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ORIGINAL ARTICLE

Serum Chemokines RANTES and Monocyte Chemoattractant Protein-1 in Egyptian Patients with Atopic Asthma: Relationship to Disease Severity

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Background and Aims. Asthma is a highly prevalent, complex inflammatory disease of the airways often associated with bronchial hyperreactivity and atopy. The chemokine RANTES (regulated upon activation, normal T-cell expressed and secreted) is an important element for the chemotaxis at the site of allergic inflammation. This study aimed to assess the serum levels of the chemokines RANTES and monocyte chemoattractant protein-1 (MCP-1) in Egyptian patients with atopic asthma and to evaluate their possible relation t the severity of airway obstruction.

Methods. The study included 60 Egyptian patients with atopic asthma and 20 healthy volunteers. Serum levels of the chemokines RANTES and MCP-1 were measured. Total serum IgE level and absolute eosinophil counts were determined. The severity of airway obstruction was assessed using spirometric measurement (FEV₁).

Results. The serum levels of RANTES were significantly higher in all asthmatic patients than the controls ($p < 0.001$). Moreover, RANTES levels were significantly increased in patients with moderate and severe asthma as compared to those with mild asthma ($p < 0.001$). Serum RANTES correlated positively with absolute eosinophil counts and total serum IgE and negatively with FEV₁, whereas there was no significant correlation with serum MCP-1 in all asthmatic patients.

Conclusions. Serum RANTES may be used as a useful noninvasive marker of airway obstruction and a potential diagnostic tool for monitoring asthma severity. In this regard, identification and blocking of this chemokine and/or its receptor may be a promising therapeutic approach to asthmatic patients. © 2012 IMSS. Published by Elsevier Inc.

Key Words: Asthma, Chemokines, MCP-1, RANTES, Severity.

Introduction

Asthma is a chronic inflammatory disorder of the airways characterized by bronchial hyperresponsiveness, reversible airflow obstruction, mucus hypersecretion, inflammatory cell migration into airways, bronchial epithelial desquamation and structural remodeling. The pathogenesis and etiology of asthma is very complex and not fully understood, although interactions between genetic and environmental

factors have been suggested as important determinants of this disease (1,2).

The immune response underlying the development of asthma has attracted considerable interest because many immune mediators have been shown to play a role in mediating the bronchial airway inflammation that accompanies allergic asthma (3). Chemokines are low molecular weight chemotactic cytokines comprised of four structurally different families—CXC, CC, CX3C, and C—on the basis of arrangement of the amino terminal cysteine residues (4). CC chemokines are involved in the initiation and amplification of allergic diseases such as atopic dermatitis, rhinitis, and asthma (5–10).

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A strong argument can be made for the CC chemokine ligand 5 (CCL5) known as RANTES (regulated upon activation, normal T-cell expressed and secreted) as a candidate mediator in asthma. It is produced by T lymphocytes, endothelial cells, fibroblasts, eosinophils, platelets and other cells. This mediator has the ability to attract several types of inflammatory cells including eosinophils, monocytes and memory T helper (Th) cells to the site of inflammation (11,12). In addition, increased levels of RANTES have been detected in the nasal secretions of atopic patients exposed to local allergen challenge (13) and were found in higher levels in the bronchoalveolar lavage fluid (14) and exhaled breath condensate (15) of asthmatic patients. Furthermore, blocking antibodies to RANTES inhibit airway inflammation in a murine model of allergic airway disease (16).

In addition, polymorphisms in RANTES gene have been identified. Some studies indicated that some polymorphisms were associated with an increased risk of asthma. However, results from different studies were apparently conflicting. Lachheb et al. (17) suggested that -28 C/G and -403 G/A polymorphisms within the RANTES promoter region play an important role in asthma predisposition and in the severity of airway obstruction. Fryer et al. (18) reported that the -403 G/A polymorphism was associated with asthma risk. In contrast, there was no evidence of association between RANTES gene promoter polymorphisms and asthma in a Spanish population (19). Overall results are inconclusive. Therefore, a meta-analysis was carried out (20). However, larger studies including different ethnic groups with a careful matching between asthma patients and controls should be considered in the future. Monocyte chemoattractant protein-1 (MCP-1); the CC chemokine ligand 2 (CCL2), may play a significant role in allergic responses because of its ability to induce mast cell activation and leukotriene C4 release into the airway, which directly induces airway hyperresponsiveness (21). Neutralization of MCP-1 drastically reduces bronchial hyperactivity, lymphocyte-derived inflammatory mediators, and T-cell and eosinophil recruitment to the lung (22).

In many studies performed using invasive (such as bronchoalveolar lavage -BAL) as well as semi-invasive (e.g., induced sputum) methods in asthmatic patients, an increased expression of local and systemic CC chemokines has been demonstrated (23,24). However, these relatively invasive approaches are unsuitable for repeated monitoring of airway inflammation. Therefore, establishing a simple monitoring system of airway inflammation would be useful for asthma management. Given the importance of RANTES and MCP-1 in allergic inflammation, this study was conducted to assess their serum levels in Egyptian patients with atopic asthma and to evaluate their possible relation to the severity of airway obstruction.

Materials and Methods

Subjects

Sixty Egyptian patients with atopic asthma were selected from those admitted to the Internal Medicine and Respiratory Medicine Departments in Tanta University Hospital (35 men and 25 women) with a mean age of 38.7 ± 12.5 years. Twenty healthy age- and gender-matched non-smoker volunteers (10 men and 10 women) with a mean age of 38.1 ± 14.3 years were recruited for participation as controls. They were selected from medical and paramedical staff and from blood donors in Tanta University Hospital. None of the control subjects had ever suffered from asthma or chronic respiratory symptoms. They had not undergone any respiratory tract infection during the month preceding the study. The study was approved by the scientific and ethics committees of the Tanta University Hospital, Tanta University, Tanta, Egypt, and informed consent was obtained from each subject before the start of this study. Diagnosis of asthma was based on the guidelines proposed by the American Thoracic Society (25). The clinical severity of asthma was assessed according to the Global Initiative for Asthma (GINA) guidelines (26). Positivity of the skin prick test (wheal diameter ≥ 3 mm greater than saline control) to one or more of common aeroallergen extracts (house dust mite, house dust, cotton dust, mixed pollens, cat fur, dog fur, feathers) and/or total serum IgE levels ≥ 100 IU/mL were used to define atopic status in all asthmatic patients (27). Also, positive family history of atopic dermatitis, rhinitis and asthma was taken into consideration. All asthmatic patients were stable and had been without regular asthma treatment including steroid therapy prior to the study, but rescue use of short-acting bronchodilator as needed for symptom relief was permitted. All patients were lifelong nonsmokers and none had suffered respiratory tract infection for at least 8 weeks prior to the study. All cases included in this study were subjected to careful history taking, complete clinical examination, laboratory investigations, radiological assessment, and pulmonary function tests.

Blood Collection and Laboratory Analyses

Peripheral venous blood was collected aseptically from patients and control subjects. Absolute eosinophil counts were performed on an aliquot of the whole venous blood using a Sysmex SE-9000 Automated Hematology Analyzer (TOA Medical Electronics Co, Kobe, Japan). The remaining blood sample was allowed to clot at room temperature for 30 min and centrifuged at $3000 \times g$ for 10 min. Serum was then aspirated and divided into aliquots in small plastic tubes. One aliquot was used for determination of total serum IgE levels using fluorescent enzyme immunoassay (AutoCAP System; Pharmacia Diagnostics AB, Uppsala, Sweden). The other aliquots were stored

at -70°C until used for measurements of RANTES and MCP-1 levels.

Measurement of Serum Level of RANTES and MCP-1

Serum RANTES and MCP-1 levels were measured with quantitative sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (28), using commercially available kits for both RANTES (Quantikine, Human RANTES Immunoassay Kit, DRN00B, R&D Systems, Minneapolis, MN) and MCP-1 (Quantikine, Human MCP-1/CCL2 Immunoassay Kit, DCP00, R&D Systems).

Pulmonary Function Tests

Spirometric study was done for all cases using Morgan's Q1 transfer apparatus with Della computer (England) according to the American Thoracic Society standards (29). Forced expiratory volume in 1 sec (FEV_1), forced vital capacity (FVC), and the FEV_1/FVC ratio were measured before and 15 min after the inhalation of salbutamol (Glaxo Operations Ltd., Greenford, UK). The results were recorded as the percentage of the predicted values. Asthmatic patients were categorized into severity groups by using level of FEV_1 % predicted. The patients were classified as having mild ($\text{FEV}_1 > 80\%$), moderate ($\text{FEV}_1 60\text{--}80\%$), or severe ($\text{FEV}_1 < 60\%$) airflow obstruction.

Statistical Analysis

Results were expressed as mean $\pm$ standard deviation (SD). Comparisons between groups were made using Student's t-test for continuous variables. Correlation between parameters was determined by Pearson's correlation coefficient (r); p value < 0.05 was considered statistically significant.

Results

The main clinical and laboratory parameters of asthmatic patients are shown in Table 1. There was significant lowering of pulmonary function parameters (FEV_1 , FVC and FEV_1/FVC) in asthmatic patients as compared with control subjects. The severity of asthma in the studied patients—according to the GINA guidelines based on daytime and nocturnal symptoms—and lung function, was as follows: intermittent in seven patients (11.67%), mildly persistent in 12 patients (20%), moderately persistent in 24 patients (40%), and severely persistent in 17 patients (28.33%).

Compared with control subjects, total serum IgE concentrations and absolute eosinophil counts were significantly increased in all asthmatic patients ($p < 0.001$, for both); however, no significant difference was found among the different grades of asthma severity (Tables 1 and 2). Serum

Table 1. Clinical and laboratory parameters of asthmatics and control subjects

Parameters	Controls ($n = 20$)	Asthmatic patients ($n = 60$)
Age (years)	38.1 ± 14.3	38.7 ± 12.5
Gender (M/F)	10/10	35/25
FEV_1 (% predicted)	97.6 ± 7.96	77.5 ± 5.2^a
FVC (% predicted)	89.7 ± 2.4	85.6 ± 4.1^a
FEV_1/FVC (%)	84.3 ± 0.5	78.5 ± 0.8^a
Total serum IgE (IU/mL)	33.2 ± 12.4	480.7 ± 102^a
Absolute eosinophil counts (cells/mm ³)	50 ± 15	172 ± 60^a
Serum RANTES (pg/mL)	2930 ± 515.2	8139.6 ± 3543.8^a
Serum MCP-1 (pg/mL)	173.6 ± 85.2	226.5 ± 93.8^b

FEV_1 , forced expiratory volume in one second; FVC, forced vital capacity; RANTES, regulated upon activation, normal T-cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1.

Data are expressed as mean $\pm$ standard deviation.

^a $p < 0.001$.

^b $p < 0.05$ asthmatic patients vs. controls.

levels of CC chemokine RANTES were significantly higher in all asthmatic patients than the control subjects ($p < 0.001$) as shown in Table 1. Moreover, RANTES levels were significantly increased in patients with moderate and severe asthma as compared to those with mild asthma ($p < 0.001$, for both) (Table 2). Regarding MCP-1 in asthmatic patients, there was an increase in MCP-1 serum levels in all asthmatics as compared to the controls ($p < 0.05$), whereas there was no significant difference among the different grades of asthma severity (Tables 1 and 2).

In the present study, serum RANTES in all asthmatic patients was positively correlated with absolute eosinophil counts ($r = 0.611$; $p < 0.001$) and total serum IgE ($r = 0.298$; $p < 0.05$) and negatively correlated with FEV_1 ($r = -0.385$; $p < 0.01$), whereas there was no significant correlation with serum MCP-1 as shown in Table 3. On the other hand, no significant correlations were found between serum MCP-1 and absolute eosinophil counts, total serum IgE, or FEV_1 .

Discussion

Asthma is a complex disorder of the airways that is increasing in prevalence and can result in significant morbidity and mortality (30). Much attention has recently been focused on the role of allergic inflammatory reaction in asthma. Chemokines are a family of cytokines that are believed to be involved in the pathogenesis of asthma, possibly by recruiting leukocytes to the inflammatory site (31). CC chemokine RANTES has been implicated in allergic inflammation of asthma by promoting migration and activation of inflammatory cells, including eosinophils (32). A growing body of evidence suggests that many cell types present in asthmatic airways have the capacity to

Table 2. Laboratory parameters according to grades of asthma severity

Parameters	Mild asthma (n = 19)	Moderate asthma (n = 24)	Severe asthma (n = 17)
Total serum IgE (IU/mL)	479 ± 107	489 ± 104	495 ± 67
Absolute eosinophil counts (cells/mm ³)	130 ± 52	156 ± 43	178 ± 87
Serum RANTES (pg/mL)	4498.3 ± 2625	7818.2 ± 3253.8 ^a	10740.6 ± 3410.2 ^{b,c}
Serum MCP-1 (pg/mL)	204.1 ± 81.6	220.4 ± 68.5	238.7 ± 72.2

RANTES, regulated upon activation, normal T-cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1.

Data are expressed as mean ± standard deviation.

^ap < 0.001 moderate asthma vs. mild asthma.

^bp < 0.001 severe asthma vs. mild asthma.

^cp < 0.01 severe asthma vs. moderate asthma.

generate RANTES (6). However, the exact role of RANTES in airway allergic inflammation has been somewhat controversial.

This study was designed to investigate the potential contribution of serum levels of the CC chemokines RANTES and MCP-1 to the pathogenesis of atopic asthma and to evaluate their relationship with the severity of airway obstruction. The study revealed that serum levels of RANTES were significantly higher in all asthmatic patients than in control subjects and RANTES levels were significantly increased in patients with moderate and severe asthma as compared to those with mild asthma. These results are in agreement with the findings of Lun et al. (33) who observed that the plasma concentration of RANTES was significantly higher in both atopic and onatopic asthmatic patients than the controls and correlated positively with the GINA severity score in all asthmatic patients. This suggested its involvement in the severity of the disease. RANTES has also been found at significantly higher levels in patients during asthma attacks than in those with asymptomatic stages or in control subjects (34). This observation is supported by Ying et al. (35) who showed that RANTES expression was higher in bronchial mucosa from both atopic and nonatopic asthmatics.

Also of note, Berkman et al. (36) reported that RANTES was constitutively expressed in the airways and RANTES mRNA was elevated in patients with asthma. Moreover, increased expression of mRNA encoding RANTES in the bronchial mucosa in atopic asthma was found by Powell et al. (37). Zietkowski et al. (15) reported that RANTES levels in exhaled breath condensate were significantly higher in asthmatic patients than in healthy controls and higher in patients with unstable asthma than in those with stable

asthma, indicating that RANTES can be used as a diagnostic tool for detection and monitoring of asthma. This observation is confirmed by previous studies performed by Tillie-Leblond et al. (38) who revealed that CC chemokines (including RANTES) were present at high levels in bronchoalveolar lavage (BAL) fluid from patients with severe asthmatic exacerbation than in patients with mild asthma. Conversely, Erten et al. (39) found no difference in RANTES levels in asthmatic patients compared to the control group. This finding may be attributed to the usage of inhaled corticosteroids in these patients, which was shown to repress RANTES by inhibition of NF-κB-dependent transcription (40).

Our current data also showed that serum RANTES in all asthmatic patients was positively correlated with absolute eosinophil counts, and total serum IgE and negatively correlated with FEV₁, which is an index of airflow limitation. Meanwhile, Teran et al. (41) reported that RANTES is a major eosinophil attractant in BAL fluid of asthmatic patients exposed to an allergen challenge, and that the concentration of RANTES strongly correlated with eosinophil count. Also, a previous report showed that RANTES-positive sputum eosinophils and the percentage of FEV₁ after allergen challenge were significantly correlated in asthmatic patients, which is compatible with our results (42). Eosinophils cause tissue damage and promote allergic disease in the lung through the release of toxic proteases, lipid mediators, cytokines, and oxygen free radicals (43).

In the context of the development of persistent inflammation that underlies asthma-related physiological dysfunction, we postulate that because eosinophils recruited by RANTES are a major source of the reactive oxygen species released during the inflammatory process, chronically

Table 3. Correlation between serum RANTES, serum MCP-1 and other parameters in asthmatic patients

	FEV ₁ (% predicted)	Absolute eosinophil counts (cells/mm ³)	Total serum IgE (IU/mL)	Serum MCP-1 (pg/mL)
Serum RANTES (pg/mL)	r = -0.385 p < 0.01	r = 0.611 p < 0.001	r = 0.298 p < 0.05	r = 0.192 p > 0.05
Serum MCP-1 (pg/mL)	r = -0.214 p > 0.05	r = 0.221 p > 0.05	r = 0.127 p > 0.05	-

RANTES, regulated upon activation, normal T-cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; FEV₁, forced expiratory volume in one second.

p value < 0.05 was considered significant.

elevated levels of the chemokine RANTES may at least in part result in significant damage to the airway wall necessitating airway remodeling. Thus, RANTES may be important in determining the severity of inflammation leading to airway remodeling and bronchial response to inhaled allergen. These findings may be exploited clinically as RANTES antagonists could be of considerable therapeutic efficacy in modifying the underlying inflammation present in these patients.

In addition to targeting the eosinophil, it has been demonstrated that RANTES rapidly degranulates basophils and releases histamine (44), selectively enhances B-cell IgE production (45), and also participates in T-lymphocyte chemotaxis and activation (46). Therefore, RANTES may be involved in inflammatory cell recruitment and the induction of bronchoconstrictive mediators from cells, resulting in airflow limitation. These RANTES characteristics further support the proposed role in asthma pathogenesis.

Considering these observations, many studies have also made an interesting link between RANTES polymorphisms and asthma, showing that the variant in the promoter region is associated with a high risk of asthma and severe airway obstruction (47,48). All of these results indicate that RANTES is an attractive gene candidate for the genetic dissection of asthma.

As regards MCP-1 in our study, there was an increase in MCP-1 serum levels in all asthmatic patients as compared to the controls, whereas there was no significant difference among the different grades of asthma severity. It is possible that MCP-1 is secreted by alveolar macrophages when they are activated by toxic particles and attracts monocytes that can differentiate into macrophages, playing a key role in monocyte migration. Therefore, an increased serum MCP-1 may contribute to an increase in macrophages, increased monocyte differentiation, and more severe airway inflammation as in patients with COPD (49).

Our results are in accordance to the findings of Chan et al. (50) who reported that serum MCP-1 levels were significantly higher during the asymptomatic phase of asthma than that of normal controls and were significantly higher during acute asthma attacks, suggesting a possible role of MCP-1 in the pathogenesis of asthma. Furthermore, increased MCP-1 expression has been demonstrated in the bronchial epithelium of asthmatic patients (51), with marked elevation of sputum MCP-1 levels preceding exacerbation of acute asthma attacks in comparison to the asymptomatic state (52).

By investigating BAL fluid, Alam et al. (14) observed that MCP-1 levels were significantly higher in asthma patients than in control subjects. Moreover, it has been demonstrated that the levels of MCP-1 were increased in BAL fluid of ventilated subjects with status asthmaticus compared to mild to moderate asthmatics (38), suggesting that this CC chemokine may be an important factor in life-threatening asthma attacks. On the contrary, the

findings of Holgate et al. (53) and Folkard et al. (54) failed to support the findings of Alam et al. that this chemokine was increased in BAL fluid from patients with stable asthma.

Interestingly, Szalai et al. (55) found an association between the MCP-1 -2518G allele and asthma susceptibility and severity. Although these results are encouraging, reproducibility is critical in gene association studies and confirmatory data from different ethnic populations are needed.

Together, chemokines have now emerged as potentially critical cytokines involved in the pathogenesis of asthma. This has provided the rationale for increased scientific investigation in the field and for the development of new therapeutic strategies targeting chemokine and/or chemokine receptor pathways involved in this socioeconomically important disease.

In conclusion, serum RANTES may be used as a useful noninvasive marker of airway obstruction and as a potential diagnostic tool for monitoring asthma severity. In this regard, identification and blocking of this chemokine and/or its receptor may be a promising therapeutic approach to asthmatic patients. However, future well-designed large studies are required to elucidate its therapeutic role in asthma.

References

- Maddox L, Schwartz DA. The pathophysiology of asthma. *Annu Rev Med* 2002;53:477–498.
- Sengler C, Lau S, Wahn U, et al. Interactions between genes and environmental factors in asthma and atopy: new developments. *Respir Res* 2002;3:7.
- Luster AD. Chemokines: chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–445.
- Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000;12:121–127.
- Baggiolini M, Dahinden CA. CC chemokines in allergic inflammation. *Immunol Today* 1994;15:127–133.
- Teran LM, Davies DE. The chemokines: their potential role in allergic inflammation. *Clin Exp Allergy* 1996;26:1005–1019.
- Alam R. Chemokines in allergic inflammation. *J Allergy Clin Immunol* 1997;99:273–277.
- Homey B, Zlotnik A. Chemokines in allergy. *Curr Opin Immunol* 1999;11:626–634.
- Teran LM. CCL chemokines and asthma. *Immunol Today* 2000;21:235–242.
- Bisset LR, Schmid-Grendelmeier P. Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. *Curr Opin Pulm Med* 2005;11:35–42.
- Alam R, Stafford S, Forsythe P, et al. RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* 1993;150:3442–3447.
- Schall TJ, Bacon K, Toy KJ, et al. Selective attraction of monocytes and T-lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 1990;347:669–671.
- Sim TC, Reece LM, Hilsmeier KA, et al. Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995;152:927–933.

14. Alam R, York J, Boyars M, et al. Increased MCP-1, RANTES and MIP-1 alpha in bronchoalveolar lavage fluid of allergic asthmatic patients. *Am J Respir Crit Care Med* 1996;153:1398–1404.
15. Zietkowski Z, Tomasiak MM, Skiepo R, et al. RANTES in exhaled breath condensate of stable and unstable asthma patients. *Respir Med* 2008;102:1198–1202.
16. Lukacs NW, Strieter RM, Warmington K, et al. Differential recruitment of leukocyte populations and alteration of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. *J Immunol* 1997;158:4398–4404.
17. Lachheb J, Chelbi H, Hamzaoui K, et al. Association between RANTES polymorphisms and asthma severity among Tunisian children. *Hum Immunol* 2007;68:675–680.
- EQ2 18. Fryer AA, Spiteri MA, Bianco A, et al. The -403 G → A promoter polymorphism in the RANTES gene is associated with atopy and asthma. *Genes Immun* 2000;1:509–514.
19. Muro M, Marin L, Torio A, et al. CCL5/RANTES chemokine gene promoter polymorphisms are not associated with atopic and nonatopic asthma in a Spanish population. *Int J Immunogenet* 2008;35:19–23.
20. Zhang YG, Huang J, Zhang J, et al. RANTES gene polymorphisms and asthma risk: a meta-analysis. *Arch Med Res* 2010;41:50–58.
21. Campbell EM, Charo IF, Kunkel SL, et al. Monocyte chemoattractant protein-1 mediates cockroach allergen-induced bronchial hyperreactivity in normal but not CCR2-/- mice: the role of mast cells. *J Immunol* 1999;163:2160–2167.
22. Gonzalo JA, Lloyd CM, Wen D, et al. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J Exp Med* 1998;188:157–167.
23. Smith DL, Deshazo RD. Bronchial lavage in asthma. An update and perspective. *Am Rev Respir Dis* 1993;148:523–532.
24. Pin I, Gibson PG, Kolendowicz R. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992;47:25–29.
25. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225–244.
26. National Institutes for Health and National Heart, Lung, and Blood Institute. Global Initiative for Asthma. Global strategy for asthma management and prevention. Bethesda, MD: National Institutes of Health; 2002. NIH publication 02–3659.
27. Brown WG, Halonen MJ, Kaltenborn WT, et al. The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 1979;63:328–335.
28. Locati M, Bonecchi R, Corsi MM. Chemokines and their receptors: roles in specific clinical conditions and measurement in the clinical laboratory. *Am J Clin Pathol* 2005;(123 Suppl):S82–95.
29. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–1136.
30. Garcia G, Adler M, Humber M. Difficult asthma. *Allergy* 2003;58:114–121.
31. Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999;54:825–857.
32. Barnes PJ. Cytokine modulators for allergic diseases. *Curr Opin Allergy Clin Immunol* 2001;1:555–560.
33. Lun SW, Wong CK, Ko FW, et al. Aberrant expression of CC and CXC chemokines and their receptors in patients with asthma. *J Clin Immunol* 2006;26:145–152.
34. Chihara J, Yasuba H, Tsuda A, et al. Elevation of the plasma level of RANTES during asthma attacks. *J Allergy Clin Immunol* 1997;100:S52–S55.
35. Ying S, Meng Q, Zeibecoglou K, et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999;163:6321–6329.
36. Berkman N, Krishnan VL, Gilbey T, et al. Expression of RANTES mRNA and protein in airways of patients with mild asthma. *Am J Respir Crit Care Med* 1996;154:1804–1811.
37. Powell N, Humbert M, Durham SR, et al. Increased expression of mRNA encoding RANTES and MCP-3 in the bronchial mucosa in atopic asthma. *Eur Respir J* 1996;9:2454–2460.
38. Tillie-Leblond I, Hammad H, Desurmont S, et al. CC chemokines and interleukin-5 in bronchial lavage fluid from patients with status asthmaticus. Potential implication in eosinophil recruitment. *Am J Respir Crit Care Med* 2000;162:586–592.
39. Erten G, Musellim B, Tutluoglu B, et al. Peripheral blood lymphocyte activation and RANTES levels in asthma. *Turk J Med Sci* 2008;38:531–536.
40. John M, Oltmanns U, Binder C, et al. Inhibition of chemokine production from human airway smooth muscle cells by fluticasone, budesonide and beclomethasone. *Pulm Pharmacol Ther* 2004;17:41–47.
41. Teran LM, Noso N, Carroll M, et al. Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. *J Immunol* 1996;157:1806–1812.
42. Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640–647.
43. Boyce JA. The pathobiology of eosinophilic inflammation. *Allergy Asthma Proc* 1997;18:293–300.
44. Kuna P, Reddigari SR, Schall TJ, et al. RANTES, a monocyte and T lymphocyte chemotactic cytokine releases histamine from human basophils. *J Immunol* 1992;149:636–642.
45. Kimata BH, Yoshida A, Ishioka C, et al. Furusho K. RANTES and macrophage inflammatory protein 1 α selectively enhance immunoglobulin (IgE) and IgG4 production by human B cells. *J Exp Med* 1996;183:2397–2402.
46. Bacon KB, Premack BA, Gardner P, et al. Activation of dual T cell signaling pathways by the chemokine RANTES. *Science* 1995;269:1727–1729.
47. Yao TC, Kuo ML, See LC, et al. The RANTES promoter polymorphism: a genetic risk factor for near-fatal asthma in Chinese children. *J Allergy Clin Immunol* 2003;111:1285–1292.
48. Sohn MH, Kim SH, Kim KW, et al. RANTES gene promoter polymorphisms are associated with bronchial hyperresponsiveness in Korean children with asthma. *Lung* 2008;186:37–43.
49. de Boer WI, Sont JK, van Schadowijk A, et al. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in COPD. *J Pathol* 2000;190:619–626.
50. Chan CK, Kuo ML, Yeh KW, et al. Sequential evaluation of serum monocyte chemotactic protein 1 among asymptomatic state and acute exacerbation and remission of asthma in children. *J Asthma* 2009;46:225–228.
51. Sousa AR, Lane SJ, Nakhosteen JA, et al. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;10:142–147.
52. Kurashima K, Mukaida N, Fujimura M, et al. Increase of chemokine levels in sputum precedes exacerbation of acute asthma attacks. *J Leukoc Biol* 1996;59:313–316.
53. Holgate ST, Bodey KS, Janezic A, et al. Release of RANTES, MIP-1 α , and MCP-1 into asthmatic airways following endobronchial allergen challenge. *Am J Respir Crit Care Med* 1997;156:1377–1383.
54. Folkard SG, Westwick J, Millar AB. Production of interleukin-8, RANTES and MCP-1 in intrinsic and extrinsic asthmatics. *Eur Respir J* 1997;10:2097–2104.
55. Szalai C, Kozma GT, Nagy A, et al. Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity. *J Allergy Clin Immunol* 2001;108:375–381.