

Aberrant Expression of CC and CXC Chemokines and Their Receptors in Patients with Asthma

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This study further elucidates the roles of selected chemokines (IP-10, MIG, and RANTES) and their receptors (CCR3, CCR5, and CXCR3) in asthma. We compared their profiles in six groups of participants—atopic cohort and nonatopic cohort (each including controls and asthmatic patients with or without steroid therapy). Plasma concentration of IP-10 was significantly lower while that of RANTES and the expression of CCR3 were higher in asthmatic patients (all $p < 0.05$). Plasma RANTES correlated positively with the GINA severity score in all asthmatic patients ($r = 0.27$, $p < 0.05$), and with IL-13 in nonatopic asthmatic patients ($r = 0.46$, $p < 0.05$). In asthmatic patients, the *ex vivo* release of IP-10 and MIG was attenuated in PBMC activated with allergen, mitogens and IL-18 ($p < 0.05$). In conclusion, plasma RANTES may be a surrogate marker for asthma and the diminished Th1 related CXC chemokine production may contribute to Th2 predominance in asthma.

KEY WORDS: Asthma; chemokines; cytokines; peripheral blood mononuclear cells.

INTRODUCTION

Atopic asthma is characterized by infiltration into the airway submucosa of leukocytes, which contributes to airway obstruction and bronchial hyperresponsiveness (1). Leukocyte infiltration is mediated by the interaction of chemokines and their corresponding receptors. Chemokines are subclassified into four groups—CXC, CC, CX3C, and C—on the basis of arrangement of the

amino terminal cysteine residues. They act by binding to cell surface receptors differentially expressed on diverse cell types (2). These chemokine receptors are selectively expressed on human lymphocytes which are highly polarized towards a helper T cell type (Th) 1 or Th2 phenotype (3, 4). Th1 cells preferentially express CC chemokine receptor (CCR) 5 and CXC chemokine receptor (CXCR) 3, while CCR3, CCR4, and CCR8 are expressed on Th2 cells (5). CC chemokines are involved in the initiation and amplification of allergic diseases such as atopic skin inflammation, rhinitis, and asthma, while CXC chemokines and receptors are involved in autoimmune diseases like autoimmune thyroiditis and encephalomyelitis (6). Thus, the regulated production of specific chemokines and distinct subsets of receptor expression are likely to affect the recruitment of Th1 and Th2 lymphocytes, thereby controlling the intensity and severity of an immune response. Numerous studies have demonstrated the contribution of a biased Th2 response to the pathogenesis of allergic diseases. Wong *et al.* (7) previously demonstrated a decrease in the ratio of Th1/Th2 cells in asthmatic patients and consequently a predominance of Th2 cell response. However, recent studies have shown an increase in interferon- γ producing T cells in asthmatic patients and they are capable of inducing airway hyperresponsiveness independent of interleukin (IL)-4 and IL-13 (8, 9). Interestingly, IL-18, which is an interferon- γ inducing cytokine, is also increased in the plasma of atopic asthmatic patients. Although it is well known that CC chemokines and their receptors critically participate in atopic asthma by recruiting eosinophils and Th2 lymphocytes, the role of CXC chemokines is of lesser knowledge. The significance of elevated IL-18 in atopic asthma is also not well defined.

Therefore, we compared the profiles of selected chemokines and their receptors in six groups of participants—atopic and nonatopic cohorts consisting of control subjects, nonsteroid-treated asthmatic patients

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and steroid-treated asthmatic patients. We measured the plasma concentrations of CC chemokine regulated upon activation, normal T cell expressed and secreted (RANTES), CXC chemokines interferon- γ inducible protein (IP)-10 and monokine inducible by interferon- γ (MIG). In addition we examined the correlation between these chemokines and Th2 cytokine IL-13. We also investigated the cell surface expression of CCR3, CCR5, and CXCR3 on lymphocytes. The clinical correlations of the above parameters with asthmatic exacerbation were investigated. *Ex vivo* activated release of chemokines by house dust mite (*Dermatophagoides pteronyssinus*) allergen protein (Der p 1), phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) in whole blood were studied. To further elucidate the possible regulation of the selected chemokines in atopic asthma, we examined the effect of IL-18 on the *ex vivo* release of chemokines.

METHODS

Asthmatic Patients, Control Subjects, and Blood Samples

Sixty-nine Chinese adult patients with asthma were recruited from the Asthma Clinic of the Prince of Wales Hospital, Hong Kong. Diagnosis of asthma was based on the guidelines of the American Thoracic Society (10). Lung function of the subjects was assessed by spirometry (Model S, Vitalograph, Buckingham, UK) according to the American Thoracic Society standards (11). Forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), and the FEV₁/FVC ratio were measured before and 15 min after the inhalation of salbutamol (Glaxo Operations Ltd., Greenford, UK). The results were compared with the local predicted age- and sex-matched values (12). The severity of asthma in these patients was assessed according to the Global Initiative for Asthma (GINA) guidelines (13, 14). All our studied asthmatic patients were on short acting bronchodilator as needed. Some of them were on inhaled steroids such as beclomethasone dipropionate (Becloforte; Glaxo Wellcome, Research Triangle Park, NC) or budesonide (Pulmicort; AstraZeneca, London, UK). All patients and control subjects had no oral intake of steroid or change of asthma medications 4 weeks before recruitment of study. They were also not on theophylline or anti-leukotriene therapy, and only 6 (8.7%) patients were taking long acting β -2 agonist. Forty-seven sex- and age-matched healthy Chinese volunteers were recruited as control subjects. All subjects were nonsmoker and free from upper respiratory tract infection for 4 weeks before recruitment. Nine milliliters of venous peripheral ethylenediaminetetraacetic acid blood and 5 mL of clot-

ted blood was collected from each participant. Aliquots of whole blood were processed immediately for *ex vivo* study and peripheral blood mononuclear cell (PBMC) isolation. Plasma and serum samples were preserved at -70°C for subsequent assays. The above protocol was approved by our joint university and hospital clinical research ethics committee, and informed consent was obtained from all participants.

Assays on Serum Immunoglobulin (Ig) E and Eosinophilic Cationic Protein (ECP)

The atopic status of all participants was ascertained by positive serum specific IgE assays to house dust mite (Der p 1), cat, dog, mixed cockroaches and mixed moulds by fluorescence enzyme immunoassay (AutoCAP analyzer, Pharmacia Diagnostics AB, Uppsala, Sweden) (15). Subjects were classified as atopic if they had at least one positive result on testing for allergen specific IgE. Serum ECP was similarly measured (AutoCAP analyzer).

Assays for Chemokines and Cytokines in Plasma

The concentrations of IP-10, MIG, IL-8, monocyte chemotactic protein (MCP)-1 and RANTES in plasma were measured simultaneously using human chemokine cytometric bead array (CBA) reagent (Becton Dickinson Biosciences Pharmingen, CA). Samples were analysed on a multifuorescence BD FACSCaliburTM flow cytometer using BD CellQuestTM software and BDTM CBA Software. The assay sensitivities of these five chemokines were 2.8, 2.5, 2.7, 1.0, and 0.2 ng/L, respectively. The coefficients of variation of all chemokine assays were less than 10%. Plasma IL-18, and IL-13 concentrations were measured by enzyme-linked immunosorbant assay (ELISA) (R&D Systems Inc., MN).

Ex Vivo Production of Chemokines

The method of Viallard *et al.* (16) was adopted. Whole blood was diluted 1:1 with culture medium RPMI 1640 (Gibco Laboratories, NY), and 1 mL aliquots were dispensed in each well of a 24-well plate (Nalge Nunc International, IL). The blood culture was then incubated with or without T cell mitogen PHA (Sigma Co, MO, USA) at 5 $\mu\text{g/mL}$ and B cell and macrophage mitogen LPS at 25 $\mu\text{g/mL}$ (Sigma), Der p 1 recombinant protein (Indoor Biotechnologies Ltd., Manchester, UK) at 3 $\mu\text{g/mL}$ and recombinant protein IL-18 (R&D Systems) at 100 ng/mL for 24 h at 37°C in a 5% CO₂ atmosphere. The cell-free supernatant was harvested and stored at -70°C for subsequent assays.

Table I. Characteristics of the Study Populations^a

	Atopic			Nonatopic		
	Asthmatic patients on treatment with steroid (AS) (n = 23)	Asthmatic patients not on steroid therapy (ANS) (n = 13)	Control subjects (AC) (n = 18)	Asthmatic patients on treatment with steroid (NAS) (n = 15)	Asthmatic patients not on steroid therapy (NANS) (n = 18)	Control subjects (NAC) (n = 29)
Sex						
Male	15	15	9	4	8	7
Female	8	8	9	11	10	22
Age (year) mean $\pm$ SD (range)	38.57 $\pm$ 13.35 (20–63)	38.62 $\pm$ 12.93 (22–61)	38.18 $\pm$ 15.36 (22–66)	55.80 $\pm$ 12.35 (34–80)	40.94 $\pm$ 13.29 (20–63)	42.73 $\pm$ 9.03 (23–55)
Lung functions						
FEV ₁ (L/min)	2.55 $\pm$ 0.82 [80.45 $\pm$ 17.62]	2.57 $\pm$ 0.98 [86.28 $\pm$ 32.84]	NE	1.88 $\pm$ 0.62 [89.08 $\pm$ 19.11]	2.60 $\pm$ 0.79 [90.66 $\pm$ 16.17]	NE
FEV ₁ /FVC ratio (%)	79.79 $\pm$ 8.60	85.17 $\pm$ 10.05	NE	84.44 $\pm$ 7.62	85.47 $\pm$ 10.26	NE
Daily dosage of inhaled steroid (μ g)	1152.2 $\pm$ 657.0	0	0	1317.9 $\pm$ 600.5	0	0
Atopy (%)	100	100	100	0	0	0
Serum total IgE (kU/L) ^b	175.0* (55.6–477.5)	260.0* (95.6–454.5)	151.5* (70.0–550.5)	70.5 (10.3–129.0)	93.8** (47.1–209.0)	37.2 (5.7–78.0)
Serum ECP (μ g/L) ^b	4.18* (2.23–6.17)	2.00 (2.00–3.06)	2.00 (2.00–4.28)	3.55* (2.80–10.35)	2.55* (2.02–5.83)	2.00 (2.00–2.05)

Note. NE: not examined. Higher than nonatopic control subjects [^{*} $p < 0.05$, ^{**} $p < 0.01$, ^{***} $p < 0.001$ (Mann–Whitney rank sum test)].

^aValues given as mean $\pm$ SD, unless otherwise indicated.

^bValues given as median (IQR).

Expression of Chemokine Receptors on PBMC

PBMC were prepared by centrifugation of blood, using a density gradient (Ficoll-Paque Plus; Amersham Pharmacia Biotech Ltd., Uppsala, Sweden). The cells were incubated with fluorescein isothiocyanate (FITC)-conjugated CCR3-specific monoclonal antibody (mAb), purified CCR5 and CXCR3-specific mAb, followed by FITC-conjugated secondary antibody (Becton Dickinson Biosciences Pharmingen). FITC-conjugated rat IgG2a, purified mouse IgG2a, and mouse IgG1 were used as isotypic controls for CCR3, CCR5, and CXCR3, respectively. The typical forward and side scatter gates for lymphocytes were set to exclude contaminating monocytes from the analysis. Altogether, 5000 events were collected in the log mode and were expressed as mean fluorescence intensity (MFI).

Statistical Analysis

The Mann–Whitney *U* test was used to assess the differences of data between the asthmatic patients and the control subjects. All analyses were performed using statistical software (SPSS for Windows, version 9.0, SPSS Inc., Chicago, IL). Probability (*p*) values of less than 0.05 were considered significant. Unless otherwise specified, results are expressed as the median (interquartile range) (IQR).

RESULTS

Asthma Severity, Atopic Status, and Serum ECP

The characteristics of the study groups are summarized in Table I. These include six groups of participants—atopic controls (AC), atopic asthmatic patients treated with steroid (AS), atopic asthmatic patients without steroid therapy (ANS), nonatopic controls (NAC), nonatopic asthmatic patients treated with steroid (NAS), and nonatopic asthmatic patients not on steroid therapy (NANS). The severity of asthma in the recruited patients, according to the GINA guideline based on daytime and nocturnal symptoms, and lung function, was as follows: intermittent asthma 13 patients (19%), mild persistent asthma 11 patients (16%), moderate persistent asthma 21 patients (30%), and severe persistent asthma 24 patients (35%). Compared with NAC, the serum total IgE concentration was significantly increased in NANS and all atopic study groups (Table I). In the nonatopic cohorts, concentration of ECP in serum was significantly increased in all asthmatic patients when compared with the controls. The AS group showed significantly increased ECP concentration compared with NAC (Table I). Positive correlations were found between the total IgE concentration against FEV₁ ($r = 0.33$, $p < 0.01$), and ECP concentration against GINA severity score ($r = 0.53$, $p < 0.001$) in all asthmatic patients.

Table II. Plasma Chemokines in Asthmatic Patients and Normal Control Subjects

Chemokine/ cytokine	Group	Median (IQR) (pg/mL)	Group	Median (IQR) (pg/mL)
IP-10	NAC	1288 (986–1494)	AC	1424 (1257–1909)
	NANS	999 (733–1305)	ANS	1229 (756–1519)
	NAS	775 (644–955)***	AS	792 (627–1028)***###
MIG	NAC	234 (176–411)	AC	272 (231–424)
	NANS	232 (173–352)	ANS	439 (211–613)
	NAS	297 (255–370)	AS	257 (160–289)
RANTES	NAC	2927 (732–14480)	AC	1963 (138–17260)
	NANS	11630 (4803–16970)*	ANS	16030 (5868–20390)***
	NAS	12650 (8914–19090)**	AS	14100 (4930–21390)***
IL-18	NAC	188 (146–259)	AC	176 (144–204)
	NANS	204 (138–334)	ANS	216 (199–323) [#]
	NAS	240 (174–298)	AS	235 (178–324) [#]
IL-13	NAC	107 (101–113)	AC	107 (103–113)
	NANS	106 (101–120)	ANS	112 (104–123)
	NAS	119 (106–127)*	AS	118 (102–129) [#]

Note. The Mann–Whitney rank sum test was used to assess differences in concentration with reference to the NAC group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and the AC group ([#] $p < 0.05$, ^{##} $p < 0.01$, ^{###} $p < 0.001$).

Chemokines in Plasma

Concentration of CXC chemokine IP-10 was significantly decreased in AS and NAS compared to their control counterparts (Table II). MIG concentrations were similar between asthmatic groups and the control groups regardless of atopic status. The plasma concentration of CC chemokine RANTES was significantly higher in both atopic and nonatopic asthmatic patients than the control subjects (Table II), which correlated positively with the GINA severity score in all asthmatic patients ($r = 0.27$, $p < 0.05$). Plasma RANTES also correlated positively with IL-18 in the ANS group ($r = 0.77$, $p < 0.01$) and with IL-13 in the asthmatic patients with nonatopic status ($r = 0.46$, $p < 0.05$). Concentration of IL-18 was significantly increased only in the atopic asthmatic patients when compared with the atopic control subjects (Table II), while that of IL-13 was significantly increased in both AS and NAS when compared with their control counterparts (Table II). However, there was no similar correlation observed with other chemokines. For MCP-1 and IL-8, the plasma concentrations were similar between different groups.

Expression of Chemokine Receptors on Cell Surface of PBMC

The differential analysis of Th1 and Th2 subsets in PBMC was studied via the cell surface expression of Th2 marker CCR3 and Th1 markers CCR5 and CXCR3. CCR3 expression of all steroid-treated patients was significantly increased when compared to control subjects (Table III). CCR3 expression correlated positively with the GINA

severity score in all asthmatic patients ($r = 0.30$, $p < 0.05$). CXCR3 expression was significantly increased in the NAS group, while significantly decreased in all atopic asthmatic patients when compared to their corresponding controls (Table III). We observed a significant increase in CXCR3 expression in the AC group when compared with the NAC group (Table III). For steroid-treated asthmatic patients, CXCR3 expression was significantly lower in the AS group when compared with the NAS group [2.3 (1.2–3.5) versus 3.5 (2.6–4.0) MFI, $p < 0.05$]. In addition, CXCR3 expression correlated negatively with IL-13 in all nonatopic patients but not in atopic patients ($r = -0.44$, $p < 0.05$). CCR5 expression was similar among the different groups.

Ex Vivo Production of Chemokines Upon Activation

The basal releases of IP-10, MIG and RANTES from nonactivated whole blood were very low. The *ex vivo* releases of IP-10 and MIG were significantly enhanced after PHA and LPS stimulation when compared with their medium control in all study groups, while that of RANTES was only observed in the ANS group (Table IV). By analyzing the percentage of induction, IP-10 was similar among all groups. The percentage of MIG induction was significantly decreased in the AS group when compared with the AC or NAC group (Table IV). It was also significantly decreased when compared with the ANS group [80 (11–159)% vs. 494 (185–1337)%, $p < 0.01$]. The percentage of both IP-10 and MIG induction from Der p 1 activated whole blood from asthmatic patients were significantly decreased compared with corresponding

Table III. Expression of Chemokine Receptors in Asthmatic Patients and Normal Control Subjects

Chemokine receptor	Group	Median (IQR) (MFI)	Group	Median (IQR) (MFI)
CCR3	NAC	0.47 (0.27–0.98)	AC	0.50 (0.42–0.80)
	NANS	0.73 (0.28–1.66)	ANS	0.63 (0.45–0.94)
	NAS	1.13 (0.54–1.73)**	AS	1.00 (0.57–1.83)**#
CCR5	NAC	2.93 (2.22–5.40)	AC	4.77 (4.04–7.39)
	NANS	3.95 (2.34–5.76)	ANS	3.00 (2.47–4.72)
	NAS	3.78 (2.65–4.78)	AS	3.37 (2.42–4.87)
CXCR3	NAC	2.42 (1.09–3.03)	AC	3.96 (2.72–4.78)**
	NANS	2.79 (1.44–6.04)	ANS	1.93 (1.37–3.18)#
	NAS	3.45 (2.55–4.01)*	AS	2.28 (1.18–3.52)#

Note. The Mann–Whitney rank sum test was used to assess differences in concentration with reference to the NAC group (* $p < 0.05$, ** $p < 0.01$) and the AC group (# $p < 0.05$).

control groups (Table V). On the contrary, the percentage of RANTES induction from whole blood after PHA and LPS stimulation was significantly decreased in the AS group when compared with the NAC group (Table IV), while that after Der p 1 activation showed no significant changes (Table V).

The *ex vivo* release of IL-18 from nonactivated whole blood was also very low. However, only the NAC, NAS, and ANS groups showed significant increases upon activation by PHA and LPS (Table IV). The percentage induction of IL-18 showed significant decreases in all atopic

asthmatic patients when compared to their corresponding controls (Table IV). The percentage induction of IP-10 and MIG were attenuated from whole blood activated by IL-18 in asthmatic patients but not in the control subjects (Table VI).

DISCUSSION

This study shows for the first time that CC chemokine RANTES may be related to airway hyperresponsiveness

Table IV. *Ex Vivo* Production of Chemokines and IL-18 from Mitogen-Activated PbmC

Chemokines/cytokines	Groups	Medium (pg/mL)	PHA LPS (pg/mL)	Percentage change (%)
IP-10	NAC	504 (371–665)	14650 (7978–26180)***	2765 (1222–4593)
	NANS	501 (284–643)	9567 (4544–15230)***	2048 (779–3494)
	NAS	273 (113–489)	4570 (2192–12210)***	1807 (1137–4656)
	AC	497 (366–599)	11600 (6680–12030)***	2466 (1175–2726)
	ANS	382 (268–573)	9933 (8399–16420)***	2726 (2098–3601)
	AS	336 (245–372)	3585 (1926–6797)***	1091 (400–1760)
MIG	NAC	224 (159–308)	1891 (740–9817)***	1670 (202–4403)
	NANS	131 (85–176)	724 (247–4529)*	292 (95–702)
	NAS	120 (81–178)	296 (188–2013)***	146 (40–2487)
	AC	183 (130–272)	1816 (409–5114)***	833 (195–2575)
	ANS	175 (130–326)	1082 (614–2446)***	494 (185–1337)
	AS	110 (80–212)	260 (155–282)**	80 (11–159) ^{a,d}
RANTES	NAC	494 (236–1503)	993 (482–2616)	88 (71–108)
	NANS	1233 (741–3000)	3446 (1572–5678)	75 (55–115)
	NAS	869 (422–1481)	1428 (772–2184)	57 (29–149)
	AC	1178 (872–2411)	2315 (1286–3175)	38 (19–127)
	ANS	1226 (730–2327)	2909 (1242–5569)*	67 (48–128)
	AS	1279 (743–2632)	2409 (1043–3914)	45 (32–71) ^b
IL-18	NAC	413 (332–601)	752 (588–874)**	66 (36–92)
	NANS	411 (203–1016)	484 (243–1145)	29 (–23–74)
	NAS	429 (338–896)	945 (442–1268)*	53 (37–74)
	AC	299 (137–760)	561 (392–1139)	94 (62–176)
	ANS	451 (356–784)	659 (580–1018)*	50 (25–78) ^c
	AS	681 (332–915)	903 (569–1395)	41 (24–74) ^d

Note. The Mann–Whitney rank sum test was used to assess the differences of production between PHA-LPS treated and medium control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), and the percentage change with reference to the NAC group (^a $p < 0.05$, ^b $p < 0.01$) and the AC group (^c $p < 0.05$, ^d $p < 0.01$).

Table V. *Ex Vivo* Production Of Chemokines from Derp1 Activated PBMC

Chemokines	Groups	Medium (pg/mL)	DerP1 (pg/mL)	Percentage change (%)
IP-10	C	94 (94–149)	255 (131–1441)*	170 (–12–1430)
	A	103 (82–167)	74 (59–143)	–22 (–33–16) [#]
MIG	C	74 (43–99)	95 (52–117)	21 (–17–65)
	A	71 (56–117)	62 (47–123)	–12 [–18–(–4)] [#]
RANTES	C	101 (65–246)	89 (62–210)	–5 (–26–9)
	A	583 (431–1629)	507 (418–1588)	–10 (–28–17)

Note. Groups: Control subjects (C), Asthmatic patients (A). The Mann–Whitney rank sum test was used to assess the differences of production between Der p 1-activated and medium control group (* $p < 0.05$), and the percentage change with reference to the controls ([#] $p < 0.05$).

in nonatopic asthma and together with its receptor CCR3 contributes to the severity of asthma. We also demonstrated a decrease in production of Th1 related CXC chemokine such as IP-10 and MIG in asthmatic patients which may contribute to the diminished antagonizing effect on Th2 cytokine production and hence Th2 predominance.

Plasma concentration of RANTES and expression of CCR3 on lymphocytes correlated positively with GINA severity score in all asthmatic patients. This suggested their involvement in the severity of the disease. CCR3 is an eotaxin receptor, but also allows the binding of other CC chemokines such as RANTES (17). In the present study, flow cytometric immunophenotyping was used to identify the lymphocyte population from the mixture of PBMC. Thus, the detected CCR3 expression serves as a marker for Th2 lymphocytes, while CCR5 and CXCR3 for Th1 (18). It is therefore speculated that the increase in plasma RANTES and CCR3 expression may result in a possible enhancement in Th2 lymphocyte recruitment in both atopic and nonatopic asthmatic patients. The present findings also suggest that patients with higher severity may be more responsive to CCR3 ligands. This RANTES-CCR3 interaction likely contributes to Th2 predominance in asthmatic patients and correlate with the disease severity.

Plasma RANTES correlated positively with IL-13 in the nonatopic asthmatic patients, suggesting its involvement with airway immunopathogenesis in this patient group. The Th2 cytokine IL-13 in plasma showed significant increase in the more severe asthmatic groups regardless of atopic status. Interestingly, RANTES correlated positively with IL-13 in the nonatopic asthmatic patients. It has been well documented on RANTES and eosinophil chemotaxis, and ECP being utilized to monitor the activation of eosinophils (19). However, ECP does not correlate with airway hyperresponsiveness such as in eosinophilic bronchitis characterized by the presence of eosinophilic inflammation but not airflow obstruction (20).

By correlating with IL-13 which relates to both airway inflammation and hyperresponsiveness (21), RANTES may act as a surrogate marker in nonatopic asthma. Although unable to directly assess the pathological condition of the lung, the use of peripheral blood may be another method to monitor asthma-related changes. Interestingly, we previously found significant elevation of Th1 chemokine IP-10 but not RANTES in the severe acute respiratory syndrome, a novel virus-induced pulmonary inflammatory disease with Th1 predominance (22). The present findings support RANTES as a surrogate marker in asthma characterized by Th2 predominance.

Table VI. *Ex Vivo* Production of Chemokines from IL-18 Activated PBMC

Chemokines	Groups	Medium (pg/mL)	IL-18 (pg/mL)	Percentage change (%)
IP-10	C	94 (94–149)	620 (142–1461)*	338 (13–1451)
	A	103 (82–167)	112 (68–235)	13 (–27–35) [#]
MIG	C	74 (43–99)	115 (45–223)	56 (–4–124)
	A	71 (56–117)	63 (51–105)	–9 (–16–4) [#]
RANTES	C	101 (65–246)	99 (64–229)	–2 (–34–16)
	A	583 (431–1629)	482 (365–1370)	–17 [–32–(–5)]

Note. Groups: Control subjects (C), Asthmatic patients (A). The Mann–Whitney rank sum test was used to assess the differences of production between IL-18-activated and medium control group (* $p < 0.05$), and the percentage change with reference to the controls ([#] $p < 0.05$).

This study demonstrated a Th1 suppressed response in asthma. Asthma was originally regarded as a Th2 mediated inflammatory response. In this study, we observed a significantly lower plasma concentration of IP-10, which is a Th1 related chemokine, in asthmatic patients with higher severity and under steroid treatment. The plasma level of another CXC chemokine MIG, a Th1 cell chemoattractant inducible by γ -interferon, did not show significant differences among the groups. However, the *ex vivo* production of MIG upon activation is significantly decreased in the asthmatic patients. These results indicate that asthmatic patients are unlikely to release chemokines for Th1 cell attraction upon allergen or cytokine stimulations.

The profiles of chemokine and chemokine receptor were found to be different between atopic and nonatopic asthma. Although it has been suggested that both atopic and nonatopic asthma shares similar immunopathological entity which shows a Th2 cytokine profile, the mechanism for nonatopic asthma was suggested to be autoallergy or autoimmunity triggered by viral infection (23). Th1 response is typically induced by viral respiratory tract infections despite the final Th2 response in asthma (24). Therefore we compared the asthmatic patients according to their atopic status in order to discuss the possible difference in chemokine patterns between the two types of asthma. Expression of CXCR3 was found to be less in atopic than nonatopic patients with asthma. CXCR3 is the receptor for both IP-10 and MIG signaling, thereby enhancing the polarization of Th1 lymphocyte (2). The observation from the atopic study groups suggests less Th1 cells were recruited in those with asthma, which is in line to the observation on the decrease in CXC chemokine expression in plasma. However for the nonatopic study groups, more Th1 lymphocytes were present in asthmatic patients, which suggests a differential role of Th1 lymphocytes in atopic and nonatopic asthma. The differential role of Th1 lymphocytes can be further seen from the negative correlations of IL-13 against IP-10 and MIG found only in atopic patients but not in nonatopic patients.

The proinflammatory cytokine IL-18 exhibited a Th2 effect in atopic asthma and attenuated the release of CXC chemokines. IL-18 is an important regulator of both Th1 and Th2 cytokine production (25). It is predominantly produced by activated macrophages, and synergizes with IL-12 and IL-2 to induce interferon- γ synthesis, thereby promoting Th1 cytokine response. Paradoxically, IL-18 by itself strongly induces IgE and allergic inflammation, indicating a role for IL-18 in promoting Th2 response (26). The plasma concentration of IL-18 correlated positively with the CC chemokine RANTES in the atopic asthmatic patients without steroid treatment, suggesting

that IL-18 plays a more critical role in the atopic patients than nonatopic patients. Since there was an elevation in IL-18 concentration in the plasma of asthmatic patients, we further investigated if IL-18 promoted or suppressed the release of chemokines in asthma. In line with the postulated Th2 role of IL-18, we found both the releases of IP-10 and MIG from IL-18 activated PBMC were suppressed in the asthmatic patients. IL-18 can increase allergic sensitization, serum IgE, Th2 cytokines and airway eosinophilia in murine model of allergic asthma (27, 28) and the present study also suggest IL-18 contribute to Th2 predominance possibly by suppressing the release of CXC chemokines, hence the recruitment of Th1 cell.

Herein, we have chosen to use whole blood for *ex vivo* induction to mimic the T lymphocytes in their *in vivo* environment where cytokines and other immune cell types are already present. We also found that Der p 1, IL-18 or PHA, and LPS could not enhance the release of CC chemokine RANTES in asthmatic patients, but caused a suppressed release of CXC chemokines IP-10 and MIG. We therefore suggest that the Th2 predominant profile seen in asthma is caused by a decreased production of Th1 related CXC chemokine, hence a deficiency in the chemoattraction of Th1 cells and counter inhibition on Th2 cells chemoattraction.

CONCLUSIONS

In summary, plasma RANTES may be a possible surrogate marker for monitoring asthma severity. In this regard, the identification and blocking of this chemokine or related chemokine receptors may be the novel therapeutic approach to both atopic and nonatopic asthma (29). Our study also demonstrated a diminished production of CXC chemokine IP-10 and MIG upon allergen activation in asthmatic patients, which contributes to a skewed Th2 profile. Chemokines interact in a complex network and so far no chemokine-based therapeutics has been approved for use in humans (30). The dual roles of IL-18 and differential expression of CXC chemokines in atopic and nonatopic asthma should be taken cautiously when considering Th1 vaccination on treating asthma (31). Future studies are required to further elucidate the therapeutic roles of chemokines and proinflammatory cytokine IL-18 in asthma.

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