

Basophilic cell progenitors, nasal metachromatic cells, and peripheral blood basophils in ragweed-allergic patients

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Circulating hematopoietic progenitor cells giving rise to colonies containing basophilic cells (basophilic cell colony-forming units in culture [CFU-c]), nasal epithelial metachromatic cells (basophils and/or mast cells) (NMC), and blood basophils were enumerated before, during, and after a ragweed-pollen season in patients with ragweed hayfever and patients with perennial allergic rhinitis who were not ragweed allergic. In the patients with seasonal hay fever, the following was observed: basophilic cell CFU-c, measured as basophilic cell or histamine-containing colonies, were significantly reduced during the ragweed season compared to before ($p < 0.005$) or after ($p < 0.025$) the season in the ragweed-allergic group only. Conversely, peripheral blood basophils were higher during the ragweed season than before or after ($p < 0.001$) in the ragweed-allergic group, whereas the number of NMC was higher during the season than before the season in this group ($p < 0.05$). There were no such changes during the season in the group of patients with perennial allergic rhinitis. The observed seasonal changes in both NMC and circulating basophilic cell CFU-c are discussed in the context of lineage relationships among metachromatic cell types. (J ALLERGY CLIN IMMUNOL 78:365-71, 1986.)

The mechanism of accumulation of basophils and mast cells at tissue sites in hypersensitivity reactions¹ may involve chemotaxis of mature cells from circulation²⁻⁴ and/or proliferation of progenitors in situ.^{5,6} We have recently demonstrated that there are increased numbers of circulating hematopoietic progenitors capable of forming colonies containing basophilic cells in vitro (basophilic cell CFU-c) in atopic patients.⁷ Moreover, we have demonstrated that in a mixed group of patients with allergic rhinitis, the number of peripheral blood basophilic cell CFU-c is inversely related to the number of formalin-sensitive nasal epithelial NMC.⁸ In the present studies we examined the question of whether or not the inverse relation demonstrated between circulating basophilic cell CFU-c and NMC is simply related to the level of sensitivity or to an ongoing IgE-dependent reaction. Accordingly, we have enumerated circulating baso-

Abbreviations used

CFU-c: Colony-forming unit in culture
NMC: Nasal metachromatic cells

philic cell CFU-c, mature basophils, and NMC in patients with pure seasonal, ragweed-induced, allergic rhinitis before, during, and after antigen exposure and compared the findings with those in a group of patients with perennial allergic rhinitis who were not ragweed allergic.

MATERIAL AND METHODS

Subjects

Seven patients with a positive skin test and positive IgE RAST to ragweed antigen and 10 patients with perennial allergic rhinitis, who had a negative skin test to ragweed and a positive skin test to house dust mite or animal dander, were initially selected. One patient from each group was excluded because of unwillingness to continue to participate. There were six women in the ragweed-allergic patient group (group A) with a mean age of 40.6 years ($SD \pm 9.2$, range 30 to 57 years) and four men and five women in the perennial allergic-patient group (group B) with a mean age of 35.6 years ($SD \pm 11.6$, range 17 to 58 years). Medications used by the six ragweed-allergic subjects were as

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follows: H_1 antihistamine in three, ophthalmic cromoglycate in two, nasal beclomethasone (one or two sprays up to twice daily midseason only; stopped 1 week before tests) in two, and inhaled salbutamol in two. Medications used by the nine subjects with perennial-allergic rhinitis were H_1 antihistamine in three, ophthalmic cromoglycate in two, nasal steroid (stopped 1 week before the tests) in two, inhaled salbutamol in three, and inhaled beclomethasone in two. No patient was treated by injection treatment.

Symptoms were assessed by questionnaire in which sneezing, nose blowing, and nasal stuffiness were graded from 0 to 3 according to the severity of each symptom. Scoring was calculated by adding grade of nasal stuffiness together with the higher of either nasal sneezing or nose blowing as previously described.¹⁰

Cell culture and assessment of granulocyte colonies

Cell culture and assessment of granulocyte colonies from peripheral blood of patients were performed as previously described.^{7,8} Heparinized venous blood was layered over Ficol-Hypaque (specific gravity 1.077, Pharmacia Fine Chemicals, Piscataway, N. J.), and methylcellulose (0.9%) cultures of interface mononuclear rich cells (1×10^6 cells per dish) were performed in Iscove's modified Dulbecco's medium supplemented with 20% fetal calf serum, 1% penicillin-streptomycin, and 5×10^{-5} mol/L of 2-mercaptoethanol in the presence of 5% v/v conditioned medium derived from a human T cell line (Mo, kindly supplied by Dr. D. Golde, University of California-Los Angeles).

Granulocyte colonies of ≥ 40 cells were enumerated and classified into neutrophil-macrophage (GM) or eosinophil (Eo) types as previously described.^{7,8} In any cell culture from a given subject, six Eo type and six GM-type colonies were picked at random from replicate dishes and placed in 400 μ l of phosphate-buffered saline for histamine assay; a further 12 Eo-type and six GM-type colonies were placed on slides for differential counting. All assessments were performed after 14 days of culture. A colony with $\geq 5\%$ metachromatic granule-containing polymorphonuclear leukocytes on differential counting was considered "basophilic cell positive." The progenitor of such a colony was defined as a basophilic cell CFU-c.⁸ Since the basophilic cells found in these colonies more closely resembled basophils than mast cells morphologically, they have previously been referred to as basophils.^{7,8}

The absolute number of basophilic cell CFU-c was calculated by multiplying the total number of Eo- or GM-type colonies (per 10^6 cells plated) by the proportion found to contain $\geq 5\%$ basophilic cells by use of criteria described below.⁸

Histochemical techniques

Scraping specimens were taken from anterior surface of the inferior turbinate by a small steel curette (Nagashima, Japan; 2 by 3 mm) as previously described.^{8,10} A total of four small (approximately 4 mm² by 40 μ m) scrapings, two from each nasal cavity, were smeared thinly on the glass slides. After fixation in Mota's basic lead acetate or 10%

buffered formalin (pH 6.9), cell smears were stained with 0.5% toluidine blue^{8,10} in 0.7 N HCl at pH 0.5. The NMC count is the total number of cells in smears from both nasal cavities counted with a magnification of 500. To characterize cell morphology and size, 200 NMC were characterized in each specimen under a magnification of 1250.

Reproducibility, without significant differences in the number of NMC, has previously been established between paired scrapings from the right and left nasal cavities and among three scraping samples collected sequentially from one nasal cavity, processed by Mota's fixation and toluidine blue staining.⁹

Cell types were enumerated as previously described.^{7,10} Briefly, the total number of NMC was determined from the preparation fixed with Mota's basic lead acetate. Two types of NMC, mast cell and basophil, in this preparation, were classified morphologically. Total NMC counts were determined from the preparation fixed with 10% buffered formalin (formalin-resistant mast cell). Formalin-sensitive mast cells were enumerated by subtracting the number of mast cells in the formalin-fixed preparation from those in the smear prepared by Mota's fixation.

For differential counting of cells in individual colonies picked from the methylcellulose cultures, smeared cells were stained with May-Grunwald-Giesma. To confirm the metachromatic nature of the granules, 0.5% toluidine blue at pH 0.5 was used after fixation in Mota's basic lead acetate. Cells in which all granules were metachromatic were counted as basophilic cells; cells with mixed basophilic-eosinophilic granulation were excluded. Direct counting of peripheral blood basophil leukocytes was performed as described by Kimura et al.¹¹

Peripheral blood and nasal secretions were taken from all subjects during the summer and fall of 1984. July 13 to August 13 was considered as pre-season (I), August 27 to September 21 as in-season (II), and October 1 to October 26 as off-season (III). Thus, the seasonal (group A) and perennial (group B) allergic patients each fell into three subgroups: I, II, and III, depending on the time of sampling.

Histamine assay

Histamine assay was performed by automated spectrofluorometric methods.^{12,13} An individual colony was considered histamine positive if it contained ≥ 0.20 ng on this assay.^{7,8,13}

Statistical analysis

For statistical comparisons among groups, F analysis was conducted to determine if variances were equal. If this were true, paired t tests were used; if this were not true, a non-parametric Mann-Whitney U statistic was used.

RESULTS

Pollen counts

The ragweed-pollen count in Hamilton, Ontario, in 1984 demonstrated a mild first peak (58 pollen per cubic meter) on August 15, moderate second peak (203) on August 21, maximal count (480) on August

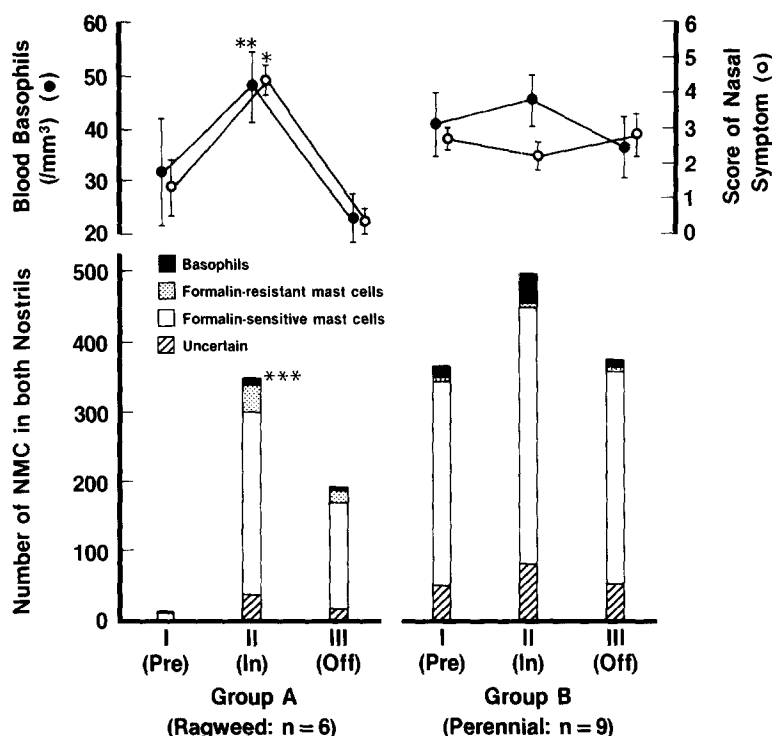


FIG. 1. Nasal symptoms and seasonal kinetics of peripheral basophils and NMC in ragweed and perennial allergic-rhinitis patients. Nasal score was calculated by an index of nasal stuffiness and either nasal sneezing or nose blowing, from 0 to 3, according to the severity of each symptom (see text). *Significantly higher than preseason (group A-I) and off-season (group III) ($p < 0.01$). **Significantly higher than preseason (group A-I) and off-season (group III) ($p < 0.001$). ***Total number of NMC in group A-II was significantly higher than in group A-I ($p < 0.05$) but not group A-III. Number of formalin-sensitive NMC in group A-II was significantly higher than in group A-I ($p < 0.025$) but not group A-III.

27, and last peak (231) on September 7, gradually decreasing to nil by late in September.

Clinical nasal symptoms

Mean ($\pm$ SE) nasal symptom score in group B patients was 2.7 ± 0.4 in group B-I, 2.2 ± 0.4 in group B-II, and 2.9 ± 0.6 in group B-III (not significant); in group A patients, values were: 1.3 ± 0.9 for A-I, 4.3 ± 0.6 for A-II ($p < 0.01$), and 0.3 ± 0.3 for group A-III (Fig. 1).

Kinetics of peripheral blood basophil and NMC

Peripheral blood basophils were significantly higher in group A-II than in groups A-I or A-III ($p < 0.001$); no such changes occurred in group B patients (Fig. 1).

The number of NMC in group A-II was significantly higher than in A-I ($p < 0.05$) but not significantly different from group A-III. There were no differences in NMC in group B subjects at the different times. The number of formalin-sensitive NMC was significantly higher in group A-II than in group A-I ($p < 0.025$) but not significantly different from group

A-III; again, there were no differences in group B subjects. Nasal basophil or formalin-resistant mast cell values were not significantly different at the different times in groups A and B (Fig. 1).

Total colony counts

The total number of Eo-type colonies (per 10^6 cells plated) in group A was 31.5 ± 8.6 for A-I and decreased significantly for A-II ($p < 0.01$) and for A-III ($p < 0.05$); group B values did not differ significantly from each other (Table I). There were no significant differences in numbers of GM-type colonies among the different times of sampling for group A subjects nor in numbers of either Eo- or GM-type colonies for group B subjects (Table I).

Proportion of basophilic cells in colonies and frequency of basophilic cell-positive colonies (basophilic cell CFU-c)

The mean percent of basophils in colonies and frequency of basophilic cell CFU-c in groups A and B subjects are presented in Table II. The proportion of basophilic cells in Eo-type colonies and the frequency

TABLE I. Total and basophilic cell-positive granulocyte colony-forming cells in ragweed and perennial allergic-rhinitis patients

	Total colonies		Basophilic-positive (>5%) colonies	
	Eo	GM	Eo	GM
Group A (n = 6)				
I	31.5 ± 8.6*	5.1 ± 1.1	4.8 ± 1.0	0.6 ± 0.4
II	16.8 ± 4.9	5.8 ± 1.8	0.8 ± 0.4†	0.2 ± 0.2
III	19.1 ± 6.0	6.8 ± 1.0	6.9 ± 2.4	0.2 ± 0.2
Group B (n = 9)				
I	14.1 ± 3.7	3.3 ± 1.3	4.6 ± 1.9	0.8 ± 0.3
II	12.3 ± 3.7	5.5 ± 1.3	2.8 ± 1.7	0.5 ± 0.3
III	9.2 ± 2.6	3.4 ± 0.8	5.9 ± 1.7	0.6 ± 0.2

Values indicate the number of colonies per 10⁶ mononuclear-rich cells plated. Mean ± standard error. A = ragweed-allergic group; B = perennial allergic-rhinitis group; I = preseason; II = in-season; III = off-season.

*Significantly higher than group II and group III ($p < 0.05$).

†Significantly lower than group I ($p < 0.005$) and group III ($p < 0.025$).

TABLE II. Percent basophils and percent basophil CFU-c in granulocyte colonies

	% Basophilic cells in colonies		% Basophilic cell CFU-c*	
	Eo	GM	Eo	GM
Group A (n = 6)				
I	3.0 ± 0.8	1.5 ± 0.5	19.7 ± 5.7	11.1 ± 5.1
II	0.6 ± 0.2†	0.8 ± 0.5	2.5 ± 1.6*	2.7 ± 2.7
III	6.0 ± 2.1	0.9 ± 0.4	34.2 ± 10.9	5.5 ± 3.5
Group B (n = 9)				
I	4.8 ± 1.0	4.3 ± 1.8	35.9 ± 8.6	24.8 ± 9.1
II	3.5 ± 1.6	2.7 ± 1.6	29.9 ± 8.4	10.3 ± 6.6
III	8.7 ± 2.9	2.3 ± 1.0	52.0 ± 9.5	22.2 ± 10.7

Mean ± standard error. Proportion of basophilic cells was calculated from average percent of basophils in picked colonies.

*Cells forming colonies containing >5% basophils.

†Significantly lower than preseason and off-season ($p < 0.05$).

of Eo-type basophilic cell CFU-c were significantly lower in group A-II than in either of groups A-I ($p < 0.05$) or A-III ($p < 0.05$). There were no such differences in GM-type colonies. There were no significant differences among the three sampling times in basophilic cell CFU-c, either Eo- or GM-type, among group B subjects.

Absolute numbers of basophilic cell CFU-c

Mean (±SE) calculated basophilic cell CFU-c numbers (see Methods) according to colony type for each patient group and subgroup are also presented in Table I. The number of Eo-type basophilic cell CFU-c in group A-II was significantly lower than in groups A-I ($p < 0.005$) or A-III ($p < 0.025$); no differences in basophilic cell CFU-c at the different sampling times were noted in group B.

Frequency and number of histamine-positive colonies

The frequency and number of histamine-positive Eo-type colonies in group A-II were likewise significantly lower than in group A-I and group A-III ($p < 0.05$); for GM-type colonies, the percent for group A-II was significantly lower than that of group A-I ($p < 0.05$) but not when compared to group A-III. In contrast, the number of histamine-positive GM-type colonies was similar among the three B subgroups (Tables III and IV). When colony histamine positivity was defined, alternatively, by a calculated value of ≥ 0.5 pg of histamine per colony cell, the frequency of histamine-positive Eo-type colonies in group A-II was also found to be significantly lower than in A-I ($p < 0.025$) and in A-III ($p < 0.05$), and the number of histamine-positive Eo-type colonies in

TABLE III. Frequency of histamine-positive colonies in ragweed and perennial allergic-rhinitis patients

	Histamine			
	≥ 0.20 ng/Colony		≥ 0.5 pg/Colony cell	
	Eo (%)	GM (%)	Eo (%)	GM (%)
Group A (n = 6)				
I	36.8 $\pm$ 6.1	12.7 $\pm$ 4.0	42 $\pm$ 7	19 $\pm$ 4
II	26.3 $\pm$ 8.1*	2.6 $\pm$ 2.6†	19 $\pm$ 4‡	5 $\pm$ 5
III	49.8 $\pm$ 12.5	13.8 $\pm$ 6.6	39 $\pm$ 8	10 $\pm$ 5
Group B (n = 9)				
I	38.3 $\pm$ 7.7	15.1 $\pm$ 10.9	54 $\pm$ 8	19 $\pm$ 10
II	44.1 $\pm$ 9.2	5.9 $\pm$ 4.0	46 $\pm$ 7	5 $\pm$ 3
III	29.6 $\pm$ 6.6	9.8 $\pm$ 6.1	46 $\pm$ 7	6 $\pm$ 4

Mean $\pm$ standard error.*Significantly lower than group I and group III ($p < 0.05$).†Significantly lower than group I ($p < 0.05$) but not group III.‡Significantly lower than group I ($p < 0.025$) and group III ($p < 0.05$).**TABLE IV.** Number of histamine-positive colonies in ragweed and perennial allergic-rhinitis patients

	Histamine-positive colonies (per 10^6 cells plated)			
	$(\geq 0.2$ ng/Colony)		$(\geq 0.5$ pg/Cell)	
	Eo	GM	Eo	GM
Group A (n = 6)				
I	11.2 $\pm$ 4.1	0.8 $\pm$ 0.2	11.7 $\pm$ 3.0	0.9 $\pm$ 0.2
II	5.1 $\pm$ 1.9*	0.1 $\pm$ 0.2	3.9 $\pm$ 1.2†	0.1 $\pm$ 0.1
III	10.0 $\pm$ 4.2	1.1 $\pm$ 0.6	6.9 $\pm$ 2.5	0.8 $\pm$ 0.5
Group B (n = 9)				
I	6.5 $\pm$ 2.6	0.4 $\pm$ 0.4	7.2 $\pm$ 1.9	0.5 $\pm$ 0.2
II	7.2 $\pm$ 3.0	0.2 $\pm$ 0.2	5.8 $\pm$ 1.7	0.3 $\pm$ 0.2
III	3.5 $\pm$ 1.3	0.3 $\pm$ 0.2	3.9 $\pm$ 1.0	0.2 $\pm$ 0.1

Mean $\pm$ standard error.*Significantly lower than group I and group III ($p < 0.05$).†Significantly lower than group I ($p < 0.05$) but not group III.

group A-II was significantly lower than in group A-I ($p < 0.05$) but not in group A-III. There were no significant differences among all three group A subgroups for histamine-positive GM-type colonies (Tables III and IV).

There were no significant differences in the frequency and number of histamine-positive Eo- or GM-type colonies among the three B subgroups.

DISCUSSION

The number and type of NMC in nasal secretions and scrapings in allergic rhinitis have been previously reported.¹⁰ These cells, as well as blood basophils, can be observed to increase during seasonal pollenosis.¹⁴⁻¹⁶ The present studies demonstrate that the

number of mature basophils, basophilic cell CFU-c, and NMC change significantly in ragweed-allergic subjects during the ragweed season; these fluctuations are not observed in a comparable group of subjects with perennial allergic rhinitis who are not allergic to ragweed.

Our finding of fluctuating levels of circulating human hematopoietic progenitors capable of basophilic differentiation during an ongoing antigen-IgE antibody reaction raises the question of the fate of such progenitors in vivo. Both Guy-Grand et al.⁶ and Crapper and Schrader¹⁷ and Crapper et al.¹⁸ have demonstrated evidence for traffic of intestinal mast cell precursors in the mouse. We have previously demonstrated a general increase of circulating basophilic cell

CFU-c in atopic patients compared to nonatopic individuals⁷ and have recently found that the level of peripheral blood basophilic cell CFU-c is inversely related to the number of NMC in nasal scrapings taken from randomly selected allergic-rhinitis patients, including those with seasonal and perennial symptoms.⁸

In the present studies, we investigated relationships among basophilic cell CFU-c, NMC, and blood basophils in nasal allergic patients with pure ragweed-allergic rhinitis, compared to a group of patients with nonragweed, perennial allergic rhinitis. The number of basophilic cell CFU-c in ragweed-allergic subjects decreased dramatically during the ragweed season ($p < 0.005$) compared to before or after the season but remained unchanged in perennial allergic-rhinitis patients and higher than in nonatopic patients.^{7,8} Similar data were obtained with histamine rather than morphology as a marker of basophilic cell-containing granulocyte colonies. These results were found by analysis of colonies at day 14 in vitro, when maximum colony growth is observed for all groups of patients. Earlier than day 14, inadequate colony growth precludes analysis; later than day 14, cells in colonies begin to die, and analysis is likewise difficult. As further support of a relationship between progenitors and tissue basophil/mast cells, we have observed that in some patients there are high levels of circulating basophilic cell CFU-c in the peripheral blood in the presence of positive allergy skin tests, a normal level of blood basophils, but in the absence of allergic symptomatology (data not presented). These patients may represent that subpopulation of atopic subjects in whom specific IgE levels do not correlate with symptoms¹⁹; one explanation for the lack of symptoms may be inhibition of access to tissue sites and/or of differentiation of basophilic cell CFU-c within nasal mucosa.

Recently, others and we have demonstrated that supernatants released from activated T cells stimulated by mitogen or antigen are required for elaboration of interleukin-3 and growth of subtypes of mast cells or basophils in animals and man.²⁰⁻²⁵ In addition, human keratinocytes,²⁶ circulating human T cells taken from patients undergoing chronic antigen exposure locally in the skin,²⁷ and nasal polyp epithelial cells²⁸ produce basophil and eosinophil colony-stimulating activities. The relative roles of basophil/mast cell growth and differentiation^{7,8} and chemotaxis^{2-4,29} in allergy need to be evaluated further.

Whether or not basophilic cell CFU-c migrate from peripheral blood into the nasal mucosa and differentiate into a subpopulation of NMC in the nasal mucosa also needs to be further investigated. Histochemical

evidence we have recently obtained has demonstrated that the basophilic cells in cultured colonies are invariably formalin sensitive,⁸ suggesting a lineage relationship to formalin-sensitive NMC.

Studies of resident nasal basophilic cell CFU-c and basophil colony-stimulating activity^{21,28} as well as functional profiles of NMC and colony cells are now in progress.

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