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Rat IL-3 stimulates the growth of rat mucosal mast cells in culture

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SUMMARY

Rat mast cells with the properties of mucosal mast cells (MMC) proliferate in cultures of haemopoietic tissue in the presence of conditioned medium (CM) from antigen- or mitogen-activated T lymphocytes. The present study shows that recombinant rat interleukin-3 (rIL-3) both stimulated the development of MMC from bone marrow (BM) precursors and maintained the proliferation of rat MMC lines in an identical manner to that of CM. The content per cell of the MMC granule-specific proteinase RMCPII was similar in both IL-3- and CM-stimulated cultures. Passage of CM through DEAE–cellulose separated two active peaks that stimulated autologous MMC proliferation. The biochemical properties of peak 1 were similar to those of murine IL-3 and stimulated multi-potential stem cell development in soft agar cultures of BM cells from rats treated with 5-fluorouracil (which enriches for haemopoietic stem cells). RIL-3 was also active in this assay whereas peak 2 was not, demonstrating that peak 1 contained IL-3 activity. The presence of MMC in the majority of multi-potential colonies in the soft agar cultures confirmed the early stem cell origin of the MMC lineage. The cultured BM-derived mast cells in the rat are analogues of the MMC subset that is most readily observed proliferating in the gastrointestinal tract in response to helminth parasite infection. The demonstration that IL-3 is responsible for the development and proliferation of MMC should lead to a better understanding of the functional roles of these cells.

INTRODUCTION

We have described previously an *in vitro* culture system in which rat haemopoietic progenitor cells can be stimulated to produce, within 2–3 weeks, pure populations of T-dependent (mucosal) mast cells (MMC) in the presence of medium conditioned by antigen- or migoten-activated T cells (Haig *et al.*, 1982, 1983). Further studies have shown that the MMC growth activity is produced by a subset of T cells bearing the $OX19^+$, $W3/25^+$, OX8-helper/inducer markers (McMenamin *et al.*, 1985). In mice, T-dependent mast cells proliferate in the presence of the haemopoietic growth factor interleukin-3 (IL-3; Ihle, Pepersack & Rebar, 1981), also known as multi-colony-stimulating factor (multi-CSF) (Metcalf, 1984), mast-cell growth factor (Yung *et al.*, 1981), histamine-stimulating cell factor (Dy *et al.*, 1981), haemopoietic cell-growth factor (Bazill *et al.*, 1983) and burst-promoting activity (Iscove *et al.*, 1983). IL-3 has been purified to homogeneity (Ihle *et al.*, 1982; Schrader *et al.*, 1983; Bazill *et al.*, 1983), the cDNA cloned (Yokota *et al.*, 1984; Fung *et al.*, 1984) and the pure or expressed product shown to support the proliferation of not only T-dependent murine mast cells but also multi-potential haemopoietic progenitor cells (Ihle *et al.*, 1983; Hapel *et al.*, 1985). Recently, the gene for rat IL-3 has been characterized and the expressed product shown to stimulate multi-potential stem cells (Cohen, Hapel & Young, 1986).

In this study we show that rIL-3 and partially purified material from mesenteric lymph node-conditioned medium stimulate both the development and proliferation of MMC from cultured BM precursors.

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MATERIALS AND METHODS

Animals and parasites. F344 and Wistar rats aged 2·5–3·5 months were obtained from inbred colonies at the University of Glasgow Veterinary School and the Moredun Research Institute, Edinburgh. Inbred F344 rats were originally from Olac Ltd (Bicester, Oxon). Techniques for the culture of the intestinal parasite *Nippostrongylus brasiliensis*, maintained at Glasgow University, were as described previously (McMenamin *et al.*, 1985). Each rat was infected with 4000 larvae by subcutaneous injection.

Preparation of conditioned medium (CM)

CM was prepared from mesenteric lymph node cells (MLN) of infected rats using a modification of the original method (Haig *et al.*, 1983). Aliquots (200 ml) of 4×10^6 per ml viable nucleated MLN cells were prepared in Iscoves' serum-free medium, which contained 5×10^{-5}M 2-mercaptoethanol (2-ME), 100 U/ml

penicillin, 50 μg/ml streptomycin, 100 μg/ml bovine serum albumin (BSA; Sigma Ltd, Poole, Dorset), 80 μg/ml phosphatidyl choline (Sigma), 25 μg/ml transferrin (Sigma) and 2 μg/ml concanavalin A (Con A; Miles, Stoke Poges, Slough). Cultures were incubated for 48 hr at 37° in 120 cm^2 tissue culture flasks (Nunc, Paisley, Renfrewshire). CM was harvested by centrifugation and sterilized by filtration through a 0·22-μm filter (Millipore, Harrow, Middlesex) prior to storage at −30°. Batches of CM and dilutions of them were assessed for their ability to stimulate growth of MMC in cultures of normal syngeneic BM (see below) and were used in experiments at the optimal concentration (20–50%).

Partial purification of MMC growth activity from CM

The method of Yung *et al.* (1981) was followed. Solid ultrapure ammonium sulphate (Aristar, BDH Ltd, Poole, Dorset) was added to 2–5 l of CM with stirring to give a final 85% saturation at room temperature. After gentle stirring in the cold for 2 hr, the precipitate was pelleted by centrifugation at 30,000 *g* for 30 min and redissolved in a minimal volume of distilled water. The sample was then exhaustively dialysed against 5 mM sodium phosphate buffer (pH 8·0) + 0·001% polyethylene glycol 6000 (PEG, BDH Ltd) at 4°. An aliquot of the sample (the volume depending on the protein concentration) was then added to a column of DEAE-cellulose (DE-52, Whatman Ltd, Maidstone, Kent) equilibrated with the same phosphate buffer and the unbound protein fractions plus two column volumes of eluate were collected prior to eluting bound protein with a 0–0·5 M NaCl gradient in 5 mM phosphate buffer, pH 8·0. Fractions of 8-ml volume were collected and assayed for activity against autologous rat BM-derived mast cells. The unbound fractions were pooled, concentrated over an Amicon 5000 MW cut-off filtre and made isotonic with × 10 Hanks' balanced salt solution (HBSS) + 1% BSA. Gradient-eluted fractions were equilibrated with phosphate-buffered saline (PBS), pH 7·2, containing 0·001% PEG using prepacked PD-10 Sephadex columns (Pharmacia Ltd, Uppsala, Sweden). All fractions were sterilized by filtration through 0·22-μm filters prior to testing in proliferation assays.

Haemopoietic cell culture

Liquid BM cultures were prepared as described previously by Haig *et al.* (1982, 1983). A final concentration of $2·5 \times 10^5$ viable nucleated BM cells/ml was suspended in Iscove's medium together with 5×10^{-5}M 2-ME, penicillin, streptomycin (see above), 20% heat-inactivated horse serum (Flow Labs, Irvine, Ayrshire) an optimal amount of CM (usually 25%). For routine batch testing, 24- or 48-well plates (Costar Ltd, Northumbria Biologicals, Cramlington, Northumberland) were used to culture 1-ml aliquots of BM in the presence of dilutions of CM and, for experimental studies, 120 cm^2 flasks (Nunc, Gibco Ltd, Paisley, Renfrewshire) were used. These bulk cultures were refed and restimulated with medium and CM every 4–6 days. Plate cultures were kept at 37° in a humidified incubator with 5% CO_2 in air, for 6–8 days.

Soft agar cultures were prepared as described previously (Haig, Jarrett & Tas, 1984). One-millilitre cultures in Iscove's medium containing 0·3% agar (Bactor Agar, Difco Ltd, Detroit, MI), 20% heat-inactivated horse serum, 5×10^{-5}M 2-ME, penicillin, streptomycin and optimal concentrations of CM and BM were plated in three cm^2 tissue culture dishes (Flow Labs) and incubated for 14 days at 37° in a humidified atmosphere of 7·5% CO_2 in air. Colonies (>40 cells) and clusters (3–40 cells) were counted using an Olympus CX inverted microscope. The phenotype of individual colonies was analysed by fixation and staining *in situ* using a modification of the method of Salmon & Buick (1979). Briefly, 2 ml methanol were added to the cultures for 30 min to fix the cells. The agar discs were rinsed several times *in situ* with distilled water to clarify the agar and remove the methanol. The entire agar discs were tipped out onto glass slides (3·5 × 7 cm), covered with nitrocellulose filtration membranes (0·8 μm, Millipore Ltd) and allowed to dry overnight. The preparations were stained with 0·05% toluidine blue, pH 0·5, for 15 min or Giemsa at 1:15 dilution for 30 min.

Proliferation assay

CM and DE-52 fractions were assayed for their ability to stimulate incorporation of [^{3}H]thymidine ([^{3}H]TdR) into pure populations of 2·5–3-week-old cultured rat MMC. After thorough washing in HBSS, 10^6 MMC ml were prepared in Iscove's complete medium and 100-μl aliquots of cells were added to 100-μl sample dilutions in 96-well plates (Nunc). These were incubated at 37° in a humidified atmosphere of 5% CO_2 in air for 24–72 hr with a final 18-hr pulse of 0·5 μCi per well of [^{3}H]TdR (5 Ci/mM Amersham, Amersham, Bucks). Cells were harvested onto glass fibre mats using a cell harvester (Flow Ltd) and activity from replicate wells was counted in a scintillation counter.

5-Fluorouracil treatment

Normal rats were treated with 5-fluorouracil (5-FU; Sigma) by intravenous injection of 150 mg 5-FU/kg body weight (30 mg per rat in 3 ml saline). Four days later, BM cells were harvested and washed prior to culture (Hodgson & Bradley, 1979).

Interleukin-3

The rat IL-3 gene derived from a genomic library of (PVG × DA) rat DNA was expressed in COS-1 monkey cells as described previously (Cohen *et al.*, 1986). COS-1 supernatants containing recombinant IL-3 were used with mock-transfected COS-1 cell supernatants as controls. One unit of activity is defined as that which stimulates half maximal TdR incorporation in a BM proliferation assay (Cohen *et al.*, 1986).

RMCPII analysis

Cell pellets were resuspended in 0·15 M KCl then frozen and thawed three times to release proteinases, and RMCPII concentrations were assayed by ELISA as described previously (Miller *et al.*, 1983). The cellular content of RMCPII was visualized in cytocentrifuged preparations using affinity-purified rabbit $F(ab')_2$ anti-RMCPII cross-absorbed with RMCPI (Gibson & Miller, 1986). Sheep Fab anti-rabbit $F(ab')_2$-peroxidase conjugate and the substrate 3-3-diaminobenzidine were used to develop the reaction.

Statistical analysis

All experiments were repeated at least once. Statistical analysis, where appropriate, was by Student's *t*-test.

Table 1. Development of BM cells in the presence of rIL-3 and CM

Sample	Total viable cells $\times 10^{-4}$/ml	MMC* $\times 10^{-3}$/ml	N* $\times 10^{-3}$/ml	M* $\times 10^{-3}$/ml	EOS* $\times 10^{-3}$/ml	Others $\times 10^{-3}$/ml
IL-3 100 U/ml	155	490	288	518	145	110
IL-3 50 U/ml	132	401	304	418	142	55
IL-3 25 U/ml	78	250	170	234	83	44
IL-3 12·5 U/ml	61	146	192	140	105	27
IL-3 6·2 U/ml	41	123	95	78	45	69
IL-3 3·1 U/ml	14	42	39	32	12	15
IL-3 1·6 U/ml	9	ND	ND	ND	ND	ND
CM 50%	103	494	160	216	56	104
CM 25%	100	445	280	102	110	63
CM 12·5%	74	182	300	84	133	41
CM 6·2%	37	97	103	66	85	20
CM 3·1%	35	79	67	80	50	74
COS snt 50%	4					
medium*	3					

$2{\cdot}5 \times 10^5$ viable Wistar rat BM cells per ml medium were seeded into wells of 48-well plate together with dilutions of IL-3 and CM. On Day 7 of culture, cells were harvested, pooled from replicate wells and a total viable and differential cell count performed.

* MMC, mucosal mast cells; N, neutrophil lineage; M, monocyte/macrophage lineage; EOS, eosinophil lineage; other, mainly haemopoietic undifferentiated blasts, but also some megakaryocytes and lymphocytes; COS snt, supernatant from mock transfected COS monkey cells.

RESULTS

Recombinant rat IL-3 stimulates RMCPII-positive MMC development

In two experiments, dilutions of rIL-3 and CM were tested against normal rat BM cells in liquid culture. One experiment was performed in replicate wells of 24-well plates, the other in 48-well plates. Both gave qualitatively similar results, so only one set of data is presented in Table 1. RIL-3 was active in liquid culture, with between 3 and 100 U/ml stimulating increases in numbers of MMC, neutrophils, macrophages and eosinophils in a dose-dependent fashion over the 7 days of the assay period. In this respect the behaviour of rIL-3 was identical to that of CM. The majority of the remaining cells were haemopoietic blasts with a few megakarocytes and lymphocytes. There were no apparent differences between cultures stimulated with rIL-3 or CM. The mast cells stimulated by both rIL-3 and CM were of MMC phenotype since, in cytocentrifuged preparations, they were intensely stained after immunoperoxidase labelling with anti-RMCPII (Fig. 1).

Recombinant rat IL-3 stimulates the proliferation of RMCPII-positive MMC

Mast cell lines of greater than 90% homogeneity were obtained from 2·5-week-old BM cultures and tested for proliferation in the presence of dilutions of rIL-3 and CM. Proliferation was determined over a 4-day period, at which time cell pellets were analysed for their RMCPII content. Table 2 shows a representative experiment in which a dose-dependent proliferation of MMC was induced by both rIL-3 and CM. In this and the repeated experiment, there was evidence of a high dose inhibition of proliferation with a rIL-3 concentration of 50 U/ml and with CM at concentrations greater than 12·5% v/v. The accumulation of granule-associated RMCPII was inhibited only by a concentration of CM at 50% v/v. A differential cell count on all samples at the end of the experiments showed that the proportion of MMC remained constant at 90% or had increased slightly. Macrophages were the only contaminating cell type.

Fractionation of MMC growth-stimulatory activity from CM

Two litres of CM containing 380 mg protein/l were treated as described in the Material and Methods. Unadsorbed protein passing through a DE-52 column in 5 mM phosphate, pH 8·0, was collected, pooled, concentrated and sterilized by filtration (peak 1). Adsorbed protein was eluted by the application of a 0–0·5 M NaCl gradient in buffer. These unconcentrated fractions were desalted and sterilized. The various samples were tested for their ability to stimulate rat BM-derived MMC from 3-week-old cultures in a 48-hr proliferation assay. Unfractionated CM was used as a reference control. Two major stimulatory peaks were obtained (Fig. 2), one in the pooled unadsorbed fraction (peak 1) and a second that was eluted at NaCl concentrations of between 20 and 80 mM (peak 2) in advance of the major protein peak. Unfractionated CM was estimated to contain 52 units of activity/mg protein where 1 unit is defined as the activity in 1 ml that induces 50% maximal proliferation of MMC. Peak 1 contained 2100 units of activity in 100 μg protein representing a 400-fold purification of MMC growth activity. Samples from the second active peak were pooled (peak 2) and tested together with peak 1, whole CM and rIL-3 for activity against autologous rat BM-derived MMC. The results are shown in Fig. 3. Whole CM, peak 1 and rIL-3 stimulated dose-dependent MMC

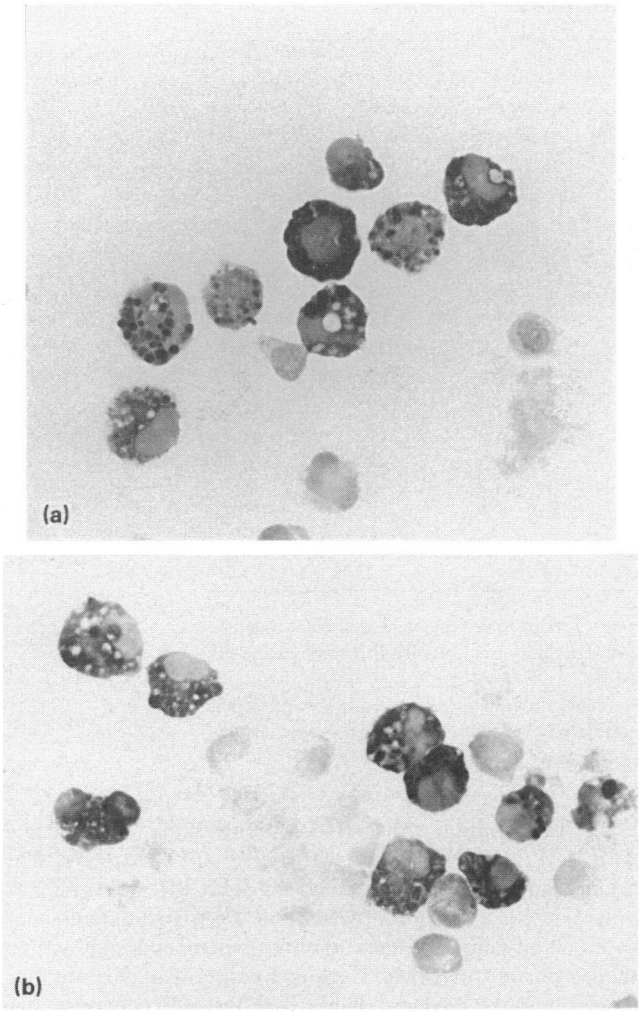

Figure 1. Immunohistochemical detection of RMCPII in BM cultures stimulated with rIL-3 (a) or with conditioned medium (b). Analysed on Day 7. Magnification × 1700.

proliferation in both a 24- and 72-hr assay. Peak 2, however, was active only in the 24-hr assay, having no effect in the 72-hr assay. In addition to mast cell-stimulating activity, peak 2 contained both granulocyte–macrophage colony-stimulating factor (GM-CSF) activity and T-cell blast proliferative activity, whereas peak 1 contained neither activity (results not shown but see below).

The growth of MMC in BM cultures treated with 5-FU.

Table 3 shows the effect of peak 1, peak 2, rIL-3 and unfractionated CM at optimal concentrations on cells surviving treatment with 5-FU, which is known to deplete cycling cells (Hodgson & Bradley, 1979). Colonies were counted on Day 14. Colonies containing MMC developed from cells in BM surviving 5-FU treatment. RIL-3 and peak 1 supported the growth of multi-potential colonies whereas peak 2 did not. In control cultures, peak 2 stimulated granulocyte–macrophage colony formation and therefore contained GM-CSF activity.

Rat BM cells treated with 5-FU gave rise to a higher proportion of multi-potential colonies than untreated marrow,

Table 2. The growth and differentiation of MMC in the presence of IL-3 and CM

Sample	Total MMC $\times 10^{-4}$/ml	RMCPII per pellet (g)	RMCPII per MMC (pg)
IL-3 50 U/ml	55	15·6	28
IL-3 25 U/ml	66	12·3	19
IL-3 12·5 U/ml	48	9·3	19
IL-3 6·2 U/ml	29	8·4	28
IL-3 3·1 U/ml	25	6·1	24
IL-3 1·6 U/ml	15	4·1	27
IL-3 0·8 U/ml	13	4·4	34
CM 50%	35	5·7	16
CM 25%	55	12·0	22
CM 12·5%	64	10·6	16·5
CM 6·2%	58	10·6	18·2
CM 3·1%	54	10·2	19
CM 1·6%	41	6·7	16
CM 0·8%	25	3·8	15
COS snt 50%	10	1·8	18
medium	12	2·1	18

MMC (90%) were harvested from a 2·5-week-old Wistar rat BM culture and thoroughly washed. Viable cells ($1·5 \times 10^5$/well medium were incubated in replicate wells of a 96-well plate together with dilution of the samples. After 4 days cells were harvested and total viable cell counts and differential cell counts were performed. All samples were > 90% mast cells after 4 days with macrophages as the sole-contaminating cell type.

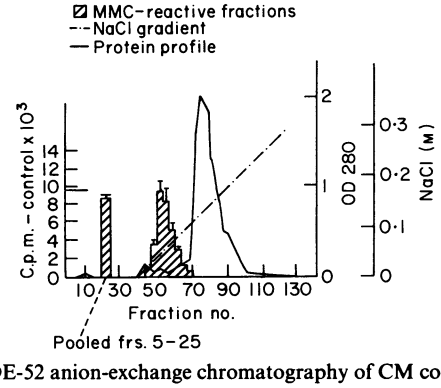

Figure 2. DE-52 anion-exchange chromatography of CM concentrated by ammonium sulphate precipitation. Each fraction (fr.) contained 8 ml eluate. Pooled frs 5–25 = peak 1. Pooled frs 52–60 = peak 2. →The plateau activity of CM (at 25% v/v) prior to fractionation.

confirming that enrichment for early stem cells had occurred. MMC were most often associated with macrophages in mixed colonies, or with blast cells that could be distinguished morphologically by an undifferentiated basophilic cytoplasm. Multipotential colonies were identified by their content of more than two cell types. Frequently such colonies contained MMC, monocytes/macrophages, blast cells and early erythroid progenitors, as well as occasional neutrophils, eosinophils and megakaryocytes. The cell types of the various colonies are

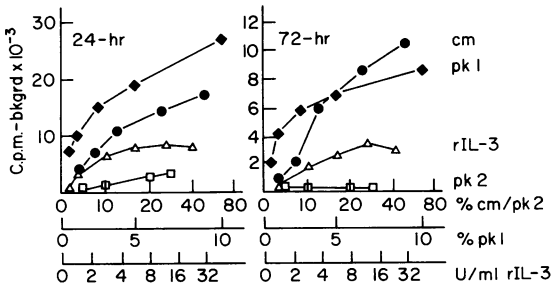

Figure 3. Stimulation of rat MMC by CM; Peak 1 (pk 1); peak 2 (pk 2) and rIL-3. COS monkey cell supernatant was not active in these assays. U/ml, units per ml rIL-3. Wistar rat BM-C derived MMC taken after 2·5 weeks of culture with CM and consisting of >99% MMC. c.p.m. background (bkgd) for 24-hr assay = 416 ± 32; c.p.m. background for 72-hr assay = 161 ± 12.

shown in Table 3. No exogenous erythropoietin was added to the cultures, so the number of colonies containing erythroid cells is probably an underestimate. Few neutrophils were observed in these cultures, which could be due to poor growth support by horse serum or disappearance and death of this cell type by Day 14 of culture. However, colonies containing neutrophils can be seen in 7-day-old cultures (data not shown).

DISCUSSION

This study has shown that: (i) BM-derived MMC develop from haemopoietic cells and proliferate in the presence of recombinant or partially purified rat IL-3; (ii) rIL-3 can substitute for the activity in MLN Cm; (iii) MMC develop from multi-potential haemopoietic stem cells in culture.

Recently, the gene for rat IL-3 was cloned and the expressed product shown to stimulate both BM cell proliferation and multi-potential stem cell development in soft agar cultures (Cohen *et al.*, 1986). The present work further defines the activities of rIL-3 to include the development of MMC, neutrophils, macrophages and eosinophils from bone marrow precursors and the stimulation of MMC proliferation. In these assays rIL-3 and unfractionated CM behaved in the same fashion, suggesting that rIL-3 solely or predominantly may be responsible for these activities in spite of the presence, in CM, of the factors identified in peak 2.

Cultured BM-derived rat mast cells are *in vitro* analogues of the MMC phenotype (McMenamin *et al.*, 1987) and it is this population which responded to rIL-3 in the experiments described in this paper. Both rIL-3 and CM stimulated the proliferation of MMC containing similar amounts of the granule-specific proteinase RMCPII, and there was no differential effect on MMC maturation. High doses of both IL-3 and whole CM inhibited MMC proliferation. This effect occurred in repeated experiments. High concentrations of cytokines may down-regulate their own receptors as is the case with IL-2 (Smith & Cantrell, 1985). Li & Johnson (1984) reported that low concentrations (2·5%) of mouse spleen-CM maintained BM-derived mast cell progenitor cells in a proliferative mode better than higher concentrations (20%) which induced terminal differentiation. Clearly a more detailed examination of the interaction of rIL-3 with its receptors on cultured MMC is required to identify any dose-dependent effect on differentiation (and function) versus proliferation.

Table 3 Soft agar cultures of BM cells surviving treatment with 5-FU

			Colony analysis (%)†						
Cells	Stimulus	Colonies*	MMC	GM	M	N	EOS	Multi‡	BLASTS
5-FU BM§	MLN CM¶	10±2	35	—	11	—	—	44	6
	Peak 1**	26±4	41	—	6	—	—	48	5
	Peak 2††	—	—	—	—	—	—	—	—
	rIL-3‡‡	18±4	26	—	14	—	1	46	7
	COS snt§§	—	—	—	—	—	—	—	
Control BM	MLN CM	65±4	51	6	31	—	3	4	—
	Peak 1	133±13	58	—	34	1	1	6	—
	Peak 2	14±2	—	16	82	—	2	—	—
	rIL-3	121±7	31	11	51	—	1	6	—
	COS snt	5±1	—	—	100	—	—	—	—

* Values per 10^5 cells seeded. Mean ± range for triplicate cultures using Wistar rats, four per group. Cultures analysed on Day 14.

† A minimum of 50 colonies per group analysed. See the Materials and Methods.

‡ Multi = multi-potential colonies containing three or more cell types, usually erythroid MMC, macrophage (M), myeloid precursors and sometimes neutrophils (N), eosinophil (EOS) and megakaryocytes. No pure erythroid or megakaryocyte colonies were seen. Over 70% of multi-potential colonies contained MMC. GM = granulocyte–macrophage colonies which contain neutrophils and macrophages.

§ BM from rats treated with 5-FU.

¶ CM from mesenteric lymph node cells at 20% v/v.

** Unadsorbed peak from DE-52–cellulose fractionation of CM at 10% v/v.

†† Salt-eluted peak from DE-52–cellulose fractionation of CM at 10% v/v.

‡‡ Recombinant rat IL-3 at 25 u/ml.

§§ COS monkey cell supernatant at 10% v/v.

In order to characterize the MMC growth-stimulatory activity in CM further, MLN CM was fractionated over DEAE-cellulose. This procedure revealed two peaks of activity stimulating MMC proliferation. Peak 1 stimulated MMC growth at least as well as whole CM and rIL-3, whereas peak 2 was only transiently active in 24- and 48-hr assays. In addition peak 1 was able to stimulate multi-potential colony formation as well as rIL-3 and expressed virtually all the activity of unfractionated CM whereas peak 2 contained no such activity. Peak 1 would therefore appear to contain rat IL-3 activity with little contaminating GM-CSF. Peaks 1 and 2 are being purified further. Murine IL-3, which stimulates multi-potential stem cell development and mast cell proliferation *in vitro* (Ihle *et al.*, 1982; Hapel *et al.*, 1985; Rennick *et al.*, 1985), elutes (like peak 1) in the unadsorbed fraction from a DEAE-cellulose column equilibrated with low ionic strength buffer at pH 8·0 (Yung *et al.*, 1981; Clark-Lewis & Schrader, 1981).

These studies also show that MMC develop from early multi-potential stem cells. The BM of rats treated with 5-fluorouracil is depleted of cycling cells and is therefore enriched for early haemopoietic stem cells (Hodgson & Bradley, 1979). Stimulation of these cells in semi-solid culture with CM, rIL-3 or the IL-3-enriched peak 1 preparation induced an increase in the number of multi-potential colonies compared to cultures of BM from untreated rats. More than 70% of the colonies that contained at least three distinct cell types also contained MMC. As each colony is the product of a single cell, MMC have developed from multi-potential haemopoietic stem cells. Murine-cultured mast cells, which are thought to be MMC-like, and connective tissue mast cells (CTMC) also have been shown to develop from multi-potential stem cells (Pharr *et al.*, 1984; Garland & Crompton, 1983; Schrader *et al.*, 1983; Prytowsky *et al.*, 1984; Kitamura *et al.*, 1981). However, unlike the putative murine MMC, morphologically recognizable CTMC do not proliferate in the presence of IL-3 alone (Nakahata *et al.*, 1986), even though it has been suggested that murine IL-3-dependent mast cells may be precursors of CTMC (Nakano *et al.*, 1985; Levi-Schaffer *et al.*, 1986).

The rat is still the model system of choice for studying mast cell heterogeneity. The present findings should facilitate a better understanding of MMC differentiation and function.

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