Eur Respir J 2003; 21: 616–620
DOI: 10.1183/09031936.03.00083303
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European Respiratory Journal
ISSN 0903-1936

Plasma TARC concentration may be a useful marker for asthmatic exacerbation in children

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ABSTRACT: Recent studies suggested the T-helper cells type-2 lymphocytes-specific thymus and activation-regulated chemokine (TARC) and monocyte-derived chemokine (MDC) are useful inflammatory markers for chronic asthma. However, their roles in assessing the severity of acute asthma are unknown. This study aims to evaluate the serial changes of plasma TARC and MDC concentrations in children with asthmatic exacerbation.

All patients with acute asthma were treated with systemic corticosteroid for 5 days. The severity of asthmatic exacerbation was classified according to the Global Initiative for Asthma guidelines. Plasma TARC and MDC concentrations were measured by sandwich enzyme immunoassays.

Sixteen children, with a median (interquartile range) age of 9.3 (7.2–10.6) yrs and asthmatic exacerbation, were recruited. Plasma TARC concentration showed inverse correlation with peak expiratory flow rate at presentation. The median plasma TARC concentration was highest during the acute attacks (46 pg·mL^{-1}) as compared to those levels at 1 (31 pg·mL^{-1}) and 5 weeks (32 pg·mL^{-1}) following treatment. The median plasma MDC level similarly decreased from 698 pg·mL^{-1} at baseline to 261 pg·mL^{-1} 1 week later, but increased back to 574 pg·mL^{-1} at 5 weeks.

These results suggest that plasma T-helper cells type-2 lymphocytes-specific thymus and activation-regulated chemokine but not monocyte-derived chemokine concentration may be a useful inflammatory marker in assessing asthmatic exacerbation in children. *Eur Respir J 2003; 21: 616–620.*

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Keywords: Asthma
children
monocyte-derived chemokine
thymus and activation-regulated chemokine

Received: September 7 2002
Accepted after revision: November 14 2002

The prevalence rates of asthma and wheezing illnesses have been increasing in children and young adults worldwide [1]. The International Study of Asthma and Allergies in Childhood found that 7.7% of 3,110 10-yr-old children in Hong Kong suffered from asthma [2]. Atopy, as defined by elevated serum total and allergen-specific immunoglobulin (Ig)E concentrations, is a major component of asthma [3]. It is characterised by an overproduction of cytokines from T-helper cells type-2 (Th2) lymphocytes [4]. IMAI *et al.* [5] isolated and characterised a new chemokine designated as thymus and activation-regulated chemokine (TARC). The gene encoding TARC is located on chromosome region 16q13 [6]. The gene product is a basic protein with 71 amino acids and a predicted mass of 8 kDa. It acts on the chemokine receptor CCR4, which is expressed on peripheral blood (PB) mononuclear cells and human T-lymphocytes [7]. TARC-induced selective migration of Th2 lymphocytes *in vitro* [7–9]. The expression of CCR4 on Th2 lymphocytes and CCR4-specific ligand TARC on airway epithelial cells were strongly upregulated in endobronchial biopsies from asthmatic patients following allergen challenge [10]. Monocyte-derived chemokine (MDC) is another CC chemokine that has close homology to TARC [11, 12]. Similar to TARC, the gene encoding human MDC is located on chromosome 16q13 [13], and the biological actions of MDC are also mediated mainly through CCR4. MDC is a potent chemoattractant for Th2 lymphocytes, dendritic cells and natural killer cells [11, 12, 14]. Th2 cytokines, including interleukin (IL)-4 and IL-13, were potent stimulators of monocyte MDC production [15]. The interactions between CCR4, TARC and MDC are important in regulating the trafficking of Th2 lymphocytes into sites of allergic inflammation. The authors have recently reported that plasma TARC concentration was increased in children with chronic asthma [16]. This inflammatory marker also showed positive correlation with plasma total IgE concentration. Two other groups have observed similar findings in serum [17, 18] and induced sputum [18] from adult asthmatics. The aim of this study was to investigate whether plasma TARC and MDC concentrations would be useful inflammatory markers in evaluating the disease severity of asthmatic exacerbation in children.

Subjects and methods

Study population

Asthmatic patients aged 6–15 yrs and hospitalised for acute attacks were recruited. The diagnosis of asthma was made according to the American Thoracic Society criteria [19]. Asthmatics that had exacerbation or received oral corticosteroid (CS) within the preceding 3 months were excluded. At presentation, the severity of asthma attacks was classified according to the Global Initiative for Asthma (GINA) guidelines [20]. All patients were treated by nebulised Salbutamol and a 5-day course of systemic CS. The anti-asthma treatment, including inhaled CS, for these patients in the following 4 weeks was individualised according to the severity of current

asthma attacks. Their peak expiratory flow rates (PEFR) were measured using mini-Wright peak-flow meters (Clement Clarke, Essex, England, UK) at presentation (visit one) and 1 (visit two) and 5 weeks (visit three) later. During these visits, plasma samples were extracted within 12 h of collection from ethylenediamine tetraacetic acid (EDTA)-anticoagulated PB stored at 4°C according to the procedures described below. Parents of all patients gave informed written consent and the Clinical Research Ethics Committee of The Chinese University of Hong Kong approved this study.

Plasma total and allergen-specific immunoglobulin E concentrations

EDTA-anticoagulated PB was centrifuged at 4°C at 2,000×*g* for 10 min. Plasma samples thus obtained were stored at -70°C until analysis. The measurement of total and aeroallergen-specific IgE antibodies was made in plasma samples taken at baseline. Total IgE concentration in plasma was measured by microparticle immunoassay (IMx analyser; Abbott Laboratories, Abbott Park, IL, USA) and analysed as a quantitative trait following logarithmic transformation (IgE_{log}). Specific IgE antibodies to *Dermatophagoides pteronyssinus*, cat, dog, mixed cockroaches and mixed moulds were measured by fluorescent enzyme immunoassay (AutoCAP system; Pharmacia Diagnostics AB, Uppsala, Sweden), with IgE concentration $\geqslant 0.35$ kIU·L^{-1} classified as positive. Subjects were defined as atopic if they had at least one positive allergen-specific IgE.

Plasma thymus and activation-regulated chemokine and monocyte-derived chemokine concentrations

Plasma samples were analysed for TARC and MDC in batches, using 96-well polystyrene microplates coated separately with murine monoclonal antibodies against these human chemokines. The levels of TARC and MDC were measured in duplicate by sandwich enzyme immunoassays (R&D Systems, Minneapolis, MN, USA) and mean values were recorded. Immunoassay sensitivities for TARC and MDC detection were 7 pg·mL^{-1} and 62.5 pg·mL^{-1} respectively, whereas the coefficient of variation was 7.1% for TARC and 2.9% for MDC measurements.

Statistical analysis

Data were presented as median and interquartile range (IQR). The longitudinal changes in plasma TARC and MDC levels as well as PEFR in subjects with asthmatic exacerbation were analysed by Wilcoxon signed-ranks test. The correlation between plasma chemokine concentrations and initial PEFR and IgE_{log} was determined using Spearman rank correlation test. All comparisons were made two-sided. A $p<0.05$ was considered significant.

Results

Sixteen patients having asthmatic exacerbation were studied longitudinally. The clinical and laboratory characteristics of these subjects are summarised in table 1. More than 80% of subjects were atopic. Upon completion of the 5-day course of systemic CS, 13 (81%) patients were started on inhaled CS prophylaxis (five with beclomethasone dipropionate and eight with budesonide) at the second visit. All patients were asymptomatic at the two follow-up visits. The median (IQR) PEFR of patients at presentation was 53% (40–57%) of predicted, which increased to 80% (75–92%) predicted at visit two (p=0.001). PEFR showed a further marginal improvement between visit two and visit three (median, IQR: 91%, 84–95%; p=0.046).

Plasma TARC concentration showed significant negative correlation with PEFR of patients at presentation (fig. 1a; r=-0.644, p=0.007). This relationship was not observed for plasma MDC level (fig. 1b). A higher plasma TARC concentration was observed in patients having GINA class 2 or 3 attacks, compared to those with class 1 exacerbation (median TARC levels 52 pg·mL^{-1} and 39 pg·mL^{-1} respectively; p=0.065). The longitudinal changes in plasma TARC and MDC levels over the 5-week follow-up are summarised in figure 2. Plasma TARC concentrations decreased significantly at 1 (p=0.0004) and 5 weeks (p=0.001) following systemic CS treatment. This marker remained suppressed between 1–5 weeks after asthma attack (p=0.776). Similarly, plasma MDC concentrations dropped at 1 week (p=0.0004) following treatment. However, plasma MDC levels increased back towards the baseline values at the third visit (p=0.0004), when systemic CS was stopped.

Discussion

In the present study, plasma concentrations of both TARC and MDC could be suppressed following systemic CS treatment in children with asthmatic exacerbation. This study shows a significant inverse correlation between plasma TARC measurement and PEFR in these patients at presentation. Throughout the 5 weeks of follow-up, all patients were free from asthma symptoms and their PEFR measurements remained normal. Despite this stable asthma control, plasma MDC concentrations in patients increased towards the baseline values again with the discontinuation of systemic CS whereas TARC levels in plasma remained suppressed. The above findings suggest that plasma TARC measurement may

Table 1. – Clinical and laboratory data of the 16 subjects

Characteristics	
Clinical information	
Age at evaluation yrs	9.3 (7.2–10.6)
Sex male:female	11:5
Allergic rhinitis in preceding 12 months n %	9 (56)[#]
Atopic dermatitis in preceding 12 months n %	2 (13)[#]
Prior treatment with inhaled steroid n %	3 (19)[¶]
PEFR at presentation % pred	53 (40–57)
Severity of asthma attacks as classified by GINA class 1:2:3	8:7:1
Laboratory investigations	
Eosinophil count in PB×10^9 L^{-1}	0.58 (0.09–0.90)
Eosinophil % in PB	5.5 (1.0–10.8)
Plasma total IgE concentration kIU·L^{-1}[+]	419 (183–834)
Presence of at least one positive allergen-specific IgE n %[+]	12 (86)[§]

Data are expressed as median (interquartile range) or n (%) unless otherwise stated. PEFR: peak expiratory flow rate; % pred: % predicted; GINA: Global Initiative for Asthma; PB: peripheral blood; IgE: immunoglobulin E. [#]: diseases remained stable throughout the study period; [¶]: inhaled budesonide at 200 μg daily for two patients and 400 μg daily in one patient; [+]: results were available for 14 patients only; [§]: included IgE to *D. pteronyssinus* in 12 patients, IgE to cockroaches in three patients and IgE to cat in one patient.

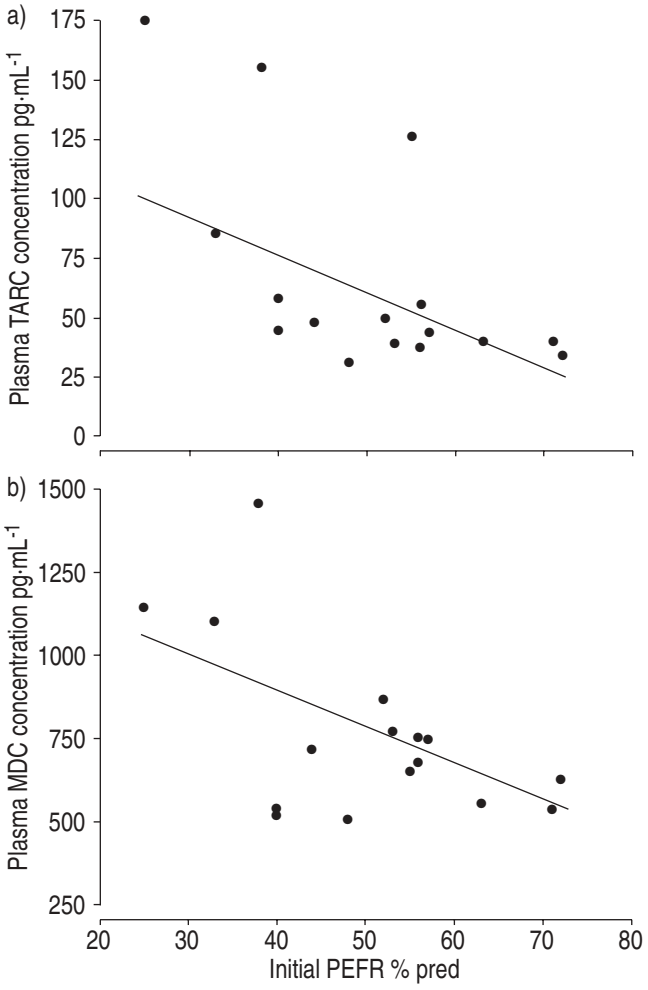

Fig. 1. – The relationship between plasma a) thymus and activation-regulated chemokine (TARC) (r=-0.644, p=0.007) and b) monocyte-derived chemokine (MDC) (r=-0.368, p=0.16) concentrations and peak expiratory flow rates (PEFR) of subjects at presentation.

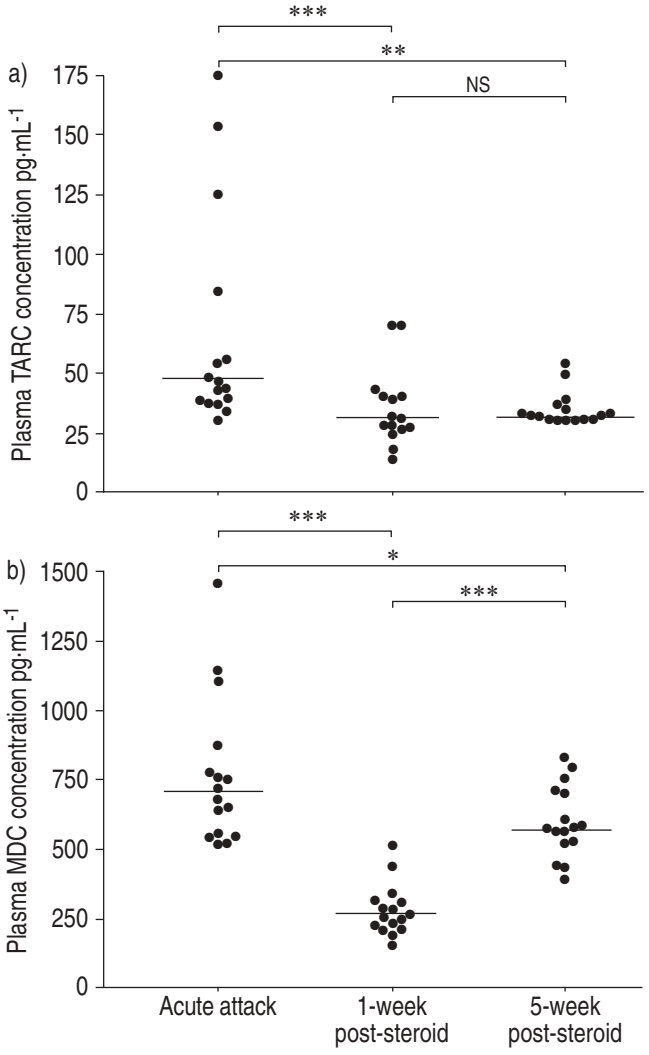

Fig. 2. – Plasma concentrations of a) thymus and activation-regulated chemokine (TARC) and b) monocyte-derived chemokine (MDC) of the patients at acute attack, and 1 and 5 weeks following systemic steroid treatment. The median (interquartile range) plasma TARC levels at these visits were 46 (38–77) pg·mL^{-1}, 31 (27–41) pg·mL^{-1} and 32 (30–36) pg·mL^{-1} respectively. The corresponding values for MDC were 698 (547–845) pg·mL^{-1}, 261 (219–318) pg·mL^{-1} and 574 (517–704) pg·mL^{-1}. The horizontal bars represent median levels of plasma chemokines. NS: nonsignificant. *: p<0.01; **: p<0.005; ***: p<0.0005.

be a better inflammatory marker for evaluating asthmatic exacerbation in children than plasma MDC level.

TARC and MDC play crucial roles in attracting Th2 cells into the inflamed asthmatic airway. The pathogenesis of asthma is mediated by CD4$^+$ T-lymphocytes that produced a Th2 cytokine profile [21]. TARC and MDC are important chemokines responsible for the selective trafficking of Th2 lymphocytes into sites of allergic inflammation [7–9, 11, 12, 14]. Asthmatic patients exposed to a relevant allergen released large quantities of TARC in bronchoalveolar lavage fluid [22]. On the other hand, the use of AMD3100, a specific inhibitor of CXCR4 preferentially expressed on Th2 cells, down-regulated airway inflammation and reduced local production of TARC and MDC as well as Th2 cytokines [23]. Pretreatment of sensitised mice with an anti-TARC antibody has also been shown to prevent airway eosinophilia induced by ovalbumin and decreased infiltrating CD4$^+$ lymphocytes and levels of Th2-type cytokines [24]. These studies highlighted the potential roles of TARC and MDC in the pathogenesis of asthma.

Plasma TARC concentration was also increased in children with chronic asthma. The authors previous study showed increased plasma TARC concentration in steroid-naive patients with mild-intermittent asthma when compared to control children and patients with persistent asthma who received inhaled CS treatment [16]. Plasma TARC concentration also correlated with plasma levels of total and cat-specific IgE antibodies. SUGAWARA *et al.* [17] also reported recently in a small study that plasma TARC levels were elevated in patients with asthma. However, the authors did not study the relationship between TARC and atopy-related traits or lung function parameters. SEKIYA *et al.* [18] found that TARC but not MDC concentrations were increased in sera and induced sputum samples in asthmatics. Bronchial epithelium from asthmatics expressed significantly more intense TARC but not MDC protein compared to that of control subjects [25]. Furthermore, tumour necrosis factor-alpha, IL-4 and interferon-gamma enhanced TARC but not MDC expression in bronchial epithelial cell cultures. The present study, involving patients with asthmatic exacerbation, further support that plasma TARC but not MDC level may be a useful inflammatory marker reflecting the disease activity of asthma.

The results of this study suggest the possible use of TARC measurement in a number of clinical situations. Plasma TARC measurement may be a sensitive indicator of subtle

changes in the extent of airway inflammation in asthmatics before they have clinical deterioration. It would be interesting and important to study the correlation between TARC and other inflammatory markers of asthma as well as to investigate whether TARC can be used to predict asthmatic exacerbation. Further prospective studies involving serial measurements of plasma TARC concentrations in asthmatic children are required to clarify the potential use of TARC measurement in asthmatic patients. TARC may also have a potential role for the study of basic mechanisms of allergic diseases. Further, this marker can be included as an outcome variable in the evaluation of anti-inflammatory properties of therapeutic drugs.

TARC and MDC have also been implicated in the pathogenesis of other allergic diseases. Nasal epithelial cells derived from allergic individuals released larger amount of TARC than those derived from normal subjects [26]. Elevated levels of TARC [21] and MDC [21, 27] were also found in the serum of atopic dermatitis (AD) patients compared with controls. In addition, serum TARC and MDC levels correlated with the clinical and laboratory markers for AD severity [17, 21, 28]. These results further substantiate that these Th2 chemokines play important roles in the pathogenesis of allergic diseases.

TARC measurements were not affected by storing the plasma samples at 4°C for as long as 12 h before processing. Because of logistic difficulties, EDTA-anticoagulated PB from patients with asthmatic exacerbation was kept at 4°C for up to 12 h prior to the extraction and storage of plasma. There was concern whether the delay in sample processing might have changed the plasma chemokine concentrations. The authors addressed this issue in a separate experiment in which blood samples were collected from 12 subjects for TARC measurement. Each sample was then divided into six aliquots and stored at 4°C and 21°C for 0–24 h. The authors found that the TARC level in plasma samples remained unchanged at 4°C, even when stored for up to 24 h. However, TARC concentrations significantly increased in plasma samples that were stored at 21°C for as little as 4 h (unpublished data). Similarly, FUJISAWA *et al*. [27] reported a rapid surge in TARC levels within 10 min when serum samples were allowed to clot at 37°C. They also found TARC levels to be much higher in serum in comparison to plasma samples [27]. Further studies are necessary to clarify the effects of various processing regimens on the measurement of chemokines in PB.

In conclusion, plasma T-helper cells type-2 lymphocytes-specific thymus and activation-regulated chemokine but not monocyte-derived chemokine concentration may be a useful inflammatory marker for the monitoring of asthmatic exacerbation in children. Further studies are necessary to clearly define the role of T-helper cells type-2 lymphocytes-specific thymus and activation-regulated chemokine measurement in routine asthma management.

References

1. Lai CK, Douglass C, Ho SS, *et al.* Asthma epidemiology in the Far East. *Clin Exp Allergy* 1996; 26: 5–12.
2. Wong GW, Hui DS, Chan HH, *et al.* Prevalence of respiratory and atopic disorders in Chinese schoolchildren. *Clin Exp Allergy* 2001; 31: 1225–1231.
3. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989; 320: 271–277.
4. Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000; 105: 399–408.
5. Imai T, Yoshida T, Baba M, Nishimura M, Kakizaki M, Yoshie O. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein-Barr virus vector. *J Biol Chem* 1996; 271: 21514–21521.
6. Nomiyama H, Imai T, Kusuda J, Miura R, Callen DF, Yoshie O. Assignment of the human CC chemokine gene TARC (SCYA17) to chromosome 16q13. *Genomics* 1997; 40: 211–213.
7. Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Yoshie O. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. *J Biol Chem* 1997; 272: 15036–15042.
8. Bonecchi R, Bianchi G, Bordignon PP, *et al.* Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998; 187: 129–134.
9. Imai T, Nagira M, Takagi S, *et al.* Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemokine. *Int Immunol* 1999; 11: 81–88.
10. Panina-Bordignon P, Papi A, Mariani M, *et al.* The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001; 107: 1357–1364.
11. Chantry D, DeMaggio AJ, Brammer H, *et al.* Profile of human macrophage transcripts: insights into macrophage biology and identification of novel chemokines. *J Leuk Biol* 1998; 64: 49–54.
12. Godiska R, Chantry D, Raport CJ, *et al.* Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. *J Exp Med* 1997; 185: 1595–1604.
13. Nomiyama H, Imai T, Kusuda J, Miura R, Callen DF, Yoshie O. Human chemokines fractalkine (SCYD1), MDC (SCYA22) and TARC (SCYA17) are clustered on chromosome 16q13. *Cytogenet Cell Genet* 1998; 81: 10–11.
14. Chang M, McNinch J, Elias C 3rd, *et al.* Molecular cloning and functional characterization of a novel CC chemokine, stimulated T cell chemotactic protein (STCP-1) that specifically acts on activated T lymphocytes. *J Biol Chem* 1997; 272: 25229–25237.
15. Andrew DP, Chang MS, McNinch J, *et al.* STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13. *J Immunol* 1998; 161: 5027–5038.
16. Leung TF, Wong CK, Chan IHS, Ip WK, Lam CWK, Wong GWK. Plasma concentration of thymus and activation-regulated chemokine is elevated in childhood asthma. *J Allergy Clin Immunol* 2002; 110: 404–409.
17. Sugawara N, Yamashita T, Ote Y, Miura M, Terada N, Kurosawa M. TARC in allergic disease. *Allergy* 2002; 57: 180–181.
18. Sekiya T, Yamada H, Yamaguchi M, *et al.* Increased levels of a TH2-type CC chemokine thymus and activation-regulated chemokine (TARC) in serum and induced sputum of asthmatics. *Allergy* 2002; 57: 173–177.
19. American Thoracic Society. Medical section of the American Lung Association. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136: 225–244.
20. National Institutes of Health, National Heart Lung and Blood Institute. Global initiative for asthma. Publication No. 96-3659B. Bethesda: NHLBI, 1998.
21. Kakinuma T, Nakamura K, Wakugawa M, *et al.* Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001; 107: 535–541.
22. Berin MC, Eckmann L, Broide DH, Kagnoff MF. Regulated production of the T helper 2-type T-cell chemoattractant TARC by human bronchial epithelial cells in vitro and in human lung xenografts. *Am J Respir Cell Mol Biol* 2001; 24: 382–389.

23. Lukacs NW, Berlin A, Schols D, Skerlj RT, Bridger GJ. AMD3100, a CxCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am J Pathol* 2002; 160: 1353–1360.
24. Kawasaki S, Takizawa H, Yoneyama H, *et al.* Intervention of thymus and activation-regulated chemokine attenuates the development of allergic airway inflammation and hyperresponsiveness in mice. *J Immunol* 2001; 166: 2055–2062.
25. Sekiya T, Miyamasu M, Imanishi M, *et al.* Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000; 165: 2205–2213.
26. Terada N, Nomura T, Kim WJ, *et al.* Expression of C-C chemokine TARC in human nasal mucosa and its regulation by cytokines. *Clin Exp Allergy* 2001; 31: 1923–1931.
27. Fujisawa T, Fujisawa R, Kato Y, *et al.* Presence of high contents of thymus and activation-regulated chemokine in platelets and elevated plasma levels of thymus and activation-regulated chemokine and macrophage-derived chemokine in patients with atopic dermatitis. *J Allergy Clin Immunol* 2002; 110: 139–146.
28. Kakinuma T, Nakamura K, Wakugawa M, *et al.* Serum macrophage-derived chemokine (MDC) levels are closely related with the disease activity of atopic dermatitis. *Clin Exp Immunol* 2002; 127: 270–273.