

Further characterization and biologic activity of ragweed antigen—induced neutrophil chemotactic activity in man

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We have previously described the appearance in serum of increased neutrophil chemotactic activity (NCA) during bronchospasm induced by inhalation of ragweed antigen in ragweed-sensitive subjects. This NCA is non-complement derived, appears within 1 min after antigen inhalation, and is not seen after methacholine-induced bronchospasm. This article describes further characterization of this chemotactic activity and correlation with in vivo leukocytosis. NCA consistently eluted in the void volume (fraction I) after Sephadex G-150 chromatography of patient serum obtained 10 min postchallenge. Fraction I contained 94% of the NCA of postchallenge whole serum. Both postchallenge whole serum and fraction I deactivated neutrophils to autologous chemoattractants and complement-derived chemotactic factors, but not serum-independent chemotactic factors. NCA was chemotactic for neither human nor guinea pig eosinophils, nor for human mononuclear cells. A significant increase of circulating neutrophils was seen only after antigen-induced bronchospasm and correlated with the increase in NCA. Thus, NCA represents another inflammatory mediator of probable mast cell origin that may explain, at least partially, the accumulation of neutrophils observed in the peripheral blood, skin, and bronchial wall after immediate hypersensitivity reactions.

It has been observed by others¹ and confirmed by us² that neutrophils are the first cells to infiltrate the skin of ragweed-sensitive subjects in response to the intradermal injection of ragweed antigen. In addition, neutrophils are also prominent in the late-phase skin test response seen after intradermal ragweed injection in highly sensitive subjects.³ To further investigate the mechanism of appearance of these cells in immediate hypersensitivity reactions, we have employed ragweed inhalation challenge as a model of antigen-

induced asthma. We previously reported the occurrence of a heat-stable, non-complement dependent neutrophil chemotactic activity (NCA) after antigen, but not after methacholine-induced bronchospasm.⁴ This activity was present as early as 1 min and persisted for 24 hr after positive antigen inhalation challenge. NCA correlated with both the dose of antigen administered and the amount of bronchospasm produced.⁵ It could also be inhibited by prior administration of disodium cromoglycate, a substance thought to block mast cell activation.⁵ This article focuses on the further characterization and specificity of NCA, and attempts to correlate this in vitro activity with in vivo measurement of circulating leukocytes.

METHODS

Materials

Dextran (Sigma Chemical Co., St. Louis, Mo.); Hanks' balanced salt solution, medium 199, horse serum (Microbiological Associates, Bethesda, Md.); fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.); eosin

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Supported by Grants RO1 AI 14332 and 5 MO1 RROOO40 from the National Institutes of Health.

Received for publication Feb. 23, 1979.

Accepted for publication May 29, 1979.

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(Paragon C and Co., Inc., Bronx, N. Y.); hematoxylin (Harris alum hematoxylin, Harleco, Gibbstown, N. J.); chromotrope 2R (Matheson, Coleman and Bell, Norwood, Ohio); inulin (P Fanstiehl, Waukegan, Ill.); Hypaque (diatrizoate, Winthrop Laboratories, Inc., Miles Research Products, N. Y.); Ficoll, Sephadex G-150, dextran blue, aldolase, and hemoglobin (Pharmacia Fine Chemicals, Division of Pharmacia Inc., Piscataway, N. J.) were purchased from the manufacturers.

Characterization studies

These studies were carried out, after informed consent was obtained, in five ragweed-sensitive subjects in whom a decrease in FEV₁ of $\geq 20\%$ and an increase in serum NCA had been observed following inhalation of ragweed extract (Greer Laboratories, Lenoir, N. C.). After baseline blood specimens were obtained, each subject inhaled the lowest concentration of ragweed extract previously observed to induce a 20% fall in FEV₁; the extract was administered by a dosimeter* in a standard fashion, as previously described.⁴ Blood specimens were obtained 10 min after inhalation, at the time of the peak increase in serum NCA.⁵ Blood was allowed to clot at 4° C for 60 min, and the serum separated and tested either immediately or after snap freezing and storage at -70° C. All sera were heat inactivated at 56° C for 30 min and then tested at final concentrations of 20% (v/v) unless otherwise indicated.

Neutrophil chemotaxis was measured by use of a modification of the Boyden chamber assay with 3.0 μ Millipore filters (Millipore Corp., Bedford, Mass.), as previously described.⁴ In brief, peripheral blood neutrophils were obtained from healthy volunteers, separated by dextran sedimentation, washed and resuspended in buffer (medium 199 and 10% heated fetal calf serum) at 2.5×10^6 neutrophils/ml, and then exposed to various chemoattractants for 3 hr at 37° C.

Every experiment included duplicate chambers incubated with bacterial filtrate† (a positive chemotactic control⁶), buffer alone, and paired patient sera before and after challenge, as previously described.⁴ After staining, the filters were counted in a blind fashion using a minor modification we have adapted for greater reproducibility.⁵ This consists of multiplying the total number of cells in a high-power field (hpf) that have migrated the furthest distance into the filter, times the mean distance of penetration into the filter, as estimated by the micrometer on the microscope. This product was averaged for a total of 10 hpf in duplicate filters and was defined as the chemotactic index. Control responses for our laboratory have been published⁵ and are: buffer = 422 ± 44 ; normal serum = $1,441 \pm 106$; bacterial filtrate = $2,173 \pm 112$.

Simultaneous fractionation of pre- and postchallenge patient sera was performed by Sephadex G-150 chromatography on paired, previously calibrated columns (2.2 by 40 cm), employing phosphate-buffered saline in 0.01 M edetate (EDTA), pH 7.2, as the buffer. Columns were run at a flow rate of 4 to 6 ml/hr at 4° C. Two milliliters of serum were applied and 2- to 3-ml fractions were collected. After preliminary screening of individual tubes for NCA, it was determined that the most consistent results were obtained by pooling and concentrating several fractions to achieve the original volume as follows: (1) fraction I, 49 to 60 ml (void volume); (2) fraction II, 61 to 72 ml; (3) fraction III, 73 to 84 ml; and fraction IV, 85 to 96 ml. Concentration was performed by ultrafiltration (Amicon Corp., Scientific Systems Division, Lexington, Mass.). Fractions were tested immediately or after snap freezing and storage at -70° C. They were diluted with buffer to 20% final concentration (v/v) unless otherwise noted. Calibration of the column was accomplished with dextran blue (mw = 2×10^6), aldolase (mw = 1.6×10^5), and hemoglobin (mw = 6.4×10^3).

Chemotactic deactivation

The capacity of NCA to deactivate neutrophils when incubated in minimally chemotactic doses was examined.⁷ Neutrophils were preincubated for 30 min at 37° C in 10% postchallenge whole sera or fraction I (100 μ l), known to contain NCA. The cells were then washed, resuspended in buffer, and exposed to various chemoattractants. Control preincubations were performed with the same concentrations of prechallenge sera, normal sera, or buffer. Chemoattractants included: (1) normal serum activated by bovine serum albumin/anti-bovine serum albumin (BSA-anti-BSA) immune complexes formed at equivalence,⁴ (2) normal sera activated by 13 μ g/ml inulin,⁸ (3) bacterial filtrate, and (4) postchallenge whole sera diluted at 1:5 in buffer or fraction I (200 μ l), known to contain NCA. Percent of inhibition was calculated as:

$$1 - \frac{\text{chemotactic index cells preincubated in NCA}}{\text{chemotactic index cells preincubated in buffer}} \times 100$$

Eosinophil and mononuclear leukocyte chemotaxis

Eosinophil chemotaxis resembled neutrophil chemotaxis with the following modifications: (1) human eosinophils were obtained from each of three hypereosinophilic donors who had 60% to 70% peripheral blood eosinophilia (one with visceral larva migrans, two with idiopathic hypereosinophilia). Dextran sedimentation yielded 70% to 80% eosinophils with less than 10% neutrophil contamination; (2) guinea pig eosinophils were obtained by peritoneal lavage after weekly intraperitoneal injections of horse serum.⁹ This resulted in peritoneal exudates with 40% to 60% eosinophils and less than 5% neutrophil contamination; (3) Millipore filters of 5- μ size were used and were stained with chromotrope 2R¹⁰; (4) the positive control chemoattractant

*Provided by Richard Rosenthal, M.D., Johns Hopkins University Medical School.

†Broth filtrate of *E. coli* kindly provided by Harry S. Morton, Sc.D., Professor of Microbiology, University of Pennsylvania School of Medicine.

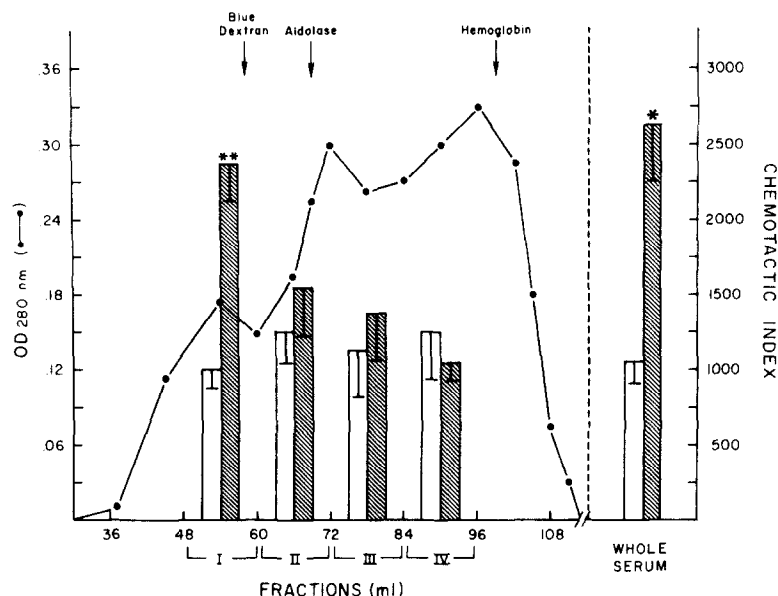


FIG 1. Sephadex G-150 chromatography of patient sera before (*open bars*) and after (*crosshatched bars*) positive ragweed inhalation challenge. The height of the bars gives the mean and the vertical line, the SEM for five patient experiments. Whole patient serum on the right is compared to pooled and concentrated fractions of the column effluent (see text). Protein concentration is assessed by optical density at 280 nm and is superimposed by lines connecting the solid circles. Arrows indicate the elution of molecular markers, dextran blue (2×10^6), aldolase (180,000), and hemoglobin (64,000). * $p < 0.01$ (Student's test for paired values), compared to pre-fraction I. ** $p < 0.001$ (Student's test for paired values), compared to pre-whole serum.

consisted of guinea pig lung homogenate.¹¹ Chemotactic indices were determined as already described.

Monocyte chemotaxis was performed as previously described¹² with these modifications: (1) Sykes Moore tissue culture chambers were used; (2) 5.0- μ Millipore filters were employed instead of polycarbonate filters; (3) the positive chemoattractant used was normal-serum activated by BSA-anti-BSA complexes.

In vivo correlation of NCA with leukocytosis

Seventeen antigen-sensitive subjects exhibiting bronchospasm ($\geq 20\%$ $\downarrow$ FEV₁) after inhalation of ragweed antigen underwent repeat ragweed inhalation challenge, after informed consent had been obtained. Serial total and differential leukocyte counts were performed* and compared to serum NCA measured 10 min after antigen inhalation. The same procedures were performed on 15 subjects who did not develop bronchospasm ($< 10\%$ $\downarrow$ FEV₁) after inhalation of ragweed extract (in 12, ragweed skin test was positive; in three, it was negative). In addition, 12 of 17 ragweed-sensitive subjects with positive inhalation challenge to ragweed were rechallenged with methacholine, as previously described.⁴ Six subjects developed bronchospasm ($\geq 20\%$ $\downarrow$ FEV₁) and six subjects did not ($\leq 10\%$ $\downarrow$ FEV₁). Total

and differential leukocyte counts were determined* serially after these challenges.

RESULTS

Characterization

Column chromatography. The results of simultaneous Sephadex G-150 chromatography performed on patients' sera obtained immediately before (pre-sera) and 10 min after (post-sera) a positive ragweed inhalation challenge are depicted in Fig. 1. NCA was significantly greater in post-sera ($2,618 \pm 617$) compared to pre-sera ($1,124 \pm 276$, $p < 0.01$). Likewise, fraction I of postchallenge sera also exhibited significantly increased NCA when compared to fraction I of prechallenge sera ($2,472 \pm 158$ vs. $1,117 \pm 132$, $p < 0.001$). Indeed, fraction I contained 94% of the NCA found in postchallenge sera, a fact that could not be explained by the difference in protein concentration. In addition, the chemotactic activity rechromatographed in the same position when the columns were recycled. NCA in all other serum fractions, before and after challenge, varied between

*Performed by the Hematology Laboratory of the Hospital of the University of Pennsylvania.

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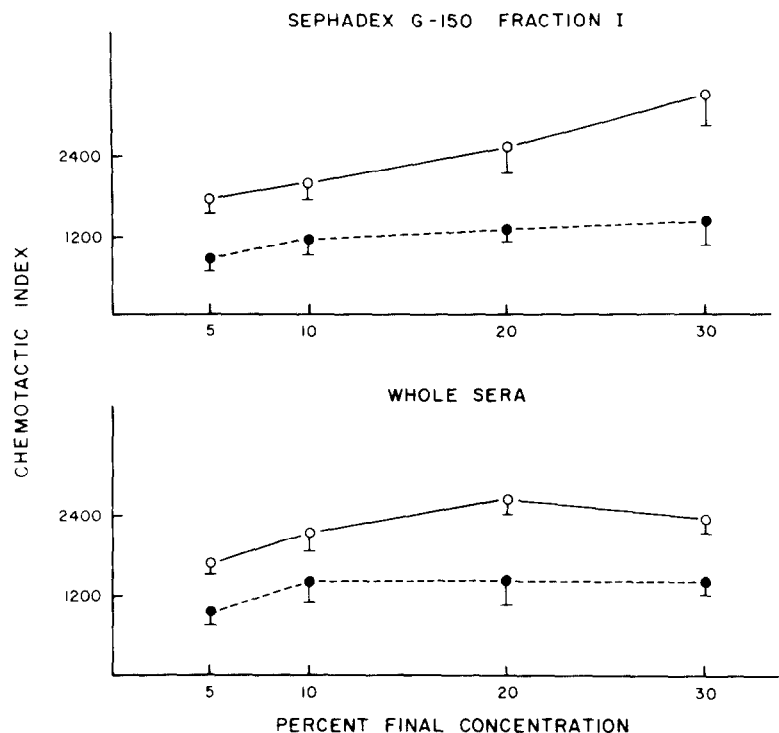


FIG. 2. Dose-response of whole sera and Sephadex G-150 fraction I before and after ragweed inhalation challenge. The chemotactic activity (mean chemotactic index SEM) of increasing concentrations of whole sera (*bottom*) of pooled fraction I after Sephadex G-150 fractionation (*top*) before (●---●) or after (○---○) positive ragweed inhalation challenge in five subjects.

1,000 and 1,600, not significantly different from the activity in normal sera as tested in our assay ($1,441 \pm 106$).

The dose-response curves for NCA assessed at 5% to 30% concentrations in whole serum and fraction I were compared for pre- and postchallenge specimens (Fig. 2). NCA increased over a concentration range of 5% to 20% in both whole and fractionated postchallenge sera. NCA after challenge was approximately twice the chemotactic activity observed in prechallenge sera at all concentrations. Maximum chemotactic activity was expressed at a 20% concentration (200 μ l) for postchallenge whole sera, and at a 30% concentration (300 μ l) for postchallenge fraction I.

Deactivation by NCA. Preliminary experiments revealed that preincubation of neutrophils with 10% (v/v) concentration of postchallenge sera, followed by washing, markedly reduced the chemotactic response of those cells when exposed subsequently to 20% concentrations of the same sera (525 ± 130 , 73% deactivation). Such reduced responses did not follow preincubation of the cells with buffer ($1,939 \pm 382$), pooled normal sera ($2,019 \pm 641$), or prechallenge sera ($2,577 \pm 165$).

The results of further experiments on fractionated postchallenge sera are listed in Table I. Preincubation

of neutrophils with 100 μ l fraction I of postchallenge sera resulted in (a) 81% reduction in the subsequent chemotactic response to 200 μ l of the same fraction and (b) 62% and 68% reductions in chemotactic responses to immune-complex and inulin-activated sera, respectively. However, such preincubation did not lead to any significant reduction of chemotactic activity in response to bacterial filtrate. No significant reductions in responses were seen when cells were preincubated with buffer solutions.

Cell specificity of NCA. The results of experiments testing postchallenge serum and fraction I for chemotactic activity of eosinophils are shown in Table II. No significant increase of chemotactic activity was found in either postchallenge whole sera or fraction I compared to prechallenge sera or fraction I when either human or guinea pig eosinophils were responding cells.

In addition, the chemotactic activities of postchallenge serum and fraction I for peripheral blood monocytes were not significantly different from those of buffer and all three produced 30% of the activity found in immune complex-activated serum.

Comparison of NCA and leukocytosis after inhalation challenge. To determine if the systemic increase in NCA had any biologic effect, we compared the total leukocyte and differential counts at 10 to 60 min

TABLE I. Chemotactic deactivation by NCA*

Cellular compartment †	Chemoattractants				
	Post-sera	Post-fraction I	Immune complex-activated sera ‡	Inulin-activated sera ‡	Bacterial filtrate ‡
Neutrophils and buffer	1,929 ± 382	4,572 ± 1,071	2,910 ± 432	4,273 ± 754	2,392 ± 165
Neutrophils and post-fraction I	Not done	879 ± 270	1,119 ± 240	1,533 ± 200	2,321 ± 117

* Mean chemotactic index ± SEM of four experiments.

† 0.9 ml neutrophils (5×10^6 /ml + 0.1 ml test factor or buffer) preincubated at 37 ° C for 30 min; the cells were then washed and resuspended in buffer at original concentration and exposed to various chemoattractants.

‡ For details of preparation, see text.

TABLE II. Eosinophil chemotaxis

Cells	Pooled normal sera	Buffer	Guinea pig lung homogenate*	Prechallenge sera	Postchallenge sera	Prechallenge fraction I	Postchallenge fraction I
Guinea pig*†	865‡	322	2,657	932	1,238	1,150	1,340
Eosinophils	± 186	± 42	± 412	± 108	± 478	± 205	± 308
Human	898	462	2,535	1,037	859	Not done	Not done
Eosinophils*§	± 270	± 179	± 277	± 341	± 255		

* See text for details of preparation.

† Six experiments.

‡ Mean chemotactic index ± SEM.

§ Three experiments.

TABLE III. Change in total leukocytes after inhalation challenge

Inhalation*	n	10 min	30 min	60 min
<i>Antigen</i>				
Positive	17	400 ± 250†	325 ± 275	575 ± 250‡
Negative	15	-25 ± 75	20 ± 120	200 ± 125
<i>Mecholyl</i>				
Positive	6	-150 ± 325	-150 ± 125	-150 ± 100
Negative	6	-300 ± 200	-400 ± 200	-200 ± 100

* Positive, $\geq 20\%$ ↓ FEV₁; negative, $\leq 10\%$ ↓ FEV₁.

† Mean (± SEM). Differences between leukocyte levels in blood obtained after inhalation challenge and the leukocyte levels in prechallenge blood specimens from the same subjects.

‡ Significantly different ($p < 0.001$, Student's *t* test) from the mean changes in blood leukocyte levels 60 min after methacholine challenge.

after inhalation of antigen and Mecholyl, in subjects with and without bronchospasm. Table III categorizes the change in total leukocytes after inhalation challenge. An increase in leukocytes of 400 to 575 cells/mm³ was seen from 10 to 60 min after inhalation of antigen in positive challenges. In contrast, during negative antigen inhalation challenges, there was a much smaller increase (200 cells/mm³) seen only at 60 min. Furthermore, after both positive and negative Mecholyl inhalation challenges (in 12 of the antigen-sensitive subjects), a decrease in total leukocyte counts was observed (150 to 400 cells/mm³) which was significantly different at 60 min from counts in

antigen-positive challenges. Since we had determined that NCA was specific for neutrophilic leukocytes, we attempted to correlate the change in NCA with the change in neutrophil levels after inhalation challenge. Neutrophil levels increased (128 to 1,033) beginning at 10 min and continued to rise for 60 min after inhalation of antigen in subjects exhibiting bronchospasm (Table IV). These increased levels at 60 min were significantly different from neutrophil levels following (a) antigen-negative and (b) Mecholyl-positive and negative challenges. Indeed, there was no significant increase of neutrophil levels at any time after antigen-negative challenges, while a decrease was ob-

TABLE IV. Change in NCA and neutrophilic leukocytes after inhalation challenge

Inhalation *	n	NCA	10 min	30 min	60 min
<i>Ragweed</i>					
Positive	17	1,112 ± 273†	128 ± 291‡	480 ± 305	1,033 ± 372§
Negative	15	-18 ± 102	-91 ± 178	-208 ± 243	270 ± 215
<i>β-Acetyl methacholine</i>					
Positive	6	Not done	-408 ± 175	-370 ± 116	-391 ± 211
Negative	6	Not done	-172 ± 182	148 ± 223	-161 ± 289

*Positive, ≥20% ↓ FEV₁; negative, ≤10% ↓ FEV₁.
†Mean difference ± SEM of chemotactic index after minus before inhalation challenge; p < 0.001 compared to negative antigen challenge (Student's t test).
‡Mean difference ± SEM of neutrophils per microliter after minus before inhalation challenge.
§p < 0.01 compared to 60 min after negative antigen challenge; p < 0.001 compared to either positive or negative Mecholyl challenge (Student's t test).

TABLE V. Correlation of neutrophilic leukocytosis and NCA* after positive inhalation challenges

Subject	Increase in NCA †	Increase in neutrophils at 60 min ‡
1	-210	-755
2	2,119	144
3	548	2,410
4	839	692
5	153	1,022
6	3,621	5,818
7	1,250	259
8	1,898	539
9	722	123
10	1,342	316
11	1,740	1,758
12	694	-288
13	1,023	870
14	1,143	990
15	1,492	930
16	596	2,790
17	-63	-51

*Correlation of NCA and neutrophilia: r = 0.61, p < 0.01.
†Chemotactic index post/prechallenge.
‡Neutrophils/mm³ 60 min after minus before challenge.

served after Mecholyl inhalation challenge. In 14 of the 17 subjects with antigen-induced bronchospasm, neutrophil levels were increased at 60 min (123 to 5,818). In three of 17 a decrease occurred (-51, -288, -755), and NCA increased in 15 of 17 subjects with antigen-induced bronchospasm (596 to 3,621), with a mean increase of 1,112 ± 523. The increase in NCA significantly correlated with the increased level of neutrophils seen after inhalation challenge in those subjects exhibiting bronchospasm (r = 0.61, p < 0.01), Table V. No significant correlation existed between chemotactic activity and neu-

trophil levels after antigen-negative challenges (r = -0.44, p > 0.1).

In contrast, neutrophil levels at 60 min were increased in only six of 15 subjects who did not manifest bronchospasm after antigen inhalation (55 to 1,258). The change in NCA in 15 subjects ranged from -911 to +779 (mean change, -18 ± 102), significantly different from the increase in NCA after positive challenges (t = 4.74, p < 0.0001). NCA was not measured after Mecholyl inhalation challenges since we had previously found no increase in NCA after such challenges⁴ and we did not observe an increase in white blood cells or neutrophils. The quantitative changes in peripheral blood lymphocytes and eosinophils after each of the challenges were also determined (not shown). A small (nonsignificant) reduction in lymphocyte levels occurred in antigen-induced bronchospasm, concomitant with the rise in neutrophil levels. Lymphocyte counts did not change following any of the other challenges. Eosinophil levels, as determined by differential cell staining, also did not change significantly during the 60-min period following any of the challenges.

DISCUSSION

Herein we report further in vitro and in vivo characterization of a serum-derived chemotactic activity for neutrophils (NCA) appearing after ragweed-induced bronchospasm. First, we have demonstrated that virtually all of the antigen-induced NCA elutes in the void volume of Sephadex G-150, comprising a molecular weight of ≥150,000 and confirming our original observations using amicon ultrafiltration.⁴ While chemotactic activity is found in other eluates during the column separation, in no other fraction of post-challenge serum was there significantly greater chemotactic activity than seen in the corresponding frac-

tion of pre-challenge serum. The "background" chemotactic activity of these other G-150 column eluates was similar to that observed in normal sera in this and another study.¹³ We cannot determine from these studies whether NCA is present alone or is bound to immune complexes or antibodies, accounting for its separation in this column fraction. Indeed, recently, investigators have been able to bind *in vitro* a low-molecular-weight peptide to IgG or IgM antibodies and it still retained its neutrophil chemotactic properties.¹⁴ Our attempts at further molecular separation of this activity by Sepharose 6B chromatography and QAE Sephadex ion exchange chromatography have been frustrated by the degree of background activity of normal and prechallenge sera. This possible activation of all sera by Sepharose 6B and QAE Sephadex has to date limited our attempts to further localize the NCA of postchallenge fraction I. Additional studies are underway in an attempt to limit this "background" activation. Despite our inability to further purify NCA, we have documented several distinct characteristics. Thus, NCA does function in a dose-dependent fashion in both sera and fraction I. Chemotactic activities in whole sera have traditionally been determined at 5% to 20% concentrations, since at >20% concentrations, various naturally occurring serum inhibitors can mask chemotactic effects.¹⁵ The enhancement of chemotactic activity in postchallenge fraction I at 30% concentration is probably due to a loss of some serum inhibitors as well as concentration of the NCA. It is probable that chemotactic inhibitors were still present in our fraction I, since at concentrations >30% a decrease of activity was observed. Till and Ward¹⁶ reported two naturally occurring inhibitors of chemotaxis eluting at or near the void volume on Sephadex G-150 chromatography. In addition, one of us (M. N.) has observed an increase of serum inhibitory activity occurring during 56° heating.¹⁷ It is likely that the combination of multiple, naturally occurring serum chemotactic factors and inhibitors has contributed to our difficulty in further molecular characterization of NCA. Despite these problems, our other *in vitro* investigations have demonstrated further specificities of NCA. The demonstration of the ability to deactivate NCA only in postchallenge sera is another measure of the occurrence of a unique chemotactic activity demonstrable in post- but not in prechallenge sera. Furthermore, the specificity of the deactivation (demonstrated against complement-derived but not non-complement derived chemotactic factors) argues against this reflecting merely the presence of high-molecular-weight inhibitors, since Till and Ward¹⁶ had obtained different results in their *in*

vitro studies of inactivation of their inhibitors. We had previously determined that NCA was not complement derived. The ability of complement-chemotactic factors to cross-deactivate, and inability of bacterial filtrate to do so, may provide a useful characteristic of NCA in future studies.

Finally, our cell specificity studies indicate that NCA is chemotactic for neutrophilic leukocytes, but not eosinophils or mononuclear cells. This was true of both our whole serum and fraction I activities. Although data are shown at 20% concentration, experiments in which doses of 5% to 100% concentrations were used also failed to reveal any difference in chemotactic activity between pre- and postchallenge specimens.

Although we have confirmed our earlier observations of an antigen-induced *in vitro* chemotactic activity, its *in vivo* biologic role still remains to be determined. Thus, our observations of systemic leukocytosis consisting mostly of neutrophils are pertinent in this regard, since they occur only after antigen-induced bronchospastic responses and correlate with the appearance and cell specificity of NCA. The relationship to antigen-induced bronchospasm is strengthened by the fact that Mecholyl-induced bronchospasm caused neither an increase of NCA⁴ nor an increase in neutrophils. The timing of appearance of *in vivo* neutrophil increases agrees with *in vivo* studies in animal models. Thus, O'Flaherty et al.¹⁸ demonstrated an initial decrease in neutrophil levels after infusion of C5a (over 1 to 3 min), followed by a two to threefold increase in neutrophil levels at 60 to 120 min. This is consistent with our findings of a significant neutrophilia 1 hr after antigen inhalation.

We have previously demonstrated that NCA appears rapidly after induced bronchospasm, and its appearance is significantly related to the amount of antigen inhaled and resultant bronchospasm induced. In addition, its appearance can be inhibited *in vivo* by prior administration of disodium cromoglycate.⁵ These characteristics suggest an anaphylactic origin (presumably the lung mast cells). Another neutrophil chemotactic factor of presumable anaphylactic origin has recently been described as appearing in the serum after cold challenge in patients with idiopathic cold urticaria.⁷ Similarities to NCA include: (1) onset of appearance within 1 min; (2) large molecular size (>750,000 daltons), (3) specificity for neutrophils, and (4) properties of deactivation and concentration dependency suggesting true chemotactic activity.

Whether there are one or more neutrophil chemotactic activities involved, several lines of evidence suggest the need for continued investigation of the

role and mechanism of appearance of neutrophils in IgE-mediated reactions. (1) Peripheral blood neutrophil levels increase after positive ragweed inhalation challenge. (2) Neutrophils are prominent in early^{1, 2} and late-onset,³ immediate hypersensitivity reactions to ragweed antigen. (3) Neutrophils themselves have the ability to modulate the appearance of other inflammatory cells.^{19, 20} (4) The most effective therapeutic agents in the treatment of bronchial asthma, corticosteroids, are also potent antiinflammatory agents and may derive at least some of their beneficial effects by ameliorating neutrophil-dependent, inflammatory lesions.²¹

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