IgE-Mediated Anaphylactic Degranulation of Isolated Human Skin Mast Cells
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Isolated human skin mast cells (HSMC) were prepared and cultured overnight before functional and electron microscopic studies. Mast cell suspensions were examined after stimulation with anti-IgE to produce anaphylactic degranulation or examined in buffer-incubated controls. Histamine release was measured in replicate samples. Control, isolated HSMC studied by electron microscopy were well preserved and fully granulated. Although all granule patterns reported for human mast cells were found, crystal granules were the most prevalent, as is true for HSMC in situ. Individual mast cells containing both crystal and scroll granules occurred. Lipid bodies were rare, as in HSMC in situ. Control, isolated mast cells did not express granule changes associated with either piecemeal degranulation or recovery during wound healing in situ; nor were morphologic changes of anaphylactic degranulation present. Spontaneous histamine release was 0% in control samples. Anaphylactic degranulation of isolated HSMC was accompanied by 24% maximum histamine release and characteristically showed extrusion of altered, membrane-free granules through multiple pores in the plasma membrane to the exterior of the cell. Other morphologic aspects of anaphylactic degranulation, as expressed in isolated human lung mast cells, were also present. These events included granule swelling, fusion, alteration of matrix contents, degranulation channel formation, pore formation, and shedding of granules, membranes, and surface processes. The ultrastructural morphology of isolated HSMC and their IgE-mediated degranulation shows some differences from similar studies of isolated human lung mast cells and of human lung and gut mast cells in biopsy samples. These differences include crystal granules as the predominant granule pattern, minor numbers of lipid bodies, and extrusion of granules during anaphylactic degranulation as characteristic for HSMC. By contrast, isolated human lung and gut mast cells have more scroll granules and particle granules, respectively, and more lipid bodies. In isolated human lung mast cells, anaphylactic degranulation is almost exclusively an intracellular fusion event characterized by the formation of complex degranulation channels within which altered granule matrix materials solubilize. In addition to morphologic differences between mast cells of skin, lung, or gut origin, functional differences have also been reported among mast cells of these organs. The ultrastructural morphology of isolated HSMC is identical to that of skin mast cells in biopsy samples, thereby validating the usefulness of this new source of HSMC for correlative functional and morphologic studies.

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FUNCTIONAL STUDIES of human skin mast cells (HSMC) using excised slices of skin have been available for some time.1-13 Recently, efforts to improve on these studies have focused on the preparation of isolated skin cells from which variably pure mast cells of skin origin have been obtained.14-18 These functional studies, in concert with similar studies using isolated mast cells of lung or gut origin, have shown differences in eicosanoid production and in responsiveness to various secretory stimuli among the mast cells of skin, lung, and gut origin.6,5

Ultrastructural morphologic studies of HSMC in situ using skin biopsy material are also available.9,22 The results of these in situ studies are fourfold: (1) HSMC granule architecture is generally crystalloid; lipid bodies are infrequent. Other granule patterns, such as mixed granules as well as cells with granules of several patterns, also occur in HSMC.12 (2) HSMC associated with a variety of pathologic conditions express a morphologic release reaction, termed piecemeal degranulation, characterized by focal and complete emptying of granule containers.20-22 (3) HSMC may refill granule containers during wound healing.22 (4) HSMC, stimulated in situ by a variety of stimuli including mechanical, antigen, or complement, express anaphylactic degranulation characterized by granule extrusion.13-15,18,19,21

In the studies presented here, we used isolated HSMC preparations6 and ultrastructural methods7 to analyze isolated HSMC after overnight culture, in buffer controls, and following stimulation with anti-IgE to compare their morphology with skin mast cells in vivo.22 We found that such isolated HSMC were well preserved, and, like their in situ counterparts, the predominant granule pattern contained crystals. We also found that extrusion of granules was the prime morphologic feature of isolated HSMC, a finding also true for HSMC stimulated in vivo.13-15,16,21,22 The comparative studies of HSMC to their in situ counterparts in biopsy material, made possible by newly developed isolation techniques,20,24 show that morphologic structure and events (of which HSMC are capable) are similar, regardless of whether they are studied in situ or as isolated mast cells. Moreover, both functional and morphologic studies suggest that key differences exist which distinguish HSMC from at least two other organ sources of human mast cells, lung and gut.9,23-25

MATERIALS AND METHODS

Isolation of adult HSMC. Adult skin from reduction mammo- plasty specimens was placed immediately in Eagle's minimum essential media at 4°C and chopped to 2-mm fragments in PAG (a buffer consisting of 25 mmol/L PIPES [piperazine-N,N'-bis(2-ethane-sulfonic acid)], 110 mmol/L NaCl, 5 mmol/L KCl, 0.1% dextrose, and 0.003% human serum albumin). After rinsing twice, the tissue fragments were incubated in a solution of PAG contain-

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ing 2,500 U collagenase/g wet weight of tissue (Worthington Biochemical, Freehold, NJ), 4 mg hyaluronidase/g wet weight (Sigma, St Louis, MO), and 2,500 U/mL DNase (Calbiochem, San Diego, CA) for 1 hour at 37°C with constant stirring. The supernatant was collected and centrifuged at 23°C, 800 rpm for 8 minutes. The cells obtained were then washed twice in PAG.

**Short-term culture of adult skin mast cells.** Cells obtained from the digestion procedure were resuspended in RPMI 1640 medium with 25 mmol/L HEPES, 2 mmol/L L-glutamine, 1% penicillin, 1% gentamicin (Whitaker, M.A. Bioproducts, Walkersville, MD) and 5% fetal calf serum (FCS; GIBCO, Grand Island, NY). The suspension was placed in 5-mL aliquots (2 x 10⁶ mast cells/mL) into 25-cm² tissue culture flasks and incubated in humidified 95% air, 5% CO₂ at 37°C overnight. Mast cells were harvested, washed in PAG at 23°C, counted by using alcian blue staining at pH 1.0, and stained with erythrosin B to determine viability. Mast cell purities of the two experiments were 30% and 15%.

**Histamine release assay.** Cells were suspended in PAGCM (PAG supplemented with 1 mmol/L CaCl₂ and 1 mmol/L MgCl₂) at a concentration of 2 x 10⁶ mast cells/tube and challenged in duplicate with 1 or 3 μg/mL goat antihuman anti-IgE in a volume of 0.1 mL. Control cells were incubated in PAGCM alone. Supernatants were assayed for histamine by the automated fluorometric technique of Siraganian. Results were expressed as the percentage of total histamine content, determined by lysis of cells in 2% perchloric acid and corrected for spontaneous histamine release, as determined from buffer control tubes. Maximum histamine release was 24% with 0% spontaneous histamine release from buffer controls.

**Fixation and preparation for electron microscopy.** Replicate samples of either buffer-incubated control mast cells or mast cells 3 minutes after stimulation with anti-IgE were prepared for electron microscopy as described. Specifically, cell suspensions were fixed by diluting them in a 10-fold excess of a mixture of 1% paraformaldehyde, 1.25% glutaraldehyde, and 0.025% CaCl₂ in 0.1 mol/L sodium cacodylate buffer, pH 7.4. Cells were fixed for 1 hour at room temperature, washed, and resuspended in 0.1 mol/L sodium cacodylate buffer, pH 7.4, 4°C. Cell samples were suspended in warm 2% agar, centrifuged to form agar pellets of cells, postfixed for 2 hours at room temperature in 2% collidine-buffered osmium tetroxide and stained en bloc with uranyl acetate for 2 hours at room temperature. Agar blocks containing mast cell pellets were dehydrated in a graded series of alcohols at room temperature and infiltrated and embedded in a propylene oxide-Epon sequence. Thin sections were stained with lead citrate and viewed in a Philips 400 transmission electron microscope (Philips NA, Mahwah, NJ).

**RESULTS AND DISCUSSION**

**Control, isolated adult HSMC.** Isolated, adult HSMC, obtained from surgically removed reduction mammoplasty specimens, were well preserved in the electron microscopic samples studied after overnight cultures and showed no spontaneous histamine release in 3-minute buffer-incubated samples (Fig 1A). These mononuclear cells had a full complement of completely filled cytoplasmic granules, numerous narrow surface folds, rare intracytoplasmic canalicular structures, nonmembrane-bound osmiophilic lipid bodies, free ribosomes, vesicles, and mitochondria. The principal contaminating cell in the electron microscopic samples was the squamous epithelial cell (Fig 1B). Epithelial cells were present as single cells of approximately the same size as mast cells and as focal cellular aggregates still connected by desmosomes. Squamous epithelial cells are also mononuclear cells with narrow surface folds. These typical epidermal cells may be definitively identified by desmosomes and

![Fig 1. Medium magnification micrographs of control, isolated HSMC preparations show a well-preserved, fully granulated mast cell with narrow surface folds (A) and a squamous epithelial cell (B) with monolobed nucleus, extensive, dense cytoplasmic tonofilaments and keratin, and narrow surface folds. Original magnification (A) ×13,500; (B) ×12,500.](image-url)
by large aggregates of tonofilaments and keratin in their cytoplasm. Lipid bodies are nonmembrane-bound cytoplasmic organelles that are ubiquitous in mammalian cells. We have described the incorporation of an important precursor for the production of prostaglandins and leukotrienes (arachidonic acid) in cytoplasmic lipid bodies of a wide variety of cells. These cells include human mast cells isolated from lung,\(^{25,28}\) macrophages of human, guinea pig, and mouse origin,\(^{28}\) human eosinophils,\(^{39}\) human ciliated epithelial cells of respiratory origin,\(^{32}\) guinea pig biliary adenocarcinoma cells,\(^{32}\) and human neutrophils.\(^{35}\) Lipid bodies may be important intracellular storage and/or synthetic sites for the production of the various eicosanoids of which these cells are capable. In situ samples of human skin, mast cell lipid bodies are generally rare, regardless of the underlying pathology for which the biopsy was obtained.\(^{3,11,22}\) In contrast, lipid bodies are frequently found in human mast cells of lung,\(^{25,24,26,32}\) and gut,\(^{23,27}\) origin. They are particularly numerous in recovering, isolated mast cells of lung origin after anaphylactic degranulation.\(^{9,26,31}\) We also noted release of small numbers of these structures into intracytoplasmic degranulation channels during anaphylactic degranulation of isolated human lung mast cells.\(^{9,25,26}\)

Isolated, adult HSMC have greater numbers of lipid bodies (Figs 2 and 3) than do HSMC in situ. Lipid body numbers in isolated skin mast cells still remain small as compared with the large numbers of lipid bodies regularly present in mast cells of lung or gut origin, examined either in situ\(^{30,31}\) or as isolated cell preparations.\(^{23,25,26,31}\) These regularly observed differences in arachidonic acid-rich cytoplasmic structures among human mast cells of skin, lung, or gut origin are of particular interest when compared with the different capabilities for eicosanoid production of which isolated mast cells from these sites are capable.\(^{3,34-44}\) Thus, Lawrence et al.\(^{9}\) have found that appropriately stimulated HSMC produce prostaglandin D\(_2\) (PGD\(_2\)) and less leukotriene C\(_4\) (LTC\(_4\)), whereas human lung and gut mast cells make substantial quantities of PGD\(_2\) and LTC\(_4\).\(^{3,34-41}\)

The granules of isolated, control, adult HSMC were examined at higher magnifications (Figs 2 and 3). As in HSMC in situ,\(^{9,12,22}\) the crystal granule\(^{3,16,20,22,42,45}\) was the predominant granule pattern in these well-preserved isolated cells. Crystal granules (Fig 2) displayed extremely regular parallel arrays of several periodicities that appeared as hexagonal arrays when viewed in cross-section. Scroll granules\(^{20,22,23,42,44}\) were also regularly present in isolated HSMC (Fig 3). The particle granule\(^{25}\) was the least frequent pattern present (\(\approx 1\%\) of HSMC granules). Mixed granules\(^{9,23}\) were regularly found. These granule patterns were identical to those described for human mast cells present in situ in multiple sites.\(^{9}\) However, the frequency of the various patterns in HSMC differed. For example, the crystal granule, the most frequent pattern in HSMC,\(^{9,20,21}\) was less frequent in human lung mast cells or human gut mast cells in situ or in isolated cell preparations,\(^{9,23,25,36,31,37}\) whereas the scroll granule is the most frequent granule pattern present in human lung mast cells and human gut mast cells in situ or in isolated cell preparations of lung or colon origin.\(^{9,23,25,26,36,37}\) The particle granule, the least frequent granule pattern in mast cells from all sources, is more generally found in human gut mast cells than in mast cells from other sites.\(^{25,37}\) Thus, granule pattern differences regularly distinguish HSMC from human lung mast cells or human gut mast cells, but the difference is one of magnitude. That is, it is possible to find human mast cells with crystal granules in sites other than skin, although they occur most frequently in skin. For example, \(\approx 80\%\) of the isolated adult skin mast cells in this study primarily had crystal granules. Moreover, as in HSMC in situ,\(^{25}\) we regularly found mast cells with both crystal and scroll granules in the same cell (Figs 2 and 3) in isolated, adult HSMC.

The ultrastructural morphologic distinctiveness of many HSMC as compared with human lung mast cells and human gut mast cells, whether studied in situ or as isolated cell preparations,\(^{9,20,22,25,26,32,37}\) complements functional and immunomorphologic studies that show differences among these three mast cell populations in humans. For example, immunomorphologic studies of human mast cell neutral proteases have shown that most HSMC contain both chymase and tryptase activities, whereas most human lung mast cells and human small intestinal mucosal mast cells contain tryptase activity alone.\(^{45-47}\) Functional studies have also shown important differences among human mast cell populations. For example, HSMC release histamine when stimulated with morphine, substance P, 48/80, f-Met peptide, C5a, poly-L-lysine, or bradykinin analogues; human lung and gut mast cells do not.\(^{5,48}\) Thus, ultrastructural, neutral protease, and functional criteria exist that define adult HSMC as different from those of other anatomic sites similarly studied. The mechanism(s) responsible for these differences is (are) not yet known, but could be the result, singly or in combination, of environmental, developmental, functional, and recovery influences on these versatile cells with a wide morphologic repertoire.\(^{3,29}\)

Isolated, control HSMC did not show morphologic changes regularly associated with piecemeal degranulation,\(^{22}\) recovery during wound healing,\(^{22}\) anaphylactic degranulation in situ,\(^{13,15,17-19,21,46,51}\) or anaphylactic degranulation when studied as isolated cells,\(^{9,23,24,25,28}\) or recovery from anaphylactic degranulation of isolated cells.\(^{29,32}\) In particular, we did not see focal or complete losses of granule contents as are present in piecemeal degranulation,\(^{22}\) nor did we see increased focal and irregular dense materials accumulating in either empty or partially empty granule containers, as are present in HSMC recovering during wound healing in situ.\(^{22}\) Control HSMC did not show granule swelling, fusion, or degranulation channel formation, all characteristic for anaphylactic degranulation in human mast cells.\(^{23,24}\) Thus, these completely granulated cells typical of HSMC in situ provide excellent material with which to study classically induced anaphylactic degranulation. Cytoplasmic canaliculi were rarely evident.\(^{29,30}\) These are structures that occur infrequently in in situ mast cell populations and increase dramatically in the recovery phase from anaphylactic degranulation of isolated lung mast cells.\(^{29,30}\) Extracellular tracers used after fixation showed that canaliculi originate from the
Fig 2. High magnification micrographs of granule types in cytoplasm of a single mast cell show regular parallel arrays of crystal granules (closed arrow, A and B) and scroll granule (open arrowhead, A). Homogeneous dense material and peripheral parallel lamellae comprise several mixed granules in (A) (closed arrowheads), and one osmiophilic, non-membrane-bound lipid body is present in (A) (open arrow). Original magnification (A) ×37,000; (B) ×70,000.

cell surface and are internalized with increasing frequency with time after degranulation.\textsuperscript{29,30} Therefore, we think their presence indicates membrane recovery following an antecedent degranulation event but does not necessarily reflect ongoing anaphylactic degranulation in the control mast cell population.

\textit{Anti–IgE-mediated anaphylactic degranulation of isolated, adult HSMC.} We studied the ultrastructural morphology of anti–IgE-mediated anaphylactic degranulation of isolated HSMC (24\% histamine release at 3 minutes poststimulation). We found that the morphology of anaphylactic degranulation expressed by this population of mast cells isolated from skin was basically similar to anaphylactic degranulation expressed by mast cells isolated from human lung.\textsuperscript{29,24} While the overall morphologic expression of this secretory event was similar in isolated lung and skin mast cells, we did note certain differences. For example, we rarely found extrusion of altered, membrane-free granule matrices in a large number of kinetic release experiments using isolated lung mast cells,\textsuperscript{9,22,24} but granule extrusion was the generally observed event using isolated skin mast cells. Whether this reflects, in part, a difference in the amount of granule materials between these two granule populations or not is a possibility that requires further investigation.
Anaphylactic degranulation of isolated HSMC showed the following morphologic events: (1) granule swelling and matrix alteration; (2) granule to granule membrane fusions; (3) degranulation channel formation; (4) pore formation; (5) granule matrix extrusion; and (6) shedding of cytoplasmic processes and membranes. Degranulation channels (Fig 4), like those frequently formed in human lung mast cells undergoing anaphylactic degranulation, were interconnected spaces formed from fusion of granule membranes that permeated the cytoplasm. These channels contained altered matrix material (Figs 4 and 5) or appeared empty of contents. Such degranulation channels occurred sometimes in cells that also contained canalicular structures (Fig 4). Degranulation channels opened to the exterior of cells through pores in the plasma membrane (Fig 5) and were associated with increased cell processes as granule-channel membranes were exteriorized (Figs 5B and 6A). Many mast cells with visible, extruded, altered granule matrix materials adjacent to elongated cell surface folds were evident in stimulated samples (Figs 5B and 6A). Mast cells extruded granules individually or multiply through numerous pores in the plasma membrane (Fig 5B). As in
anaphylactic degranulation of isolated human lung mast cells,\textsuperscript{23,24} we also noted shedding of cell surface folds, cell membranes, and focal pieces of cytoplasm (Fig 6). This general release process accompanied shedding of extruded granule materials, such that mast cells with decreased numbers of cytoplasmic granules and massive shedding were evident. Identification of these actively releasing mast cells was often possible by the presence of a single, residual mast cell granule. Some mast cells with virtually no granules and smooth surfaces, resulting from shedding, still contained focal degranulation channels that also aided in their identification (Fig 6B). Subsequently, the size of such degranulated mast cells was reduced (Fig 6B) from the size of mature, fully granulated, unstimulated mast cells (Fig 1A). In some small mast cells, nuclear condensation was extreme; in others, nucleoli formed, and increased numbers of cytoplasmic mitochondria and vesicles were evident, changes that we have described as an integral event in the synthetic phase of the recovery from anaphylactic degranulation of isolated human lung mast cells.\textsuperscript{30,31}

The ultrastructural morphology of anaphylactic degranulation expressed by isolated HSMC, while similar overall to that of isolated human lung mast cells,\textsuperscript{23,24} differed in showing, more frequently, extrusion of altered granule matrix materials directly to the exterior and in showing, less frequently, cytoplasmic degranulation channel formation and solubilization of altered granule matrix materials before pore formation and resolution of degranulation chan-
Fig 5. HSMC 3 minutes after stimulation with anti-IgE. (A) The cytoplasm contains a mixture of dense, unaltered granules and degranulation channels filled with altered granule matrix materials. One of these is open to the exterior and is in the process of releasing altered matrix (arrowhead). (B) A different mast cell is extruding swollen, membrane-free granules through the cell membrane at four separate points. A few unaltered granules remain in the cytoplasm. Original magnification (A) X 20,000; (B) X 17,000.

nels. The secretory stimulus in each instance was the same, and in each case, studies were performed on isolated cells that had been cultured overnight. The morphologic differences in anaphylactic degranulation, of which HSMC and human lung mast cells are capable, may, then, reflect inherent differences between these two populations of human mast cells. This possibility is supported by a number of ultrastructural studies done on HSMC in situ. These studies used a variety of stimuli, including mechanical, antigen, C5a, or C5a-desArg, either in skin of patients with urticaria pigmentosa or in normal skin. In each instance, and like our studies of isolated HSMC, extrusion of altered granules was illustrated as the primary mechanism of anaphylactic degranulation of HSMC in situ.

Shedding of mast cell membranes and processes, in conjunction with extrusion and shedding of granules during anaphylactic degranulation, occurs in both isolated human mast cell preparations that we have studied in detail. Possible functional significance of such a process, of which many cells are capable, in the biology of mast cells is suggested by the recent study by Dreskin et al. Using a
Fig 6. HSMC 3 minutes after anti-IgE stimulation. (A) A large mast cell that has released nearly all cytoplasmic granules shows several extruded, membrane-free, swollen, residual granule matrices adjacent to the cell surface (arrowheads) among numerous elongated, narrow surface folds. Note that all folds maintain their connections to the cell. (B) A small mast cell with a few residual degranulation channels (D) and three unaltered granules (arrows) shows a relatively smooth surface, several large, irregular surface processes and numerous cell and membrane fragments adjacent, but unattached, to the cell. Original magnification (A) × 18,000; (B) × 17,000.
closely related cell, the rat basophil leukemia cell, these investigators have produced cell cytoplasts and have studied their functional capacities. Essentially, cytoplasts are fragments of cytoplasm that are devoid of nuclei and bounded by resealed plasma membranes. These structures are somewhat analogous to shed processes from mast cells, although much larger in size. Using these preparations, Dreskin et al have shown that these anuclear rat basophil leukemia cytoplasmic fragments are capable of IgE receptor-mediated hydrolysis of phosphoinositides, a biochemical correlate of anaphylactic degranulation.54

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