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Mast Cell Clones: A Model for the Analysis of Cellular Maturation

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ABSTRACT Cloned mouse mast cells resemble, by ultrastructure, immature mast cells observed in vivo. These mast cell clones can be grown in the absence of any other cells, facilitating direct investigations of their biochemistry and function. We find that cloned mast cells express plasma membrane receptors (Fc\(_R\)) that bind mouse IgE with an equilibrium constant (K\(_A\)) similar to that of normal mouse peritoneal mast cells. In addition, cloned mast cells do not display detectable la antigens and cannot enhance Ig secretion when added to lymphocyte cultures or mediate natural killer lysis. In the presence of 1 mM sodium butyrate, cloned mast cells stop dividing and acquire abundant electron-dense cytoplasmic granules similar to those of mature mast cells. Their histamine content increases concomitant with cytoplasmic granule maturation and may exceed that of untreated mast cells by 50-fold. Unlike peritoneal mast cells, cloned mast cells incorporate \(^{35}S\)O\(_4\) into chondroitin sulfates rather than heparin. These findings demonstrate that, unlike fully differentiated mouse peritoneal mast cells, cloned immature mouse mast cells contain no heparin and low levels of histamine. In addition, they establish that high-affinity Fc\(_R\) are expressed early in mast cell maturation, well before completion of cytoplasmic granule synthesis and mediator storage.

We have previously reported methods to clone mast cells with normal karyotypes from mouse hematopoietic tissue in vitro (30). Our cloned mast cells contain less histamine than normal mouse peritoneal mast cells and resemble immature mast cells by morphology. Others have described similar findings with uncloned cells (22, 32, 38, 42, 43, 51, 53). Although some mast cells synthesize heparin when fully differentiated (23, 54), certain mast cell tumors are devoid of heparin and incorporate \(^{35}S\)O\(_4\) into chondroitin sulfate exclusively (18). Histochemical evidence suggests that normal immature connective tissue mast cells (6) and the mast cells in the intestinal lamina propria of rodents (25, 50) also may synthesize glycosaminoglycans other than heparin, but this notion has not been confirmed directly.

In this report, we show that cloned mast cells closely resemble, by ultrastructure, immature mast cells found in vivo. Cloned mast cells incorporate \(^{35}S\)O\(_4\) preferentially into chondroitin sulfates, confirming histochemical evidence that immature mast cells contain little heparin. In addition, cloned mast cell proliferation, cytoplasmic granule synthesis, and mediator storage can be modulated in vitro, permitting direct analysis of mast cell maturation.

MATERIALS AND METHODS

Antisera

Lyt-1.2 and Lyt-2.2 antisera, prepared as described (45), were kindly donated by F. W. Shen1; monoclonal antibody against Thy-1.2 (mc-a-Thy-1.2) was donated by Ed Clark2; and mc-a-Lyt-1 and mc-a-Lyt-2 were gifts from J. 1 Memorial Sloan-Kettering Cancer Center, New York, NY
2 University of Washington, Seattle, WA
Ladetton and J. Herzenberg. Mast cells were examined for IgE antibodies using AT 4-ATL alloantisera (16) or mc-a-Ia (10-3.6, reference 35) generously provided by John Freid and J. M. Kupnna.

**Mast Cells**

Procedures for the isolation, growth, and cloning of mast cells from mouse hematopoietic tissue have been previously described (30). Briefly, cells from the livers of 13-d-old mouse fetuses from one A/J female mated with one C57BL/6 male were incubated in Dulbecco's modified Eagle's medium (DME) with 4% heat-inactivated fetal calf serum (FCS), 5 X 10^{-5} M 2-mercaptoethanol and 2 mM glutamine (DME-FCs) conditioned by concanavalin A (Con A)-activated BALB/c spleen cells (Con A-CM). 10 d later, cells were distributed at limiting dilutions in wells containing irradiated (2,000 R) syngeneic bone marrow cells.

Colonies appeared at 10-14 d with a cloning efficiency of ~10%. All colonies grown under these conditions were composed of cells that resembled mast cells by light microscopy because of their prominent metachromatic cytoplasmic granules. All colonies expressed the Ly 5.1 Thy 1.1 12' phenotype of surface membrane glycoproteins. No growth of mast cells was observed from preparations of irradiated bone marrow cells. Mast cell lines were cloned by micromanipulation (30) and have been designated CLMC/1-14. The cloned cells have normal karyotypes and can be cultivated in large numbers (>10^6) with doubling times in Con A-CM of 36-48 h. Clones are stable for at least 24 mo, either alone or with an irradiated spleen cell feeder layer, although doubling times of cells maintained in Con A-CM may progressively increase. Using similar conditions, mast cell colonies also may be isolated from adult mouse spleen or bone marrow cells. Treatment of spleen or marrow cells with antiserum to Thy 1.2 and complement facilitates growth of mast cell colonies, which by electron microscopy contain >95% (usually >99%) mast cells after 4-6 wk of culture. We have successfully maintained our cultures with medium conditioned by Con A-stimulated splenocytes (Con A-CM), cloned Ly 12' inducer T lymphocytes (31), or WEHI-3 cells (32). All the cells described in this report were maintained in Con A-CM unless otherwise specified. The active growth factor in the supernatant of cloned Ly 12' inducer T lymphocytes has been purified to apparent homogeneity. It has a pl of ~6.0 and is of 45,000 (30) (Nabel et al., manuscript in preparation).

**Effect of Sodium Butyrate on Mast Cells**

Mori et al. reported that p-815 murine mastocyteoma cells, ordinarily devoid of cytoplasmic granules, underwent granulogenesis when exposed to sodium butyrate over 3-4 d in vitro (26). Butyrate-induced granulogenesis was accompanied by a prolongation of mastocytoma doubling time and an increase in cell size, changes that suggested a differentiation effect. We evaluated whether sodium butyrate might exert similar effects on our mast cell clones. In these experiments, mast cells (1 X 10^6 cell/ml) were cultured in Con A-CM containing 10-20% medium conditioned by Ly 12' inducer T lymphocytes (31) supplemented with various concentrations of sodium butyrate (Pfaltz and Bauer, Stamford, CT) for 4 d. In some experiments, 1-mi aliquots of cells were cultured in 24-well plates (Costar, Data Packaging, Cambridge, MA). In other experiments, 5- to 10-mi aliquots of cells were seeded into 25 cm^2 tissue culture flasks (Falcon Labware, Oxnard, CA). The medium, including butyrate, was replaced after 48 h of culture. After 4 d, cells were recovered for electron microscopy and for determination of cell number and histamine content.

**Transmission Electron Microscopy (TEM) and Autoradiography**

Cells were fixed in suspension for 1 h at room temperature in a mixture of paraformaldehyde and glutaraldehyde (9), were washed twice in 0.1 M sodium cacodylate buffer, pH 7.4, and were centrifuged through soft agar in a microfuge. The embedded pellets were then processed for electron microscopy either by the osmium-potassium ferrocyanide (OPF) or osmium-collidine uranyl en bloc (Oaub) techniques (9). They were then dehydrated in a graded series of alcohols and infiltrated and embedded in a propylene oxide-Epon sequence. For thin Epon sections were cut with an LKB 5-12 ultratome (LKB Instruments, Rockville, MD), were placed on copper grids and looped with llford L-4 emulsion. Sections were examined in a Philips 400 electron microscope.

**Histamine Determination**

Histamine was routinely measured fluorometrically with an Autoanalyzer II

**IgE Receptors**

The methods used to enumerate and characterize the binding properties of FcER have been described in detail (17, 48). Briefly, washed cells were re sus- pended in MEM or RPMI-1640 medium with 0.01 M EDTA and 10% FCS. pH 7.4, at 1 X 10^6 cells/ml. Cells at 0.4 ml received 0.05 ml of additional medium or medium containing 10 mg/ml noniodinated, affinity purified mouse monocy- clonal IgE antibody from hybridoma H 1 DNP-c-26 (20). After 15 min at 37°C, 125-IgE, 80% bindable to the rat basophilic leukemia cell line (16), at 100 μg/ml, was added in 0.05 ml for 90 min at 37°C with constant shaking. Duplicate determinations of binding of 125-IgE to cells were made by layering 0.2 ml aliquots of cells onto 0.2 ml of heat-inactivated FCS and centrifuging the tubes in a microfuge. The radioactivity in the cell pellets was measured in a gamma counter and the number of 125-IgE molecules bound per cell calculated according to the formula:

\[
\text{Mean cpm in cells without excess noniodinated IgE} = \frac{\text{cpm of 1 ng 125-IgE}}{\text{cpm of 1 ng 125-IgE}} \cdot \text{cell number}
\]

The forward rate constant (k_f) of IgE binding to MC/9 was measured by incubating prewarmed cells (1.3 X 10^6/ml) with 3 μg/ml of 125-I-labeled mouse monoclonal IgE at 37°C in Dulbecco's modified Eagle's medium containing 10% FCS and 0.01 M EDTA with constant shaking. The binding reaction was stopped at various intervals by adding a 133-fold excess of unlabelled IgE. Cells in control tubes were incubated with a 133-fold excess of unlabelled IgE before addition of 125-IgE. Nonspecific binding of radioactivity to control cells was subtracted from experimental tubes to determine specific binding. The number of IgE receptors per cell was determined separately by incubating the cells with 10 μg/ml of 125-IgE for 90 min as described above. To measure the dissociation rate (k_d) of IgE bound to MC/9, cells (1.3 X 10^6/ml) were incubated with 3 μg/ml of 125-I-labeled mouse monoclonal IgE at 37°C for 1 h in Ly 12' T lymphocyte-conditioned Dulbecco's modified Eagle's medium as described above. Cells were then divided into two tubes, centrifuged, and resuspended in fresh medium. A 133-fold molar excess of unlabelled IgE or an equal volume of culture medium was added to each tube at t_0. The cells were incubated at 37°C with constant slow rotation and residual cell-bound 125-IgE was determined at various intervals.

**Synthesis of Sulfated Glicosaminoglycans**

Procedures reported previously for the characterization of guinea pig basophil glycosaminoglycans were used with minor modifications (36).

**Labeling of mast cells with 35S-Sulfate**: Briefly, cloned mast cells (CLMC/9 X 10^6 cells in 5 ml DME 4% FCS containing 25% (v/v) Con A-CM) were labeled with 1 mCi sodium 35S-Sulfate (New England Nuclear, NEX-041) for 4-21 h at 37°C. After labeling, cells were washed three times in DME, an uncloned population was taken for TEM (see above). Then the medium was replaced with DME containing 0.5-10 ng/ml range (46). The histamine content of some samples was also determined before and after treatment with diamine oxidase by the isotropic- enzymatic method (44), with similar results.
highly purified mucopolysaccharidases of established substrate specificities.

Thawed aliquots of frozen 35S-labeled cells were heated in boiling water for 5 min to inactivate endogenous degradative enzymes (34), and then exhaustively digested with pronase P, which solubilized >90% of incorporated [35S]sulfate. Glycosaminoglycans were chromatographed on DEAE-Sephadex A-25 (0.8 × 6.9 cm column) previously equilibrated with 0.15 M NaCl, 0.01 M Tris, pH 7.5. Fractions were eluted in 0.5-ml volumes with a two-stage linear gradient of NaCl (0.15–2.0 M; 2.0–4.0 M) (36). Glycosaminoglycans were then incubated with specific mucopolysaccharidases and the products were chromatographed on Sephadex G-25 (0.6 × 24.5 cm column) equilibrated with 0.15 M NaCl, 0.01 M Tris, pH 7.5. Flow rate was 8 ml/h and fraction volume was 0.3 ml.

Chondroitin AC and ABC lyases (Miles-Yeda, Elkhartd, Indiana) (41), and heparinase (purified from Flavobacterium [19]) were employed according to published methods. National Institutes of Health glycosaminoglycan reference standards were generously provided by J. A. Cifonelli, University of Chicago, Chicago, IL.

Nonlabeled glycosaminoglycans and degradation products were detected spectrophotometrically by the carbazole method of Bitter and Muir (3) with glucuronolactone as the standard.

**Evaluation of Mast Cells for Accessory Cell and Natural Killer Function**

Mast cell clones were examined as previously described for the ability to enhance Ig secretion (31) or to mediate NK lysis (29).

**RESULTS**

**Ultrastructure**

By TEM, clones MC/5 and MC/9 (Figs. 1 and 2B) consisted entirely of mast cells exhibiting various degrees of maturity, as judged both by nuclear and cytoplasmic criteria. Uncloned mast cell populations (Fig. 2A, C, and D) contained >99% mast cells. Mast cell nuclei were round with occasional nuclear membrane indentations, dispersed nuclear chromatin, and occasional small nucleoli. Rare cells exhibited bilobed nuclei. The plasma membrane displayed uniformly distributed, narrow short processes and folds. The cells' most prominent ultrastructural feature was cytoplasmic granules similar to those described in immature mast cells in vivo (4-6, 24) and in uncloned populations in vitro (5, 13, 51). These appeared to develop from large empty vacuoles in the active Golgi area (Fig. 3A). Vacuoles filled progressively with small vesicles (Figs. 2 and 3B) and then acquired dense matrix, some in the form of progranulelike (4-6, 24) structures (Figs. 2D, 3A and C). All cultures or clones contained at least a few very immature-appearing cells that were devoid of dense granule matrix.

**FIGURE 1** Typical immature mast cells from clone MC/5 derived from fetal liver. These mononuclear cells exhibit numerous immature granules that appear as large, membrane bounded, focally electron lucent structures containing small vesicles and/or dense progranules. Cells also contain mitochondria, cytoplasmic vesicles, and a prominent Golgi area. The cell surfaces are covered by short, narrow, uniform processes. Bar, 5 µm. × 2,000.
FIGURE 2 Mast cells from mouse bone marrow (line 3: A and C) fetal liver (MC/9: B), and spleen (line 1: D). The mast cells from bone marrow and spleen were maintained in WEHI-3 conditioned medium; MC/9 mast cells were maintained in Con A-CM. All cells contain a single eccentric nucleus and display short, narrow surface processes. The most immature cells (A) had cytoplasmic vacuoles containing only a few vesicles. In more mature cells (B, C), the cytoplasmic vacuoles acquired the features of immature mast cell granules. These contained variable proportions of fine, granular material; multiple small, round, extremely dense, progranules, and numerous small vesicles. The most mature cells (D) contained many granules rich in progranules. Although all populations contained cells of various maturity, line 3 appeared most immature and was comprised predominantly of cells like that shown in A. Vesicles and larger vacuoles appeared to originate in the active Golgi areas (indicated by arrows in C). Bars, 4 μm. A, × 9,000; B, × 5,500; C, × 6,500; D, × 8,500.

and some were comprised predominantly of such cells (Fig. 2A). The cytoplasm also contained mitochondria and small vesicles. The cells had no cytoplasmic glycogen particles or aggregates, which are a feature of basophilic leukocytes and other granulocytes but not of mast cells.

**Receptors for IgE and Histamine Content**

Mast cell cultures (>99% mast cells by TEM) from bone marrow or spleen maintained with WEHI-3 conditioned medium, and cloned mast cells (MC/9) from fetal liver maintained in Con A-CM or in 10% Con A-CM conditioned by Ly 1*2* inducer T lymphocytes, expressed approximately half as many plasma membrane receptors for IgE as normal peritoneal mast cells (Table I). The MC/9 Fc,R bound mouse IgE with an equilibrium constant at 37°C similar to that of mature mouse peritoneal mast cells (Table II). The histamine content of our mast cells was much less than that of mature peritoneal mast cells.
Effect of Sodium Butyrate on Mast Cell Proliferation, Ultrastructure, and Histamine Content

Cloned mast cells (MC/9) were grown in Ly 1⁺2⁻ T lymphocyte-conditioned medium with or without 1 mM sodium butyrate. After 4 d, mast cells cultured without butyrate had increased sevenfold ($8 \times 10^6$ to $5.4 \times 10^6$), contained 0.03 pg histamine/cell, and had a viability by trypan blue exclusion of 96%. By ultrastructure, these cells appeared very immature, with a few large cytoplasmic vacuoles that contained occasional small vesicles but no electron-dense granule matrix (Fig. 5A). By contrast, mast cells in 1 mM sodium butyrate ceased dividing, remained viable (87–96% viable cells by trypan blue exclusion) and developed numerous cytoplasmic granules containing abundant electron-dense matrix material (Fig. 5B) similar to that of mature mast cells in vivo. Furthermore, butyrate-treated cells stored 50-fold more histamine (1.4 pg/cell) than untreated controls.

We investigated the effects of sodium butyrate over an ~100-fold dose range (Table III). While mast cells grown with 1 mM butyrate exhibited profoundly diminished proliferation and markedly increased histamine content, mast cells grown in the presence of 0.3 mM butyrate were minimally affected. Sodium butyrate was toxic to cloned mast cells at concentrations ≥2 mM.
Sulfated Glycosaminoglycan Synthesis

Cellular Localization of Incorporated Sodium $^{35}$Sulfate: TEM autoradiography localized silver grains to the mast cells' cytoplasmic granules (Fig. 6). Although the majority of cells were labeled, the number of grains/cell varied widely. In general, the mast cells with the

**Table I**

| Cell Type                        | Histamine (pg/cell) | $F_{c}R \times 10^{-4}$/cell |
|----------------------------------|---------------------|------------------------------|
| Normal mouse peritoneal mast cells |                     |                              |
| CBA/J                            | 9.4-10.4            | 32.1 ± 7.3                   |
| BALB/c                           | 5.2-10.6            | 23.0 ± 5.9                   |
| MC/9 mast cell clone (fetal liver) | 0.1-0.4             | 12.5                         |
| Mast cell line 1 (spleen)        | 0.1-0.4             | 10.4                         |
| Mast cell line 2 (bone marrow)   | 0.1-0.7             | 7.0                          |
| Ly $1^{+}2^{-}/9$ (inducer T lymphocytes) | 0                 | 0                            |
| YAC-1 (mouse lymphoma)           | ND                  | 0                            |

* Normal mouse peritoneal mast cells were purified to ≈88% on a metrizamide gradient as described in reference 48. Cultured cells were maintained as described in the text.

§ Range of 3 to 12 determinations performed over a period of weeks (spleen and bone marrow lines maintained in WEHI-3 conditioned medium) to a year (MC/9 clones maintained in Con A conditioned medium). Replicate simultaneous determinations of histamine content varied by <10%.

¶ Values for CBA/J or BALB/c mast cells are M ± SEM of five or six duplicate determinations, respectively. Values for cultured cells are means of duplicate determinations.

**Figure 4** Forward rate constant ($k_1$) of IgE binding to MC/9 (left). Dissociation rate ($k_1$) of IgE bound to MC/9 (right). The data were normalized by taking the amount of IgE bound at $t_0 = 1.0$. Each point is the mean of duplicate measurements.

**Table II**

| Cell Type                        | $k_1$ | $k_1$ | $K_a$ |
|----------------------------------|-------|-------|-------|
| Normal mouse peritoneal mast cells |       | 0.79  | 1.04  |
| Rat peritoneal mast cells (49)    | 1.65  | 0.21  | 7.86  |

* Association ($k_1$), dissociation ($k_2$), and equilibrium ($K_a$) constants were determined as described in Materials and Methods.

**Figure 5** Cloned mast cells derived from fetal liver (MC/9) grown in Con A-CM supplemented with 15% Ly $1^{+}2^{-}$ inducer T lymphocyte-conditioned medium. The cell in A, cultured without sodium butyrate, contains cytoplasmic vacuoles with a few small vesicles but no electron-dense granule matrix. The cell in B, cultured for 4 d with 1 mM sodium butyrate, has numerous cytoplasmic granules rich in electron-dense granule matrix. Bars, 2 μm. x 8,000.
most mature granule morphology were most heavily labeled. **CHARACTERIZATION OF 35S-GLYCOSAMINOGLYCANS.** Assessment of the relative charge of the cloned mast cell (MC/9) 35S-glycosaminoglycans, as determined by chromatography on DEAE cellulose, revealed a macromolecular peak which appeared coincident with the chondroitin sulfate reference standard (Fig. 7). Chromatography on Sepharose 4B established that the 35S-glycosaminoglycans had an average $M_r$ of 20,000 daltons (Fig. 8). These 35S-macromolecules, and the chondroitin sulfate standard, were susceptible to complete hydrolysis to disaccharides by chondroitin ABC or AC lyase, but were insensitive to heparinase (Fig. 8), indicating that they represented chondroitin ABC or AC lyase-sensitive chondroitin-sulfates without detectable heparin or dermatan sulfate (chondroitin sulfate B). Similar sulfated glycosaminoglycans were synthesized by mast cells incubated with $^{35}$SO$_4$ for 4, 12 (data not shown), and 21 h. By single dimensional electrophoresis (49), these molecules had charge characteristics similar to those of the chondroitins. Mast cells from spleen (line 1) or bone marrow (line 2 and line 3) also incorporated $^{35}$SO$_4$ into chondroitin sulfates, not heparin. MC/9 or bone marrow line 3 mast cells, cultured for 4 d with 1 mM sodium butyrate, synthesized 35S-glycosaminoglycans identical to those of mast cells grown without butyrate, as judged by DEAE cellulose chromatography and susceptibility to enzymatic degradation. Although it did not induce synthesis of heparin, butyrate augmented mast cell storage of chondroitin sulfate. For example, butyrate-treated bone marrow mast cells (line 3) contained 81% more $^{35}$S-chondroitin sulfates after an 18-h incubation with $^{35}$SO$_4$ than untreated controls. Chromatographic (DEAE) analysis of mast cell culture medium failed to detect secreted 35S-macromolecules.

In contrast to cloned mast cells, uncloned peritoneal mast cells incorporated $^{35}$SO$_4$ predominantly into heparin. These glycosaminoglycans eluted from DEAE cellulose as a single peak between 1.0 and 2.0 M NaCl. These 35S-macromolecules, and the heparin reference standard, were susceptible to hydrolysis to disaccharides by heparinase but were insensitive to chondroitin ABC or AC lyase.

**TABLE III**

| Sodium butyrate concentration (mM) | Doubling time (h) | Histamine content (pg/cell) |
|-----------------------------------|-------------------|-----------------------------|
| 0                                 | 20.4              | 0.07                        |
| 0.01                              | 20.5              | 0.06                        |
| 0.04                              | 20.5              | 0.06                        |
| 0.11                              | 21.5              | 0.06                        |
| 0.33                              | 25.8              | 0.08                        |
| 1.00                              | 132.0             | 0.50                        |

Cloned mast cells (MC/9) were cultured for 4 d with or without sodium butyrate as described in the text.

**FIGURE 7** DEAE-Sephadex A-25 column (0.8 X 6.9 cm) chromatography of pronase-digested cloned mast cells (MC/9) labeled with $^{35}$S-sulfate for 4 or 21 h (see text). The bar indicates the elution pattern of the chondroitin sulfate reference standard.

**FIGURE 8** Sepharose 4B column (0.6 X 110 cm) chromatography of pronase-digested cloned mast cells (MC/9) labeled with $^{35}$S-sulfate for 21 h (see text). The column was calibrated with Dextran Blue, National Institutes of Health reference standard glycosaminoglycans (Ch-6-S, chondroitin-6-sulfate; HS, heparan sulfate; Hep, heparin), and NaCl.
cytes. Data from a typical experiment are shown in Table IV. Unable to alter Ig secretion when added to enriched B lymphocytes, these clones contained cytoplasmic granules with immature mast cells found in vivo (4-6, 24). Like immature mast cells, these clones expressed nearly as many Fc×R as mature peritoneal mast cells. Uncloned rat mast cells also express Fc×R when their histamine content is very low (14), supporting the notion that incorporation of Fc×R into the plasma membrane represents an early event in the program of mast cell differentiation. While some mast cell-like lines reportedly bear Ia antigens (42, 51), mouse (7, 52) and rat (28) mature peritoneal mast cells do not express Ia antigens detectable by alloantisera. We have not detected Ia antigens on the surface membranes of cloned mast cells by immunofluorescence with monoclonal reagents. Although expression may vary according to culture conditions, degree of differentiation (47), or source of antisera, we have not observed Ia expression at any time with the cells described in this report.

The relationship of mast cells to basophils has been widely discussed (12). Basophils are bone marrow-derived granulocytes that normally circulate in the blood and, unlike mast cells, are not ordinarily found in connective tissues. Although basophils and mast cells express certain biochemical and functional similarities, they are not identical. Electron microscopy can be used to distinguish basophils from mast cells in all mammalian species, including the mouse (8, 10). Ultrastructurally, our cloned mast cells resemble immature mast cells found in vivo, not mouse basophils. Mouse basophils lack the uniform surface processes of normal and cloned mast cells, have a more central and polylobed nucleus, and have cytoplasmic granules that are fewer, larger, and different in ultrastructure than those of mast cells (10). Mouse tissue mast cells and granulocytes may share a common hematopoietic precursor (15), although the morphology of this precursor cell, and the factor(s) that control its division and differentiation, have not been described. We do not know whether our cloned cells are committed to the mast cell lineage alone; however, we have not observed the development of other cell types using the culture conditions described in this study.

We have recently reported that a cloned mouse cell line mediating natural killer lysis (29) resembles immature basophils by ultrastructure and expresses high affinity FC×R (11). In the present study, cloned immature mast cells were devoid of natural killer cell activity. We have also tested the ability of mast cell clones to regulate other immune functions. Mast cell clones do not enhance B cell Ig secretion. To date, we have not observed inhibition of Ig secretion by cloned mast cells (data not shown). However, histamine (37, 39) and perhaps other mediators released by mast cells may influence T lymphocyte activity, and we are currently testing whether Ig secretion is affected by cloned mast cells sensitized with IgE and degru-lated with antigen.

We have been able to regulate mast cell proliferation and induce maturation in vitro. Cloned mast cells grown in medium conditioned by Ly 1+2− T lymphocytes (see Materials and Methods) divided with a doubling time of ~20 h. These cells

**Accessory Cell Function and Natural Killer Lysis**

Mast cells from three clones derived from fetal liver were unable to alter Ig secretion when added to enriched B lymphocytes. Data from a typical experiment are shown in Table IV. Analysis of mast cell clones with monoclonal antisera failed to detect Ia antigens.

Three mast cell clones from fetal liver or bone marrow did not lyse the YAC-1, EL-4, RL-12, and MBL-2 lymphomas and the P815 mastocytoma. A representative experiment is shown in Table V.

**DISCUSSION**

Cloned mast cells resembled by ultrastructure proliferating immature mast cells found in vivo (4-6, 24). Like immature mast cells, these clones contained cytoplasmic granules with numerous small vesicles and various amounts of electron dense progranulelike structures. Despite their immature appearance by ultrastructure and their low histamine content, cloned mast cells expressed nearly as many Fc×R as mature peritoneal mast cells. In addition, the Fc×R of cloned mast cells bound IgE with an equilibrium constant similar to that of normal mouse peritoneal mast cells. Uncloned rat mast cells also express Fc×R when their histamine content is very low (14), supporting the notion that incorporation of Fc×R into the plasma membrane represents an early event in the program of mast cell differentiation. While some mast cell-like lines reportedly bear Ia antigens (42, 51), mouse (7, 52) and rat (28) mature peritoneal mast cells do not express Ia antigens detectable by alloantisera. We have not detected Ia antigens on the surface membranes of cloned mast cells by immunofluorescence with monoclonal reagents. Although expression may vary according to culture conditions, degree of differentiation (47), or source of antisera, we have not observed Ia expression at any time with the cells described in this report.

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appeared very immature by ultrastructure and contained only a few cytoplasmic vacuoles devoid of electron-dense granule matrix. Their histamine content was very low. By contrast, mast cells grown in the same medium supplemented with 1 mM sodium butyrate stopped dividing, developed more mature cytoplasmic granules containing increased levels of 35S-glycosaminoglycans, and stored up to 50 times more histamine than control mast cells without butyrate. These findings are consistent with earlier work identifying the cytoplasmic granule as the site of histamine storage in mature mast cells (1).

The cloned mast cells described in this report may represent a subset which grows under selective culture conditions. Although there is no proof that mast cells in vivo are composed of distinct subpopulations, variations in the morphology and histochemistry of mast cells in different tissues have suggested this possibility. For example, microspectrophotometric analysis suggests that the sulfated glycosaminoglycans of rat intestinal mucosal mast cells, like those of our clones, are similar to chondroitin sulfates (50). Mucosal mast cells also resemble mast cell clones in their low histamine content (2, 25) and responsiveness to T lymphocyte regulation (21, 33, 40). However, mouse mucosal mast cells have not been isolated, and their glycosaminoglycans have not been analyzed by hydrolysis with purified mucopolysaccharidases. The number and IgE binding affinity of their FcεR have not been determined. Although the extent and causes of mast cell phenotypic variation remain to be fully defined, some differences among mast cell populations may be regulated by microenvironmental factors affecting morphology, mediator content, or maturation. In this view, the ultrastructure and mediator content of cloned mast cells may reflect their immaturity or the influence of their culture conditions, rather than their derivation from a distinct mast cell subpopulation. In support of this notion, histochemical evidence has suggested that, like mucosal mast cells, immature connective tissue mast cells in vivo contain little or no heparin (6). In addition, cloned mast cells resemble immature connective tissue mast cells by ultrastructure (4–6, 24). On the other hand, certain mastocytoma sublines reportedly synthesize heparin, albeit in small quantities, while they are rapidly dividing and their mediator content is very low (27). By contrast, we have not detected heparin synthesis in our cloned mast cells even after using butyrate to halt proliferation and induce maturation. Whether other agents may induce further maturation and synthesis of heparin remains to be determined.

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