Human Mast Cells Synthesize New Granules During Recovery From Degranulation. In Vitro Studies With Mast Cells Purified From Human Lungs

By Ann M. Dvorak, Robert P. Schleimer, and Lawrence M. Lichtenstein

Secretory cells undergoing release and recovery events related to constitutive and/or stimulus-initiated secretion might be expected to undergo distinctive changes in morphology as well. We studied the release and recovery events of human mast cell secretion stimulated by antibody to immunoglobulin E. We used enzymatically digested mast cells from human lung specimens further purified by countercurrent centrifugation elutriation. Release kinetics were like those reported for isolated human lung mast cells. In two complete kinetic experiments we restudied these early release patterns (0 to 30 minutes). Mast cells, either stimulated or controls, were then cultured and sampled for electronmicroscopic studies at periodic intervals (3 to 48 hours). We describe events of the late recovery period here, although some overlap with processes seen in early recovery samples occurred. Mast cells that released nearly all their cytoplasmic granules and exteriorized the containers, eg, granule-channel membranes, underwent progressive enlargement of Golgi structures and development of numerous small cytoplasmic vesicles and small, membrane-bound granules filled with particulate and dense content. Ultimately, new mature cytoplasmic granules of all substructural patterns occurred. Nuclear blast changes and expansion of cytoplasmic mass accompanied this period of new granule synthesis. Mixed recovery patterns were present in individual cells. These represented the morphological expression of a variety of recovery events. Thus, some cells showed a combination of channel recovery and remodeling to form new granule containers within which condensation of content produced crystalline patterns, as well as synthesis of new granules, as described here. This morphological versatility resulted in multiple mast cell morphological phenotypes during these release and recovery processes.

MATURE HUMAN LUNG mast cells are readily identifiable, granule-filled secretory cells. Their secretory events can be elicited by exposure to antibody to cell-bound immunoglobulin E. The biochemical and ultrastructural correlates of this release response can be studied in replicate samples of isolated, partially purified mast cells.[1-4] These preparations can also be cultured for short times (up to 48 hours), thus allowing analysis of recovery events that follow stimulation of their secretory processes.[5]

We have studied isolated human lung mast cells, after all steps of the described isolation procedures, in culture for various times up to 48 hours, during the entire kinetics of histamine release and after various recovery times from these release reactions.[1-5] These studies confirmed the previously described morphology of human lung mast cells and their fine structural granule patterns.[6] We delineated the presence of four basic granule patterns (scroll, crystal, particle, mixed) and lipid bodies in isolated human lung mast cells. We described the different ultrastructural morphology of lipid bodies and granules,[1,2] the different contents of lipid bodies and granules by ultrastructural autoradiography (sulfur in granules; arachidonic acid in lipid bodies),[1,2] the different mode of generation of lipid bodies and granules[4] and the different behavior of lipid bodies and granules during degranulation[2,3] and early recovery from degranulation.[5] The differences in the behavior of these two subcellular organelles can now be extended to late recovery of human mast cells from degranulation.

The recovery of human lung mast cells from degranulation, or a massive secretory event, is complex. In general, these processes could be related either to early recovery times (3 to 24 hours) or to later ones (18 to 48 hours), but we found that considerable overlap existed. Some of this overlap might be related to time after stimulus but some might also be related to the completeness of the release event in individual cells. We separated recovery events into those more generally present early and/or after incomplete release from cells, and those more generally present late and/or after complete release from cells. The early events involved conservation principles whereby granule membranes were reused to repackage newly condensing, crystalline granule contents.[5] The later events followed externalization of nearly all of the cells' granules and the membranes they contributed to degranulation channels. They were characterized by various synthetic events which we describe here.

MATERIALS AND METHODS

Isolation of mast cells. Mast cells were purified from human lung as previously described.[7] In brief, grossly normal lung fragments were obtained from pneumonectomy or lobectomy specimens removed from lung tumors. Single cells were dispersed from lung fragments by two 30-minute incubations in solutions containing pronase (2 mg/mL) and chymopapain (0.5 mg/mL) followed by two incubations in solutions containing collagenase (1 mg/mL) and elastase (10 U/mL). Lung cell suspensions containing mast cells were enriched by countercurrent centrifugation elutriation.[7] Four such preparations with mast cell purities of 59%, 66%, 35%, and 15% were selected for the release and recovery experiments described here.

Mast cell degranulation. Mast cell release experiments identical to those we have previously reported were repeated.[2,3] Thus, mast

From the Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston; and the Department of Medicine, Division of Clinical Immunology, The Johns Hopkins School of Medicine at the Good Samaritan Hospital, Baltimore.

Submitted June 19, 1987; accepted August 25, 1987.

Supported by US Public Health Service Grants No. CA28834, AI 07290, and HL 23586.

Address reprint requests to Dr Ann M. Dvorak, Department of Pathology, Beth Israel Hospital, 330 Brookline Ave, Boston, MA 02215.

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0006-4971/88/7101-0010$03.00/0

cells were stimulated to degranulate by exposure to antibody to IgE (goat anti-human IgE, kindly provided by Dr K. Ishizaka), 2 μg/mL in 25 mmol/L piperazine-N_1N^1*bis*-2-ethane sulfonic acid, 140 mmol/L NaCl, 6 mmol/L KCl, 0.003% human serum albumin, and 0.1% glucose buffer (PAG) plus 1 mmol/L $CaCl_2$ at 37°C.[8] In four separate experiments, mast cells were incubated with anti-IgE or an equivalent concentration (2 μg/mL) normal goat serum (control) and fixed for ultrastructural studies at time 0, 3, 5, and 20 minutes thereafter. To measure the extent of mast cell degranulation, the supernatants of parallel aliquots of anti-IgE or control mast cells were analyzed for histamine content by the automated fluorometric technique of Siraganian.[9] Total mast cell-associated histamine was determined by lysing the cells with 0.4N perchloric acid, and specific histamine release was determined. In the four kinetic studies, maximal histamine release occurred at 20 minutes poststimulus (72%, 45%, 84%, and 95%).

Mast cell recovery. After challenge with anti-IgE or goat serum (control), cells were centrifuged, supernatants were removed and cells were resuspended in 12 mL medium RPMI with 25 mmol/L HEPES, 10% heat-inactivated fetal calf serum (FCS), 250 U/mL penicillin, 250 μg/mL streptomycin, and 50 μg/mL gentamycin. Cells were aliquoted into 24-well plates (1 mL/well) and incubated pending harvest at later times. Recovery times in four experiments were 3, 6, 12, 18, 24, 36, and 48 hours.

Electron microscopy. Cell suspensions were fixed by diluting them in a tenfold excess of a mixture of 1% paraformaldehyde, 1.25% glutaraldehyde, and 0.025% $CaCl_2$ in 0.1 mol/L sodium cacodylate buffer, pH 7.4.[10] Cells were fixed at 1 hour at room temperature, washed, and then suspended in 0.1 mol/L sodium cacodylate buffer, pH 7.4 at 4 °C.

To facilitate determination of whether cytoplasmic channels and other structures were open to the cell exterior, aliquots of fixed mast cells were resuspended in 1 mL Hanks' balanced salt solution (HBSS) containing 50 μL (0.5 mg) of cationized ferritin (Miles Laboratories), an electron-dense tracer that binds to accessible negatively charged membranes. After incubation with cationized ferritin on a rotary shaker set at low speed for 30 minutes at room temperature,[11,12] the samples were washed again in 0.1 mol/L sodium cacodylate buffer, transferred to microtubes, and centrifuged at 1,500 g for 1 minute. The cells were suspended in warm 2% agar, centrifuged again, and then postfixed as a pellet in agar from 2 hours at room temperature in 2% collidine-buffered osmium tetroxide and stained en bloc with uranyl acetate for 2 hours at room temperature.[10] Cell pellets were then dehydrated in a graded series of alcohols and embedded in a propylene-Epon sequence. Sections were cut with diamond knives, placed on copper grids, stained with lead citrate, and viewed in a Philips 400 transmission electron microscope. A total of 64 thin sections of various time points in four kinetic release and recovery experiments were examined by electron-microscopy. All mast cells in each grid (~100 cells) were examined at medium-to-high magnifications (×2,800 to ×30,000).

RESULTS

Control mast cells. Well-preserved control mast cells (Fig 1) were round, mononuclear cells with uniformly distributed short, narrow surface processes and a full complement of typical cytoplasmic granules.[3] Granule patterns included scrolls, crystals, particles, and mixtures of these patterns.[2] Golgi complexes and membrane-bound and free ribosomes were minor cytoplasmic components. Large lipid bodies enmeshed in intermediate filaments were routinely found in the cytoplasm. Canalicular structures, consisting of internalized surface folds, were present.

Aside from morphological evidence of minor granule perturbation accompanied by spontaneous histamine release values of 1% to 5%,[3] we did not see morphological images which routinely accompany anti-IgE–mediated degranulation and histamine release[2,3] in controls (Fig 1).

Mast cells stimulated by anti-IgE. Previously described morphological patterns of anaphylactic degranulation of human lung mast cells[2,3] were present in the early release period (0–20 minutes) of four kinetic studies. In brief, we saw swollen granules and granule fusions that formed tortuous, connected chains of granules. These chains enlarged, widened, and elongated to become degranulation channels filled with altered granule matrix material. These channels permeated the cytoplasm of the cell, somewhat analogous to

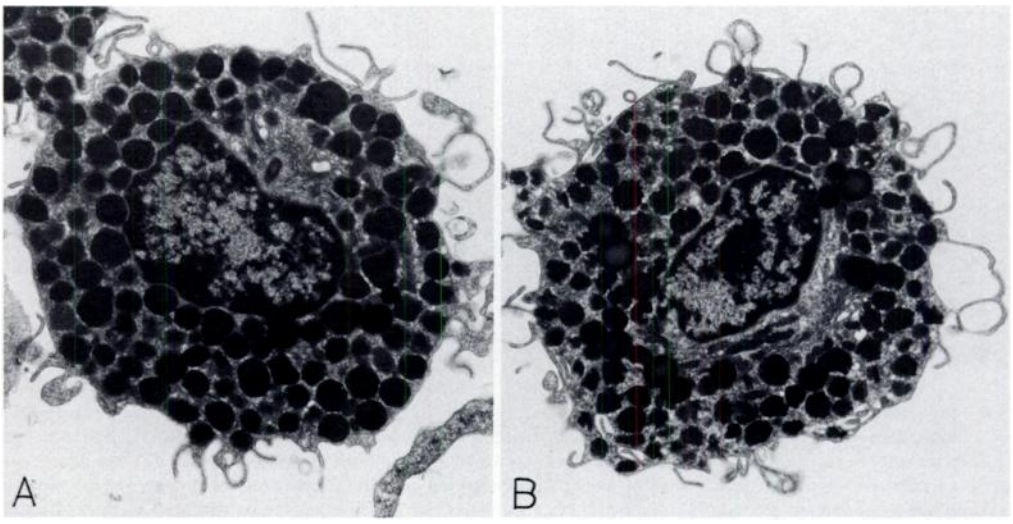

Fig 1. Control unstimulated mast cells studied in replicate with stimulated mast cells at 24-hour (A) and 36-hour (B) intervals. These well-granulated, mononuclear mast cells are filled with granules and display numerous surface folds. A. Original magnification ×9,000. B. magnification ×8,000.

a sponge. Many channels eventually opened by a number of pores to the exterior and lost the altered granule matrix material. Rarely, extrusion of identifiable membrane-free granules was identified in one of these kinetic experiments.

Mast cell recovery from degranulation. Mast cells recovering in short-term cultures after a degranulation stimulatory event showed a number of morphological processes. Although some overlap existed in the precise timing of such recovery changes in the four kinetic experiments that we examined in detail, certain recovery patterns were more prevalent in the early recovery period (3 to 24 hours), whereas others were more prevalent in the later times (18 to 48 hours). In this study, we concentrated on changes of the late recovery period.

In the early recovery period, resolution of channels and their altered granule matrix materials predominated.[5] In brief, conservation of channel-granule membranes occurred. Remodeled channels separated into new granule spaces (Fig 2). Simultaneously, condensation and crystallization of content took place as granules reformed and became smaller. This apparent reversal of granule swelling and channel formation that characterized the early release period resulted in reutilization of the original containers (granule membranes) and perhaps some of their contents as well.

Resolution of degranulation channels involved a different route in many cells (Figs 3 through 6). After release of all visible altered granule matrix materials to the exterior by pores through the plasma membrane, most of these open

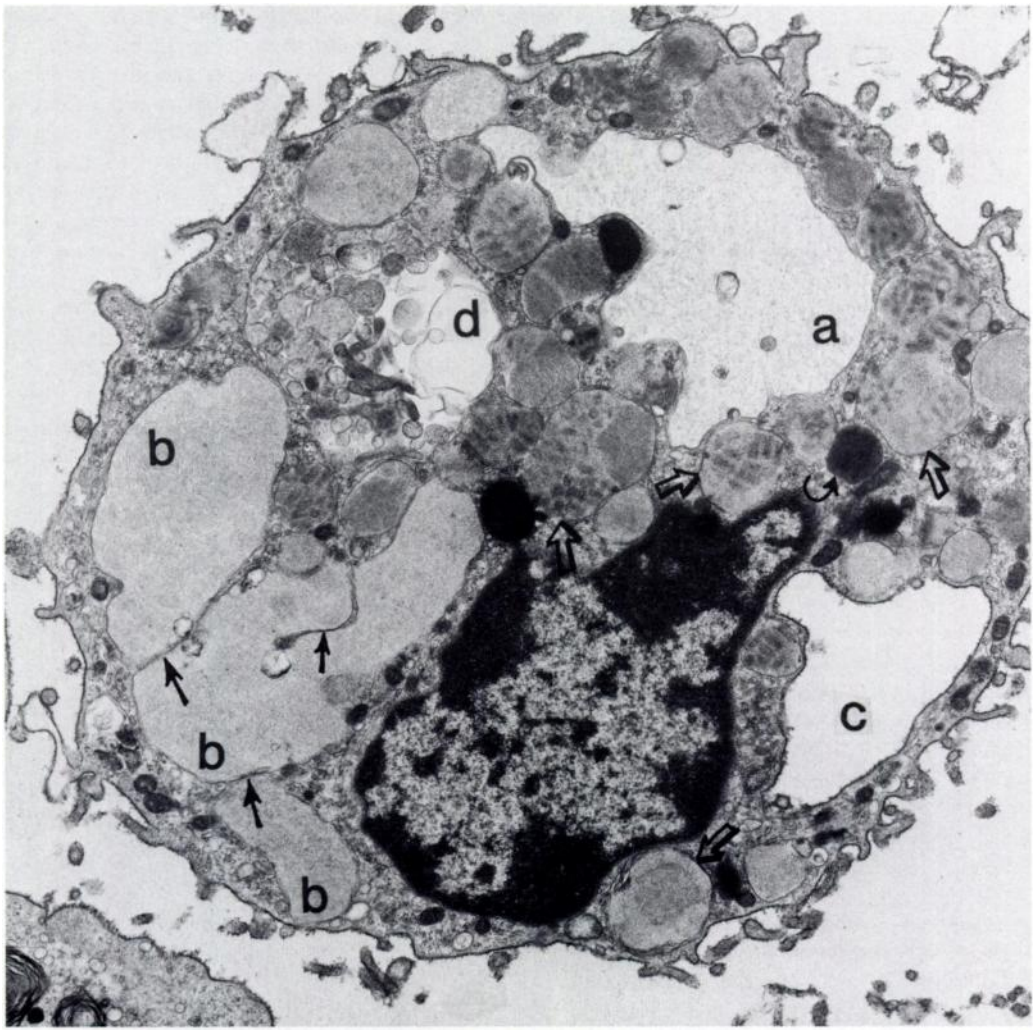

Fig 2. Human lung mast cell recovered 3 hours poststimulus. Recovering degranulation channels show increasing condensation of contents (a,b). Channels show vesicles (a,b) and membranes protruding into channels and separating more condensed channels (b) into large granule-shaped structures (arrows, b). More condensed granules with crystalline and scroll contents lie adjacent to these separating channel-granules (open arrows). Some of these large granules show multiple granule-sized domains of different contents (open arrows). One unaltered, completely condensed granule is present (curved arrow). Four dense lipid bodies are also present. One channel (c) has emptied its entire altered granule matrix content. It is lucent and contains no vesicles or membranous partitions. The membrane is stained with a uniform layer of cationized ferritin, indicating continuity with the similarly stained plasma membrane. A canalicular structure (d) is filled with elongated, dense surface folds, vesicles, and multiple cytoplasmic fragments. Cationized ferritin does not bind to these membranes. magnification ×14,500.

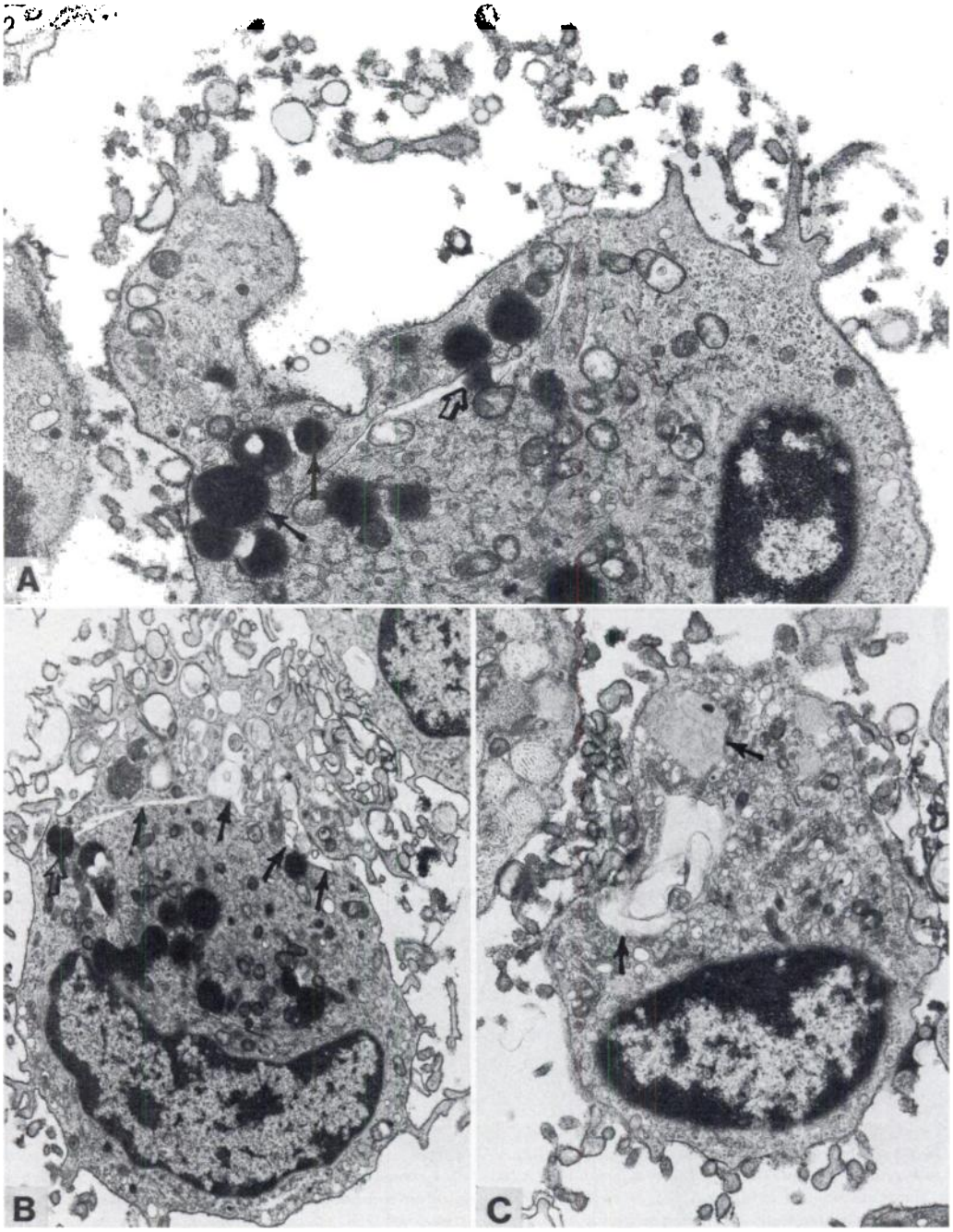

Fig. 3. Mast cells recovered 3 hours (A,B) and 6 hours (C) after degranulation. Numerous shed cell processes surround the mast cell in (A), which has released all granules. Numerous lipid bodies remain (arrows). One lipid body is within a linear open space in the cytoplasm (open arrow). A large area of cytoplasm is separating along a membrane-lined linear zone in (B) (arrows). A large lipid body is located at one end of this separation line (open arrow). The mast cell in (C) shows shedding of surface processes and membranes. A resolving channel and several separated granules are apparent (arrows). The cytoplasm is filled with vesicles. A: original magnification ×19,000. B: magnification ×11,000. C: magnification ×13,000.

channels exteriorized their granule-channel membranes. This resulted in a marked increase in surface activity with increased numbers of elongated surface folds.[3] Cationized ferritin routinely bound to the membranes of these open channels as this exteriorization process continued. In some instances, the entire cell was permeated by an interconnected channel system whose membranes all stained with cationized ferritin. These spongelike cells at this point provided numerous channels for free circulation of external materials throughout the cytoplasmic area. Despite this, we generally did not find such materials in these spaces. In contrast, canalicular structures routinely contained recognizable surface folds, vesicles, and portions of membrane-bound cytoplasm but did not contain recognizable granules or altered

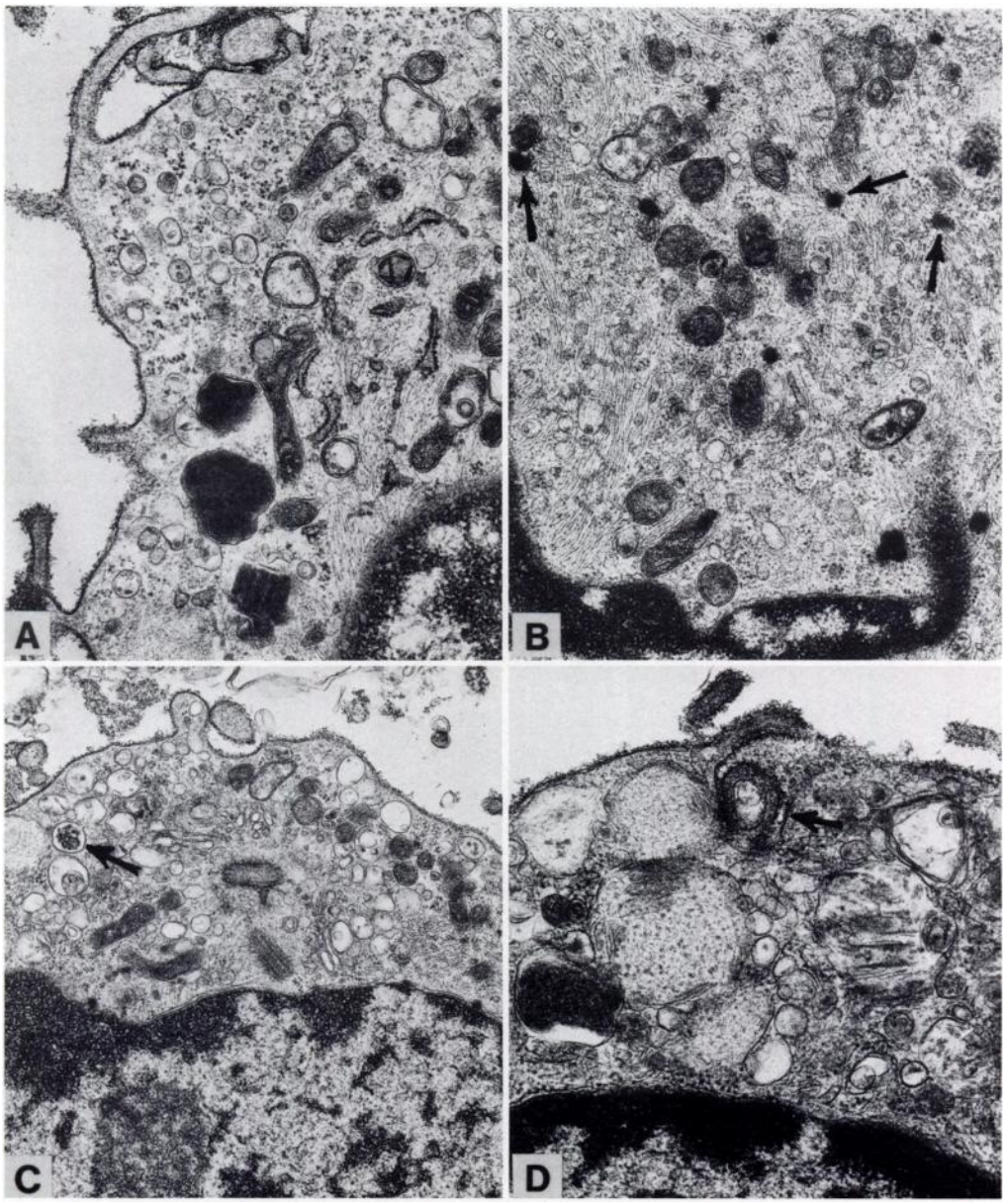

Fig 4. Mast cells recovered 12 (A,C), 24 (B) and 48 hours (D) after degranulation are seen at higher magnification. Clusters of polyribosomes surround vesicles in the peripheral cytoplasm in (A). A few strands of rough endoplasmic reticulum are also seen. Numerous intermediate filaments fill the cytoplasm (B). Note small, irregular, nonmembrane-bound lipid bodies enmeshed in the intermediate filaments (arrows). An active Golgi area has centrioles and numerous vesicles in (C). Some vesicles contain granule particles (arrow). CF stains the plasma membrane but does not stain the membranes of the cytoplasmic vesicles and vacuoles. At 48 hours poststimulus (D), this mast cell has granules with particles and with scrolls. Numerous chains of vesicles surround the granules filled with particles. One surface canalicular structure is forming by internalization of CF-stained surface folds (arrow). A. magnification ×27,000. B. magnification ×26,500. C. magnification ×21,000. D. magnification ×36,500.

granule matrix materials (Figs 2 and 5). Neither did canaliculi participate visibly in the granule partitioning and content condensation described in the early recovery period.[5]

Some mast cells that externalized their channels released a mixture of membranes and surface folds[3,13] (Fig 3). These membrane fragments were often more dense than their counterparts that remained attached to cell bodies. Mast cells undergoing these surface events developed smooth, fold-free surface contours (Fig 6A). This smooth surface appearance is an unusual one for unstimulated mast cells. Small mast cells with smooth surfaces were routinely found at late recovery times (Fig 6A). These lymphocyte-sized cells could be identified as mast cells by the presence of a few typical mast cell granules in their cytoplasm.

Mast cells that released virtually all their granules and exteriorized all their degranulation channels underwent a dramatic series of morphological events (Figs 4-7). The

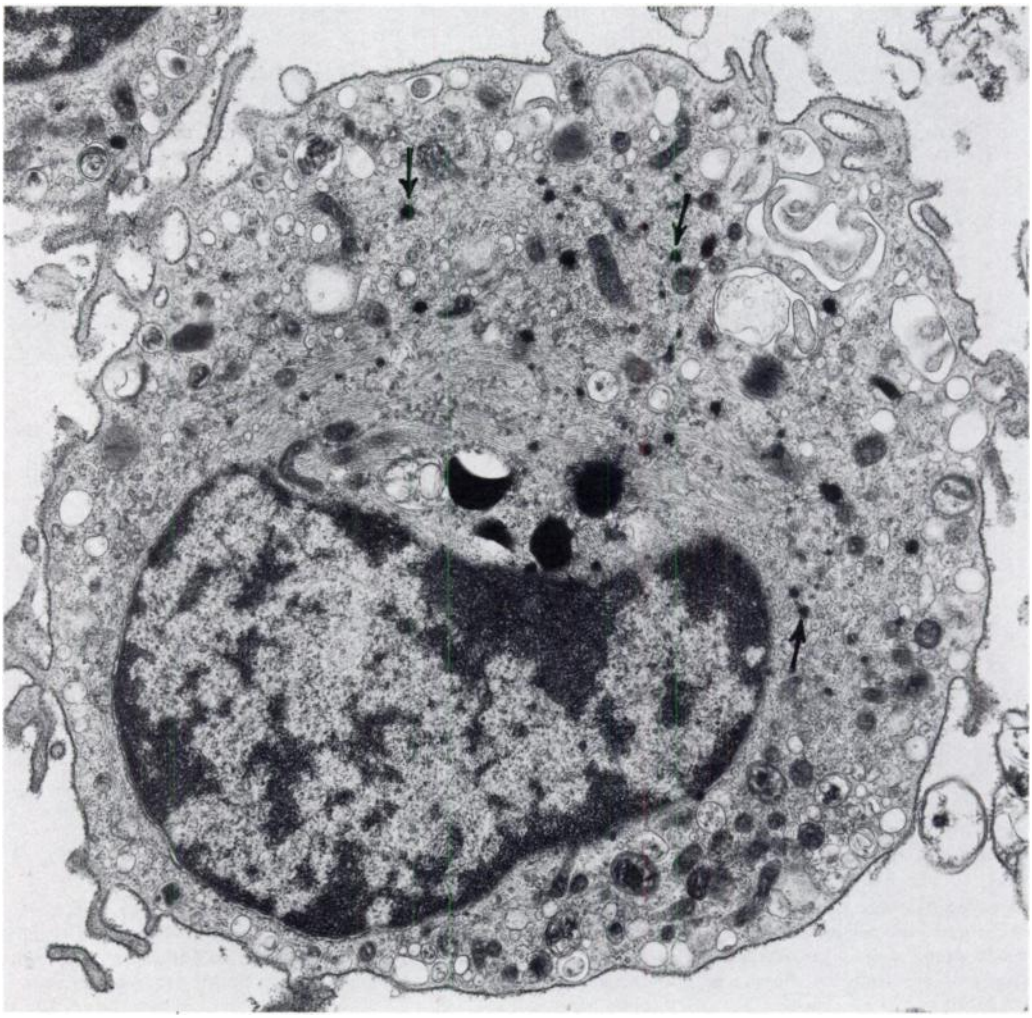

Fig 5. Low magnification view of a mast cell recovered 24 hours after degranulation. Active formation of numerous peripheral cytoplasmic vesicles and internalized surface folds in canalicular structures is present. The central cytoplasm is filled with intermediate filaments. Several large lipid bodies are present, and numerous small lipid bodies are enmeshed in intermediate filaments (arrows). Magnification ×18,500.

result of these synthetic events was the renewal of fully granulated mast cells of normal size (Fig 8).

Initially, we noted progressive expansion of the Golgi synthetic apparatus (Figs 4C, 6B and C, and 7). This expansion was primarily related to an increase in Golgi area vesicles. Ultimately, the entire cytoplasmic area became filled with vesicles (Figs 3C and 6C). Polysomes appeared in cytoplasmic areas adjacent to vesicles (Fig 4A). Some strands of rough endoplasmic reticulum also appeared. These were few in number, and their cisternae were not dilated (Fig 4A). As Golgi vesicles and vacuoles enlarged, particulate and dense granule content appeared in many, and these new small granules were located in peripheral cytoplasmic areas (Figs 4C and D, 6B, and 7). Although many of the Golgi vesicles contained particulate material, some containing single scrolls were also observed. As synthetic structures appeared and granulogenesis progressed, nuclear enlargement and appearance of large nucleoli occurred (Fig 4C). Fully granulated mast cells present 48 hours after stimulus contained granules of all types—scrolls, particles, crystals, and mixed patterns.

We also noted changes in lipid bodies at late times after a releasing stimulus. Lipid bodies of minute size became apparent. These numerous, dense, nonmembrane-bound structures were enmeshed in intermediate filaments that filled the central cytoplasmic area (Figs 4B and 5). Lipid bodies did not appear to originate from the Golgi apparatus. They were particularly prominent in larger mast cells that had released nearly all their granules and showed active internalization of surface folds in canalicular structures in the peripheral cytoplasmic area (Fig 5). Newly forming scroll granules were also more commonly located in the peripheral cytoplasmic areas.

The morphological complexity of recovery from degranulation was compounded by cells undergoing simultaneous closure (or failure to open), remodeling, and refilling of degranulation channels; exteriorization of channel membranes, with concomitant internalization of surface folds in

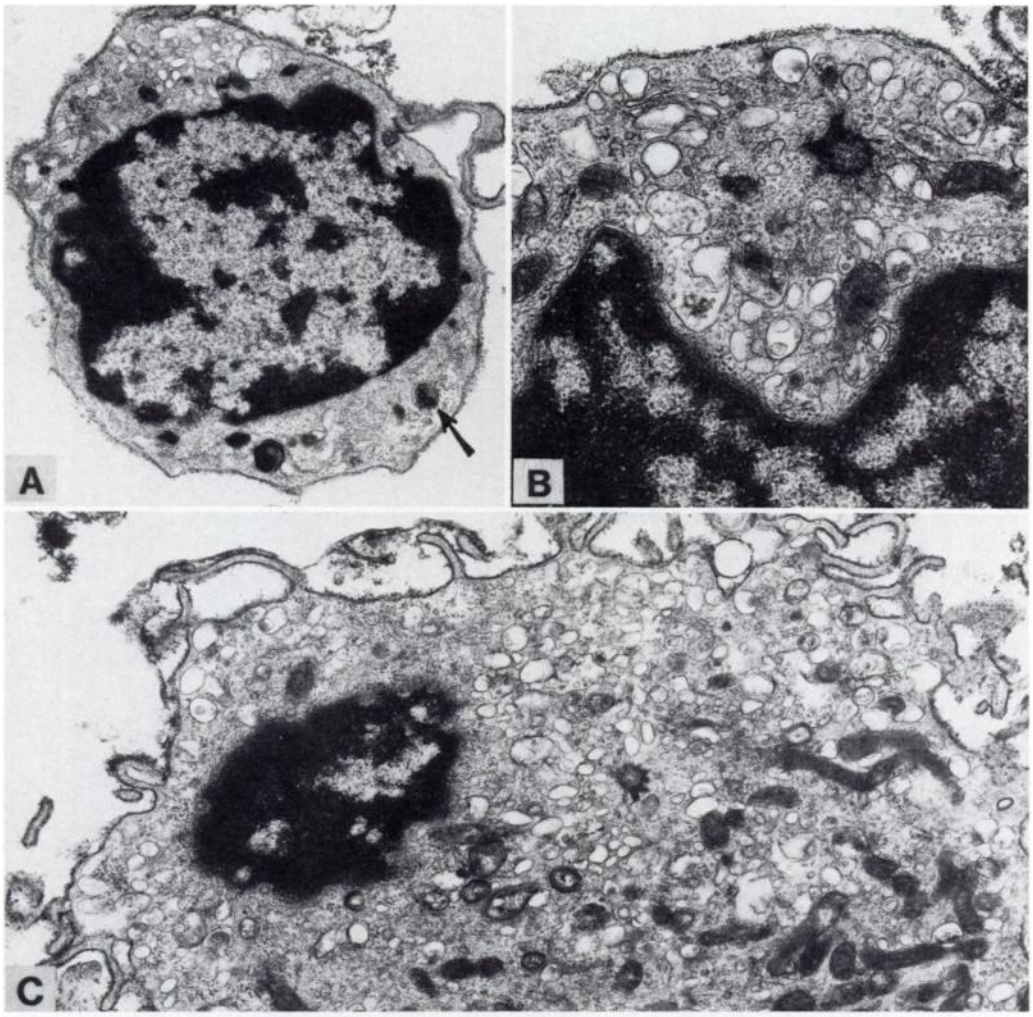

Fig 6. Mast cells studied at 48 hours poststimulus. A small lymphocyte-sized, smooth-surfaced mast cell with a single remaining granule (arrow) shows numerous vesicles adjacent to the Golgi area (A). An active Golgi zone of a similar small mast cell is shown at higher magnification in (B). Many vesicles contain granule particles. In (C), a recovering, granule-free mast cell shows a cytoplasm packed with vesicles radiating outward from the Golgi area that displays a centriole. A: magnification ×14,000. B: magnification ×32,000. C: magnification ×19,000.

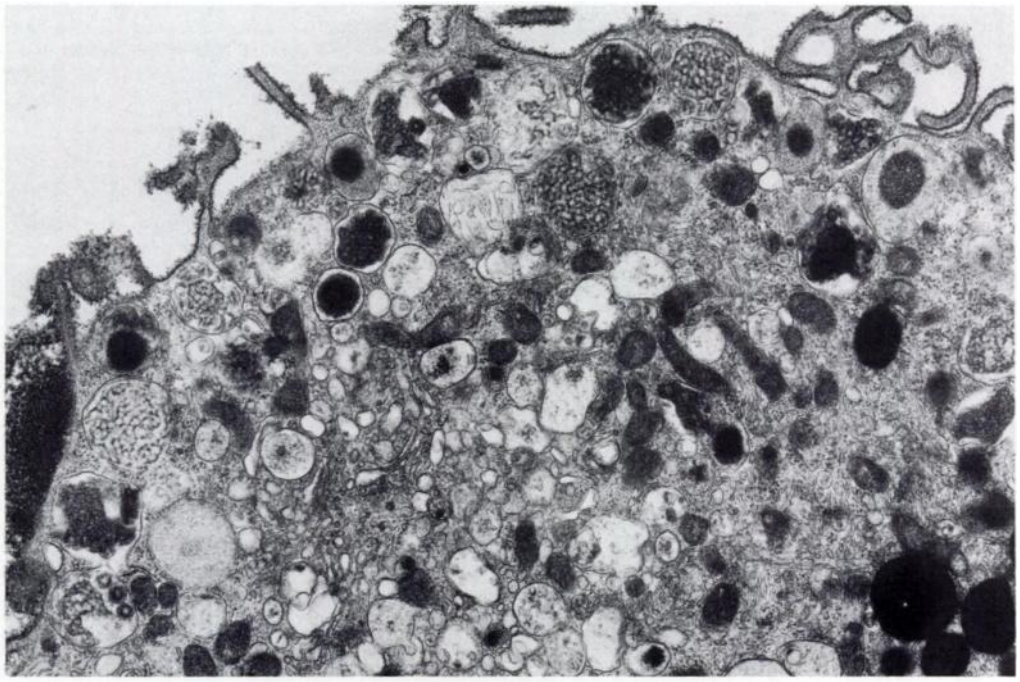

Fig 7. Higher magnification micrograph of a mast cell recovered at 48 hours poststimulus shows the actively synthesizing Golgi area with numerous particle-filled progranules. Larger granules in the peripheral cytoplasm contain particles, scrolls, and mixed patterns. magnification ×23,500.

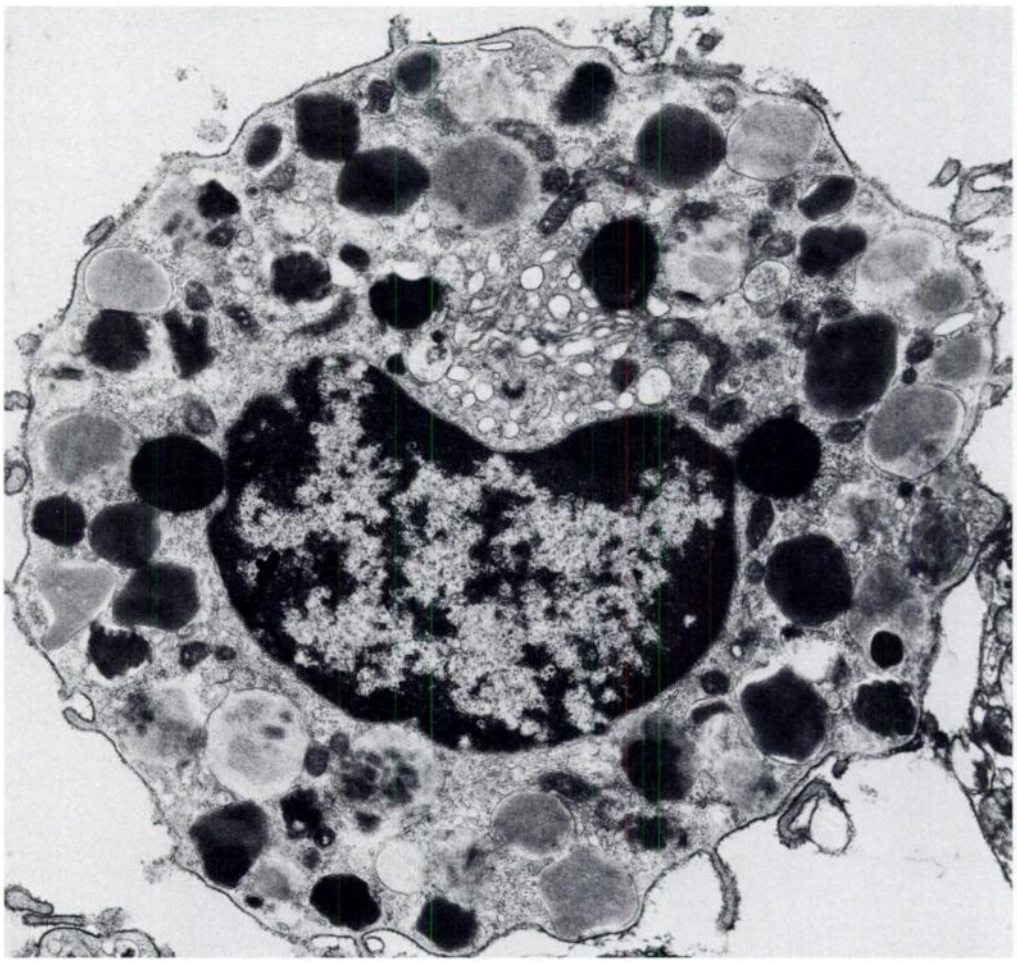

Fig 8. Mast cell recovered at 48 hours postdegranulation shows a nearly complete complement of granules. The Golgi area is still actively producing vesicles and progranules. The surface is nearly smooth, but some typical mast cell surface folds are present. Cationized ferritin is uniformly bound to the cell surface. magnification ×19,000.

canalicular structures; and release of exteriorized membranes and cytoplasmic processes, with subsequent synthesis of new granules. Once the individual recovery patterns were identified, the various combinations of these recovery events in single cells could also be delineated. We refer to these patterns as mixed recovery patterns for ease of discussion.

DISCUSSION

The principal findings of this study are as follows. Human lung mast cells can be induced by exposure to anti-IgE to release nearly all their cytoplasmic granules. At late recovery intervals following this nearly complete morphological release reaction, human lung mast cells use synthetic mechanisms to reconstitute their cytoplasmic granules. Mixed recovery patterns are commonly observed over a wide time span (3 to 48 hours) following exposure of human lung mast cells to a release stimulus.

Mast cells that released nearly all their granules and large amounts of exteriorized membranes as well[3] were similar to small lymphocytes in size and morphology[13] except for the presence of specific mast cell granules. These small mast cells[13] were routinely present during the late recovery period but generally not during control cultures studied at similar times. The release process left these cells without surface folds, which typify mast cells. We have observed similar losses of cell membranes in concert with granule extrusion to result in viable, small, immature murine mast cells with smooth surface contours.[14] Shedding of cell components is not without precedent in biology, having been reported in numerous systems. For example, we have identified procoagulant-containing vesicles shed from tumor cells in vivo and in vitro.[15-17]

The late recovery events we describe in previously degranulated human mast cells are dependent on the progressive appearance of activated Golgi areas, numerous cytoplasmic vesicles, and foci of free, cytoplasmic polyribosomes. Rough endoplasmic reticulum did not appear in large amounts in these recovering, synthesizing human lung mast cells. Cells undergoing these active cytoplasmic synthetic events also underwent blast changes of their nuclei. Nuclei expanded, their chromatin dispersed, and large nucleoli appeared. As granules rebuilt, cytoplasmic area also expanded.

With appropriate stimuli, the typical resting, mature mast

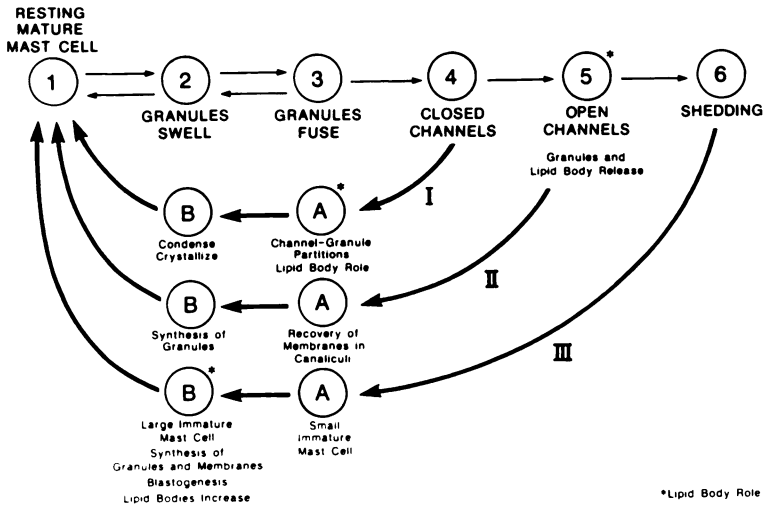

Fig 9. Composite schematic diagram (numbers and letters refer to diagram) of major events in anaphylactic degranulation and recovery of human lung mast cells. See text for explanation.

cell (1 in Fig 9) undergoes granule swelling (2 in Fig 9) and fusion (3 in Fig 9), which precede the development of numerous closed cytoplasmic degranulation channels filled with altered granule matrix (4 in Fig 9). Minor amounts of granule swelling (2 in Fig 9) and fusion (3 in Fig 9) accompany spontaneous histamine release associated with the cell handling of isolation and purification. By reversal of granule swelling and fusion, these morphological changes recover completely after a brief culture interval. Degranulation channels (4 in Fig 9) undergo resolution by three pathways (I, II, III in Fig 9). Individual cells can simultaneously display portions of each recovery pathway, giving rise to the expression of considerable morphological phenotypic variation in recovering human mast cells.

Recovery pathway I in Fig 9 (Lab Invest 54:663, 1986) involves the formation of partitions (IA in Fig 9) within the degranulation channels, a process facilitated by small lipid bodies and vesicles (IA). These newly partitioned containers undergo content condensation and crystallization (IB in Fig 9) to produce morphologically mature mast cells (1 in Fig 9).

Recovery pathway II in Fig 9 follows the externalization of degranulation channel membranes and release of granule and lipid body contents (5 in Fig 9) (J Cell Biol 99:1678, 1984). Membranes and surface processes are internalized within canaliculi (IIA in Fig 9). Simultaneously, new granules are synthesized (IIB in Fig 9) to produce morphologically mature mast cells (1 in Fig 9)

Recovery pathway III in Fig 9 follows the exteriorization of granules and channels accompanied by shedding of all cytoplasmic granules and variable amounts of surface-related structures (6 in Fig 9) (Lab Invest 53:45, 1985). This explosive, extensive event is noncytotoxic and results in the production of small, immature mast cells devoid of nearly all granules (IIIA in Fig 9) (Cell Immunol 105:199, 1987). Blastogenesis of nuclei and expansion of cytoplasmic synthetic machinery results in large, immature mast cells (IIIB in Fig 9). These cells are filled with lipid bodies and small, immature granules. Further maturation and granulogenesis gives rise to morphologically mature mast cells (1 in Fig 9).

Human mast cell recovery patterns may differ considerably (Fig 9). We generally could relate early recovery events to mast cells that failed to release all their altered granule matrix materials from cytoplasmic degranulation channels (I in Fig 9).[5] These early events included reuse of channel-granule membranes, remodeling, and partitioning of channel-granules and condensation and crystallization of granule products in these reforming granule spaces.[5] The idea of sealing of degranulation sacs or channels is not new. We[5,18] and other investigators[19-21] have demonstrated or postulated sealing of cytoplasmic degranulation spaces in previously degranulated guinea pig basophils,[18] human lung mast cells,[5] and rat mast cells.[19-21]

Later recovery events involved cells undergoing release of virtually all granule contents. Massive additions to the plasma membrane occurred as the granule containers (membranes) were exteriorized.[3] Recovery of these membranes within canaliculi (II in Fig 9) served as one mechanism of resolution of these membrane shifts. Shedding of these membranes in concert with granule release (III in Fig 9) served as another mechanism of resolution of these explosive degranulation events.[3,13,14] Such losses were not cytotoxic and resulted in increased numbers of small, immature mast cells (IIIA in Fig 9) following a degranulation stimulus.[3,13,14] These membrane shifts and losses which accompanied anaphylactic degranulation stimulated the appearance of synthetic structures, nuclear blast changes, and ultimately new granules. These large, immature mast cells (IIIB in Fig 9) typically contained large numbers of small and large lipid bodies and smaller numbers of small, immature granules.

Some cells could be found that simultaneously bridged these two main recovery events, such that channel content and container recovery and reorganization and Golgi area synthesis of new granules occurred simultaneously. The morphological expression of recovery from degranulation of these isolated human lung mast cells resulted in numerous morphological phenotypes. These patterns and processes (Fig 9) must be recognized in order to identify their appearance eventually in in vivo material.

The findings we report represent the first such studies in human mast cells recovering from anaphylactic degranulation. In certain features, they resemble recovery events previously described in guinea pig basophils[18] and rat mast cells.[19-28]

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