

Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity

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Background: Chemokines play an important role in the pathophysiology of asthma and allergy. Recently, polymorphisms in the gene regulatory region of monocyte chemoattractant protein 1 (MCP-1) and in the promoter region of RANTES have been found; these polymorphisms increase the expression of the chemokines.

Objective: We investigated whether the presence of the polymorphisms was associated with atopy or asthma and whether these alleles influenced the severity of asthma in affected individuals.

Methods: Three groups of subjects—160 children with asthma (disease severity being classified according to the Global Initiative for Asthma guidelines, modified for children), 151 children with nonasthmatic but allergic phenotype, and 303 children without allergic or asthmatic disorders—were screened with a PCR-based assay for genotyping.

Results: The frequency of the -2518G polymorphism in the gene regulatory region of MCP-1 was significantly higher in asthmatic children than in controls ($P < .001$; odds ratio [OR] = 2.0 [1.4-2.6]) and nonasthmatic atopic children ($P < .001$; OR = 2.0 [1.4-2.9]). The MCP-1 G/G genotype correlated with asthma severity. In asthmatic children, the MCP-1 -2518G allele was also associated with an increased blood eosinophil level. The promoter polymorphisms in the RANTES gene did not have a detectable effect on the susceptibility to asthma or allergy or on the blood eosinophil count.

Conclusion: In this cohort of children, there are associations between carrying G at -2518 of the MCP-1 gene regulatory region and the presence of asthma as well as between asthma severity and homozygosity for the G allele. In asthmatic children, the MCP-1 -2518G polymorphism correlated with increased eosinophil levels. This variant of MCP-1 might

belong to the predictor gene set for asthma. (*J Allergy Clin Immunol* 2001;108:375-81.)

Key words: Asthma, atopy, polymorphism, chemokines, MCP-1, RANTES, eosinophils

Asthma is a pulmonary disease characterized by increased bronchial responsiveness to a variety of stimuli. It is the most common chronic disease of childhood and the most frequent reason for pediatric hospital admission, and its incidence is on the rise.¹ Previous studies have suggested that asthma is a multifactorial disease influenced by genetic and environmental factors.²⁻⁴

Chemotactic cytokines, or *chemokines*, are small signaling proteins that are deeply involved in the physiology and pathophysiology of acute and chronic inflammatory processes; they attract and stimulate specific subsets of leukocytes.⁵ There have been identified in human asthma a number of chemokines whose production appears to be related to the severity of asthmatic inflammation and reactive airway responses.^{6,7}

Monocyte chemoattractant protein 1 (MCP-1) might 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.⁸ Neutralization of MCP-1 drastically reduces bronchial hyperactivity, lymphocyte-derived inflammatory mediators, and T-cell and eosinophil recruitment to the lung.⁹

Recently, a biallelic A/G polymorphism in the MCP-1 distal gene regulatory region at position -2518 (numbers indicate nucleotide positions relative to the major transcription start sites) has been found that affects the level of MCP-1 expression in response to an inflammatory stimulus.¹⁰ Monocytes from individuals carrying a G allele at -2518 produce more MCP-1 after treatment with IL-1 β than do monocytes from A/A homozygous subjects. The effect of the G allele appears to be dose-dependent, inasmuch as cells from individuals homozygous for G at -2518 produced more MCP-1 than cells from G/A heterozygotes.¹⁰

RANTES is one of the most extensively studied chemokines in allergic and infectious diseases. RANTES is likely to be important in airway inflammation because blocking antibodies to RANTES inhibit airway inflammation in a murine model of allergic airway disease.¹¹

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Abbreviations used

GINA: Global Initiative for Asthma
 HWE: Hardy-Weinberg equilibrium
 MCP-1: Monocyte chemoattractant protein 1
 PEF: Peak expiratory flow

Furthermore, eosinophil chemotactic activity that appears in the bronchoalveolar lavage fluid of patients with asthma after allergen challenge was found to be due to RANTES.¹²

Two polymorphisms in the RANTES promoter region (–28 C/G and –403 G/A) have been found to affect the transcription of the RANTES gene.^{13,14} In human cell lines, the –28G and the –403A were shown to increase promoter activity of RANTES in comparison with the more frequent –28C and –403G, respectively, suggesting that these polymorphisms increase RANTES expression in the human body.^{13,14}

Given the importance of MCP-1 and RANTES in allergic inflammation, we hypothesized that polymorphisms that influence the levels of these chemokines might play a role in the susceptibility to asthma or allergy, and we determined the prevalence of the MCP-1 –2518G and RANTES –28G and –403A polymorphisms in 3 groups of Hungarian children. The first group consisted of children with asthma; the second, of children with a nonasthmatic but atopic phenotype with various clinical signs of severe allergy; the third, of children without allergic or asthmatic disorders. We investigated whether the presence of the polymorphisms was associated with atopy or asthma and whether the allele influenced the severity of asthma in affected individuals.

METHODS

Subjects

In all, 160 asthmatic subjects 3 to 18 years of age, 151 nonasthmatic allergic subjects 1 to 18 years of age (mean, 5.6 years [SD, 3.8]), and 303 subjects of comparable age without atopic or allergic disorders were enrolled in the study. All of the children were from Budapest.

The subjects with asthma attended the Allergic Outpatient Consultation of the Budai Children's Hospital. All of these asthmatic children had specialist physician–diagnosed asthma with the following characteristics: (1) recurrent breathlessness and expiratory dyspnea requiring treatment; (2) physician-diagnosed wheeze; and (3) reversibility of the wheezing and dyspnea by bronchodilator treatment (measured as FEV₁ by means of a spirometer [Piston]).^{15,16} The asthmatic children (or their parents) were instructed to record accurately for 2 weeks their symptoms, treatment, and, twice daily (in the evening and in the morning), peak expiratory flow (PEF). PEF 100% was determined by calculation from the personal best value and the expected value according to the height of the subject.¹⁷

If a child is less than 5 years old, the determination of lung function tests (PEF or FEV₁) is usually not possible. In such cases, the diagnosis and classification of the disease were made according to the frequency and severity of other symptoms.

The treatment of the asthmatic subjects remained unchanged before the blood was drawn. None of these children had experienced an exacerbation or a respiratory infection, as indicated by increased symptoms, for at least 4 weeks. There were no differences in PEF between days 1 and 14 of the period during which disease activity was assessed.

We used the recommendations for classification of asthma severity of the Global Initiative for Asthma (GINA) guidelines (National Heart, Lung, and Blood Institute/World Health Organisation Workshop on the Global Strategy for Asthma)¹⁵ and modified them for children. We classified our asthmatic subjects into 5 categories (rather than 4), as follows: intermittent (1), seasonal (1.5), mild (2), moderate (3), and severe persistent (4) asthma. We created the seasonal asthma category to include patients who show symptoms characteristic of mild asthma in the allergic period while showing symptoms characteristic of the intermittent category during the rest of the year. Our classification of asthma severity is presented in Table I; some characteristics of the asthmatic subjects are presented in Table II.

The nonasthmatic allergic subjects attended the Allergic Outpatient Consultation of Heim Pál Pediatric Hospital. All of these children had specialist physician–diagnosed allergy with the following criteria: (1) clinical signs of severe allergy, (including allergic rhinitis, atopic dermatitis, food allergy, and urticaria) and (2) atopy (see below).

The control children were randomly selected from patients in the Heim Pál Pediatric Hospital who met both of the following criteria: (1) no symptoms or history of allergic diseases and (2) total serum IgE levels below the general population means for their ages.

Informed parental consent was obtained for each subject, and the study was approved by an institutional review committee.

Laboratory analysis

Total genomic DNA was extracted from white blood cells through use of the method of Miller et al.¹⁸ The MCP-1 –2518 A/G, RANTES –28 C/G, and –403 G/A polymorphisms were determined as described previously.¹⁹

Total serum IgE levels and specific IgE levels to more than 100 allergens were determined by means of the Pharmacia CAP System. IgE levels were defined as high when total serum IgE exceeded 100 kU/L; specific IgE was considered positive in those having detectable allergen-specific IgE (>0.35 kU/L). Atopy was defined as a skin prick test result positive for at least 1 allergen (wheal diameter 3 mm greater than saline control) and/or positive total or specific IgE levels.

The white blood cell counts and the eosinophil cell counts were measured by means of a Coulter MAXM Analyser.

Statistical methods

Allele frequencies were calculated by allele counting. Data were analyzed through use of MedCalc and Arlequin software. Hardy-Weinberg equilibrium was determined through use of a χ^2 goodness-of-fit test. The Fisher exact test was used to detect differences in allele distribution between the groups. Exact-test-of-population differentiation was used to determine whether the genotypic composition of the groups was significantly heterogeneous. CIs were calculated at the 95% level. ANOVA was used to estimate the impact of the polymorphism on the quantitative traits. Two-tailed *P* values less than .05 were considered statistically significant.

RESULTS

Table III shows the prevalence of the polymorphisms in the 3 groups of subjects. Exact-test-of-population differentiation indicated that the distributions of genotype frequencies at –2518 in the gene regulatory region of the MCP-1 were significantly different among the subject groups (*P* < .001). The differences were significant in the frequencies of the –2518G allele between asthmatic subjects and controls (*P* < .001; odds ratio [OR] = 2.0 [95% CI, 1.4–2.6]) and between asthmatic subjects and nonasthmatic atopic subjects (*P* < .001; OR = 2.0 [1.4–2.9]).

TABLE I. Classification* of asthma severity according to the modified recommendations of GINA

Severity (grade)	Symptoms	Nocturnal asthma	PEF or FEV ₁ (variability†)
Intermittent (1)	<1-2 per week	<2 per month	>80% (<20%)
Seasonal asthma (1.5)	MP/I	MP/I	MP/I
Mild persistent (2)	1-2 per week but <1 per day	1-2 per month	60% to 80% (20% to 30%)
Moderate persistent (3)	> 1-2 per week	> 1-2 per week	60% to 80% (>30%)
Severe persistent (4)	Continuous	Frequent	≤60% (>30%)

MP/I, Like mild persistent asthma in the allergic period and like intermittent asthma in the nonallergic period.

*Meeting 1 criteria is enough for classification.

†Ratio of PEF or FEV₁ value measured in the morning to PEF or FEV₁ value measured in the evening (%).

TABLE II. Characteristics of asthmatic subjects according to MCP-1 –2518 genotype

Clinical or biological characteristic	All asthmatic subjects	MCP-1 –2518 genotype		
Genotype		A/A	A/G	G/G
Number	160	68	74	18
Age (y) (range, 3-18)	10.1 ± 3.5	9.8 ± 3.1	8.7 ± 4.5	13.3 ± 1.8
Age at first diagnosis (y) (range, 0.5-9)	3.5 ± 2.2	3.7 ± 2.3	3.4 ± 2.7	3.6 ± 1.5
Sex: male/female	90/70	34/34	46/28	10/8
Atopy: no. (%)	125 (78.1)	52 (76.5)	58 (78.4)	15 (83.3)
Total IgE (kU/L) (range, 9-1001)	266 ± 231	243 ± 227	278 ± 256	301 ± 228
White blood cell count (10 ⁶ /mL)	6.9 ± 1.5	6.7 ± 1.5	7.1 ± 1.7	7.2 ± 1.0
Eosinophil (%)	5.4 ± 3.1	3.9 ± 2.2*	5.9 ± 3.7*	9.3 ± 4.8*
Eosinophil cell count (10 ⁶ /mL)	0.35 ± 0.24	0.27 ± 0.20†	0.36 ± 0.27†	0.64 ± 0.26†

* $P < .001$ for AA vs AG; $P < .001$ for AA vs GG; $P = .001$ for AG vs GG.

† $P = .03$ for AA vs AG; $P < .001$ for AA vs GG; $P < .001$ for AG vs GG.

TABLE III. Genotype and allele frequencies for MCP-1 –2518, RANTES –28, and –403 in the 3 groups of children

Polymorphism 1/2*	Population	Genotype 1/1*	Genotype 1/2*	Genotype 2/2*	Total	Allele 2 frequencies
MCP-1						
–2518 A/G						
	Asthmatic	68 (42.5%)	74 (46.3%)	18 (11.3%)	160	34.4%‡
	Allergic†	100 (66.2%)	39 (25.8%)	12 (8.0%)	151	20.9%‡
	Control	194 (64.0%)	90 (29.7%)	19 (6.3%)	303	21.1%‡
RANTES						
–28 C/G						
	Asthmatic	144 (90%)	16 (10%)	0 (0%)	160	5.0%
	Allergic†	136(90.1%)	14(9.3%)	1 (0.7%)	151	5.3%
	Control	284 (93.7%)	19 (6.3%)	0 (0%)	303	3.1%
RANTES						
–403 G/A						
	Asthmatic	122 (76.3%)	32 (20.0%)	6 (3.8%)	160	13.8%
	Allergic†	100 (66.2%)	47 (31.1%)	4 (2.7%)	151	18.2%
	Control	211 (69.6%)	84 (27.7%)	8 (2.6%)	303	16.5%

*1 = frequent allele; 2 = rare allele.

†Nonasthmatic allergic children

‡ $P < .001$ and OR = 2.0 (1.4-2.9) for asthmatic vs nonasthmatic allergic children; $P < .001$ and OR = 2.0 (1.4-2.6) for asthmatic vs control children. In nonasthmatic allergic children, there was a deviation from Hardy-Weinberg equilibrium: $\chi^2 = 7.15$; $P < .01$.

To investigate whether there is difference in susceptibility to asthma between children homozygous and children heterozygous for the MCP-1 –2518G relative to controls, we compared the prevalence in these genotypes with the prevalence in the controls. In both genotypes, there were significant differences between the asthmatic and the control children. In the heterozygotes, the significance level of the difference was $P < .001$ and the OR was 2.4

(1.6-3.6) (46.3% and 29.7% in asthmatic and control children, respectively); in the homozygotes, the significance level of the difference was $P < .001$ and the OR was 2.7 (1.4-5.5) (11.3% vs 6.3%). The similarity of the ORs indicates that there is no difference in relative susceptibility to asthma between the heterozygous and homozygous carriers, suggesting a dominant mode of action of the MCP-1 –2518 polymorphism in the development of asthma.

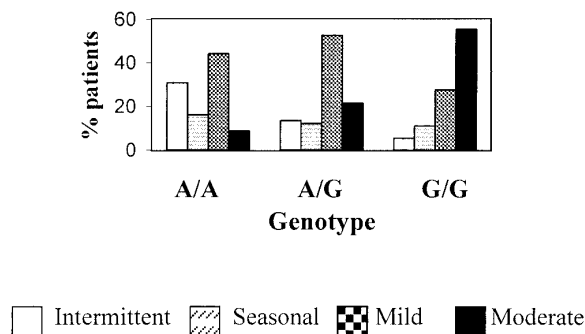


FIG 1. Distribution of asthmatic children with different severity scores according to their genotypes at -2518 of the MCP-1 gene regulatory region. Each column represents the percent of subjects having the given genotype.

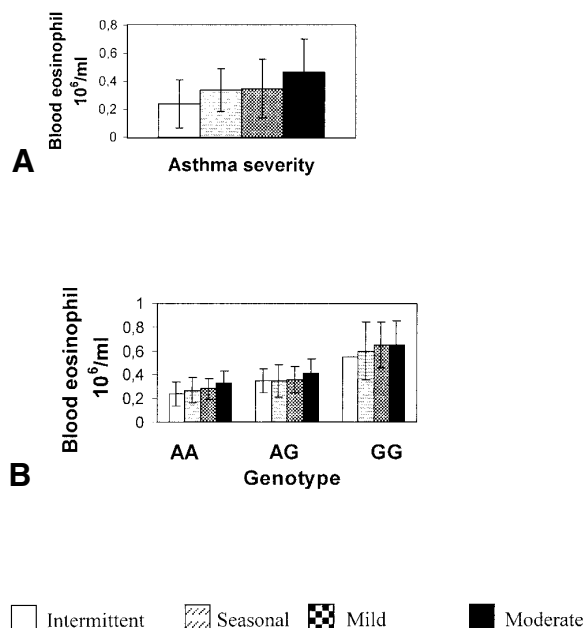


FIG 2. A, Means of blood eosinophil counts in the different asthma severity groups. There are significant differences between the intermittent and moderate asthma groups ($P = .003$). **B,** Means of blood eosinophil counts in the different asthma severity groups with a given genotype at -2518 of the MCP-1 gene regulatory region. The differences are significant ($P < .05$) between subjects with A/A vs G/G and A/G vs G/G in the seasonal, mild, and moderate groups. Numbers of subjects in the subgroups were as follows: intermittent: AA, 21; AG, 10; GG, 1; seasonal: AA, 11; AG, 9; GG, 2; mild: AA, 30; AG, 39; GG, 5; moderate: AA, 6; AG, 16; GG, 10.

No significant differences were observed in the genotype and allele frequencies of the RANTES promoter polymorphisms between the groups.

The overall results were in Hardy-Weinberg equilibrium with the exception of the MCP-1 genotype distribution in the nonasthmatic atopic subjects, in whom the χ^2 goodness-of-fit test revealed deviation from it ($\chi^2 = 7.15$; $P <$

.01). Analysis of the genotype distribution in this group revealed that the deviation from Hardy-Weinberg equilibrium was due mainly to a high number of the G/G homozygotes. However, comparing the frequencies of G/G homozygotes in the nonasthmatic allergic children and the controls by means of the Fisher exact test, we found no differences ($P = .6$; OR = 1.3 [0.6-2.7]). It is possible that larger population studies would be needed to explore the exact biological role of this polymorphism in allergy.

To determine the role of these polymorphisms in asthma, some clinical and biological characteristics of the asthmatic subjects were compared between the different genotypes. The results for the MCP-1 genotypes are presented in Table II. Of the 160 subjects with asthma, 125 (78.1%) were atopic and 35 (21.9%) were nonatopic. There were no significant differences either in the prevalence of the MCP-1 -2518G allele or in the genotype distributions between atopic and nonatopic asthmatic subjects (the allele frequencies were 35.2% and 31.4%, respectively). The frequency of the G allele was significantly higher in subjects with atopic asthma than in allergic but nonasthmatic subjects ($P < .001$; OR = 2.1 [1.4-3.0]). Although the frequency of the G allele was higher in nonatopic asthmatic subjects than in controls (31.4% vs 21.1%), the difference—because of the relative small number of nonatopic asthmatic children—was not statistically significant ($P < .07$; OR = 1.7 [1.0-2.9]). These data indicate an association between the MCP-1 -2518G allele and the asthmatic phenotype, whereas there is no association between this allele and the atopic phenotype.

There were significant differences in both the relative and the absolute eosinophil cell counts between the genotypes. The highest eosinophil counts were in the MCP-1 G/G homozygotes, the lowest in the A/A homozygotes. The values in the heterozygotes fell between those of the other 2 genotypes, indicating that in this respect the effect of the G allele was additive. To investigate the impact of this genotype on the eosinophil levels, we compared the eosinophil counts for the different MCP-1 -2518 genotypes in the nonasthmatic allergic group. In this group, a similar trend could be seen, but the differences were not statistically significant; the relative eosinophil counts were $4.9\% \pm 4.1\%$, $5.3\% \pm 4.0\%$, and $5.7\% \pm 3.7\%$ in subjects with the A/A, A/G, and G/G genotypes, respectively. The P values were as follows: $P = .6$ for A/A vs A/G; $P = .5$ for A/A vs G/G. The absolute eosinophil counts were as follows: subjects with the A/A genotype, $0.41 \pm 0.34 \times 10^6/\text{mL}$; subjects with the A/G genotype, $0.52 \pm 0.48 \times 10^6/\text{mL}$; subjects with the G/G genotype, $0.55 \pm 0.35 \times 10^6/\text{mL}$. The P values were as follows: $P = .1$ for A/A vs A/G; $P = .2$ for A/A vs G/G.

Mean patient age at the first diagnosis of asthma, total IgE concentration, and white blood cell count did not differ between the genotypes.

No clinical or laboratory characteristics were associated with a given RANTES genotype (data not shown).

The asthmatic subjects were classified into categories according to the severity of their asthma. Of the 160 asthmatic subjects, 32 (20%) had intermittent, 22 (13.8%) had seasonal, 74 (46.3%) had mild, and 32

(20%) had moderate asthma. There were no subjects with severe persistent asthma in this population. The subjects with different asthma severity scores were then separated by their genotypes at the MCP-1 -2518 locus (Fig 1). The genotype distributions were different among the severity groups ($P < .001$). The prevalence of the G allele increased in parallel with the severity scores: 18.8%, 29.5%, 33.1%, and 56.3% in the intermittent (1), seasonal (1.5), mild (2), and moderate (3) groups, respectively. The differences were significant between 1 and 2 ($P < .05$); 1 and 3 ($P < .001$), between 1.5 and 3 ($P = .01$), and between 2 and 3 ($P = .002$).

To assess the effect of the MCP-1 -2518 genotypes on the severity of asthma, we compared the prevalence of the children with intermittent and moderate asthma in the different genotype groups. In the group homozygous for the G allele, 55.6% (10/18) had moderate asthma and only 5.6% (1/18) had intermittent asthma ($P = .003$); in the group homozygous for the A allele, 30.9% (21/68) had intermittent asthma and 8.8% (6/68) had moderate asthma ($P = .002$); in the group with the A/G genotype, there was no significant difference between the prevalence of children with intermittent (13.5%; 10/74) and the prevalence of children with moderate (21.6%; 16/74) asthma. These findings suggest a significant association between asthma severity and homozygosity for the MCP-1 -2518G allele, whereas the lack of the G allele seems to be beneficial with respect to the symptoms of asthma.

According to these results, the MCP-1 -2518G allele influences both the blood eosinophil count and the asthma severity. To investigate whether there was a connection between blood eosinophil counts and asthma severity in our asthmatic patients, we compared the different severity groups with respect to blood eosinophil levels. As can be seen in Fig 2, A, the eosinophil cell counts increased in parallel with the severity of the asthma. The difference was statistically significant between the intermittent and the moderate asthma ($P = .003$). To determine whether increased eosinophil count is associated with severity of asthma or with the genotype, eosinophil count and genotype were compared within the same severity groups (Fig 2, B). In each severity group, the mean eosinophil count was lowest in subjects with the A/A genotype and highest in subjects with the G/G genotype. The mean eosinophil count also increased with the severity within a genotype group. It must be noted, however, that because of the small numbers of children in the subgroups, significant differences in the mean eosinophil counts ($P < .05$) were found only between those with A/A vs G/G and A/G vs G/G genotypes in the seasonal, mild, and moderate groups. The differences were not significant between the severity groups within a genotype.

DISCUSSION

Family and twin studies have demonstrated an involvement of genetic components in the development of atopy and asthma, and much work has been done to find the responsible genes.^{20,21} Several loci linked to

atopy and asthma have been suggested through genome-wide linkage studies.³ The present study is the first to implicate a chemokine gene variant in susceptibility to and symptoms of asthma. Analysis of polymorphisms in the chemokine system indicated that children carrying G at -2518 of the MCP-1 gene regulatory region were at increased risk for asthma. The current findings also demonstrate that the presence of the G allele correlates with asthma severity and blood eosinophil count in asthmatic children and thus acts as a disease modifier. However, according to this study, the MCP-1 G allele is not associated with atopy in general, inasmuch as the prevalence of this polymorphism in nonasthmatic but atopic children did not differ from that in a control population; moreover, the prevalence was significantly lower than that in asthmatic children. Furthermore, as in several other studies,^{22,23} the circulating level of eosinophils also correlated with the asthma severity in our group of asthmatic subjects, which is proof of the important role of eosinophils in asthma.

Recent years have witnessed a great deal of interest in the role that some chemokines might play in the initiation and perpetuation of asthma and allergic inflammation by facilitating selective leukocyte recruitment to inflammatory sites—regulating IgE synthesis, promoting T_H2-type cytokine responses, and directly activating leukocytes to release inflammatory mediators.²⁴⁻²⁶ Other chemokines might have a beneficial role in nonatopic subjects by reinforcing the maintenance of a T_H1-type cytokine-dominated response commonly associated with clinical unresponsiveness to allergens.²⁷

MCP-1 was found to mediate airway hyperreactivity in normal and allergic mice and directly induced pulmonary mast cell degranulation *in vitro*.⁸ Several human studies have demonstrated that MCP-1 is upregulated during asthmatic responses.^{28,29} The expression of MCP-1 protein in bronchial tissue from asthmatic subjects was significantly increased in comparison with that in nonasthmatic subjects, in that >50% and <8%, respectively, of the bronchial epithelial cells stained positive.²⁸

A few reports demonstrated considerable interindividual variability in MCP-1 production in response to stimuli such as cytokines.^{10,30} Human MCP-1 gene transcription appears to be under the control of 2 distinct areas of the 5'-flanking region of the gene.³¹ The distal regulatory region is located 1.8 through 2.7 kb upstream of the transcriptional start site. This segment contains 2 nuclear factor- κ B binding motifs essential for cytokine induction of MCP-1 expression.³¹ The -2518 A to G polymorphism was found to affect the transcriptional activity of this region and correlated with individual differences in monocyte MCP-1 production.¹⁰ Our findings that individuals carriers for the high MCP-1 producer G allele are at increased risk for asthma and that the presence of the G allele correlates with asthma severity are in good correlation with the findings that MCP-1 administration to normal mice induced a significant increase in airway resistance that was dose-dependent. This ability of MCP-1 to induce a hyperreactive response was associated with

increased levels of histamine. It has been suggested that MCP-1 can activate basophils to release histamine and induce leukotriene C₄-mediated pathways. Both mediators play important contributory roles in the exacerbation of asthmatic responses.^{8,23} Furthermore, inhibition of MCP-1 in a murine model of induced allergic airway inflammation attenuated the associated mononuclear cell infiltration and airway hyperreactivity.¹¹ Our finding that polymorphism in the regulatory region of the MCP-1 is associated with asthma but not with atopy is also in good correlation with the findings that there is a linkage between asthma and the chemokine cluster 17p11.1-q11.2 (the MCP-1 is located on chromosome 17q11.2) but that no such linkage exists between atopy and this chromosome region.^{3,4}

It is more difficult to explain that the MCP-1 2518G allele in asthmatics is associated with higher blood eosinophil counts, because MCP-1 does not directly induce the chemotaxis and release of eosinophils. However, the finding that neutralization of MCP-1 drastically reduced eosinophil recruitment to the lung suggests a role of MCP-1 in the eosinophil functions.⁹ The main receptor of MCP-1 is CCR2, which is not expressed by eosinophils isolated from the peripheral circulation. Yet eosinophils elicited and isolated from the peritoneum of sensitised mice had detectable surface expression of CCR2⁷; theoretically, therefore, MCP-1 could have a direct effect on eosinophils. MCP-1 can drive undifferentiated T-lymphocyte populations toward an IL-4-producing T_H2-type cell.³² IL-4 can upregulate eotaxin, which is the most potent chemoattractant for eosinophils.^{7,23,33} This suggests that MCP-1 also can act indirectly on eosinophils. Furthermore, T_H2 cells also produce IL-5, which is one of the main activators for eotaxin and also causes eosinophil release from the bone marrow.²² This could be responsible for the increased level of eosinophils in the circulation. However, given the complexity and robustness of the chemokine system, several other pathways could be hypothesized. Moreover, at present it cannot be ruled out that the effect of the MCP-1 -2518G allele is mediated through other, indirect mechanisms, such as alteration of the expression of other chemokines, or that it is simply a marker linked to some other nearby gene responsible.

It must be noted that in our nonasthmatic atopic subjects, a similar but not significant trend was detectable between the presence of the G allele and the eosinophil count, which could suggest that the effect of the MCP-1 -2518G allele was less pronounced in the nonasthmatic subjects. Given that the level of blood eosinophils is controlled by a series of complex processes, it cannot be stated that the higher eosinophil levels in individuals with asthma are due to the MCP-1 G/G phenotype, but it can be suggested that the phenotype might play a role in the eosinophil level elevation. Large population studies incorporating asthmatic and nonasthmatic individuals might explore the exact biological role of this polymorphism.

Our study failed to detect an association between polymorphisms within the promoter region of the RANTES

and asthma or atopy, and these polymorphisms had no detectable influence on the blood eosinophil level in either group and no detectable influence on other investigated parameters of asthma. The expression of RANTES is differentially regulated in various cell types, and a large number of putative cis-acting elements have been described in the promoter region.³⁴ According to our results, these polymorphisms in the promoter region of the RANTES gene do not influence significantly either the development of asthma or atopy or the eosinophil count. It can be assumed that these more inducible RANTES genes do not have a detectable effect on the investigated parameters or that, more simply, in those cells (eg, airway epithelial cells) that participate in the pathomechanism of these diseases, other cis-regulating elements are responsible for the expression of RANTES.

In conclusion, this is the first report illustrating important associations between a polymorphism in the chemokine system and susceptibility to and symptoms of asthma. According to the present findings in this cohort of children, there are associations between carrying G at -2518 of the MCP-1 gene regulatory region and the presence of asthma and between asthma severity and homozygosity for the G allele. In asthmatic children, the MCP-1 -2518G allele was also associated with increased eosinophil level. This means that this variant of MCP-1 might belong to the predictor gene set for asthma and could be used in genomic analysis. Furthermore, if these associations can be proved in other groups of patients, and if the higher level of MCP-1 is really responsible for these effects, development of therapeutic drugs for asthma specifically targeted against MCP-1 and/or its receptor, CCR2, might prove useful in the prevention of the disease and/or in the alleviation of its symptoms.

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