

Short communication

Increased levels of a TH2-type CC chemokine thymus and activation-regulated chemokine (TARC) in serum and induced sputum of asthmatics

Background: Cytokines liberated by TH2 cells play crucial roles in the pathogenesis of bronchial asthma. Recent studies have demonstrated that CC chemokine receptor (CCR)4 is preferentially expressed by TH2 cells. These facts suggest possible involvement of two CCR4-specific ligands i.e., thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), in the pathogenesis of bronchial asthma via recruitment of TH2 cells to inflammatory sites. We investigated the levels of TARC and MDC in the serum and induced sputum of asthmatics.

Methods: The levels of TARC in the serum (46 asthmatics and 26 healthy subjects) and induced sputum (30 asthmatics and 6 healthy subjects) were measured using a highly sensitive ELISA system. The levels of eotaxin and MDC were also measured by ELISA.

Results: TARC, but not MDC, was significantly increased in asthmatic sera ($P < 0.001$). Although MDC was undetectable in the sputum of most cases by our assay system, sputum TARC was significantly increased ($P = 0.027$).

Conclusions: The elevated TARC levels in asthmatics might be involved in the pathophysiology of asthma.

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Substantial evidence has indicated that cytokines liberated by TH2 cells play crucial roles in the pathogenesis of bronchial asthma. Originally, TH2 subsets were functionally identified based on their profiles of cytokine production, but recent studies have clearly demonstrated the preferential expression of CC chemokine receptor (CCR)4 in this subpopulation (1). Furthermore, CCR4-specific ligands, i.e., thymus and activation-regulated chemokine (TARC)/CC chemo-

kine ligand (CCL)17 and macrophage-derived chemokine (MDC)/CCL22, have been shown to elicit selective migratory responses in TH2 cells (2). These facts suggest possible involvement of both of these chemokines in accumulation of TH2 cells at inflammatory sites in bronchial asthma (3). In fact, increased expression of MDC and TARC was reported in murine experimental models of asthma (3, 4). In these models, neutralization of MDC and TARC resulted in attenuation of pulmonary allergic inflammation (3, 4). Furthermore, by using immunohistochemistry, we recently demonstrated increased expression of TARC in the bronchial epithelium of asthmatics (5). To determine whether TARC and

Abbreviations. CCR: CC chemokine receptor; CCL: CC chemokine ligand; TARC: thymus and activation-regulated chemokine; MDC: macrophage-derived chemokine.

Table 1. Subjects included in this study, and measured indices of their sputa and sera

	Sputum		Serum	
	Normal	asthmatics	Normal	Asthmatics
n (male/female)	6 (2/4)	30 (16/14)	26 (13/13)	46 (18/28)
Age (years)	36.0 ± 8.3	57.3 ± 11.0	36.0 ± 6.7	42.7 ± 14.1
FEV1.0 (L)	—	1.82 ± 0.71 (n = 29)	—	2.43 ± 0.95 (n = 44)
FEV1.0 (% predicted)	—	66.8 ± 14.1 (n = 29)	—	80.8 ± 12.7 (n = 44)
SD ₂₀ histamine (μg/ml)	—	632.0 ± 3327 (n = 25)	—	1358.0 ± 8226 (n = 30)
Eosinophils in sputum(%)	0.25 ± 0.47	15.3 ± 23.8**	—	—
Eosinophils in blood (/μl)	—	389.0 ± 228	168.0 ± 102	263.0 ± 251
Serum total IgE (IU/ml)	—	537.2 ± 970.5 (n = 29)	121.6 ± 130.7	527.0 ± 1186.2**
Eotaxin/CCL11 (pg/ml)	14.9 ± 19.3	111.8 ± 184.0*	76.9 ± 32.2	74.5 ± 29.9
TARC/CCL17 (pg/ml)	31.8 ± 39.1	117.7 ± 139.9*	88.8 ± 58.2	192.0 ± 143.6**
MDC/CCL22 (pg/ml)	< 750.0 (6/6) ^a	< 750.0 (26/30) ^a	673.8 ± 175.5	635.2 ± 193.7
ECP in sputum (ng/ml)	7.0 ± 4.6	260.3 ± 407.0**(n = 27)	—	—

Data expressed as the mean ± SD, except for sputum levels of MDC, which are expressed as median values. Statistical significance assessed by the Mann–Whitney U-test is shown as follows: *P < 0.05, **P < 0.01, vs. corresponding value of the healthy controls. Numbers of patients assessed for FEV1.0 (L), FEV1.0 (% predicted), PC₂₀ histamine or serum IgE are indicated in parentheses. ^a: Numbers of undetectable cases.

MDC proteins are increased locally and systemically in human asthmatics, we investigated the levels of TARC and MDC proteins in the serum and sputum of asthmatics by using ELISA systems.

an equal volume of Dulbecco's phosphate buffered saline (D-PBS) and centrifuged. Serum preparations were obtained from 46 consenting outpatients with asthma at Doai Memorial Hospital. Non-allergic control sera were obtained from 26 staff members (healthy subjects) of Doai Memorial Hospital and University of Tokyo Hospital

Material and methods

Subjects

Patients with bronchial asthma were diagnosed according to the American Thoracic Society criteria (6). Induced sputum samples (6 controls and 30 asthma) were from subjects reported previously (7). Sputa were obtained following hypertonic saline inhalation, as described by Pin et al. (8) Homogenized sputum was treated with 10 mM dithioerythritol and filtered. The sample was then mixed with

Assay of TARC, MDC, eotaxin, eosinophil cationic protein (ECP) and IgE

Immunoreactive TARC was quantified by a sandwich immunoassay using two different newly established mouse mAbs (clones 4A3 and 5F12) against human TARC. Samples and standards were incubated overnight in ELISA microtiter plates (Nunc, Roskilde, Denmark) precoated with mAb 4A3, which were then washed and incubated with HRPO-conjugated mAb 5F12 Fab. The enzyme activity was

Table 2. Correlation coefficients and statistical significance in (A) serum of asthmatics and (B) sputum of asthmatics.

A		Serum of asthmatics (n = 46)						
		FEV1.0 (L)	% predicted	PC ₂₀ histamine (μg/ml)	Blood eosinophils (/μl)	Serum IgE (RIST) (U/ml)	Serum TARC/CCL17 (pg/ml)	Serum MDC/CCL22 (pg/ml)
vs								
		(n = 44)		(n = 30)				
Eotaxin	rs	− 0.217	− 0.041	0.182	− 0.473	− 0.473	0.392	− 0.037
	p	0.170	0.796	0.335	0.049*	0.002**	0.011*	0.809
TARC	rs	− 0.294	− 0.300	0.229	0.045	0.023	1.000	0.466
	p	0.052	0.055	0.218	0.771	0.882	—	0.003**
MDC	rs	< 0.001	− 0.039	0.096	0.379	0.429	0.466	1.000
	p	0.999	0.806	0.604	0.014*	0.005**	0.003**	—
B		Sputum of asthmatics						
		FEV1.0 (L)	% predicted	PC ₂₀ histamine (μg/ml)	Sputum eosinophils (%)	Serum IgE (RIST) (IU/ml)	Sputum TARC/CCL17 (pg/ml)	Sputum ECP (ng/ml)
vs								
(n = 30)		(n = 29)		(n = 25)		(n = 29)		(n = 27)
Eotaxin	rs	− 0.446	− 0.349	0.060	− 0.071	− 0.215	0.509	0.454
	p	0.018*	0.065	0.770	0.702	0.256	0.006**	0.021*
TARC	rs	− 0.083	− 0.263	− 0.245	0.062	− 0.239	1.000	0.327
	p	0.659	0.163	0.230	0.737	0.207	—	0.096

Correlation coefficient (rs) and statistical significance (P) were assessed by Spearman's rank correlation test. **P < 0.01. The number of patients assessed for FEV1.0 (L), FEV1.0 (%predicted), PC₂₀ histamine or serum IgE are indicated in parenthesis.

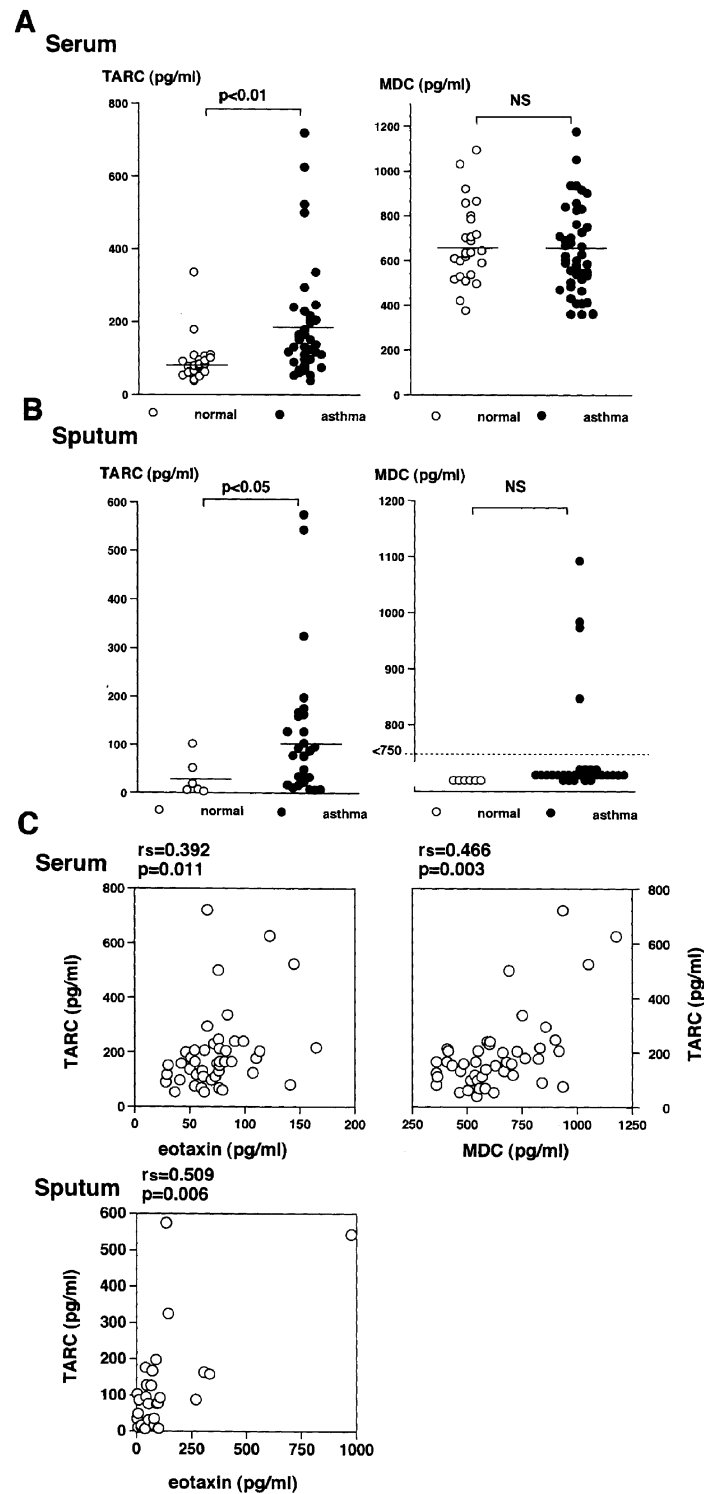


Figure 1. A and 1B: Concentrations of TARC and MDC in serum or sputum of normal controls and asthmatics. Each symbol represents the measured value from a single sputum or serum sample obtained from healthy (open circle) or asthmatic (closed circle) subjects. Statistical significance was assessed by the Mann–Whitney U-test; P-values are indicated in the figure. Horizontal bars show the mean values. NS: not significant. Figure 1C: Correlation coefficients and statistical significance in serum or sputum of asthmatics. Correlation coefficients (r_s) and statistical significance (p) were assessed by Spearman's rank correlation test.

finally determined using tetramethyl-benzidine as a substrate. This ELISA method detected TARC concentrations of as low as 0.6 pg/ml. Immunoreactive TARC was stable during our processing of sputum samples, and the dilution curves of sputum and serum showed good linearity (data not shown). Immunoreactive eotaxin/CCL11 was quantified as described previously (9). Immunoreactive MDC was measured using a commercially available ELISA kit for MDC (R&D Systems, Minneapolis, MN, USA; detection limit: 62.5 pg/ml). The levels of TARC and MDC in sputa were measured after diluting samples 6 times and 12 times, respectively. ECP was assayed using an RIA kit (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). Total IgE levels were determined using Lumiward System (Shionogi, Osaka, Japan).

Statistics

Statistical comparison of values between groups was made by the Mann-Whitney U-test. Tests for correlations between variables were performed by Spearman's rank correlation test. A P-value of <0.05 was considered significant.

Results

As shown in Fig. 1(A and B), TARC levels in both serum and sputum of asthmatics were increased with statistical significance compared with the healthy control subjects ($P < 0.001$ and $P = 0.027$ for serum and sputum, respectively). In contrast, no significant differences were observed for the serum concentration of MDC between the asthmatics and healthy subjects ($P = 0.305$). Furthermore, the concentration of MDC in the sputum was below the detection limit (<750 pg/ml) in most of the cases with our assay system (Table 1).

Correlation analysis showed positive correlations among the levels of TARC, MDC and eotaxin in the serum of asthmatics (Table 2A and Fig. 1C). Although the serum IgE level was significantly elevated in the asthmatics compared with the healthy subjects, no correlation was observed between the serum levels of TARC and IgE. On the other hand, the serum IgE level and the number of blood eosinophils correlated significantly with the serum MDC level (Table 2A). Correlation analysis of the sputum of asthmatics showed a positive correlation between the levels of TARC and eotaxin (Fig. 1C). However, in contrast to eotaxin, which showed a strong positive correlation with the ECP concentration, TARC did not correlate with the level of ECP (Table 2B).

Discussion

Although it has been argued that TH0 cells may also express CCR4 (10), the evidence accumulated to date strongly supports that both MDC and TARC play dominant roles in TH2-type disease conditions by recruiting TH2 cells to inflammatory sites (11). This study has for the first time investigated the levels of TARC and MDC in asthmatics. The TARC levels in

both the sputum and serum were significantly increased in asthmatics compared with healthy subjects. The elevated levels of circulating TARC in asthmatics may be a reflection of increased TARC expression at inflammatory sites in the asthmatic airway. We have previously shown increased expression of TARC in the bronchial epithelium of asthmatics. Furthermore, our in vitro study demonstrated that bronchial epithelial cells are capable of generating large amounts of TARC but virtually no MDC (4). Since bronchial epithelial cells are known to represent an important cellular source of chemokines fundamental to bronchial asthma, the predominance of TARC rather than MDC in asthmatics might be explained by an increase in epithelial cell-derived TARC.

Since the low sensitivity of our MDC assay (~100 times lower than that for TARC) might have affected the statistical results, we should be careful about drawing a final conclusion regarding MDC from our results. It has been clearly demonstrated that blocking of MDC attenuates asthmatic reactions in mice (3). In fact, although the MDC level in asthmatics did not increase with statistical significance in our study, it did correlate with some parameters. Thus, a more sensitive assay for MDC is needed to clarify the possible role of MDC in the pathogenesis of asthma, which remains unclear in the present study.

The significantly elevated TARC levels in asthmatics strongly suggest that this chemokine is actively involved in the pathogenesis of the disease. Since in vitro expression of TARC is up-regulated by TH2-derived cytokines such as IL-4 (5), it is likely that TARC plays a role in exacerbation and maintenance of local TH2 responses in asthma by recruiting additional TH2 cells to allergic inflammatory sites. However, this study included only a limited number of cases for correlation analysis between various parameters. In this context, further investigation employing a larger number of asthmatic cases will clarify the importance of TARC and MDC TH2-specific chemokines in the still unexplored inflammatory cell networks involved in asthma. Comparative studies between serum and sputum employing single donors will shed light on the in vivo kinetics of these molecules. The modes of expression of these chemokines in atopic and non-atopic asthma are also intriguing questions meriting further studies.

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