

CD4 T-lymphocyte activation is associated with peak expiratory flow variability in childhood asthma

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Background: Asthma has been recognized as a chronic inflammatory disorder of the airway. We have investigated the relationships among the activation markers on lymphocytes, eosinophils, their serum products in the peripheral blood, and the variability of airway obstruction in childhood asthma.

Methods: Twenty-two patients with atopic asthma (mean age, 12 years) were treated regularly and asked to measure their peak expiratory flow (PEF) twice daily for 7 days. Peripheral venous blood was obtained on day 8.

Results: The absolute counts of CD4 T lymphocytes expressing the activation marker CD25 in the peripheral blood on day 8 correlated significantly with the values of the coefficient of variation (CV) of both morning PEF ($p < 0.01$) and night PEF ($p < 0.05$) obtained over 7 days, but those of CD8+/CD25+ T lymphocytes, those of CD23+ B lymphocytes, and the serum concentrations of soluble CD25 did not. The absolute counts of peripheral blood eosinophils also demonstrated a significant correlation with the CV values of both morning PEF ($p < 0.01$) and night PEF ($p < 0.05$).

Conclusion: CD4 T-lymphocyte activation and increased counts of eosinophils in peripheral blood correlate with CV of PEF in patients with asthma, suggesting that CV of PEF is a good marker for assessing not only the variability of airway obstruction but also the degree of airway inflammation. (*J ALLERGY CLIN IMMUNOL* 1996;97:955-62.)

Key words: Asthma, children, T-lymphocyte activation, peak expiratory flow, variability

Bronchial mucosal inflammation has been recognized as an important feature of the pathogenesis of asthma.¹ Bronchial biopsy specimens from patients with symptomatic asthma revealed increased numbers of irregularly shaped lymphocytes² and CD25 (the α chain of the IL-2 receptor) positive cells,³ which is suggestive of T-lymphocyte activation in the lamina propria and submucosa. IL-5, formerly termed *eosinophil differentiation factor* or *B-cell growth factor II*, is capable of priming eosinophils for increased activity.⁴ With in situ

Abbreviations used

CV:	Coefficient of variation
FEV ₁ %:	Ratio of forced expiratory volume in 1 second to forced vital capacity
FITC:	Fluorescein isothiocyanate
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PEF:	Peak expiratory flow

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Received for publication Dec. 2, 1994; revised May 19, 1995; accepted for publication June 1, 1995.

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0091-6749/96 \$5.00 + 0 1/1/66680

hybridization, messenger RNA encoding IL-5 can be demonstrated in the bronchial mucosa of patients with asthma but not in that of normal control subjects.⁵ Bronchoalveolar lavage cells that were positive for not only IL-5 but also IL-3, IL-4, and granulocyte-macrophage colony-stimulating factor were increased in atopic asthma,⁶ suggesting a predominant activation of T-lymphocyte subset similar to mouse CD4_{H2} clones.⁷

Additional evidence of T-lymphocyte activation

TABLE I. Patient characteristics and therapy for asthma

Patient No.	Sex	Age (yr)	Duration (yr)	Therapy		Symptoms during the study period
				Inhaled	Oral	
1	M	13	11	Bec, Sal	Theo	Yes
2	M	14	9	Bec, Sal	Theo	Yes
3	F	10	7	Bec, Sal	Theo	Yes
4	F	10	6	Bec, Sal	Theo	Yes
5	M	13	8	Bec		No
6	F	12	8	DSCG	Theo	No
7	M	13	5	Bec, Sal	Theo	Yes
8	F	10	5	Bec, Sal		No
9	M	14	13	Bec	Theo, Clen	Yes
10	M	12	6	Bec, Pro	Theo	No
11	M	13	8	Bec, Sal	Theo, Clen	No
12	F	12	11	Bec	Theo, Clen	Yes
13	F	15	14	Bec	Clen	No
14	F	8	4	DSCG	Theo	No
15	M	9	7	DSCG	Theo	No
16	F	9	3	DSCG	Theo	Yes
17	F	12	9	Bec	Theo	No
18	F	14	13	Bec, Pro	Theo	Yes
19	F	14	12	Bec	Theo	No
20	F	14	11	Bec, Pro	Theo	No
21	M	8	7	Bec	Theo	No
22	F	13	12	DSCG		No

Bec, Beclomethasone dipropionate; *DSCG*, disodium cromoglycate; *Theo*, theophylline; *Sal*, salbutamol; *Clen*, clenbuterol; *Pro*, procaterol.

is that CD4⁺ lymphocytes expressing CD25 were increased in peripheral blood of patients with acute severe asthma and accompanied by increased serum concentrations of IL-5.⁸ Peripheral blood mononuclear cells (PBMCs) of patients with acute exacerbation of asthma demonstrated elevated expression of mRNA encoding IL-5, but not IL-2, IL-4, or interferon- γ by polymerase chain reaction quantification.⁹ These findings in both the bronchial mucosa and peripheral blood might be of importance for clinicians to consider in the choice of local and systemic antiinflammatory therapies.

Serial measurement of peak expiratory flow (PEF) has been recommended as a reliable indicator of the severity of variable airflow obstruction and is widely used in the clinical management of asthma. The coefficient of variation (CV) of PEF readings has been recognized as one of the best means of expressing PEF variability.^{10, 11}

Having shown that activated CD4 T lymphocytes were detectable in the peripheral blood of patients with acute severe asthma,⁸ we hypothesized that

CD4 T-lymphocyte activation is accompanied by PEF variability in patients with asthma. Our aim therefore was to examine the correlation between CD4 T-lymphocyte activation in PBMCs and CV of PEF in patients with asthma who were treated regularly during the study period.

METHODS

Patients

Twenty-two children (9 boys and 13 girls with a mean age of 12 years; range, 8 to 15 years), with a diagnosis of asthma as defined by the International Consensus Report on the Diagnosis and Management of Asthma,¹² formed the study group. All patients demonstrated at least 20% improvement in their FEV₁ measurement after β_2 -agonist inhalation, and all were atopic as defined on the basis of one or more positive skin scratch test responses to a range of 12 common aeroallergens in the presence of a positive histamine control and a negative vehicle control. Patient characteristics are shown in Table I. Seventeen patients were taking inhaled glucocorticoids, and 18 were taking theophylline orally in a dose attaining therapeutic serum levels between 5 and 20 mg/l. Thirteen patients were taking

inhaled and/or oral β_2 -agonists regularly. The doses of these medications were maintained during the study period. No patient received oral or intravenous glucocorticoids within 1 month before the study. If oral or intravenous glucocorticoids were prescribed because of increasing symptoms, patients were withdrawn from further participation in the study. However, patients were allowed to use additional β_2 -agonists if needed. All patients remained afebrile throughout the study period, and none demonstrated overt radiologic or bacteriologic evidence of infection. Informed consent was obtained from parents of all patients before entry into the study, which was approved by the Human Subjects Committee of the Osaka Prefectural Habikino Hospital.

Study protocol

PEF recordings were made with mini-Wright peak flow meters (Clement Clarke International, Essex, U.K.). Patients were instructed in the correct use of the instrument and asked to measure their PEF twice daily (7:00 AM and 8:00 PM) before they took morning and bedtime medications for 7 days. On day 8, peripheral venous blood was obtained from the patients for isolation of serum and mononuclear cells between 9:00 and 9:30 AM, 2 hours after their morning medications. Ratio of FEV₁ to forced vital capacity (FEV₁%) was measured with a spirometer no later than 30 minutes after blood was drawn. We calculated CV of morning PEF and CV of night PEF with records obtained over 7 days to analyze PEF variability as follows:

$$\text{CV of PEF (\%)} = 100 \times \text{PEF SD} \div \text{mean PEF}$$

Analyses of leukocyte and lymphocyte subsets

A full blood count, including differential leukocyte count, was performed by the standard hospital diagnosis service. PBMCs from the patients were obtained by centrifugation of heparinized venous blood on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) and washed three times in phosphate-buffered saline (PBS).

PBMCs were double-stained with the following murine IgG monoclonal antibodies: anti-CD4 (helper T cells), anti-CD8 (cytotoxic/suppressor T cells), and anti-CD23 (Fc ϵ RII) coupled to phycoerythrin; anti-CD25 (the α chain of IL-2 receptor, a marker of T-cell activation), IgG₁ control, and anti-CD19 (B cells) coupled to fluorescein isothiocyanate (FITC) (Becton-Dickinson, San Jose, Calif.). Aliquots of 500,000 cells in 50 μ l of PBS containing 0.1% wt/vol sodium azide were incubated with 10 μ l of one of the FITC-labeled antibodies along with 10 μ l of one of the phycoerythrin-labeled antibodies for 30 minutes on ice. After washing twice in PBS at 4° C, the cells were resuspended in 500 μ l of PBS and analyzed by flow cytometry (FACScan, Becton-Dickinson). For each sample, cells were gated on volume/90-degree light scatter plots, and 10,000 lymphocytes were counted. B (CD19) lymphocytes expressing CD23, CD4 T lymphocytes expressing CD25, and CD8 T

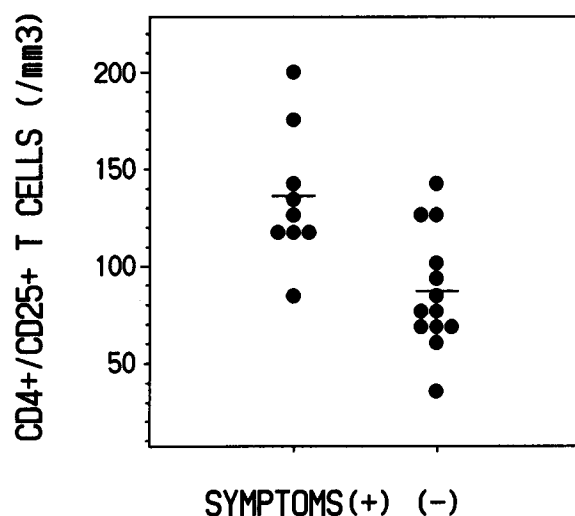


FIG. 1. Comparison of absolute counts of CD4+/CD25+ T lymphocytes in peripheral blood on day 8 between patients with asthmatic symptoms during 7 days of study and those without symptoms. Mean values are represented as horizontal lines. Statistical comparison was done by Mann-Whitney test ($p < 0.01$).

lymphocytes expressing CD25 were determined by using Consort 30 software (Becton-Dickinson). Monocytes that stained weakly with anti-CD4 and natural killer cells that stained weakly with anti-CD8 were excluded from analysis. The percentages of CD25⁺ cells were obtained by subtracting the fluorescence histogram of cells stained with the control IgG₁-FITC antibody from those of the specifically stained cells.⁸ Absolute counts of double-stained cells were calculated by multiplying the fractions of these cells by the total counts of peripheral blood lymphocytes.

Analyses of serum soluble CD25 and IL-5

Soluble CD25 concentrations in serum were measured by an ELISA with a commercially available kit (Immunotech, Marseille, France).

Serum concentrations of IL-5 were measured by an ELISA. Appropriate standards (recombinant IL-5; R&D, Minneapolis, Minn.) and samples were incubated in the wells of Maxisorp 96-well microtiter plates (Nunc, Inc., Naperville, Ill.) coated with the monoclonal antibody (anti-human IL-5/TRFK5 clone; Pharmingen, San Diego, Calif.). After washing, a biotinylated monoclonal antibody (anti-human IL-5/JES15A10 clone, Pharmingen) was added to the well and incubated. Subsequently, the plates were washed, supplemented with avidin-peroxidase, and then incubated. Finally, a substrate solution (o-phenylenediamine-H₂O₂) was added to the wells. The optical densities were read with an automated plate reader. The lower limit of sensitivity of the assay was 20 pg/ml.

TABLE II. Absolute numbers of activated lymphocytes and eosinophils, serum concentrations of soluble CD25 (sCD25), and pulmonary function in patients with asthma

Patient No.	CD4/CD25 (/mm ³)	CD8/CD25 (/mm ³)	CD19/CD23 (/mm ³)	sCD25 (pmol/L)	Eosinophils (/mm ³)	FEV ₁ % on day 8	CV of PEF (%)	
							Morning	Night
1	121	3.3	367	44.2	244	86	8.82	4.69
2	82	4.6	155	36.2	131	90	9.85	8.85
3	204	7.9	548	42.2	553	96	24.3	29.3
4	130	7.2	179	51.3	353	80	9.45	21.7
5	69	39.9	108	50.0	170	88	3.06	0.99
6	96	42.5	180	31.2	528	91	5.24	4.43
7	123	8.1	431	65.6	0	75	4.63	2.69
8	57	4.1	93	81.9	187	97	4.43	5.57
9	146	11.5	62	38.8	265	84	15.4	10.5
10	139	6.6	284	32.6	173	84	3.83	2.93
11	64	2.4	262	58.8	275	76	4.74	3.30
12	118	5.8	149	47.0	840	93	11.0	5.02
13	32	3.0	247	NT	156	88	4.09	6.50
14	83	6.0	114	50.0	285	85	7.33	10.9
15	105	6.1	131	75.6	203	71	8.32	4.39
16	179	11.4	318	55.9	320	92	19.4	8.24
17	128	13.1	117	81.9	1232	93	10.6	12.4
18	122	10.0	215	40.7	669	67	24.4	16.4
19	75	4.4	107	41.6	174	95	4.14	5.91
20	79	2.9	170	37.5	145	94	6.55	7.58
21	127	6.2	213	55.2	1050	90	15.4	18.9
22	72	3.4	76	44.2	152	73	15.4	10.7
Mean	107	9.6	206	50.6	368	86	10.0	9.04
SD	39.9	10.4	120	14.6	311	8.5	6.32	6.92

Statistical analysis

Comparisons of unpaired data were analyzed with the Mann-Whitney U test. Differences associated with *p* values of less than 0.05 were considered significant. Correlation coefficients and their statistical significance were determined with Spearman's rank correlation analysis.

RESULTS

Asthmatic symptoms, lymphocyte activation, and eosinophils

Twenty-one of the patients demonstrated FEV₁% above 70%, and only one patient demonstrated FEV₁% below 70% (patient 18), although all patients were free of symptoms between 9:00 and 10:00 AM on day 8. Nine patients had asthmatic symptoms, such as wheeze and dyspnea, at least once during the study period of 7 days (Table I), although symptoms were not severe. The absolute counts of CD4 T lymphocytes expressing CD25 in the peripheral blood on day 8 were significantly elevated in the patients with symptoms compared with those in patients without symptoms during 7 days of the study (*p* < 0.01, Fig. 1). On the other hand, the absolute counts of CD8 T lymphocytes expressing CD25, B lymphocytes expressing CD23, and peripheral blood

eosinophils and serum concentrations of soluble CD25 did not show any difference between the two groups with and without symptoms. Data for each patient are shown in Table II. Seventeen patients were taking inhaled corticosteroids, and the daily dose varied from 450 to 1200 µg (Table III). There was no correlation between the dose of inhaled corticosteroid (or the dose per body surface area) and any markers of inflammation.

In addition, no correlation was observed between the absolute counts of CD4 T lymphocytes expressing CD25 and the serum concentrations of soluble CD25 (Fig. 2, A). Serum concentrations of IL-5 were less than 20 pg/ml in most of the patients. Only two patients had serum concentrations of IL-5 that were above the lower limit of detection as determined by ELISA (patient 17, 32 pg/ml and patient 19, 21 pg/ml).

Correlation between CV of PEF and activated lymphocytes

CV of PEF was calculated to assess the variability of airway obstruction in patients by using records obtained during 7 days of the study. No

TABLE III. Doses of inhaled corticosteroids and doses per body surface area administered to 17 patients

Patient No.	Dose of Bec ($\mu\text{g/day}$)	Dose of Bec/BSA ($\mu\text{g/m}^2$ BSA/day)
1	800	650
2	1200	686
3	1000	990
4	600	444
5	450	385
7	1200	923
8	600	536
9	600	429
10	800	563
11	1200	795
12	1200	863
13	450	333
17	900	763
18	800	620
19	600	420
20	900	634
21	600	659
Mean	818	629
SD	259	188

Bec, Beclomethasone dipropionate; BSA, body surface area.

correlation was observed between the values of PEF CV and those of FEV₁ on day 8. The absolute counts of CD4 T lymphocytes expressing CD25 in the peripheral blood on day 8 correlated significantly with the values of CV of both morning PEF ($p < 0.01$, Fig. 3, A) and night PEF ($p < 0.05$, Fig. 3, C) obtained during 7 days but did not correlate with FEV₁ on day 8. In contrast, the absolute counts of CD8 T lymphocytes expressing CD25 and B lymphocytes expressing CD23 and soluble CD25 concentrations in serum did not have any correlation with CV values of either morning PEF or night PEF and did not correlate with FEV₁ on day 8 either.

Correlation between CV of PEF and eosinophils

The absolute counts of peripheral blood eosinophils demonstrated a significant positive correlation with the values of CV of both morning PEF ($p < 0.01$, Fig. 3, B) and night PEF ($p < 0.05$, Fig. 3, D) obtained over 7 days. The absolute counts of peripheral blood eosinophils also correlated significantly with absolute counts of CD4 T lymphocytes expressing CD25 ($p < 0.05$, Fig. 2, B) but did not correlate with absolute counts of CD8 T lymphocytes expressing CD25 and B lymphocytes express-

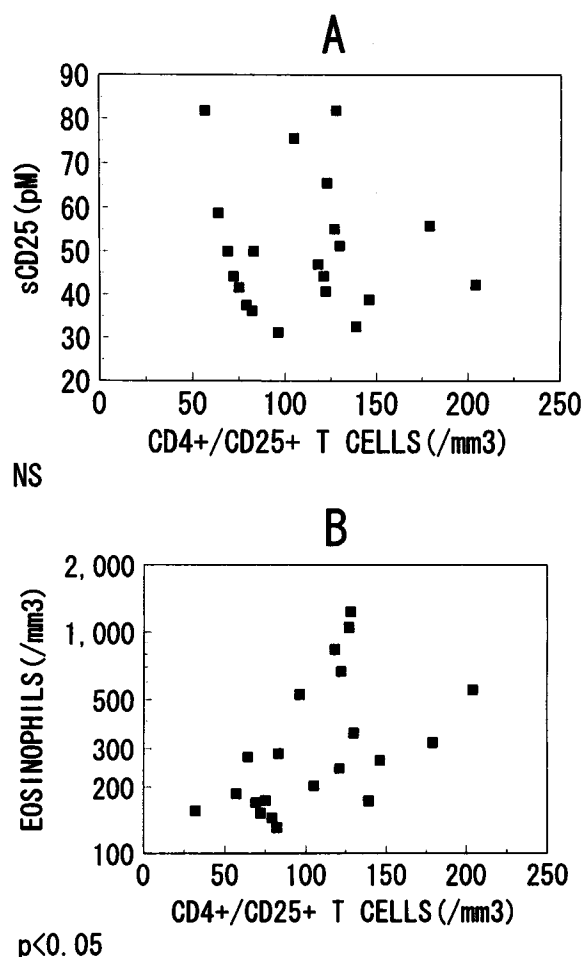


FIG. 2. A, Correlation between absolute counts of CD4+/CD25+ T lymphocytes in peripheral blood and serum concentrations of soluble CD25 ($n = 21$; $p = \text{NS}$). B, Correlation between absolute counts of CD4+/CD25+ T lymphocytes in peripheral blood and those of peripheral eosinophils ($r_s = 0.490$, $n = 22$; $p < 0.05$).

ing CD23 or soluble CD25 concentrations in serum in the patients.

DISCUSSION

This study clearly demonstrates that CV values of PEF correlated significantly with the absolute counts of CD25⁺ CD4 T lymphocytes and eosinophils in peripheral blood of children with atopic asthma. It is interesting that only CD25⁺ CD4 T-lymphocyte counts were relevant to PEF variability among lymphocytes with activation markers. This is in accordance with the findings reported by Virchow et al.,¹³ that CD25⁺ CD4 T-lymphocyte counts were inversely correlated with the change in airway obstruction when FEV₁ was observed lon-

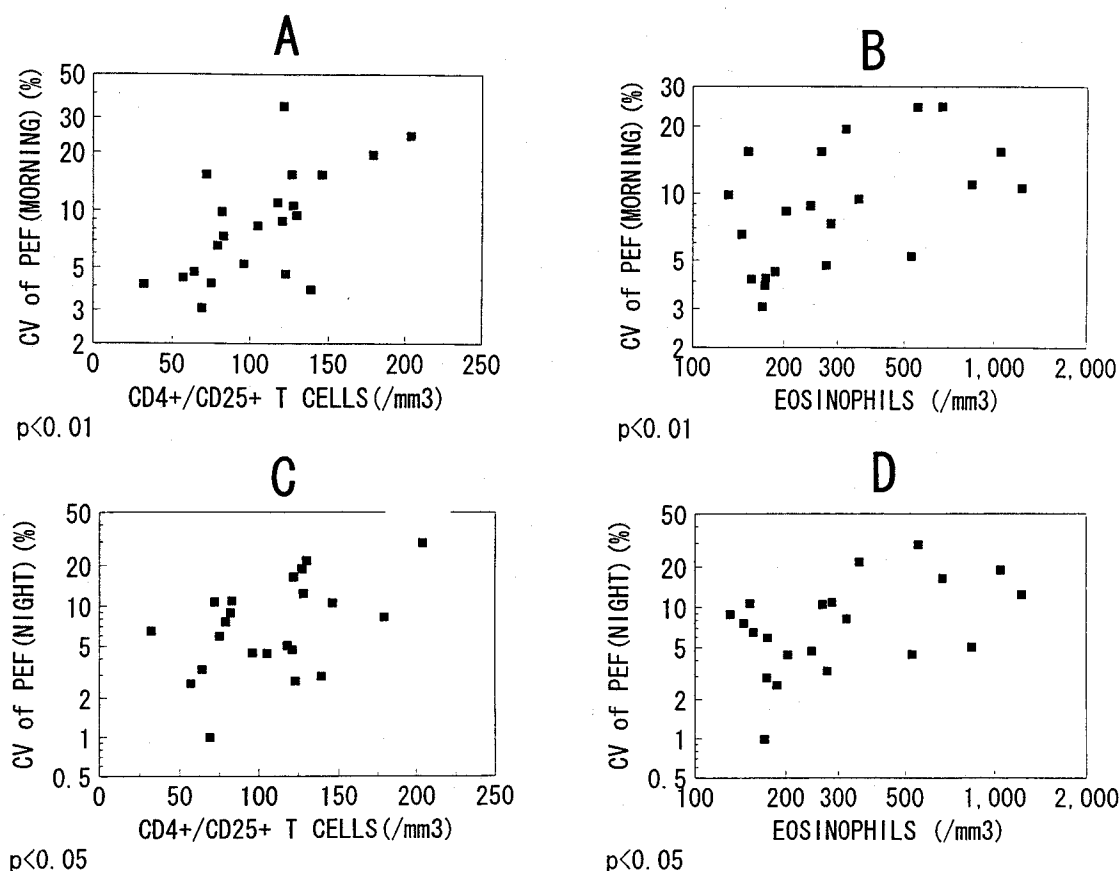


FIG. 3. A, Correlation between absolute counts of CD4+/CD25+ T lymphocytes in peripheral blood on day 8 and CV values of PEF (morning) obtained during 7 days ($r_s = 0.579$, $n = 22$; $p < 0.01$). B, Correlation between absolute counts of eosinophils in peripheral blood on day 8 and CV values of PEF (morning) obtained during 7 days ($r_s = 0.557$, $n = 22$; $p < 0.01$). C, Correlation between absolute counts of CD4+/CD25+ T lymphocytes in peripheral blood on day 8 and CV values of PEF (night) obtained during 7 days ($r_s = 0.462$, $n = 22$; $p < 0.05$). D, Correlation between absolute counts of eosinophils in peripheral blood on day 8 and CV values of PEF (night) obtained during 7 days ($r_s = 0.500$, $n = 22$; $p < 0.05$).

gitudinally for 21 days. We used daily PEF records of 7 days accompanied by CV calculation to assess the variability of airway obstruction more sensitively than FEV₁, because all patients were free of symptoms and mean FEV₁% was 86% between 9:00 and 10:00 AM on day 8.

Corrigan and Kay¹⁴ have reported that serum soluble CD25 concentrations tended to be higher in nonatopic patients with acute severe asthma. In this study the serum concentrations of soluble CD25 did not correlate with CV of PEF. This may be attributable to the fact that all patients in this study were atopic and did not have severe asthmatic symptoms throughout the study. Serum concentrations of IL-5 were increased in eight of 15 patients with acute severe asthma, in addition to increased numbers of activated CD4 T lympho-

cytes in the peripheral blood.⁸ In contrast, only two of 22 patients in this study had serum concentrations of more than 20 pg/ml of IL-5, which was the lower limit of detection by ELISA. This may be simply because the patients did not have severe symptoms throughout the study.

Peripheral blood CD23⁺ B-lymphocyte counts are known to be elevated in atopic patients.¹⁵ However, in this study development of asthmatic symptoms and CV of PEF showed no relevance to peripheral blood CD23⁺ B-lymphocyte counts. This may indicate that CD23⁺ B lymphocytes are associated with atopy but not with airway inflammation.

It is well known that eosinophils also play an important role in the inflammation of the airway in asthma. T_{H2}-like CD4 T lymphocytes stimulated by

specific allergens secrete IL-5 that induces proliferation, activation, and prolonged survival of eosinophils. Thus it is reasonable to consider that eosinophil counts correlate with activated CD4 T-lymphocyte counts in the peripheral blood. This might cause good correlations between CV of PEF and not only the absolute counts of CD25⁺ CD4 T lymphocytes but also those of eosinophils. Development of asthmatic symptoms correlated with the absolute counts of CD25⁺ CD4 T lymphocytes but did not correlate with those of eosinophils. Besides the fact that CV of PEF is more sensitive for analyzing the variability of airway obstruction than symptoms, this may result from different patterns of migration from blood vessels between activated T lymphocytes and eosinophils, which may have a different pathway of adhesion to endothelium.¹⁶ Hence, in the peripheral blood a "spillover" effect from the inflammatory process in the bronchial mucosa may be different between activated T lymphocytes and eosinophils. The other reasons may be that CD25 expression on T lymphocytes is transient,¹⁷ but survival of eosinophils stimulated by cytokines is prolonged.¹⁸

Because asthma has been recognized as a chronic inflammatory disorder of the airway, the early use of antiinflammatory agents, one of which is inhaled corticosteroids, is advocated in the treatment of chronic asthma. Administration of corticosteroids to human beings results in eosinopenia, and it has been reported that corticosteroids inhibit IL-5-enhanced in vitro survival of human eosinophils.¹⁹ The relative amounts of IL-5 mRNA were reduced after oral glucocorticoid therapy in patients with asthma,⁹ suggesting that corticosteroids contribute to inhibition of IL-5 transcription of T lymphocytes in vivo. In addition, treatment with inhaled corticosteroids reduced the expression of the activation markers in both T lymphocytes recovered by bronchoalveolar lavage and peripheral blood T lymphocytes, along with improvement of symptoms.²⁰ However, nocturnal serum cortisol production was significantly reduced by inhaled corticosteroids in asthmatic children, which emphasized the importance of using the minimum dose compatible with good control of asthma.²¹ In this study there was no correlation between the dose of inhaled corticosteroid (or the dose per body surface area) and any markers of inflammation. Our results suggest that activation markers of T lymphocytes may be an indicator of control rather than of severity in asthma. In our study some of the patients may remain undertreated because of the high counts

of activated T lymphocytes and large variability of PEF.

On the other hand, after treatment with theophylline, there was a significant reduction in the number of EG2-positive activated eosinophils in the bronchial mucosa of patients with asthma, suggesting that theophylline may be an antiinflammatory agent.²² Further characterization by the use of antiinflammatory agents in various clinical settings may allow a pathophysiologic classification of the disease and may point the way toward novel therapeutic strategies.

We thank Dr. Susumu Kishimoto, Honorary Director of Osaka Prefectural Habikino Hospital, for his careful review of the manuscript and many helpful suggestions.

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