

Functional interleukin-5 activity in peripheral blood mononuclear cells from adolescents with mite antigen asthma in remission

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Summary

Background Asthma improves in most children during adolescence such that a small minority of patients exhibit clinically significant symptoms by the age of 20 years.

Objective and methods To investigate late allergic reactions, including eosinophil inflammation, associated with outgrowing mite antigen-induced bronchial asthma during adolescence, the relationship between clinical status and functional activity of interleukin (IL)-5 produced by *Dermatophagoides farinae* (Df)-stimulated peripheral mononuclear cells (PBMCs) in culture was assessed in mouse IL-3-dependent cells transfected with the human IL-5 receptor gene.

Results Activity of IL-5 spontaneously produced by PBMCs from either patients with mite-sensitive bronchial asthma or nonatopic control subjects was low. The activity of IL-5 produced by PBMCs stimulated with concanavalin A was significantly higher. Upon challenge with specific allergens, such as Df antigen, but not with irrelevant antigens, including ovalbumin, the *in vitro* activity was increased in patients with active disease and decreased in patients in remission.

Conclusion Results suggest that the antigen-specific up-regulation of functional IL-5 activity in late allergic reactions is reduced in patients in remission and likely to result in an improvement in clinical status. The Df antigen may suppress Df-induced responses in patients with asthma in remission.

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Introduction

Allergen-activated T-cell subsets, possibly equivalent to TH2, predominate in allergy and express both interleukin (IL)-4, an essential cofactor for IgE production, and IL-5, which induces terminal differentiation and activation of eosinophils [1,2]. In patients with mite-sensitive bronchial asthma, mononuclear cells and T cells cultured with IL-2 and *Dermatophagoides farinae* (Df) release eosinophil colony-stimulating factor [3,4]. Expression of IL-5 messenger RNA has also been detected in the bronchial mucosa of patients with asthma [5–8] and cellular localization of the mRNA in the airway supports the hypothesis that IL-5 is involved in late asthmatic responses.

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Production of IL-5 by peripheral blood mononuclear cells (PBMCs) is significantly increased by phytohaemagglutinin stimulation [8]. Upon challenge with a specific allergen, such as mite antigen, IL-5 is produced *in vitro* by PBMCs from patients with mite-sensitive atopy [9]. Incubation of epithelial cells and dendritic cells from asthma patients with autologous resting CD4-positive T cells and mite antigen induces T-cell activation and release of high levels of IL-5 [10]. However, the amount of IL-5 released varies among patients [3,11]. IL-5 in serum was detected in all asthmatic and disease-free individuals. Its values during asthma exacerbation were significantly higher than during remission of asthma [12].

Asthma improves in most children during adolescence such that a small minority of patients exhibit clinically significant symptoms by the age of 20 years [13–15]. Up-regulated interferon (IFN)- γ production in children

with asthma after exposure to Df antigen might reduce IL-4 secretion by Df-stimulated cells and result in suppression of IgE production and clinical improvement [16]. To further investigate late allergic reactions, including eosinophil inflammation, associated with outgrowing mite antigen-induced bronchial asthma during adolescence, we studied the relationship between clinical status and relevant antigen-induced functional, but not antigenic, activity of IL-5 produced in culture by Df-stimulated PBMCs from patients with atopy, assessed with mouse IL-3-dependent cells, FDC-P1, transfected with the human IL-5 receptor gene (FDC-h5R) [17].

Materials and methods

Subjects

The subjects of the study were 13 Japanese patients with mite-sensitized bronchial asthma (Table 1) and seven non-allergic volunteers who served as control subjects. Patients with active disease had recurrent asthma attacks and a positive immediate skin reaction (defined as an immediate weal response ≥ 10 mm in mean diameter on skin prick test) to house dust mite and Df antigen (mite 1, Torii & Co. Ltd, Tokyo, Japan), but not hen's egg antigen (Sigma, St Louis MO, USA). Serum IgE scores, as determined with radio-allergosorbent tests, for mite antigen [18] ranged from 3 to 5 and those for ovalbumin (Sigma) were 0.3 ± 0.2 . The patients did not suffer from other allergic diseases, such as atopic dermatitis. The diagnostic criteria used for bronchial asthma were those of the American Thoracic Society. None of the patients had acute asthma at the time of examination or was receiving oral or inhaled corticosteroids or anti-allergic agents, such as ketotifen [19]. Patients were identified prospectively and followed-up until they could be divided

into two groups: patients in remission, i.e. disease-free for 2 years without medication; and patients not in remission, i.e. more than 10 mild to moderate attacks per year despite use of medications, such as theophylline (15–20 mg/kg/day) and β_2 -agonists. The severity of acute exacerbations of asthma and the overall disease severity were estimated according to the Guidelines for the Diagnosis and Management of Asthma (the National Asthma Education Program; Expert Panel Report) released by the National Institutes of Health, USA. A mild attack was defined as normal alertness, absent or mild dyspnea (patient was able to speak in complete sentences), no or mild intercostal retraction, good skin colour, end-expiratory wheezing only, and 70 to 90% of predicted or personal-best PEFR or FEV₁. The FEV₁ values of both patient groups were within normal limits between acute asthma attacks and in the 4 to 6 weeks preceding examination and did not differ significantly. The severity of asthma for more than 2 years before the study period was mild to moderate in both groups (more than 10 mild or moderate attacks per year; medication, intermittent or persistent). Clinical characteristics of the two patient groups are shown in Table 1. Five children allergic to hen's egg who served as disease controls had recurrent eczema and positive skin reactions to egg white, but not to house dust mite or Df antigen. The patients also had positive responses (immediate, late, and/or delayed response; eruptions, angioedema, or exacerbation of atopic dermatitis) to the oral provocation test to raw hen's egg. Serum IgE RAST scores against ovalbumin ranged from 2 to 3 and those for Df were 0. The patients did not suffer from bronchial asthma. Diagnostic criteria for atopic dermatitis were based on those of Hanifin and Rajka [20].

Informed consent was obtained either from the patient, or in the case of minors, from the parents.

Table 1. Characteristics of subjects used in the study

	Controls	Remission	Active
No. of patients	7	7	6
Median age in years (range)	18 (7–35)	11 (9–13)	10 (6–12)
Sex (M : F)	4 : 3	6 : 1	3 : 3
Disease severity	(Nonallergy)	Disease-free	Mild
Documented attacks*		0	More than 10 mild or moderate attacks
Medication		–	+ (intermittent or persistent)
Serum IgE (U/mL)	$132 \pm 69^\dagger$	952 ± 294	588 ± 95.3
Df-IgE RAST score	0	4.6 ± 0.9	4.7 ± 0.3
OVA-IgE RAST score	0	0.2 ± 0.2	0.3 ± 0.2
Eosinophil count(/mL)	NT	779 ± 178	1161 ± 167
Skin prick test (Df)	–	+	+

*per year over the previous 2 years. † mean value $\pm$ SE.

IL-5 production and measurement of the activity

Blood samples were obtained from patients and control subjects, after which PBMCs were isolated by centrifugation on Ficoll-Hypaque (Pharmacia Diagnostics, Uppsala, Sweden) gradients. The PBMCs ($10^6/\text{mL}$) were cultured with $1\text{ }\mu\text{g/mL}$ Df antigen (Torii & Co. Ltd), $5\text{ }\mu\text{g/mL}$ concanavalin A (Con A; Sigma) or $10\text{ }\mu\text{g/mL}$ ovalbumin (Sigma) in RPMI 1640 medium containing $40\text{ }\mu\text{g/mL}$

gentamycin and 5% fetal calf serum (FCS; Sigma). After 3 days' incubation, supernatants were collected and IL-5 activity was determined with a bioassay using FDC-h5R cells [17], which were kindly provided by Dr Kiyoshi Takatsu, Institute for Medical Science, Tokyo University. Briefly, FDC-5 h cells were treated with 100 U/mL murine recombinant IL-3 (Genzyme Corp., Boston MA, USA) and then cultured for 2 days in RPMI 1640 medium supplemented with 5% FCS in a 96-well microplate (NUNC, Roskilde, Denmark) (37°C , 10% CO_2). Next, 10^4 FDC-5 h cells were added to the supernatants and then cultured for 3 days. Then, $0.5\text{ Ci }^3\text{H-TdR}$ was added to each well, after which cultures were incubated for an additional 24 h before harvest. The $^3\text{H-TdR}$ uptake was quantified with a liquid scintillation counter. Recombinant IL-5 (Genzyme) was used as a standard. Results are expressed with reference to a standard IL-5. The sensitivity of this assay was 2 pg/mL .

To verify the IL-5-dependent proliferation of FDC-h5R cells, 10^4 FDC-5 h cells were added to recombinant IL-5 or the culture supernatants obtained from Df-stimulated patient PBMCs and then cultured for 3 days with or without rat antihuman IL-5 antibody (Pharmingen, San Diego CA, USA). Then, the $^3\text{H-TdR}$ uptake for an additional 24 h was quantified.

Statistical analysis

Data were analysed with the two-tailed Student's *t*-test.

Results*IL-5 activity*

The FDC-h5R cells proliferated in response to human recombinant IL-5 (Fig. 1a), but this IL-5-dependent proliferation was suppressed by co-incubation with 100 ng/mL rat antihuman IL-5 antibody (Fig. 1bi), but not with rat IgG

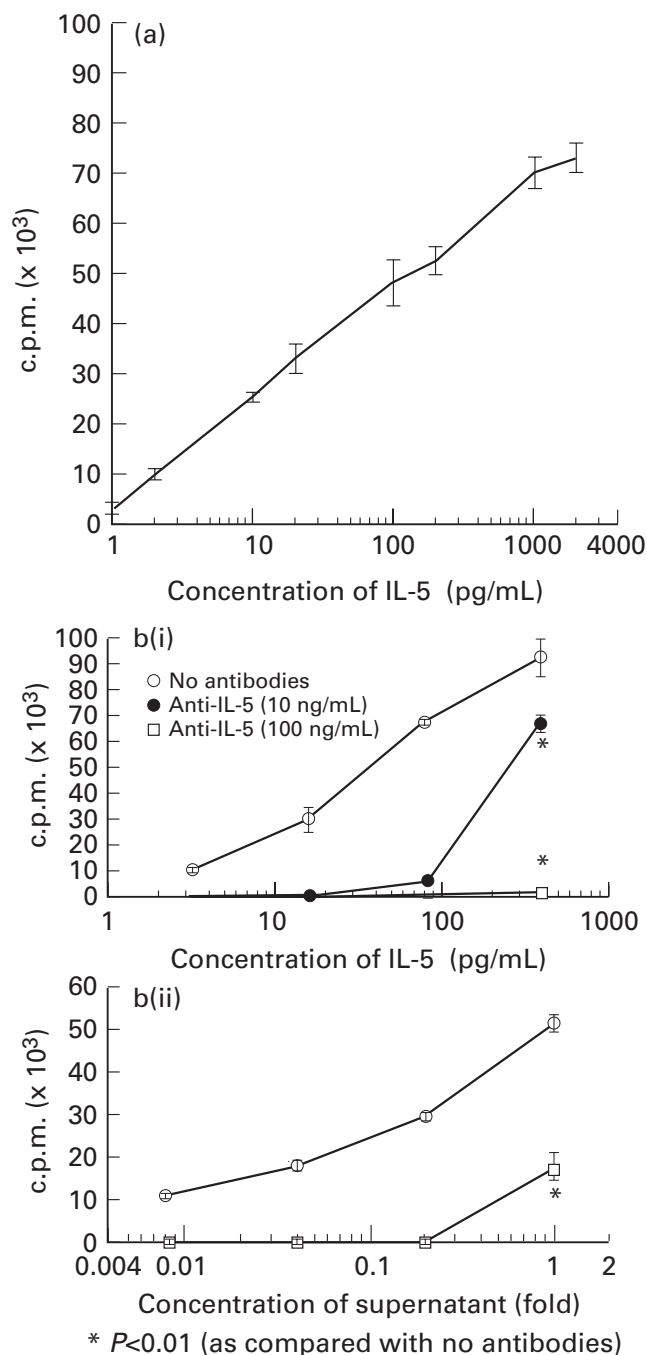


Fig. 1. (a) Proliferation of FDC-h5R cells in response to human recombinant IL-5. As each independent experiment showed the same trends in the proliferation of FDC-h5R cells in response to human recombinant IL-5, data are expressed as the mean standard errors of three representative experiments of more than 10 independent experiments. (b) The IL-5-dependent proliferation of FDC-h5R cells was blocked by anti-IL-5 antibody. FDC-5 h cells (10^4) were added to recombinant IL-5 (i) or the culture supernatants obtained from Df-stimulated patient PBMCs (ii) and then cultured for 3 days with or without rat antihuman IL-5 antibody. Then, the $^3\text{H-TdR}$ uptake for an additional 24 h was quantified. Data are expressed as the mean SE of triplicate trials in a representative experiment of three independent experiments. * $P < 0.01$ (as compared with no antibodies).

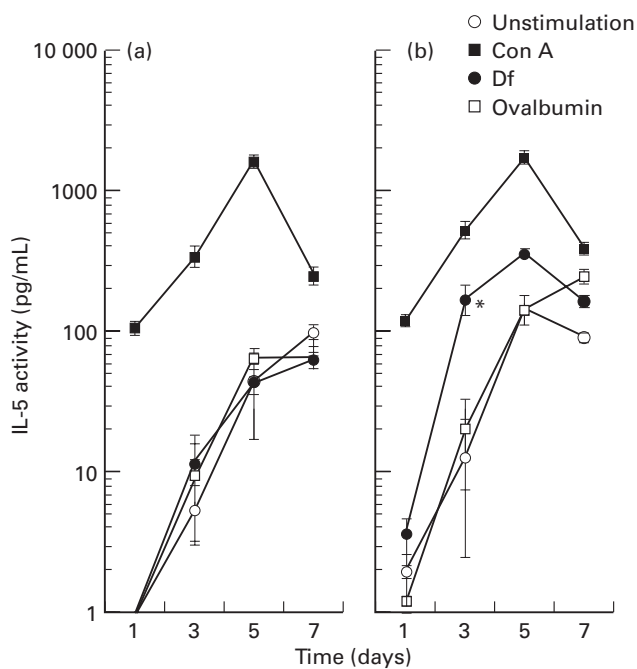


Fig. 2. Kinetics of IL-5 production by antigen-stimulated PBMCs. PBMCs from (a) nonallergic subjects ($n = 4$) or (b) patients ($n = 3$) were stimulated with 1 $\mu\text{g/mL}$ Df, 10 $\mu\text{g/mL}$ ovalbumin, or 5 $\mu\text{g/mL}$ Con A. After 1–7 days culture, IL-5 activity of the supernatants was determined with a bioassay using FDC-h5R cells. Data are expressed as the mean $\pm$ SE of three to four representative experiments. * $P < 0.01$ (as compared with no antibodies).

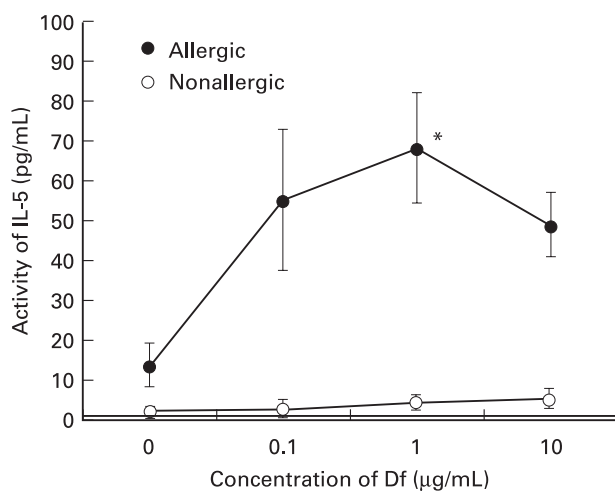


Fig. 3. Df-induced IL-5 activity in patients with mite-sensitive asthma. PBMCs from patients were cultured with serial dilutions of Df. After 3 days' incubation, supernatants were collected and IL-5 activity was determined with a bioassay using FDC-h5R cells. Data shown are the mean $\pm$ SE of four independent experiments (one active and three remitted patients). * $P < 0.01$ (as compared with no antibodies).

(data not shown). These results indicate that functional IL-5 activity can be assayed with FDC-h5R cells. The FCS concentration of 5% used for cultures was optimal for evaluation of IL-5 activity in supernatants of PBMCs stimulated with 1 $\mu\text{g/mL}$ Df or 5 $\mu\text{g/mL}$ Con A in both patients and control subjects. Anti-human IL-5 antibody (100 ng/mL) blocked the proliferation of FDC-h5R cells in response to these supernatants, a finding that indicates the supernatant used for assay has functional IL-5 activity (Fig. 1bii).

Upon stimulation with Con A, the activity of IL-5 in culture supernatants was highest on day 5 of FDC-h5R cell cultures in both controls and patients (Fig. 2). Production of Df-induced IL-5 was detected in patients on days 3 and 5 of cell culture (Fig. 2). One milligram of Df-induced maximal IL-5 production by PBMCs from patients (Fig. 3). In control subjects, IL-5 activity did not differ significantly between PBMCs stimulated with Df and those that were not (Fig. 2). Furthermore, ovalbumin failed to induce significant IL-5 production either in controls or in patients allergic to Df antigen alone (Fig. 2).

Antigen-specific IL-5 production in patients

PBMCs obtained from patients with bronchial asthma allergic to mite antigen alone, patients with atopic dermatitis allergic to hen's egg alone and nonallergic subjects were cultured with Df antigen ovalbumin or concanavalin A. IL-5 activity in the supernatants after 3 days' incubation was evaluated. Spontaneous IL-5 production by PBMCs from patients and nonatopic subjects was low (Fig. 4). However, production was markedly increased when PBMCs were stimulated with Con A. Stimulation with Df significantly increased IL-5 production by PBMCs in patients with bronchial asthma but not in normal subjects. The production of IL-5 was not affected by stimulation with irrelevant ovalbumin. In contrast in patients allergic to hen's egg production of IL-5 was significantly increased by stimulation with relevant ovalbumin but not Df antigen (Fig. 4). The results indicate IL-5 production induced by Df antigen in patients with bronchial asthma was specific for the relevant antigen.

To further investigate the relationship between IL-5 activity and clinical status, the activity of IL-5 in the supernatants of Df-stimulated PBMCs after 3 days' incubation was also evaluated in subjects outgrowing mite antigen-induced bronchial asthma. As shown in Fig. 5 production of IL-5 was lower in the patients in remission than in patients with active disease.

In addition, IL-5 production induced by Df in patients was not correlated with either serum IgE or eosinophil counts in peripheral blood (data not shown).

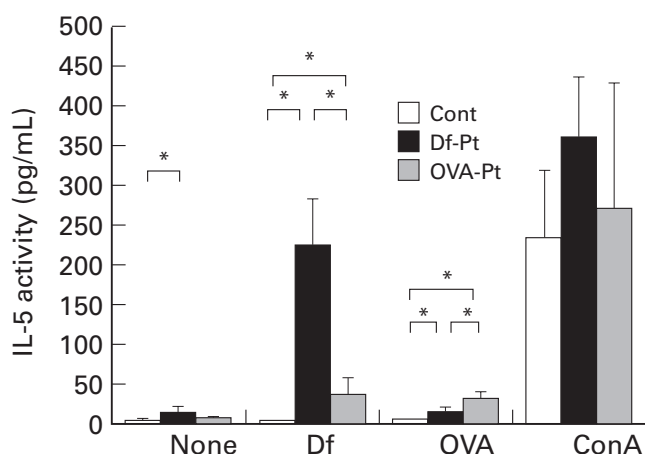


Fig. 4. Df-induced IL-5 production is specific for antigen. PBMCs from patients allergic to mite antigen alone or patients allergic to hen's egg alone were cultured with the relevant antigen. IL-5 activity in the supernatants after 3 days' incubation was evaluated with a bioassay using FDC-h5R cells. Data shown are the mean $\pm$ SE of five to seven experiments. * $P < 0.05$.

Discussion

To measure IL-5, both a bioassay based on the ability of IL-5 to induce IgM secretion by murine B cell lymphoma BCL1 cells and sandwich enzyme-linked immunosorbent assay (ELISA) have been used. However, the bioassay using BCL1 cells has low sensitivity: the detection limit for human IL-5 is about 500 ng/mL compared with 10 pg/mL with ELISA. The bioassay using FDC-h5R cells was more sensitive than ELISA; its detection limit was 2 pg/mL, as shown in Fig. 1(a). Every standard curve showed the same tendency. We conclude that our assay system is useful for quantifying functional human IL-5 activity in various biological fluids, unlike ELISA, which measures antigenicity against the IL-5 protein.

To evaluate the ability of Df-stimulated lymphocytes to produce IL-5, PBMCs from patients with bronchial asthma were cultured with graded doses of Df antigen for 3 days, after which functional activity of IL-5 was measured. The IL-5 activity increased in a dose-dependent manner until 1 g/mL, then decreased at 10 μ g/mL Df antigen (Fig. 3). The IL-5 activity in patients was significantly higher than that in nonallergic control subjects (Fig. 3). Moreover, activity was not observed when PBMCs from patients, whose bronchial asthma was due to Df antigen, were stimulated with ovalbumin antigen (Fig. 4). Similarly, IL-5 activity in PBMCs from patients with atopic dermatitis due to ovalbumin antigen was observed after ovalbumin stimulation (Fig. 4). However, the IL-5 activity in cultures treated with Con A did not differ between patients and normal subjects. Thus,

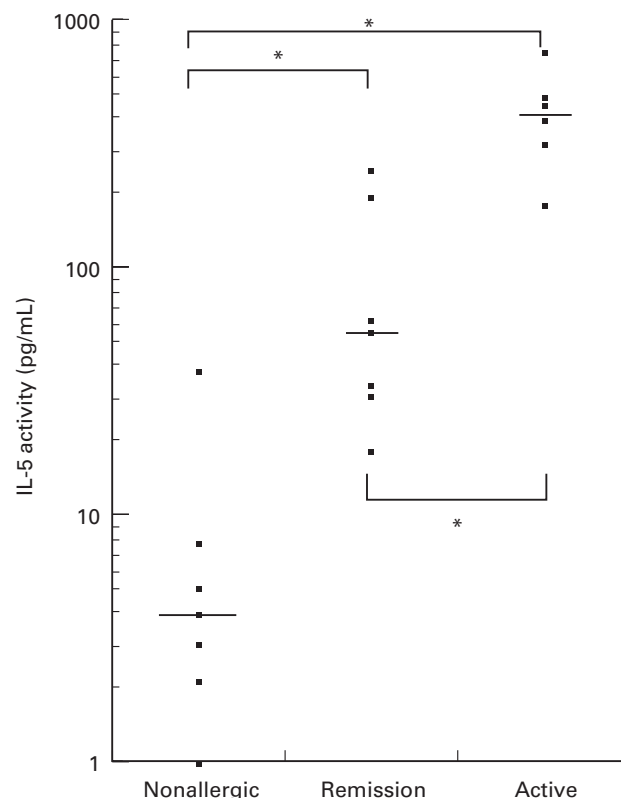


Fig. 5. Df-induced IL-5 activity in patients in remission. PBMCs were cultured with Df antigen for 3 days, and IL-5 activity in the supernatant was determined with a bioassay using FDC-h5R cells. Recombinant IL-5 was used as a standard. Results shown are data of individual patients and the median. Nonallergic, nonallergic subjects; Remission, patients in remission; Active, patients with active disease. * $P < 0.03$.

IL-5 activity produced by Df-stimulated lymphocytes was specific for the relevant antigen.

IL-5 is produced not only by T cells, but also by mast cells, eosinophils and other immune cells. Depletion of CD4-bearing cells completely removes IL-5-producing cells in patients with mite-sensitive asthma [9]. In this study, production of IL-5 induced by Df was not correlated with the number of eosinophils in peripheral blood, a finding that is consistent with reports that *in vitro* IL-5 production by PBMCs are not correlated with blood eosinophil counts in either patients with asthma or in normal subjects [8]. Peripheral levels of IL-5 likely reflect terminal differentiation and activation of eosinophils by IL-5, but not the number of eosinophils in the bronchial mucosa of patients. Together, these findings suggest that T cells are a principal source of IL-5 in PBMCs and that Df-induced IL-5 production by T cells might be related to augmentation of eosinophil inflammation in the airway in Df-sensitized bronchial asthma.

As previously reported, Df-induced IL-4 production by PBMCs is up-regulated in patients with Df-sensitive bronchial asthma whereas IFN- γ production is down-regulated [16]. The cytokine imbalance was restored, and IFN- γ production increased markedly in patients in remission [16]. This study found that Df-induced production of IL-5, a principal TH2 cytokine, was also increased in patients with active Df-sensitive bronchial asthma and was significantly decreased in patients in remission after *in vitro* stimulation with Df antigen (Fig. 5). Thus, the ability of lymphocytes from patients in remission to secrete functional IL-5 in response to Df antigen, which likely reflects late allergic responses, was down-regulated. Changes in the production of these cytokines related to both immediate and late allergic responses are likely involved in outgrowing of mite-sensitive bronchial asthma.

In this study it was extremely difficult to obtain any objective evidence that the severity of asthma in patients in remission was originally equivalent to that in patients with active disease. Therefore, we studied patients whose asthma had been mild and nearly stable for more than 2 years before the study period. The results of this cross-sectional study suggest that IL-5 activity reflects the rate of asthma exacerbation.

Perhaps a more logical approach to studying IL-5 activity would be to perform a longitudinal study of patients who did and did not outgrow their asthma; however, such a study would take an extremely long time to complete.

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