

Chemokines in seasonal allergic rhinitis

Piotr Kuna, Mark Lazarovich, and Allen P. Kaplan Stony Brook, N.Y.

Monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 (MCAF/MCP-1), RANTES, and macrophage inflammatory protein (MIP)-1 α are chemokines known to activate basophils (MCAF/RANTES) and eosinophils (RANTES/MIP-1 α). IL-8 inhibits MCAF-induced histamine release from basophils. We questioned whether a relationship exists between the levels of these chemokines and various inflammatory mediators released from mast cells, eosinophils, and basophils as assessed in nasal secretions obtained from patients during the allergy season and out of season. Samples were assessed for MCAF/MCP-1, RANTES, MIP-1 α , IL-8, histamine, tryptase and eosinophil cationic protein (ECP) in three subject groups: subjects with allergic rhinitis (n = 18), atopic subjects without rhinitis (n = 9), and healthy individuals (n = 6). Statistically significant differences were apparent only in the subjects with symptoms as follows. MCAF/MCP-1 increased during the season from 336 ± 47 pg/ml to 829 ± 137 pg/ml ($p < 0.001$), whereas IL-8 decreased from a baseline of 1932 ± 335 pg/ml to 1070 ± 202 pg/ml ($p < 0.028$). The ratio of IL-8 to MCAF/MCP-1 decreased during the symptomatic season from the baseline of 6.66 ± 1.06 seen during winter to 1.3 ± 0.22 during ragweed season ($p < 0.001$). Histamine increased from 6.3 ± 1.5 to 89 ± 15.5 ng/ml ($p < 0.001$), ECP increased from 20.6 ± 6.4 to 237.1 ± 50.2 ng/ml ($p < 0.001$), and tryptase increased from 2.34 ± 0.6 to 9.7 ± 2.3 U/ml ($p < 0.001$). Most samples did not have detectable quantities of MIP-1 α or RANTES. We also found a correlation between the level of MCAF/MCP-1 and IL-8 and the level of histamine or IL-8 and ECP. Our results suggest that the chemokines MCAF/MCP-1 and IL-8 may participate in the pathogenesis of allergic rhinitis, contributing to the attraction of the proinflammatory cells and mediator release, which might be very important during the late phase of the allergic reaction. Furthermore, the ratio of certain chemokines, such as MCAF/MCP-1 and IL-8 may reflect the magnitude of the reaction, as does the presence of histamine and ECP. (J ALLERGY CLIN IMMUNOL 1996;97:104-12.)

Key words: Monocyte chemotactic protein-1, IL-8, RANTES, rhinitis, histamine releasing factor, chemokines

Histamine releasing factors (HRFs) are defined as cytokine-like molecules that induce basophil and/or mast cell secretion of histamine in the absence of a particular antigen or anti-IgE antibody. Much of the activity appears to be due to type β chemokines (C-C family), which include monocyte chemotactic and activating factor/monocyte chemoattractant factor-1 (MCAF/MCP-1),^{1,2} RANTES,^{3,4} and human macrophage inflammatory proteins (MIP)-1 α and β .^{5,6} Priming basophils

with either IL-3, IL-5, or granulocyte-macrophage colony-stimulating factor augments secretion in response to any of these chemokines. Group α chemokines, such as connective tissue activating peptide III/neutrophil activating peptide-2 (CTAP III/NAP-2), are either less potent as secretagogues or dependent on prior cell priming for activity as reported with IL-8.^{7,8} Preincubation of basophils with various chemokines, IL-8 in particular, can inhibit histamine release caused by β chemokines and represents the activity termed histamine release inhibitory factor.^{9,10}

The activity of HRF has been characterized primarily by using basophils as the target cells; studies of mast cells, particularly in human beings, have been limited but suggest a lesser responsiveness to chemokines.⁵ Both MCAF/MCP-1 and RANTES have been shown to be chemotactic for basophils, and RANTES is chemotactic for eosinophils.^{4,11,12} Infiltration with these cells is associ-

From the Division of Allergy, Rheumatology and Clinical Immunology, Department of Medicine, State University of New York-Stony Brook, Health Sciences Center.

Received for publication Dec. 5, 1994; revised Mar. 30, 1995; accepted for publication Apr. 10, 1995.

Reprint requests: Allen P. Kaplan, MD, Department of Medicine, SUNY-Stony Brook, HSC T-16, Stony Brook, NY 11794-8160.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/65634

Abbreviations used

CTAB:	Cetyltrimethylammonium bromide
CTAP-III/NAP-2:	Connective tissue activating peptide III/neutrophil activating peptide-2
ECP:	Eosinophil cationic protein
HRF:	Histamine releasing factor
MCAF/MCP-1:	Monocyte chemotactic and activating factor/monocyte chemoattractant protein-1
MIP:	Macrophage inflammatory protein
PBS:	Phosphate-buffered saline
PMSF:	Phenylmethylsulfonyl fluoride

ated with the late-phase allergic reaction and may account, in part, for the protracted histamine release seen, particularly in studies of allergic rhinitis.^{13, 14}

HRF has been shown to be present in nasal washings of patients with allergic rhinitis,¹⁵ and the quantity present, based on functional assessment, appears to be diminished by treatment with either topical corticosteroid¹⁶ or allergen-specific immunotherapy.¹⁷ However, there has been no assessment of any of the identified molecular forms of HRF in nasal washings. In this article we focus on the group β chemokines, as well as IL-8, and relate their presence to the subjects' atopic status, as well as to secretion of mediators derived from basophils, mast cells, and eosinophils.

METHODS

Materials

Tissue culture flasks were purchased from Corning Glass Works (Corning, N.Y.); MIP-1 α and IL-8 ELISA kits were from R&D Systems (Minneapolis, Minn.); and Tryptase RIACT assay and eosinophil cationic protein (ECP) radioimmunoassay were purchased from Kabi Pharmacia Diagnostics (Piscataway, N.J.). Recombinant human MCAF/MCP-1 (rh-MCAF/MCP-1), rh IL-8, rh RANTES, and rh MIP-1 α were purchased from Pepro Tech Inc. (Rocky Hill, N.J.); and purified goat anti-mouse and rabbit anti-goat IgG conjugated to alkaline phosphatase were purchased from Jackson Immuno-research Laboratories, (West Grove, Pa.). Microtiter plates (Immulon-2 and Immulon-4) were from Dynatech Laboratories Inc. (Alexandria, Va.), and chloroform and sodium hydroxide were obtained from Fisher Scientific (Springfield, N.J.); Dulbecco's phosphate-buffered saline (PBS) without Ca⁺⁺ and Mg⁺⁺, penicillin, streptomycin, and RPMI-1640 were from Gibco Lab. Life Technologies, Inc. (Grand Island, N.Y.). Spectrapor

dialysis tubing was obtained from Spectrum Medical Industries (Los Angeles, Calif.); affinity-purified goat anti-human IgE antibody was from Kirkegaard & Perry Laboratories Inc. (Gaithersburg, Md.); tritiated histamine and [¹⁴C]S-adenosyl methionine were from New England Nuclear Corp. (Boston, Mass.); and scintillation fluid was from ICN Radiochemicals (Irvine, Calif.). The following reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.): ethylenediaminetetraacetic acid, sodium phosphate monobasic, sodium chloride, piperazine-N, N'-bis[2-ethanesulfonic acid], human serum albumin, histamine hydrochloride, calcium ionophore A 23187, cetyltrimethylammonium bromide (CTAB), and phenylmethylsulfonyl fluoride (PMSF).

Subjects

Subjects included 18 atopic individuals with seasonal allergic rhinitis caused by ragweed pollen only, nine subjects without allergic rhinitis but with positive skin test responses to one or more common allergens (20-allergen panel; Greer Laboratories, Lenoir, N.C.) and six healthy, nonatopic individuals. The subjects with positive skin test responses had no symptoms of asthma, urticaria, or eczema but had positive skin test responses to a variety of allergens (e.g., dust mite, *Dermatophagoides pteronyssinus*, cat) but not ragweed. Allergic rhinitis was defined by a positive seasonal history and a positive skin test response (5 mm or greater wheal at 15 minutes) to ragweed (prick test, 4000 PNU/ml; Greer Laboratories). All donors with allergic rhinitis reported symptoms present during the allergy season for at least 3 years before the study. None of the atopic subjects had asthma, urticaria, or eczema nor did they ever undergo immunotherapy; and none had any nasal infection within 1 month before the study. The nonatopic subjects had no history of seasonal or perennial rhinitis, asthma, or urticaria and had negative prick skin test responses to a panel of allergens. None of the subjects had received any medication for at least 3 days before the study. Patients had been taking antihistamines, such as chlorpheniramine or brompheniramine, but not long-acting, non-sedating ones. Administration was stopped 3 days before the study. No patient had used systemic steroids, and none had begun use of topical cromolyn or steroids when the season started, because the study was planned prospectively. These drugs had been used during the prior season. Blood was drawn for determination of total IgE.

Nasal washings collection

Subjects were asked to exhale forcefully through the nose to clear excess mucus. Then, preservative-free PBS (150 mmol/L NaCl, 50 mmol/L NaH₂PO₄), prewarmed to 37° C, was aerosolized into the nostrils sequentially with a manually operated metered-dose pump, delivering 200 μ l per activation. To collect the nasal washings, the subject allowed the fluid to decant passively into a

sterile plastic 50 ml tube submerged to the rim in crushed ice, containing 5 mmol/L ethylenediaminetetraacetic acid and 0.5 mmol/L PMSF to inactivate proteolytic enzymes. The procedure was repeated, alternating nostrils, until a total of 3.0 ml of liquid was collected. The individual variation in the recovered versus introduced volume (the mean recovery of lavage fluid was $58\% \pm 14\%$) prompted us to base our studies on constant recovered volumes rather than volume introduced. This method increases consistency of comparisons between individuals. The sample was centrifuged at 400 g for 10 minutes at 4° C. Next, three glass beads (diameter, 2 mm) were added to the collected supernatant and vigorously vortexed for 10 minutes at maximal velocity, 1800 to 2000 rpm/min, in order to disrupt mucus clumps. The sample was then filtered through a 0.22 μ m membrane, aliquoted in cryotubes, and stored at -80° C.

Chemokine recovery

The percentage of cytokine recovery was evaluated with two different approaches. First, we determined cytokine recovery in our immunoassay by the addition of a known amount of recombinant cytokine to the nasal lavage fluid. Second, we determined cytokine recovery from the nose. Six volunteers, including the authors, participated in the experiment. Participants cleared their noses of excess mucus, and 100 μ l of a mixture of IL-8, MCAF, MIP-1 α , and RANTES at a concentration of 30 ng/ml each (prepared in PBS immediately before the experiment) was subsequently introduced into one nostril with an automatic pipette. An equivalent volume of PBS alone was introduced into the second nostril. After 30 seconds, preservative-free PBS at 37° C was aerosolized into the first nostril and allowed to decant passively into a 50 ml plastic tube, containing 0.5 mmol/L PMSF, submerged to the rim in crushed ice until a volume of 1.5 ml was collected. Thereafter, identically prepared PBS from a new pump was aerosolized into the second nostril and allowed to decant into a second, iced, plastic 50 ml tube with 0.5 mmol/L PMSF to a volume of 1.5 ml. Both samples were processed (as described above) and aliquoted into cryotubes and stored at -80° C. The recovery of chemokines from the nasal fluid was in the range of 86% to 112%, (mean $\pm$ SEM, $96.7\% \pm 3.9\%$). The recovery of chemokines from the nose was in the range of 56% to 83% (mean $\pm$ SEM, $68.8\% \pm 3.8\%$).

Mediator assay

Histamine assay. The radioenzyme assay for histamine was performed in duplicate for each serial dilution (1:1, 1:5, 1:10, 1:50, and 1:100) of nasal washing by using a modification of the double radioenzymatic method of Beaven et al.¹⁸ as described previously.⁶

Tryptase. Tryptase in the lavage fluid was analyzed with a modified Pharmacia Tryptase RIACT assay with two monoclonal antibodies for tryptase.¹⁹ In the RIACT, 50 μ l of nasal lavage fluid, 50 μ l of sample diluent, and

50 μ l of iodine 125-labeled anti-tryptase antibody were simultaneously added to plastic tubes coated with anti-tryptase antibody. After incubation overnight, the tubes were washed three times and then counted in a gamma counter.

ECP. The radioimmunoassay for ECP was performed in duplicate for each serial dilution (1:1, 1:10, and 1:100) of nasal washings. Nasal washes were mixed with 0.5% CTAB in 0.15 mmol/L NaCl. Fifty microliters of nasal fluid with CTAB was assayed for ECP with a commercially available radioimmunoassay kit from Pharmacia, according to the manufacturer's directions.

IL-8 and MIP-1 α ELISA. ELISAs of IL-8 and MIP-1 α were performed with specific ELISA kits. Study samples and standard dilutions of a specific cytokine were assayed separately in duplicate according to the manufacturer's instructions. The color intensity of the ELISA reaction was read at a wavelength of 450 nm with the correction set to 570 nm by using a Thermo max microplate autoreader (Molecular Devices Corp., Menlo Park, Calif.). The average net optical density of the standards was plotted, and the amount of a specific cytokine in each sample was determined by interpolation from the standard curve. Results were the means of duplicate samples. None of the assay had demonstrated any measurable cross-reactivity to other cytokines. The sensitivity of the ELISA for MIP-1 α was 2 pg/ml, and for IL-8, 4.7 pg/ml.

Preparation of monoclonal anti-MCAF antibodies. BALB/c mice were immunized with purified human recombinant MCAF/MCP-1. The presence of MCAF/MCP-1-specific antibody in serum was determined by indirect ELISA and Western blot. The lymphocytes were fused with P3X63-Ag 8.653 mouse myeloma cell line according to a standard fusion protocol. The initial clone selection and subsequent subclone selection were done on the basis of reaction with human recombinant MCAF/MCP-1 as determined by ELISA and Western blot. The subclone that yielded the strongest signal termed (no. 40-2) was grown in 2 L of RPMI-1640 medium. Cells were removed, the supernatant was concentrated, and IgG was purified with a protein G Sepharose HiTrap column (Pharmacia). A sodium dodecylsulfate-polyacrylamide gel electrophoresis was performed to assess purity; more than 99% of the total protein migrated as IgG. Antibody class and subclass were determined by ELISA and shown to be IgG 2b kappa. The antibodies were specific and did not react with RANTES, HuMIP-1 α , and - β , HC-14, MCP-2, MCP-3, I-309, IP-10, IL-8, CTAP III/NAP-2, and platelet factor 4.

Anti-MCAF antibody preparation. A polyclonal anti-MCAF antibody was raised in a rabbit. Recombinant human MCAF/MCP-1 (100 μ g in 200 μ l of PBS) was diluted in 1.0 ml of 1:4 mixture of Freund's complete and incomplete adjuvant and injected at three sites: one subcutaneous and two deep intramuscular. Booster injections were given as described previously every 2

weeks; however, the third and all subsequent injections were given in incomplete adjuvant. After 3 months, the rabbit was bled, and antiserum was collected and assayed (ELISA) against purified recombinant human MCAF/MCP-1, IL-8, RANTES, HuMIP-1 α , HuMIP-1 β , I-309, IP-10, CTAP III, and platelet factor 4. The antiserum was specific and reacted only with MCAF/MCP-1. The antiserum and the preimmune rabbit serum were made 45% in ammonium sulfate and centrifuged at 10,000 *g* for 15 minutes; the supernatant was discarded, and the precipitate was dissolved in PBS and dialyzed against PBS. IgG was purified with a protein G Sepharose HiTrap column. The purified protein migrated as IgG on sodium dodecylsulfate–polyacrylamide gel electrophoresis.

Sandwich ELISA for MCAF/MCP-1 and RANTES. ELISAs were performed according to standard procedures with 96-well microtiter plates (Immulon-4). The wells were coated with 5 μ g/ml of the rabbit anti-MCAF/MCP-1 IgG in 100 μ l of 0.05 mmol/L NaHCO₃ buffer (pH 9.6) for 24 hours at 4° C. The wells were washed, and unbound sites were blocked by incubation for 60 minutes at 37° C with 1% nonfat milk in PBS. After washing, triplicate samples of 100 μ l MCAF/MCP-1 standards or unknowns (nasal lavages) at dilutions of 1:1 and 1:10 in PBS containing 0.05% Tween were added and incubated for 2 hours at 37° C. The plates were washed again with PBS/Tween, filled with 100 μ l/well of anti-MCAF 40-2 monoclonal antibody at a concentration of 10 μ g/ml, and incubated for 2 hours at 37° C. The wells were washed, and goat anti-mouse IgG conjugated to alkaline phosphatase, diluted 1:2000 in PBS/Tween, was added and incubated for 2 hours at 37° C. After washing, 100 μ l of p-nitrophenyl phosphate (1 mg/ml) in 10% diethanolamine-HCL buffer (pH 9.6) was added to the wells. The absorbance was read after 30 minutes at 405 nm on a Thermo max microplate reader. RANTES concentrations were measured by an analogous sandwich ELISA.²⁰ The assay uses two anti-RANTES monoclonal antibodies that recognize different, noncompeting determinants. The specificity of the ELISA was tested with other chemokines of the C-X-C and C-C families, none of which were shown to cross-react in the assay. All experiments were repeated twice. Using this procedure, we were able to quantitatively measure human MCAF/MCP-1 and RANTES at concentrations of 100 pg/ml and higher.

Total IgE measurements

Total IgE was determined in serum samples from each individual donor with a commercially available radioimmunoassay kit from Pharmacia according to the manufacturer's instructions.

Protein

Protein concentration was determined according to the method of Bradford²¹ with human IgG as the reference protein.

Statistical analysis

Results are expressed as means $\pm$ SEM. Paired data were analyzed by Student's paired *t* test and the Mann-Whitney U test. Unpaired data were analyzed by Student's *t* test. Spearman's rank correlation was used to evaluate relation between parameters studied with the StatWorks computer program (Cricket Software, Philadelphia, Pa.).

RESULTS

The results of atopic, ragweed-sensitive subjects compared with atopic subjects without symptoms and nonatopic control subjects are shown in Fig. 1. Total proteins in micrograms per milliliter were measured in the subjects with symptoms and increased from a mean of 583.7 ± 106.2 to 1287.8 ± 252 when the winter season was compared with ragweed season. The comparable mean protein values for the atopic subjects without symptoms were 201.8 ± 61.6 and 208.4 ± 17.1 , and those for normal control subjects were 193.3 ± 71.7 and 205.3 ± 77.2 . Thus the baseline protein level was increased in the symptomatic group, and it increased further during ragweed season. No such change was seen in the other control subjects (Fig. 1, *A*). Histamine, ECP, and tryptase levels increased only in the subjects with symptoms (Fig. 1, *B*, *C*, and *D*) as follows. Histamine levels increased from 6.3 ± 1.5 to 89.1 ± 15.5 ng/ml, ECP levels increased from 20.6 ± 6.4 to 237.1 ± 50.2 ng/ml, and tryptase levels increased from 2.34 ± 0.6 to 9.7 ± 2.3 U/ml; whereas no significant change was observed in the other groups. The changes observed in protein content or mediator levels in the symptomatic group were significant when compared with the baseline value obtained during winter (protein, $p < 0.01$; histamine, $p < 0.001$; ECP, $p < 0.001$; tryptase, $p < 0.001$). The data are similarly significant when the levels of patients with symptoms during the late summer are compared with levels of either of the other two groups during the ragweed season.

We next assessed the levels of MCAF/MCP-1, RANTES, MIP-1 α , and IL-8 in nasal washings of the same subjects. As shown in Fig. 1, *E*, there is a marked increase in MCAF/MCP-1 from 336 ± 47 to 829 ± 137 pg/ml ($p < 0.001$) in the patients with symptoms during their allergy season. We thought it would be interesting to separate our normal control subjects (who have not only no ragweed-induced hay fever but no allergic symptoms whatsoever) into those who did and those who did not have a positive skin test result, assuming that these might indicate an atopic background, which might

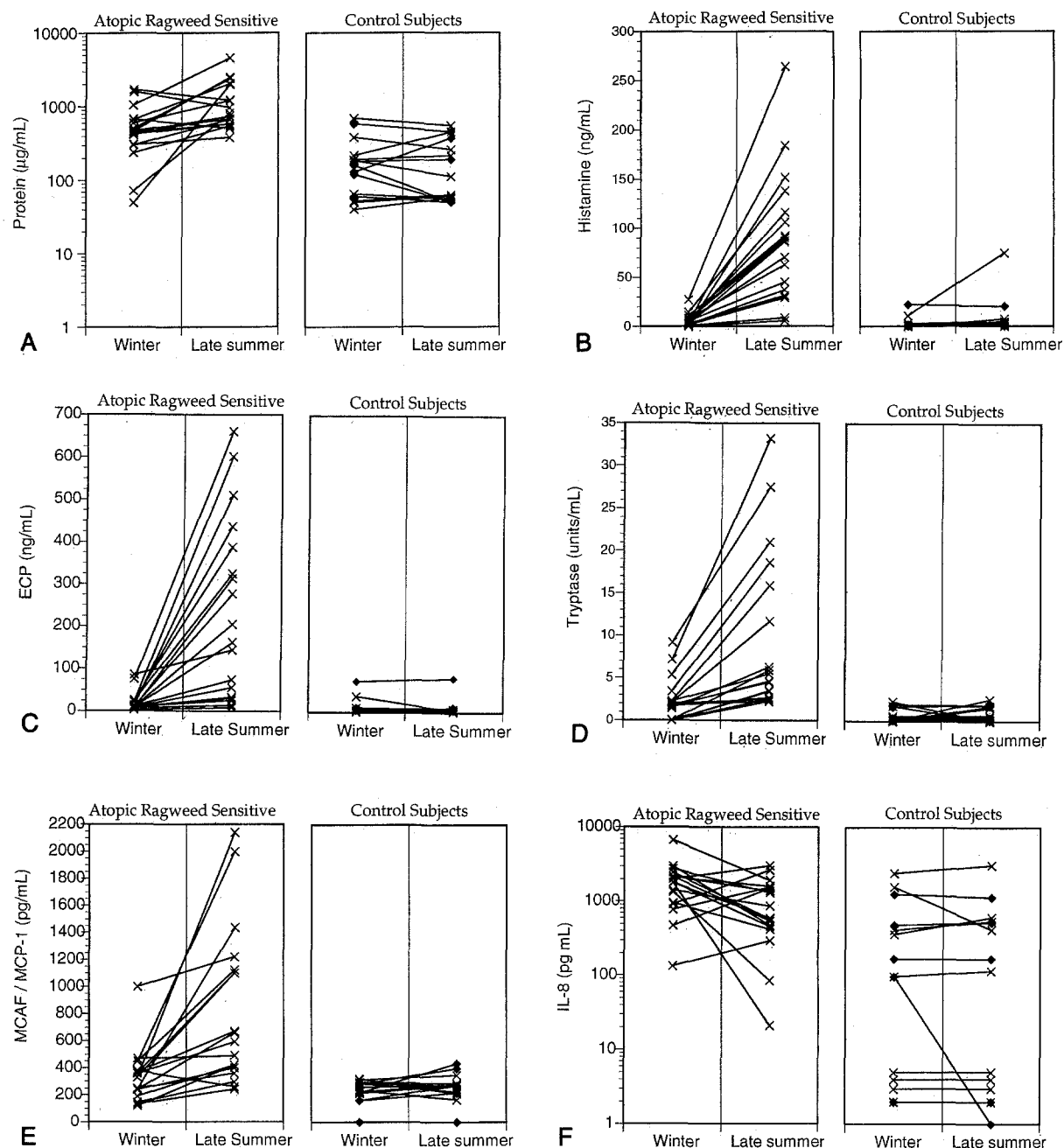


FIG. 1. Seasonal comparison of total protein (A); histamine (B); ECP (C); tryptase (D); MCP-1 (E), and IL-8 (F) levels between ragweed-sensitive subjects ($n = 18$) and control subjects ($n = 15$). The latter group consists of nine atopic subjects without symptoms and six nonatopic subjects.

some day be evident clinically. Values among the healthy subjects (negative skin test results) varied from 135 ± 46 pg/ml in winter to 219 ± 76 pg/ml in summer, and values in the group with positive skin test results and no symptoms ranged from 270 ± 13 to 258 ± 17 pg/ml in summer. Combined they are 216 ± 24 pg/ml in winter and 243 ± 31 pg/ml in summer. No statistically significant difference is

seen (Mann-Whitney U test or Student's paired t test) in both groups between winter and summer. Thus MCAF/MCP-1 behaved in a fashion similar to the products of mast cells (or basophils) or eosinophils. On the other hand, IL-8 decreased in atopic subjects with symptoms during the season from a baseline of 1932.1 ± 334.8 pg/ml to 1070 ± 202 pg/ml ($p < 0.028$). Although a decrease is seen,

the baseline IL-8 level is greater than that of MCAF/MCP-1, and values are similar in magnitude during the symptomatic period. No significant change in IL-8 level is seen in the other groups, although the baseline mean level was lower at 534.3 ± 333.8 pg/ml for atopic subjects without symptoms and 336.7 ± 200.6 pg/ml for healthy donors with a broader range of variability (Fig. 1, F).

Most samples did not have detectable quantities of MIP-1 α or RANTES. Of the 33 subjects tested, only six had detectable MIP-1 α levels. Four were in the symptomatic group, and the level of MIP-1 α varied from 57 ± 10 pg/ml in winter to 62 ± 11 pg/ml in summer. Two were in the asymptomatic atopic group, and the concentration of MIP-1 α varied from 48 ± 9 pg/ml in winter to 53 ± 10 pg/ml in summer. Seven subjects had detectable RANTES levels. Five were in the symptomatic group; and the level of RANTES, detectable only during the ragweed season, was 204 ± 37 pg/ml. Two were in the asymptomatic atopic group, and the mean concentration of RANTES was 147 ± 12 pg/ml.

We also examined the data for correlations between the levels of histamine, tryptase, ECP, and protein for the atopic subjects with symptoms. As shown in Fig. 2, there is a correlation of histamine and ECP levels for atopic subjects with symptoms during the summer ($r = 0.59$, $p < 0.01$). There was no correlation between histamine levels and either protein ($r = -0.14$) or tryptase levels ($r = 0.37$), although the trend was an increase in each of them during the symptomatic season. The level of MCAF/MCP-1 did not correlate with values for histamine, protein, or tryptase; whereas correlation was seen between IL-8 and ECP ($r = 0.7$, $p < 0.001$), but only during the off season. There was no correlation of IL-8 levels and histamine, protein, tryptase, or ECP during the ragweed season. The changes in MCAF and IL-8 observed when compared in the winter (symptom-free period) to the summer (ragweed season) were negatively correlated because the mean levels of MCAF rose, and the mean IL-8 level diminished. Thus the ratio of the IL-8 level to the MCAF/MCP-1 level decreased during the season from a baseline of 6.66 ± 1.06 to 1.3 ± 0.22 ($p < 0.001$). Symptom scores are not given; however, the subjects with rhinitis all had many symptoms including sneezing, rhinorrhea, and congestion that correlated with the timing of the ragweed season, whereas control subjects were totally free of symptoms.

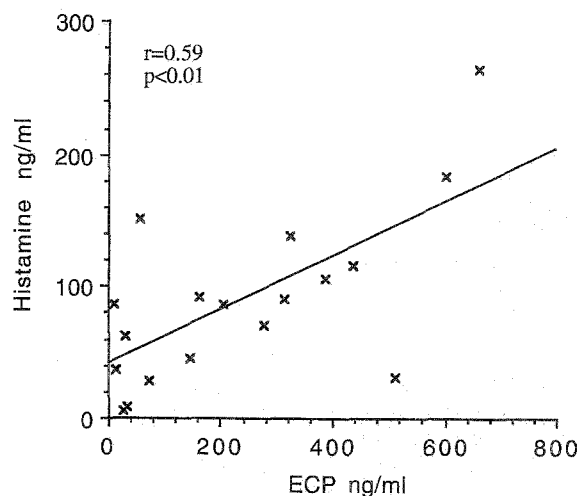


FIG. 2. The correlation of histamine and ECP levels in atopic subjects with symptoms during the ragweed season.

DISCUSSION

Chemokines such as MCAF/MCP-1, RANTES, and MIP-1 α/β are known to activate basophils and eosinophils and are responsible in large part for "HRF" activity.⁶ They are also potent chemotactic factors for basophils and eosinophils, as well as mononuclear cells, and may therefore contribute to the cellular infiltrate that characterizes the allergic late-phase reaction.²²⁻²⁶ Recently described, MCP-2 is a weak basophil agonist, whereas MCP-3 is a potent basophil activator and acts on eosinophils also; these were not examined in this study, and monospecific antisera are not yet available.^{27, 28} However, our anti-MCP-1 antibody does not recognize either.

One model used to study the allergic late-phase reaction in the nose is antigen challenge administered after a standardized protocol of nasal washing and vasoconstriction.^{29, 30} Postchallenge nasal washes can then be assayed for a variety of mediators of allergy symptoms. For example, histamine, prostaglandin D₂, leukotriene C₄, platelet activating factor, and kinin-generating enzymes (toluene-sulfonyl arginine methyl ester esterase) can be detected during the immediate reaction, and a similar array of substances, except for prostaglandin D₂, are seen during the late phase.³⁰ Histamine has been particularly difficult to measure, with results ranging from an inability to detect it to specific release as a result of antigen challenge.^{29, 31} This same model can be used to study other substances, such as cytokines, chemokines, and basic proteins secreted by eosinophils.^{32, 33} In this study, we have sampled nasal secretions during the ragweed season and during a controlled, asymp-

tomatic period rather than by an antigen challenge, hoping to more closely approximate the natural condition. We also chose not to wash out the nose in any preliminary fashion but to monitor the initial washing during the season versus out of season, even though the timing of the secretion obtained cannot be inferred.

One of the surprising results was the ready determination of histamine levels, which were considerably higher during the ragweed season than during the control period or when assessed in nonatopic subjects. We therefore believe that this is a valid assessment of allergy mediators, which may in fact more closely relate to the pathogenesis of the disease than do *in vivo* antigen challenge protocols. ECP and tryptase levels were similarly increased in the subjects with symptoms and histamine and ECP levels were correlated in this group. Our results also focused on the chemokines MCAF/MCP-1, RANTES, and IL-8 because these are believed to modulate the presence and activation state of basophils and eosinophils.³⁴ MCAF/MCP-1 was clearly elevated in allergic subjects, whereas IL-8 levels, although high in all groups, diminished during the allergy season. Thus the ratio of MCAF/MCP-1 to IL-8 rose during the ragweed season. The ratio of C-X-C chemokines to C-C chemokines may be critical to the pathogenesis of a wide variety of allergic or rheumatoid diseases. Because MCAF/MCP-1 is the most potent and plentiful form of HRF and IL-8 inhibits histamine release, the increase in MCAF/MCP-1 and simultaneous decrease in IL-8 level may lead to basophil recruitment and activation during the allergy season and account for the protracted histamine release seen during studies of the late-phase reaction.³⁵ This observation is also consistent with recent data indicating reciprocal control of C-X-C and C-C chemokines at the messenger RNA level on stimulation with IL-4 or interferon- γ , which are regulatory cytokines of allergic reactions.³⁶ Interaction of various chemokines with receptors on inflammatory cells is, however, very complex. Recently, we have reported the phenomenon of receptor class desensitization on human basophils for C-X-C and C-C chemokines, showing that low concentrations of various chemokines desensitized basophils to subsequent challenge with an optimal concentration of MCAF/MCP-1.³⁷

In most of the subjects studied, we were unable to quantitate RANTES and MIP-1 α values because the levels were too low and we could therefore not infer their relationship to eosinophil chemotaxis and activation. However, the close association of ECP

levels with histamine levels implies the presence of eosinophils along with basophils.

MCAF/MCP-1 and IL-8 have been examined in other inflammatory conditions. For example, IL-8 levels are elevated in empyema and parapneumonic pleural effusions and correlate with neutrophil counts, but IL-8 is undetectable in pleural fluid transudates.³⁸ On the other hand, malignant pleural fluid had particularly high levels of MCAF/MCP-1, which correlated with the presence of monocytes; basophils were not examined.³⁸ Studies of nasal fluid or nasal washings of allergic subjects have examined HRF activity as assessed by basophil histamine release and have determined the effect of corticosteroid therapy on HRF levels. However, the specific constituents of HRF have not been examined. The results indicate that HRF activity of allergic and nonallergic subjects varied, depending on the atopic status of donor basophils, and that background histamine release present in all nasal washings, whether donors are atopic or not, is dependent on membrane-bound IgE.¹⁵ Clearly, quantitation of particular substances is necessary to interpret the data. Other cytokines have been quantitated in the allergic late-phase response in the nose and are clearly elevated. These include IL-1 β , IL-5, IL-6, and granulocyte-macrophage colony-stimulating factor.³³ Although we did not examine IL-5 titers, it is interesting that Kimmitt et al.³⁹ recently demonstrated that the level of IL-5 varies with asthma symptoms but not with a skin test, which is similar to our observation regarding MCP-1 level. Basophil and eosinophil influx into the nose has been demonstrated after antigen challenge, their presence is inhibited by topical corticosteroid treatment,^{13, 40} and functional HRF activity is significantly diminished.¹⁶ Thus inhibition of leukocyte-endothelial cell interactions concomitant with cell migration (chemotaxis) is inferred. Our observations suggest that the chemokine concentrations in the nose during a symptomatic period relate to basophil influx and the protracted release of histamine seen during late-phase reactions.^{41, 42}

REFERENCES

1. Kuna P, Reddigari SR, Rucinski D, Oppenheim JJ, Kaplan AP. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for human basophils. *J Exp Med* 1992;175:489-93.
2. Alam R, Lett-Brown MA, Forsythe PA, et al. Monocyte chemotactic and activating factor is a potent histamine releasing factor for basophils. *J Clin Invest* 1992;89:723-8.
3. Kuna P, Reddigari SR, Schall TJ, Rucinski D, Viksman MY, Kaplan AP. RANTES a monocyte and T lymphocyte

- chemotactic cytokine releases histamine from human basophils. *J Immunol* 1992;149:636-42.
4. Bischoff SC, Krieger M, Brunner T, et al. RANTES and related chemokines activate human basophil granulocytes through different G protein-coupled receptors. *Eur J Immunol* 1993;23:761-7.
5. Alam R, Forsythe PA, Stafford S, Lett-Brown MA, Grant JA. Macrophage inflammatory protein-1 α activates basophils and mast cells. *J Exp Med* 1992;176:781-6.
6. Kuna P, Reddigari SR, Schall TJ, Rucinski D, Sadick M, Kaplan AP. Characterization of the human basophil response to cytokines, growth factors, and histamine releasing factors of the intercrine/chemokine family. *J Immunol* 1993;150:1932-43.
7. Dahinden CA, Kurimoto Y, De Weck AL, Lindley I, Dewald B, Baggiolini M. The neutrophil-activating peptide NAF/NAP-1 induces histamine release and leukotriene release by interleukin 3-primed basophils. *J Exp Med* 1989;170:1787-92.
8. Reddigari SR, Kuna P, Miragliotta GF, et al. Connective tissue-activating peptide-III and its derivative, neutrophil activating peptide-2, release histamine from human basophils. *J ALLERGY CLIN IMMUNOL* 1992;89:666-72.
9. Kuna P, Reddigari SR, Kornfeld D, Kaplan AP. IL-8 inhibits histamine release from human basophils induced by histamine releasing factors, connective tissue activating peptide III, and IL-3. *J Immunol* 1991;147:1920-4.
10. Alam R, Forsythe PA, Lett-Brown MA, Grant JA. Interleukin-8 and RANTES inhibit basophil histamine release induced with monocyte chemotactic and activating factor/monocyte chemoattractant peptide-1 and histamine releasing factor. *Am J Respir Cell Mol Biol* 1992;7:427-33.
11. Leonard ED, Skeel A, Yoshimura T. Biological aspects of monocyte chemoattractant protein-1 (MCP-1). In: Westwick J, Kunkel SL, Lindley I, eds. Chemotactic cytokines: biology of the inflammatory peptide supergene family. New York: Plenum, 1991:57-61.
12. Kameyoshi Y, Dorschner A, Mallet AI, Christophers E, Schroder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176:587-92.
13. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil Influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J ALLERGY CLIN IMMUNOL* 1988;81:580-9.
14. Juliusson S, Pipkorn U, Karlsson G, Enerback L. Mast cells and eosinophils in the allergic mucosal response to allergen challenge: changes in distribution and signs of activation in relation to symptoms. *J ALLERGY CLIN IMMUNOL* 1992;90:898-909.
15. Sim TC, Alam R, Forsythe PA, Welter JB, Lett-Brown MA, Grant JA. Measurements of histamine releasing factor activity in individual nasal washings: relationship with atopy, basophil response, and membrane-bound IgE. *J ALLERGY CLIN IMMUNOL* 1992;89:1157-65.
16. Sim TC, Hilsmeier KA, Alam R, Allen RK, Lett-Brown MA, Grant JA. Effect of topical corticosteroids on the recovery of histamine releasing factors in nasal washings of patients with allergic rhinitis. A double-blind, randomized, placebo-controlled study. *Am Rev Respir Dis* 1992;145:1316-20.
17. Brunet C, Bedard PM, Lavoie A, Jobin M, Hebert J. Allergic rhinitis to ragweed pollen. II. Modulation of histamine-releasing factor production by specific immunotherapy. *J ALLERGY CLIN IMMUNOL* 1992;89:87-94.
18. Beaven MA, Jacobsen A, Horakova Z. Modification of an enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum, and urine. *Clin Chem Acta* 1972;37:91-103.
19. Wenzell SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* 1988;137:1002-6.
20. Devergne O, Marfaing-Koka A, Schall TJ, et al. Production of the RANTES chemokine in delayed-type hypersensitivity reactions: involvement of macrophages and endothelial cells. *J Exp Med* 1994;179:1689-94.
21. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
22. Alam R, Stafford S, Forsythe O, et al. RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* 1993;150:3442-7.
23. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* 1990;347:669-71.
24. Schall TJ, Bacon K, Camp RDD, Kaspari J, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1 α) and MIP-1 β chemokines attract distinct population of lymphocyte. *J Exp Med* 1993;177:1763-74.
25. Schall TJ. Biology of the RANTES/SIS cytokine family. *Cytokine* 1991;3:165-83.
26. Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 α and MIP-1 β . *Science* 1993;260:355-8.
27. Alam R, Forsythe P, Stafford S, et al. Monocyte chemotactic protein-2, monocyte chemotactic protein-3, and fibroblast-induced cytokine. Three new chemokines induce chemotaxis and activation of basophils. *J Immunol* 1994;153:3155-9.
28. Dahinden CA, Geiser T, Brunner T, et al. Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 1994;176:751-6.
29. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
30. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
31. Linder A, Strandberg K, Deuschl H. Variations in histamine concentrations in nasal secretion in patients with allergic rhinitis. *Allergy* 1988;43:119-26.
32. Linder A, Venge P, Deuschl H. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. *Allergy* 1988;42:583-90.
33. Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994;149:339-44.
34. Kaplan AP, Kuna P, Reddigari SR. Chemokines as allergic mediators: relationship to histamine-releasing factors. *Allergy* 1994;49:495-501.
35. Naclerio RM. The role of histamine in allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1990;86:628-32.
36. Rathanaswami P, Hachicha M, Sadick M, Schall TJ, Mc-

- Coll SR. Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. *J Biol Chem* 1993;268:5834-9.
37. Kuna P, Reddigari SR, Rucinski D, Schall TJ, Kaplan AP. Chemokines of the α - β -subclass inhibit human basophils' responsiveness to monocyte chemotactic and activating factor/monocyte chemoattractant protein-1. *J ALLERGY CLIN IMMUNOL* 1995;95:574-86.
38. Antony VB, Godbey SW, Kunkel SL, et al. Recruitment of inflammatory cells to the pleural space: chemotactic cytokines, IL8, and monocyte chemotactic peptide-1 in human pleural fluids. *J Immunol* 1993;151:7216-23.
39. Kimmit P, Humbert M, Durham SR, Kay AB, Corrigan CJ. Elevated expression of IL-5 mRNA in the bronchial mucosa is a reflection of asthmatic, but not atopic status [Abstract]. *J ALLERGY CLIN IMMUNOL* 1995;95:222.
40. Bisgaard H, Grenburg H, Mygind N, Dahl R, Lindqvist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. *J ALLERGY CLIN IMMUNOL* 1990;85:891-5.
41. Talbot SF, Atkins PC, Valenzano M, Zweiman B. Correlations of in vivo mediator release with late cutaneous allergic responses in humans. I. Kinetics of histamine release. *J ALLERGY CLIN IMMUNOL* 1984;74:819-26.
42. Reshef A, Kagey-Sobotka A, Adkinson NF Jr, Lichtenstein LM, Norman PS. The pattern and kinetics in human skin of erythema and mediators during the acute and late-phase response (LPR). *J ALLERGY CLIN IMMUNOL* 1989;84:678-87.

AVAILABLE NOW! The FIVE-YEAR (1986-1990) CUMULATIVE INDEX TO THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher for \$48.00. This comprehensive, 188-page reference guide is a current presentation of all topics included in the JOURNAL from January 1986 to December 1990 (volumes 77-86). It incorporates complete references to over 1500 original articles, abstracts, case reports, letters, editorials, and CME articles. It features 1662 subject headings, under which there are 5863 entries. Each subject entry lists the complete article title, author(s), volume, page, and year of publication. It also includes 5801 author entries, listing contributors, along with their respective titles, author-to-author referral, volume, page, and publication date.

To purchase, call or write: Mosby, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE, Subscription Services, 1 (800) 453-4351 or 314-453-4351. PREPAYMENT REQUIRED. Make checks payable to Mosby. (All payments must be in US funds drawn on a US bank.) Price: \$48.00 in the US, \$54.35 in Canada, and \$51.00 international (price includes mailing charges).