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Murine Basophil–Mast Differentiation: Toward Optimal Conditions for Selective Growth and Maturation of Basophil–Mast or Allied Cells

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Recent investigations revealed that basophil-mast cells were related to the hemopoietic system. Strikingly, murine bone marrow showed a singular paucity in cells with basophil-mast features; moreover in clonogenic assays (methylcellulose, agarose) bone marrow was found to be manifestly poor in basophil-mast progenitor cells. Our work brought to light several new facts concerning the culture and differentiation of this cell type: 1° pure and mixed mast clones can be derived in large numbers from bone marrow, provided progenitors are cultured in collagen matrix. Up to 1,382 hemopoietic clones were analysed in situ after staining: 30% contained mast cells (34 per 10^5 cells), thus the basophil-mast lineage was one of the most frequent. We concluded that other cloning media were noticeably nonoptimal for the growth and/or maturation of mast cells. We suggested that collagen and the molecular edifices derived from it, both found in variable amounts in the natural mast environments, should play essential roles in mast phenotype expression. 2° Cholera toxin (CT) selectively eradicated nonmast progenies: mast progenitors and mast progenies were resistant. In this way, pure and rapidly expanding mast cell clones were obtained at a frequency never reported before. CT possibly acts both directly, as a stimulator of mast cell proliferation, or indirectly on marrow subpopulations which repress basophil-mast cell growth and maturation. In vitro culture conditions, specifically designed for basophil-mast lineage, should prove of interest in the search for an unifying hypothesis concerning the multiple forms of mast cells found in various tissues.

The characterization of the multilineage hemopoietic growth factor (13 various other names have been used; for a review see Watson and Prestige, 1983; Dy and Lebel, 1981; Yung et al, 1981; Iscove et al., 1982; Bazill et al., 1983; Ilhe et al., 1983) recently opened the way for cloning murine pluripotent hemopoietic progenitor cells (Metcalf et al., 1979; Johnson, 1980; Johnson et al., 1982). This regulator also ensured the growth of various factor-dependent cell lines such as basophil–mast and/or P cell (Nagao et al., 1981; Tertian et al., 1981; Hastorpe, 1980; Schrader, 1981), granulocytes (Dexter et al., 1980), mast/erythroid progenitors (Wendling et al., 1983) and pluripotent progenitor lines (Greenberger et al., 1983). These new findings and others (Kitamura et al., 1981; Sonoda et al., 1982, 1983) contributed to demonstrating that basophil–mast cell progenitors are related to hemopoietic lineages and pointed chiefly to the multipotent hemopoietic stem cell as the primary basophil–mast ancestor.

The obtention of mast cell lines (Tertian et al., 1981) or mast progenitor cell lines (Wendling et al., 1983), from bone marrow or bone marrow cultures became an easy task; this contrasted with the reported paucity of colonies containing mast cells obtained in direct clonal assays (Nakahata et al., 1982; Suda et al., 1985). In addition, it was striking to observe that in the recent analysis of bone marrow multipotent progenitor cells, the systematic screening of progenies revealed that mast cell progenitor clones were negligible or assimilated to a minor component (Metcalf et al., 1979; Johnson, 1980; Johnson et al., 1982; Nakahata et al., 1982; Suda et al., 1985). In contrast, Crapper and Schrader (1983) and Guy-Grand et al. (1984) demonstrated the abundance of basophil–mast progenitor cells in this tissue, using a limiting dilution method.

Several proposals should account for the rarity of marrow mast progenitor cells detectable by clonogenic assays: 1° Undifferentiated mast cells migrate from bone marrow to other hemopoietic organs or connective tissues, where they find more conducive environments for growth and differentiation; 2° The clonal assays so far

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used are not optimal for this particular cell type, the maturation of which has specific requirements; 3° marrow bystander cells, as well as the micromilieu generated in culture in the colony and its vicinity, suppressed mast cell growth.

The above considerations reinforced the contention that the potential of bone marrow for mast lineage has to be reevaluated using new approaches. This paper reports on the growth of mast cell clones in collagen matrix and the positive selection of mast progenitors by treatment of bone marrow with increasing doses of cholera toxin.

MATERIALS AND METHODS

Animals

Twelve-week-old mice of either sex were used. BDF1 mice (C57 Bl/6 × DBA/2)F1 were obtained from Iffa-Credo (France).

Conditioned media from the tumor cell line WEHI (WEHI-CM)

The WEHI-3 myelomonocytic leukemia (formerly adapted to culture by P. Ralph et al., 1976 was obtained from TM Dexter, (Manchester, England) and was cultured in Fischer medium supplemented with 5% horse serum (HS) at 2.10^5 cell/ml for 5 days, then grown to plateau after 7 days. Crude culture media were centrifuged at 15,000g for 60 min and filtered through prewashed filters (Nalgen 22 μm), then stored at −30°C.

Cell cultures and CFUc assays

Long-term bone marrow cultures were performed as described by Dexter et al. (1977) with the modification introduced by Greenberger (1978). Cultures were fed each week by a half-replacement of the medium. When adherent cells were used the totality of the medium was discarded, the adherent layer was gently washed with warmed medium, the adherent cells were detached with a teflon scraper, and they were dissociated by three to five passages through a syringe needle (23 gauze). The stromal cell population was then separated from hemopoietic cells by adherence on plastic flask for 1 h in culture medium, at 37°C.

CFUc assays

Agar culture assays were performed using a modification of the method described by Bradley and Metcalf (1966). Briefly, one part of 3% bacto-agar (Difco) was added to nine parts of warm Iscove modified medium (IMDM) (Iscove and Melchers, 1978) containing 15% HS (Flow, Scotland). Fresh and cultured bone marrow cells were plated at a concentration of 10^5 cell and 5.10^4 cells respectively, in 1 ml vol, in 35 mn Petri dishes (Nunc). Colony-stimulating factor (CSF) was provided by the addition of 0.15 ml pretested WEHI-CM. The cultures were incubated for 7 days and 15 days at 37°C in a 5% CO_2 humidified atmosphere. Colonies were observed at 50 × magnification.

Collagen culture assays were performed as previously described (Lanotte et al., 1981, 1982). Briefly, 1 volume of aqueous collagen was mixed at 4°C with 3 volumes of concentrated media (1 volume × 2 IMDM, 2 volumes made up by HS (15% final), and WEHI-CM (15% final) and 1 × IMDM). Cells (in 0.5 ml) were added to the resulting isotonic solution of collagen. One ml of cell suspension was plated in 35mm Petri dishes before the gelation occurred. Incubation was carried out as above. In situ morphological identification of colonies and enyzme histochemistry were performed as previously reported (Lanotte, 1984).

Culture subcloning

Colonies were individually subcloned from agar cultures in 100 μl of medium supplemented with 15% (v/v) WEHI-CM for 2 to 4 days in 96-well plates. Cells from positive wells were then transferred to 1 ml fresh culture medium in 24 well plates for 4 to 7 days. Then, 2.10^5 cells/ml cultures were expanded in 5 ml media in T25 culture flasks and cultured for up to several months.

Cholera toxin treatment

Cholera toxin and B chain subunits (toxoid) were generously donated by Dr. Tayot (Institut Merieux, Lyon). Toxin was purified by affinity chromatography on Dextran-lyso GM_1 ganglioside (Tayot et al., 1981). Several batches of CT, toxoid, and A chain were also provided by Sigma. Stock solution was stored at 4°C and diluted aliquots were always used fresh. Toxin and toxoid (10% v/v) were added to liquid culture media and CFU-c assays media during the incubation period.

Histological and immunofluorescence procedures for basophil-mast identification

The May-Grunwald, alcian blue, and toluidine blue stainings and the paraformaldehyde fluorescence were carried out using the classical procedures without modifications.

Histamine in mast cells was detected using the di-orthophtalaldehyde (OPT) fluorescence reaction described by Ehinger and Thunberg (1967) with the modification introduced by Guy-Grand. Visualization was carried out on both cell smears and in situ in cultured basophil–mast clones. Dried collagen gel culture preparations (unfixed) were put into a glass jar containing a few mg of OPT powder (Fluka, Switzerland) for 30 sec at 80°C. Slides were then immediately humidified under a moist air stream (5 sec); preparations were allowed to cool down to room temperature for few minutes before mounting in xylene and cytological examinations using UV light fluorescence microscopy (Leitz).

IgE receptors on basophil–mast cells were visualized using a immunofluorescence technique as described by Guy-Grand et al. (1984). Briefly, a monoclonal mouse IgG (from Seralab, Sussex, U.K.) was applied (1/50 diluted) for 3 h on cultured cells. Controls were incubated with normal mouse serum (1/5 diluted). IgE was detected by the fluorescence positivity with a goat anti-mouse IgE (Miles, Elkhart, IN).

RESULTS

Growth of mast progenitor cells in collagen gels

Mast cell progenies were identified in situ in collagen gel cultures using the classical or specific staining (see Materials and Methods). Basophil–mast clones were unequivocally identified in the cultures (Fig. 1a–e) by their basophilic, alcianophilic, and metachromatic granules with histamine and serotonine contents. Others hemopoietic lineage clones (such as monocyte or neutrophilic granulocyte clones) furnished negative controls on the same culture slide. Pure mast cell clones as well as

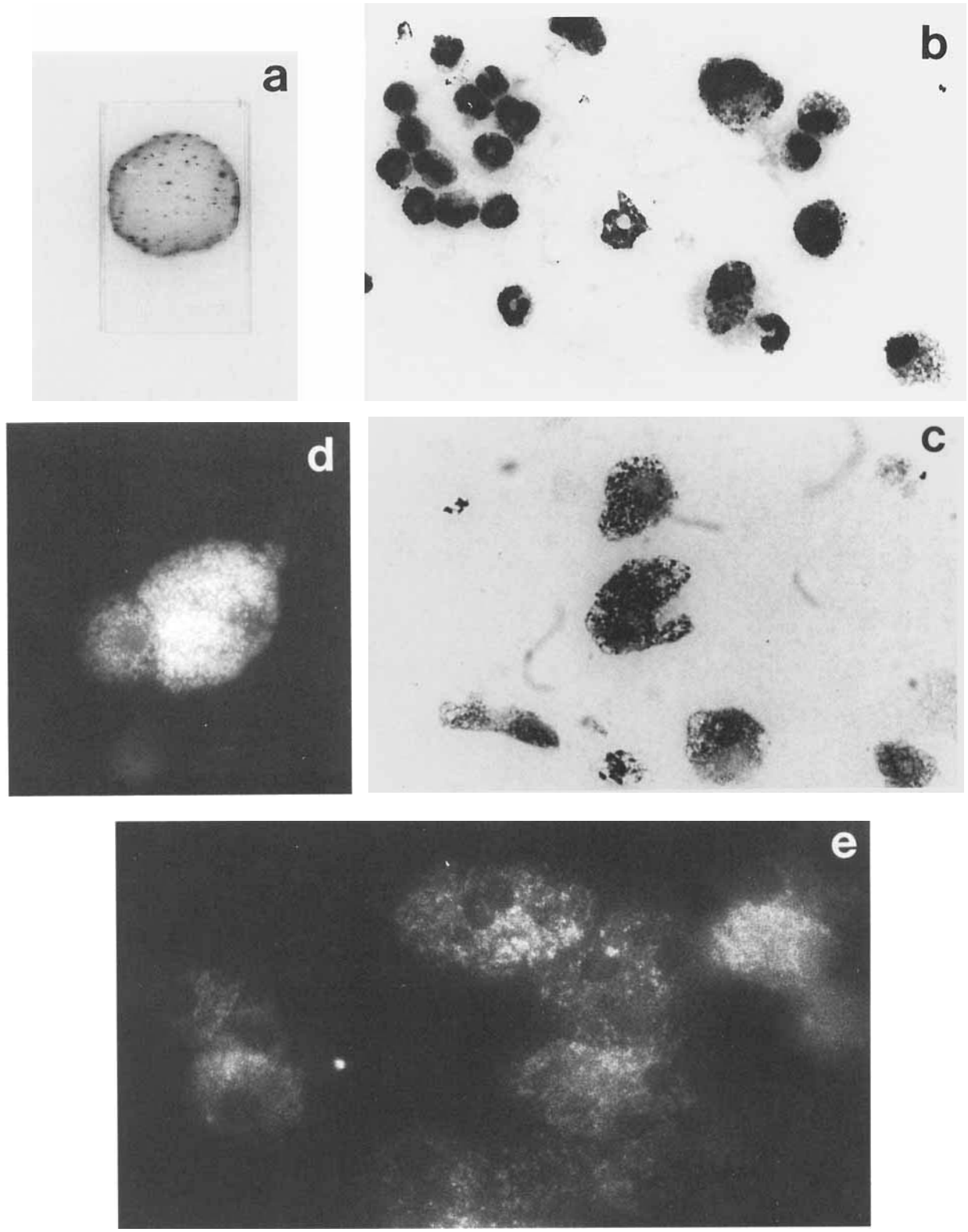

Fig. 1. In situ morphological identification of mast cell clones in bone marrow cultures grown in collagen matrix. a) Gigantic mast cell clones in 10 day old cultures (May-Grunwald stain). b) May-Grundwald stain. c) Metachromatic staining by toluidine blue. d) Identification of serotonine and dopamine in cytoplasmic granules by formaldehyde fluroescence. e) Identification of histamine producing cells by OPT fluorescence in bone marrow basophil–mast cell clones.

mixed colonies containing variable amounts of mast cells were found in collagen gel cultures after 8 days. When incubation was prolonged up to 15 days, the mast moiety of mixed colonies continued to expand. After 3 weeks most of the "persisting" clones achieved a homogenous mast pattern. The frequencies of mast cell progenitors in bone marrow and in 8-week-old bone marrow cultures were evaluated (Table 1). It is shown that mast clones were either pure or mixed. Plating efficiencies were respectively 35, 52, and 106 per 10^5 cells for normal marrow, floating cell and adherent cell populations in bone marrow cultures. Very high PE values featured cell populations associated with the hematopoietic microenvironment in the adherent layer: this was not particular to mast progenitor cells, that culture compartment being the privileged site of progenitor homing. In fact, the frequency of mast progenitors was steady and independent of the origin of the cells (27.4% to 30.5% of total CFCs). However, PE were indeed very high and such high values had never been reported previously for bone marrow, except for limiting dilution analysis.

A detailed analysis of mast association in multilineage clones was carried out. Mast cell subclones were frequent in mixed colonies (Table 2). Monocytes and granulocytes were the predominant associations in mixed clones but associations with megakaryocytes were also frequent (about 3–6% of total CFCs).

Selective growth effect of cholera toxin in collagen cultures

Bone marrow cells were cultured in the presence of CT for 8 days (10^{-7}M to 10^{-12}M toxin). A study which accounts for the in situ analysis of 1,382 clones is summarized in Table 3. It is shown that increasing doses of CT eradicated hemopoietic clones, with the exception of pure mast cell clones and mast-containing clones. Moreover, mast clones were found enlarged in these cultures (especially between 10^{-9}M 10^{-8}M). All clones grown in culture were analysed for their lineage content and classified according to 16 combinations of pure and mixed clones of neutrophil granulocytes monocytes, basophil–mast cells, and megakaryocytes. It was found that without CT a significant proportion of mast clones grown from bone marrow were related to the multipotent hemopoietic progenitors (see Table 3, control). It also transpired that the numbers of pure mast clones were increased by toxin treatments, whereas in the same conditions, binar associations of mast cells with monocytes or granulocytes were greatly reduced. Clones associating megakaryocytes and mast cells alone or with a third lineage were significantly more resistant. The total number of basophil–mast cell clones remained constant. Finally, the eradication of nonmast clones facilitated the evaluation of mast cells, thus indicating that

TABLE 1. Frequency of mast progenitor cells in fresh bone marrow and long-term cultures

CFC per 10^5 cells[a]	Pure mast clones	Mixed clones containing mast cells (20%)	Other	No. of CFC (total)	Mast progenitor cells (%)	Plating efficiency (per 10^5 cell)
Normal bone marrow cells (BDF_1 10 wks, femurs)	17	17	90	124	27.4	34
Dexter's cultures (BDF_1)						
8 wks in culture						
Floating cells	9	43	129	181	28.7	52
Adherent layer	36	79	242	348	30.5	106

[a]Cells were plated at a concentration of 5.10^4 cells/ml. WEHI-3b-CM (15% v/v) was used as a source of growth factor. All colonies grown after 7 days of culture (>50 cells) were identified in situ after staining (653 clones were scored).

TABLE 2. Lineage associations of mast cells in multilineage clones

	Mast cells associated with (%)[a]		
		Marrow long-term cultures	
	Fresh bone marrow	Adherent layer	Floating cells
Meg. Gr. Mono	1.07	3.4	1.1
Meg. Gr	0.5	0.5	2.2
Mono. Gr	5.9	12.6	17.1
Mono. Meg	1.6	0.5	0.5
Gr.	0.5	1.7	0.5
Mono	3.75	0.5	2.2
Meg	0.5	2.3	0.5
Pure mast cell clones	13.7	10.35	4.9
Total (% of colonies containing mast cells)	27.4	30.5	28.7

[a]Data shown here correspond to the analysis of the 16 lineage combinations found in the experiment reported Table 1 (653 clones scored).

TABLE 3. Selection of basophil-mast progenies from lineage restricted and multipotential colony-forming cells by cholera toxin

Lineage associations in hemopoietic clones	Toxin (log molar dilutions)					
	−7	−8	−9	−11	−12	0
Gr.Meg.Mo.**Ms**:Gr.Meg.Mo	0:0	0:0	0:0	4:1	2:2	5:2
Gr.Meg.**Ms**:Gr.Meg	2:0	3:0	10:2	3:6	12:10	3:2
Meg.Mo.**Ms**:Meg.Mo	0:0	0:0	0:0	4:0	6:0	0:12
Gr.Mo.**Ms**:Gr.Mo	0:3	0:0	1:0	2:6	0:7	23:106
Meg.**Ms**:Meg	7:0	19:3	8:4	22:16	6:34	2:5
Gr.**Ms**:Gr	0:11	3:14	17:57	10:61	11:137	2:58
Mo.**Ms**:Mo	0:0	0:0	0:0	1:4	0:6	42:87
Ms	104	106	79	60	95	52
Mixed-CFCs containing **Ms** (total)	9	25	36	46	37	77
CFCs with **Ms** (total)	113	131	115	106	132	129
Other CFCs	4	17	63	94	196	272
Total CFCs	127	148	178	200	328	401

Values represented the total number of hemopoietic clones grown from 3.10^5 bone marrow cells cultured in four 1 ml culture gels (4 × 75,000 cells). For the six culture conditions studied in this experiment 1,382 colonies were analysed in situ for their hemopoietic lineage content (abbreviations: Gr, granulocytes; Meg, megakaryocytes; Mo, monocytes; **Ms**, basophil–mast cells).

the mast cell progenitor population was not overestimated when the various hemopoietic lineages were cocultured (compare Tables 1 and 3).

Role of marrow bystander cells on renewal and development of primary basophil–mast progenitors

A typical example of the evolution of mast and granulomonocytic clones during treatment with log-molar dilutions of toxin is shown in Figure 2. The total mast colony numbers were roughly constant, but the colony size increased. The fact that mast clones persisted when the growth of other lineages was inhibited by toxin provided a useful tool for evaluating the role of bystander cells on mast cell growth and their long-term maintenance in culture.

In our experience, cloning mast cells in semi-solid agar media has always been unreliable. Most frequently mast progeny was low, and proliferation vanished after 10 days in agar cultures (Li and Johnson, 1984). It was tempting to elucidate whether the low performance obtained in agar was somehow related to the fact that the media favored other lineages at the expense of mast progenies. Agar cultures were treated with toxin to eliminate nonmast lineages: we found that toxin treatment increased the number of mast clones growing in agar and significantly increased the self-renewal potential of primary clones containing mast cells. Table 4 shows results obtained in a typical experiment. When toxin (6.10^{-9}M), was added to the culture, the PE of mast cells on D15 was roughly similar in agar or in collagen, whereas no mast clones (more than 50 cells) could be identified alive in untreated agar cultures (phase-contrast microscopy). We confirmed this result by the individual recloning of 10-day-old colonies from toxin treated or untreated agar cultures (Table 4). Clones from untreated cultures inevitably became extinct, whereas clones from cultures initially treated with toxin achieved an extensive rate of self-renewal up to 5 weeks: six cloned cultures chosen at random were then passaged up to 8 months.

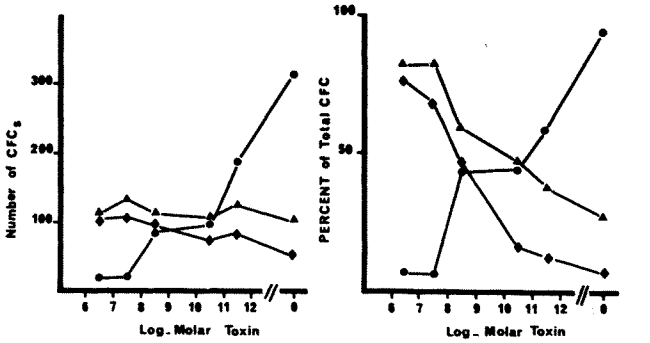

Fig. 2. Comparative evolution of mast and monocytic clones following the treatment of cultures by cholera toxin (Monocyte clones ●—●; pure mast cell clones ♦; total of all classes of hemopoietic clones containing masts, ▲).

DISCUSSION

This paper offers a reappraisal of the potentiality of murine bone marrow for developing mast cells. We have reported on a survey that accounted for 1,382 bone marrow hemopoietic colonies grown in collagen matrix submitted in situ morphological analysis after specific histochemical staining. Mast cells showed the typical morphology of this lineage; basophil granules stained specifically by toluidine or alcian blue contained serotonin and histamine. These techniques allowed unequivoqual identification of basophil–mast cells in hemopoietic clones, even when these cells were in a minority. We failed, for technical reasons, to identify the IgE receptors on mast cells in situ in primary clones; however, when mast colonies were expanded for 3 weeks in liquid cultures, mast cells were uniformly positive for IgE receptors. Data related to three main parameters influencing the growth and differentiation of marrow basophil–mast progenitor cells are discussed.

TABLE 4. Effect of cholera toxin on clonal growth and recloning potential of mast colonies grown in agar

	Primary cultures (day of culture)		Ten-day agar culture clones[a] (day after recloning)						
	7	15	3	6	8	11	14	35	8 mths
Collagen									
Control	0	0							
WEHI-CM	132 ± 26	39 ± 9							
WEHI-CM ± toxin	54 ± 4.5	20 ± 1.8							
Agar									
Control	0	0							
WEHI-CM	108 ± 27	0[c]	24	10[d]	!				
WEHI-CM ± toxin	36 ± 8	28.6–12	21	19	17	16	16	16	Six mast cell lines[b]

[a]Ten-day-old colonies from agar primary cultures were individually expended as described in Materials and Methods. The number of positive well or culture flask was recorded, at each feeding day, during 6 weeks. Toxin was added in the primary cultures, when indicated (toxin 6.10^{-9} M) and omitted afterwards in expended cultures.
[b]Among the 16 positive cultures on D35, six randomly selected cultures gave six WEHI-CM-dependent mast cell lines.
[c]Few nonproliferating macrophage clones and few mast cell clusters (< 20cells) were present.
[d]No proliferation, dead cultures.

Cultured in collagen for 7 days, bone marrow cells formed pure mast clones but also mixed lineage clones (25–30% of all CFC). The population identified morphologically as a mast cell progeny in such cultures was much more abundant in collagen than in agar cultures. The paucity of bone marrow-derived mast progenitors, reported by others (Nakahata et al., 1982; Suda et al., 1985) was due, in our opinion, to nonoptimal cloning conditions. In collagen gels, mast progenitors were readily identified from the first week of culture as one of the most frequent hemopoietic progenitors. A similar result had recently been observed in serum-free collagen cultures stimulated with purified stimulating factors (Cormier et al., 1985). Several hypotheses can be formulated to explain the difficulty of cloning marrow mast progenitors in agar or methylcellulose: 1) Mast progenitors form colonies in such semisolid media but terminal maturation is abnormal and the progenies are not identifiable as basophil–mast cells (deficient formation of granules or degranulation); 2) Bone marrow mast progenitors differed from progenitors found in other organs (spleen, lymph node, thymus, or serosa). Mast cells required some molecules representative of their natural environment for optimal terminal maturation. In this case, mast cell proliferation obtained in liquid culture (Nagao et al., 1981; Tertian et al., 1981, Schrader and Nossal, 1980; Schrader et al., 1981) should result from a sustained proliferation of late or committed mast progenitors, not necessarily representative of all classes of mast progenitors (Li and Johnson, 1984).

At present, it is not clear whether collagen itself played a direct role in mast cell maturation. Possibly this molecule, under certain conditions, is essential for the maturation of basophil–mast granules. Thus, aside from the soluble, circulating mast cell growth factor involved in self-renewal and mast clone development, mast cells should find in collagen matrix a conducive environment for terminal maturation to some extent related to the environment of mucosa and connective tissues (i.e., a local insoluble regulator). Native collagen formed branched molecular superstructures with fibronectin, mucopolysaccharides, and several peptidoglycans. Such molecular edifices provided promising models for investigating the role of the extracellular matrix on the maturation of basophil–mast cells. These molecules should be responsible for the phenotypical variations of mast-like cells, albeit born of the same ancestor cell: herein should be found a unified hypothesis concerning the debated origin of mast cells and basophil granulocytes (Schrader and Nossal, 1980, Schrader et al., 1981; Schrader, 1981; Kitamura et al., 1981; Phaar et al., 1984), serosal masts (peritoneum), connective tissue and intramucosal mast cells (Guy-Grand et al., 1984; Mayrhofer and Fischer, 1979; Schrader et al., 1983).

It was clear from previous reports that CT strongly inhibited granulomonocytic lineage cells (Lenz et al., 1982; Izumi et al., 1984). The selective inhibition of the proliferation of the various murine hemopoietic progenitor cells by cholera toxin (Lanotte et al., 1986) offered the possibility of selecting mast cell clones simply by treatment of cultures. Mast progenitors generating either pure mast clones or multilineage colonies are especially resistant to cholera toxin. This method furnished a direct and precise evaluation of mast progenitor cells. Unlike Saito et al. (1985), we found no increase of the total mast clone number after CT treatment. In contrast, the size of pure mast clones was increased and the mixed clones had a larger mast progeny. Like mast progenitors, mast cell lines (Tertian et al., 1981; Wendling et al., 1983) were resistant to CT (unpublished data). In spite of the fact that CT binding raised c-AMP levels, we failed to find any strong evidence of a enhanced growth rate of factor-dependent mast cell lines. It is appropriate to mention here that c-AMP elevating agents are potent inhibitors of mast cell degranulation. It is likely that this phenomenon occurred in cultures (Dy and Lebel, 1981): toxin treatment should help to identify mast cells since basophilic granules accumulated in their cytoplasms.

Our results also raised the question as to whether bystander cells or minor populations of the marrow played inhibitory effects on the growth and maturation of mast cells: CT treatment possibly eradicated this suppressive population. The assumption of the existence of some inhibitory population in the marrow is strengthened by data reported by Schrader's group (1983) and

Guy-Grand et al. (1984) who found a similarly high frequency of mast progenitors in bone marrow using a limiting dilution analysis in a microculture system—in this system (6.10^2 to 6.10^3 cells per culture) the effect of marrow bystander cells was lower than for cultures with 4.10^4 to 10^5 cells. In this matter, the stimulatory role of spleen feeder cells in mast cell growth (Guy-Grand et al., 1984) should also be unravelled. It is thus interesting to compare the low frequency values reported by Ogawa's group (Nakahata et al., 1982; Suda et al., 1985), to the high frequencies obtained by Schrader et al. (1983), Guy-Grand et al. (1984), and ourselves.

We have previously shown that fibroblastic stromal cells, and cloned preadipocyte cell lines produced substantial amounts of M-CSF in culture and triggered monocyte-macrophage colonies (Lanotte et al., 1982). Moreover, the M-CSF produced by stromal cells enhanced the size of colonies stimulated by multi-CSF (Lanotte et al., 1982); giant macroscopic colonies were observed. Stromal fibroblastic cells from the marrow or cells from the adherent layer of Dexter's cultures played similar roles (unpublished data). In these giant colonies, the monocytic macrophage growth was increased and mast cell growth significantly suppressed: mast progeny became a minor component in mixed colonies (result not shown). Interestingly, the treatment of these cultures with toxin resulted in a complete elimination of monocytic macrophage colonies which in turn restored the size of mast clones to its original level. Finally, several authors noticed that the establishment of mast cell lines in liquid culture was facilitated when adherent stromal cells and macrophages were periodically removed from the culture (Wendling et al., 1984; also our unpublished data). Marrow adherent cells produced specific inhibitors or contributed to purging the cultures of growth factors (Toksoz et al., 1980; Wright et al., 1980; Zipori and Sasson, 1982; Heard et al., 1982).

In conclusion, our work evidences that the complete maturation of basophil–mast cells requires both complex humoral (Li and Johnson, 1984) and microenvironmental stimuli for which further investigations are needed. Supramolecular edifices built up from collagen matrices should furnish a means to create realistic surrogates of mast cell microenvironments in vitro. Such "milieux" would benefit investigations regarding the cell type(s) and factor(s) influencing the terminal expression of the various mast phenotypes.

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