

Comparative ultrastructural morphology of human basophils stimulated to release histamine by anti-IgE, recombinant IgE-dependent histamine-releasing factor, or monocyte chemotactic protein-1

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An ultrastructural analysis of human basophils stimulated with anti-IgE, recombinant histamine-releasing factor (rHRF), or monocyte chemotactic protein-1 (MCP-1) (compared with unstimulated cells) was performed. Partially purified peripheral blood basophils were prepared for electron microscopy at time points known to precede histamine release and at half-maximum histamine release times for each secretagogue. Activation morphologies associated with stimulation included granule-vesicle attachments, piecemeal degranulation, anaphylactic degranulation, and uropod formation. These features were qualitatively similar in the stimulated samples. Quantitative differences were evident, however, when stimulated samples were compared with controls or at different time points after stimulation with a single agent or when individual secretagogues were compared. All stimulated samples differed quantitatively from the control samples. Rank orders for morphologic activation events revealed that the most effective trigger for anaphylactic degranulation was anti-IgE > MCP-1 > rHRF, whereas the most effective trigger for uropod formation was rHRF > anti-IgE > MCP-1. Rank orders for piecemeal degranulation and granule-vesicle attachments were the same: MCP-1 > anti-IgE > rHRF. Important relationships among these anatomic events reveal that the development of motile configurations is not associated with the development of secretion morphologies and that piecemeal degranulation precedes and is inversely related to anaphylactic degranulation in stimulated samples. (J Allergy Clin Immunol 1996;98:355-70.)

Key words: Human basophil, secretion, electron microscopy, anti-IgE, recombinant IgE-dependent histamine-releasing factor, monocyte chemotactic protein-1, piecemeal degranulation, anaphylactic degranulation, motile structures, vesicle transport

Biochemical mechanisms responsible for histamine secretion from human basophils have undergone intensive study, exceeding reports of associ-

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Abbreviations used

AND:	Anaphylactic degranulation
CLC:	Charcot-Leyden crystal
FMLP:	formyl-methionyl-leucyl-phenylalanine
GVA:	Granule-vesicle attachment
HRA:	Histamine-releasing activity
HRF:	Histamine-releasing factor
MCP-1:	Monocyte chemotactic protein-1
PMD:	Piecemeal degranulation

ated morphologic mechanisms. Studies of the direct stimulation of histamine release from basophils include those with IgE, small peptides,

calcium ionophore, or phorbol esters. Agents that influence the stimulation of histamine release may act indirectly by priming for histamine release, which is induced later by exposure to a direct secretagogue,¹ and IgE-dependent histamine release may vary as a function of IgE heterogeneity.^{1,2} Morphologic mechanisms associated with the release of histamine from stimulated basophils include the direct extrusion of individual, membrane-free secretory granules to the extracellular milieu or extrusion of granules in aggregate into newly constructed cytoplasmic degranulation chambers. Both of these secretory events require antecedent granule-to-granule and/or granule-to-plasma membrane fusions. Another morphologic mechanism associated with secretion is the gradual emptying of cytoplasmic secretory granules in the absence of granule-to-granule or granule-to-plasma membrane fusions.³ The former mechanism is generically termed *anaphylactic degranulation* (AND), and the latter is termed *piecemeal degranulation* (PMD). The ultrastructural morphology of AND and PMD has been well defined and constitutes the anatomic extremes of a continuum, which was postulated as a general degranulation model for basophils in 1975.⁴ This model states that basophils may release small packets of granule materials over time—a process that is effected by the trafficking of small vesicles and leads to the piecemeal losses of granule contents that are characteristic of PMD. If the rate of vesicular traffic increases sufficiently, vesicles may form tubules (by vesicle-to-vesicle fusions, as vesicles bud from granules)—structures that facilitate granule-to-granule or granule-to-plasma membrane fusions leading to the morphologic release pattern termed *AND*. This continuum has been illustrated in human basophils reacting to stimulation with formyl-methionyl-leucyl-phenylalanine (FMLP).⁵ Documentation of this morphologic continuum in the repertoire for histamine release from basophils and instances giving rise primarily to either PMD (as in contact allergy⁶) or AND (as in IgE-mediated stimuli⁷) suggest that therapeutically useful interventions should target both forms of anatomic release. To do so, more knowledge of the anatomy of these release reactions and of the stimuli that produce them is required.

In the early 1970s, extensive morphologic studies of basophil-rich tissue reactions led to the suggestion that products of specific mononuclear cells might attract and activate nonspecific basophils in these sites.⁸ Such reactions, termed *cutaneous basophil hypersensitivity* in guinea pig models,⁹ were

associated with PMD exclusively. The first reports of mononuclear cell products with histamine-releasing activity (HRA)^{10,11} supported the suggestion that mononuclear cell products activate human basophils. The HRA of histamine-releasing factor (HRF)-containing cellular products is now attributed to multiple cytokines.¹² Two of these include the chemokine, monocyte chemotactic protein-1 (MCP-1)¹³⁻¹⁶ (also called monocyte chemotactic and activating factor) and a newly subcloned protein, rp21, an IgE-dependent HRF.¹⁷ MCP-1, a low molecular weight chemokine, is the most potent histamine-releasing cytokine described, with a potency equivalent to that of anti-IgE.¹⁸⁻²⁰ It acts rapidly, with approximately 80% histamine release by 30 seconds of stimulation¹⁹ without further release after 1 minute.²⁰ Recombinant IgE-dependent histamine-releasing factor (rHRF) (e.g., protein rp21) causes histamine release similar to that caused by the previously described HRF found in vivo.² Histamine release stimulated by rHRF has the same kinetics as that of anti-IgE (i.e., half-maximum release is at 7 minutes), and rHRF also acts as an IgE-dependent trigger for histamine release from basophils.¹⁷

In this study, we examined the ultrastructural morphology of isolated, partially purified peripheral blood human basophils, which were stimulated to release histamine with MCP-1 or rHRF (rp21), and compared these events with those induced by anti-IgE and control basophils incubated without secretagogues. Two time points for each secretagogue were selected for study: one at the half-maximum histamine release interval and another at an earlier time, known to precede biochemically detectable histamine release. Four ultrastructurally defined activation events were identified and quantitated in the responding and control basophils as follows: (1) granule-vesicle attachments (GVAs), as first identified in human contact allergy skin lesions⁶; (2) PMD³; (3) AND³; and (4) uropod formation.²¹ Uropods (tails) are motile structures that have been identified in the basophils of guinea pigs²² and human beings.^{7,23,24} These activated structures were numerous in preparations of basophils stimulated with HRA-containing mononuclear cell supernatants,²⁴ and they were generally found in basophils that lacked ultrastructural evidence of secretion.^{7,23,24}

We found both similarities and differences in the populations of basophils responding to the three secretagogues in this study. Qualitatively, AND, PMD, GVA, and uropod formation were identical, when present. Quantitative differences were, how-

ever, evident. For example, the most effective trigger for AND was anti-IgE, whereas MCP-1 was the most effective stimulant for GVA and PMD; uropods predominated in rHRF-stimulated samples. An inverse relationship of the extent of PMD and AND in stimulated samples occurred, and the development of motile structures in basophils was associated with less morphologic evidence of secretion in the cell population.

These findings define, for the first time, the ultrastructural events that are stimulated in basophils by two new secretagogues, MCP-1 and rHRF, and support the following conclusions. (1) The development of motile structures (uropods) is extensive in rHRF-stimulated samples but not in MCP-1-stimulated samples; (2) evidence of secretion from uropod-containing cells is minor; (3) MCP-1 is a potent stimulant of GVAs, structures important for the vesicular transport of granule materials in PMD; and (4) MCP-1 stimulated the greatest amount of PMD and did so extensively at times preceding the established time of half-maximum histamine release.

METHODS

Peripheral blood basophils were purified according to previously reported procedures.^{1, 2, 25} MCP-1 was purchased from Peprotech, Inc. (Rocky Hills, N.J.), and the subcloning of rp21 was reported.¹⁷ Briefly, for the preparation of recombinant (r)HRF, the complementary DNA for mouse rp21 was kindly supplied by Drs. Margaret Koots and George Brawerman of Tufts University (Boston, Mass.). Primers were designed and the polymerase chain reaction product was expressed in *Escherichia coli* as a protein fused to glutathione S transferase and affinity-purified on immobilized glutathione. The same primers were used to generate the human rp23 from the U937 cell line. This was also expressed as a glutathione S transferase fusion protein and affinity-purified. We have previously shown that both rp21 and rp23 fusion proteins and their cleaved products induced similar IgE-dependent histamine release and that glutathione S transferase did not cause histamine release from human basophils. Furthermore, a polyclonal antibody to cleaved human rp23 showed a band of identity among rp23, rp21, and native material from cultured peripheral blood mononuclear cells.¹⁷ For the purpose of this study, we used cleaved rp21 as rHRF. This recombinant material is produced in *E. coli* and has no posttranslational modifications, such as glycosylation and myristylation. This could explain the necessity of using higher concentrations of rHRF (114 $\mu\text{g/ml}$ or 5×10^{-6} mol/L). Aliquots of cells containing 19% basophils were used as unstimulated controls, sampled at 30 seconds and 7 minutes, or were stimulated with MCP-1 10^{-7} mol/L (0.8 $\mu\text{g/ml}$) for 5 seconds or 30 seconds, with

rHRF (114 $\mu\text{g/ml}$) for 2 minutes or 7 minutes, or with anti-IgE (0.1 $\mu\text{g/ml}$) for 2 minutes or 7 minutes. The late times for each secretagogue were selected to coincide with the known half-maximum histamine release point for each stimulant; the early time points were selected to precede detectable histamine release for each agent. Cell samples that contained basophils after stimulation (as well as unstimulated cells) were each fixed in suspension in a mixture of aldehydes and processed for electron microscopy, as in previous studies.^{5, 7} Random 1 μm plastic sections of each sample were examined, from which random areas were selected for thin sections. Photographs of all cells displaying morphologic evidence of change from control cells were obtained in the electron microscope by examining one or two thin sections of each sample. Additional photographs of unaltered basophils from each sample were obtained when present. Four activation morphologies (GVAs, PMD, AND, and uropod formation) were identified and quantitated for each sample. These data were then analyzed for significance by using the chi square test.

RESULTS

Control basophils

Control cells (Fig. 1), incubated in buffer for 30 seconds or 7 minutes, displayed the previously recorded ultrastructural morphology of unstimulated human basophils^{3, 21}; that is, they had polylobed nuclei, short, irregular surface processes, and membrane-bound, particle-filled secretory granules. Single particles and aggregates of glycogen were present in the cytoplasm, as were a few small, smooth membrane-bound vesicles. Several small, homogeneously dense paranuclear granules were evident. Golgi structures were poorly developed. Some glycogen aggregates were clustered at the poles or edges of granules. Focal electron-lucent areas reflected release of pieces of granule particles adjacent to some of the glycogen collections. Homogeneously dense Charcot-Leyden crystals (CLCs) were embedded within the particulate content of some granules.²⁶ Some granules revealed enclosed or attached dense concentric membranes. Rarely, one or two individual granules had altered, electron-lucent contents; these granules were not enlarged or fused. The presence of one such granule was sufficient to include the cell in the category of PMD (such a finding being the minimum requirement for entry into this category) (Table I). For example, 9% (30 seconds) and 18% (7 minutes) of the photographed cells had one or two such altered granules and were classified in the PMD category ($p = \text{NS}$). These values were significantly less than those of the 2-minute sample stimulated with anti-IgE (40%) ($p < 0.005$, $p <$

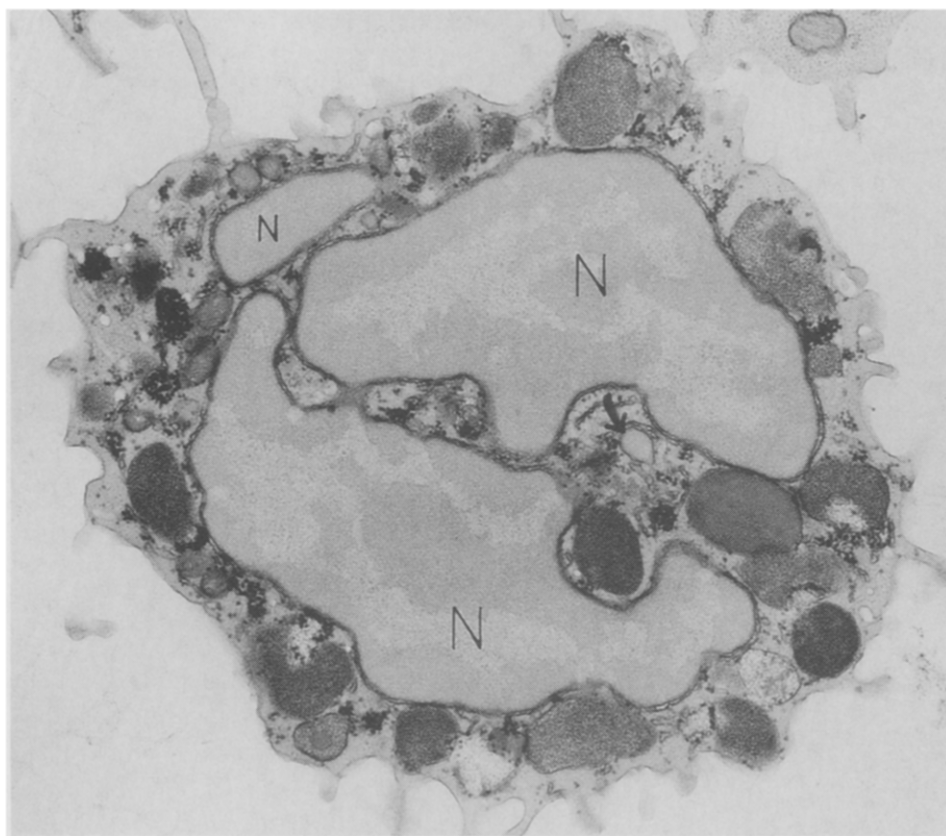


FIG. 1. Control human basophil shows a polylobed nucleus (N) and numerous large secretory granules filled with electron-dense particles. Another granule type is small, rests near the nucleus, and contains homogeneous, poorly electron-dense particles (arrow). Focal electron-dense particles and aggregates of glycogen are present in the cytoplasm. (Original magnification $\times 26,500$.)

TABLE I. Ultrastructural morphology of human basophils stimulated with anti-IgE, rHRF, or MCP-1

Stimulant	Time	Total no. of cells	Granule-vesicle attachments		PMD		AND		Motile structures	
			No.	%	No.	%	No.	%	No.	%
Control	30 sec	35	5	14	3	9	0	0	2	6
Control	7 min	67	6	9	12	18	0	0	2	3
Anti-IgE	2 min	30	12	40	12	40	4	13	1	3
Anti-IgE	7 min	52	1	2	6	12	35	67	8	15
rHRF	2 min	36	7	19	9	25	0	0	5	14
rHRF	7 min	57	12	21	5	9	9	16	28	49
MCP-1	5 sec	341	108	32	189	55	0	0	2	0.6
MCP-1	30 sec	150	71	47	42	28	49	33	8	5

0.025) and the 5-second sample stimulated with MCP-1 (55%) ($p < 0.001$, $p < 0.001$). The 30-second sample stimulated with MCP-1 (28%) had significantly more PMD than the 30-second control sample (9%) ($p < 0.05$). The 7-minute control sample had PMD values similar to both 7-minute samples stim-

ulated either with anti-IgE (12%) or with rHRF (9%) ($p = \text{NS}$, $p = \text{NS}$). GVAs (see description under "Recombinant HRF-stimulated basophils" and "MCP-1-stimulated basophils"), proposed as the mechanism for effecting PMD,⁶ were similar in both control samples (14%, 9%, $p = \text{NS}$).

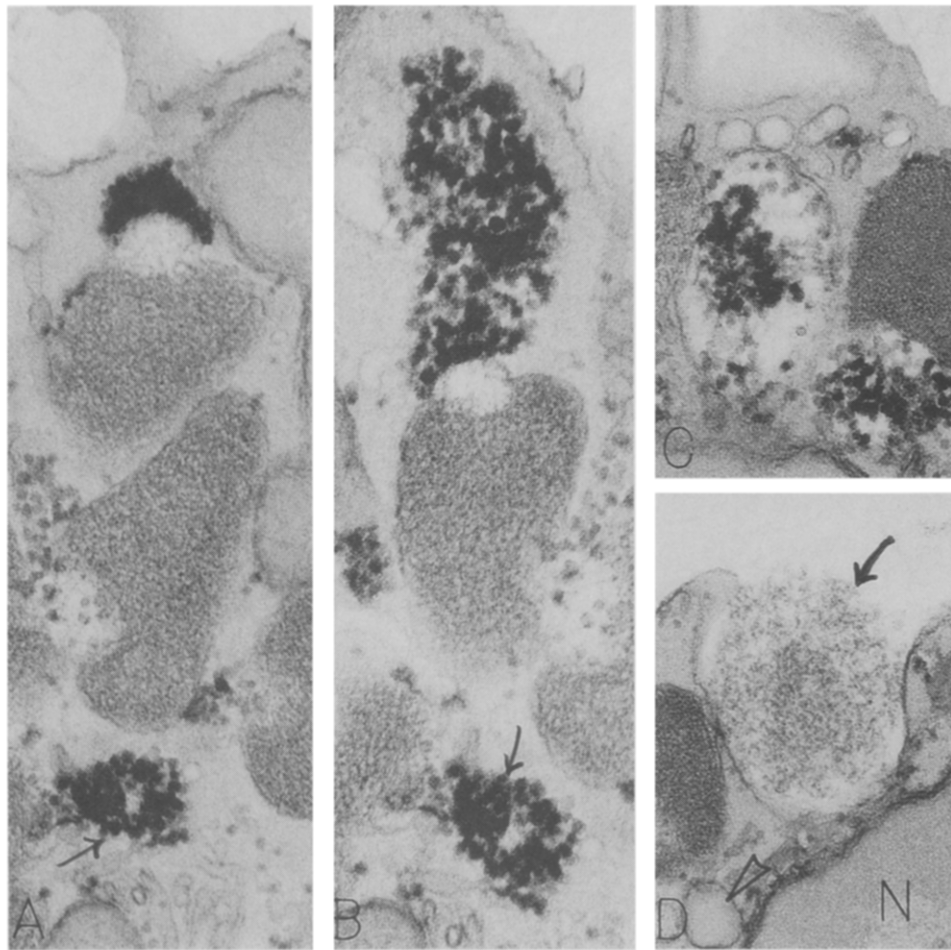


FIG. 2. Portions of basophils (2 minutes after stimulation with anti-IgE) show granule-vesicle attachments (**A** and **B**), cytoplasmic perigranular vesicles (**C**), and exocytosis of a single granule (**D**). In **A** and **B**, vesicles attached to granules contain altered granule contents, and granules contain particles. Granule-attached vesicles are surrounded by large, electron-dense aggregates of glycogen and are oriented toward the overlying plasma membrane. Similar glycogen clusters, which are free in the cytoplasm (*arrows*), enclose electron-lucent vesicles. In **C**, one granule has completely released its dense particle contents and contains larger, electron-dense glycogen particles. Small, smooth membrane-bound, electron-lucent vesicles surround this granule pole, just beneath the overlying plasma membrane. Two granule types of human basophils are shown in **D**. One particle-containing granule, devoid of its membrane, has been extruded to the cell surface (*arrow*); a small, homogeneous granule (*arrowhead*) rests beside the nucleus (*N*). (Original magnifications: **A**, $\times 62,500$; **B**, $\times 76,000$; **C**, $\times 58,000$; **D**, $\times 50,000$.)

We found no AND in either control sample, regardless of incubation time. Small numbers of cells displayed uropods (motile configurations) (6% at 30 seconds and 3% at 7 minutes; $p = \text{NS}$). Virtually all of the cells were round and demonstrated neither tails nor electron-lucent granules.

Anti-IgE-stimulated basophils

Forty percent of the basophils stimulated with anti-IgE for 2 minutes showed PMD, generally characterized by two to three empty or partially empty, nonfused, nonswollen granules ($p < 0.005$

compared with the 30-second control, 9%; $p < 0.025$ compared with the 7-minute control, 18%). This finding was associated with glycogen-rich GVAs (40%), a finding that was evident significantly more frequently than in controls obtained at 30 seconds (14%, $p < 0.025$) or 7 minutes (9%, $p < 0.001$) (Fig. 2, *A* and *B*). Some empty granules contained glycogen and were surrounded by electron-lucent vesicles (Fig. 2, *C*). Thirteen percent of the basophils that were stimulated with anti-IgE for 2 minutes showed AND. Generally, AND consisted of extrusion of one or several granules to

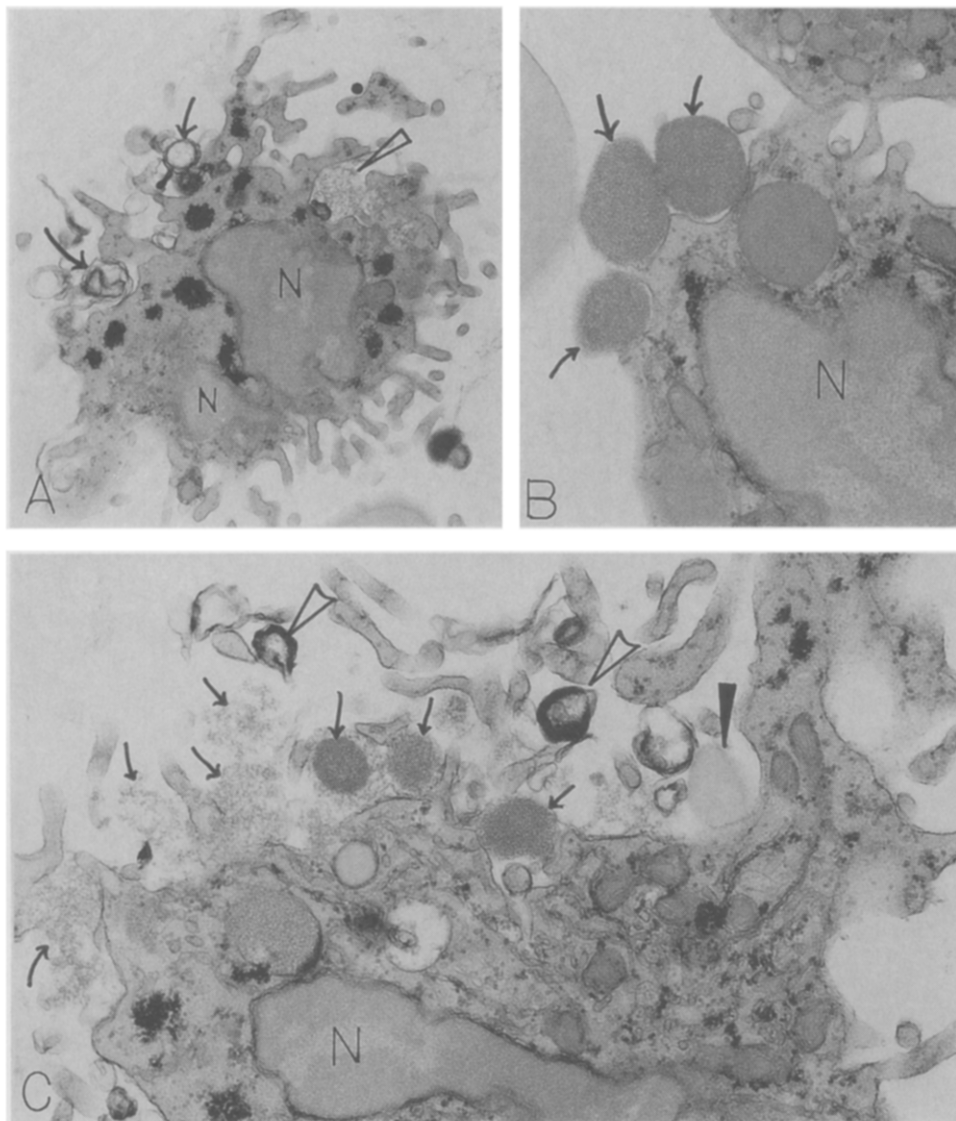


FIG. 3. Human basophils, stimulated for 7 minutes with anti-IgE, show AND. At low magnification (**A**), a completely degranulated basophil displays a polylobed nucleus (*N*), marked shape change with numerous elongated cell processes, focal dense aggregates of cytoplasmic glycogen, and extruded granules (*arrowhead*) and dense concentric membranes (*arrows*) adjacent to the cell surface. At higher magnifications (**B** and **C**), exocytosis of membrane-free granules (*arrows*), dense concentric membranes (*open arrowheads*), and a CLC (*closed arrowhead*) are evident. Note that several unaltered, large granules remain in the cytoplasm of these degranulating basophils. (Original magnifications: **A**, $\times 18,000$; **B**, $\times 30,000$; **C**, $\times 33,000$.)

the cell surface (Fig. 2, *D*) through different openings in the plasma membrane; many unaltered granules remained in these responding cells; some granules showed piecemeal granule losses. Three percent of the cells stimulated with anti-IgE for 2 minutes had motile structures—a value similar to that of both control samples ($p = \text{NS}$, $p = \text{NS}$).

Basophils that were stimulated for 7 minutes with anti-IgE showed 67% AND (Fig. 3), a value significantly different from other samples with

AND (i.e., 13% at 2 minutes with anti-IgE, $p < 0.001$; 16% at 7 minutes with rHRF, $p < 0.001$; 33% at 30 seconds with MCP-1, $p < 0.001$). PMD (12%) and GVA (2%) were similar to those in controls but were less than those in the 2-minute anti-IgE-stimulated cells ($p < 0.005$ [12% vs 40%], $p < 0.001$ [2% vs 40%]). AND in the anti-IgE-stimulated cells resembled that previously reported for regulated secretion by human basophils; that is, we found evidence of extrusion of mem-

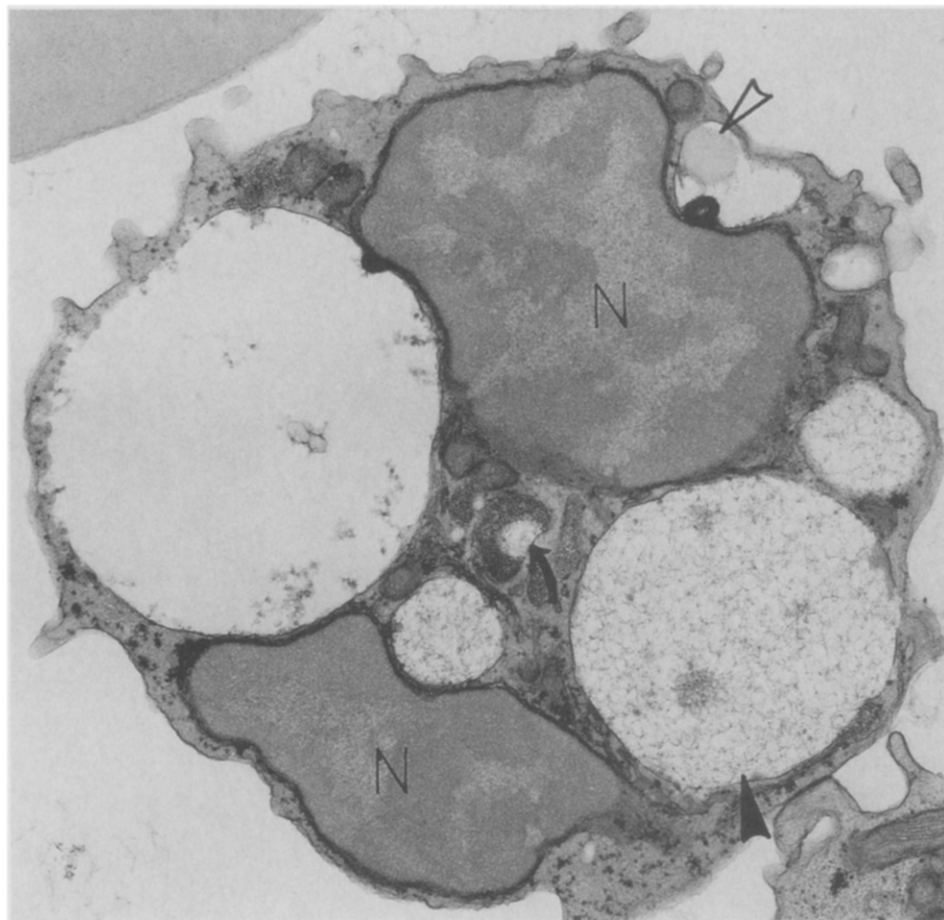


FIG. 4. This basophil (anti-IgE, 7-minute stimulation) shows PMD characterized by secretion of granule contents with retention and enlargement of granule containers. Some residual granule particles remain in one enlarged granule (*closed arrowhead*); another granule shows only focal piecemeal loss (*arrow*) of its granule particles. One empty granule contains a CLC (*open arrowhead*). Note that this cell is round and lacks the extensive shape change and elongation of surface processes that characterize cells undergoing AND (compare with Fig. 3, A). N, Nucleus. (Original magnification $\times 25,500$.)

brane-free granule contents (particles, CLCs, dense concentric membranes) (Fig. 3) through multiple openings in the plasma membrane. Many of these granule materials remained loosely associated with cell surfaces, often amidst shed membranes and surface processes (Fig. 3). These actively responding cells had markedly elongated surface processes and irregular shapes (Fig. 3, A). They were devoid of motile structures (although we found uropods in nondegranulating cells in the same samples, 15%) and often were nearly devoid of granules (Fig. 3, A). Some cells retained no cytoplasmic granules at all. They contained glycogen aggregates but virtually no cytoplasmic vesicles and retained polylobed nuclei (Fig. 3, A). They did not show any ultrastructural criteria of injury, necrosis, or apoptosis. A few basophils formed

intracellular degranulation chambers, which were filled with extruded, membrane-free granule particles. These membrane-bound intracytoplasmic chambers were formed de novo from individual granule fusions and opened to the cell surface through one degranulation pore.

A small number of basophils that were stimulated for 7 minutes with anti-IgE showed PMD. These cells were characterized by grossly swollen, electron-lucent, empty granules (Fig. 4). (Cells similar to these were absent in the 7-minute rHRF sample). Other granules present in these cells showed no alterations, and AND was absent.

Recombinant HRF-stimulated basophils

Recombinant HRF-stimulated basophils showed a trend toward more PMD at 2 minutes (25%) than

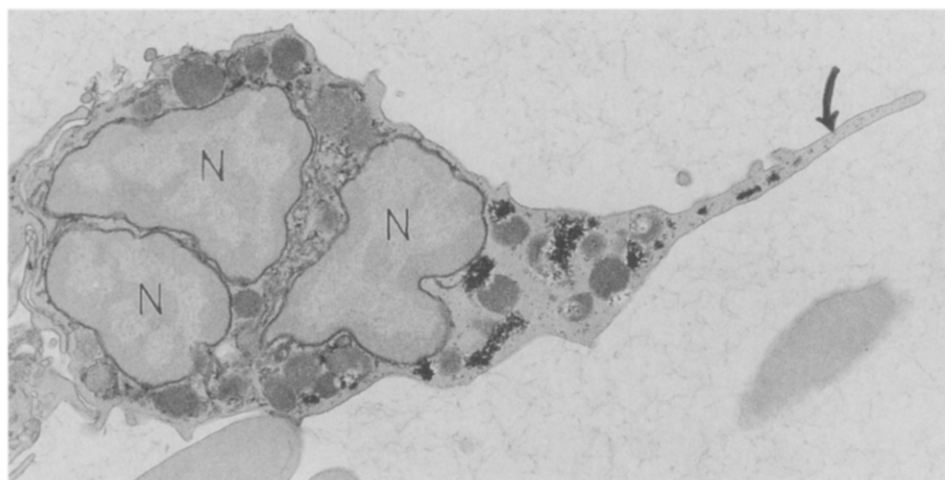


FIG. 5. This basophil (rHRF, 7 minutes) shows a motile configuration without morphologic evidence of PMD or AND. A full complement of unaltered, particle-filled granules is present. The nucleus (*N*) has several lobes. An elongated uropod (*arrow*) is evident. (Original magnification $\times 13,500$.)

control values of PMD by 30 seconds (9%) but did not achieve statistical significance ($p = \text{NS}$). AND was absent at 2 minutes. The extent of AND at 7 minutes (16%) was equivalent to that induced by anti-IgE at 2 minutes (13%, $p = \text{NS}$) but was far less than anti-IgE induced at 7 minutes (67%, $p < 0.001$). Recombinant HRF produced the largest amount of motile configurations in basophils (Fig. 5), 49% by 7 minutes ($p < 0.001$ compared with anti-IgE at 7 minutes, 15%; $p < 0.001$ compared with MCP-1 at 30 seconds, 5%; $p < 0.001$ compared with controls at 30 seconds, 6%; and $p < 0.001$ compared with controls at 7 minutes, 3%). Motile structures in the 7-minute rHRF sample (49%) exceeded those seen at 2 minutes (14%) ($p < 0.001$), whereas motile basophils in control samples did not differ between 30 seconds (6%) and 7 minutes (3%) ($p = \text{NS}$). The morphology of AND induced by rHRF was identical to that seen in anti-IgE-stimulated cells (Fig. 6, *F*). The morphology of PMD in rHRF-stimulated samples differed from the PMD morphology in the anti-IgE 7-minute sample in that basophils with grossly enlarged, nonfused, empty granules were not present. Rather, individual cells showed one to two focally or completely empty, nonfused granule containers, which were not swollen, in their cytoplasm.

Attached, glycogen-rich granule vesicles similar to those of all stimulated samples (except for the 7-minute anti-IgE sample) were seen in the rHRF-containing samples (Fig. 6). For example, large masses of glycogen focally contacted individual

altered granules (Fig. 6, *E*), and these masses capped vesicular protrusions containing altered granule materials (Fig. 6, *A-D*). Some of the latter structures were attached at granule poles oriented toward plasma membranes (Fig. 6 *A* and *B*).

The large number of uropod-bearing basophils that were stimulated with a 7-minute exposure to rHRF showed no morphologic evidence of AND; however, some had focal PMD.

MCP-1-stimulated basophils

Fifty-five percent of the basophils exposed for 5 seconds to MCP-1 showed PMD (Fig. 7, *A*) ($p < 0.001$, $p < 0.001$ compared with controls; $p = \text{NS}$ compared with anti-IgE, 2 minutes, 40%; $p < 0.001$ compared with anti-IgE, 7 minutes, 12%; $p < 0.001$ compared with rHRF 2 minutes, 25%; $p < 0.001$ compared with rHRF, 7 minutes, 9%), a value that was decreased significantly by a 30-second exposure to MCP-1 (28%) (Fig. 7, *B*) ($p < 0.001$), at which point AND began (33% compared with none in the 5-second MCP-1 sample). Also, the extent of PMD in individual cells exceeded that of controls in that altered granules were more prevalent. By 30 seconds after MCP-1 stimulation, 28% of the cells exhibited PMD similar to the 2-minute samples stimulated with anti-IgE (40%) and rHRF (25%) ($p = \text{NS}$, $p = \text{NS}$). Some basophils demonstrated extensive PMD, involving nearly all granules, which were markedly enlarged and had not fused with adjacent granule or plasma membranes (Fig. 7, *B*). These cells resembled the small number of cells undergoing PMD that re-

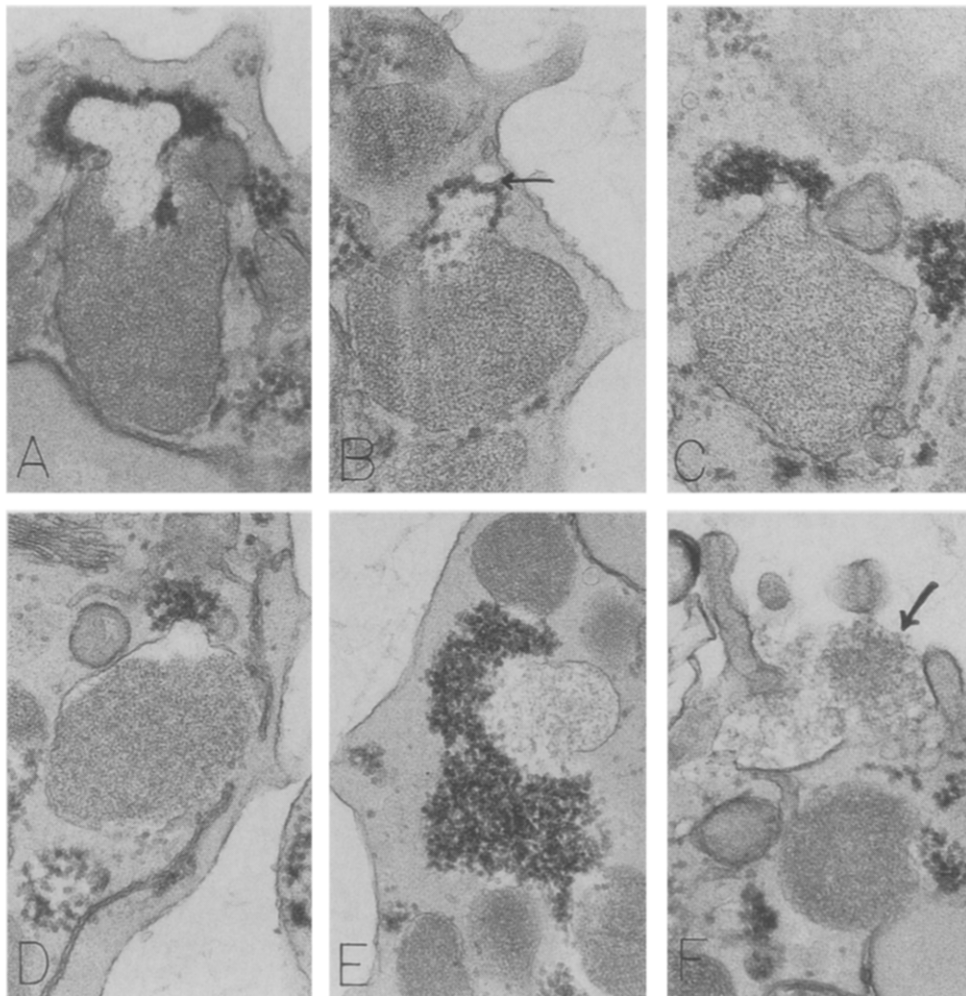


FIG. 6. Higher magnification views of human basophils (2 minutes [A and B] or 7 minutes [C to F] after stimulation with rHRF) are shown. Glycogen-rich GVAs are illustrated (A to D). Attached vesicles show a variable degree of altered contents similar to that underlying them in the particulate granule material, and vesicles are encased with dense glycogen aggregates. Some of these attached vesicles are oriented toward the cell surface (A,B). Note the separate vesicle adjacent to one of the attached vesicles, which spans the cytoplasm to abut on the plasma membrane (arrow, B). The entire content of one granule is altered (E), as in PMD, and an extensive glycogen aggregate surrounds this granule. In F, granule exocytosis, as in AND, is illustrated (arrow). (Original magnifications: A, $\times 53,500$; B, $\times 56,000$; C, $\times 52,000$; D, $\times 42,000$; E, $\times 35,500$; F, $\times 51,500$.)

mained in the anti-IgE 7-minute sample (Fig. 4), a sample showing primarily AND (67%). PMD in the 30-second MCP-1 sample (28%) significantly exceeded that in either the 7-minute anti-IgE (12%) and rHRF (5%) samples ($p < 0.05$, $p < 0.05$).

The images of basophil AND (Fig. 8) evident at 30 seconds after exposure to MCP-1 resembled those reported for other samples. For example, there was cellular evidence of extensive degranulation, with many extruded granules resting on the cell surface after emerging through multiple openings in the plasma membrane. Granule particles,

CLCs, and dense concentric membranous arrays amidst shed cellular processes were clustered around these secreting, viable cells (Fig. 8, B). Many basophils showed extrusion of a single granule with little to no alteration of the remaining granules. Some cells released multiple, nonswollen, membrane-free granules through a single plasma membrane pore, which had widened to permit their passage (Fig. 8, A). A small number of basophils undergoing AND had formed cytoplasmic degranulation chambers from fused granule membranes; these structures were filled with ex-

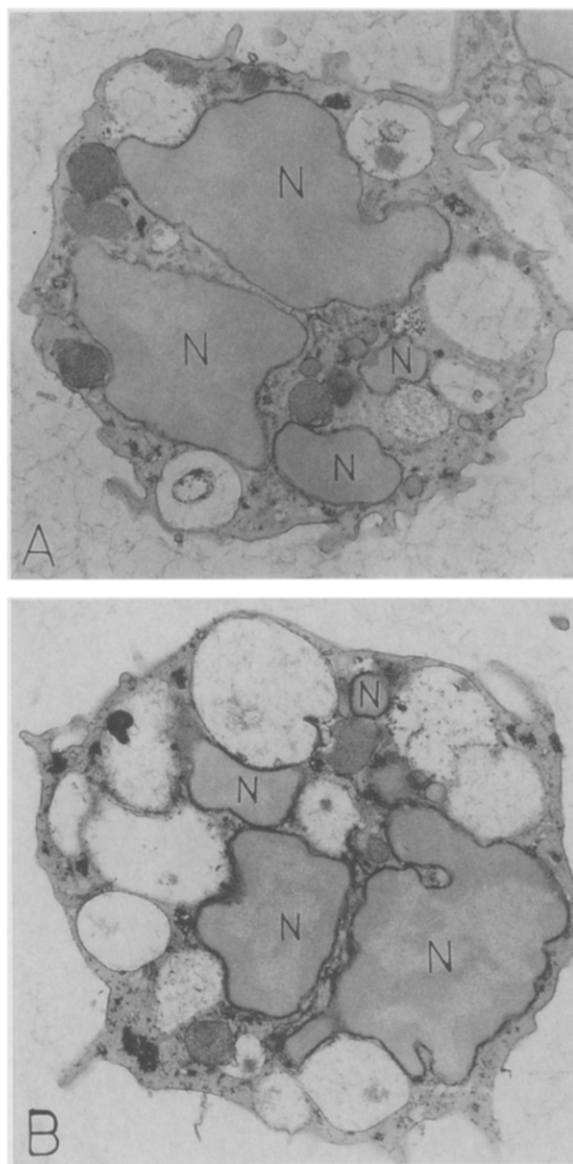


FIG. 7. MCP-1-stimulated basophils are shown. In **A** (5 seconds after stimulation), a basophil with a polylobed nucleus (*N*) displays PMD with granule particle losses from approximately 50% of visible granules. Partially and completely empty granules are moderately increased in size and have not fused with adjacent granules or plasma membrane. In **B** (30 seconds after stimulation), another cell with a polylobed nucleus (*N*) shows extensive PMD involving nearly all secretory granules. Empty granule chambers are swollen and remain in the cytoplasm. Amplification of surface processes and shape changes are absent from these cells, which are actively undergoing PMD. (Original magnifications: **A**, $\times 14,500$; **B**, $\times 15,000$.)

truded, membrane-free granules. Although there were no basophils undergoing AND in the MCP-1 5-second sample, 33% of the basophils in the 30-second MCP-1 sample were involved in AND.

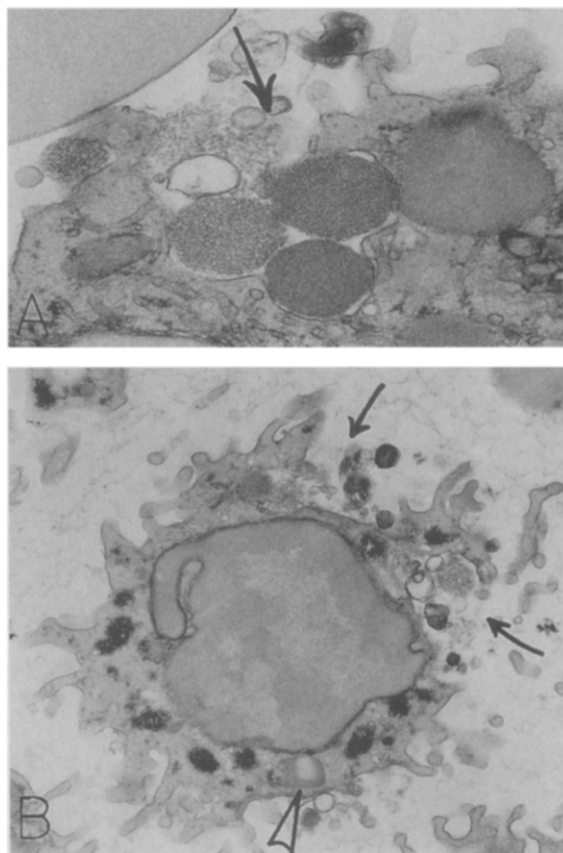


FIG. 8. Basophils (30 seconds, MCP-1 stimulation) display AND. In **A** several membrane-free granules are being extruded through a single wide opening in the plasma membrane (arrow). In **B** extensive shape change and surface process amplification are evident. Electron-dense cytoplasmic aggregates of glycogen are present. Exocytosis of granules and concentric dense membranes has occurred at several openings in the cell surface (arrows). A single unaltered granule, containing dense particles and a CLC, remains in the cytoplasm (arrowhead). (Original magnifications: **A**, $\times 37,500$; **B**, $\times 16,500$.)

The extent of AND in the MCP-1 30-second sample (33%) was significantly greater than that present in the rHRF 7-minute sample (16%, $p < 0.025$) and in the anti-IgE 2-minute sample (13%, $p < 0.05$) but was less than that found in the anti-IgE 7-minute sample (67%, $p < 0.001$).

The number of MCP-1-stimulated motile structures (5-second sample, 0.6%) was significantly less ($p < 0.005$) than those formed in the 30-second control sample (6%) but did not differ from the 7-minute control sample (3%, $p = \text{NS}$). The 30-second MCP-1-stimulated cells had motile structure formation similar to both controls ($p = \text{NS}$).

The PMD images induced by MCP-1 in basophils were accompanied by numerous glycogen-associated GVAs (Fig. 9) (32%, 5 seconds; 47%,

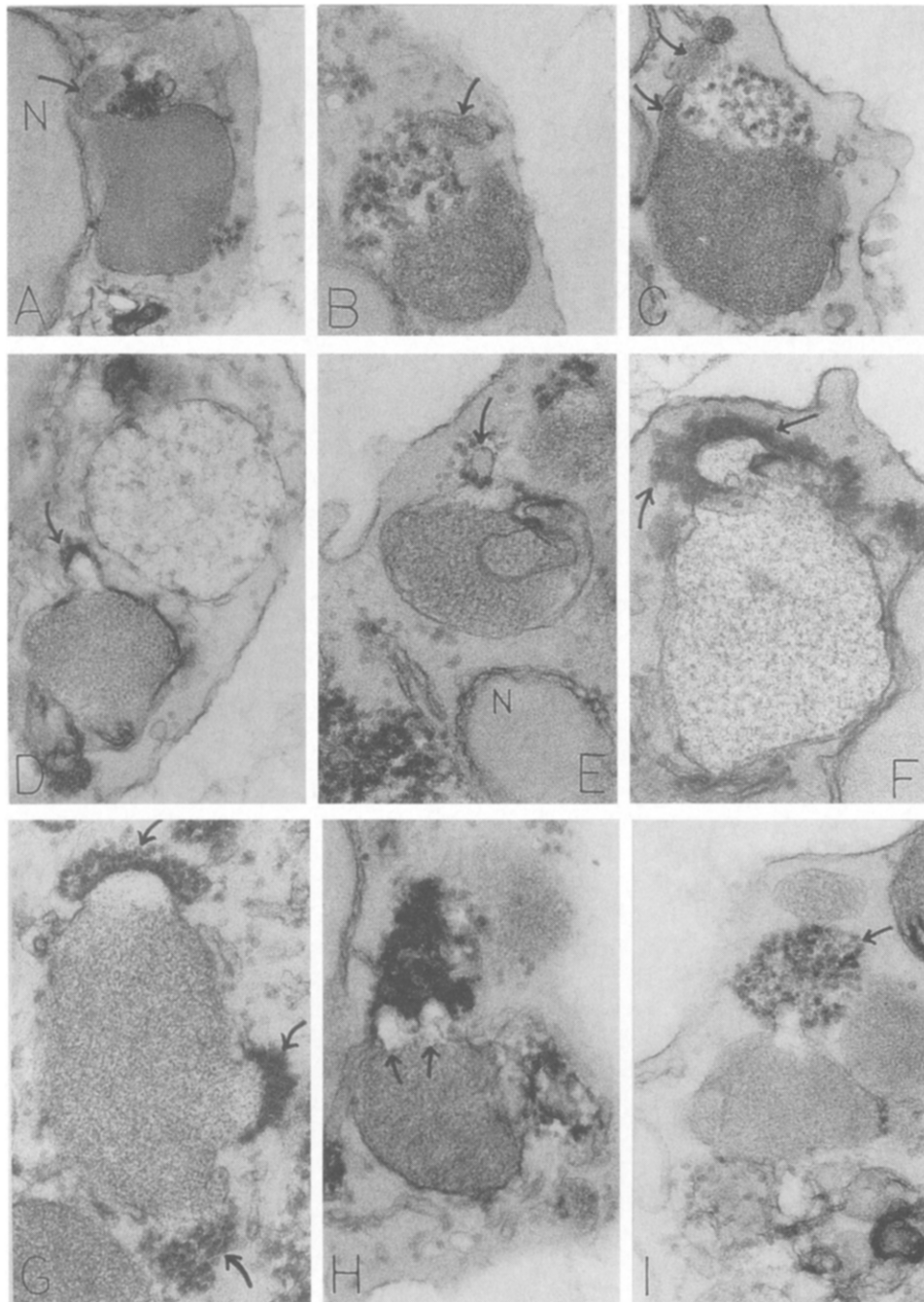


FIG. 9. A higher magnification montage of basophils, stimulated with MCP-1 for 5 seconds (**A** to **G**) or 30 seconds (**H** and **I**), shows glycogen-rich, granule-vesicle attachments that accompany PMD. Some of the attached, elongated vesicles contain electron-dense granule particles (arrows in **A** to **C**); some attached vesicles contain less dense (or electron-lucent) altered materials (arrows in **D** to **I**). Larger, electron-dense glycogen particles are interposed at the necks of tubular-vesicular granule extensions (**A**, **B**, and **E**) or encase the outer surface of attached vesicles (**D**, **F** to **I**). Also noted are single (**A** to **C**, **E**, **F**, and **I**), double (**H**), and triple (**G**) glycogen-rich vesicles attached to granules. Some granules, with vesicles attached, have a full complement of dense particles (**A** to **D**), focal, underlying piecemeal losses of granule particles (**E**, **G** to **I**), or diminished particles throughout (**F**). One empty granule (**D**) has individual glycogen particles within it. **A**, $\times 50,000$; **B**, $\times 57,500$; **C** and **D**, $\times 49,000$; **E**, $\times 70,000$; **F** and **G**, $\times 63,000$; **H**, $\times 51,000$; **I**, $\times 54,500$.)

30 seconds). In fact, the largest number of these structures of all samples examined were identified in the 30-second MCP-1 sample ($p < 0.001$, $p < 0.001$ vs controls 14%, 9%; $p = \text{NS}$, $p < 0.001$ vs anti-IgE at 2 minutes, 40%, and 7 minutes, 2%; $p < 0.005$, $p < 0.001$ vs rHRF at 2 minutes, 19%, 7 minutes, 21%; and $p < 0.001$ vs MCP-1 at 5 seconds, 32%). GVAs in the 5-second MCP-1 sample (Fig. 9, A-G) (32%) also exceeded those for 30-second (14%) and 7-minute (9%) control samples ($p < 0.05$, $p < 0.001$). The extent of these structures in the 5-second MCP-1 sample (32%) was similar to that in the 2-minute anti-IgE (40%) or the 2-minute (19%) and 7-minute (21%) rHRF samples ($p = \text{NS}$, $p = \text{NS}$, $p = \text{NS}$). The extent of granule-attached vesicles in the 5-second MCP-1 sample significantly exceeded that in the 7-minute anti-IgE-stimulated cells (2%, $p < 0.001$).

Granule-attached vesicles were often uniquely encased in glycogen aggregates; many of these glycogen-encased, granule-attached vesicles were oriented on granule poles or sides facing plasma membranes (Fig. 9). Some granules had up to three attached vesicles (Fig. 9, G). Focal piecemeal losses of granule particles were regularly noted adjacent to these attached vesicles (Fig. 9, D-I). Some particle-filled vesicles were similarly hooked to granules at glycogen-rich areas (Fig. 9, A to C). Glycogen aggregates that were near granules and those that were apparently not near granules surrounded cytoplasmic, electron-lucent, smooth membrane-bound vesicles and larger vacuoles. Some of these structures were filled with material similar to the altered granule substance adjacent to areas of vesicle attachments—areas that are referred to as focal PMD.

The relationships of four activated morphologic features in basophils as a function of unstimulated or stimulated cells are compared graphically in Fig. 10.

DISCUSSION

Control basophils display morphologic features qualitatively similar to peripheral blood basophils not subjected to isolation procedures and quantitatively dissimilar to those induced by anti-IgE, rHRF, or MCP-1

The unstimulated control samples of partially purified human basophils that were examined in these studies qualitatively resembled peripheral blood basophils that were prepared for ultrastructural analysis without exposure to isolation proce-

dures.^{21,27} The changes recorded in the unstimulated basophils in this study differed quantitatively from the morphologic changes of cells associated with exposure to any of the three secretagogues examined. Each of these triggers was associated with variable amounts of PMD, AND, GVA, for example, and two were associated with the formation of motility configurations, termed *uropods*. Controls did not display AND at all, and the amount of PMD, GVA, and uropods expressed was small and similar in the early (30-second) and late (7-minute) control samples. Approximately 13% of the basophils in the control samples had morphologies consistent with PMD. However, the extent of this process in the control cell populations examined at two time points, as well as within individual cells, was far less than it was in basophils undergoing PMD in stimulated samples.

Quantitative differences of several ultrastructural activation morphologies are induced in human basophils by exposure to anti-IgE, rHRF, or MCP-1

We selected two time points at which to examine ultrastructural changes for each secretagogue: one sample was taken at approximately one-half maximum histamine release,^{17-20,28} and the other sample was selected to precede histamine release. Thus the known kinetics for either anti-IgE- or rHRF-stimulated histamine release dictated sample collections at 7 minutes (one-half maximum histamine release) and 2 minutes (before histamine release). MCP-1 samples were collected at 30 seconds (one-half maximum histamine release) and at 5 seconds (before histamine release). Four morphologic processes (i.e., PMD, AND, GVA, and uropod formation) were markedly different quantitatively in differently stimulated samples. Qualitatively, the processes were similar, with few exceptions (see later). When these events were examined in terms of time needed for half-maximum histamine release for each trigger, anti-IgE was clearly the most effective in producing AND images, surpassing the next most effective trigger, MCP-1, by 34% ($p < 0.001$). Thus a rank order for the elicitation of AND is as follows: anti-IgE > MCP-1 > rHRF, when examined at times comparable to half-maximum histamine release. The only other sample that showed AND was the 2-minute anti-IgE sample, confirming our previous observation that the ultrastructural detection of AND, stimulated by an IgE-mediated mechanism, may precede the biochemical detection of histamine release.⁷

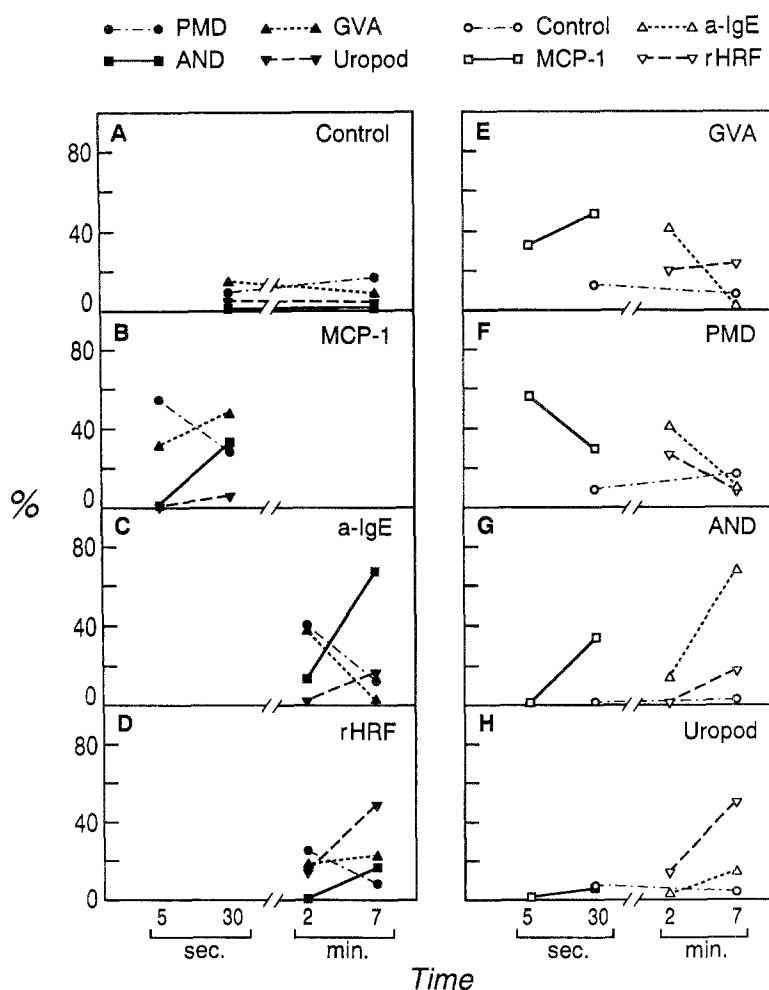


FIG. 10. Kinetic and quantitative relationships of four activated morphologic features induced in human basophils by anti-IgE, rHRF, or MCP-1 compared with unstimulated human basophils.

PMD, at the time point required for half-maximum histamine release, did not exceed that in controls with either anti-IgE or rHRF stimulation. Stimulation with MCP-1 at the half-maximum histamine release time point did, however, produce PMD exceeding that of controls. Moreover, all triggers elicited extensive PMD in samples obtained before histamine release. A rank order for the effectiveness of producing PMD images is as follows: MCP-1 > anti-IgE > rHRF.

GVA showed interesting differences at half-maximum histamine release times. For anti-IgE, a trigger that functioned primarily by inducing AND, the GVA formation percent (2%) was the lowest of all samples examined; whereas for MCP-1, a trigger that induced large amounts of PMD, the greatest amount of GVA was recorded (47%). Thus GVAs are implicated by association as a mechanism for effecting mediator release by vesicular transport from granules that empty and re-

main in place in the cytoplasm (e.g., as in PMD) rather than by release from granules that are physically extruded to the cells' exterior (e.g., as in AND).

Uropods are motile structures that were previously recorded in quantity in basophils stimulated with HRA-containing mononuclear cell supernatants.²⁴ As before,²⁴ we noted no basophils with uropods undergoing AND in any preparations, and instances of PMD were minor, involving only focal portions of granules (or only several granules) among many granules that showed no alterations. Of the triggers examined here, rHRF produced 49% basophils with uropods at the half-maximum histamine release interval. The rank order of uropod production was: rHRF > anti-IgE > MCP-1. Clearly, this newly defined recombinant protein¹⁷ produces considerable ultrastructural evidence of the anatomic structures needed for basophil migration, a feature not shared by stimulation of

basophils with MCP-1. Some motile structures were detected in the half-maximum histamine release interval after anti-IgE stimulation, similar to our previous report of anti-IgE-stimulated basophils.⁷

PMD is featured during the early exposure of human basophils to several histamine-releasing secretagogues

PMD of basophils was first described in tissue basophils after their migration from the vascular space in response to experimentally produced and sequentially biopsied contact allergy skin lesions.^{6,8} The secretory process termed *PMD* is defined ultrastructurally by the progressive emptying of electron-dense granule contents without fusion to adjacent granules or to the plasma membrane. Since the early description in basophils, this secretory process has been documented in basophils and mast cells of numerous species in a variety of experimental models and diseases.^{5,29-33} PMD is the single most frequently encountered ultrastructural event in activated tissue basophils and mast cells in human disease.^{3,33}

In contact allergy, a crescendo of basophil PMD evolves over a 3-day period in sequential biopsy specimens, a secretory process that does not evolve into AND. In 1975, we postulated a degranulation model for basophils that encompassed a continuum of PMD→AMD, dependent on the rate of vesicular traffic.⁴ This continuum has since been documented in a kinetic morphologic study of FMLP-stimulated basophils.⁵ An evolving continuum of PMD→AND suggests that one might find ultrastructural evidence of PMD before AND after stimulation. Such a precursor relationship to AND, documented for FMLP, was also suggested by studies of tetradecanoylphorbol acetate-stimulated basophils.²⁹ In this case, a forme fruste of AND prevailed (at later times), which was associated with infrequent images of fully developed AND. In this report we also found that PMD preceded AND after stimulation with each of the three triggers we examined. Moreover, as the extent of PMD diminished at half-maximum histamine release times, the extent of AND increased for all three triggers. This finding and our previous similar findings for FMLP and tetradecanoylphorbol acetate indicate a precursor relationship of PMD to AND, supporting the general degranulation model proposed in 1975.⁴ Because the actual triggers of basophil PMD in contact allergy tissue sites are not known, one cannot yet determine whether an incomplete trigger or an inhibitor

functions to halt the progression of PMD to AND in basophils responding to contact allergens in the skin, for example. The ability to examine these processes carefully, *ex vivo*, with well-defined, purified stimulants,¹⁷⁻²⁰ as they become available, should aid in the resolution of such questions and perhaps contribute to therapeutic regulation of unwanted secretory events in human basophils during disease.

The ultrastructural expression of secretion in human basophils is qualitatively similar after exposure to anti-IgE, rHRF, or MCP-1

The general morphologic processes of PMD and AND expressed by human basophils after stimulation with anti-IgE, rHRF, or MCP-1 did not differ qualitatively. In previous studies we have called attention to two morphologic variants (one having components of the morphology of AND and the other, of PMD) that were evident on examination of mannitol-stimulated cells³⁴ or FMLP-stimulated cells,⁵ respectively. It is important to recognize that these variants exist in secreting basophils, because by light microscopy, one cannot distinguish them, and they evolve by quite different ultrastructural mechanisms. In the first case, basophils stimulated with hyperosmolar agents, such as mannitol,³⁴ developed degranulation sacs or channels within the cytoplasm that contained numerous membrane-free, extruded granules. This newly developed, membrane-delimited space is formed from the fused membranes of the extruded granules and ultimately opens to the extracellular space through a single pore in the plasma membrane. Such structures are integral features of AND in mast cells and basophils of many species.²¹ They are, for example, extensively formed during AND in stimulated guinea pig basophils³⁵ but are quite rare in human basophils stimulated to undergo AND.⁷

The second morphologic variant occurs in PMD when inward vesicular traffic (endocytosis) exceeds outward vesicular traffic, leading to extensive enlargement of individual, nonfused granule containers.⁵ These single, giant granule containers are often completely devoid of granule matrix; that is, they do not arise by granule-to-granule membrane fusions nor do they contain extruded granules. The expanded granule container results from the addition of vesicle membranes that traffic to individual granules from the cell surface. In this study, intracytoplasmic degranulation chamber formation was exceedingly rare as a component of AND induced

by anti-IgE, rHRF, or MCP-1. Small amounts of PMD with enlarged, empty containers were evident at 7 minutes after exposure to anti-IgE or at 30 seconds after exposure to MCP-1.

A comparative analysis of the qualitative morphologic changes prevailing after stimulation of basophils with several triggers shows that stimulation with anti-IgE produced extensive evidence of AND, analogous to previous IgE-mediated morphologic studies,⁷ and that stimulation with rHRF produced less AND than did anti-IgE, with abundant evidence of motile structure formation. These findings are similar to those previously recorded for cytokine-containing mononuclear cell supernatants.²⁴ On the other hand, by 5 seconds, MCP-1 produced extensive evidence of PMD that preceded AND. PMD (produced by MCP-1) exceeded that found in controls or in samples stimulated by rHRF or present 7 minutes after exposure to anti-IgE; thus, MCP-1 was the most potent trigger for eliciting the morphology of PMD in these studies.

GVA's support a role for vesicle transport in stimulated secretion from human basophils

Significantly increased numbers of GVAs were present in both basophil samples stimulated with MCP-1 and in the 2-minute anti-IgE-stimulated sample. We first described vesicles attached to basophil granules in contact allergy skin biopsy specimens and noted that these attached structures were often connected to glycogen particles and aggregates.⁶ These associations were striking in the MCP-1-stimulated basophils studied here, suggesting a role for GVAs in effecting the extensive emptying of granules, characteristic of PMD. The closely associated glycogen aggregates may provide an energy source for vesicular motion. Vesicular traffic from the extracellular milieu has been documented in both human and guinea pig basophils by using electron-dense horseradish peroxidase or eosinophil peroxidase, and release of previously granule-loaded horseradish peroxidase from guinea pig basophils in cytoplasmic vesicles was noted in washout studies.²¹ Morphologically similar cytoplasmic vesicles, present in FMLP-stimulated basophils during active histamine release, contain histamine and CLC protein, as determined by an enzyme-affinity gold method³⁶ or by an immunogold method,²⁶ respectively. Thus ample evidence exists for a vesicular role in uptake into and secretion from human basophils.^{21,37} The marked increase in vesicles attached to granules, encased in glycogen, and oriented to plasma mem-

branes (in basophils displaying significant amounts of PMD after stimulation with MCP-1 [and anti-IgE, at 2 minutes]) supports this interpretation. Basophil samples expressing extensive AND (anti-IgE, 7 minutes) and/or motile structures (anti-IgE, 7 minutes; rHRF, 2 minutes and 7 minutes) did not have GVAs exceeding those present in controls, further supporting their effector role in PMD, as well as the precursor or nonobligatory relationships of PMD to AND and the lack of either PMD or AND in human basophils that form uropods after stimulation.

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REFERENCES

1. MacDonald SM, Schleimer RP, Kagey-Sobotka A, Gillis S, Lichtenstein LM. Recombinant IL-3 induces histamine release from human basophils. *J Immunol* 1989;142:3527-32.
2. MacDonald SM, Lichtenstein LM, Proud D, et al. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987;139:506-12.
3. Dvorak AM. Ultrastructural analysis of anaphylactic and piecemeal degranulation of human mast cells and basophils. In: Foreman JC, editor. *Immunopharmacology of mast cells and basophils*. London: Academic Press, 1993: 89-113. In: Page C, editor. *The handbook of immunopharmacology*.
4. Dvorak HF, Dvorak AM. Basophilic leucocytes: structure, function and role in disease. In: Lichtman MA, editor. *Granulocyte and monocyte abnormalities* London: W B Saunders Company, Ltd., 1975:651-83. *Clinics in haematology*. Volume 4, no. 3.
5. Dvorak AM, Warner JA, Kissell S, Lichtenstein LM, MacGlashan DW Jr. F-Met peptide-induced degranulation of human basophils. *Lab Invest* 1991;64:234-53.
6. Dvorak AM, Mihm MC Jr, Dvorak HF. Degranulation of basophilic leukocytes in allergic contact dermatitis reactions in man. *J Immunol* 1976;116:687-95.
7. Dvorak AM, Newball HH, Dvorak HF, Lichtenstein LM. Antigen-induced IgE-mediated degranulation of human basophils. *Lab Invest* 1980;43:126-39.
8. Dvorak AM, Dvorak HF. Cutaneous basophil hypersensitivity—A 20-year perspective, 1970-1990. In: Foreman JC, editor. *Immunopharmacology of mast cells and basophils* London: Academic Press, 1993:153-80. In: Page C, editor. *The handbook of immunopharmacology*.
9. Dvorak HF, Dvorak AM, Simpson BA, Richerson HB, Leskowitz S, Karnovsky MJ. Cutaneous basophil hypersensitivity. II. A light and electron microscopic description. *J Exp Med* 1970;132:558-82.
10. Thuesen DO, Speck LS, Lett-Brown MA, Grant JA. Histamine-releasing activity (HRA). I. Production by mitogen- or antigen-stimulated human mononuclear cells. *J Immunol* 1979;123:626-32.
11. Thuesen DO, Speck LS, Lett-Brown MA, Grant JA. Histamine-releasing activity (HRA). II. Interaction with basophils and physicochemical characterization. *J Immunol* 1979;123:633-9.

12. MacDonald SM. Histamine releasing factors and IgE heterogeneity. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 4th edition. St. Louis: Mosby, 1993:1-11.
13. Yoshimura T, Robinson EA, Tanaka S, Appella E, Leonard EJ. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. *J Immunol* 1989;142:1956-62.
14. Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ. Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J Exp Med* 1989;169:1485-90.
15. Zachariae CO, Anderson AO, Thompson HL, et al. Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J Exp Med* 1990;171:2177-82.
16. Furutani Y, Nomura H, Notake M, et al. Cloning and sequencing of the cDNA for human monocyte chemotactic and activating factor (MCAF). *Biochem Biophys Res Commun* 1989;159:249-55.
17. MacDonald SM, Rafnar T, Langdon J, Lichtenstein LM. Molecular identification of an IgE-dependent histamine releasing factor (HRF). *Science* 1995;269:688-90.
18. Bischoff SC, Krieger M, Brunner T, Dahinden CA. Monocyte chemotactic protein 1 is a potent activator of human basophils. *J Exp Med* 1992;175:1271-5.
19. 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.
20. 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.
21. Dvorak AM, editor. *Basophil and mast cell degranulation and recovery*. New York: Plenum Press, 1991. In: Harris JR, editor. *Blood cell biochemistry*. Volume 4.
22. Galli SJ, Dvorak AM, Hammond ME, Morgan E, Galli AS, Dvorak HF. Guinea pig basophil morphology *in vitro*. I. Ultrastructure of uropod-bearing (motile) basophils and modulation of motile structures by serum and substrate effects. *J Immunol* 1981;126:1066-74.
23. Dvorak AM, Lett-Brown M, Thueson D, Grant JA. Complement-induced degranulation of human basophils. *J Immunol* 1981;126:523-8.
24. Dvorak AM, Lett-Brown MA, Thueson DO, et al. Histamine-releasing activity (HRA). III. HRA induces human basophil histamine release by provoking noncytotoxic granule exocytosis. *Clin Immunol Immunopathol* 1984;32:142-50.
25. Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM. IgE-dependent IL-4 secretion by human basophils. The relationship between cytokine production and histamine release in mixed leukocyte cultures. *J Immunol* 1994;153:1808-17.
26. Dvorak AM, Ackerman SJ. Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to granules and intragranular crystals in mature human basophils. *Lab Invest* 1989;60:557-67.
27. Dvorak AM. Morphologic and immunologic characterization of human basophils, 1879 to 1985. *Riv Immunol Immunofarmacol* 1988;8:50-83.
28. Warner JA, Peters SP, Lichtenstein LM, et al. Differential release of mediators from human basophils: differences in arachidonic acid metabolism following activation by unrelated stimuli. *J Leukoc Biol* 1989;45:558-71.
29. Dvorak AM, Warner JA, Morgan E, Kissell-Rainville S, Lichtenstein LM, MacGlashan DW Jr. An ultrastructural analysis of tumor-promoting phorbol diester-induced degranulation of human basophils. *Am J Pathol* 1992;141:1309-22.
30. Dvorak AM, McLeod RS, Onderdonk A, et al. Ultrastructural evidence for piecemeal and anaphylactic degranulation of human gut mucosal mast cells *in vivo*. *Int Arch Allergy Immunol* 1992;99:74-83.
31. Dvorak AM, Mitsui H, Ishizaka T. Ultrastructural morphology of immature mast cells in sequential suspension cultures of human cord blood cells supplemented with *c-kit* ligand: distinction from mature basophilic leukocytes undergoing secretion in the same cultures. *J Leukoc Biol* 1993;54:465-85.
32. Dvorak AM, Tepper RI, Weller PF, et al. Piecemeal degranulation of mast cells in the inflammatory eyelid lesions of interleukin-4 transgenic mice. Evidence of mast cell histamine release *in vivo* by diamine oxidase-gold enzyme-affinity ultrastructural cytochemistry. *Blood* 1994;83:3600-12.
33. Dvorak AM. Basophils and mast cells: piecemeal degranulation *in situ* and *ex vivo*—a possible mechanism for cytokine-induced function in disease. In: Coffey RG, editor. *Granulocyte responses to cytokines. Basic and clinical research*. New York: Marcel Dekker, 1992:169-271.
34. Findlay SR, Dvorak AM, Kagey-Sobotka A, Lichtenstein LM. Hyperosmolar triggering of histamine release from human basophils. *J Clin Invest* 1981;67:1604-13.
35. Dvorak AM, Galli SJ, Morgan E, Galli AS, Hammond ME, Dvorak HF. Anaphylactic degranulation of guinea pig basophilic leukocytes. I. Fusion of granule membranes and cytoplasmic vesicles: formation and resolution of degranulation sacs. *Lab Invest* 1981;44:174-91.
36. Dvorak AM, Morgan ES, Lichtenstein LM, MacGlashan DW Jr. Activated human basophils contain histamine in cytoplasmic vesicles. *Int Arch Allergy Immunol* 1994;105:8-11.
37. Dvorak AM. Ultrastructural evidence for vesicular transport in basophil secretion. *Fundam Clin Immunol* 1995;3:17-28.