AN ELECTRON MICROSCOPE STUDY OF MOUSE MAST CELLS ARISING IN VIVO AND IN VITRO

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INTRODUCTION

Selective differentiation of cells morphologically identical with tissue mast cells at the light microscope level has been described by Ginsburg and Sachs (5) and Ginsburg and Lagunoff (4) in a mouse tissue culture system composed of a C3H feeder layer overplated with horse serum-sensitized C57Bl lymphoid cells. Histochemical evaluation indicates that the granules of the tissue culture-derived mast cells, like the granules of mast cells from normal mouse connective tissue, contain strongly acidic mucopolysaccharides and a chymotrypsin-like enzyme (4), further suggesting a homology between the tissue culture-derived mast cells and native mast cells. In this report, the degree of homology between mast cells arising in
normal mouse tissues and those arising from mouse lymphoid cells in tissue culture has been further examined with the electron microscope.

The mast cell has a unique ultrastructural appearance in the rat (1) and in the mouse (vide infra). In the rat, the fine structure of the mast cell has been studied with the electron microscope in developing and in mature animals, and the process of rat mast cell differentiation and mast cell granule formation has been arranged into a postulated sequence of events (1, 2). This sequence of developmental morphologic alterations, and the distinctive appearance of the mature rat mast cells, form a useful framework for the analysis of differentiation of mast cells arising in mouse connective tissue, and for comparison of these native mast cells with tissue culture–derived mast cells.

MATERIALS AND METHODS

Mast cell cultures were obtained according to the method of Ginsburg and Lagunoff (4) by plating cells, teased from the pooled lymph nodes from C57Bl mice “hyperimmunized” by repeated injections of horse serum, onto confluent monolayers derived from minced and trypsinized C3H mouse embryos. (Platings were done only on feeder layers judged by microscopic examination to be confluent monolayers in optimal condition.) The C57Bl mice (all males) received 0.1 ml of horse serum intraperitoneally every week for 6 wk. They then had routine care until 14 days prior to sacrifice for the commencement of a culture series, when they received another 0.1 ml of horse serum intraperitoneally. The C57Bl mice were then killed by cervical dislocation, and skinned from the ventral aspect; the lymph nodes (easily located because of their moderate hypertrophy) were dissected, pooled in phosphate-buffered saline at pH 7.4, teased, trypsinized, and plated onto the feeder monolayer in a density of 1 x 10⁴ cells in 10 ml of culture medium per 5 cm dish with a small volume of tissue culture fluid. The free quadrants of culture layer were lifted with fine forceps. As it was lifted, the specimen rolled naturally into an elongated cylindrical mass about 1 mm in diameter. These “cylinders” were placed immediately in 6% glutaraldehyde in 0.06 m Sorensen’s phosphate buffer (final pH 7.40), to which 0.1% wt/vol of Alcian blue was added (1). Fixation was carried out in an ice bath for 4–6 hr followed by a brief rinse in phosphate buffer and postfixation for 1 hr in 2% osmium tetroxide in s-collidine buffer. The specimens were then embedded in Epon according to Luft (9), sectioned on the Porter-Blum microtome, captured on unsupported 150 X 75 mesh per inch grids, stained with uranyl acetate followed by Millonig’s lead stain (10), and examined in an RCA EMU3G microscope.

Native mast cells were examined in tissue specimens from the tails of newborn mice, and from the tongues of nonimmunized, adult male C57Bl mice. Tissue specimens were fixed, embedded, sectioned, stained, and examined in the same way as the tissue culture specimens.

OBSERVATIONS AND DISCUSSION

The details of the differentiation and maturation of mast cells seen in electron micrographs of both native and tissue culture specimens were morphologically identical. Further, the changes in morphology observed in differentiating mouse mast cells as they accumulate increasing numbers of recognizable mast cell granules were similar in most respects to those previously described for the rat mast cell (1). This differentiation process consisted of the formation and sequestration of increasing numbers of the dense granules that eventually fill the cytoplasm of the mature mast cell.

In the description that follows, the process of differentiation and granule formation is described without reference to the source of the cells (native vs. tissue culture). Formative steps are represented by pairs of micrographs from both native tissues (denoted by * on the micrographs) and tissue culture (noted in the figure legends for the micrographs). The number of days between plating and harvest is indicated in the legends for the micrographs from tissue culture specimens.

The nuclei in the earliest recognizable mast cells contained a variety of chromatin patterns, probably reflecting their proliferative character.
The cytoplasmic membrane system was rudimentary in immature mast cells, except for an abundant perinuclear Golgi apparatus. In addition to the usual smooth membrane contours, the Golgi apparatus contained small dense “progranules” (1) ranging up to about 70 μm in diameter, inside both bristle-coated and smooth membranes. A few recognizable but incompletely formed mast cell granules and mitochondria were scattered in the peripheral cytoplasm. The ribosomal complement of the cytoplasm consisted mainly of aggregates of ribosomes not associated with membranes. The plasmalemma was relatively unspecialized but usually displayed a few distinct, short microvilli. Invaginations resembling “pinocytotic vesicles” were rarely seen in native mast cells but were occasionally seen in mast cells arising in tissue culture (Fig. 1).

As the cells became more mature, the cytoplasm contained increasing numbers of specific granules with their limiting membranes. In intermediate stages of development, the Golgi apparatus with its associated progranules remained prominent, and many of the granules appeared to be in formative stages. Maturation was completed by the elaboration of a sufficient number of completely condensed mast cell granules to crowd the cytoplasm. In the fully differentiated mast cell, the intergranular cytoplasm contained a rare, rough endoplasmic reticulum contour, a few mitochondria, and scattered ribosomal aggregates; the Golgi apparatus was present, but reduced in size (Fig. 3).

The morphology of individual developing granules was similar in many details to that observed in rats (1). The process of granule formation in mouse mast cells can be analyzed in the hypothetical sequence previously reported for the formation of rat mast cell granules (1). Dense progranules encased in vesicles appeared to form in the Golgi apparatus. These progranules then appeared to aggregate inside a single limiting membrane near the Golgi apparatus, although occasionally single progranules and progranular aggregates were found in cytoplasm remote from the Golgi apparatus. Inside these larger vacuoles the progranules fused to form ropy cords and clusters of dense material. The accretion and fusion of the progranular material into smaller strands appeared to occur at this stage in the rat, but in the mouse mast cell the granule seemed merely to “condense” to form a homogeneous, intensely osmiophilic, spherical mass wrapped in a perigranular membrane (Figs. 1 and 2). Possible macromolecular correlates of these morphologic events were reported previously (1).

In the tissue culture specimens the feeder layer cells near mast cells were predominantly morphologic analogues to the macrophages of native tissues. Native mast cells, both immature and mature, were also often spatially associated with distinctive macrophages. Further, the detailed appearance of the macrophages and the character of their apposition were strikingly similar in native and tissue culture material (Fig. 4). The microvilli of the mast cells and neighboring macrophages were intertwined. The cytoplasm of the macrophages abutting mast cells contained membrane-limited contours filled by aggregates of particulate material. These macrophage “granules” were similar to mast cell granules in size, but contained less electron-opaque material. In addition, the macrophage cytoplasm underlying the zone of apposition contained irregularly

Key to symbols
- A, aggregates of progranules
- cap, capillary
- en, endoplasmic reticulum
- G, Golgi zone
- Gr, mast cell granules
- Mr, perigranular membrane
- mae, macrophage
- mr, microvillus
- N, nucleus
- n, native tissue
- p, progranules

**Figure 1** Immature mast cells from (a) newborn mouse tail, and (b) tissue culture (45 days). The plasmalemma has relatively few microvilli. Numerous granules in various states of accretion and condensation rim the cytoplasm. Cisternae of rough endoplasmic reticulum are sparse, but the cytoplasm is dotted with ribosomal aggregates. The Golgi apparatus is prominent and contains dense progranules in both smooth and bristle-coated vesicles. X 12,000.
Figure 2  Higher magnification of the Golgi apparatus in immature mast cells; (a) newborn mouse tail, and (b) tissue culture (31 days). Progranules can be seen in association with Golgi cisternae and in bristle-coated and smooth vesicles. Larger, more peripheral vesicles contain several progranules, apparently in the process of accretion. × 38,000.
Figure 3  Mature mast cells from (a) adult mouse tongue, and (b) tissue culture (45 days). Numerous microvilli extend from the plasmalemma. Fully formed, uniformly dense granules pack the cytoplasm. The intergranular cytoplasm is relatively unspecialized, but contains scattered rough endoplasmic reticulum, ribosomal aggregates, and mitochondria. In native mouse tissues, mast cells are often in close association with elements of the microcirculation and with macrophages. The Golgi apparatus, depicted in the mast cell from tissue culture (b), is reduced in size, but contains progranules. X 7600.
shaped multivesicular bodies, large numbers of bristle-coated vesicles of relatively uniform size containing material of intermediate density, and "empty" smooth membrane vesicles ranging widely in size. Macrophages displaying this cytoplasmic conformation seem to be specifically associated with mast cells in native mouse and rat tissues.

The association of mast cells from both species with distinctive macrophages raises the possibility of some functional relationship reflected in the spatial apposition of the two types of cells. Mast cell granules have often been reported lying inside neighboring cells of various types (12, 13), but, except for their suggestive morphology, the granules seen in the macrophages (Fig. 4) have not been identified as coming from mast cells. The reports from light microscope studies of phagocytosis of mast cell granules by other cell types (12) usually involved prior degranulation of the mast cell; no such injury to the mast cell appears in micrographs from either native or tissue culture specimens. If the "granules" in mast cell-associated macrophages did indeed originate in neighboring mast cells, it is possible that granules were secreted by both developing and mature mast cells. Such a process might indicate a dynamic role for the mast cell in normal connective tissue, as opposed to the widely held view that mast cells lie dormant until an appropriate stimulus causes them to degranulate (12).

The development of mast cells under tissue culture conditions has also been reported by Pluznik and Sachs, using mouse as the tissue source (10), and Csaba and Olah, using rat thymus as tissue source (3). Pluznik and Sachs, after further study, favored the view that the cells they termed "mast cells" were actually macrophages, with cytoplasmic "granules" composed of phagocytosed agar medium (3).

1 Sachs, L. and D. Lagunoff. Personal communication.

The report by Csaba and Olah, of mast cells arising in cultured rat thymus cells, contains a series of electron micrographs depicting what the authors interpret as mast cell granule formation (3). On the basis of their experiments they advance a hypothesis that histamine and/or serotonin binds heparin, forming the mast cell granule matrix. The process of "mast cell granule formation" depicted in their micrographs in no way resembles that seen in electron micrographs of native tissues of the rat (1). Further, there is no resemblance between the process of mast cell formation they report in their culture system and the differentiation of mast cells seen in native mouse tissue or the mouse tissue culture system described in this report. Further, there is ample evidence that heparin is firmly bound to the protein fraction of the granule early in granule formation, whereas the amines are loosely associated with the sulfate moiety of heparin late in the evolution of the granule (5-8), casting doubt on the hypothesis that low molecular weight amines can bind heparin into the mast cell granule.

The comparisons made in this study and the evidence presented by Ginsburg and Lagunoff (4) indicate that the tissue culture system described by Ginsburg and Lagunoff produces bona fide mast cells. Other in vitro systems reported thus far have failed to produce cells that meet any adequate definition of the mast cell.

SUMMARY

A mouse tissue culture system composed of antigen-stimulated C57Bl lymphoid cells grown on a feeder monolayer of C3H embryo fibroblasts produces cells identical to mast cells at the light microscope level. These tissue culture mast cells arising in vitro were compared at the ultrastructural level with those present in newborn and adult mouse connective tissue. The tissue culture-derived and native tissue mast cells were equivalent in all respects, including frequent association of mast cells with a distinctive type of macrophage. Further, the most prominent process in mast cell

**Figure 4** Mast cells in close apposition to macrophages, from (a) adult mouse tongue, and (b) tissue culture (31 days). The microvilli from the two cell types are in intimate contact. The mast cell cytoplasm is typical, but the macrophages contain a characteristic complement of smooth and bristle-coated vesicles near the plasmalemma, and a population of membrane-limited "granules" of various density and size. The granules in the macrophages are less dense and osmiophilic than those in the mast cells. X 20,000.
differentiation, i.e. granule formation, is morphologically identical in tissue culture and native mouse mast cells, and closely parallels granule formation in rat mast cells. The detailed ultrastructural homology between mouse mast cells in vivo and those produced in vitro indicates that the tissue culture system produces bona fide mast cells. A comparison between mast cell differentiation in the mouse and that in the rat documents the basic similarity of the formative process of mast cells from these two species.

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