

Immunotherapy suppresses the production of monocyte chemotactic and activating factor and augments the production of IL-8 in children with asthma

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Background: Histamine-releasing factor consists of a group of cytokines that can cause basophils or mast cells to release histamine. However, the composition of histamine-releasing factor remains undefined.

Objective: This study was done to measure the concentrations, in plasma and mononuclear cell culture supernatants from children with asthma, of chemokines that are known to contribute to histamine-releasing factor activity.

Results: Plasma and mononuclear cell culture supernatants were obtained from 25 children newly diagnosed with asthma, 25 good responders to immunotherapy, 23 poor responders, 25 patients with acute attacks, and 13 normal subjects. All the patient groups produced, spontaneously and after stimulation with phytohemagglutinin and mite allergen, greater amounts of monocyte chemotactic and activating factor, macrophage inflammatory protein-1 α , and RANTES (β chemokines) and IL-8 and growth-related gene α (α chemokines) than did normal subjects. Successful immunotherapy resulted in decreased production, especially the spontaneous type, of β chemokines that cause histamine release and in increased production of α chemokines that inhibit histamine release.

Conclusion: Abnormal chemokine production may contribute to the pathogenesis of bronchial asthma, and restoration of normal chemokine production may be used to explain, in part, the clinical efficacy of immunotherapy. (*J Allergy Clin Immunol* 1996;98:580-7.)

Key words: HRF, MCAF, MIP-1 α , RANTES, IL-8, GRO α , mite, immunotherapy, asthma, children

The role of basophils and mast cells in allergic diseases has been appreciated for many years. Both the interaction of antigen with surface-bound IgE antibody and stimulation by a wide variety of endogenous and exogenous substances cause these cells to release a number of inflammatory mediators such as histamine, leukotrienes, and platelet-activating factor. These in turn act on specific target organs and induce various allergic symptoms.¹

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Abbreviations used

GRO:	Growth-related gene
HRF:	Histamine-releasing factor
MCAF:	Monocyte chemotactic and activating factor
MCP:	Monocyte chemoattractant protein
MIP:	Macrophage inflammatory protein
MNCs:	Mononuclear cells
PHA:	Phytohemagglutinin

T lymphocytes play a crucial role in the immediate-type allergic reaction in regard to the regulation of IgE production by B lymphocytes, as well as the induction of maturation and differentiation of basophils and mast cells.² It was Dvorak et al.³ who, in 1974, were the first to postulate that these lymphocytes might directly activate basophils and mast cells in the delayed-type hypersensitivity re-

action. Subsequently, Thuesen et al.⁴ reported the presence of a factor or factors in the supernatants of streptokinase-streptodornase-stimulated human mononuclear cells (MNCs) capable of causing basophils to release histamine and defined these elements as histamine-releasing factor (HRF) or activity. In addition, it was subsequently shown that T lymphocytes, B lymphocytes, pulmonary macrophages, neutrophils, platelets, and endothelial cells can also produce HRF.⁵ Such activity can also be found in the nasal washings and bronchoalveolar lavage fluids of patients with allergic asthma.⁵

A number of studies have been conducted to characterize HRF. However, until now all the results have clearly indicated that HRF activity in the culture supernatants of stimulated MNCs was the net result of interactions among a group of cytokines present in the supernatants. Examples include HRF, histamine release enhancing factor, and histamine release inhibitory factor, such as IL-8.^{6, 7}

Recently, a group of cell products, which possess both the characteristics of chemoattractant and cytokine activity, the so-called *chemokines*, has been studied extensively. There are now a total of 18 human chemokines that can be divided into two subfamilies (α or CXC and β or CC) on the basis of overall sequence homology, the disposition of the first two of four canonical cysteine residues, and the chromosomal location of the corresponding genes.^{8, 9} In human beings most α chemokines attract neutrophils but not monocytes, whereas β chemokines attract monocytes but not neutrophils.

A previous study¹⁰ showed that immunotherapy suppresses the production of allergen-stimulated HRF activity, particularly the type of spontaneously produced HRF activity in patients experiencing clinical benefits, and our own study further demonstrated that responsiveness of basophils to HRF in children with asthma experiencing clinical benefits also decreases.¹¹ Recently, several reports,^{12, 13} including our own,¹⁴ showed that monocyte chemotactic and activating factor (MCAF) is the chemokine possessing the strongest HRF activity and that RANTES,¹⁵ macrophage inflammatory protein-1 α (MIP-1 α),¹⁶⁻¹⁸ and monocyte chemotactic protein-1 (MCP-1)¹⁹ can also cause histamine release. Therefore in this study we tried to measure the concentrations of various chemokines in plasma and MNC culture supernatants from children with asthma, hoping to clarify the nature of HRF activity in the MNC supernatants on the one hand and explore the role of chemo-

kines in the pathogenesis of bronchial asthma and in the working mechanism of immunotherapy on the other hand.

METHODS

Study populations

Twenty-five children with acute asthmatic attacks, 25 untreated children with a new diagnosis of asthma, and 25 good responders and 23 poor responders to immunotherapy were enrolled in this study. Thirteen healthy, age-matched schoolchildren with negative skin test and RAST results were included as control subjects. The "newly diagnosed" group represented those patients who visited our clinic for the first time, although they had had asthma for a number of years, they had not received immunotherapy before.

This study has been approved by the human research committee of this hospital, and informed consent was obtained from all study subjects.

All the patients were sensitive only to mites (*Dermatophagoides pteronyssinus* and *D. farinae*) in terms of having a positive history of exposure (asthma attack during house cleaning), a skin test response greater than 2+ (wheal >10 mm, by intracutaneous route with mite extract at a concentration of 10⁻⁶ gm/ml), and a positive mite RAST result (CAP system; Pharmacia Diagnostics, Uppsala, Sweden). Unlike western countries, in Taiwan, pets, such as cats and dogs, are rarely kept in houses. More than 90% of children with asthma in Taiwan are sensitive to mites,²⁰⁻²² compared with 7.4% sensitive to cat, 8.2% sensitive to dog, 45.2% sensitive to cockroach, and 22.8% sensitive to ragweed. Furthermore, more than 90% of house dust samples contain Der p 1 levels of greater than 2 mg/gm dust,²³ the proposed critical level for an asthma attack.²⁴ Routinely, a panel of 18 inhaled allergens including mites, cat, dog, pollens, cockroach, and molds was used in RAST and/or CAP testing and skin testing.

The four patient groups were comparable regarding sex, age, duration of illness, severity of asthma, skin test results, total serum IgE, and mite RAST results (Table I). Immunotherapy was started with weekly injections of 10⁻⁶ gm/ml of *D. pteronyssinus*, and the dose was increased gradually until a maximal tolerated dose was reached (usually within 6 months) and then was maintained at 4-week intervals. All the patients had been receiving immunotherapy for more than 2 years (mean, 2.5 years). The total dose of crude mite allergen given ranged from 102.6 to 187.4 mg. The maintenance dose of mite allergen varied widely depending on the individual patient, but there was no difference in the total dose of crude mite allergen given between good (142.3 $\pm$ 48.5 mg) and poor (132.9 $\pm$ 42.7 mg) responders. No oral steroid was taken for at least 2 weeks, and oral bronchodilators were withheld for at least 24 hours before blood samples were taken. Blood samples from 25 children with acute attacks triggered by house cleaning were collected when they were seen in the outpatient or

TABLE I. Demographic characteristics of study populations

	Healthy children	Good responders	Poor responders	New patients	Acute attacks
Number	13	25	23	25	25
Sex (M/F)	8/5	18/7	15/8	16/9	17/8
Age (yr)	11.0 $\pm$ 2.8	10.8 $\pm$ 2.6	10.6 $\pm$ 2.9	11.2 $\pm$ 3.0	11.5 $\pm$ 2.5
Duration of illness (yr)		3.9 $\pm$ 2.0	3.6 $\pm$ 1.9	4.1 $\pm$ 2.0	3.7 $\pm$ 2.1
Severity of asthma (Aas score)		3.1 $\pm$ 0.6	3.3 $\pm$ 0.5	3.4 $\pm$ 0.6	3.0 $\pm$ 0.8
Skin testing (mm in diameter, Der p)		18.2 $\pm$ 2.2	17.6 $\pm$ 1.8	18.0 $\pm$ 1.9	18.5 $\pm$ 2.3
Total serum IgE (IU/ml)	78.5 $\pm$ 36.5	1249.6 $\pm$ 463.4	1218.6 $\pm$ 457.7	1326.3 $\pm$ 597.4	1186.9 $\pm$ 548.3
RAST score (Pharmacia CAP system)	0	>6	>6	>6	>6

emergency department just a few hours after an attack occurred. Some of the patients had been treated in our clinic, and the rest were transferred from other hospitals. Most of them had not been receiving immunotherapy.

Patients with fever and therefore possible infections were excluded from the study. Patients with acute asthma who were taking oral steroids were also excluded. The effectiveness of immunotherapy was judged by comparing, from patient records, the sum of the frequency of asthma and the amount of medications consumed (total doses of β_2 -agonists, theophylline, and steroids) in the year before immunotherapy with that in the second year after immunotherapy. The result was considered to be excellent if the decrease of both parameters was greater than 90%, much improved for a 75% to 90% decrease, improved for a 50% to 75% decrease, slightly improved for a 25% to 50% decrease, and poor for a decrease of 25% or less.²⁵ In this study good responders were those patients who showed an improvement greater than 75%, whereas poor responders were those who showed an improvement of less than 25%.

Preparation of plasma and peripheral blood MNC culture supernatants

Thirty milliliters of heparinized blood was drawn from each patient after consent was obtained from parents. MNCs were obtained by Ficoll-Hypaque (Pharmacia Diagnostics) density gradient centrifugation. HRFs were prepared by a method described previously.^{11, 26} MNCs were washed three times with Hanks' balanced salt solution and adjusted at a concentration of 2×10^6 cells/ml in complete culture medium (RPMI-1640 supplemented with 5% fetal calf serum, 100 mg/ml streptomycin, 100 U/ml penicillin, 2 mmol/L glutamine). MNCs were then stimulated with purified phytohemagglutinin (PHA) (2 mg/ml) or crude mite extract (10 mg/ml) for 4 hours, washed three times with Hanks' balanced salt solution, and then cultured for an additional 32 hours. The culture condition was found to be optimal for both PHA- and mite-stimulated HRF production in our pre-

vious study when time course was performed between 4 hours and 7 days.¹¹ The supernatants were obtained by centrifugation at 38,000 g for 20 minutes. Both supernatants and plasma were stored in aliquots at -20°C until they were tested.

Measurement of MCAF, MIP-1 α , and RANTES (β chemokines)

MCAF was measured by ELISA. One hundred milliliters of mouse monoclonal antibody anti-human MCAF (clone 24822.11; R&D Systems, Inc., Minneapolis, Minn.) at a concentration of 10 mg/ml was added to 96-well microtiter plates and kept at 4°C overnight. After washing, 100 μl of blocking solution was added and incubated at 37°C for 1 hour. The plates were washed again, and 100 μl of serum or supernatant was added and incubated at room temperature for 2 hours. After washing three times, 100 μl of rabbit anti-human MCAF polyclonal antibody (Code 2371-01; Genzyme, Cambridge, Mass.) at a concentration of 10 mg/ml in blocking solution was added and incubated for 1 hour at room temperature. After washing, 100 μl of biotin-anti-rabbit polyclonal antibody (1:1000 dilution, Sigma Chemical Co., St. Louis, Mo.) was added and incubated at room temperature for 1 hour. After washing, 100 μl of ExtrAvidin-peroxidase (1:5000 dilution, Sigma Chemical Co.) was added and incubated at room temperature for 1 hour. After washing, the color was developed by adding 100 μl of o-phenylenediamine dihydrochloride at a concentration of 2 mg/3 ml, and the reaction was stopped by 2N H_2SO_4 30 minutes later. The color was read at an OD450. Recombinant human MCAF standard was purchased from Pepro Tech Inc. (United States). The sensitivity of this method was 0.25 pg/ml. All the measurements were done in duplicate, and the intraexperimental variance was 5% to 10%.

MIP-1 α and RANTES in plasma and supernatants were measured by ELISA test kits purchased from R&D Systems, Inc. Basically, the assay uses the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for the target protein was coated

TABLE II. Production of MCAF among various studied groups

Study group	Plasma	Spontaneous production	Stimulated with	
			PHA	Mite
Healthy children (<i>n</i> = 13)	2.0 ± 2.4	106.2 ± 53.7	11,476.5 ± 2,114.2	277.4 ± 155.6
Good responders (<i>n</i> = 25)	17.5 ± 20.2*	321.8 ± 386.7†	10,054.9 ± 7,022.9	3,479.7 ± 4,957.7
Poor responders (<i>n</i> = 23)	45.7 ± 28.2*	3,260.7 ± 4,331.1†	14,348.6 ± 5,234.0	4,081.0 ± 4,405.1
New patients (<i>n</i> = 25)	7.2 ± 8.3	788.8 ± 829.8	9,325.7 ± 6,173.5	3,612.9 ± 2,766.5
Patients with acute attacks (<i>n</i> = 25)	8.5 ± 7.4	1,333.6 ± 1,265.7	10,099.8 ± 6,204.7	3,350.0 ± 3,508.6

All values are expressed as means ± SD (in picograms per milliliter).

**p* < 0.05.

†*p* < 0.01.

TABLE III. Production of MIP-1α among various studied groups

Study group	Plasma	Spontaneous production	Stimulated with	
			PHA	Mite
Healthy children (<i>n</i> = 13)	0.49 ± 0.28	0.23 ± 0.29	49.9 ± 38.6	3.8 ± 5.1
Good responders (<i>n</i> = 25)	0.49 ± 0.20	6.40 ± 6.60*	46.3 ± 37.4	18.4 ± 14.6
Poor responders (<i>n</i> = 23)	0.46 ± 0.21	9.50 ± 7.80*	48.4 ± 36.3	20.0 ± 19.6†
New patients (<i>n</i> = 25)	0.45 ± 0.23	6.70 ± 7.80	39.7 ± 27.3	12.0 ± 12.1†
Patients with acute attacks (<i>n</i> = 25)	0.46 ± 0.27	7.10 ± 7.45	44.6 ± 28.2	16.9 ± 14.8

All values are expressed as means ± SD (picograms per milliliter).

**p* < 0.01.

†*p* < 0.05.

onto the microtiter plate. Standards and samples were pipetted into the wells, and any specific protein present was bound by the immobilized antibody. After washing away any unbound proteins, an enzyme-linked polyclonal antibody specific for the target protein was added to the wells to sandwich the protein immobilized during the first incubation. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and color was developed in proportion to the amount of protein bound in the initial step. The color development was stopped, and the intensity of the color was measured. The sensitivity was 2.0 pg/ml for MIP-1α and 2.5 pg/ml for RANTES.

Measurement of IL-8 and growth-related geneα (α chemokines)

IL-8 and growth-related geneα (GROα) in plasma and supernatants were also measured by ELISA test kits purchased from R&D Systems, Inc. The assay principle was the same as that for human RANTES. The sensitivity was 3.0 pg/ml for IL-8 and 5.0 pg/ml for GROα.

Statistics

Student's *t* test was used for statistical analysis throughout the study. A *p* value of less than 0.05 was considered significant.

RESULTS

The plasma concentrations of IL-8, GROα, RANTES, and MIP-1α in healthy individuals were within the ranges described in the manuals provided by the manufacturer, indicating that the performance of the measurements in this study was correct. In addition, the spontaneous production of all chemokines, except GROα, in all patient groups was greater than that of healthy individuals, although not all of the difference reached a significant level.

Table II shows the MCAF concentrations among various studied groups. The plasma and spontaneously secreted MCAF were highest in the poor responders, and both were markedly decreased in good responders (*p* < 0.05 for plasma and <0.01 for spontaneous production). There was no difference in PHA-stimulated secretion of MCAF among five studied groups; however, all patient groups secreted a significantly greater amount of MCAF after mite stimulation than did normal subjects.

Table III compares the production of MIP-1α among various groups. The spontaneous produc-

TABLE IV. Production of RANTES among various studied groups

Study group	Plasma	Spontaneous production	Stimulated with	
			PHA	Mite
Healthy children (<i>n</i> = 13)	18,070.1 ± 7,786.3	1,059.7 ± 282.9	5,797.2 ± 1,034.2	2,575.5 ± 2,393.0
Good responders (<i>n</i> = 25)	27,566.1 ± 11,621.3	1,530.2 ± 1,314.7*	5,523.2 ± 3,868.0	4,089.7 ± 2,556.8†
Poor responders (<i>n</i> = 23)	32,041.5 ± 12,518.0	5,820.4 ± 6,535.5*†	11,561.6 ± 8,350.5*	8,288.3 ± 9,713.4*†
New patients (<i>n</i> = 25)	23,065.9 ± 16,086.6	2,364.1 ± 2,702.3†	6,104.7 ± 4,591.4*	2,470.3 ± 2,619.0
Patients with acute attacks (<i>n</i> = 25)	26,744.9 ± 15,680.4	2,395.4 ± 1,386.6	11,267.6 ± 11,007.5	3,460.1 ± 2,549.7*

All values are expressed as means ± SD (picograms per milliliter).

**p* < .001.

†*p* < 0.05.

TABLE V. Production of IL-8 among various studied groups

Study group	Plasma	Spontaneous production	Stimulated with	
			PHA	Mite
Healthy children (<i>n</i> = 13)	U	1,461.5 ± 665.6*	26,002.6 ± 24,922.5*	5,002.9 ± 1,792.5*
Good responders (<i>n</i> = 25)	U	20,031.9 ± 6,239.2*†	20,850.7 ± 6,974.0†	22,552.6 ± 5,450.8*†
Poor responders (<i>n</i> = 23)	U	14,798.5 ± 7,174.3*	14,420.9 ± 7,200.2*†	18,511.3 ± 5,857.6*
New patients (<i>n</i> = 25)	U	15,470.3 ± 8,574.9†	20,026.8 ± 20,299.1	20,586.0 ± 7,018.2†
Patients with acute attacks (<i>n</i> = 25)	U	17,310.6 ± 7,760.8	18,000.4 ± 6,758.2	19,130.4 ± 5,466.7†

All values are expressed as mean ± SD (picograms per milliliter).

U, Undetectable.

**p* < 0.01.

†*p* < 0.05.

tion of MIP-1 α was highest in the poor responder group but was significant only when compared with good responders (*p* < 0.01). The mite-stimulated production of MIP-1 α was also highest in the poor responder group but was significant only when compared with new patients (*p* < 0.05). All groups of patients with asthma produced a much greater amount of MIP-1 α than did healthy children when stimulated with mite allergen. Again, there was no difference in PHA-stimulated production of MIP-1 α among the five groups studied.

Table IV shows the production of RANTES among various groups. The spontaneous production of RANTES by the group of poor responders was statistically significantly higher than that of the other four groups. The spontaneous production of RANTES by the group of good responders was decreased to a level that was comparable to that of the healthy group, but production of RANTES by the group of patients with acute attacks and the group of new patients was still higher than that of normal subjects (*p* < 0.05). The PHA- and mite-stimulated production of RANTES was also highest in the poor responders and was significantly

lower in good responders (*p* < 0.05) and the remaining three groups (*p* < 0.01), when compared with poor responders.

IL-8 production is shown in Table V. All the spontaneous (*p* < 0.01), PHA-stimulated (*p* < 0.05), and mite-stimulated (*p* < 0.01) secretions of IL-8 were much higher in good responders than in poor responders. Normal subjects produced much less IL-8 after mite stimulation when compared with all four groups of patients with asthma (*p* < 0.01), although the PHA-stimulated IL-8 production of normal subjects (*p* < 0.01) and good responders (*p* < 0.05) was higher than that of poor responders. It is important to note that although the spontaneous, PHA-stimulated, and mite-stimulated production of IL-8 were quite comparable for each group of patients with asthma, the mite-stimulated production of IL-8 of children with asthma was much higher than that of healthy children (*p* < 0.01), suggesting that patients with asthma specifically produce increased IL-8 in response to mite stimulation.

The production of GRO α is shown in Table VI. The spontaneous production was greatest in good

TABLE VI. Production of GRO α among various studied groups

Study group	Plasma	Spontaneous production	Stimulated with	
			PHA	Mite
Healthy children (<i>n</i> = 13)	98.4 $\pm$ 42.3	2,142.4 $\pm$ 1,847.4	5,678.6 $\pm$ 3,982.5	2,008.5 $\pm$ 1,436.6
Good responders (<i>n</i> = 25)	117.0 $\pm$ 52.4	3,286.2 $\pm$ 2,285.3*	7,051.1 $\pm$ 6,244.8*	6,455.2 $\pm$ 2,181.7†
Poor responders (<i>n</i> = 23)	102.3 $\pm$ 126.1	1,419.5 $\pm$ 1,925.3	1,852.1 $\pm$ 3,003.1*	5,552.4 $\pm$ 3,235.9
New patients (<i>n</i> = 25)	158.4 $\pm$ 280.6	1,814.6 $\pm$ 1,959.4*	6,579.4 $\pm$ 2,663.9	3,663.9 $\pm$ 2,752.7
Patients with acute attacks (<i>n</i> = 25)	141.4 $\pm$ 152.7	2,068.8 $\pm$ 1,803.9	5,907.4 $\pm$ 2,443.9	3,944.5 $\pm$ 2,879.9†

All values are expressed as means $\pm$ SD (picograms per milliliter).

**p* < 0.01.

†*p* < 0.05.

responders and was significantly higher than that of poor responders (*p* < 0.01) and new patients (*p* < 0.01). PHA stimulation resulted in the greatest production of GRO α in good responders and the lowest production in poor responders (*p* < 0.01). After stimulation with mite, good responders also produced the greatest amount of GRO α , which was significantly higher than that produced by new patients (*p* < 0.05) and patients with acute attacks (*p* < 0.05).

DISCUSSION

Since its recognition by Thuesen et al.⁴ in 1979, HRF has been considered to play a very important role in the pathogenesis of allergic diseases and may be one of the mediators responsible for the increased plasma histamine level in allergic patients. The evidence includes: (1) MNCs from patients with asthma produce HRF spontaneously in cultures, and even more after specific allergen stimulation²⁷; (2) basophils from atopic subjects tend to respond to HRF more vigorously than basophils from normal subjects^{11, 28}; (3) immunotherapy suppresses allergen-stimulated HRF activity and decreases spontaneous HRF activity in patients experiencing clinical benefits^{10, 11}; and (4) patients with atopic dermatitis and food allergy produce significantly less HRF after the offending food allergen is eliminated from the diet for an extended period.²⁹ However, extensive effort has failed to identify a major component in the HRF, which can be largely responsible for its biologic activity. Thus although it is now agreed that the measurement of HRF may be used as a parameter with which to evaluate the efficacy of immunotherapy, the determination of individual cytokine activity and its different molecular species in the supernatants, such as HRF, histamine-releasing inhibitory factor, and inhibitor of HRF synthesis,

will clarify the effect of immunotherapy on mediator production and therefore its working mechanisms.

MCAF/MCP-1 was first described in 1989.³⁰ Subsequent studies showed that MCAF could be released by a wide variety of cells after stimulation with inflammatory mediators including monocytes or macrophages, fibroblasts, B cells, endothelial cells, and smooth muscle cells.⁸ However, its histamine-releasing activity was only recognized recently.¹²⁻¹⁴ Molecular cloning and sequence analysis of MCAF has just been accomplished in recent years.^{8, 30} Our preliminary data showed that MCAF-dependent histamine release required the participation of G-proteins, protein kinase C/cyclic adenosine monophosphate, and Ca⁺⁺ ions (Hsieh et al. Unpublished data). RANTES was initially thought to be specifically produced by T cells³¹; however, subsequent studies showed that it could be produced by a variety of cells including thrombin-stimulated platelets, epithelial cells of renal tubules, mesangial cells, and antigen-specific CD4⁺ and CD8⁺ T cells.^{32, 33} The histamine-releasing activity of RANTES was only recently discovered.^{14, 15, 18, 34} MIP-1 α and MIP-1 β were discovered by Oppenheim et al.⁸ and Taub et al.³⁵ Although both species belong to the β chemokines, only MIP-1 α can activate basophils, eosinophils, neutrophils, and their progenitors.¹⁶ The most exciting findings in the study of chemokines include the histamine-releasing capability of those molecules and the cloning of their receptors; the former relates to their role in the pathogenesis of allergic diseases,³⁵ and the latter relates to microbial pathogenesis.⁹

To study the working mechanisms of immunotherapy, the production of α and β chemokines were compared between groups of good and poor responders, as defined by the magnitude of im-

provement in symptom and medication scores stated in the methods section. In addition, three control groups were included: untreated, newly diagnosed patients with comparable duration of illness and disease severity; patients with acute attacks that occurred during house cleaning, which were very possibly due to exposure to critically high Der p 1 levels in dust samples^{21, 23, 24}; and age-matched healthy children.

It is very interesting to find in this study that although the spontaneous and mite-stimulated production of β (CC) chemokines such as MCAF/MCP-1, RANTES, and MIP-1 α , which possess high capability of causing histamine release, were much lower and the production of IL-8, which can inhibit histamine release, was much higher in good responders than in poor responders; and the production of α and β chemokines in the group of patients with acute attacks and the group of untreated new patients was usually in between the values for poor and good responders. These phenomena were quite similar to the recent findings by Kuna et al.³⁶ that increased MCAF and decreased IL-8 production occurred during ragweed season and that the reverse was observed during the off-season.

The reasons that poor responders have higher levels of β chemokines and lower levels of α chemokines are unknown. These levels are not likely due to the paradoxical response of poor responders to immunotherapy but may be due to disease severity. However, the decreased HRF production that reflects the net results of interaction among many cytokines present in the culture supernatants may be due to: (1) immune tolerance, resulting from repeated administration of allergen of increasing doses, as evidenced by decreased lymphoproliferation³⁷ and decreased production of IL-2³⁸ and IL-2R³⁹ after specific allergen immunotherapy; and (2) changes in cellular metabolism, resulting in decreased production and secretion of inflammatory molecules such as platelet activating factor,⁴⁰ IL-1, and tumor necrosis factor.⁴¹ However, determination of the reasons that immune tolerance and cellular changes can be induced only in good responders but not in poor responders requires further study.

In conclusion, although this report is not a prospective study (which is needed), the findings of decreased production of β chemokines, which cause histamine release, and increased production of α chemokines, which inhibit histamine release, in good responders and the reverse in poor responders may be used to account, in part, for the

clinical efficacy of immunotherapy, although the mechanisms remain to be elucidated. Investigations of changes after immunotherapy in receptor function of an individual chemokine, with respect to number and binding affinity, and messenger RNA expression of chemokines are underway.

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