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Growth of a pure population of mouse mast cells *in vitro* with conditioned medium derived from concanavalin A-stimulated splenocytes

(mast cell development/histamine content/IgE receptors)

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ABSTRACT **A pure population of mast cells was obtained after 14 days of culturing mouse bone marrow cells in the presence of medium derived from concanavalin A-stimulated mouse spleen cells. The cells were characterized as mast cells by their morphologic appearance and histologic staining, by their histamine content (450 ng per 10^6 cells) and by the demonstration of IgE receptors on their surface (150,000–440,000 receptor sites per cell). The histamine content and the number of IgE receptors remained constant for at least 4 wk of culture. These mast cells could be passively sensitized to mice hybridoma IgE. They then released 43% of their histamine content upon incubation with anti-mouse hybridoma IgE.**

Much recent interest has centered on mast cells as primary mediators of allergic reactions. Granules of the mast cells contain the body's major source of histamine, serotonin, bradykinin, slow reacting substance of anaphylaxis, and other mediators of allergic response (1). A persistent problem that one faces in studying mast cell function has been the extreme difficulty experienced in obtaining large numbers of purified cells uncontaminated by other cell types.

One approach to this problem would be to develop tissue culture methods for growing mast cells. Ginsburg and Sachs (2) enriched mast cell populations by seeding mouse thymus cells on mouse embryonic fibroblasts. Ishizaka *et al.* (3, 4) obtained rat mast cells by long-term culture of rat thymus cells on fibroblast monolayers. However, their methods still have the potential problem of contamination of the mast cell population with fibroblasts. In this report we describe successful growth of mast cells from bone marrow precursors in an *in vitro* system. The principle of our method involves treatment of mouse bone marrow cells with conditioned medium obtained from mouse spleen cells treated with concanavalin A (Con A). The stem cells then appear to develop into mast cells after 2 wk in culture.

MATERIALS AND METHODS

Animals. Bone marrow cells obtained from femurs of BALB/c mice, and spleen cells were obtained from C3H and C57Bl/6J mice. All mice used were 2-mo-old males obtained from Memorial Sloan-Kettering Cancer Center breeding colony.

Culture Medium. Cells were cultured in a 1:1 (vol/vol) mixture of enriched medium [RPMI-1640 supplemented with 10% (vol/vol) fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 0.1 mM nonessential amino acids] and conditioned medium (see below). Penicillin and streptomycin (each 100 units/ml) were included in the culture medium, which was pH 7.1–7.3. All reagents were purchased from GIBCO.

Conditioned Medium. Spleen cells from C57Bl/6J and C3H mice (0.5×10^6 cells per ml from each strain) were cultured together in enriched medium (see above) containing Con A (2 μg/ml) in 75-cm^2 tissue culture flasks (Falcon). The cells were incubated at 37°C in humidified 5% CO_2/95% air.

After incubation for 45 hr, the medium was collected, centrifuged for 20 min at $1000 \times g$, filtered through a 0.45-μm Millipore filter (Nalge Sybron Corp., Rochester, NY), and used as conditioned medium.

IgE and Anti-IgE. Mouse hybridoma IgE with specific binding to trinitrophenyl was a gift of D. Katz (Scripps Clinic and Research Foundation, La Jolla, CA). Goat anti-mouse hybridoma IgE (anti-IgE) was a gift of H. Metzger (National Institutes of Health).

Cell Cultures. Bone marrow cells (4×10^6) were suspended in 40 ml of cultured medium (see above) in the 75-cm^2 tissue culture flasks and maintained at 37°C in humidified 5% CO_2/95% air. The cells were washed on day 7 of culture and recultured in fresh medium for an additional 7 days.

Microscopic Examination. Cells for histological examination were resuspended in a small volume of fetal calf serum and stained with 0.1% toluidine blue in 50% (vol/vol) ethanol (pH 3.0). The number of viable cells was counted by trypan blue dye exclusion. Macrophages were identified by nonspecific esterase staining (5).

Iodination of IgE with ^{125}I. IgE protein (1 mg) was labeled with ^{125}I (2 mCi; 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) by the method of Greenwood *et al.* (6). To see the specific binding function of the protein after the labeling process, the proportion of ^{125}I-labeled IgE (^{125}I-IgE) binding specifically to rat basophilic leukemia cells was measured and found to be 60%.

Measurement of IgE Binding to Cultured Mast Cells. The binding of ^{125}I-IgE to cultured mast cells was measured by the method of Kulczycki *et al.* (7). In all of the tests the concentration of ^{125}I-IgE for incubation was expressed in terms of the protein bindable to rat basophilic leukemia cells. Two aliquots of a cell suspension containing 1×10^6 mast cells in 0.5 ml of culture medium were incubated with 1 μg of ^{125}I-IgE. A control tube containing a 100-fold excess of unlabeled IgE mixed with the cell suspension prior to the addition of the ^{125}I-IgE was prepared for every sample. After incubation at 37°C, the two samples were pooled, and three sets of 0.2 ml were layered on 0.2 ml of inactivated fetal calf serum in conical microtubes,

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Abbreviations: Con A, concanavalin A; anti-IgE, goat anti-mouse hybridoma IgE.

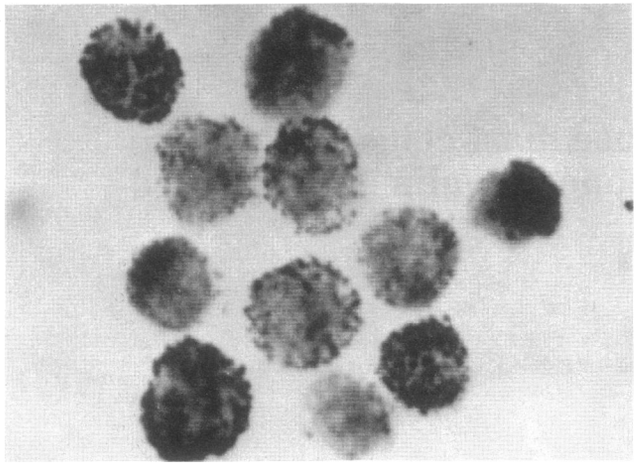

FIG. 1. Mast cells obtained from bone marrow culture in the presence of Con A-treated spleen cell conditioned media. Note the pure mast cell culture after 14 days of incubation. (Toluidine blue; ×1000.)

which were then centrifuged for 1 min in a Beckman Microfuge. Radioactivity in the cell pellets, which contained 30% of the initial cells layered on the fetal calf serum, was assayed in an automatic gamma counter. Variation of radioactivity in triplicate samples was less than 7%. The radioactivity in cell pellets from a control tube that contained unlabeled IgE and ^{125}I-IgE was subtracted from the count of the cells that had been incubated with the same concentration of ^{125}I-IgE alone.

The number of IgE molecules bound per mast cell was calculated from the net value, based on the assumption that the molecular weight of the protein is 190,000 (8).

Assay of Histamine. One million cells were washed three times in phosphate-buffered saline and then homogenized in 0.7 ml of distilled water. After centrifugation at 1000 × *g* for 15 min, the supernatant was stored frozen until assayed for histamine by the spectrophotofluorometric technique of Anton *et al.* (9).

Histamine Release from Cultured Mast Cells. Cultured mast cells (1×10^6 cells in 0.5 ml of medium) were passively sensitized with 1 μg of IgE for 60 min at 37°C. The cells then were washed three times with saline, suspended in 0.5 ml of saline containing 1 μg of anti-IgE, and incubated at 37°C. A control was made in the same way but without IgE or anti-IgE. After a 30-min incubation, the cell suspensions were centrifuged, and the histamine content in the supernatants was measured. The percentage of histamine released was calculated by using the formula:

$$\% \text{ release} = 100 \times \frac{\text{supernatant histamine (experimental} - \text{control)}}{\text{total cellular histamine}}$$

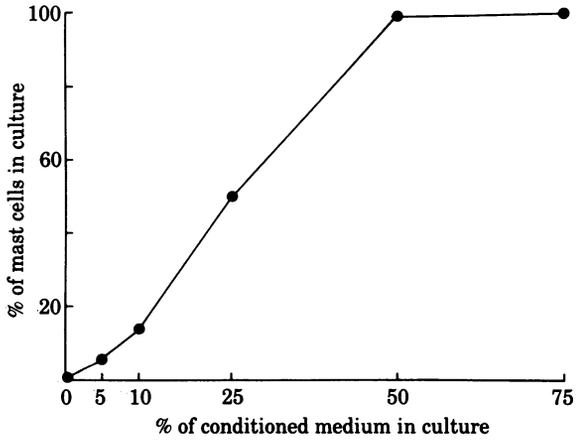

FIG. 2. Dose–response curve of mast cells in culture induced by different concentrations of conditioned medium.

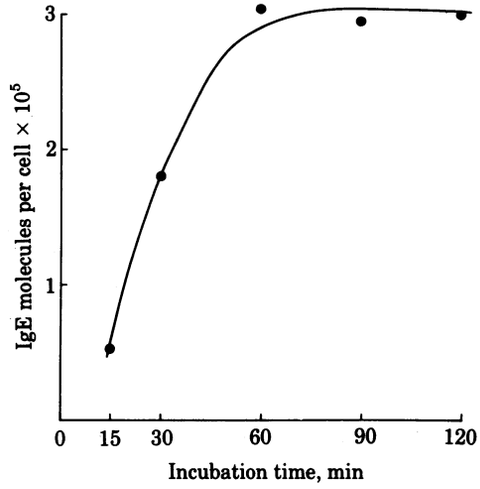

FIG. 3. Kinetics of IgE binding with cultured mast cells. Mast cells were obtained after a 14-day culture, and the cell suspension (1×10^6 cells per 0.5 ml) was incubated in the presence of 1 μg of ^{125}I-IgE at 37°C. Aliquots of the cell suspension were taken at various intervals and centrifuged to determine cell-bound IgE. Variation among triplicate tubes in such experiments was within ±7%.

RESULTS

Seven days after seeding the mouse bone marrow cells in culture, the cytoplasm of approximately 20% of the cells began to show definite metachromasia on staining with toluidine blue, which suggests that these cells are mast cells or mastoblasts. After 14 days, most cells (98%) floated free in the culture medium; they were round in shape but quite heterogeneous with respect to size (10–20 μm) and density of metachromatic granules. In almost all of the cells, the cytoplasm was filled with metachromatic granules, and the nucleus was obscured by the granules (Fig. 1). A small number of macrophages (1–2%), lymphocytes (0.5–1%), and unidentified cells (0.1%) were present in the cultures, even when incubated for an additional 14 days. Dose–response analysis of the influence of conditioned medium was made to find the optimal concentration needed to obtain the population most enriched in mast cells. A pure population of mast cells was achieved in the presence of 50% or more conditioned medium in the cultures (Fig. 2). The yield was 4 million mast cells per culture and was obtained after a 2-wk incubation. To confirm the presence of IgE receptors and to calculate the number of receptors, mast cells that had been cultured for 14 and 28 days were exposed to mouse hybridoma ^{125}I-IgE. The kinetics of the binding reaction was studied by measuring cell-bound IgE in triplicate samples of cell suspensions at various times after exposure to the ^{125}I-IgE. A representative result for

Table 1. Histamine content in cultured mast cells

Period of culture, days	Histamine content, ng per 10^6 cells
14	449 ± 89*
21	461 ± 94
28	473 ± 76

Bone marrow cells (0.1×10^6 cells per ml) from BALB/c male mice were maintained in the described culture medium. Every 7 days the cells were recultured in fresh medium.
* Mean of six cultures ± SEM.

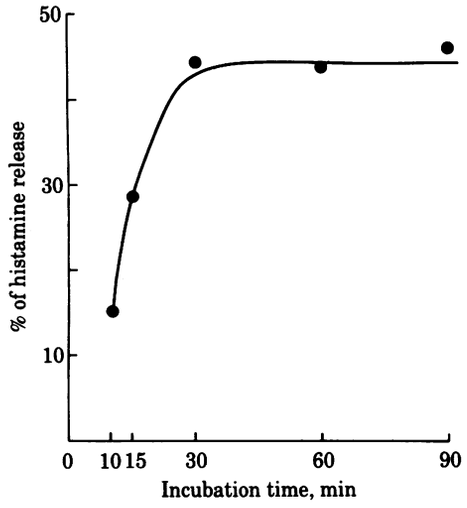

FIG. 4. Kinetics of anti-IgE-induced histamine release from mast cells. Cultured mast cells (1×10^6) were sensitized with 1 μg of IgE for 60 min and, after washing, were treated with 1 μg of anti-IgE. Cells were pooled from four different cultures after 14 days of incubation.

the kinetics of binding of IgE at 1 μg/0.5 ml of culture (cells cultured for 14 days) is shown in Fig. 3.

An equilibrium was reached between bound and unbound IgE after 60 min. In repeated experiments of the same design, the cells obtained from the 14-day and 28-day cultures gave similar binding curves.

The maximum number of IgE molecules bound per cell ranged from 1.5 to 4.4×10^5, and the number of receptors remained constant through the longer culture period to 28 days.

The mean histamine content of the cultured mast cells was 0.45 μg per 10^6 cells after 14–28 days of culture (Table 1).

Because the cells bear receptors for IgE and contain histamine, it was expected that cells sensitized by IgE might be triggered to release histamine after treatment with anti-IgE antiserum. As can be seen from Fig. 4, mast cells that were treated with 1 μg of IgE, released 44% of their total histamine after 30 min of incubation with 1 μg of anti-IgE antiserum.

DISCUSSION

The origin of tissue mast cells has been a subject of controversy for nearly a century since the first description of the cells by Ehrlich (10, 11). Evidence supports the origin of mast cells from connective tissue cells (10–13) and lymphoid cells (2–4, 14, 15). Burnet has presented the hypothesis that mast cells or some subpopulation of mast cells are postmitotic derivatives of thymus-derived (T) lymphocytes (16).

Recently, Kitamura *et al.* (17–21), using mice with a genetic absence of mast cells, followed the increase of mast cell numbers in various organs after injection of hematopoietic and lymphopoietic cells from congenic mice. Their results suggested that the main source of mast cell precursors was hematopoietic rather than lymphopoietic.

The present report does not discriminate between hematopoietic or lymphopoietic precursors. The development of mast cells from bone marrow cells incubated with medium derived from Con A-stimulated spleen cells suggests, however, that a factor, or factors, released from T cells can participate in the differentiation of mast cells.

Concerning IgE receptors, our data show that when cells were incubated at 37°C with 1 μg or more (data not shown) of ^{125}I-IgE per 10^6 cells, the cell-bound IgE reached a maximum within 60 min (Fig. 3). Because the number and viability of mast cells did not change during the 60-min incubation, it appears that the binding of IgE with the cells reached equilibrium within 60 min. The results indicate that the average number of total receptors per cell is in the order of 1.5 to 4.4×10^5. The number is close to that for free receptors for IgE on rat peritoneal mast cells (22).

The histamine content in the cultured mast cells (0.45 μg per 10^6 cells) was somewhat lower than that reported for mouse mast cells (15) (1–7 μg per 10^6 cells). The lower histamine content of the cultured cells may be attributable to the presence of Con A in the culture medium because Con A is known to trigger degranulation of mast cells (23). It is reasonable to assume that the difference in the histamine assays used also may contribute to variation in the results.

The release of histamine present in the cells by anti-IgE indicates that the surface IgE receptors of the cells are functional. The present study indicates that mast cells obtained during culture from hematopoietic tissue of the mouse have typical characteristics of mast cells and should be suitable for the analysis of the biochemistry of mast cells and the regulation of mast cell function.

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