Two populations of mast cells on fibroblast monolayers: correlation of quantitative microscopy and functional activity

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Summary
We have previously shown that a confluent layer of mature mast cells is obtained when lymph node cells are grown on embryonic fibroblast monolayers. Two populations of mast cells may be observed, depending on treatment of the mice from which the lymph node cells are derived. We report now on the morphometric evaluation of these two mast cell entities, and we correlate this with cellular biochemistry and secretory behaviour. The first type of mast cell is small (265 ± 20 μm³). It arises from the embryonic monolayer and the cells have feathers resembling those of connective tissue mast cells. These cultured cells are filled with about 1000 homogeneous electron-dense granules, which usually range in diameter from 0.05 to 0.2 μm. The second type of mast cell arises from precursors originating from the lymph node and they have feathers of mucosal mast cells. These cells are larger (480 ± 40 μm³) and contain about 300 heterogeneous granules, which range from 0.1 to 0.8 μm. Both cell entities contain about equal amounts of histamine, serotonin and chymase. Biologically, the two cell entities secrete soluble mediators (histamine and serotonin) at different rates compared to the rate at which they secrete chymase. We suggest that such a pattern of secretion exhibits a form of degranulation that permits the release of freely diffusible mediators that are loosely bound to granules, but only partially permits the secretion of insoluble mediators, which are stored in the granules. Alternatively, there might be a mechanism that rapidly inactivates or binds chymase so that only the vasoamines will be free.

Key words: mast cells, granule formation, degranulation, piecemeal degranulation.

Introduction
Mast cells play an important role in allergic syndromes. The cells proliferate mainly in connective tissues (Galli et al. 1984). In recent years, several methods were developed for growing these cells in tissue culture. The methodology is based on growing stem cells in media (Ginsburg, 1963; Ginsburg & Lagunoff, 1967; Nabel et al. 1981; Razin et al. 1981; Schrader, 1981) containing differentiating factors. Those growth modulators may be obtained either from previously prepared conditioned media (Nabel et al. 1981; Razin et al. 1981; Schrader, 1981) or they may be secreted directly into the growth medium by other cell types present in the culture (Ginsburg, 1963; Ginsburg & Lagunoff, 1967). In recent years we have used mainly the latter approach. We have found that two different cell populations may be present in those cultures (Ginsburg et al. 1978; Barrett & Metcalfe, 1984). If lymphoid cells are derived from an immunized mouse and are grown (in the presence of the antigen) on a fibroblast monolayer, mast cells appear, in which, as seen by electron microscopy, granules have a heterogeneous electron density. These mast cells originate from precursors in the lymph node and resemble mucosal mast cells (MMC). By contrast, when cells were derived from non-immunized mice the granules were homogeneous. These mast cells proliferate from the embryonic skin fibroblast monolayer and resemble connective tissue mast cells (CTMC). In this paper we shall refer to the two cell cultured mast cell populations as MMC-like and CTMC-like cells. In addition, MMC-like have almost 10 times more IgE receptors than CTMC-like cells and have a significantly shorter life span in tissue culture (Ginsburg et al. 1982).

It is well established that murine mast cell granules contain various pharmacologically active mediators, such...
as histamine, serotonin, proteoglycans, proteolytic enzymes, chemotactic factors, and so on (Galli et al. 1984). In previous papers we demonstrated that cultured mast cells secrete histamine, even in the absence of known stimuli (Ginsburg et al. 1978). The rate of basal histamine secretion is dependent on the cell of origin, MMC-like secrete histamine faster than CTMC-like cells. Fast secretion of mast cell granule content (i.e. anaphylactic degranulation) may be lethal, and it therefore has been suggested in recent years that the role of slow mediator release (by mast cells in vivo) might represent an immuno-physiological control of local homeostasis. It is the purpose of this study to correlate the structure of the granules with the function of those cells in tissue culture.

Materials and methods

The mice and procedures for immunization of mice with horse serum and tissue culture techniques employing fibroblast monolayers were as described (Ginsburg, 1963; Ginsburg et al. 1978, 1982). The vasoamine content of the monolayers was estimated by radioenzymic methylation of histamine (Taylor et al. 1980) or of N-acetylated serotonin (Hammel & Langunoff, 1978) using S-[3H]adenosyl-methionine (Amersham). In either method, 1 ng ml\(^{-1}\) of vasoamine could be detected (5% accuracy). Chymotrypsin-like activity was assayed using N-benzyloxymethyl-lysine ethyl ester (BTEE) as a substrate (Lagunoff & Benditt, 1963). One unit will hydrolyse 1 umol BTEE per min at pH 7.8 at room temperature (23°C ± 2°C).

The detailed methodology and procedures used for electron microscopy (Ginsburg et al. 1978) and morphometry of mast cells (Hammel et al. 1983, 1987; Hammel, 1986) have been described. In brief, monolayers were rinsed with phosphate-buffered saline (PBS) and fixed with 1% glutaraldehyde in PBS (pH 7.0) for 2 h at room temperature. The monolayers were then rinsed in PBS and post-fixed with 1% osmium tetroxide (in 0.1 M-phosphate buffer, pH 7.0) for 60 min at room temperature. Following dehydration in ethanol, the monolayers were detached from the culture dishes by addition of propylene glycol and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined under a Jeol-100B electron microscope.

Morphometry was performed on electron micrographs (×12,000) of mast cells from five independent experiments. Granule measurements were carried out using a MOP-3 digitizer (Carl Zeiss, Thornwood, NY) interfaced to an HP-86 microcomputer (Hewlett Packard Co., Palo Alto, CA) for data transformation and analysis. The numerical granule density (N), was estimated by \( N = N_n / A \) (Hammel, 1986) (\( N_n \), density of granules per 1 μm\(^2\) of cytoplasm area; \( A \), mean granule profile area). The cell cytoplasm area was determined by point counting using a screen of 1 point cm\(^{-2}\) (Hammel et al. 1983, 1987). Recently, we described a new computer-assisted approach that may assist in evaluation of granule volume data (Hammel et al. 1983). The method is based on simple measurement of each granule profile area \( (a) \), which is transformed to its equivalent granule profile volume \( (v = 4/3(a/π)^{3/2}) \). A histogram of the equivalent volumes is constructed and the noise in the distribution is smoothed by a continuous averaging method. Studies employing this method have shown that there is a granule volume periodic distribution in mast cells (Hammel et al. 1983), pancreatic acinar cells (Hammel & Lagunoff, 1982; Hammel et al. 1987), and other cells (Hammel & Lagunoff, 1982).

The follow-up of initial basal release rate was performed by washing the plates three times with fresh PBS and addition of fresh Dulbecco's medium (2 ml) to each of the plates. Medium was collected from each plate (triplicates) at different times of incubation (humidified incubator, 37°C, 5% CO\(_2\)). It was centrifuged for 3 min (1000 g). The supernatant was collected and acidified by the addition of 10 μl of 10 M-HCl. Basal release was constant during at least the first 3 h (Ginsburg et al. 1978). Degranulation was induced by the addition of 780 μl of mouse anti-horse serum (Ginsburg et al. 1978) diluted with fresh Dulbecco's medium (1:40, v/v). This procedure for mast cell activation induces about 40% release of vasoamine. After 3 min of incubation (humidified incubator, 37°C, 5% CO\(_2\)) with 2 ml of the antiserum, 20 μl of decomplemented horse serum was added, and the plates were incubated for another 5 min. The post-degranulation supernatant and one rinsing of the plates were pooled. The cells were broken by addition of 1 ml of 0.01 M-HCl, followed by three freezing and thawing cycles to release residual histamine.

Results

The ultrastructure of cultured mast cells derived from precursors in non-immunized mice resembled that of resting connective tissue mast cells (Ginsburg et al. 1982; Barrett & Metcalfe, 1984). Numerous granules with highly electron-dense homogeneous contents were observed throughout the cell cytoplasm (Fig. 1). In cultured mast cells arising from precursor cells of immunized mice, the granular morphology was considerably different. The secretory granules were fewer and much larger. The granular substructure was highly heterogeneous. In many granules, the electron-dense contents were interspaced with regions of low density (Fig. 2). The granules were often positioned in proximity to the cell membrane.

Morphometric measurements distinguish quantitatively between the two types of mast cell cultures (Table 1). The CTMC-like mean cell volume (265 ± 20 μm\(^3\)) is 45% smaller than for the MMC-like cells (480 ± 40 μm\(^3\)). There are no statistically significant differences in nuclear volume (40–50 μm\(^3\)) and in the total granule volume (70–90 μm\(^3\)) between the two cell types. However, the variation in granule size is evident from the mean granule volume and unit granule volume data. The CTMC-like mean granule volume is 0.063 ± 0.009 μm\(^3\), while in the MMC-like cells the volume is about five times larger, namely 0.309 ± 0.05 μm\(^3\). Representative micrographs of granules of different sizes in CTMC-like and MMC-like cells are shown in Figs 3 and 4. In both micrographs, granules that consist of 1, 2, 3, and 4 unit granules equivalent volumes were identified by a number that indicates the unit content of the granule. As the final magnification for both micrographs is the same, the difference in granule dimensions between the two cell types is evident.

The granule area histograms (Fig. 5) demonstrate the differences in size distribution quantitatively. Granules of up to 2 μm\(^2\) may be detected in the MMC-like plate, while the largest CTMC-like mast cell granule profile is less than 1 μm\(^2\). The moving-bin analysis of granules is shown in Fig. 6. The unit granule volume is 0.011 μm\(^3\) for CTMC-like and 0.044 μm\(^3\) for MMC-like cells. We
have termed the intermodal spacing unit a unit granule. Recently, Mroz & Lechene (1986) confirmed our observation by using biochemical methods to quantify single granule content. The ratio of total granule volume to unit granule volume yields the number of unit granules in one cell. There are about three times more unit granules ($6.4 \times 10^3$) in the CTMC-like compared to the MMC-like cells ($2.1 \times 10^3$). The number of 'mean' granules in one MMC-like is about 300 while there are more than 1000 in a CTMC-like cell.

The mediator content of the mast cells in both cultures seems to be the same (Table 2). The histamine content in those cultures is $7 \text{pg cell}^{-1}$. The second vasoamine concentration in those mast cells is significantly less; in both cultures there is about $0.5 \text{ pg serotonin in one cell}$. The cells also have some chymotrypsin-like activity ($0.8 \text{unit} / 10^6 \text{ cells}$). The combination of mast cell volume and contents may be an estimate of the total vasoamine concentration in the granules. If granular histamine and serotonin were unbound in the granule, then histamine molarity would be in the range of $0.7-0.9 \text{M}$, which is more than twice the cell osmolarity ($0.3 \text{M}$). The serotonin concentration is significantly less ($0.04 \text{M}$).

We have demonstrated that mast cell cultures may be induced to secrete histamine actively at a rate faster than mast cell culture basal release of histamine (Ginsburg et al. 1978). The basal release of vasoamine in the MMC-like plates is about five to six times faster than in the CTMC-like plates (Table 3). In both cultures the basal release of vasoamine is at least 10 times faster than the basal release of chymase (in the CTMC-like almost no chymotrypsin-like secretion could be detected in the

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**Fig. 1.** Electron micrograph of connective tissue-like mast cell in culture. Secretory granules (g) are numerous with highly homogeneous electron-dense contents. The granules occupy most of the available cytoplasmic space. n, nucleus; f, fibroblast feeding of layer. $\times 12000$.

**Fig. 2.** Mucosal-like mast cell in culture. Secretory granules (g) are fewer but larger. Most of the mature granules are arrayed one to four rows deep along the cell membrane. The granular content is highly heterogeneous. n, nucleus; f, fibroblast feeding of layer. $\times 12000$. 

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medium). When mast cell cultures were induced to degranulate, two to five times more vasoamine granule equivalents (120-400 granules) were estimated to be secreted, compared to the chymase value (50 granules).

**Discussion**

The morphology of mast cells, correlated with histamine release processes, has been studied mainly in rats. In recent years, several different methods of growing mouse mast cells in tissue culture have been developed (Ginsburg, 1963; Ginsburg & Lagunoff, 1967; Nabel et al. 1981; Razin et al. 1981; Schrader, 1981). We use cell cultures grown on embryonic skin monolayers. Two entities of mature mast cells can be differentiated in these cultures (Ginsburg et al. 1982; Barrett & Metcalfe, 1984). The first arises from precursors present in the skin

### Table 1. Morphometric characterization of tissue culture mast cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>CTMC-like*</th>
<th>MMC-like†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell volume (µm³)</td>
<td>265 ± 20</td>
<td>480 ± 40</td>
</tr>
<tr>
<td>Nuclear volume (µm³)</td>
<td>40 ± 4</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>Total granule volume (µm³)</td>
<td>71 ± 10</td>
<td>93 ± 12</td>
</tr>
<tr>
<td>Unit granule volume (µm³)</td>
<td>0±11</td>
<td>0±44</td>
</tr>
<tr>
<td>Granules per cell</td>
<td>1±1×10⁵</td>
<td>0±3×10³</td>
</tr>
<tr>
<td>Unit granules per cell</td>
<td>6±4×10³</td>
<td>2±1×10³</td>
</tr>
<tr>
<td>Mean granule volume (µm³)</td>
<td>0±633 ± 0±090</td>
<td>0±309 ± 0±059</td>
</tr>
<tr>
<td>Unit granules per mean granule</td>
<td>5±75</td>
<td>7±02</td>
</tr>
<tr>
<td>Range of unit granules per granule</td>
<td>1±10</td>
<td>1±48</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean.

*24 cell profiles.
†21 cell profiles.
††Data extrapolated from Fig. 5.

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**Fig. 4.** Higher magnification of secretory granules in a MMC-like cell. Granules that consist of 1, 2, 3 and 4 unit granules are identified by the appropriate number (I-IV). The actual size of each of these granules is: I, 0.14 µm² = 0.9·V(1); II, 0.23 µm² = 1.9·V(1); III, 0.29 µm² = 2.7·V(1); IV, 0.38 µm² = 4.0·V(1) (see Fig. 6). x21000.

**Fig. 5.** Mast cell granule-area histogram. The narrow size distribution of 486 CTMC-like granules is indicated by the continuous line. The broken line represents similar data for 436 MMC-like granule area distribution.
The value of the first mode, calculated from the intermodal spacing, is indicated in each histogram.

**Fig. 6. Moving-bin histogram demonstrating the periodic multimodal distributions of 486 CTMC-like granules (A) and 436 MMC-like granules observed in electron micrographs.** The value of the first mode, calculated from the intermodal spacing, is indicated in each histogram.

**Table 2. Mast cell granule constituents**

<table>
<thead>
<tr>
<th>Mediator</th>
<th>CTMC-like</th>
<th>MMC-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (pg cell)</td>
<td>7 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Serotonin (pg cell)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Chymase (units/10^6 cells)</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Histamine ([M]/granule)</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Serotonin ([M]/granule)</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean.

*One unit equals the amount of enzyme that yields 1 µmol of ethanol in 1 min of incubation at 25°C (substrate is benzoyl-L-tyrosine ethyl ester, 5X10^-4 M).**

**Table 3. Characteristics of mast cell mediator release**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Basal release</th>
<th>Chymase (granule cell^-1 h^-1)</th>
<th>Vasoamine (granule cell^-1 per 10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTMC-like</td>
<td>1.0 ± 0.2</td>
<td>6.1 ± 1.1</td>
<td>10.0 ± 2.0</td>
</tr>
<tr>
<td>MMC-like</td>
<td>1.0 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td>10.0 ± 2.0</td>
</tr>
</tbody>
</table>

**Table 4. Characteristics of mast cell mediator release**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Chymase (granule cell^-1 h^-1)</th>
<th>Vasoamine (granule cell^-1 per 10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTMC-like</td>
<td>4.0 ± 10^4</td>
<td>1.2 ± 10^4</td>
</tr>
<tr>
<td>MMC-like</td>
<td>0.6 ± 10^4</td>
<td>0.5 ± 10^4</td>
</tr>
</tbody>
</table>

Sixteen-day-old mast cells (4X10^8) in one plate; three to five plates of each of the five different cultures. No lactic dehydrogenase activity was found in pooled media. Data are presented as mean ± standard error of the mean.

The two cell types by using quantitative morphometric methods correlated with biochemical data.

The histamine content in the mast cells grown on a monolayer is equal to that of mature mouse peritoneal mast cells (1-7 pg cell^-1); however, it is up to 100 times greater than that for cloned cells grown in conditioned media without a feeder layer (Galli et al., 1984). The serotonin content is the same in all types of mast cells grown in tissue culture (0.3-0.7 pg cell^-1), but it is 10 times more than the amount found in peritoneal mast cells in vivo (Weitzman et al., 1985). This might be due to the presence of serotonin in tissue culture media (Weitzman et al., 1985).

On a morphometric basis, the main differences between the two cell populations are the cell size and the granule-size distribution. The MMC-like cell is about twice as large and contains less granules (1/4) compared to the CTMC-like cell. A similar striking difference is found in unit granule volume. A 'unit granule' was suggested as being the basic amount produced by the Golgi complex (Hammel et al., 1983, 1987). It is the product of fusion of many progranules (Combs, 1971), so that each cell type has its own granule unit volume (Hammel et al., 1983, 1987). The facts that these two populations are morphometrically dissimilar and arise from cell culture precursors derived from mice treated differently (Ginsburg et al., 1978) suggest that we are dealing not with a process of cell hypertrophy, but with two different entities of mast cells (Ginsburg et al., 1982; Barrett & Metcalfe, 1984). Morphometric data from CTMC-like cells and those of mouse ears (Hammel et al., 1987) have some similarities. The nuclear, cytoplasmic and aggregate granule volumes of CTMC-like cells are compatible with those of mouse ear. However, the unit granule volume of CTMC-like cells is about three times larger than for mouse ear mast cells. Since in many cell systems it is well known that intragranule biochemical modifications and biophysical processes occur (Farquhar & Palade, 1981; Orci, 1985), we cannot exclude the

Monolayer. We term these connective tissue-like (or resting; Ginsburg et al., 1978) mast cells (Ginsburg et al., 1982; Barrett & Metcalfe, 1984). The second group develops from lymph node precursors and are termed by us mucosal-like mast cells (Ginsburg et al., 1982; Barrett & Metcalfe, 1984). They were previously called mast cells in the active state (Ginsburg et al., 1978), due to their high basal release of histamine and their changing ultrastructure. In this study we tried to distinguish between
may either represent two mast cell lineages or different vice versa). Thus the two populations we have observed result in the change from CTMC-like to MMC-like (or possibility that intracellular molecular modifications may regulate such a phenomenon have been reported in any mast cell.

The calculated granule histamine concentration in both cultures is above cell osmolarity; it is in the range of concentration estimated for rat mast cell granules (Hellander & Bloom, 1974). If most of the cellular histamine is located in the mast cell granule (Åborg & Uvnäs, 1968; Lagunoff, 1974), then it should be bound to the granule matrix (Hellander & Bloom, 1974) to reduce intergranule osmotic pressure, so that it will be near the physiological cytoplasmic pressure (about 300 mM). The high histamine concentration in the granule and the basal release of histamine may produce a gradient between the cell microenvironment and the blood vessels. Near the cell the histamine concentration will be at the intragranule level. The plasma value is much less (10^{-8} M; Lorenz & Doenicke, 1978; Taylor et al., 1980; Taylor & Sharpe, 1981). That range covers all the histamine concentrations needed in various experiments that were done to induce immunophysiological changes. For example, human bronchus and parenchymal strip preparations responded to histamine in the range of 10^{-7}-10^{-4} M (Vincenc et al. 1983). The influence of histamine on the oxidative metabolism of eosinophils is in the range of 10^{-9}-10^{-5} M (Pincus et al. 1982). Similar gradient response curves were found in other cellular systems such as fibroblasts (Russel et al. 1977) and vascular smooth muscle (Lorenz & Doenicke, 1978). The availability of two anemic mouse strains (Kitamura et al. 1979) that lack mast cells argues against a major mast cell contribution to the physiological regulation of homeostasis.

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References


Mast cell entities


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