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Connective Tissue Mast Cells Exhibit Time-Dependent Degranulation Heterogeneity

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Previous studies have identified two ultrastructurally distinct forms of mast cell (MC) degranulation following activation. Immunoglobulin E (IgE)-mediated reactions are characterized by a very rapid swelling and fusion of MC granules and abrupt mediator release. In certain chronic disease states (e.g., bullous pemphigoid), there is "piecemeal" degranulation with a more-gradual mediator release effected by microvesicular transport of "pieces" of granules to the cell surface. It is unclear whether these two degranulation patterns are determined by the different natures of the stimuli, heterogeneity among responding MC granules, or temporal factors. To investigate these issues, we have carried out electron microscopic studies with skin biopsies obtained from ragweed-sensitive subjects 15 and 30 s and 1, 3, 5, and 10 min after intradermal ragweed injection. "Anaphylactic"-type granule changes began by 15 s after ragweed injection and were complete by 5 min; unaffected granules were juxtaposed with granules that were swollen and fused. The remaining granules subsequently underwent changes in appearance similar to those seen in piecemeal degranulation. However, microvesicular transport of granule components to the surface was not observed. These findings indicate that skin MC changes in sites of IgE-mediated reactions include not only the typical very rapid anaphylactic degranulation but also a slower onset of gradual alteration of other granules, frequently within the same MC. These different patterns could reflect MC granule heterogeneity with attendant different responses to IgE-mediated stimuli.

It is generally recognized that local mast cell (MC) alterations are seen in a number of skin reactions. Two ultrastructural patterns of MC degranulation during the release of granule contents to the extracellular environment have been described. In "anaphylactic" degranulation there is swelling and homogenization of the entire granule matrix and fusion of granules with each other and the plasma membrane to form conduits for the release of granule contents into the pericellular space (14). This pattern has been seen in immediate immunoglobulin E (IgE)-mediated reactions and with opiates, compound 48/80, and calcium ionophore (5–13). In a second pattern, "piecemeal" degranulation, there is a partial loss of the MC granule matrix at random time points, possibly occurring via a microvesicular transport of granule contents to the pericellular space (4, 10). Piecemeal degranulation has been observed in several chronic processes (e.g., bullous pemphigoid and wound healing) with postulated slow, ongoing mediator release (2–5, 8, 10). However, it is not clear whether both degranulation patterns occur in immediate hypersensitivity reactions. We report here sequential studies that suggest that some of the MC granule alterations seen in piecemeal degranulation occur, in addition to anaphylactic changes, in IgE-mediated skin reactions.

MATERIALS AND METHODS

Five atopic subjects with strong clinical and immediate whealing skin test reactivities to ragweed were studied after informed consent and Institutional Review Board approval were obtained. Following multiple intradermal injections of 20 PNU of ragweed extract (Greer Labs, Lenoir, N.C.), the extent of whealing was recorded, and 3-mm-diameter punch biopsies were obtained precisely at the injection sites at 0.25, 0.5, 1, 3, 5, and 10 min, as previously described (13). Control sites were (i) noninjected contralateral skin and (ii) biopsies obtained 10 min after intradermal injection of the buffered saline diluent (phosphate-buffered saline [PBS]).

Histologic studies. Specimens were bisected and immediately processed for electron microscopy. They were divided by scalpel incisions, with selection of tissue blocks at the rim of the biopsy to be sure that fixation was rapidly complete in the chosen tissue. These blocks were washed in 0.1 M cacodylate buffer for 2 h at room temperature, postfixed in 2% (wt/vol) osmium tetroxide for 1 h, dehydrated in a graded cold ethanol solution to propylene oxide, and then embedded in Epon. One-micrometer sections, stained with 0.5% toluidine blue, were examined by light microscopy for MC content. Ultrathin sections were examined with a Hitachi 7000 transmission electron microscope after being stained with uranyl acetate and lead citrate or bismuth subnitrate. All viable MCs were photographed at a low magnification, and representative MC constituents were photographed at higher magnifications.

Fifteen to 25 mast cells were examined randomly in three to five tissue blocks obtained from each biopsy by using numerous ultrathin (65-nm) sections, each separated by 20 to 30 μm to ensure that sequential sections of single MCs were not analyzed. Each coded slide was examined independently by three experienced observers.

RESULTS

In biopsies of uninjected skin and of skin 10 min after intradermal PBS injection, MCs all contained numerous membrane-bound intracytoplasmic granules with no evidence of degranulation (Fig. 1A). The ultrastructural appearance in over 95% of the mast cells was that of the tryptase-positive, chymase-positive MC, with cores of electron-dense crystal lattice or more electron-dense amorphous material surrounded by rims of curvilinear lamellae oriented circumferentially around the periphery of the granule. However, the "scroll pattern" typical of tryptase-positive, chymase-negative MCs was seen in occasional cells (Fig. 1C).

In biopsies obtained 15 s after antigen injection, there was subtle evidence of degranulation, with global swelling and disorganization (solubilization) of all granule matrix constituents and fusion of granules with each other and the plasma membrane to form conduits to the extracellular space (anaphylactic

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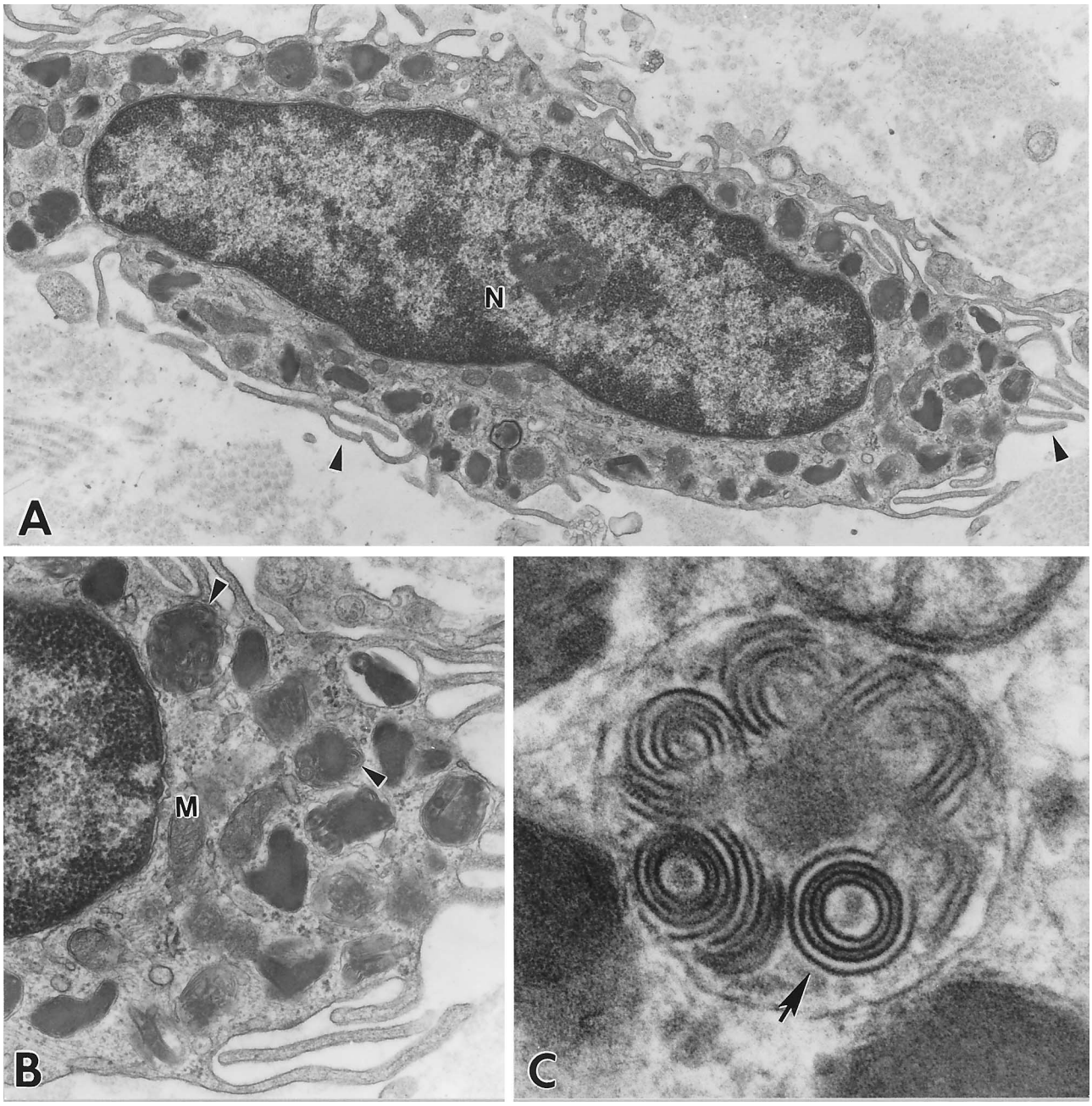

FIG. 1. Human connective tissue MC from adult control skin. (A) The MC has numerous cytoplasmic processes (arrowheads), a nucleus (N)/cytoplasm ratio of >1, and cytoplasm filled with round-to-oval granules. Magnification, ×13,500. (B) Granules exhibit cores with various electron densities, with peripheral rims of curvilinear lamellae (arrowheads). M, mitochondria. Magnification, ×26,000. (C) Membrane-bound connective tissue MC granule with a well-formed scroll substructure (arrow). Magnification, ×160,000.

degranulation) (Fig. 2A and B). These changes were seen in a majority of the granules.

Further granule changes occurred over the next 5 min. Externalized granule contents were identified in the pericellular space as round, non-membrane-bound, amorphous structures retaining the ultrastructural appearance of internally solubilized MC granules. These fine structural features were similar to those described for morphine-degranulated tryptase-positive, chymase-positive MCs in a skin organ culture system (9). By 5 min after ragweed injection, the contents of many of the granules were discharged. However, 20 to 40% of the granules per MC were still present in the cytoplasm, with an appearance similar to that in resting, unstimulated MCs (Fig. 2C). Frequently, these intact granules were adjacent to granules which were markedly swollen with matrix solubilization (Fig. 2C).

All remaining intact granules began to exhibit features of degranulation between 5 and 10 min after antigen challenge (Fig. 3). Ultrastructural changes in these granules were characterized by swelling and segmental solubilization of the granule matrix to give a "moth-eaten" appearance similar to that described for piecemeal degranulation (4, 10). Affected granules were characterized by amorphous, zonal, electron-dense

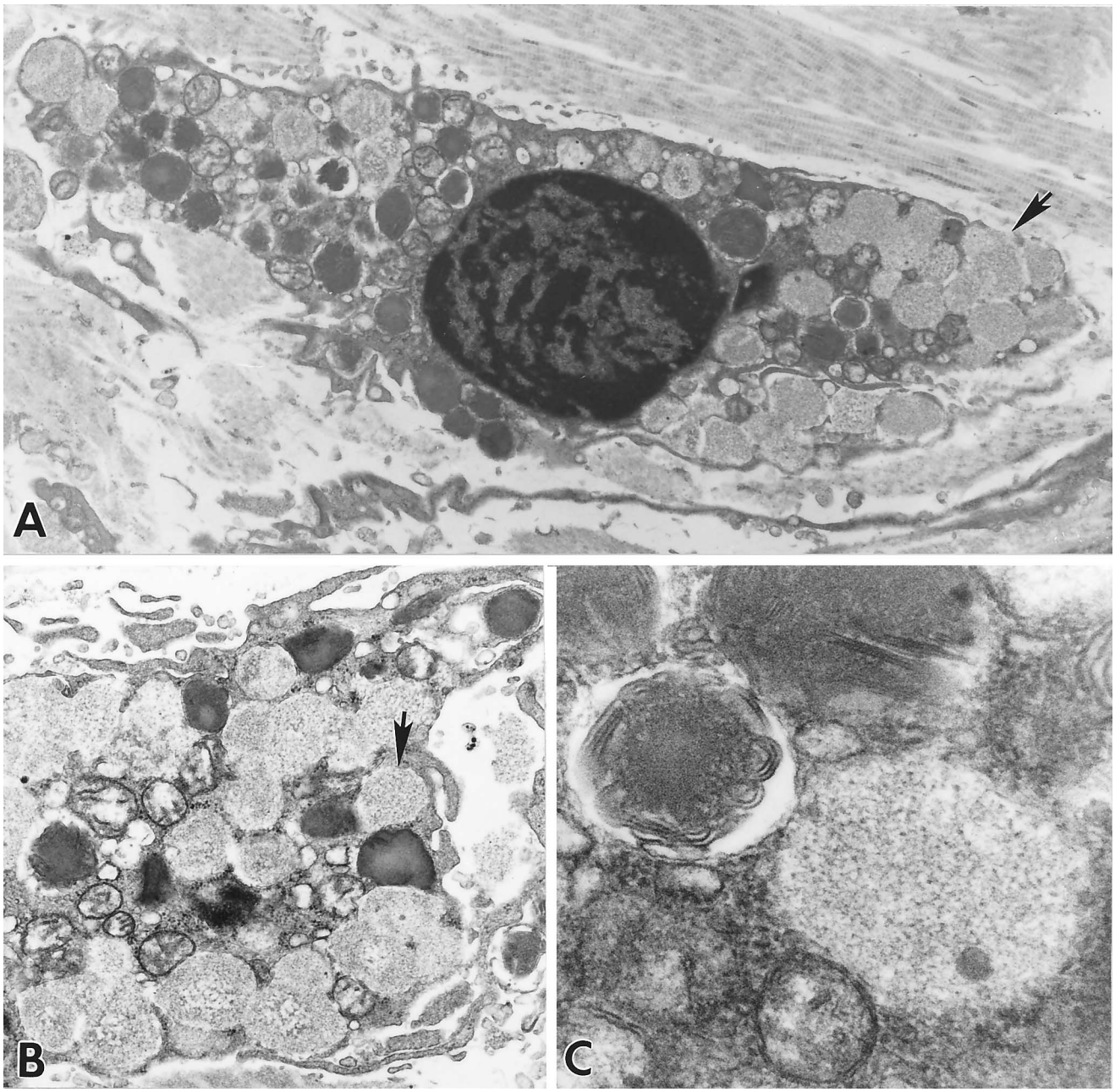

FIG. 2. Human connective tissue MC 15 s after intradermal injection of ragweed extract, exhibiting global degranulation. (A) Degranulation is characterized by solubilization and swelling of the entire granule matrix, fusion of granule membranes with each other, and fusion of granule membranes with the plasma membrane to form conduits to the extracellular space (arrow). Magnification, ×12,500. (B) The matrix of affected granules is completely solubilized as evidenced by disorganization of the granule ultrastructure and an increase in electron lucency (arrow). Magnification, ×19,800. (C) Granules actively involved in degranulation are juxtaposed with granules that do not exhibit ultrastructural features of degranulation. Magnification, ×78,000.

regions juxtaposed with more lucent areas. The occasional fusion of individual granule membranes with one another, and/or fusion of granule membranes with the plasma membrane, was noted after this pattern of granule swelling. However, this membrane fusion occurred much less frequently than it did in biopsies obtained during the first 5 min after injection, when anaphylactic degranulation was exclusively observed. Microvesicular transport of granule material was not observed at any time during the degranulation process, although small, empty, membrane-bound vesicles were present. Externalized non-membrane-bound granule contents with ultrastructural features similar to those of the piecemeal-type degranulation were seen in the extracellular space 10 min following ragweed injection (Fig. 3), suggesting that extrusion of intact whole granules did not occur during this piecemeal-like granule alteration between 5 and 10 min. All MC granules displayed features of degranulation by 10 min after antigen challenge.

DISCUSSION

The findings in this study indicate that in vivo IgE-mediated activation of skin MCs results in some of the granule alter-

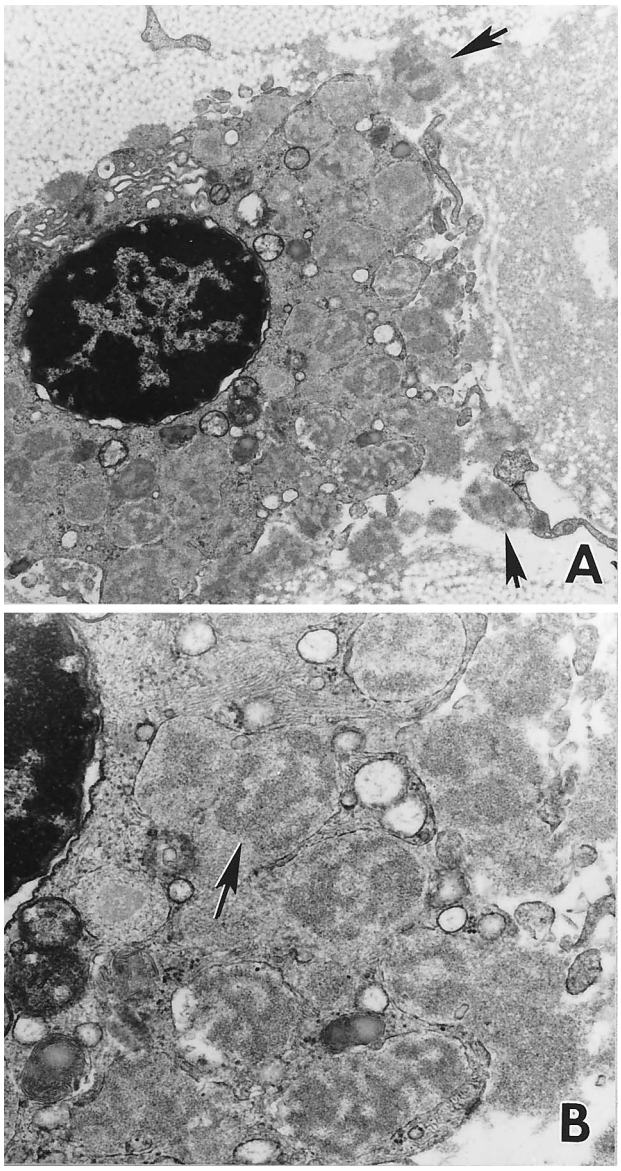

FIG. 3. Segmental degranulation in a human connective tissue MC 10 min after intradermal injection of ragweed extract. (A) The majority of MC granules exhibit features of degranulation at this later time point. Swelling and occasional fusion of granules accompanied by fusion of granule membranes with the plasma membrane facilitate the release of granule contents. Extracellular non-membrane-bound granule matrix is present in the pericellular space (arrow). Magnification, ×9,300. (B) The granule ultrastructure at this later time point reveals partial solubilization of granule matrix to impart a segmental appearance (arrow). Magnification, ×25,000.

ations seen in both the reported anaphylactic and piecemeal patterns of degranulation. Of particular interest is that the changes suggesting anaphylactic degranulation occurred first, starting in most granules within 15 s after intradermal antigen injection in these sensitive subjects. However, granules without any alterations were also present in these MCs at 5 min, often located adjacent to prominently altered granules. At 10 min, almost all of the granules had undergone changes, some of which resembled those seen in piecemeal degranulation. By this time, most of the granules either were extruded into the pericellular space or had a solubilized matrix within the cytoplasm. However, no evidence of microvesicular transport of granule matrix materials was seen. The study design used here, with biopsies obtained sequentially at different sites, did not permit any definite conclusions as to whether the anaphylactic-type and piecemeal-type granule alterations occurred sequentially in the same individual MC.

In reviewing these findings, we prefer to use terminology other than anaphylactic and piecemeal to describe these degranulation events, for the following reasons. (i) The term anaphylactic implies a particular immunologic cause of the rapid-onset MC granule alterations. Although that is the antigen-IgE model reported here, this pattern of events appears to be very similar to that previously observed by us in skin MCs activated by morphine, a nonimmunologic agonist (14). (ii) The term piecemeal degranulation has been utilized for MC alterations in chronic inflammatory skin diseases such as bullous pemphigoid (4). We found granule changes similar to those seen in piecemeal degranulation occurring between 5 and 10 min after intradermal antigen challenge in the sites of IgE-mediated skin reactions. However, we did not see the microvesicular transport picture (granule matrix within microvesicles) reported previously as a component of piecemeal degranulation (4, 10). Rather, we identified MC granules in the pericellular space; such granules showed a pattern of segmental degranulation.

Therefore, we suggest the use of the terms global degranulation and segmental degranulation to describe these events. The term global refers to the complete solubilization of MC granule contents within individual organelles. The morphologic correlate of global degranulation has been extremely well characterized and involves the eventual fusion of granule membranes with one another and with the plasma membrane (6, 11). Such changes have been observed following incubation of MCs with various types of stimuli (6, 9, 11). The term segmental describes the solubilization of demarcated regions of the granule matrix, while other regions of the granule remain relatively intact and electron dense. The mechanism(s) whereby certain regions of the granule are solubilized more than others is unknown. Our finding of MC granules with features of segmental degranulation which had been extruded into the pericellular space leads us to speculate that one mechanism by which MC granule contents are released in segmental degranulation may be similar to that seen in global degranulation (9).

Although the ultramicroscopic appearance of the sequential degranulation seen in this study was very similar to that seen in some chronic diseases (2, 4, 10), it is not known whether the biochemical events and mediator release patterns in these granules are the same. It would also be of considerable interest to see the MC events in sites of IgE-mediated reactions persisting over hours and days. The functional significance of the two MC granule alteration patterns occurring in IgE-mediated reactions is also not yet defined. Our studies utilizing skin chambers appended to denuded blister bases have characterized the temporal patterns of mediator release (1, 12). However, these findings cannot be compared closely with changes occurring in the MC granules within the first 10 min after antigen challenge because of the additional time required for diffusion from the reaction site in the underlying dermis into the overlying skin chambers (1). Semiquantitative immunoelectron microscopic studies may allow a more direct comparison of the temporal patterns of granule alterations and granule mediator content.

It has become increasingly apparent that MCs are key regulatory elements in the inflammatory cascade and potentially are significant contributors to tissue homeostasis. The mechanisms by which they influence their surrounding microenviron-

ment in a target-specific manner remain elusive, but the ultrastructural and temporal heterogeneities of MC degranulation outlined in this study should provide a basis for further investigation of these issues.

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