

Recovery of human basophils after FMLP-stimulated secretion

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Summary

Background Basophils are circulating, secretory granulocytes that are generally considered to be end-stage cells. In one species of guinea-pigs, basophilic leucocytes have been shown to recover from stimulated secretion in short-term cultures. Similar studies have not been done using human basophils.

Objective The purpose of this study was to examine human basophils in short-term recovery intervals following stimulation of secretion to determine whether visual evidence of recovery occurred.

Methods We examined the ultrastructural morphology of early recovery (10 min–6 h) of human basophils following secretion stimulated by formyl-methionyl-leucyl-phenyl-alanine (FMLP). A combined technique for electron microscopy consisted of post-fixation exposure to cationized ferritin and reduced osmium, providing maximum quality images and allowing identification of intracellular spaces/organelles that opened to the cell surface, often out of the plane of section.

Results The ultrastructural evaluation revealed that control basophils (0 time–6 h) did not undergo regulated secretion or develop the morphologies associated with recovery following secretion. FMLP-stimulated basophils underwent an overlapping continuum of piecemeal degranulation → anaphylactic degranulation (0 time–1 min), producing vesicle- and granule-free, completely degranulated, viable, mature basophils with polylobed nuclei. The early recovery period (10 min–6 h) following FMLP stimulation was characterized by reconstitution of granules. Morphological mechanisms for granule reconstitution included a mixture of conservation, condensation, and synthetic events.

Conclusion Human basophils, like guinea pig basophils, have the potential to recover from regulated secretion.

Keywords: granulogenesis, granule condensation, granule conservation, granule synthesis, guinea-pig basophil, mast cells

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Introduction

Basophils, like other granulocytes, are generally viewed as end-stage cells with no potential for renewal or prolonged functional life, particularly after migration

to tissues from the peripheral blood. Basophils are secretory cells characterized by synthetic product storage in prominent cytoplasmic granules and regulated secretion of these products by exocytosis of granules [1]. Many secretory cells with similar granule storage sites and secretory programs (i.e. numerous endocrine and exocrine cells) are not end-stage cells and characteristically replenish secreted materials as needed [2]. Evidence

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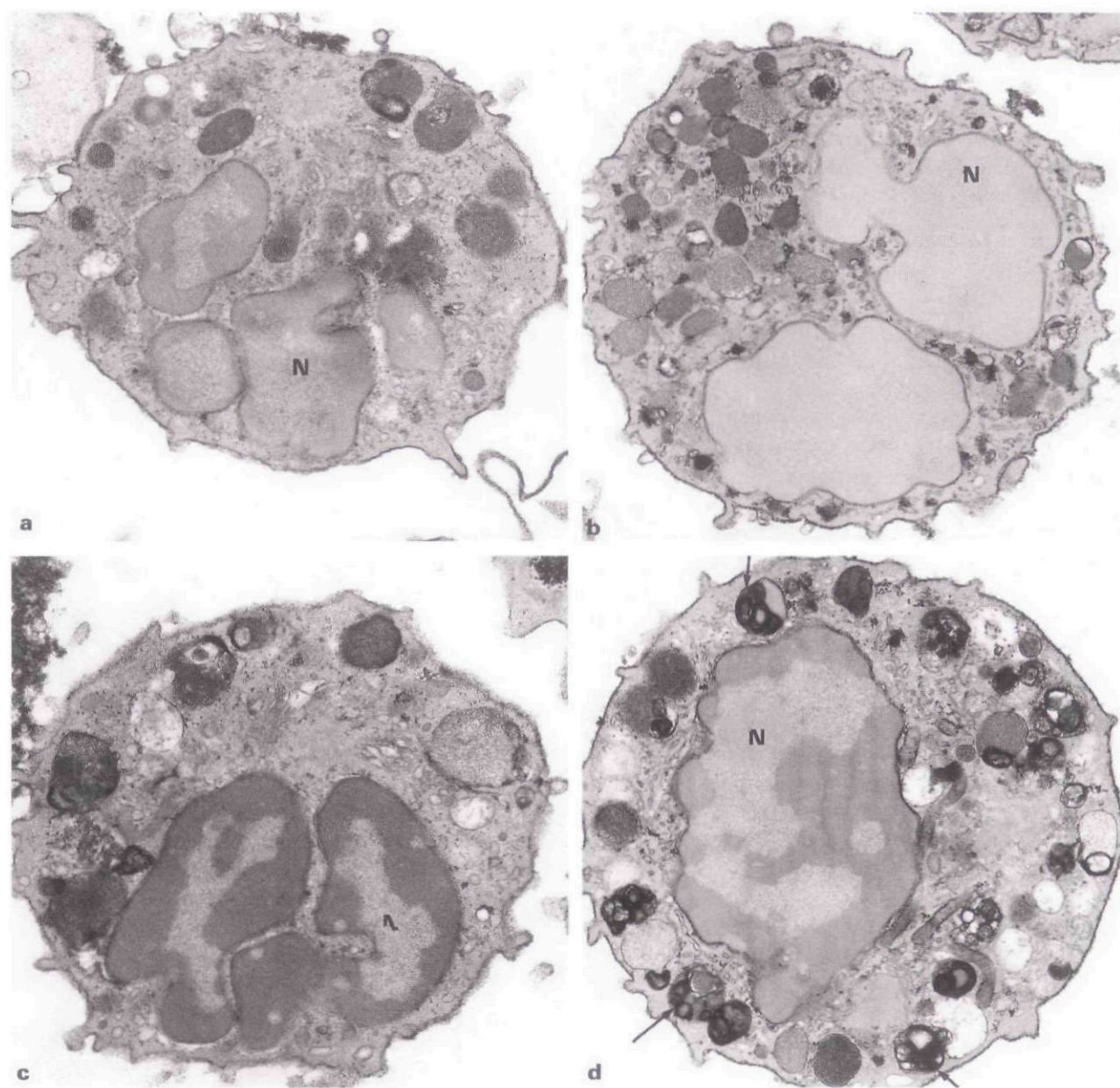


Fig. 1. Unstimulated basophils, incubated in buffer for 1 min (panel a), 5 min (panel b), 1 h (panel c), or 6 h (panel d), are round, have irregular, short surface processes, and retain cytoplasmic particle-filled secretory granules. Some granules contain concentric dense membranes (panel d, arrows); others are partially devoid of dense material, particularly by 6 h (panel d). The surface of unstimulated basophils is outlined by a uniform layer of cationized ferritin; no ferritin is present within cells or partially empty granule chambers. N = nucleus. (a,d) $\times 15\,000$; (b,c) $\times 16\,000$.

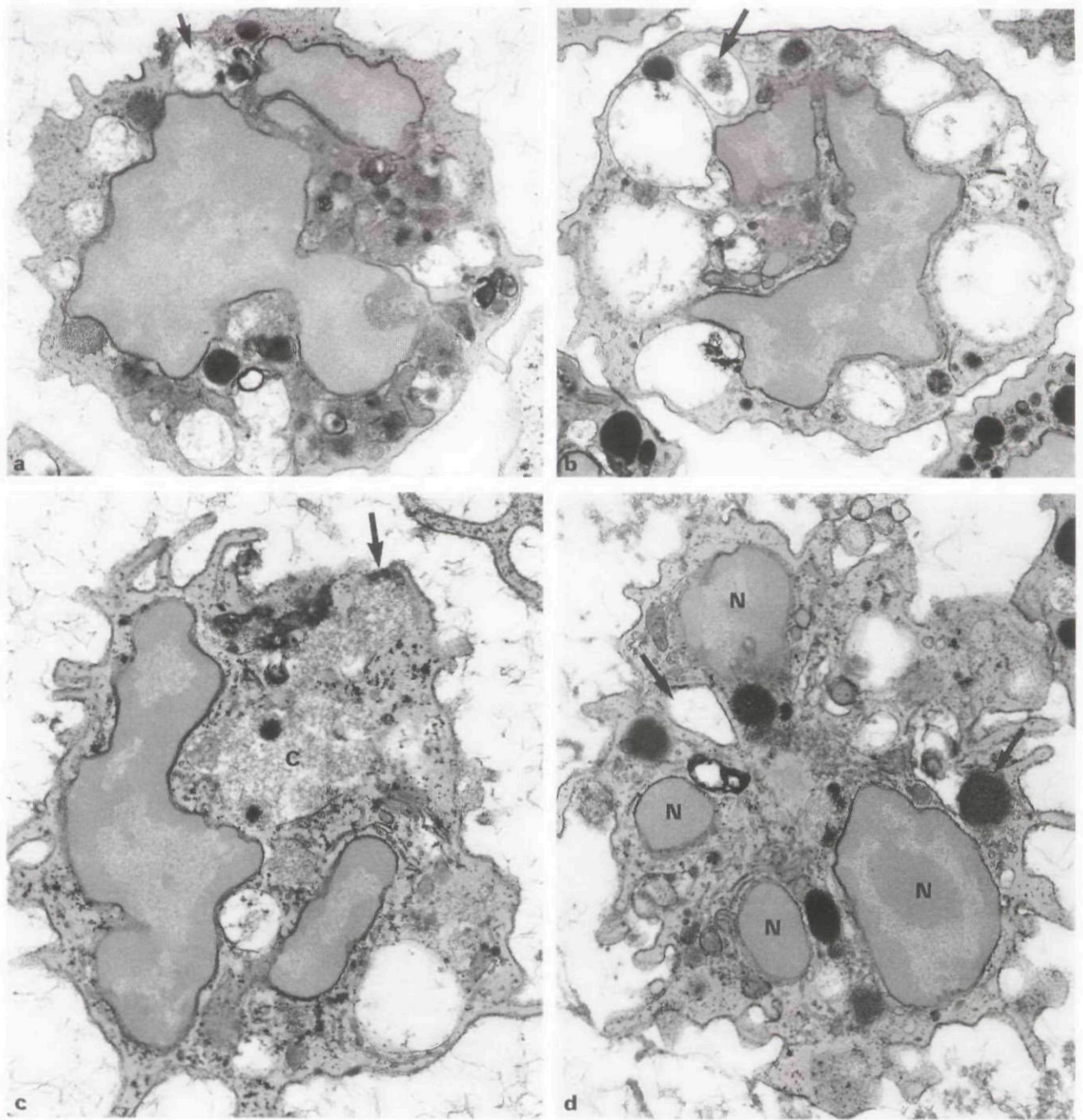


Fig. 2. FMLP-stimulated basophils, fixed for electron microscopy at 20 s (panels a–c) or 1 min (panel d) after stimulation, show morphological changes of secretion. In panels a and b, the cells retain their round shape and do not have increased surface processes. Some of the granules in panel a have released their dense granule contents and appear electron-lucent (arrow). Similar electron-lucent, empty granule chambers have markedly increased sizes in panel b. A few unaltered granules remain (panels a and b), and residual granule particles are evident in some swollen granules (panel b, arrow). Cationized ferritin is bound to the cell surface but does not enter the swollen granules (panels a and b). The changes in panels a and b are typical of piecemeal degranulation. In panel c, a large degranulation chamber (c) in the cytoplasm is filled with loosely packed granule particles. It opens to the exterior of the cell at one point where a plug of cationized ferritin is bound (arrow). Several empty granule chambers are present in the cytoplasm, and the cell surface processes have increased in length. In panel d, a few unaltered granules remain in a cell with markedly complex surface contours, where granules have been released. The nucleus (N) is polylobed. Cationized ferritin binds to the cell surface and to the membrane of several deeply invaginated portions of the surface within the cytoplasm (arrows) — one of these contains an extruded granule. The changes in panels c and d are typical of anaphylactic degranulation. (a) $\times 12\,000$; (b) $\times 10\,500$; (c) $\times 16\,000$; (d) $\times 13\,500$.

that basophils also recover from regulated degranulation by granule reconstitution was acquired through electron microscopic studies of guinea-pig basophils in short-term cultures following secretion [3]. Mast cells (cells with functional similarity to basophils) of two species (rat and human) are also known to reconstitute granules by several mechanisms following their regulated secretion by exocytosis [reviewed in ref. 2].

In the present work, we examined the ultrastructural morphology of human basophil recovery in short-term experiments (up to 6 h) following formyl-methionyl-leucyl-phenylalanine (FMLP) stimulation. Previously, FMLP stimulation of human basophils was shown to induce a rapid continuum of ultrastructural changes [4], corresponding in time to the regulated secretion of secretory products, determined biochemically [5], and characterized by early piecemeal degranulation merging into an anaphylactic degranulation phase. Piecemeal degranulation in human basophils is characterized by the development of partially empty and empty granules which do not fuse and remain in the cytoplasm [1,6]; anaphylactic degranulation in human basophils is characterized by the extrusion of individual granules to the cell's exterior following granule and plasma membrane fusion [1,2].

In the recovery period (10 min–6 h), we used a combined method — postfixation exposure to cationized ferritin [7,8] and reduced osmium [7] — to yield optimal ultrastructural information. That is, the reduced osmium postfixation technique maximizes the imaging of glycogen, granule contents and membranes [7], and the postfixation exposure to cationized ferritin allows binding of cationized ferritin to negatively charged membranes in continuity with the plasma membrane, thus defining such continuities that are present at section levels out of the plane of view [7]. These techniques together provide a view of morphological granule reconstitution in human basophils at early recovery times beyond stimulated secretion. The evident morphological mechanisms were primarily those of conservation (by re-uptake of granules and portions of granules and membrane as vesicles and vacuoles), condensation (electron-dense foci in refilling granules), and synthesis (Golgi expansion and progranule formation).

Materials and methods

Basophil purification

Buffy coat cells, obtained from normal donors undergoing hemapheresis, were partially purified by counter-current centrifugal elutriation and then placed on Percoll

density gradients (1.075 and 1.066 g/mL). Purified basophils were recovered from the interface between the two Percoll layers [5,9]. Two final preparations containing 52% and 28% basophils were used for these studies.

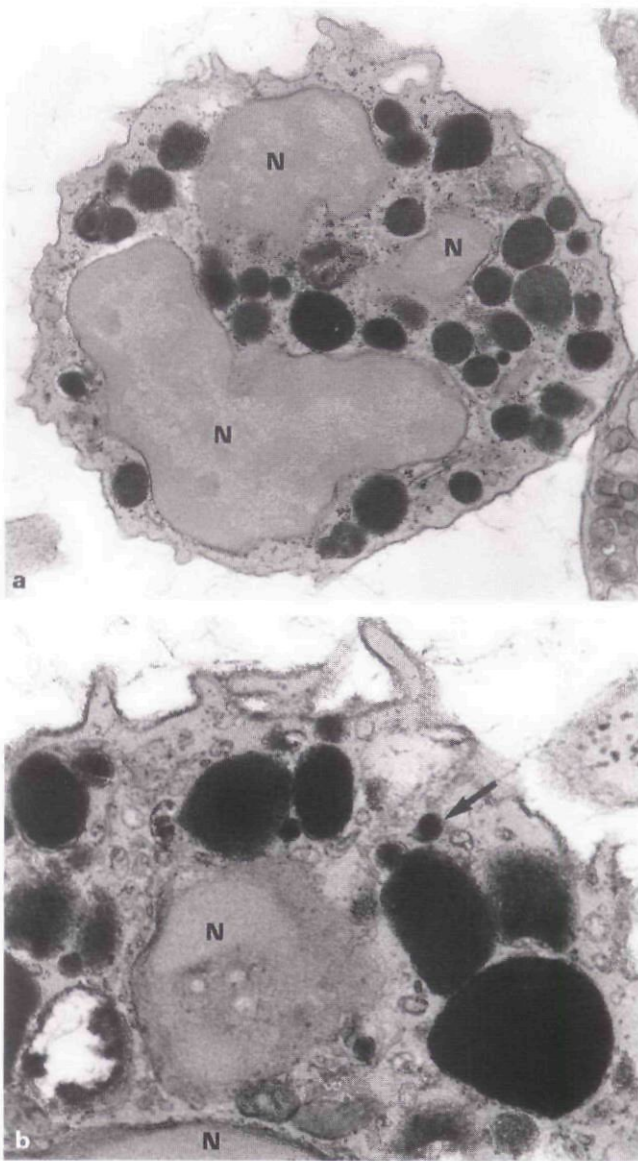


Fig. 3. Basophils, in the recovery phase following FMLP stimulation (6 h), have a full complement of uniformly electron-dense granules. The cell shape and surface contour approximate those of control unstimulated cells (Fig. 1) and those undergoing piecemeal degranulation (Fig. 2a,b) and differ from those of cells undergoing anaphylactic degranulation (Fig. 2c,d). The cell nuclei (N) remain polylobed. Numerous perigranular vesicles are also electron-dense (arrow). (a) $\times 13\,500$; (b) $\times 30\,000$.

Histamine release assay

Cells were resuspended in PAGCM (PIPES [piperazine N,N' -bis-2-ethane sulfonic acid]-albumin-glucose supplemented with 1 mM CaCl_2 and 1 mM MgCl_2) and stimulated with a final concentration of 1 μM FMLP at 37°C for various periods of time (0–6 h) in a final volume of 100 μL containing ~ 1 million basophils or 2–3 million total cells. Cells not stimulated with FMLP were also examined. In both of the experiments, a portion of the cells was set up in separate tubes (including samples for total histamine content and spontaneous release), challenged similarly, and the supernatant harvested to ascertain the extent of histamine release as described previously [4,5]. For one preparation, this reaction period was 10 min, and in the second experiment, the reaction was stopped after 30 min. Net FMLP-stimulated release for the two preparations was 30% from the preparation sampled at 10 min and 39% from the preparation sampled at 30 min.

Electron microscopy

Aliquoted samples of cells, either stimulated with FMLP or not, were recovered for electron microscopy by immersion fixation of suspended cells in a mixture of aldehydes, as before [7], at times 0, 20 s, 1, 10, 20, 30 min, 1, 3, 6 h. Cells from some samples were resuspended in Hanks' balanced salt solution (HBSS), containing 50 μL

(0.5 mg) cationized ferritin (Miles Laboratories, Inc.), on a rotary shaker set at low speed for 30 min at room temperature [7,8]. Further processing and embedding was as reported [7]. Thin sections were stained with lead citrate and viewed in a Philips 400 electron microscope.

Results

Human basophils, recovered in short-term cultures during the 6-h interval following FMLP-stimulated histamine release, showed ultrastructural indications of granule renewal — features differing from the ultrastructural morphology of unstimulated human basophils maintained for similar periods in short-term cultures.

Control unstimulated basophils, maintained in buffer (20 s, 1, 5, 10, 20, 30 min, 1, 3, 6 h), differed little from published descriptions of the ultrastructural anatomy of peripheral blood basophils [10]. They were small cells (~ 7 –10 μm) with condensed nuclear chromatin in polyllobed nuclei. Small, irregular surface processes and cytoplasmic membrane-bound secretory granules filled with electron-dense particles were evident (Fig. 1). The cytoplasm contained monoparticulate electron-dense glycogen particles and elongated mitochondria. Golgi structures were poorly developed, and rough endoplasmic reticulum was virtually absent. Small, membrane-bound, electron-lucent cytoplasmic vesicles were present. These structures had no apparent relationship to granules, Golgi or plasma membranes. The large secretory granules

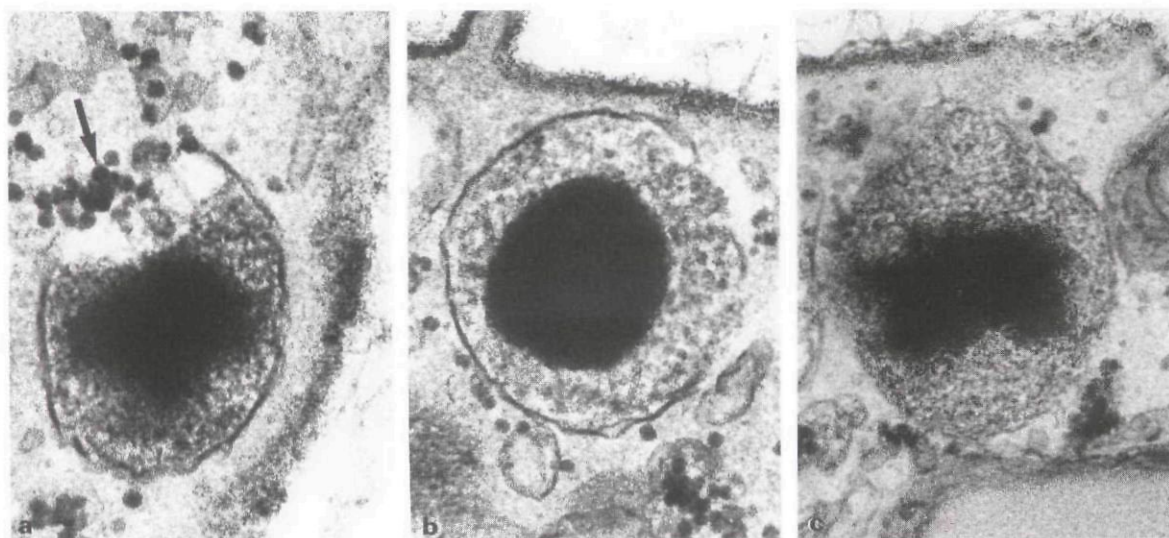


Fig. 4. Recovering basophil granules (3 h) show central, electron-dense condensation foci superimposed on less dense peripheral particles. These foci vary in shape from rectangular (panels a and c) to round (panel b). One recovering granule (panel a) retains a peripheral area of focal piecemeal loss of the peripheral granule particles adjacent to the central dense core. This area of granule release is nearly electron-lucent and is intimately associated with a cluster of electron-dense glycogen particles (arrow). (a) $\times 67\,000$; (b) $\times 60\,000$; (c) $\times 63\,000$.

($\sim 1 \mu\text{m}$) were filled with typical granule particles; some granules had multiple dense concentric membranes and homogeneously textured Charcot-Leyden crystals (CLCs) within them. At later time intervals, a portion of the secretory granules, while not enlarged, had diminished particulate content. These structures were membrane-bound and well-separated from other granules and the plasma membrane.

The ultrastructural morphology of the release reaction stimulated in human basophils by FMLP has been presented in detail [4]. These findings, comprising a kinetic study spanning 0 time stimulation to 10 min after exposure to FMLP, were recapitulated here. Briefly, we identified individual basophils expressing the morphology of piecemeal degranulation (an early event spanning the first 30 s after FMLP) and that of anaphylactic degranulation (a somewhat later event spanning 20 s to 2 min following FMLP stimulation) (Fig. 2). We have postulated that these diverse morphologies reflect a continuum of events that are produced by this trigger, leading ultimately to the release of virtually all secretory granules from these activated cells. Particularly, full granules and empty granule containers were absent from completely degranulated cells, reflecting the externalization of granule membranes as well as secretion of the contents of granule chambers.

The specific morphologies of FMLP activation in this early time frame revealed piecemeal degranulation (Fig. 2a,b). This morphology was characterized by the development of a large number of cells (at the earliest times [0', 10''] tested after stimulation) with release of electron-dense granule contents and retention of granule-sized containers (partially empty and empty) in the cytoplasm (Fig. 2a). The cells' surface was not amplified by elongated processes, and the cells clearly were mature polymorphonuclear cells without evidence of cell injury (Fig. 2a). At somewhat later times after stimulation (20'', 30''), cells undergoing piecemeal degranulation showed marked enlargement of partially empty and empty granule containers, which nearly filled the cytoplasm (Fig. 2b). As in the cells without such enlargement of releasing granules, the electron-dense tracer, cationized ferritin, used after fixation did not enter these structures, indicating that they were not open to the plasma membrane at these early times (Fig. 2a,b). Also, the cell surface did not show evidence of activation (e.g., elongation of processes) as was regularly present during active granule extrusion (Fig. 2c,d). The basophils displaying piecemeal degranulation with massive container enlargement were mature polymorphonuclear cells with no evidence of cell injury (Fig. 2b).

Later, after stimulation with FMLP, generally at 30'', 1', 2', there was extensive evidence of the morphology of

anaphylactic degranulation. This form of regulated secretion in human basophils was characterized by the formation of intracytoplasmic degranulation channels and extrusion of membrane-free granules and granule particles en masse or individually to the cell surface (Fig. 2c). These polymorphonuclear cells showed no signs of cell injury; some contained empty, granule-sized containers and enlarged containers, indicating overlap with the morphology of piecemeal degranulation in this FMLP-stimulated continuum of release activity (Fig. 2c). The surface activity of cells in the process of extruding granules and adding granule membranes to their plasma membranes was extraordinarily complex and was characterized by numerous elongated processes (Fig. 2c,d). Cells that had reached this point in release activity (1–2 minutes after FMLP) were often granule-free, undamaged

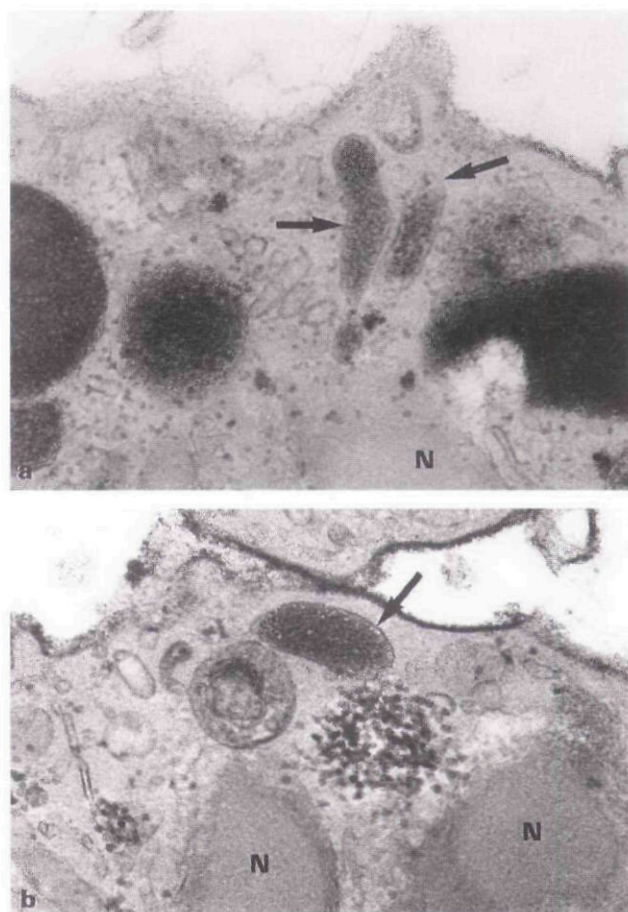


Fig. 5. The peripheral cytoplasm of these recovering basophils (30 min) shows several elongated tubules (arrows) that are filled with granule particles close to the cell surface. Cationized ferritin stains the plasma membrane but not the internal organelles. A large aggregate of glycogen particles is present (panel b). N = nucleus. (a) $\times 41\,000$; (b) $\times 37\,000$.

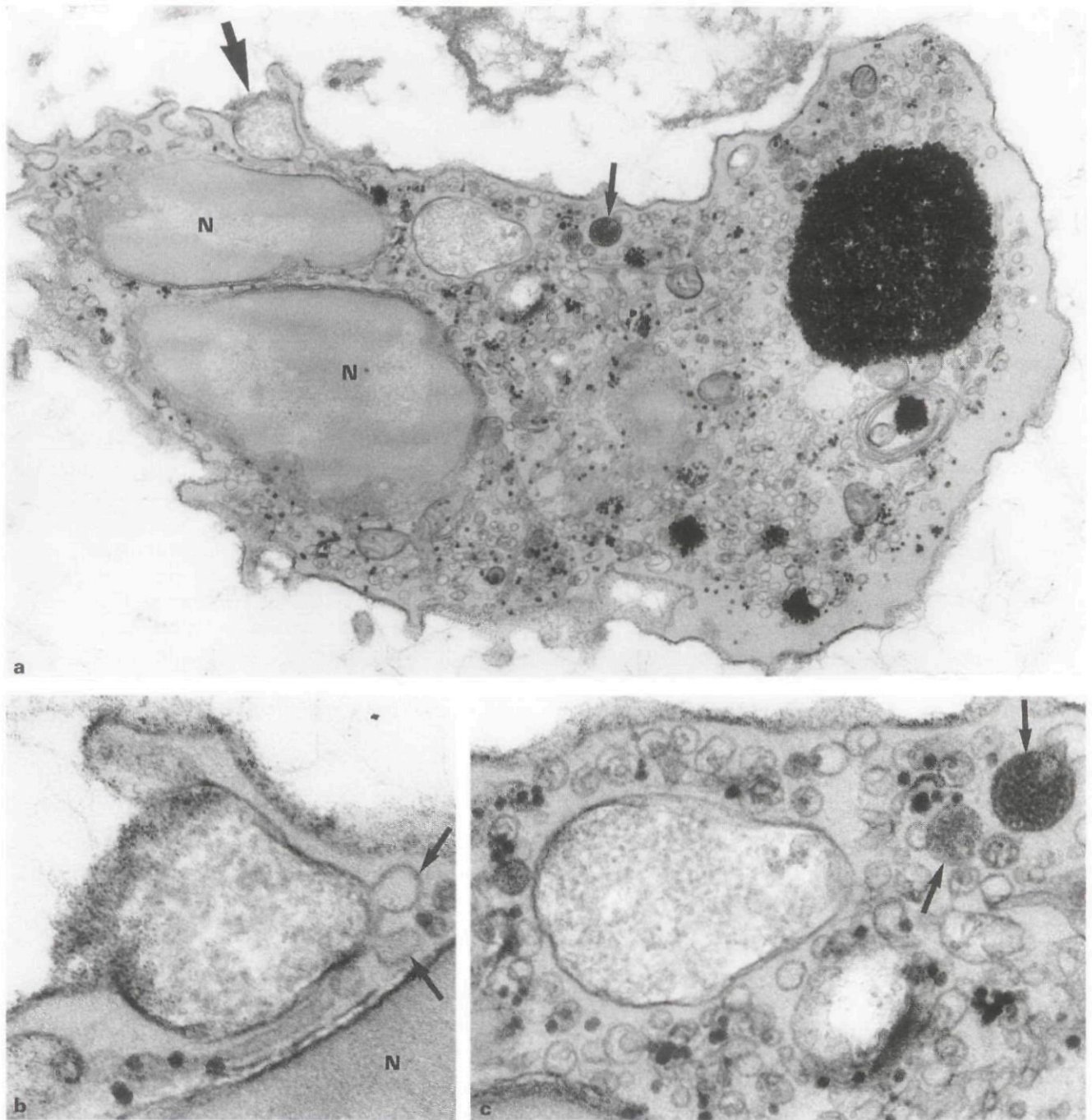


Fig. 6. A paucigranular, recovering basophil (1 h) (panel a) is elongated, has two lobes of nuclei (N) visible, and a large, round aggregate of electron-dense glycogen particles; the entire cytoplasm is virtually filled with vesicles. The cell surface is stained with cationized ferritin, which also binds to the surface of one extruded granule (large arrow, and enlarged in panel b). Another granule (small arrow) remains in the cytoplasm and contains granule particles; a large number of vesicles (which either are electron-lucent or contain electron-dense particles [arrow]) surround this one remaining cytoplasmic granule (arrow enlarged in panel c). In panel b, note that several vesicles (arrows) are attached to the plasma membrane immediately beneath the overlying extruded granule. (a) $\times 23\,000$; (b) $\times 84\,500$; (c) $\times 64\,000$.

cells with polylobed nuclei and extensive surface activation. Many cells retained several granules of normal size ($\sim 1 \mu\text{m}$) with no evidence of content losses. Large clefts into the cytoplasm from the cell surface and some empty granule containers now permitted entry and staining with the postfixation electron-dense tracer, cationized ferritin, indicating their continuity with the cell surface (Fig. 2d). The cytoplasmic vesicle content, which was high [4] in piecemeal degranulating cells examined at 0–10'' after stimulation, became low in actively extruding basophils at 30'' and 1' times after FMLP [4] (Fig. 2c,d). Moreover, residual granules that did not become altered in this early phase of release showed no evidence of central condensation and did not reside near cell surfaces (Fig. 2d). Extruded granules, on the other hand, were often closely attached to cells in an extracellular location.

Recovery from FMLP-induced human basophil secretion was characterized by a number of morphological events. First, there was little evidence of residual piecemeal degranulation, characterized by regular-sized, electron-lucent cytoplasmic containers and no evidence of piecemeal degranulation with enlarged, electron-lucent granule containers at the time intervals sampled, spanning 10, 20 and 30 min to 1, 3 and 6 h. Rather, many granule containers displayed central condensation of electron-dense materials, often superimposed on less

dense contents. Ultimately, large numbers of cytoplasmic granules were completely filled with electron-dense material, granules that revealed no evidence of focal electron-lucent areas characteristic of piecemeal degranulation. Such granules also did not generally have large collections of dense concentric membranes (Fig. 3).

Granule condensation of central dense materials on a less dense background was noted in both small and large ($\sim 1 \mu\text{m}$) particle-containing granules, many of which were located in the peripheral cytoplasmic area (Fig. 4). The central dense nucleoids resemble the general process of granule condensation seen in recovering and maturing human mast cells [11–13]. The central densities in human basophil granules should not be confused with the central dense cores that are an integral part of the anatomy of mature human eosinophil secondary granules [14]. Most of the recondensing basophil granules displayed a single dense core, but some had several. That these granules represent refilled granules, which were previously emptied by piecemeal degranulation [1,6], is suggested by the presence of some granules with central condensation cores that also retained focal electron-lucent piecemeal holes in their particulate material (Fig. 4a). We also noted elongated tubular granules filled with dense particles in the peripheral cytoplasmic area, structures that are not generally present in unstimulated cells (Fig. 5a,b).

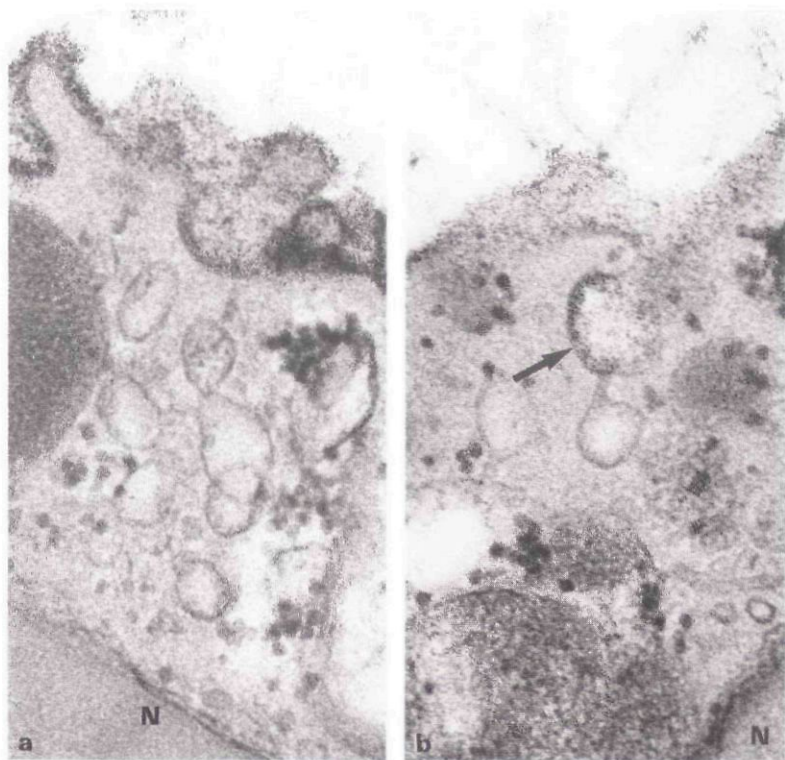


Fig. 7. The peripheral cytoplasm of two recovering basophils (3 h) shows attached chains of electron-lucent vesicles (panels a and b) and several vesicles with granule particles (panel b). Electron-dense glycogen particles are present in the cytoplasm. Cationized ferritin binds to the plasma membrane as well as to a vesicle that is open to the cell surface. This vesicle (arrow) is attached by a narrow channel to an underlying vesicle which does not contain the postfixation electron-dense tracer and, thus, is not in continuity with the surface. The narrow channel restricting this access must be less than the 11-nm size of ferritin. N = nucleus. (a) $\times 63\,000$; (b) $\times 125\,500$.

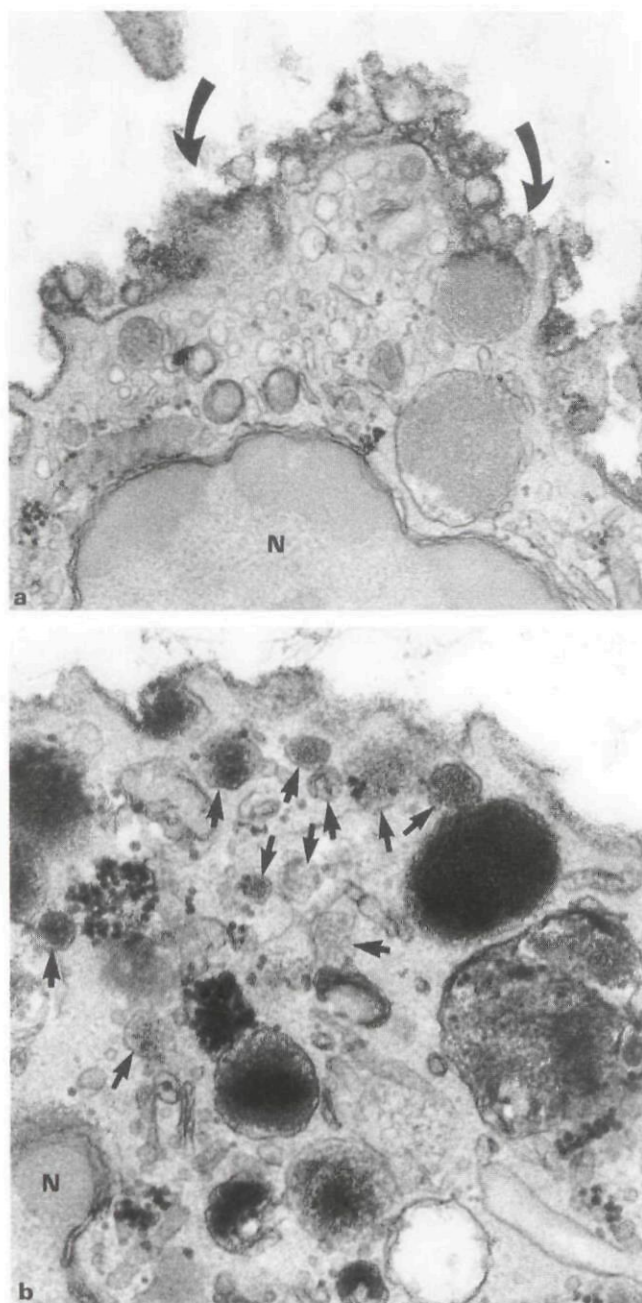


Fig. 8. Clusters of vesicles in the peripheral cytoplasm of recovering basophils (3 h) are either apparently empty (electron-lucent, panel a) or filled with electron-dense particles (arrows) similar to those in granules (panel b). Two extruded granules with overlying cationized ferritin remain attached to the cell surface (arrows in panel a). N = nucleus. (a) $\times 31\,000$; (b) $\times 41\,500$.

Paucigranular basophils were noted at recovery times. These mature cells with polylobed nuclei were not damaged. They differed from the nearly granule-free basophils, which abounded at 1 min after FMLP stimulation as a result of extrusion of nearly all secretory

granules, in that they had large numbers of cytoplasmic smooth membrane-bound tubules and vesicles (Fig. 6). Other features of these cells were large masses of aggregated glycogen in their cytoplasm.

The large number of cytoplasmic vesicles present in recovering human basophils often formed focal collections in the peripheral cytoplasm. These collections surrounded granules and were sometimes concentrated beneath surface-attached, previously released granule particles (Fig. 6b). Most of these vesicles were smooth membrane-bound, but coated pits in the plasma membrane were seen in similar locations beneath granular particles. Some vesicles that were attached to the plasma membrane and that were located in the peripheral cytoplasm at these locations contained the postfixation tracer, cationized ferritin, indicating their continuity with the cell surface (Fig. 7b). The vesicles located in these sites consisted of a mixture of electron-lucent structures and vesicles filled with dense and particulate materials analogous to adjacent cytoplasmic granules or to overlying granule particles on the plasma membrane (Figs 7–9).

Granule-sized, membrane-bound vacuoles were seen in the peripheral cytoplasm and were in continuity with the plasma membrane, as evidenced by the presence of the electron-dense postfixation tracer, cationized ferritin, within them and staining their membranes (Fig. 10). These spaces, devoid of granule content, were less frequently present than were granules in the process of being individually enclosed by short, arm-like extensions of the cytoplasm (Fig. 11). Such enclosure suggested cytoplasmic re-uptake of previously extruded granules that remained intact and attached to the cell surface. Internalization of these granules resulted in their reinvestiture with a plasma membrane-derived membrane to which the granule was attached. Initially, these released granules were coated by cationized ferritin when attached to the cell surface. As they became enclosed, this postfixation tracer no longer bound to the granules.

The majority of basophils recovering from FMLP stimulation of histamine secretion did not show extensive increases in Golgi structures. Some cells did show increased numbers of Golgi area electron-lucent, smooth membrane-bound vesicles, and others had concentrations of small vesicles and larger progranules filled with electron-dense particles (Fig. 12).

Discussion

We document here ultrastructural events that underlie granule renewal in human basophils recovering from FMLP-induced degranulation. While some unstimulated basophils, maintained over the same time frame, showed some losses of particles from granule containers, these

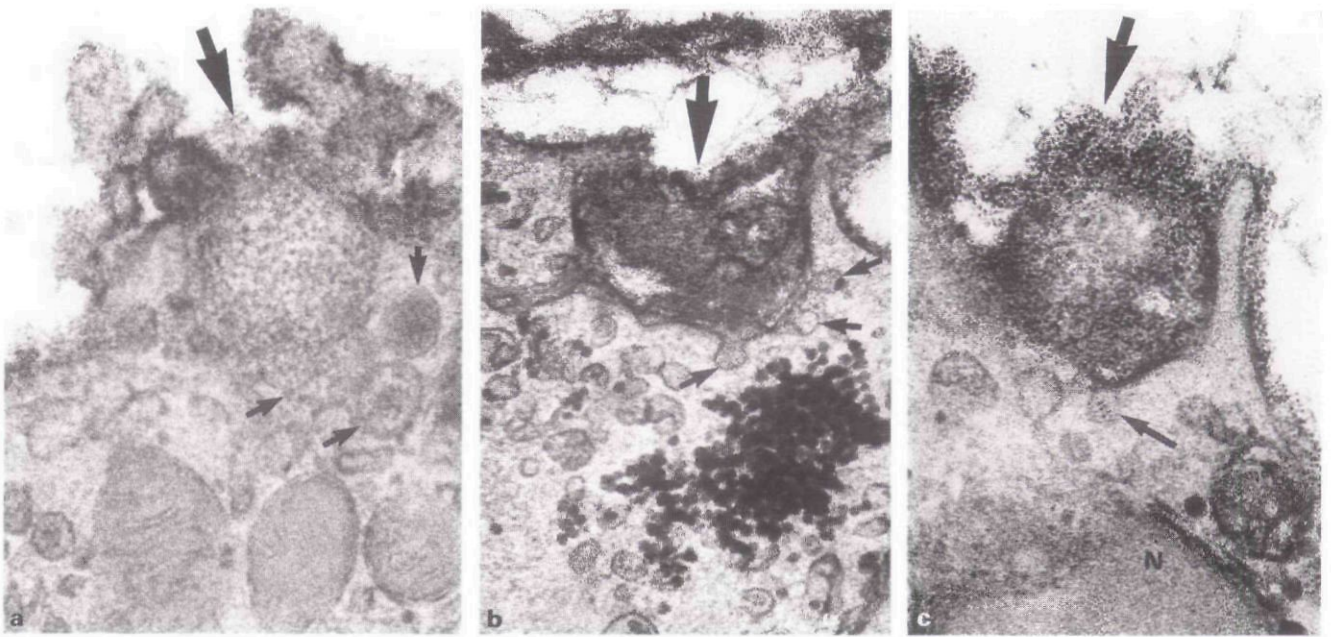


Fig. 9. The cell surfaces of these recovering basophils (3 h) have attached, extruded, membrane-free granules (arrowheads) that are heavily stained with the postfixation electron-dense tracer, cationized ferritin. Granule particles are easily seen in panel a, but their clarity is diminished in panels b and c, where cationized ferritin nearly fills the granule-occupied extracellular space. The cytoplasm immediately underlying the extruded granule contains particle-filled vesicles (arrows) in panel a. In panel b, vesicles (arrows) are attached to the plasma membrane immediately beneath the extruded granule (note the large aggregate of electron-dense glycogen particles nearby). In panel c, a vesicle (arrow), which is attached to the plasma membrane beneath the extruded granule, contains cationized ferritin, indicating continuity with the overlying extracellular area. N = nucleus. (a) $\times 70\,000$; (b) $\times 56\,500$; (c) $\times 84\,000$.

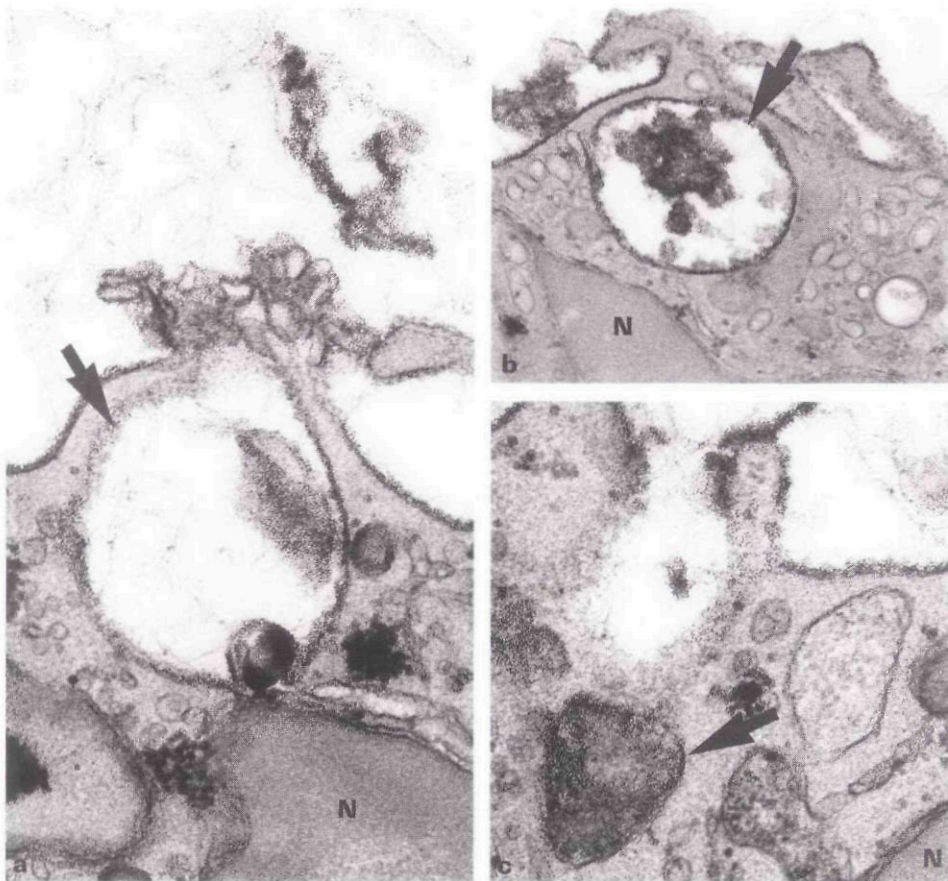


Fig. 10. Recovering basophils (3 h) contain granule-sized, membrane-bound vacuoles in the peripheral cytoplasm (arrowheads). Cationized ferritin has stained these structures, indicating continuity with the extracellular space. Some vacuoles (panels b and c) contain residual granule particles that have also bound the electron-dense postfixation tracer. N = nucleus. (a) $\times 45\,000$; (b) $\times 32\,000$; (c) $\times 49\,500$.

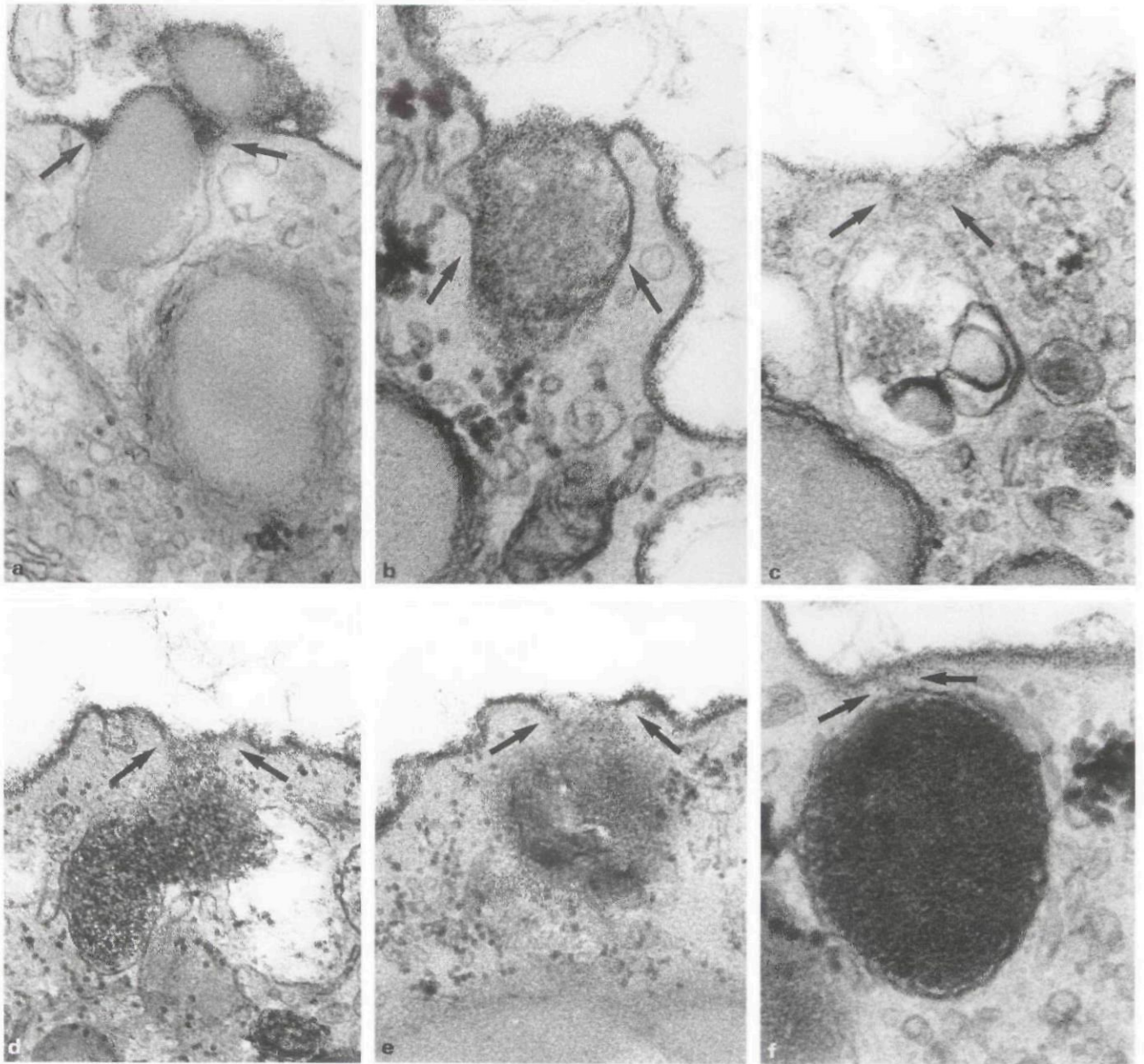


Fig. 11. Recovering basophils show extruded granules (3 h) in various stages of internalization. Note the progressive investiture by two short cytoplasmic arms (arrows) which close and exclude the extracellular tracer, cationized ferritin. Cationized ferritin binds to the cell surfaces and the surfaces of granules that still maintain continuity to the cell surfaces (panels a–d). (a) $\times 53\,000$; (b) $\times 76\,000$; (c) $\times 59\,000$; (d) $\times 45\,000$; (e) $\times 50\,000$; (f) $\times 65\,000$.

structures were not enlarged, and we did not see in the control basophils the ultrastructural changes of the continuum piecemeal degranulation $\rightarrow$ anaphylactic degranulation produced by an FMLP stimulus [4]. Characteristically, the release reaction that is stimulated in human basophils by FMLP produces empty granule containers, similar in size to full granules, and empty

granule containers that are massively enlarged. These changes, typical of piecemeal degranulation [6], do not show continuity to the cell surface at early times (seconds) as documented by the exclusion of cationized ferritin, an electron-dense postfixation tracer which makes this distinction possible. At later times (minutes) in the degranulation continuum stimulated by FMLP, extrusion of

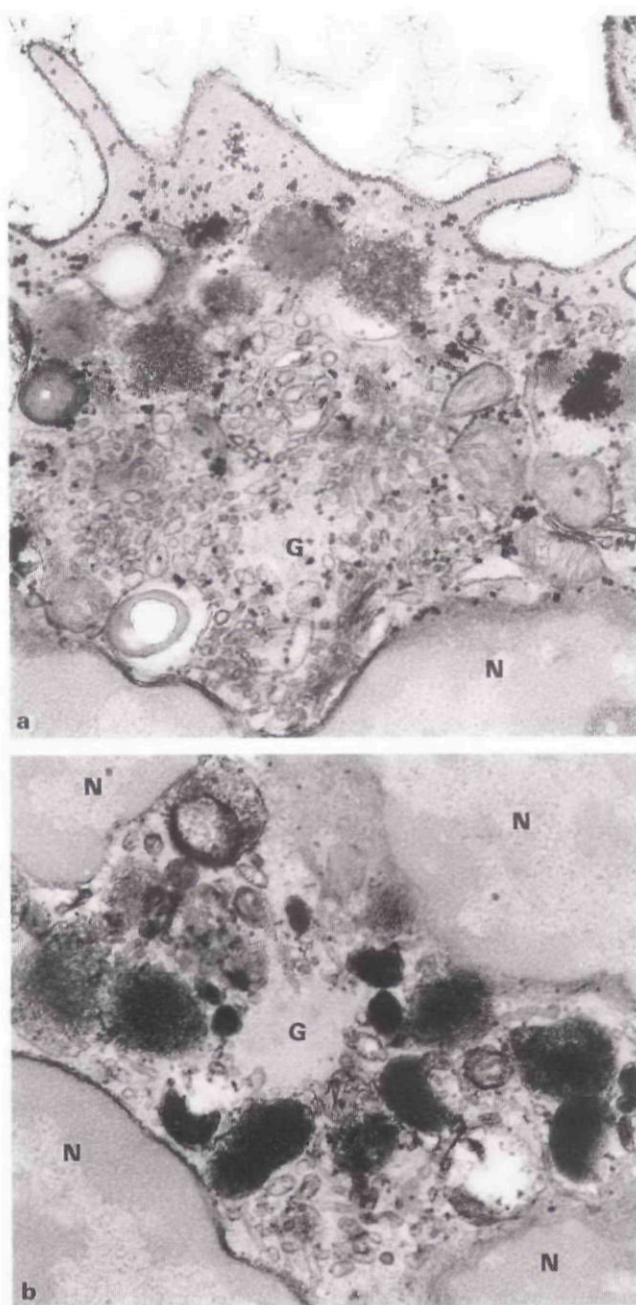


Fig. 12. Golgi areas (G) near the polylobed nuclei (N) of recovering basophils (6 h) show large numbers of vesicles (a) and electron-dense progranules (b). (a) $\times 30\,500$; (b) $\times 32\,000$.

granules and their membranes prevails, and irregularly shaped cells have deep clefts penetrating into their cytoplasm, which do stain with cationized ferritin [4]. Thus, completely degranulated cells are produced in quantity by 1–2 min after FMLP stimulation [4]. These cells are devoid of granules and empty granule containers. Some cells may retain a few granules that are not

altered. When present, such granules neither show changes in the density of particle packing nor display condensation foci. The number and distribution of cytoplasmic vesicles which characterize this degranulation continuum (0–2 min) include large numbers of perigranular vesicles in the granule emptying phase of piecemeal degranulation and a marked reduction of cytoplasmic vesicles in the granule and granule membrane extrusion phase of anaphylactic degranulation [4]. Completely degranulated basophils (1–2 min after FMLP) are viable cells having essentially no cytoplasmic vesicles or granules.

Control basophils also did not show the morphological features that accompanied granule renewal in the recovering basophils. The latter preparations were characterized primarily by conservation and condensation events; some cells had morphological evidence of enhanced synthesis. (These experiments were done in the continued presence of FMLP. If it were possible to effectively remove FMLP from its receptor and provide a medium that mimics the *in vivo* environment, the recovery by synthetic mechanisms might have been more complete). Synthesis may actually be increased at times beyond these studies, since they were terminated relatively early after degranulation (i.e. 6 h). For example, in studies of recovery following anaphylactic degranulation of isolated lung human mast cells, conservation and condensation preceded synthesis as mechanisms of granule reconstitution [11,15–18]. Conservation and condensation morphology in recovering human basophils included the development of electron-dense condensation foci in cytoplasmic granules, prominent subgranular vesicles resting beneath exocytosed, plasma membrane-attached granule particles, internalized, cationized ferritin-stained membranes forming granule-sized spaces in the cytoplasm, and extension of cytoplasmic processes (arms) that enclosed and internalized extruded granule particles. All of these events were absent from controls.

Another striking change in the recovering basophils was the presence of extensive collections of cytoplasmic vesicles in the peripheral cytoplasm and near some recovering granules — changes that were totally absent from controls and characteristically absent from the completely degranulated cells that preceded the recovery times studied. Using an enzyme-affinity method to localize histamine in ultrastructural samples [19–21], we have found that these clustered vesicles are histamine-rich [22]. While their predominant subplasma membrane location suggests that they may represent retrieval of released histamine in mature cells without enlarged Golgi structures, it is also possible that some histamine-rich vesicles originate in Golgi synthetic structures. Whatever their source, the vesicles are a prominent feature of

recovering human basophils, and, thus, a similarity to the recovery process documented earlier in guinea-pig basophils is highlighted [3].

We have previously examined the ultrastructural morphology of recovery from anaphylactic degranulation of two histamine-rich cells in short-term cultures — guinea-pig basophils [3] and human lung mast cells [11,15–18]. Recovery of rat mast cells from secretion has been examined by several groups [23–37] (and reviewed in ref. 38). These studies (spanning ~40 years) and those of human lung mast cells [11,15–18] leave little doubt that mast cells have the capacity to recover from stimulated secretion. The relative extent of such recovery *in vivo* vs recruitment of agranular mast cell precursors from the blood to various tissue sites where maturation proceeds is not known. It is possible that recovery from antecedent piecemeal degranulation, by the refilling of conserved containers, represents a major event in those disorders associated with this form of secretion [1,6,39,40].

Prior to the studies reported here, basophils of only one species — guinea-pig — had been shown to reconstitute granules following stimulated secretion [3]. The ultrastructural similarities of recovery seen in basophils from human and guinea-pigs are extensive. Whether this event takes place *in vivo* is entirely unknown. The two studies document, however, that these mature granulocytes, previously regarded as end-stage, non-synthetic cells, not only survive massive regulated secretion but also recover from their altered morphologies by reconstituting secretory granules. In the human basophils studied here, the ultrastructural morphology of recovery suggests conservation and condensation as major mechanisms, spanning 10 min to 6 h after release. There was also expansion of synthetic organelles in some cells, suggesting multiple (and/or alternative) routes of granule product recovery.

Acknowledgments

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References

- 1 Dvorak AM. Ultrastructural analysis of anaphylactic and piecemeal degranulation of human mast cells and basophils. In: Foreman JC, ed. *Immunopharmacology of Mast Cells and Basophils* (in the series *The Handbook of Immunopharmacology*; Page C, series ed.). London: Academic Press, 1993:89–113.
- 2 Dvorak AM. Basophil and Mast Cell Degranulation and Recovery. Vol 4 in the series *Blood Cell Biochemistry*; Harris JR, series ed. New York: Plenum Press, 1991.
- 3 Dvorak AM, Galli SJ, Morgan E, Galli AS, Hammond ME, Dvorak HF. Anaphylactic degranulation of guinea pig basophilic leukocytes. II. Evidence for regranulation of mature basophils during recovery from degranulation *in vitro*. *Lab Invest* 1982; 46:461–75.
- 4 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.
- 5 Warner JA, Peters SP, Lichtenstein LM, Hubbard W, Yancey KB, Stevenson HC, Miller PJ, MacGlashan DW Jr. Differential release of mediators from human basophils: Differences in arachidonic acid metabolism following activation by unrelated stimuli. *J Leukocyte Biol* 1989; 45:558–71.
- 6 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, ed. *Granulocyte Responses to Cytokines. Basic and Clinical Research*. New York: Marcel Dekker, Inc., 1992:169–271.
- 7 Dvorak AM, Newball HH, Dvorak HF, Lichtenstein LM. Antigen-induced IgE-mediated degranulation of human basophils. *Lab Invest* 1980; 43:126–39.
- 8 Danon D, Goldstein L, Marikovsky Y, Skutelsky E. Use of cationized ferritin as a label of negative charges on cell surfaces. *J Ultrastruct Res* 1972; 38:500–10.
- 9 Warner JA, Reshef A, MacGlashan DW Jr. A rapid Percoll technique for the purification of human basophils. *J Immunol Methods* 1987; 105:107–10.
- 10 Dvorak AM. Morphologic and immunologic characterization of human basophils, 1879 to 1985. *Riv Immunol Immunofarmacol* 1988; 8:50–83.
- 11 Dvorak AM, Schleimer RP, Schulman ES, Lichtenstein LM. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. *In vitro* studies with mast cells purified from human lungs. *Lab Invest* 1986; 54:663–78.
- 12 Dvorak AM, Furitsu T, Kissell-Rainville S, Ishizaka T. Ultrastructural identification of human mast cells resembling skin mast cells stimulated to develop in long-term human cord blood mononuclear cells cultured with 3T3 mouse skin fibroblasts. *J Leukocyte Biol* 1992; 51:557–69.
- 13 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 Leukocyte Biol* 1993; 54:465–85.
- 14 Dvorak AM, Ackerman SJ, Weller PF. Subcellular morphology and biochemistry of eosinophils. In: Harris JR, ed. *Megakaryocytes, Platelets, Macrophages and Eosinophils*. Vol 2 in the series *Blood Cell Biochemistry*, Harris JR, series ed. London: Plenum Publishing Corp., 1991:237–344.
- 15 Dvorak AM, Schleimer RP, Lichtenstein LM. Morphologic mast cell cycles. *Cell Immunol* 1987; 105:199–204.
- 16 Dvorak AM, Schleimer RP, Lichtenstein LM. Human mast cells synthesize new granules during recovery from

- degranulation. In vitro studies with mast cells purified from human lungs. *Blood* 1988; 71:76–85.
- 17 Dvorak AM. Recovery of human lung mast cells from anaphylactic degranulation utilizes a mixture of conservation and synthetic mechanisms. In: Galli SJ, Austen KF, eds. *Mast Cell and Basophil Differentiation and Function in Health and Disease* New York: Raven Press, 1989:119–48.
 - 18 Dvorak AM. Recovery of basophils and mast cells from degranulation. In: Dvorak AM, ed. *Basophil and Mast Cell Degranulation and Recovery*. Vol 4 in the series *Blood Cell Biochemistry*, Harris JR, series ed. New York: Plenum Press, 1991:277–367.
 - 19 Dvorak AM, Morgan ES, Schleimer RP, Lichtenstein LM. Diamine oxidase-gold labels histamine in human mast-cell granules: A new enzyme-affinity ultrastructural method. *J Histochem Cytochem* 1993; 41:787–800.
 - 20 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.
 - 21 Dvorak AM, Tepper RI, Weller PF, Morgan ES, Estrella P, Monahan-Earley RA, Galli SJ. 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.
 - 22 Dvork AM, MacGlashan DW Jr, Morgan ES, Lichtenstein LM. Histamine distribution in human basophil secretory granules undergoing FMLP-stimulated secretion and recovery. *Blood* 1995; 86:3560–6.
 - 23 Berlin G, Enerbäck L. Mast cell secretion. Rapid sealing of exocytotic cavities demonstrated by cytofluorometry. *Int Arch Allergy Appl Immunol* 1984; 73:256–62.
 - 24 Burwen SJ. Recycling of mast cells following degranulation in vitro: An ultrastructural study. *Tissue Cell* 1982; 14:125–34.
 - 25 Bytzer P, Nielsen EH, Clausen J. Surface morphology of rat peritoneal mast cells during in vitro regeneration after histamine secretion. *Cell Tissue Res* 1981; 216:647–54.
 - 26 Fawcett DW. An experimental study of mast cell degranulation and regeneration. *Anat Rec* 1955; 121:29–51.
 - 27 Hammel I, Lagunoff D, Krüger P-G. Recovery of rat mast cells after secretion: A morphometric study. *Exp Cell Res* 1989; 184:518–23.
 - 28 Krüger PG, Lagunoff D. Mast cell restoration. A study of the rat peritoneal mast cells after depletion with polymyxin B. *Int Arch Allergy Appl Immunol* 1981; 65:278–90.
 - 29 Lagunoff D. Vital staining of mast cells with ruthenium red. *J Histochem Cytochem* 1972; 20:938–44.
 - 30 Meyer SL, Saunders AM. Cytofluorometric study of mast cell polyanions. II. Adult rat peritoneal mast cells regenerating after polymyxin treatment. *J Histochem Cytochem* 1969; 17:56–61.
 - 31 Németh A, Röhlich P. Early membrane retrieval following exocytosis in rat mast cells. *Eur J Cell Biol* 1982; 28:39–46.
 - 32 Nielsen EH, Clausen J. Electron microscopic study of the regeneration in vivo of rat peritoneal mast cells after histamine secretion. *Cell Tissue Res* 1982; 224:465–8.
 - 33 Nielsen EH, Bytzer P, Clausen J, Chakravarty N. Electron microscopic study of the regeneration in vitro of rat peritoneal mast cells after histamine secretion. *Cell Tissue Res* 1981; 216:635–45.
 - 34 Nielsen EH, Clausen J, Bytzer P. Membrane retrieval in non-exocytic and exocytic rat peritoneal mast cells. *Exp Cell Res* 1981; 135:291–8.
 - 35 Padawer J. The reaction of rat mast cells to polylysine. *J Cell Biol* 1970; 47:352–72.
 - 36 Thilo L. Selective internalization of granule membrane after secretion in mast cells. *Proc Natl Acad Sci USA* 1985; 82:1711–5.
 - 37 Weil BJ, Renoux ML. Study of granule reappearance and histamine synthesis in rat mast cells maintained in short-term cultures. *Cell Immunol* 1982; 68:220–33.
 - 38 Dvorak AM. Rat mast cells. In: Dvorak AM, ed. *Basophil and Mast Cell Degranulation and Recovery*. Vol 4 in the series *Blood Cell Biochemistry*; Harris JR, series ed. New York: Plenum Press, 1991:3–25.
 - 39 Dvorak AM, Kissell S. Granule changes of human skin mast cells characteristic of piecemeal degranulation and associated with recovery during wound healing in situ. *J Leukocyte Biol* 1991; 49:197–210.
 - 40 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.

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