

Plasma concentration of thymus and activation-regulated chemokine is elevated in childhood asthma

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Background: Thymus and activation-regulated chemokine (TARC) is responsible for the trafficking of T_H2 lymphocytes into sites of allergic inflammation. Serum TARC levels correlate with the severity of atopic dermatitis. The relationship between this marker and the occurrence and severity of asthma has not been evaluated.

Objective: We tested whether plasma TARC level is a useful marker for asthma and atopy in children.

Methods: Plasma total IgE levels were measured by means of microparticle immunoassay, and specific IgE levels to common aeroallergens were measured by using a fluorescent enzyme immunoassay. We used a sandwich enzyme immunoassay to measure plasma TARC concentrations.

Results: Sixty asthmatic children and 28 age- and sex-matched control subjects were recruited, with mean logarithmic plasma total IgE levels of 2.66 ± 0.60 kIU/L and 1.74 ± 0.58 kIU/L, respectively ($P < .0001$). The median plasma TARC concentration was higher in asthmatic patients without inhaled corticosteroid treatment (131.0 pg/mL) compared with those seen in steroid-treated patients (97.5 pg/mL) and control subjects (76.0 pg/mL; $P = .01$ and $P < .0001$, respectively). Plasma TARC concentration was found to correlate with total IgE level in plasma ($r = 0.219$, $P = .04$). This marker was also increased in subjects who were sensitized to cat allergen ($P = .001$) but not in subjects sensitized to other aeroallergens. Disease severity score, FEV₁ value, and atopy were not associated with increased plasma TARC levels.

Conclusion: Our results suggest that plasma TARC concentrations are elevated in childhood asthma. This marker is also linked to plasma total IgE levels and cat allergen sensitization. (J Allergy Clin Immunol 2002;110:404-9.)

Key words: Asthma, atopy, immunoglobulin E, spirometry, thymus and activation-regulated chemokine

Asthma and wheezing illnesses have been documented to be increasing in children and young adults worldwide.¹ The prevalence of self-reported wheeze varied from 3.1% to 13.2% among Chinese children in Southeast Asian coun-

Abbreviations used

AD: Atopic dermatitis
IgE_{log}: Logarithmic total immunoglobulin E concentration
TARC: Thymus and activation-regulated chemokine

tries.² The Phase II survey of the International Study of Asthma and Allergies in Childhood found that 7.7% of 3110 10-year-old Hong Kong children had asthma.³ Atopy, as defined by elevated serum total and allergen-specific IgE concentrations, has been found to be a major component of asthma.⁴ It was characterized by an overproduction of T_H2 lymphocyte-related cytokines, such as IL-4, IL-5, and IL-13, and deficiency of T_H1-related IFN- γ .⁵

Imai et al,⁶ in 1996, first described the isolation and molecular characterization of a new chemokine designated as thymus and activation-regulated chemokine (TARC; CCL17) by cloning the *D3A* gene from PBMCs after stimulation with PHA. Thymus is the only other major source of TARC expression. The gene encoding TARC is located on chromosome region 16q13.⁷ The gene product is a basic protein with 71 amino acids and a predicted mass of 8 kd. It acts on the chemokine receptor CCR4, which is expressed on PBMCs and human T lymphocytes.⁸ In vitro studies showed that TARC could induce selective migration of lymphocytes, especially with the phenotype of T_H2 cells.⁸⁻¹⁰ The expression of CCR4 on T_H2 lymphocytes and CCR4-specific ligand TARC on airway epithelial cells was strongly upregulated in endobronchial biopsy specimens from asthmatic patients after allergen challenge.¹¹ In addition, tracking of effector T_H cells that were transplanted into naive mice revealed that these cells predominantly used the CCR4-related pathway to recruit T_H2 cells to the lung after repeated antigen stimulation.¹² CCR4/TARC interaction is thus important in regulating the trafficking of T_H2 lymphocytes into sites of allergic inflammation. TARC has also been implicated in the pathogenesis of allergic rhinitis and atopic dermatitis (AD).¹³⁻¹⁵ Elevated levels were found in the serum of patients with AD compared with those found in the sera of normal control subjects and patients with psoriasis.¹⁵ Furthermore, the serum level of TARC has been documented to correlate with the severity of AD. The aim of this study was to investigate plasma TARC concentrations in children with asthma and to investigate the correlation between this marker and allergen sensitization, as well as the clinical and spirometric indices of asthma severity.

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Supported in part by a donation from Zindart (De Zhen) Foundation Ltd, Hong Kong.

Received for publication January 16, 2002; revised May 9, 2002; accepted for publication May 9, 2002.

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0091-6749/2002 \$35.00 + 0 1/83/126378

doi:10.1067/mai.2002.126378

METHODS

Study population

Children and adolescents aged 5 to 18 years given a diagnosis of asthma according to the criteria proposed by the American Thoracic Society¹⁶ were recruited from the pediatric outpatient clinic of a university teaching hospital for study. Patients with mild intermittent asthma according to the Global Initiative for Asthma guidelines¹⁷ used as-needed inhaled β_2 -agonist only, whereas those with persistent asthma had their symptoms controlled with inhaled corticosteroid with or without a long-acting β -agonist. Antiasthma treatments in our patients were stable for at least 3 months before the study. Age-matched control children were recruited among subjects referred to the department for assessment of other minor complaints. They did not have any history of allergic or inflammatory disease and did not require any drug treatment at the time of the study. All subjects were free from respiratory tract infection, asthma exacerbation, and use of systemic corticosteroids for 4 weeks before the study. Subjects or their parents provided written informed consent, and the clinical research ethics committee of our university approved this study.

Disease severity score

The disease severity score (6-30 points) assessed exercise tolerance, the frequency of acute asthma exacerbation, the use of antiasthma medications during the 6 months before evaluation, and the occurrence of cough, wheezing, or chest tightness in the daytime and at night.¹⁸

Total and allergen-specific IgE concentrations

EDTA-anticoagulated peripheral venous blood was collected from patients and control subjects and centrifuged within 30 minutes of collection at 1000g for 10 minutes. Plasma concentration of total IgE was measured with a microparticle immunoassay (IMx analyzer; Abbott Laboratories, Abbott Park, Ill). Specific IgE antibodies to *Dermatophagoides pteronyssinus*, cat, dog, mixed cockroaches, and mixed molds were measured by means of fluorescent enzyme immunoassay (AutoCAP system; Pharmacia Diagnostics AB, Uppsala, Sweden), according to the manufacturer's instructions, with an IgE concentration of 0.35 kIU/L or greater being considered positive. Subjects were defined as atopic if they had at least one positive allergen-specific IgE level.

Spirometry

Asthmatic patients underwent spirometric assessment (Compact II; Vitalograph, Buckingham, England) to measure FEV₁ according to the American Thoracic Society standards,¹⁹ and results were compared with local age- and sex-matched predicted values.²⁰ The subjects were categorized into severity groups by using FEV₁ percent predicted (mild, FEV₁ >80%; moderate, FEV₁ 60%-80%; severe, FEV₁ <60%), as described previously.²¹

Plasma TARC concentration

EDTA plasma samples from our subjects were stored at -70°C until analysis for TARC concentrations in batch. We used a 96-well polystyrene microplate coated with a murine mAb against human TARC. The levels of TARC in plasma were measured in duplicate by using a sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, Minn). The sensitivity of this kit in detecting TARC was 7 pg/mL. The intra-assay and interassay coefficients of variance for measuring plasma TARC concentration were 4.0% and 8.3%, respectively.

Statistical analysis

Data were analyzed with the Mann-Whitney *U* test or the Kruskal-Wallis test for numeric variables. Correlation coefficients

were determined by using the Spearman rank correlation test. Because the distribution of plasma total IgE levels was skewed, all comparisons involving this marker were made after logarithmic transformation (IgE_{log}). All comparisons were 2-sided. *P* values of less than .05 were considered statistically significant.

RESULTS

Demographic data

Sixty asthmatic children and 28 control subjects were enrolled in the study, with their mean $\pm$ SD ages at evaluation being 11.5 $\pm$ 4.0 years and 10.1 $\pm$ 3.7 years, respectively (*P* = .112). All enrolled subjects were ethnic Chinese. The clinical characteristics of our asthmatic children are summarized in Table I. Twenty-six patients received inhaled corticosteroid treatment, including 6 with beclomethasone dipropionate, 13 with budesonide, and 7 with fluticasone propionate. The median daily steroid dose in these asthmatic patients was 400 μ g (interquartile range, 200-400 μ g) of beclomethasone dipropionate or equivalent. Eighteen (64%) control subjects were male, which did not differ from the asthmatic patients.

Atopic status

Table II describes the plasma total and allergen-specific IgE measurements. The mean $\pm$ SD IgE_{log} was 1.74 $\pm$ 0.58 kIU/L for control children, 2.72 $\pm$ 0.45 kIU/L for patients not receiving steroids, and 2.59 $\pm$ 0.76 kIU/L for patients receiving inhaled corticosteroids, respectively (*P* < .0001). Atopy, as defined by the presence of one or more positive specific IgE levels to aeroallergens, occurred in 93% of patients and 43% of control children (*P* < .0001). Sensitization to *D pteronyssinus* and cat allergen were significant risk factors for childhood asthma in this cohort.

Association between asthma and atopy and plasma TARC concentration

The median plasma TARC concentrations in asthmatic patients and control subjects were 109.5 pg/mL (interquartile range, 86.3-155.0 pg/mL) and 76.0 pg/mL (interquartile range, 40.3-108.8 pg/mL), respectively (*P* = .0002). Fig 1 summarizes plasma TARC levels in the control group and asthmatic children with and without inhaled corticosteroid treatment, showing that the latter group of patients had the highest plasma concentration of TARC. Plasma TARC level also weakly correlated with IgE_{log}, as shown in Fig 2 (*r* = 0.219, *P* = .040), but not with disease severity score or FEV₁. When the relationships between TARC and allergic sensitization to individual aeroallergens were analyzed, we found that plasma TARC concentration was significantly increased in subjects having specific IgE antibodies to cat allergen (*P* = .001, Fig 3).

DISCUSSION

Allergic asthma was characterized by bronchial hyperresponsiveness to a variety of specific and nonspecific stimuli, chronic pulmonary eosinophilia, elevated serum IgE levels, and excessive airway mucus production.²² The

Asthma, rhinitis,
other respiratory
diseases

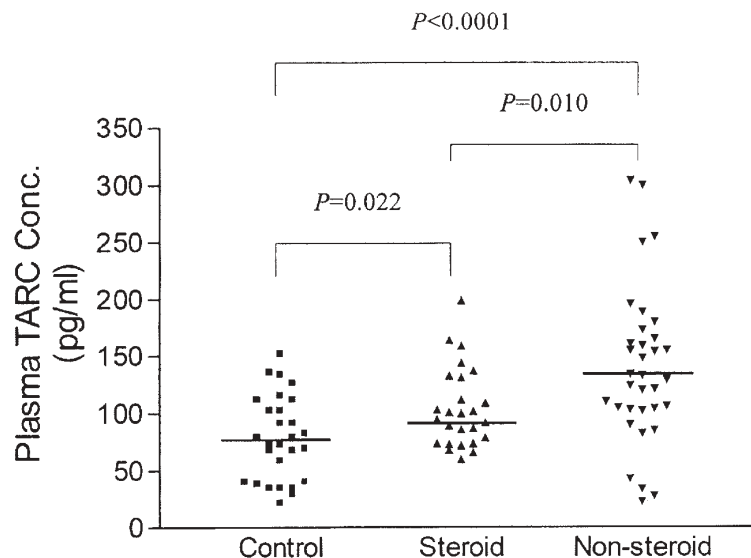


FIG 1. Plasma TARC concentrations in control subjects ($n = 28$) and asthmatic children with ($n = 26$) and without ($n = 34$) inhaled corticosteroid treatment. The medians (interquartile ranges) for these groups were 76.0 pg/mL (40.3-108.8 pg/mL), 97.5 pg/mL (74.0-131.5 pg/mL), and 131.0 pg/mL (102.8-167.0 pg/mL), respectively. Horizontal bars represent median TARC levels.

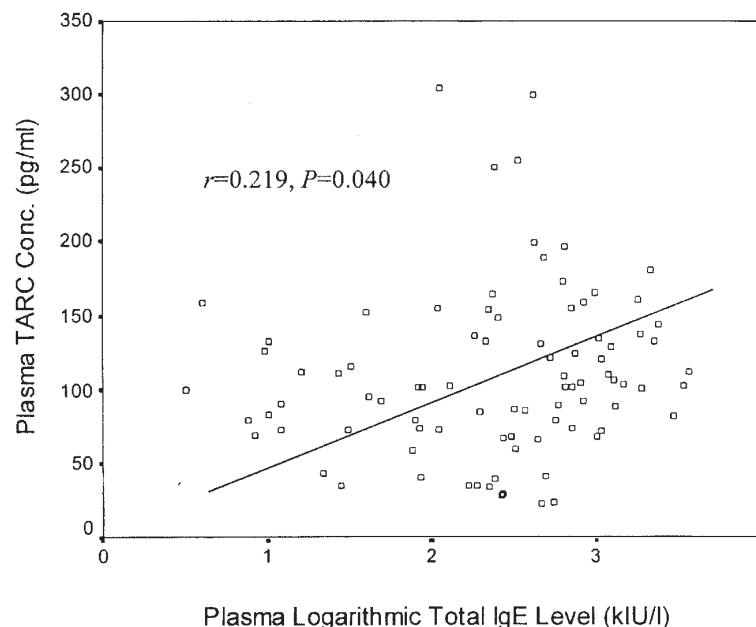


FIG 2. Relationship between plasma TARC concentrations and $\text{IgE}_{\log}$ in our 88 subjects, with correlation coefficient expressed as nonparametric Spearman r value.

pathogenesis of asthma was mediated by CD4^+ T lymphocytes that produced a $\text{T}_\text{H}2$ cytokine profile.⁵ TARC, first isolated and characterized in 1996,⁶ is an important chemokine responsible for the selective trafficking of $\text{T}_\text{H}2$ lymphocytes into sites of allergic inflammation.⁸⁻¹⁰ Kawasaki et al²³ showed that pretreatment of sensitized mice with an anti-TARC antibody prevented airway eosinophilia induced by ovalbumin and decreased infiltrating CD4^+ lymphocytes and levels of $\text{T}_\text{H}2$ -type

cytokines. It has also been observed that asthmatic patients exposed to a relevant allergen release large quantities of TARC into bronchoalveolar lavage fluid.²⁴ Furthermore, repeated allergen stimulation upregulated the expression of CCR4 on $\text{T}_\text{H}2$ cells from asthmatic subjects and also contributed to the long-term recruitment of these cells to the lung in mice with allergic airway disease.^{11,12}

This study is the first to show increased plasma TARC concentrations in asthmatic patients when compared with

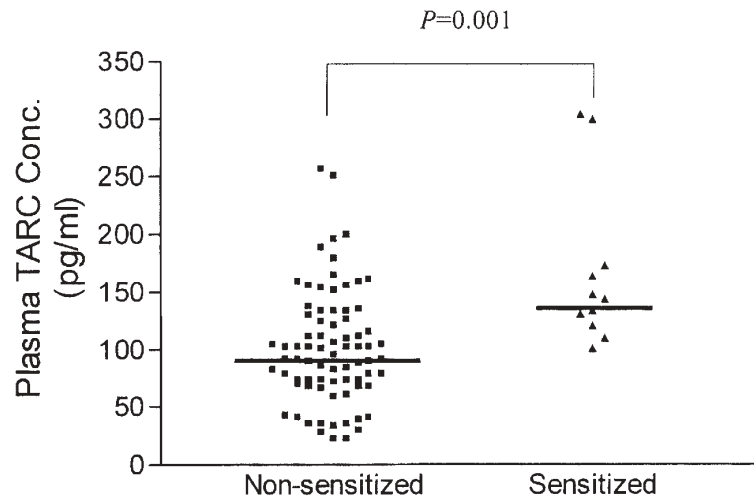


FIG 3. Plasma TARC concentrations in subjects with (sensitized, $n = 11$) and without (nonsensitized, $n = 77$) cat allergen-specific IgE in sera. The median (interquartile range) for TARC in sensitized subjects was 144.0 pg/mL (121.0-173.0 pg/mL), and that for nonsensitized subjects was 95.0 pg/mL (70.5-131.0 pg/mL). Horizontal bars represent median TARC levels.

TABLE I. Demographic data and indicators of asthma control in asthmatic children with and without inhaled corticosteroid treatment

Characteristics	Steroid group ($n = 26$)	Nonsteroid group ($n = 34$)
Background information		
Age at evaluation (y)	11.4 ± 4.0	11.7 ± 4.1
Sex (male/female)	13/13	19/15
Disease severity score	15 (12.75-19)*	10 (8-11.25)
Spirometric assessment		
FEV ₁ (% predicted)	82 ± 20 †	96 ± 22
No. of subjects with mild/moderate/severe asthma according to FEV ₁ ‡	15/6/3	25/8/0

Values are expressed as means $\pm$ SD where shown.

* $P < .0001$ between 2 groups by Mann-Whitney U test.

† $P = .015$ between 2 groups by Student t test.

‡FEV₁ results available for 24 steroid-treated patients and 33 patients without steroid treatment.

TABLE II. Plasma total IgE levels and the pattern of allergic sensitization, as determined with allergen-specific IgE assays

Characteristics	All asthmatic patients	Control subjects
Plasma IgE _{log} level (mean $\pm$ SD)	$2.66 \pm 0.60^*$	1.74 ± 0.58
Atopy (ie, ≥ 1 positive allergen-specific IgE level; %) [‡]	56 (93)*	12 (43)
Presence of specific IgE by RAST [‡]		
<i>D pteronyssinus</i> (%)	55 (92)*	10 (36)
Mixed cockroaches (%)	21 (35)	4 (14)
Cat (%)	11 (18)†	0
Dog (%)	1 (2)	0
Mixed molds (%)	6 (10)	0

* $P < .0001$ and † $P < .05$ between 2 groups.

‡Odds ratios for atopy and *D pteronyssinus* sensitization were 18.7 (95% CI, 4.7-81.7) and 19.8 (95% CI, 5.3-80.1), respectively. The odds ratio was not available for cat sensitization because none of the control subjects had specific IgE to this allergen.

those of control children. We have also observed that plasma TARC concentrations were significantly higher in children with stable, mild intermittent asthma than those of corticosteroid-treated children with persistent asthma. Our results supported the view that airway inflammation might still be present in patients with seemingly mild asthma, as assessed by means of exhaled nitric oxide levels or sputum eosinophil counts.²⁵⁻²⁷ These markers also decreased in asthmatic children after inhaled corticosteroid treatment,^{26,27} which was also consistent with our finding that plasma TARC levels were lower in corticosteroid-treated than in steroid-naïve asthmatic patients. Inhaled corticosteroids might exert anti-inflammatory effects on asthma, in part by lowering this important T_H2-chemotactic chemokine. In view of the fact that there was considerable overlap in the ranges of TARC in our 3 groups of subjects, a single measurement might not be

very useful, but serial measurements could potentially be useful in monitoring the control of asthma.

This study also found increased plasma TARC concentrations in children with cat allergen sensitization. There were 2 outliers, as shown in Fig 3, that might have skewed the data. Given the numbers of our subjects, however, this study had more than 90% power at a 5% level of significance to detect the observed difference in mean TARC levels between subjects with and without cat allergen sensitization. Thus our finding likely represented a genuine association between the 2 variables. Further study of larger sample size is necessary to confirm our preliminary findings. This marker also showed weak correlation with plasma total IgE levels. These findings were consistent with the theory that asthma and atopy are T_H2-related diseases.⁵ However, plasma TARC levels did not differ between atopic and nonatopic subjects or among asthmatic

ic patients with different degrees of flow limitation on FEV₁ measurement (data not shown). The sample size of this study was probably too small to detect a significant association between asthma and plasma TARC level. The power might have been weakened by the fact that all patients had chronic stable asthma of mild to moderate severity (Table I). Berin et al²⁴ showed increased TARC levels in bronchoalveolar lavage fluid from asthmatic patients exposed to a relevant allergen. Serum TARC levels were also markedly higher in patients with severe AD than in those with mild or moderate disease.¹⁵ It would be helpful to enroll asthmatic children with acute exacerbation to further evaluate the usefulness of TARC in assessing asthma severity because serum TARC levels would most likely be even higher in these patients. Furthermore, only 57 asthmatic patients underwent spirometry, and therefore this study possibly did not have a large enough sample to evaluate the association between plasma TARC levels and spirometric measurements. Larger studies involving population cohorts will be needed to confirm the roles of plasma TARC concentrations in assessing disease severity and control of asthma.

TARC has also been implicated in the pathogenesis of other allergic diseases. A recent report showed that nasal epithelial cells derived from allergic individuals released higher concentrations of TARC than those derived from normal subjects. TARC immunoreactivity was mainly confined to the nasal airway epithelium.¹³ By using immunohistochemical techniques, TARC was localized to keratinocytes and, to a lesser extent, to endothelial cells and dermal infiltrating cells, including CD3 lymphocytes and CD1a dendritic cells.^{14,15,28} In one study elevated levels of TARC were found in the sera of patients with AD compared with those in both normal control subjects and patients with psoriasis,¹⁵ and levels of serum TARC correlated with the severity of AD. High circulating levels of TARC have also been reported in the sera of patients with chronic hypereosinophilia.²⁹

Kakinuma et al¹⁵ also found a 10-fold increase of serum TARC level in patients with AD when compared with levels in healthy adults. The average value of serum TARC of adult patients with AD was 2338 pg/mL in that study, which was much higher than the corresponding value of 131 pg/mL, as measured in EDTA plasma from our noncorticosteroid-treated asthmatic children. As described in the package insert provided by the manufacturer, the mean TARC concentration was 2- to 3-fold higher in serum samples than those measured from EDTA plasma. This difference in TARC levels might also be explained by the fact that TARC concentration in peripheral blood might be age dependent. However, there have not been any published data regarding this maturational change in TARC level in childhood. Other explanations include the fact that AD was associated with a much larger end organ of TARC production (skin) compared with that associated with asthma (lower respiratory tract) or that epidermal keratinocytes in patients with AD might provide an extra source of TARC production apart from dendritic and endothelial cells.²⁸

Our results supported a possible linkage between childhood asthma and increased TARC level. Preadministration of CCR4-neutralizing antibody to mice before antigen challenge not only prevented eosinophil recruitment into the airways after allergen challenge but also decreased the number of CD4⁺ lymphocytes and prevented airway hyperreactivity.²³ Thus the use of small CCR4 antagonists with clinical efficacy might have an effect in treating allergic diseases.

In conclusion, this case-control study suggests that plasma TARC concentrations are elevated in asthmatic children with or without inhaled corticosteroid treatment, as well as in subjects sensitized to cat allergen. Plasma TARC concentration also correlates with plasma total IgE concentration. Further studies are necessary to investigate the potential roles of TARC as an inflammatory marker in the management and monitoring of childhood asthma and atopy.

We thank Gladys Ha for her help with spirometric assessment.

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