and the anti-inflammatory effects of montelukast and formoterol have been discussed.

Objective The purpose of this study was to define the effect of treatment with triamcinolone, montelukast and formoterol on the serum level of IL-10, eosinophil blood counts, eosinophil cationic response (ECP) and clinical parameters (symptom score, FEV₁ and PC₂₀H) in children with moderate asthma.

Methods An 8-week, placebo-controlled and randomized, double-blind trial was carried out. The subjects were 91 children with moderate atopic asthma who were allergic to dust mite. Patients were randomly allocated to receive 400 μ g triamcinolone ($n=19$), 5 or 10 mg (according to age) montelukast ($n=18$), 24 μ g formoterol ($n=18$) or placebo ($n=36$).

Results Seventy-nine children completed the study. After treatment with triamcinolone and montelukast the level of IL-10 in blood serum significantly increased, eosinophil blood counts and ECP levels significantly decreased and all clinical parameters improved; treatment with formoterol had no effect on IL-10 level, eosinophil blood counts in serum and bronchial hyper-reactivity; ECP level significantly decreased after treatment and asthma symptoms and FEV₁ improved significantly. Mean IL-10 levels in serum before and after treatment with triamcinolone were 7.23 pg/mL with 95% CI, 6.74–7.72% and 14.24 pg/mL with 95% CI, 11.6–16.88%, respectively ($P<0.001$); with montelukast they were 6.59 pg/mL with 95% CI, 6.26–7.23% and 10.94 pg/mL with 95% CI, 8.24–12.65%, respectively ($P<0.002$); with formoterol they were 7.06 pg/mL with 95% CI, 6.61–7.52% and 7.04 pg/mL with 95% CI, 6.15–7.93%. We found statistically significant correlations between serum level of IL-10 and serum level of ECP after treatment with triamcinolone and montelukast.

Conclusion This study demonstrates that one possible way by which triamcinolone and montelukast contribute to inhibition of inflammation is by increasing IL-10 levels.

Keywords childhood asthma, IL-10 levels, inflammation, lung function

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Introduction

The normal respiratory tract is characterized by elevated concentrations of IL-10 [1]. Thus, IL-10 production in the lungs of non-asthmatic patients may play a role in limiting pathology-inducing inflammatory Th2 responses [2]. IL-10 levels are reduced in asthmatic airways, potentially contributing to more intense inflammation. Subsequent studies have demonstrated IL-10 production by human Th1 and Th2 lymphocytes [3], as well as cytotoxic T cells, mast cells, monocytes, B lymphocytes, keratinocytes, eosinophils and various tumour cells [4]. This cytokine inhibits proliferation of T cells activated by anti-CD3 antibodies in the absence of antigen-presenting cells at the transcriptional level. IL-10 also inhibits pro-inflammatory cytokines

(IL-1 β , IL-6, IL-8, IL-12 and TNF- α), growth factors, ICAM-1 and chemokine (MIP-1 α) production by mononuclear phagocytes, as well as IFN- γ and TNF- α by natural killer cells. These *in vitro* findings were corroborated by *in vivo* studies in which a single intranasal dose of IL-10 concurrent with antigen challenge in previously sensitized mice specifically inhibited airway neutrophilia and eosinophilia and TNF production induced by antigenic challenge [5,6]. 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*. The fact that IL-10 inhibits cytokine production by Th2 lymphocytes has led to the concept that IL-10 might be beneficial in mitigating allergic inflammation.

There are limited data on changes in IL-10 levels in the serum of children with asthma. The presence of both pro- and anti-inflammatory activities for IL-10 points to the need to carefully

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study its role in allergic inflammation. Corticosteroids are well-established anti-inflammatory drugs with an inhibitory effect on pro-inflammatory mediators. The leukotriene modifiers are the new safe agents for the treatment of asthma and their possible anti-inflammatory properties have been studied. Some published studies showed inhibitory effect of antileukotrienes on bronchial hyper-reactivity [7] and production of IL-4, IL-5 and GM-CSF in patients with bronchial asthma [8]. An anti-inflammatory effect of α_2 -agonists has been discussed. Their possible anti-inflammatory effect is based on *in vitro* studies and provocation testing in both animals and humans [9] and has not been thoroughly studied *in vivo*.

As IL-10 inhibits cytokines associated with cellular immunity and allergic inflammation while stimulating humoral and cytotoxic immune responses, we investigated the effect of 4 weeks of treatment with triamcinolone, montelukast and formoterol on the serum level of IL-10, eosinophil blood count, ECP, bronchial hyper-reactivity, asthma symptoms severity and lung function in children with moderate asthma, allergic to dust mite.

Materials and methods

Patients

Ninety one children with moderate atopic asthma and sensitive to house dust mites, participated in an 8-week study. The study took place from May to September, when the exposure to dust was at a constant level and all children remained in the same environment. Subjects were recruited from our Clinic Center in Zgierz, Poland. Asthma symptoms were assessed using a questionnaire. Atopy was measured by skin prick tests and serum IgE. In the 4 weeks preceding the study, all patients had not been treated with inhaled corticosteroids, nor leukotriene antagonists nor long-acting β -agonists.

Study design

This was a randomized, double-blind, placebo-controlled trial comparing the effect of triamcinolone acetonide (Azmecort, Aventis, Bridgewater, NJ, USA) aerosol, one puff four times daily, 400 μ g/day, inhaled formoterol (Foradil, Novartis, Switzerland), one puff twice daily, 24 μ g/day, delivered by dry powder capsules, montelukast sodium (Singulair, MSD, Whitehouse Station, NJ, USA), 5 mg tablets in children aged 9–14 years or 10 mg tablets in children over 14 years old, and placebo, on serum levels of IL-10 and clinical parameters (score, FEV₁ and PC₂₀H) of asthma. There were three study visits. At the first visit we enrolled 91 patients and put them on β_2 -agonist (Ventolin, GlaxoWellcome, London, UK) 100 μ g four times daily for 4 weeks. They were informed of the purpose of the study and were told how to score asthma symptoms and use the inhaler. At the second visit, the subjects were randomized according to a computer-generated allocation schedule to treatment with:

- triamcinolone, formoterol placebo, montelukast placebo (19 patients, triamcinolone group);
- montelukast, triamcinolone placebo, formoterol placebo (18 patients, montelukast group);
- formoterol, triamcinolone placebo, montelukast placebo (18 patients, formoterol group);
- triamcinolone placebo, formoterol placebo, montelukast placebo (36 patients, placebo group).

The third visit was after 4 weeks of treatment. Blood sampling and lung function testing in individual patients always took place on the same day of the week and on the same time at the second and third visit. At each visit a short history was taken and the patients' diary was evaluated. At visits two and three a blood sample was taken for measurement of IL-10, ECP and eosinophil blood count. At each visit baseline spirometry was performed as well as a histamine challenge test. Before lung function testing formoterol was withheld for 24 h and ventolin was withheld for at least 6 h.

Skin prick tests

Skin prick tests for common inhaled allergens (Allergopharma, Reinbek, Germany), were performed to identify the presence of atopy. A mean weal diameter ≥ 3 mm and $\geq$ the histamine control was defined as a positive response. Patients with at least one positive response to an allergen were regarded as atopic. Skin prick tests were positive against both *D. farinae* and *D. pteronyssinus* extract in 77/91 patients, against only *D. farinae* in 8/91 and only *D. pteronyssinus* in 6/91. Skin sensitivity to house dust mites was similar in all groups. Out of the 91 children tested, six also showed positive skin prick tests to dog, and four to cat. None of these 10 patients had a cat or dog at home, nor did they have any clinical symptoms after contact with one.

Questionnaire

An asthma-screening questionnaire was based on the Paediatric Asthma Quality of Life Questionnaire and scales previously shown to have acceptable evaluative measurement properties [10,11]. Daytime asthma symptoms score and nocturnal awakenings were scored subjectively, each morning and evening, as follows: 0 = no symptoms during the day/night; 1 = symptoms, but they do not affect any activities during the day/night sleep; 2 = symptoms which affect at least one daily activity/disturb night sleep; 3 = symptoms which affect two or more daily activities/disturb sleep all night or most of the night. In addition, use of β -agonists was scored: 0 = none, 1 = once a day, 2 = two to three times a day, 3 = more than three times a day. Minimum score for each day was 0 (no symptoms daytime, no symptoms at night and no use of β -agonists) and maximum score was 9 (severe symptoms daytime and night and more than three uses of β -agonists). The mean value from a 1-week period before a visit was taken.

Lung function measurements

Forced expiratory volume in one second (FEV₁) was measured by a spirometer (Lungtest 1000) taking the highest of three successive measurements. Reference values were computed according to the recommendations of the ECSC report on standardized lung function testing in children. Histamine inhalation provocation tests were performed following the method described by Cockcroft [12], using the 1-min tidal breathing method, with a De Vilbiss nebulizer (De Vilbiss Health Care Inc., Somerset, PA, USA) with an output of 0.33 mL/min and the Lungtest 1000 spirometer. After baseline measurements of FEV₁, subjects inhaled five puffs of saline solution; this FEV₁ value was taken as the control, and if FEV₁ variations were within 10% of baseline, the patients were entered into the study. Patients then inhaled successively

Table 1. Patient baseline characteristics. All values are expressed as means $\pm$ SD

	Treatment groups			
	Triamcinolone	Montelukast	Formoterol	Placebo
No. patients	15	15	15	34
No. male	8	9	6	15
Age	12.0 $\pm$ 2.28	11.6 $\pm$ 1.7	11.6 $\pm$ 1.5	12.2 $\pm$ 1.7
Duration of asthma	3.8 $\pm$ 0.6	3.9 $\pm$ 0.5	3.7 $\pm$ 0.6	3.9 $\pm$ 0.5
Asthma symptoms (score)*	7.1 $\pm$ 0.8	6.0 $\pm$ 0.8	7.3 $\pm$ 0.7	6.5 $\pm$ 1.1
FEV ₁ (%n)	74.6 $\pm$ 4.1	76.7 $\pm$ 3.9	74.3 $\pm$ 4.3	75.3 $\pm$ 5.1

Before: before four-week treatment. After: after four-week treatment. P: statistical significance rate.

greater concentrations of histamine, ranging from 0.03 to 8 mg/mL. FEV₁ was measured 90s after each concentration step. The provocation was terminated when FEV₁ fell by at least 20% from the post-saline value or after having gone through all the concentrations of histamine. The result was expressed as the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀H).

Laboratory tests

Two-millilitre blood samples were drawn from each patient at visits two and three. Blood was collected into serum separator tubes (S-Monovette, Sarstedt, Germany) that were allowed to stand at room temperature (22 °C) for 1 h, and then centrifuged at 3000g at 4 °C for 10min. Aliquots of serum were stored for analysis. All specimens were stored at –70 °C before being analysed. In each sample, serum IL-10 and ECP were measured using a commercially available immunoassay technique according to the instructions of the manufacturer. IL-10 was measured using the ELISA kit (R & D Systems, Minneapolis, MN, USA) with sensitivity <3.9pg/mL. ECP was measured by Pharmacia CAP, Sweden kit; the intra-assay correlation coefficient was 0.97. Number of eosinophils was measured by an automated haematology analyser K-4500 (Sysmex, Kobe, Japan) and expressed as the number of cells per mm³.

Statistical methods

The results were analysed according to known statistical methods by using StatSoft Statistica for Windows, release 5.0 (StatSoft, Inc., Tulsa, OK, USA). Data were presented as mean

with 95% confidence intervals (CI), except for PC₂₀, which was assessed by geometric mean with 95% CI. On demographic data, mean with standard deviation (SD) was used. All parameters were analysed using a one-way analysis of variance (ANOVA). All analysis were determined by Student's *t*-test and confirmed by non-parametric Fisher and Wilcoxon tests. Significance of difference was determined by Wilcoxon's *w*-test. *P*-values <0.05 were considered to be significant. Test Tukey 'a' was used to determine the difference in the effect on IL-10 levels between groups. Pre-study power analysis was made to estimate the number of patients needed to demonstrate a difference between the intervention group and the control group.

Ethics

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

Results

Seventy-nine subjects completed the study: 15 in the triamcinolone group (triamcinolone, formoterol placebo, montelukast placebo), 15 in the montelukast group (montelukast, triamcinolone placebo, formoterol placebo) 15 in the formoterol group (formoterol, triamcinolone placebo, montelukast placebo) and 34 in the placebo group (triamcinolone placebo, formoterol placebo, montelukast placebo). Twelve patients

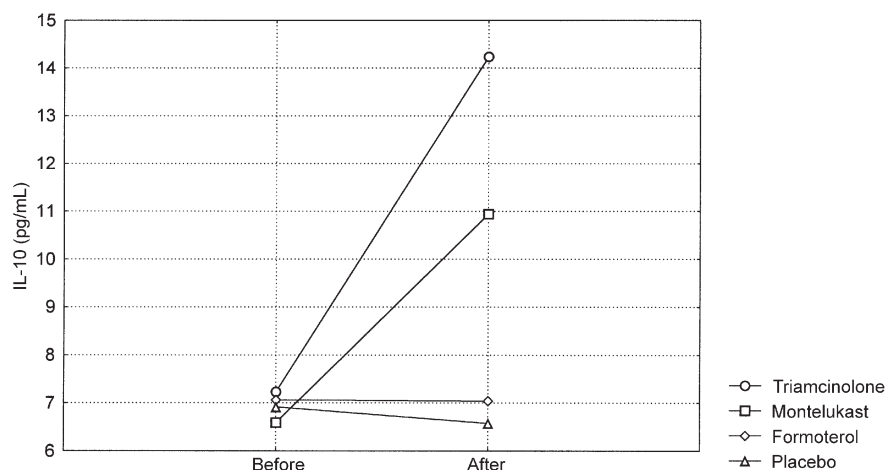


Fig. 1. IL-10 levels in serum before and after treatment with triamcinolone, montelukast, formoterol and placebo.

Table 2. Effect of treatment with glucocorticoid, antileukotriene, α_2 -agonist, placebo on clinical score, FEV₁, PC₂₀H and serum levels of IL-10, eosinophil count and ECP. Values are expressed as means

	GROUP Inhaled glucocorticoid (Triamcinolone)		Antileukotriene (Montelukast)		β_2 -agonist (Formoterol)		Placebo		P	
	Before	After	Before	After	Before	After	Before	After	Before	After
Clinical Score (Points)	7.12 95% CI 6.7–7.55 range 6–8	3.25 95% CI 2.75–3.75 range 2–5	6.0 95% CI 5.27–6.6 range 5–8	2.8 95% CI 1.69–3.48 range 1–5	7.31 95% CI 6.94–7.69 range 6–8	5.75 95% CI 5.12–6.38 range 4–8	6.53 95% CI 6.15–6.91 range 6–8	6.32 95% CI 5.87–6.77 range 5–8	< 0.001	= 0.5
FEV ₁ (%)	74.62 95% CI 72.4–76.85 range 70–82	91.19 95% CI 86.13–96.24 range 80–112	76.7 95% CI 75.87–77.1 range 70–85	94.6 95% CI 87.91–101.28 range 75–114	74.37 95% CI 72.09–76.66 range 70–82	79.75 95% CI 77.72–81.78 range 74–88	75.35 95% CI 73.62–77.08 range 70–87	72.73 95% CI 71.21–74.26 range 70–80	< 0.001	< 0.03
PC ₂₀ H (mg/mL)	2.56 95% CI 2.23–2.81 range 1.8–3.5	4.85 95% CI 4.0–5.48 range 2.2–6.3	2.8 95% CI 2.05–3.55 range 1.9–4.1	3.8 95% CI 3.03–4.58 range 2.0–5.9	2.5 95% CI 2.19–2.79 range 1.8–3.2	2.69 95% CI 2.33–2.96 range 2.1–3.8	3.03 95% CI 2.79–5.82 range 1.8–3.5	3.04 95% CI 2.76–5.8 range 1.9–3.3	> 0.05	> 0.05
IL-10 (pg/mL)	7.23 95% CI 6.74–7.72 range 6.82–12.0	14.24 95% CI 11.6–16.88 range 8.77–25.8	6.59 95% CI 6.26–7.23 range 5.9–10.8	10.94 95% CI 8.24–12.65 range 5.4–18.9	7.06 95% CI 6.61–7.52 range 6.46–14.8	7.04 95% CI 6.15–7.93 range 5.54–17.9	6.92 95% CI 6.54–7.29 range 4.4–11.69	6.57 95% CI 6.1–7.05 range 4.2–9.99	> 0.05	> 0.05
Eosinophil Count (cells/mm ³)	376.87 95% CI 324.21–429.54 range 190–560	257.19 95% CI 214.91–299.46 range 100–380	349.33 95% CI 283.99–414.67 range 220–530	309.67 95% CI 257.17–362.17 range 210–510	365.37 95% CI 311.38–419.36 range 190–580	337.5 95% CI 293.76–381.23 range 185–460	374.12 95% CI 341.57–406.66 range 200–560	381.47 95% CI 348.38–414.56 range 185–560	> 0.05	> 0.05
ECP (μ g/L)	86.47 95% CI 69.89–103.04 range 50–140	56.17 95% CI 48.27–64.06 range 35.5–80.5	74.65 95% CI 61.95–87.35 range 51.4–141	59.57 95% CI 49.33–69.81 range 25.4–87.5	94.29 95% CI 73.5–115.07 range 51.4–200	83.28 95% CI 59.54–107.02 range 45–200	78.59 95% CI 70.18–87.0 range 50.9–170.2	80.64 95% CI 72.47–88.81 range 50.11–70.2	< 0.005	> 0.05

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

were withdrawn from the study because of: asthma exacerbation (eight patients during first 4 weeks of treatment with ventolin alone), lost to follow-up (two patients) and patient withdrew consent (two patients). Characteristics of the patients who completed the study are given in Table 1.

At the beginning of the study, serum levels of IL-10 did not differ between the groups. After 4 weeks of treatment, triamcinolone and montelukast, compared with placebo, significantly increased the level of IL-10 (Fig. 1), and significantly decreased levels of ECP and eosinophil count in serum in children with moderate asthma. All parameters of clinical manifestation of asthma, FEV₁, PC₂₀H and clinical score, improved in both groups as well. Formoterol had no effect on IL-10 levels in serum; ECP and eosinophil count significantly decreased. Clinical score and lung function, but not PC₂₀, significantly improved after treatment with formoterol. Data are presented in Table 2.

Correlations

We found statistically significant correlations between serum IL-10 and serum ECP after treatment with triamcinolone ($r = -0.8069$) (Fig. 2) and montelukast ($r = -0.5418$) (Fig. 3). We found no correlations between any other measured parameters.

Discussion

Our study showed that a 4-week treatment with triamcinolone and montelukast increased serum levels of IL-10, decreased level of ECP and eosinophil blood count and was associated with clinical improvement of asthma, measured by FEV₁ and clinical score of asthmatic well-being and attenuation of bronchial hyper-reactivity. The effect of triamcinolone on IL-10 level was greater than that of montelukast. Treatment with formoterol had no effect on IL-10 level in serum and eosinophil blood count; ECP decreased significantly. Clinical score and lung function, but not PC₂₀H, improved after treatment with formoterol.

According to Borish et al., corticosteroids (GCS) inhibit (in no dose-dependent manner) production of IL-10 in peripheral blood mononuclear cells *in vitro* [1]. They suggest that although diminished IL-10 production by corticosteroids may

paradoxically enhance cellular and allergic immunity, this may be predicted to have an anti-inflammatory effect on humoral and cytotoxic influences mediated by IL-10. Another study also showed inhibition of IL-10 by glucocorticosteroids in the human monoblast cell line [13]. In contrast, Taieb et al. showed, that during steroid therapy in patients with hepatitis, plasma levels of the anti-inflammatory cytokine IL-10 increased [14]. Hodge et al., in their study, examined the effects of GCS on IL-10 production by individual cells (monocytes, T lymphocytes and NK cells) in whole blood [15]. Monocyte production of IL-10 was enhanced at low concentrations of GCS, suggesting that one anti-inflammatory mechanism of GCS action may be through upregulation of the anti-inflammatory cytokine IL-10. Another study in mild-asthmatic subjects showed that inhaled budesonide (800 µg twice daily) did not alter IL-10 from blood monocytes, but both IL-10 mRNA and protein expression from stimulated alveolar macrophages were increased, suggesting beneficial actions of steroids in asthma by enhancing IL-10; budesonide also reduced bronchial hyper-responsiveness and improved baseline FEV₁ in these patients [16]. Our study showed that treatment with corticosteroids increased levels of IL-10 in all patients and it was always associated with better clinical outcome of asthma-PC₂₀H, FEV₁ and clinical score, probably due to various anti-inflammatory effects of steroids.

In the literature there are no data on the effect of antileukotriene drugs on IL-10 levels in serum. Our study showed that treatment with montelukast significantly increased serum level of IL-10, decreased ECP, eosinophil blood count and bronchial hyper-reactivity, improved FEV₁ and provided better symptom control. These data suggest possible reasons for the anti-inflammatory effects of leukotriene modifiers.

In our study, treatment with beta-agonists had no effect on IL-10 level, eosinophil blood count and bronchial hyper-reactivity; formoterol decreased serum ECP level and improved asthma symptoms. Suberville et al., in their study, showed that isoproterenol (a specific β -adrenergic agonist) increased IL-10 release from activated mouse peritoneal macrophages [17]. Another study indicated also that production of IL-10 was increased after the cells were treated with β -agonist [18].

Some studies have shown that anti-inflammatory treatment of asthma has an effect on IL-10 [14–16], which suggests that IL-10 could be a marker in monitoring of disease. Our research

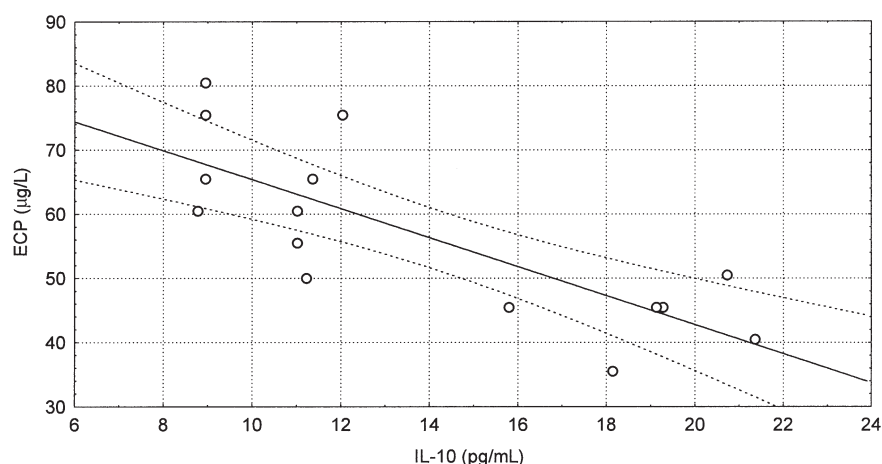


Fig. 2. Correlation between serum levels of IL-10 and serum ECP after treatment with triamcinolone. $r = -0.8069$.

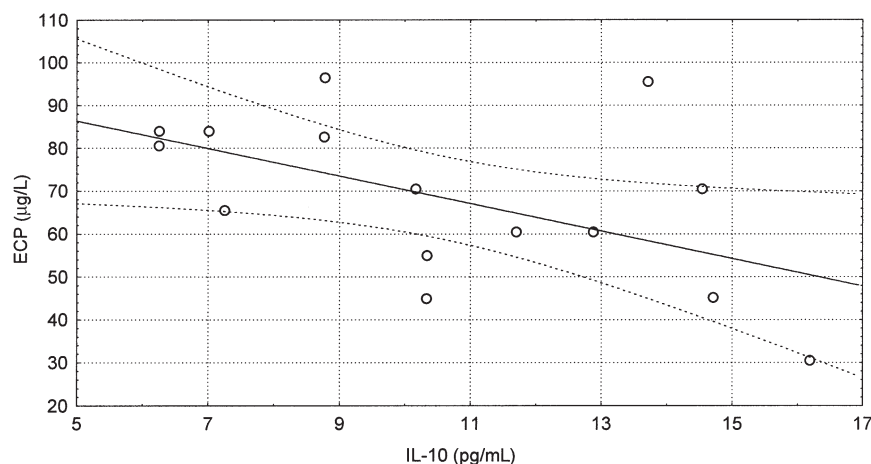


Fig. 3. Correlation between serum levels of IL-10 and serum ECP after treatment with montelukast. $r=0.5418$.

showed that patients treated with montelukast and triamcinolone had increased serum levels of IL-10 and improved asthma symptoms and airway hyper-reactivity. We cannot rule out the possibility that increased levels of IL-10 were the effect of treatment on other mediators and better asthma control and didn't necessarily reflect a direct effect of treatment on IL-10. We used ECP as a clinical marker in asthma and we found statistically significant correlation between serum IL-10 and serum ECP after treatment with triamcinolone and montelukast (Figs 2 and 3). Our data show that another possible way by which antileukotrienes and glucocorticoids contribute to inhibition of inflammation is by increasing IL-10 levels. With our understanding of the role of IL-10 in inflammation, it seems that clinical trials in asthma may show promise and help to answer the question of whether IL-10 can be a good marker of disease.

References

- Borish L, Aarons A, Rumbyrt J et al. Interleukin-10 regulation in normal and asthmatic subjects. *J Allergy Clin Immunol* 1996; 97:1288–96.
- Moore KW, Malefyt RW, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; 19:683–765.
- Yssel H, de Waal Malefyt R, Roncarolo MG et al. IL-10 is produced by subsets of human CD4⁺ T cell clones and peripheral blood T cells. *J Immunol* 1992; 149:2378–84.
- Borish L. IL-10: evolving concepts. *J Allergy Clin Immunol* 1998; 101:293–7.
- Zuany-Amorim C, Haile S, Leduc D et al. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J Clin Invest* 1995; 95:2644–51.
- Wanidworanun C, Strober W. Predominant role of tumor necrosis factor- α in human monocyte IL-10 synthesis. *J Immunol* 1993; 151:6853–61.
- Ishioaka S, Hozawa S, Haruta Y et al. Pranlukast, a cysteinyl leukotriene antagonist, reduces serum eosinophil cationic protein levels in patients with asthma. *Hiroshima J Med Sci* 1999; 48:105–10.
- Tohda Y, Nakahara H, Kubo H et al. Effects of ONO-1078 (pranlukast) on cytokine production in peripheral blood mononuclear cells of patients with bronchial asthma. *Clin Exp Allergy* 1999; 29:1532–6.
- Spoelstra FM, Postma DS, Hovenga H, Noordhoek JA, Kauffman HF. Budesonide and formoterol inhibit ICAM-1 and VCAM-1 expression of human lung fibroblasts. *Eur Respir J* 2000; 15:68–74.
- Juniper EF, Guyatt GH, Feeny DH et al. Measuring quality of life in children with asthma. *Qual Life Res* 1996; 5:35–46.
- Santanello NC, Barber BL, Reiss TF et al. Measurement characteristics of two asthma symptom diary scales for use in clinical trials. *Eur Respir J* 1997; 10:646–51.
- Cockcroft DW, Murdock KY, Mink JT. Determination of Histamine PC20. *Chest* 1983; 54:505–6.
- Garrelds IM, van Hal PT, Haakmat RC et al. Time dependent production of cytokines and eicosanoids human monocytic leukaemia U937 cells; effects of glucocorticosteroids. *Med Inflamm* 1999; 8 (4–5):229–35.
- Taieb J, Mathurin P, Elbim C et al. Blood neutrophil functions and cytokine release in severe alcoholic hepatitis: effect of corticosteroids. *J Hepatol* 2000; 32 (4):579–86.
- Hodge S, Hodge G, Flower R, Han P. Methyl-prednisolone up-regulates monocyte interleukin-10 production in stimulated whole blood. *Scand J Immunol* 1999; 49 (5):548–53.
- John M, Lim S, Seybold J, Jose P et al. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein 1 α , granulocyte-macrophage colony-stimulating factor, and interferon- γ release from alveolar macrophages in asthma. *Am J Respir Crit Care Med* 1998; 157 (1):256–62.
- Suberville S, Bellocq A, Fouqueray B et al. Regulation of interleukin-10 production by beta-adrenergic agonists. *Eur J Immunol* 1996; 26 (11):2601–5.
- Izeboud CA, Mocking JA, Monshouwer M, van Miert AS, Witkamp J. Participation of beta-adrenergic receptors on macrophages modulation of LPS-induced cytokine release. *J Recept Signal Transduct Res* 1999; 19 (1–4):191–202.