

Histologic studies of human skin test responses to ragweed, compound 48/80, and histamine*

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The patterns of mast cell and eosinophil changes at the site of intradermal injection of ragweed, compound 48/80, and histamine were studied by histologic techniques. Dermal biopsies of 23 ragweed skin test-positive subjects revealed increasing numbers of eosinophils and decreasing numbers of mast cells over a 240 minute period following injection of antigen. The eosinophil response began in the periappendigeal areas, extended into the interstitium, and occurred in sites with depressed wheal reactions in hydroxyzine-treated subjects. These cellular patterns did not occur at ragweed skin test sites in nonatopic subjects. However, compound 48/80 induced similar eosinophilic responses in both atopics and nonatopics. Histamine injection was followed by no eosinophilic or mast cell changes. These findings support the hypothesis that eosinophil responses in reagin-mediated reactions may occur secondary to release of a mediator other than histamine of mast cell origin.

The wheal and flare skin test response induced by antigen is one of the most useful diagnostic tools of the allergist. Yet relatively little is known about the histologic patterns in humans during this reaction. Kline, Cohen, and Rudolph¹ in 1932 performed dermal biopsies at various times following intradermal injections of ragweed, histamine, and other antigens in skin test-reactive subjects. They observed an eosinophil infiltration that was most prominent 30 minutes after injection, decreased gradually over the next 3 hours, and continued at a low level for the remaining 22 hours of their study. They noted a similar eosinophil response after histamine injection in their atopic but not in their nonatopic subjects. In recent years, studies of these cellular events in humans have utilized skin window techniques consisting of the application of glass slides to abraded skin sites for varying periods of time after application of antigen. Investigators²⁻⁴ using this technique on reactive subjects have demonstrated that an exudation of eosinophils begins at 6 to 8 hours and peaks at 24 hours after application of antigen. However, there has been a difference in observations as to whether histamine injection induced an eosinophilic response.

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In their more recent skin window observations, Felarca and Lowell⁵ observed both basophils and eosinophils after application of antigen to atopic subjects. They found a correlation between clinical symptoms and eosinophil response but could not correlate the basophil response with either eosinophils or clinical symptoms.

Investigations of these cellular responses in biopsy specimens would permit determinations of the temporal patterns of cellular deposition in different areas of the reaction site. In addition, responses of the stainable mast cells in these reactions can be evaluated. Therefore, we have undertaken to determine by histologic techniques: (1) the sequential patterns of eosinophil and mast cell responses after intradermal injections of ragweed, histamine, and compound 48/80 (a mast cell degranulator) and (2) the comparison of the ragweed-induced cellular responses before and during suppression of wheal formation by hydroxyzine hydrochloride.

MATERIALS AND METHODS

Twenty-three males (21 to 33 years of age) with whealing responses of greater than 8 mm. to whole ragweed extract (1,000 PNU per milliliter) were selected. None had received ragweed immunotherapy. All gave family histories of atopy; 21 had clinical symptoms of rhinitis only during the ragweed season, and 2 had perennial rhinitis with exacerbations during the ragweed season. They were considered the atopic, ragweed-sensitive population. Six males (23 to 30 years of age) without either a personal or family history of atopy and with a mean 2 mm. wheal response to ragweed served as the nonatopic population.

Replicate intradermal injections of reagents were made in the dorsal surface of the upper arms, with reading of the mean diameter of wheal response (in millimeters) 20 minutes later. Three-millimeter punch biopsies were performed as described previously⁶ exactly at the site of injection of the reagent, starting immediately after injection (time 0) and as long as 48 hours later. Just prior to the performance of each biopsy, local anesthesia was achieved by the injection of 1 per cent procaine in 0.05 ml. aliquots in a circular pattern at least 30 mm. from the biopsy site. Previous studies carried out in our laboratory have shown that local anesthesia utilized in this fashion did not alter the histologic pattern seen in the biopsies.

Biopsies were done in 23 atopic subjects after intradermal injection of 20 PNU of whole ragweed extract.* On 15 of these atopic subjects biopsies were also done after injection of 700 μ g of compound 48/80,† and biopsies were also done on 11 of the atopic subjects after injection of 1 to 11 μ g of histamine phosphate.‡ Five atopic subjects were re-tested with ragweed and biopsies were done on them during the fifth day of administration of hydroxyzine hydrochloride,§ 25 mg. postoperatively four times a day. The 6 nonatopic subjects underwent biopsies at various times after injection of 20 PNU of whole ragweed extract and 700 μ g of compound 48/80.

The biopsies were fixed in neutral buffered formalin for at least 24 hours, embedded in paraffin, sectioned, and stained in May-Greenwald-Giemsa solution (equal volumes of 200 mg. per cent May-Greenwald stain in absolute methanol and freshly prepared 3 per cent Giemsa solution). Sections were coded, arranged randomly, and read in a blind fashion for eosinophils

*0.02 ml. of 1,000 PNU per milliliter solution prepared in the Allergy and Immunology Clinic, Hospital of the University of Pennsylvania.

†0.02 ml. of 35 mg. per milliliter solution of a single lot of compound 48/80 (Burroughs Wellcome & Co., Research Triangle Park, N. C.).

‡0.02 ml. of 1:5 to 1:50 dilutions of a single lot of 2.75 mg. per milliliter solution of histamine phosphate (Eli Lilly & Co., Indianapolis, Ind.)

§Atarax (J. B. Roerig & Co., Division of Chas. Pfizer & Co., Inc., New York, N. Y.)

and mast cells. All areas in each biopsy were scanned under low power ($\times 160$) magnification, and 3 reactive areas were selected in both the periappendigeal and interstitial areas for more intensive study under high power ($\times 720$) magnification. The reactive areas were chosen under low power to be representative, geographically, of different portions of the dermis in the specimen. The degrees of infiltration in a reactive area by various cell types were not obvious under low power and were not a factor in the choice of these areas for further analysis. Highly reproducible readings in reactive areas of sequential serial sections of the same specimen suggested that examination of the single section gave representative readings. In addition, a total cell count reading of selected biopsies revealed patterns of cellular responses similar to that seen when the 3 representative reactive areas were studied. Mast cells and eosinophils were counted, and the results were expressed as number of cells per high-power field (hpf) in periappendigeal and interstitial areas. Periappendigeal areas (P.) were defined as the areas within 1 hpf of a skin appendigeal structure (sebaceous gland, sweat gland, hair follicle). Interstitial areas (Int.) were defined as areas of loose connective tissue of the dermis, excluding periappendigeal areas. Eosinophils appeared as bilobed cells with bright orange-red granules, whereas mast cells were elongated with thin filiform tails and purplish granules. The cytologic details of each cell type were confirmed under oil immersion ($\times 1600$) in all instances.

RESULTS

Whealing responses

In atopic subjects, the mean wheal responses (average diameter) 20 minutes after injection were: 16 mm. for ragweed (range, 9 to 50 mm.); 16 mm. for compound 48/80 (range, 11 to 30 mm.); and 10 mm. for histamine (range, 8 to 12 mm.). In the 6 nonatopic subjects, mean wheal responses were 2 mm. (range, 0 to 5 mm.) for ragweed and 19 mm. (range, 10 to 22 mm.) for compound 48/80. No correlation was observed between the size of the wheal response 20 minutes after injection of ragweed, compound 48/80, and histamine and subsequent eosinophil or mast cell responses at any of the time periods studied.*

The 5 atopic subjects who underwent ragweed skin test biopsies during hydroxyzine administration exhibited 77 per cent (range, 27 to 81 per cent) inhibition of their initial wheal responses. In 2 of the 5 individuals, wheal responses during hydroxyzine administration were less than 6 mm.

Cellular responses in atopic subjects

Responses after ragweed. The cellular responses of atopic subjects after ragweed administration are presented in Table I, A. Eosinophils were observed in the P. areas of the dermis 20 minutes after ragweed injection. They generally continued to increase in these areas through the 240 minute period studied, although the maximal rate of increase was observed between 20 and 60 minutes. Eosinophils in these P. areas were frequently first seen within and around vessels.

In 10 of the atopic subjects, cellular responses were studied 48 hours after injection of ragweed. No gross sign of reaction was present at this time. In 6 of the 10 individuals, eosinophilic infiltration was present in the P. areas (range, 2 to 40 per high-power field). There was no correlation between the size of the

*Tested by linear regression analysis and correlation coefficients determined by sum of the least squares.⁷

TABLE I. Cellular responses of atopic subjects

	P.					
	0*	20	60	120	240	48 hr.
A. Ragweed						
Eosinophils†	0	2.7 ± 1.0	5.4 ± 2.0	6.4 ± 1.3	6.9 ± 1.7	7.6‡ ± 3.9
Mast cells	2.2 ± 0.80	2.6 ± 0.51	1.2 ± 0.32	1.3 ± 0.30	0.83 ± 0.22	1.0 ± 0.29
Number of subjects	5	23	13	18	18	10
B. Compound 48/80						
Eosinophils†	0	2.4 ± 0.66	—	3.3 ± 1.2	5.9 ± 1.9	3.5‡ ± 1.7
Mast cells	2.0 ± 0.68	1.6 ± 0.38	—	1.8 ± 0.54	1.1 ± 0.41	1.6 ± 0.64
Number of subjects	5	15	—	6	9	9
C. Histamine						
Eosinophils†	—	0.20 ± 0.14	—	—	0.03 ± 0	—
Mast cells	—	1.4 ± 0.34	—	—	1.6 ± 0.20	—
Number of subjects	—	11	—	—	11	—

*Time in minutes after injection of antigen.

†Mean number of cells per high-power field ± S.E.M.

‡See text for interpretation.

immediate (20 minute) wheal or eosinophil response and the presence or absence of eosinophils at 48 hours.

Eosinophils were not seen in the Int. areas until 120 minutes following ragweed injection, with peak infiltrations at 240 minutes. At 48 hours, eosinophil responses were seen in the Int. areas only in 6 subjects who exhibited eosinophilic responses in the P. areas at the same time.

The successive increases in P. area eosinophils from time 0 to 20 minutes and then at 120 to 240 minutes were statistically significant. Interstitial eosinophil responses observed at 120 to 240 minutes were also significantly greater than that seen at 0, 20, and 60 minutes after ragweed injection, with a subsequent significant decrease in eosinophils in the interstitial areas of biopsies obtained at 48 hours.

There was a different pattern of changes in the mast cell population following ragweed injection. Stainable mast cells appeared to decrease in the P. areas from 60 through 240 minutes in comparison with earlier biopsies. Although these decreases were statistically significant, they were not striking, possibly as a

Int.					
0*	20	60	120	240	48 hr.
0.13	0.15	0.38	2.9	4.6	0.60†
±	±	±	±	±	±
0.13	0.09	0.20	0.83	0.98	0.27
1.4	1.2	0.58	0.77	0.60	0.83
±	±	±	±	±	±
0.75	0.23	0.24	0.26	0.22	0.23
5	23	13	18	18	10
0	0.31	—	3.2	2.8	0.50†
	±		±	±	±
	0.20		1.9	1.4	0.27
2.3	1.0	—	0.67	0.77	0.93
±	±		±	±	±
0.63	0.23		0.33	0.26	0.34
5	15	—	6	9	9
—	0	—	—	0	—
—	0.82	—	—	1.1	—
	±			±	
	0.18			0.73	
—	11	—	—	11	—

reflection of the relatively low mast cell count at time 0. Mast cell counts were still decreased at 48 hours. The number of these cells in the interstitial areas was too low at any time to allow for meaningful analysis. Mast cells were never seen within vessels.

Responses to ragweed during hydroxyzine administration. The eosinophil and mast cell responses to ragweed before and during administration of hydroxyzine are presented in Table II. Despite the previously noted 77 per cent inhibition of wheal size, prominent eosinophil responses were seen in the P. and Int. areas during hydroxyzine administration. Comparable biopsies from the same subject taken before and during hydroxyzine therapy could not be distinguished when read in a blind fashion utilizing coded slides. Modest decreases in stainable mast cells in P. areas were also noted 120 and 240 minutes after ragweed administration. Although this study group was too small to afford statistical comparisons, there appears to be a lack of inhibition of the eosinophil responses by hydroxyzine therapy.

Responses after compound 48/80. The cellular responses of atopic subjects

TABLE II. Cellular responses of atopic subjects to ragweed before and during hydroxyzine

	P.				Int.			
	20*	60	120	240	20*	60	120	240
A. Before hydroxyzine								
Eosinophils†	6.0 ± 4.3	6.7 ± 4.8	4.6 ± 2.8	4.5 ± 2.7	0.20 ± 0.12	0.70 ± 0.44	1.8 ± 0.85	2.8 ± 1.4
Mast cells	0.30 ± 0.24	1.1 ± 0.48	0.90 ± 0.53	0.80 ± 0.56	0.70 ± 0.20	0.60 ± 0.37	0.30 ± 0.12	0.80 ± 0.58
Number of subjects	5	5	5	5	5	5	5	5
B. During hydroxyzine								
Eosinophils†	5.4 ± 0.94	5.1 ± 1.7	8.7 ± 3.9	6.0 ± 1.9	0.10 ± 0.10	0.70 ± 0.58	5.2 ± 2.3	6.8 ± 3.3
Mast cells	1.1 ± 0.53	0.80 ± 0.30	0	0	0.20 ± 0.12	0.10 ± 0.10	0	0
Number of subjects	5	5	5	5	5	5	5	5

Biopsies were obtained during the 5th day of administration of 25 mg. hydroxyzine hydrochloride orally 4 times a day.

*Time in minutes after injection of antigen.

†Mean number of cells per high-power field ± S.E.M.

to compound 48/80, a mast cell degranulator, are presented in Table I, *B*. The eosinophil responses following injection of compound 48/80 were very similar to those seen after ragweed. Eosinophils increased significantly in P. areas from 20 through 240 minutes and in Int. areas from 120 through 240 minutes compared to base line concentrations. At 48 hours, 4 of 9 subjects tested exhibited eosinophils in P. and Int. areas. All 4 of these subjects had exhibited 48 hour eosinophil responses to ragweed. There were no eosinophils seen in the remaining 5 subjects. A modest decrease in stainable mast cells was observed in both P. and Int. areas after injection of compound 48/80, compared to base line levels.

Responses after histamine. The cellular responses of atopic subjects to histamine are presented in Table I, *C*. Virtually no eosinophils were seen in P. or Int. areas after injection of from one to 11 μ g of histamine. This pattern was significantly different from that occurring after injection of ragweed or compound 48/80 (described above). Stainable mast cells were virtually the same in 20 and 240 minute biopsies. In selected individuals (not shown in Table I, *C*), the mast cell levels in biopsies obtained at time 0 and at 20 minutes were also similar.

Cellular responses of nonatopic subjects

Responses after ragweed. The responses of nonatopic subjects after ragweed injection are depicted in Table III, *A*. Virtually no eosinophils were seen in P. or Int. areas at all time periods observed. Stainable mast cells did not appear to decrease after ragweed injection in these subjects. Comparison of these responses to ragweed with those of atopics showed that the latter exhibited

TABLE III. Cellular responses of nonatopic subjects

	P.				Int.			
	0*	20	120	48 hr.	0*	20	120	48 hr.
A. Ragweed								
Eosinophils†	0	0	0	0	0	0	0.083 ± 0.077	0
Mast cells	2.8 ± 1.1	2.6 ± 0.88	4.1 ± 0.80	2.75 ± 0.75	2.0 ± 0.82	1.0 ± 0.34	1.9 ± 0.20	2.4 ± 0.94
Number of subjects	4	6	6	4	4	6	6	4
B. Compound 48/80								
Eosinophils†	0	0	2.4 ± 0.69	3.2 ± 2.1	0	0	1.1 ± 0.82	1.0
Mast cells	3.3 ± 1.1	1.9 ± 1.3	2.3 ± 0.95	2.3 ± 0.76	2.4 ± 1.3	1.1 ± 0.29	0.92 ± 0.27	1.7 ± 0.64
Number of subjects	4	6	6	4	4	6	6	4

*Time in minutes after injection of antigen.

†Mean number of cells per high-power field ± S.E.M.

significantly (1) greater P. area eosinophils at 20 and 120 minutes, (2) greater Int. area eosinophils at 120 minutes, and (3) fewer stainable mast cells in both P. and Int. areas at 120 minutes.

Compound 48/80 responses. The responses of nonatopic subjects after compound 48/80 injection are presented in Table III, B. Significant increases in the eosinophils in P. areas were seen 120 minutes after injection. An eosinophil response in P. and Int. areas was observed at 48 hours in 2 of the 4 nonatopic subjects studied. Mast cells appeared to decrease from 20 through 120 minutes after compound 48/80 injection. This decrease was more obvious in the Int. than in the P. areas. These changes following compound 48/80 injection were different than those observed at ragweed skin test sites in the same subjects. Injection of compound 48/80 appeared to result in a less pronounced eosinophilic response in nonatopic than in atopic subjects; however, the pattern of mast cells responses to compound 48/80 was not significantly different for the 2 groups of subjects.

Statistical analysis of results. The statistical analysis of results is summarized in Table IV.

DISCUSSION

Eosinophils have long been recognized in allergic reactions, but their role is still not clear. Litt⁸ had demonstrated, in guinea pigs, that eosinophils are attracted to and ingest antigen-antibody complexes. Recently, Hubscher and Eisen,⁹ using I₁₂₅-labeled antigen E and fluorescein conjugated IgE, demonstrated binding of antigen and antibody to the circulating eosinophil in humans. In addition, Wicher and co-workers¹⁰ demonstrated the presence of Candida

TABLE IV. Statistically significant differences of mean cellular responses***A. Atopic subjects**

Type of cell	Location	Ragweed						
		0/20†	0/60	0/120	0/240	20/120	20/240	60/120
Eosinophils	P.	S.	S.	H.S.	H.S.	S.	S.	N.S.
	Int.	N.S.	N.S.	H.S.	H.S.	H.S.	H.S.	H.S.
Mast cells	P.	N.S.	S.	N.S.	N.S.	H.S.	H.S.	N.S.
	Int.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

B. Nonatopic subjects

Type of cell	Location	Nonatopic/atopic‡		Ragweed/compound 48/80		Compound 48/80	Nonatopic/atopic¶	
		20	120	20	120	0/120#	20	120
Eosinophils	P.	S.	H.S.	N.S.	H.S.	H.S.	H.S.	N.S.
	Int.	N.S.	H.S.	N.S.	H.S.	N.S.	N.S.	S.
Mast cells	P.	N.S.	H.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Int.	N.S.	H.S.	N.S.	H.S.	H.S.	N.S.	N.S.

S. = $p < 0.05$. H.S. = $p < 0.001$. N.S. = Not significant.

*t test analysis of means.

†Comparison of mean cellular responses between times in minutes after antigen injection.

‡Comparison of mean cellular responses after histamine with responses after ragweed or compound 48/80.

§Comparison of atopic and nonatopic responses after ragweed.

||Comparison of responses after ragweed and compound 48/80 in nonatopic subjects.

¶Comparison of nonatopic and atopic responses after compound 48/80.

#Comparison of time 0 and 120 minute responses after compound 48/80 in nonatopic subjects.

antigen-antibody complexes within the circulating eosinophils of humans. These studies suggest that eosinophils may function as specific phagocytic cells for antigen-antibody and IgE-mediated reactions. In the present study, the ragweed-induced eosinophil response was limited to the ragweed-sensitive subjects, in accord with previous investigations.¹⁻⁵ The sequential localization of eosinophils, initially in the perivascular periappendigeal areas and subsequently in the interstitial areas, is consistent with current theories that tissue eosinophils are derived from the peripheral, circulating pool. The peripheral blood origin of tissue eosinophilia has been indirectly demonstrated by Patterson, Talbot, and Booth¹¹ in the respiratory mucosa of *Ascaris*-sensitized monkeys. The timing of the dermal eosinophil response that we observed, beginning at 20 minutes and peaking 2 to 4 hours after antigen or compound 48/80 administration, is somewhat different from earlier histologic observations and skin window observations cited previously.¹⁻⁵ The significance of the eosinophils seen 48 hours after the injection of ragweed and compound 48/80 in some atopic subjects requires further investigation. However, in a previous study from this laboratory,⁶ eosinophils were not found at 48 hours after ragweed injections that elicited only a delayed-appearing reaction.

The mechanisms leading to the appearance of the eosinophils in the reactions to ragweed and compound 48/80 are not clear. Histamine release in the allergic

Compound 48/80†				Histamine/ragweed‡		Histamine/compound 48/80‡	
60/240	0/20	0/120-240	20/120-240	20	240	20	240
N.S.	H.S.	H.S.	N.S.	S.	H.S.	H.S.	H.S.
H.S.	N.S.	S.	S.	N.S.	H.S.	N.S.	S.
N.S.	N.S.	N.S.	N.S.	N.S.	S.	N.S.	N.S.
N.S.	N.S.	S.	N.S.	N.S.	N.S.	N.S.	N.S.

reaction is unlikely as a cause. There was no gross correlation between the size of the wheal response and the degree of eosinophilic infiltration. Conversely, suppression of whealing responses in hydroxyzine-treated individuals was unaccompanied by changes in the timing or localization of the eosinophilic responses in the biopsy sites. Furthermore, injection of sufficient histamine to cause a prominent wheal did not lead to eosinophilic accumulation in the same subjects. It is possible that histamine might be liberated in greater amounts or in a different way in positive wheal and flare skin reactions to antigen than would occur with direct intradermal injection of histamine. However, in preliminary studies of cellular responses in sensitized subjects to the intradermal injection of progressively smaller doses of ragweed antigen, we have found eosinophil accumulations in minimally positive reactions.

Recently, several investigators, using in vitro assay systems, have demonstrated various chemotactic factors for eosinophils. Ward¹² demonstrated a complement-dependent chemotactic factor for eosinophils and neutrophils that probably represents the trimolecular complex $C_{5,6,7}$. Kay¹³ demonstrated, in guinea pigs, a specific eosinophilotactic factor of 68,000 M. W. that is complement-dependent (ECF-C). More recently he has demonstrated, in both guinea pigs and humans, a second ECF (ECF-A) of 500-1,000 M. W. generated by incubating ragweed with human lung fragments passively sensitized with IgE from atopic, ragweed-sensitive individuals.¹⁴ He has shown that ECF-A functions independently of the complement system. Furthermore, Kay showed that neither histamine, prostaglandins, kinins, nor SRS-A were effective in the in vitro chemotaxis of eosinophils. Thus, both in vitro and in vivo investigations have demonstrated that substances other than histamine are important for eosinophil chemotaxis.

In addition to eosinophils, tissue mast cells have been investigated in reagin-mediated reactions, because of the relatively large amounts of histamine and other vasoactive substances they contain.¹⁵ Ishizaka, Ishizaka, and Tomoika,¹⁶ using I_{131} -IgE, demonstrated IgE localized to mast cells of monkey skin. Hubscher, Watson, and Goodfriend¹⁷ found localization of antigen E to dermal mast cells of monkeys. Ishizaka, Ishizaka, and Tomoika¹⁸ have recently shown that mast cells from the lungs of monkeys sensitized by IgE and IgG will release histamine and SRS-A after challenge with anti-IgE but not after challenge with anti-IgG. Patterson and Suszko,¹⁹ using bronchial mucosa of *Ascaris*-sensitive monkeys, demonstrated noncytotoxic mast cell granule dissolution, without extrusion of

granules from the cell, after exposure to antigen or compound 48/80. The timing of the degranulation was similar for antigen or compound 48/80, occurring from 5 to 12 minutes after incubation with these agents. Mast cell responses in humans in reagin-mediated reactions have been studied recently by several investigators. Callerame and associates²⁰ have found that atopic patients with seasonal allergic rhinitis have lower mast cell counts in their nasal mucosa than nonatopic patients or atopic patients with perennial rhinitis. Connell,²¹ using postmortem lung tissue from asthmatics and nonasthmatics, has observed lower mast cell counts in lung tissue from the asthmatics. Hubscher and associates²² have demonstrated in human skin that antigen E and IgE are localized to mast cells. However, at the site of wheal formation induced by antigen E, they have observed a loss of staining of mast cells. Concomitant with this loss of staining, IgE or antigen E became diffusely located in the dermis from 24 to 72 hours after antigen challenge.

We have observed a modest decrease in stainable mast cells in atopic subjects after ragweed injection coincident with an observed increase in eosinophils. The pattern of mast cell responses after ragweed in atopic subjects was significantly different from that seen in nonatopic subjects. Histamine injection did not result in any changes in mast cell concentration. Furthermore, compound 48/80 (a mast cell degranulator) produced eosinophil responses in atopic and nonatopic subjects. As noted above, the decreases in skin mast cell concentrations following ragweed and compound 48/80 were modest in the present study and require confirmation in a larger group of subjects before a definitive conclusion can be reached. The base line concentration of stainable mast cells here was low, requiring rather striking decreases to be deemed significant, as has been pointed out in a previous study by Connell.²¹ Degranulation of tissue mast cells may be a relatively late and insensitive method of assaying activation of these cells. It has been shown in animal models that histamine release can occur before degranulation of mast cells.^{23, 24}

We may, therefore, be overlooking subtle, early mast cell changes that may be as important or more important than loss of granular staining of these cells. Within these considerable limitations, it is conceivable that the eosinophil accumulations seen here occur through activation of tissue mast cells by cell-bound IgE-antigen interaction. This interaction could conceivably then release a humoral factor (possible ECF) from the mast cell, which is different from histamine. Additional studies may clarify the role of these mediators in the cellular responses.

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