

Relationship between changes in interferon- γ production by peripheral blood T cells and changes in peak expiratory flow rate in patients with chronic stable asthma

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Summary

Background Cytokines production by T helper lymphocytes (Th cells), which orchestrate the interplay of the different cells involved in airway inflammation of asthma, may be reflected in peripheral blood. Some studies have suggested that the Th cell cytokines by peripheral blood T cells correlate with asthma severity.

Objective To investigate the relationship between changes in IFN- γ production by peripheral blood T cells and changes in lung function in chronic stable asthmatics.

Methods Sixteen patients with chronic stable moderate asthma aged 35–65 years (nine women) were recruited. Morning and evening peak expiratory flow rates (PEFR) monitoring and blood sampling for peripheral blood T cell culture, total IgE and blood eosinophils were performed at baseline and week 12. Levels of IFN- γ , IL-4 and IL-5 in culture supernatants of peripheral blood T cell were determined by using enzyme-linked immunosorbent assay (ELISA) kits.

Results Patients with increased IFN- γ changes from baseline showed significantly increased changes in morning ($P = 0.02$) and evening ($P < 0.05$) PEFR compared with those with decreased IFN- γ changes. The changes in IFN- γ production and IFN- γ : IL-4 ratio significantly correlated with the changes in morning PEFR ($R_s = 0.59$, $P < 0.02$; $R_s = 0.63$, $P < 0.01$, respectively) and tended to correlate with the changes in evening PEFR ($R_s = 0.45$, $P = 0.08$; $R_s = 0.5$, $P = 0.05$, respectively). The changes in IL-4 and IL-5 did not correlate with the changes in IgE and blood eosinophils, respectively.

Conclusions These findings suggest that IFN- γ may be associated with the alteration of lung function in asthmatics and play a role in the pathophysiology of chronic stable asthma.

Keywords asthma, blood, cytokines, peak expiratory flow rates, T-lymphocytes

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Introduction

T helper lymphocytes (Th cells) orchestrate the interplay of the different cells involved in airway wall inflammation in asthma [1]. Th cells can be subdivided in two types of cells based on the profile of cytokines produced by each subtype: Th1 and Th2 cells [2]. Cytokines specifically produced by Th1 cells are IL-2 and IFN- γ , whereas Th2 cells specifically produce IL-4 and IL-5. The balance between Th1 and Th2 cells is maintained by mutual inhibition: IFN- γ inhibits Th2 cell activity, whereas IL-4 inhibits Th1 cell activity [3]. IL-4 is involved in the switch of B cells to IgE production [4] and IL-5 has been shown to cause eosinophilic infiltration into the airway wall [5]. It has been shown that T cells in bronchoalveolar lavage [6] and bronchial biopsies [7] from atopic asthmatics express mRNA

for IL-4 and IL-5. Similar observations have been made on peripheral blood T cells. Increased synthesis of mRNA encoding IL-4 and IL-5 was seen in peripheral blood CD4⁺ T cells from atopic asthmatics compared with normal controls [8, 9], suggesting that Th cell cytokine production in the bronchial mucosa of asthmatics is reflected by that in the peripheral blood.

Some cross-sectional studies [10–13] have found that Th cell cytokines produced by peripheral blood T cells correlate with asthma severity. IL-5 showed a significant inverse correlation with forced expiratory volume in one second (FEV₁) [10] and a positive relationship with airway hyper-responsiveness [11]. IFN- γ levels had a direct relationship with FEV₁ [12] and an inverse correlation with asthmatic symptom score [13]. Furthermore, in terms of IL-5, changes in IL-5 production correlated with changes in symptom score, FEV₁ % predicted, and airway hyper-responsiveness for the atopic asthmatics [14].

Because little is known about the relationship between change in IFN- γ level and change in lung function in asthma, we measured IFN- γ concentration produced by peripheral

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blood T cells and peak expiratory flow rate (PEFR) at baseline and 12 weeks later in chronic stable asthmatic patients, and the correlation between the two changes was analysed. We also determined the Th2 cell cytokines IL-4 and IL-5 in these subjects.

Materials and methods

Patients

Sixteen asthma patients aged 35–65 years (nine women and six men) were recruited from the Allergy Outpatient Clinic of the Chonnam National University Hospital (for patient information see Table 1). All patients fulfilled the American Thoracic Society criteria for asthma [15], had a PC₂₀ methacholine equal to or less than 16 mg/mL, and had moderate perennial asthma [16]. The perennial asthma was characterized by intermittent or continuous asthma symptoms without seasonal variation. They had suffered from asthma for more than 1 year and were clinically stable for at least 4 weeks before beginning the study with daily asthma medications. Ten patients were atopic, defined by one or more positive skin prick tests to 15 common aeroallergens (alder, birch, hazel, bermuda, timothy, mugwort, ragweed, alternaria, aspergillus, penicillium, *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog and cockroach; Bencard, Brentford, UK). All patients were non-smokers and had been free from clinical, symptomatic respiratory infections for at least 4 weeks before beginning the study. All patients gave written informed consent for this study, which was approved by the Chonnam University Hospital Ethics Committee.

Study design

During a 2-week run-in period, spirometry was performed using Spiro-Analyser ST-250 (Fukuda Sangyo, Japan) and twice-daily home PEFR monitoring (06.00–08.00 h and 16.00–18.00 h) with a Ferraris peak flow meter (Ferraris Medical, Temecula, CA, USA) was recorded as the best of three measurements. All patients had been well trained in the use of the peak flow meter. At the end of the baseline period,

blood was taken for peripheral blood T cell culture, total IgE and blood eosinophils. Patients were seen every 4 weeks at the outpatient clinic by the same physician. Throughout a 12-week study period, patients recorded PEFR. At week 12, blood sampling for peripheral blood T cell culture, total IgE and blood eosinophils was repeated. During the entire period of the study, doses of each asthma medication were kept constant unless exacerbations of asthma required an increase. Inhaled short-acting β_2 -agonists were used as needed.

Peripheral blood T cell culture and cytokine assays for IL-4, IL-5 and IFN- γ

Peripheral blood samples were collected in heparin anticoagulant. Peripheral blood T lymphocytes were isolated, activated and cultured using the ActiCytTM-TC reagent system (Bio-Ergonomics, St. Paul, MN, USA). Isolated lymphocytes were obtained by mixing 6 mL of blood with 6 mL of PrepaCytTM-SCTM, an immunologically based cell isolation reagent in the ActiCytTM system. Residual erythrocytes were lysed using the VitaLyseTM reagent included with the ActiCytTM kit. The cells were resuspended in the T cell Activation Media included with the ActiCytTM kit at a concentration of 1.5×10^6 cells/mL. The activation medium contained anti-CD3 monoclonal antibody (1 μ g/mL), recombinant human IL-1 α (1 ng/mL), and recombinant human IL-2 (10 ng/mL). After 20 h, the supernatant was separated and frozen at -70°C until cytokine assays. Concentrations of IL-4 (R&D Systems, Minneapolis, MN, USA), IL-5 (Endogen, Rockford, IL, USA) and IFN- γ (Endogen) were determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits with lower limits of detection of 0.13 pg/mL, 2 pg/mL and 2 pg/mL, respectively. The standard curves were generated by the standards of known IL-4, IL-5, or IFN- γ content provided.

Blood eosinophils and serum total IgE

Blood samples were drawn in Vacutainer tubes with K₃ EDTA as an anticoagulant [(366437), Becton Dickinson, Plymouth, UK]. In the anticoagulated blood samples, leucocyte count

Table 1. Patient information

Patient no.	Age (years)	Sex	Atopy	FEV ₁ (% pred)	FVC (% pred)	PC ₂₀ (mg/dL)	Asthma medication
1	41	F	+	94	95	0.17	Theo, SX, FP
2	47	M	–	84	81	0.84	Theo, PH, SX, FP
3	62	F	+	73	104	3.5	SX, FP
4	46	F	+	60	80	0.02	Theo, PH, SX, FP
5	63	F	+	83	124	8.4	Theo, SX, FP
6	49	M	–	78	86	10.1	SX, FP
7	61	F	+	72	77	0.78	Theo, PH, SX, FP
8	46	M	–	74	86	12.2	FP
9	35	M	+	82	92	7.37	Theo, SX, FP
10	59	M	+	74	91	1.86	Theo, SX, FP
11	55	F	–	100	105	0.57	Theo, FP
12	61	M	+	70	85	11.3	Theo, PH, SX, FP
13	50	F	–	60	68	12.5	Theo, PH, SX, FP
14	38	F	+	72	80	0.47	Theo, PH, SX, FP
15	57	M	+	71	81	10.1	Theo, PH, SX, FP
16	65	F	–	66	79	1.89	Theo, PH, SX, FP

PC₂₀: provocative concentration of methacholine resulting in 20% fall in FEV₁; Theo: sustained-release theophylline; PH: pranlukast hydrate; SX: salmeterol xinafolate; FP: fluticasone propionate.

and differentiation were measured. Leukocytes and leucocyte differentiation in blood samples were performed on a COULTER-STKS (Coulter Electronics Inc., Hialeah, FL, USA). Blood samples for serum preparation were drawn for measurement of total IgE concentration. After blood sampling, clotting occurs for 2 h at 37 °C. Thereafter, blood samples were centrifuged for 10 min at 1350 g. Subsequently, the serum samples were stored at –20 °C until analysis. The IgE concentration was measured by nephelometry (Dade Behring, Marburg, Germany).

Statistical analyses

PEFR was expressed as weekly averages. Baseline PEFR was determined as the averages for week 2 of the run-in period. Changes in cytokine production, PEFR, total IgE and blood eosinophils after 12 weeks within an individual were calculated as $100\% \times [(values\ at\ week\ 12 - values\ at\ baseline)/values\ at\ baseline]$. Data were expressed as median (range). The non-parametric Mann–Whitney *U*-test was used for intergroup comparisons, and the Wilcoxon's signed-rank test was used for intragroup comparisons. The relationships between the parameters were examined using Spearman's rank correlation (R_s). *P*-value of < 0.05 was taken to be significant.

Results

Median forced vital capacity and FEV₁ expressed as percentages of predicted value on baseline spirometry were 85.5 and 73.5%, respectively (Table 1). Median duration of asthma was 4.5 years. Daily doses of each asthma medication were 200–400 mg, 225 mg, 50–100 µg and 500–1000 µg for sustained-release theophylline, pranlukast hydrate, salmeterol xinafolate and fluticasone propionate, respectively. In each individual, the medication doses after 12 weeks did not change from baseline, except fluticasone propionate, daily doses of which were increased from 500 µg to 1000 µg only in three patients.

There were no significant differences in baseline concentrations of IL-4, IL-5 and IFN-γ in culture supernatants of peripheral blood T cell, total IgE and blood eosinophils between atopic and non-atopic asthmatic patients ($P > 0.05$). In all patients, the morning and evening PEFR, the concentrations of IL-4, IL-5 and IFN-γ in culture supernatants of peripheral blood T cell, and serum total IgE levels after 12 weeks did not significantly differ from baseline values ($P > 0.05$, respectively). Blood eosinophil percentages after 12 weeks were significantly decreased compared with baseline ($P < 0.05$) and blood eosinophil counts tended to be decreased ($P = 0.08$; Table 2). When all patients were classified into the groups with increased ($n = 10$) and decreased ($n = 6$) IL-4 changes after 12 weeks from baseline, there were no significant differences in the degree of changes in morning and evening PEFR between the two groups ($P > 0.05$, respectively). When all patients were divided into the two groups with increased ($n = 9$) and decreased ($n = 7$) IL-5 changes, there were no significant differences in the degree of changes in morning and evening PEFR between the two groups ($P > 0.05$, respectively). However, with regard to IFN-γ, patients with increased IFN-γ changes showed significantly increased changes in morning ($P = 0.02$) and evening ($P < 0.05$) PEFR compared with patients with decreased IFN-γ changes (Table 3). There were

Table 2. Peak expiratory flow rate (PEFR), total IgE, blood eosinophils and cytokines in culture supernatants of peripheral blood T cell at baseline and week 12

Variable	Baseline	12 weeks	<i>P</i> -values
Morning PEFR (L/min)	410 (231–679)	415 (280–689)	NS
Evening PEFR (L/min)	416 (242–697)	424 (280–706)	NS
Total IgE (IU/mL)	82.4 (29.8–920)	72.2 (30–1370)	NS
Blood eosinophil (%) (counts/µL)	4.6 (0.4–23.1)	3.8 (0.8–13.4)	0.04
	352 (37–1606)	328 (60–817)	0.08
IL-4 (pg/mL)	6.4 (3.9–14.3)	7.6 (3.7–18.3)	NS
IL-5 (pg/mL)	18.5 (3.4–148.9)	19.1 (2.1–259.0)	NS
IFN-γ (pg/mL)	555 (126–2681)	276 (41–2307)	NS

Data are expressed as median (range). NS: non-significant.

Table 3. Comparisons of changes from baseline in peak expiratory flow rate (PEFR) according to changes from baseline in IL-4, IL-5, or IFN-γ

Variable	Change in morning PEFR (%)	Change in evening PEFR (%)
Change in IL-4		
Increase† ($n = 10$)	1.9 (–4.6–33.7)	0.9 (–6.8–25.9)
Decrease‡ ($n = 6$)	–1.6 (–6.6–8.8)	–1.0 (–7.5–7.1)
Change in IL-5		
Increase† ($n = 9$)	2.4 (–5.2–33.7)	0.5 (–7.5–25.9)
Decrease‡ ($n = 7$)	–0.5 (–6.6–8.8)	–1.0 (–6.8–7.1)
Change in IFN-γ		
Increase† ($n = 5$)	7.8 (0.99–33.7)*	8.2 (–1.8–25.9)*
Decrease‡ ($n = 11$)	–1.5 (–6.6–8.8)	–1.0 (–7.5–7.1)

Data are expressed as median (range). * $P < 0.05$ compared with patients with decreased IFN-γ change. †The changes (%) from baseline in IL-4, IL-5, or IFN-γ are greater than zero. ‡The changes (%) from baseline are equal to or less than zero.

no significant differences in the changes in PEFR and the concentrations of IL-4, IL-5 and IFN-γ between the three patients in whom the dose of fluticasone propionate were increased and the rest.

The changes from baseline in IL-4 in culture supernatants of peripheral blood T cell did not correlate with the changes in serum total IgE ($R_s = 0.42$, $P = 0.12$). The changes in IL-5 significantly correlated with the changes in total IgE ($R_s = 0.60$, $P < 0.02$) and did not correlate with the changes in blood eosinophil counts ($R_s = 0.44$, $P = 0.09$). The changes in IFN-γ significantly correlated with the changes in morning PEFR ($R_s = 0.59$, $P < 0.02$; Fig. 1) and tended to correlate with the changes in evening PEFR ($R_s = 0.45$, $P = 0.08$). Moreover, the changes in IFN-γ:IL-4 ratio correlated with the changes in morning ($R_s = 0.63$, $P < 0.01$; Fig. 2) and evening PEFR ($R_s = 0.5$, $P = 0.05$). However, the changes in IL-4 or IL-5 were not related to the changes in morning and evening PEFR.

Discussion

Our longitudinal study showed that increased change in IFN-γ in culture supernatants of peripheral blood T cells was associated with the increased changes in morning and evening PEFR. Furthermore, the changes in IFN-γ had a direct relationship

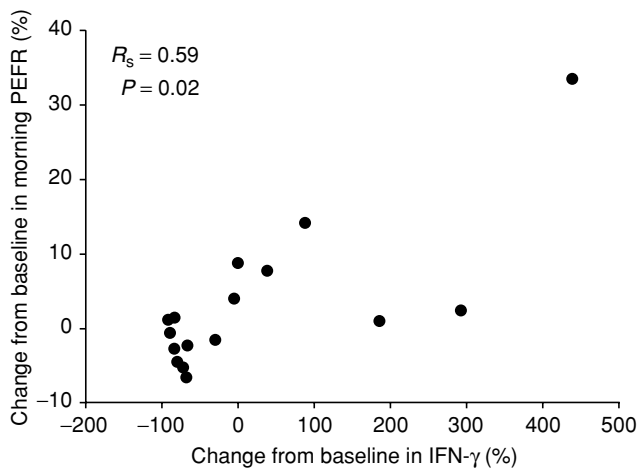


Fig. 1. Relationship between the changes after 12 weeks from baseline in IFN- γ production by peripheral blood T cell and the corresponding changes in morning peak expiratory flow rate (PEFR) in 16 patients with asthma.

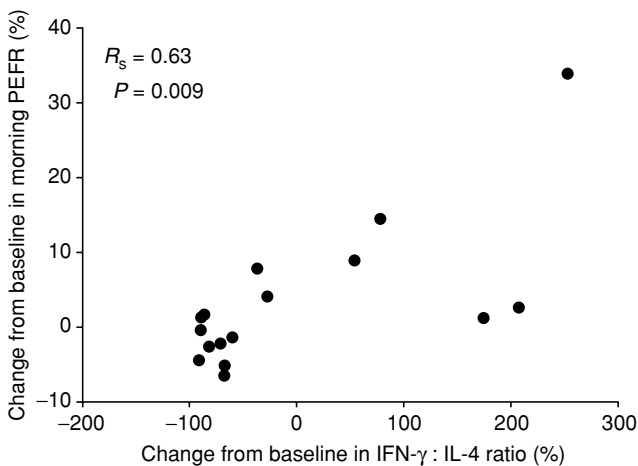


Fig. 2. Relationship between the changes after 12 weeks from baseline in IFN- γ :IL-4 ratio produced by peripheral blood T cell and the corresponding changes in morning peak expiratory flow rate (PEFR) in 16 patients with asthma.

with the changes in morning PEFR. Recently, a cross-sectional study [12] reported that the concentration of IFN- γ in supernatants of cultures of stimulated peripheral blood mononuclear cells was directly related to the FEV₁ in children with moderate atopic asthma. Our data extended their finding, and suggested that IFN- γ produced by peripheral blood T cells may be associated with the alteration of lung function in chronic stable asthmatics. Also, our finding may be supported by another study [13] showing that allergen-specific IFN- γ production by peripheral blood mononuclear cells had an inverse correlation with asthmatic symptoms score.

It is becoming clear that cytokines derived from locally activated Th cells orchestrate the interplay of the interplay of the different cells involved in airway inflammation in asthma [1]. Some studies have shown that Th cell cytokine production in the bronchial mucosa of asthmatics is reflected by that in the peripheral blood [8, 9, 17, 18], although cytokine studies using induced sputum, bronchoalveolar lavage, or bronchial biopsy may be better at revealing events in the airways.

How can we explain the direct relationship of changes in IFN- γ with changes in lung function in our chronic stable asthmatics? Our finding of the positive relationship of the changes in IFN- γ :IL-4 ratio with the changes in PEFR may support a speculation that the increased expansion of IFN- γ -producing Th1 cell as opposed to Th2 cell [3] contributes to the increased lung function. However, in the present study, the changes in IL-5 production were not related to the changes in PEFR. The fact that our asthmatic patients were clinically stable for at least 4 weeks before beginning the study with daily asthma medications is likely to explain this discrepancy. In other words, the changes in IL-5 production are likely to be too small to reflect the changes in lung function in patients with chronic stable asthma. Another possible explanation for the relationship between the changes in IFN- γ and the changes in lung function is likely to be made by a direct effect of IFN- γ on the airway smooth muscle [19, 20]. It is known that IFN- γ can induce nitric oxide (NO) production [21], which causes dilatation of the airway smooth muscle in patients with allergic asthma and reverses bronchoconstriction [19, 20]. A defect in NO production by macrophages has been observed in IFN- γ gene knockout mice [22], suggesting that the effect of IFN- γ might be mediated via stimulation of macrophage-derived NO. Further studies such as measurement of exhaled NO are needed to demonstrate the explanation. In addition, Hofstra et al. [23] showed that the airway responses of saline-challenged IFN- γ gene knockout mice were significantly higher than saline-challenged wild-type mice, indicating a role for endogenous IFN- γ in airway responsiveness, and the airway hyper-responsiveness in ovalbumin-challenged wild-type mice could be completely abolished by IFN- γ treatment.

Although the purpose of the present study was not to investigate the clinical effects of the asthma medications, the used medications including theophylline [24], pranlukast hydrate [25], salmeterol xinafoate [26] and inhaled steroids [27] are possible to affect the cytokine productions by peripheral blood T cells. In our study, however, the doses of each medication did not change between the beginning and the end of the study, except the inhaled steroids only in three patients. The changes in the concentrations of IL-4, IL-5, IFN- γ and PEFR did not differ between the three asthmatics and the rest. These indicate that the asthma medications are unlikely to affect our major finding of relationship between the changes in IFN- γ and the changes in lung function.

We found no correlation between the changes in IL-4 production by peripheral blood T cells and the changes in total IgE levels or between the changes in IL-5 production and the changes in blood eosinophil counts. IL-13, as well as IL-4 [4], has been shown to induce IgE synthesis, and IL-4 could be produced not only by T cells but also by basophils and mast cells [28]. Similarly, IL-5, the main factor responsible for chemotaxis, priming, activation of eosinophils [29], could be produced not only by T cells and but also by several other structural components present in the airways of asthmatics, including mast cells, fibroblasts, endothelial cells, epithelial cells and eosinophil itself [30].

Taken together, the present study suggests that IFN- γ may be associated with the alteration of lung function in asthmatics and play a role in the pathophysiology of chronic stable asthma. However, It is likely that many more factors than this one cytokine are important in determining lung function.

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