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Interleukin-10 and interleukin-5 balance in patients with active asthma, those in remission, and healthy controls

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Background: The immunological mechanisms of asthma remission remain unclear although several reports have suggested that balance between T helper (Th) 2 cytokines and regulatory cytokines is related.

Objective: To study the balance between interleukin (IL) 10 and IL-5 in asthma clinical remission.

Methods: We measured the numbers of IL-5 and IL-10 producing cells in peripheral blood mononuclear cells stimulated with mite antigen obtained from patients with active asthma (group A, n = 18), patients in clinical remission (group R, n = 15) and nonatopic healthy controls (group H, n = 14).

Results: The numbers of IL-5 producing cells in groups A and R were significantly higher than in group H. The number of IL-5 producing cells was lower in group R than in group A, although the difference was not statistically significant. The number of IL-10 producing cells was higher in group R than in group A, although again the difference was not statistically significant. There was a significant difference in the number of IL-10 producing cells between groups A and H but not between groups R and H. The ratio of the number of IL-10 to IL-5 producing cells was highest in group H followed by groups R and A, and the differences were statistically significant for each pair of groups.

Conclusion: Our study suggests that the IL-10/IL-5 balance is related to clinical asthma. The balance differs between patients in clinical remission and healthy controls, suggesting that allergic inflammation may continue even after clinical asthma remission.

Key words: Asthma; Child; Interleukin-10; Interleukin-5; Prognosis; Peripheral Blood Mononuclear Cell

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INTRODUCTION

Asthma is a chronic airway inflammation mediated by allergen-reactive T helper (Th) 2 cells with eosinophils and mast cells as the main effector cells that orchestrate asthmatic airway inflammation [1, 2]. The presence of eosinophils in the airway wall is a prominent feature of bronchial asthma and seems to be related to asthma severity [3]. The levels of interleukin (IL) 5, a Th2 cytokine that is important in the regulation of eosinophils and in the pathogenesis of asthma, have been shown to correlate with the severity of asthma [4, 5].

A substantial number of patients with childhood bronchial asthma improve during adolescence [6-8]. Elucidation of the mechanism of remission of child-onset asthma is quite important for predicting the prognosis of asthma, as well as for disease intervention and the development of new therapeutic strategies. Certainly, there is a possibility that changes in the immunological response to allergens are related to the severity of asthma and its remission. Decreased IL-5 production by mononuclear cells in asthma patients who are in remission has been reported [9]. Two possibilities exist regarding the suppression of Th2 cytokine response in asthma remission from the point of cytokine balance. One is the enhanced or normalized production of a Th1 cytokine, such as interferon-γ, and the second is the enhanced production of an inhibitory or regulatory cytokine, such as IL-10. While the former possibility has been proposed by several investigators [10, 11], there have been only a few studies on regulatory cytokines in asthma remission [12]. Akdis et al. [13] clearly showed that regulatory cytokines, especially IL-10, are involved in the active suppression of Th2 responses to allergens in nonatopic healthy subjects. They proposed that the balance between allergen-specific T regulatory (Treg) cells and Th2 cells is decisive in the development of allergy. However, it is not clear whether Treg/Th2 balance is related to clinical remission of child-onset asthma. Thus, we tried to address this issue by studying the numbers of IL-5 and IL-10 producing cells in mite antigen-stimulated peripheral blood mononuclear cells (PBMCs) from patients with active asthma, patients in remission and nonatopic healthy controls.

MATERIALS AND METHODS

Patients and controls

Subjects in this study are described in Table 1. Thirty-three atopic asthma patients with IgE to *Dermatophagoides farinae* were studied. The diagnostic criteria for asthma were those of the American Thoracic Society. None of the patients had acute asthma at the time of examination, and none of them were receiving oral corticosteroids. Patients were enrolled prospectively and were followed until they could be categorized into two groups (groups A and R). Group A were patients with active disease (n = 18) who experienced more than several mild-to-moderate attacks per year despite the use of medications such as inhaled corticosteroids, theophylline or leukotriene receptor antagonists. Group R, patients in remission (n = 15), were symptom-free for more than 3 years without medication. Forced expired volume values of both groups of patients were within normal limits between acute attacks of asthma and for one month prior to examination. During active period, asthma was mild-to-moderate persistent in both groups. Fourteen healthy volunteers were also included as controls (group H). Controls had no allergic symptoms, total IgE levels below 100 IU/L, and negative CAP-RAST (Pharmacia Diagnostics, Uppsala, Sweden) scores to 3 common aeroallergens, house-dust mite (*D. farinae*), Japanese cedar pollen, and cat dander. The ages of the patients in each group were: group A, 19.77 ± 3.14 years old (mean ± standard d error); group R, 20.87 ± 2.68; group H, 21.64 ± 1.74. There were no differences in age among the 3 groups. Approval from the Ethics Committee of Chiba University was obtained. Informed

Table 1. Subjects profile

Subjects	Age (yr)	Total IgE (IU/mL)	CAP-RAST* *D. farinae* (UA/mL)
Active (n = 18)	19.77 ± 3.14	1,363.8 (293.1–1,345.9)	235.0 (35.6–210.9)
Remission (n =1 5)	20.87 ± 2.68	586.9 (118.3–430.5)	50.3 (12.9–53.0)
Healthy (n = 14)	21.64 ± 1.74	30.1 (16.2–35.8)	<0.34

Values are presented as mean ± standard error or geometric mean (95% confidence interval).
D. farinae, *Dermatophagoides farina*.
*CAP-RAST: Pharmacia Diagnostics, Uppsala, Sweden.

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consent was received from all subjects.

Antigens and reagents

D. farinae extracts were kindly provided by Torii Pharmaceutical Co., Ltd. (Tokyo, Japan) and used as a mite antigen. Rat antimouse/human IL-5 monoclonal antibody (mAb), biotinylated rat antihuman IL-5 mAb, rat antihuman/viral IL-10 mAb, biotinylated rat antihuman/viral IL-10 mAb and streptavidin-alkaline phosphatase were purchased from PharMingen (San Diego, CA, USA). The BCIP/NBT Phosphatase Substrate System was obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA).

ELISPOT assays

PBMCs were separated from heparinized blood by centrifugation on a Ficoll-Paque gradient (Pharmacia). The wells of MultiScreen-HA plates (Millipore Co., Bedford, MA, USA) were coated with 100 µL of anti–IL-5 5 mAb and anti–IL-10 mAb at a concentration of 2 µg/mL. After incubation overnight at 4°C, the unbound antibody was removed, and the coated wells were blocked with 100 µL of phosphate-buffered saline (PBS) containing 5%-milk. After 2 hours incubation at room temperature, the blocking solution was discarded and 100 µL of assay medium (AIM-V, Gibco BRL, Paisley, UK) containing the indicated numbers of PBMCs (500,000 cells for IL-5 and 50,000 cells for IL-10) was added. One hundred micro litters of mite antigen at a final concentration of 10 µg/mL were added. Cells were incubated at 37°C in a 5% CO_2 water-saturated atmosphere. After 48 hours of culture, the cells were washed with distilled water containing 0.05% Tween 20. Captured cytokines were detected at the site of secretion by incubation for 2 hours at room temperature with 75 µL of biotinylated anti IL-5 mAb, biotinylated anti IL-10 mAb at a 4 µg/mL in 5% milk-PBS. After washing the wells with PBS containing 0.05% Tween (PBS/T), streptavidin conjugated to alkaline-phosphatase was added. After 1 hour of incubation, the wells were washed with PBS/T, and 100 µL of BCIP/NBT solution was added. The color reaction was stopped by washing the plates under running tap water. Each well was photographed, under a microscope, and the number of spots was counted. Mite-specific spots were calculated by subtracting the number of spots in a well containing medium from that of a well containing antigen. The assay was done in duplicate.

Statistical analysis

Because of skewed distribution, the number of IL-5 and IL-10 producing cells and the ratio of IL-10/IL-5 producing cells were converted to log scale for further statistical analysis. The Bonferroni/Dunn analysis was used for multiple comparison tests. The significance was defined as $p < 0.0167$. The StatView software (PharMingen, San Diego, CA, USA) was used for analysis.

RESULTS

Numbers of cytokine producing cells in the three groups

Fig. 1 shows the logarithm of numbers of cytokine producing cells among cells stimulated with mite antigen in the three groups. The numbers of IL-5 producing cells in groups A and R were statistically higher than those in group H ($p < 0.0001$ and $p = 0.0014$, respectively). The numbers of IL-5 producing cells in group R were lower than in group A, however, there was no statistically difference ($p = 0.1111$). The number of IL-10 producing cells in group R was higher than in group A ($p = 0.0338$), but did not reach statistical significance by The Bonferroni/Dunn analysis in which significance is defined as $p < 0.0167$. There was no statistical difference between groups R and H ($p = 0.3242$). The number of IL-10 producing cells in group H was higher than that in group A ($p = 0.0041$).

Cytokine balance in the three groups

In addition to the number of PBMCs that produce each cytokine, the balance of IL-10 to IL-5 was also compared among the three groups (Fig. 2). The ratio of the number of IL-10 to IL-5 producing cells was highest in group H, followed by groups R and A, and statistically significant among each pair of groups (A vs. R, $p = 0.0043$; R vs. H, $p = 0.0019$; A vs. H, $p < 0.0001$).

DISCUSSION

This study demonstrated the association of IL-10/IL-5 ratio with remission in asthmatics patients, and suggested that allergic inflammation may continue even after clinical asthma remission. The number of IL-5 producing cells upon stimulation with mite antigen was lower in patients in remission than in patients with active asthma as previously reported by Noma et al. [12], although the difference did not reach statistical significance. However, the number of IL-5 producing cells in healthy controls was significantly lower than in patients in remission, which is also compatible with the findings of Noma et al. [12]. Their data and ours indicate that

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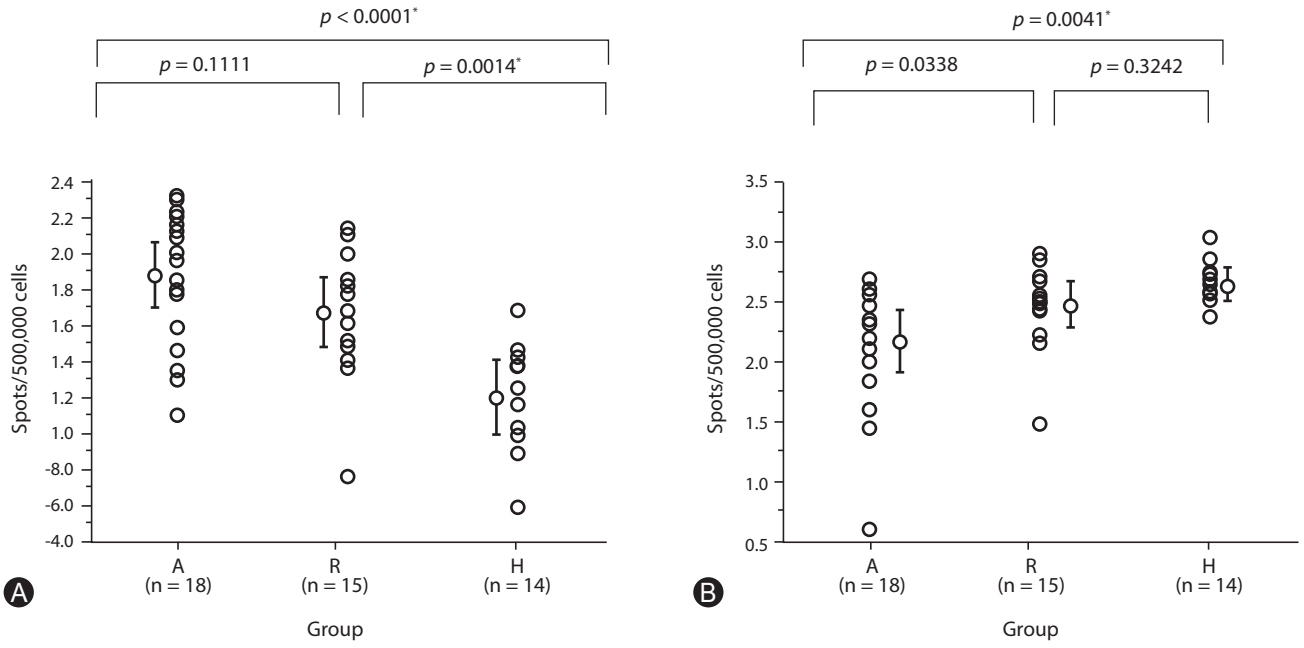

Fig. 1. (A) Numbers of interleukin (IL)-5-producing cells, and (B) numbers of IL-10-producing cells in peripheral blood mononuclear cells stimulated with mite antigen in three groups. Group A, patients with active asthma; group R, patients in emission; group H, nonatopic healthy controls. Statistical difference was evaluated by Bonferroni/Dunn analysis by using StatView software. The significance is defined as $p < 0.0167$ and shown by an asterisk. The geometric mean with 95% confidence interval is shown.

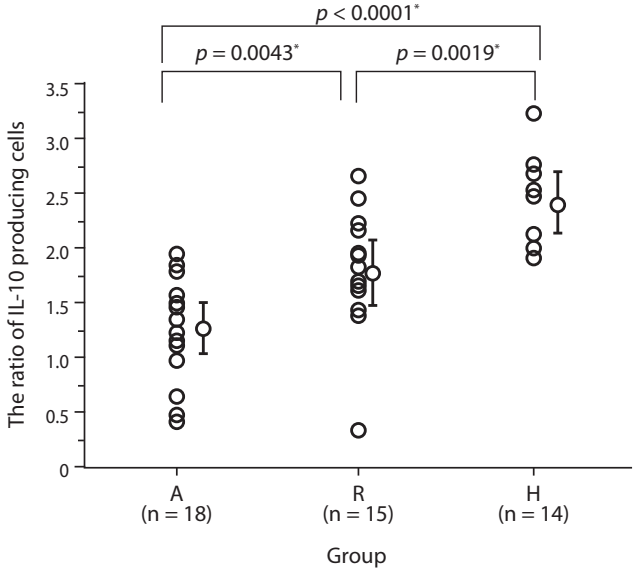

Fig. 2. Ratios of the numbers of interleukin (IL) 10 producing cells to the numbers of IL-5 producing cells in three groups. Group A, patients with active asthma; group R, patients in emission; group H, nonatopic healthy controls. Statistical difference was evaluated by Bonferroni/Dunn analysis by using StatView software. The significance is defined as $p < 0.0167$ and shown by an asterisk. The geometric mean with 95% confidence interval is shown.

allergen-specific IL-5 production does not reach normal levels in patients in remission. The findings that there are significantly fewer IL-10 producing cells in patients with asthma as compared to normal controls is compatible with previous reports showing lower production of IL-10 in asthma patients [14-17]. In contrast, no statistical difference in the numbers of IL-10 producing cells was found between patients in remission and those with active asthma, although the former tended to have more than the latter. Altogether, the present data suggest that the number of single cytokine producing cells does not necessarily discriminate asthma in remission from active asthma. On the other hand, balance of IL-10/IL-5 producing cells in PBMCs is significantly different between active asthma, asthma in remission, and healthy controls.

Akdis et al. [13] showed that regulatory cytokines actively suppress Th2 responses to allergens in nonatopic healthy subjects and proposed that the balance between allergen-specific Treg cells and Th2 cells may be decisive in the development of allergy. In the present study, we measured IL-5 and IL-10 producing cells in PBMCs. Although numbers of IL-5 producing cells in PBMCs in our study are in comparable level with those in previous studies [12, 18, 19], it is generally thought that frequency of *D. farinae*-specific

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T cells are not so high. IL-5 may be produced by bystander T cells or eosinophils in addition to *D. farinae*-specific Th2 cells. As for IL-10 producing cells, it was reported that monocytes are major source of IL-10 in PBMCs [20]. In addition it is proposed that IL-10 is also produced by regulatory B cells [21]. High frequency of IL-10 producing cells in PBMCs in our study is compatible with those observations. Thus, IL-10 not only from Treg cells but also from monocytes and B cells may be operational in suppression of allergic inflammation in asthma. Taken together, in our enzyme-linked immunospot (ELISPOT) assay we did not count only numbers of allergen-specific cytokine-producing T cells but rather enumerated all cytokine-producing cells in PBMCs upon stimulation with *D. farin*a antigen, and showed that the balance of IL-10/IL-5 producing cells in PBMCs is associated with the clinical status of child-onset asthma.

Even though asthma is a multifactorial disease and many factors are involved in its remission and relapse, the presence or absence of allergic inflammation in the bronchi is quite important in terms of prognosis. A recent report by van den Toorn et al. [22] indicated ongoing airway inflammation and airway remodeling in adolescents in clinical remission of atopic asthma. The present report confirms their data and suggests that a weak but substantial Th2-skewed response driven by antigen exposure exists in patients even after they clinically outgrow asthma. This may explain why some patients who outgrow asthma in childhood relapse in adulthood. The degree of subclinical airway inflammation may well determine the risk of asthma relapse later in life. However, in practical clinical settings, especially in the case of childhood asthma, it is difficult to perform bronchial biopsy. Thus a surrogate marker to predict the course of asthma is required. Although a larger sample size is needed to confirm our results, we propose that the ratio of IL-10/IL-5 producing cells in PBMCs may be useful for monitoring the immune status to antigens and predicting the course of asthma.

In conclusion, our study indicates the importance of the Treg/Th2 cytokine balance in the remission of asthma. Furthermore, this ratio remains lower in patients in remission than in nonatopic healthy controls, suggesting that allergic inflammation may continue even after the clinical remission of asthma in some patients.

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