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Rat mucosal mast cells: the cultured bone marrow-derived mast cell is biochemically and functionally analogous to its counterpart *in vivo*

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SUMMARY

Mast cells (MC) are biochemically and functionally heterogeneous and the mixture of MC phenotypes varies according to anatomical location. Intestinal mucosal MC (IMMC) have been used to study the mucosal MC subset in the rat, but they are difficult to isolate in sufficient numbers and with consistent purity and viability. Bone marrow-derived MC (BMMC), with an apparent mucosal MC phenotype, can be cultured in large numbers and with high purity from normal rat bone marrow using supernatants from mesenteric lymph node cells of rats infected with the nematode, *Nippostrongylus brasiliensis*. We have compared serine proteinase content, tumour necrosis factor-α (TNF-α) storage and secretion, and TNF-α-dependent cytotoxicity of IMMC and BMMC to assess the appropriateness of BMMC as *in vitro* models of mucosal MC. Two-dimensional gel electrophoretic analysis revealed that the overall protein constituents of BMMC and IMMC were highly homologous. Immunoblotting confirmed that both MC types expressed the MMC-associated enzyme, rat mast cell proteinase-2 (RMCP-2), but not RMCP-1, mast cell proteinase-5 (MCP-5) or carboxypeptidase A (CPA), which characterize the connective tissue MC in the rat and which were detected in a representative of this subset, namely, the peritoneal MC (PMC). BMMC demonstrated levels of TNF-α-dependent cytotoxicity that were equivalent to those of IMMC. Like IMMC, BMMC contained little stored TNF-α, in comparison with PMC, but both MC types generated substantial amounts of TNF-α 6 hr following IgE-mediated activation. Pretreatment of PMC with recombinant rat interferon-γ (IFN-γ) for 20 hr inhibited anti-immuoglobulin E (anti-IgE)-mediated release of the granule-associated enzyme, β-hexosaminidase, whereas identically treated BMMC were unresponsive to this cytokine. Similar results have previously been reported for IMMC. Rat BMMC, unlike their more immature and less phenotypically committed counterparts in the mouse, appear therefore to be more appropriate models for studies on the mucosal MC.

INTRODUCTION

Mast cell (MC) populations in different tissues can exhibit heterogeneity in many aspects of their phenotype, including morphology, mediator content (reflected in histochemical staining properties), sensitivity to stimulation by various secretagogues, susceptibility to MC-stabilizing drugs and responses to cytokines.[1–3] These manifestations of MC heterogeneity are most clearly seen in the rat where 'connective tissue' MC (CTMC), such as peritoneal and dermal MC, are functionally and biochemically different from mucosal MC (MMC), typified by those located within the intestinal mucosa[4,5] and about 40% of peribronchial MC.[6] The serine proteinase content of rat MC best exemplifies this tissue-specific phenotypic variation. Thus, rat MC proteinase-1 (RMCP-1), MC proteinase-5 (MCP-5) and carboxypeptidase A (CPA) characterize connective tissue MC, such as peritoneal MC (PMC), whereas rat MC proteinase-2 (RMCP-2) is selectively located to mucosal MC, including the intestinal mucosal MC (IMMC).[5–7] Rat bone marrow-derived MC (BMMC) proliferate in haemopoietic cultures in response to interleukin (IL)-3-containing supernatant from antigen- or mitogen-activated T lymphocytes.[8] BMMC are analogous in both proteinase content and staining properties to MMC, which proliferate in the rat gastrointestinal tract in response to helminth infection.[9,10] Separate studies on the functional properties of rat intestinal MMC (IMMC) and BMMC have shown further similarities between the two MC types in their responses to secretagogues, histamine and β-hexosaminidase content and the profile of arachidonic acid metabolites generated upon immunoglobulin E (IgE)-mediated activation.[11–15] IMMC are difficult to isolate in large numbers and high purity. The present study compared, for the first time, biochemical (in particular, protein profiles after electrophorectic separation) and functional properties of

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IMMC and BMMC, in the same assays, in an effort to further assess the suitability of BMMC as *in vitro* analogues of IMMC.

MATERIALS AND METHODS

Animals and parasitic infection

Outbred male Sprague-Dawley rats (weight 300–500 g) were used as the source of PMC and IMMC, and outbred male Wistar rats (weight 225–250 g) were used for the production of conditioned medium and bone marrow cultures. The BMMC culture and IMMC isolation techniques were previously established using Wistar and Sprague-Dawley rat strains, respectively.[9,10,12] The Sprague-Dawley strain was used as the source of IMMC and PMC to maintain consistency with previous studies in our laboratory, although no differences have been noted between IMMC generated using two outbred strains (Sprague-Dawley and Hooded Lister) and an inbred strain of rats (Wistar-Furth) in terms of IMMC morphology, histamine content and responses to secretagogues.[12] All rats were purchased from Charles River (Canada) Inc., St. Constant, Quebec and were maintained in an isolation room with filter-topped cages to minimize unwanted infections. With the exception of those animals used as donors of bone marrow, all rats were infected with 3000 third-stage larvae of *Nippostrongylus brasiliensis* (*Nb*), by a single subcutaneous (s.c.) injection, to stimulate mastogenesis.[12] This experimental protocol was approved by the University of Alberta Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Isolation of PMC and IMMC

Peritoneal cells were collected from rats on >day 35 postinfection with *Nb* by injecting 15 ml of HEPES-buffered Tyrode's solution, containing 0·1% bovine serum albumin (BSA), into the peritoneal cavity. Cells recovered by peritoneal lavage were layered on a two-step discontinuous gradient (30% and 80%) of Percoll (Pharmacia, Uppsala, Sweden) and centrifuged at 600 *g* for 20 min at 4°.[10] PMC were recovered from the pellet and their purity (97–99%) was determined, using morphological characteristics, by light microscopy and toluidine blue staining. IMMC were enzymatically dispersed from the small intestine of rats infected with *Nb* at least 35 days previously. The details of the procedure for IMMC isolation and subsequent enrichment using Percoll density centrifugation have recently been reviewed in detail.[16] The purity of IMMC in the present experiments ranged from 78 to 93%, with small lymphocytes being the main contaminating cells. MC viability was ≥95%.

Preparation of mesenteric lymph node-conditioned medium (CM)

Mesenteric lymph nodes were excised 15–25 days after infection of Wistar rats with *Nb*. Lymph node cells were plated at 4×10^6 viable nucleated cells per ml in Iscove's serum-free medium (ICN Biomedicals Inc., Montreal, Canada), containing 100 U/ml penicillin, 100 μg/ml streptomycin and 3 μg/ml concanavalin A, in 175 cm^2 filter-vented tissue culture flasks. Cultures were incubated for 48 hr at 37° in a humidified atmosphere of 5% CO_2 in air, and the supernatants were then harvested by centrifugation, sterilized by filtration and stored at −20°. Batches of CM were assessed for their ability to stimulate the growth of rat BMMC in culture and were used in bulk cultures at the optimal concentration (15–20%).

Culture of BMMC

Liquid cultures of normal Wistar rat bone marrow cells were prepared as previously described.[9,15] A final concentration of $2{\cdot}5 \times 10^5$ viable nucleated bone marrow cells was suspended in Iscove's medium, together with 100 U/ml penicillin, 100 μg/ml streptomycin, 10% heat-inactivated horse serum (Gibco BRL, Burlington, Ontario, Canada) and an optimal amount of CM (15–20%), in 175 cm^2 flasks. Cultures were kept at 37° in a humidified incubator with 5% CO_2 in air and were refed and restimulated with medium containing CM every 4–7 days, depending upon visual assessment of the pH of the culture medium, i.e. change in the colour of the phenol red indicator. Estimations of MC numbers in bone marrow cultures were based upon a total of 1000 cells counted on duplicate Leishman-stained cytosmear preparations. For the present experiments, BMMC were harvested from liquid cultures 21–28 days after the start of the culture and were ≥98% pure and ≥99% viable as assessed by exclusion of trypan blue dye and the cells' optical properties under phase contrast microscopy. Using flow cytometry analysis, >95% of BMMC have been shown to express surface IgE (A.J. MacDonald, unpublished observations) acquired from CM, which contains up to 2 μg IgE/ml (D.M. Haig, personal communication).

SDS–PAGE electrophoresis and immunoblot analysis

Unless otherwise stated, all reagents used for electrophoresis and immunoblot analysis were purchased from BioRad (Mississauga, Ontario, Canada). Phenylmethylsulphonyl fluoride (PMSF), aprotinin, 3-[cyclohexylamino]-1-propanesulphonic acid (CAPS), β-mercaptoethanol and HEPES were from Sigma Chemical Co., St. Louis, MO. *bis*-Acrylamide was from Boehringer Mannheim (Quebec, Canada). Goat antibodies to rabbit immunoglobulin G (IgG) (heavy and light chain), which were conjugated with horseradish peroxidase (HRP) were purchased from Gibco BRL. Affinity-purified rabbit anti-RMCP-1 and sheep anti-RMCP-2 antibodies were kindly provided by Professor Hugh Miller, University of Edinburgh, UK. Polyclonal antipeptide antibodies to MCP-5 and CPA were raised in rabbits as previously described.[7]

Analytical or electrophoresis-grade reagents and distilled and deionized water were used to prepare: Tris-buffered saline with 1% Tween-20 (TBS-T), 20 mM Tris-HCl, pH 7·6, and 137 mM NaCl; HRP developer with 1 part of 3 mg/ml HRP colour development reagent in methanol and 5 parts 0·018% hydrogen peroxide in TBS; equilibration buffer with 0·1% glycerol, 0·05% β-mercaptoethanol, 0·06 M Tris-HCl, 100 kallikrein inhibitor units of aprotinin and 2 mM PMSF, pH 6·8; HEPES-Tyrode's buffer with 0·14 M NaCl, 0·1% glucose, 3 mM KCl, 1 mM $CaCl_2.2H_2O$, 0·1% bovine serum albumin, 12 mM HEPES, 0·5 mM $NaH_2PO_4.2H_2O$; CAPS buffer with 10% methanol and 10 mM CAPS; IEF/PC buffer (for two-dimensional gel separation of proteins) with 0·5% dipalmitoyl 1-α-phosphatidylcholine, 9·5 M urea, 2% Biolyte 3/10, 5% β-mercaptoethanol, 2 mM PMSF and 100 kallikrein inhibitor units of aprotinin, pH 3·0.

As described previously,[17] for two-dimensional gel separation of PMC proteins using non-equilibrium pH gradient electrophoresis (NEPHGE), purified MC were solubilized in

IEF/PC buffer for 1 hr and then centrifuged at 160 000 *g* for 30 min. Hoeffer (San Francisco, CA) mini tube gels were poured and 10^5 MC equivalents were loaded in each, in some cases with two-dimensional protein standards (Bio-Rad). The tubes were run at 100 V for 10 min and then at 400 V for 75 min. The gels were extruded from the tubes, washed in equilibrium buffer for 10–15 min, put into the large well of a Bio-Rad apparatus with a 12% resolving and 5% stacking gel and Amersham (Oakville, Ontario, Canada) rainbow molecular weight markers were put into the reference well. The apparatus was run at 200 V for $\approx$1 hr. A Bio-Rad kit was used for all silver-stained gels according to the manufacturers' protocol.

For HRP development, the gels were equilibrated in CAPS, pH 11, for 30 min and the Hybond membranes (Bio-Rad) were dipped in methanol and then equilibrated in CAPS for 10 min. The proteins were then transferred from the gels to the membranes at 100 V for 1 hr as described previously.[17] The membranes were air-dried and stored dessicated at 4° for subsequent use. Membranes were incubated with the primary antibody for 3 hr, then washed with TBS-T. The secondary antibody was incubated with the membrane for 1 hr, then washed with TBS-T. HRP developer was added for 1 hr, followed by an H_2O wash and visualization of the reactions.

Cytotoxicity assay

MC cytotoxicity was measured using a ^{51}Cr-release assay with, as target cells, the tumour necrosis factor-α (TNF-α)-sensitive murine fibrosarcoma cell line, WEHI-164, obtained from Dr P. Ernst, University of Texas, Galveston, TX. Briefly, MC were suspended in RPMI-1640 medium supplemented with 5% fetal bovine serum and distributed in triplicate at different concentrations ($6{\cdot}25 \times 10$,^{3} $12{\cdot}5 \times 10^3$ and 25×10^3 MC) giving ratios of effector cells:target cells of 2·5:1, 5:1 and 10:1, respectively, in round-bottomed 96-well microtitre plates. WEHI-164 cells were labelled with 100 mCi of $Na^{51}CrO_4$ (ICN Pharmaceuticals Ltd, Montreal, Canada) for 90 min, washed three times, and added to the MC. Spontaneous release (SR) corresponded to the ^{51}Cr released from target cells in the presence of RPMI medium without MC. Total releasable (TR) ^{51}Cr was measured by adding 0·01% Triton-X-100 (Sigma Chemical Co.) to the target cells. Plates were incubated for 16 hr, centrifuged at 150 *g* for 5 min, and the radioactivity was determined in cell-free supernatants. The percentage cytotoxicity was calculated using the formula:

$$\frac{\text{c.p.m. in presence of MC} - \text{SR}}{\text{TR} - \text{SR}} \times \frac{100}{1}$$

Results are expressed in lytic units (LU) for 20% cytotoxicity ($LU_{20}/10^6$ MC) calculated using the equations published by Pross *et al.*[18] For control purposes, IMMC and PMC were incubated in the same medium as BMMC (i.e. containing 15–20% CM).

TNF-α assay

TNF-α activity was measured in cell-free supernatants and lysates from MC incubated for 6 hr in the presence or absence of mouse monoclonal anti-rat IgE at a concentration of 5 μg/ml (MARE-1, Serotec, Oxford, UK), using an alamarBlue (BioSource International, Camarillo, CA) assay.[19] Samples were serially diluted in microtitre plates for testing against WEHI-164 clone 13 cells (a generous gift from Dr T. Mosmann, University of Alberta, Canada). The TNF-α activity was determined by comparison with a standard curve of mouse rTNF-α cytotoxicity against WEHI-164 clone 13 cells using probit analysis.[20] The specificity of TNF-α activity in some samples was verified using an enzyme-linked immunoassay (ELISA) kit (Biosource International) for rat TNF-α and the results confirmed our values from the bioassay.

β-Hexosaminidase assay

β-Hexosaminidase (β-hex) activity was assayed by hydrolysis of the substrate *p*-nitrophenol-β-D-2-acetamido-2-deoxy-glucopyranoside (Sigma). One unit of this enzyme cleaves 1 μmol of substrate/hr at 37°.[21] Samples (20 μl) were added to duplicate wells in a microtitre plate on ice, followed by 40 μl of 5 mM substrate in 0·15 M disodium citrate buffer titrated to pH 4·5, and the mixture was incubated at 37° for 45 min. The reaction was terminated by the addition of 150 μl of ice-cold 0·2 M glycine-NaOH buffer, pH 10·7, and the absorbance was read at 405 nm. The A_{405} for blank (control) wells, where samples were replaced with buffer, were automatically subtracted from the sample A_{405} and the percentage β-hex released calculated by the formula:

$$\frac{A_{405} \text{ of supernatant samples}}{A_{405} \text{ of pellet samples} + A_{405} \text{ of supernatant samples}} \times \frac{100}{1}$$

β-Hex release from BMMC and PMC was stimulated by incubation with 5 μg/ml monclonal anti-rat IgE for 15 min at 37°. The reaction was terminated by rapid cooling in iced water and centrifugation (6000 *g*; 4 min; 4°). Recombinant rat interferon-γ (rrIFN-γ), for incubation with BMMC and PMC, was purchased from Immunocorp (Montreal, Quebec, Canada).

Statistical analyses

Analysis of variance (ANOVA) and Student's *t*-test for paired samples were applied using Microsoft Excel software. A value of $P < 0{\cdot}05$ was taken as significant for paired comparisons and $P < 0{\cdot}025$ was taken as significant for multiple comparisons. Results are expressed as the mean ± standard deviation (SD).

RESULTS

Comparison of BMMC and IMMC proteins

The spectrum of silver-stained proteins in BMMC and IMMC following separation by NEPHGE in the first dimension and SDS–PAGE in the second dimension is shown in Fig. 1. For comparison, PMC proteins were also analysed. The protein profiles of PMC and IMMC were very similar to those previously published by Abe *et al.*[17]

The patterns of basic proteins in BMMC and IMMC were strikingly similar and were in marked contrast to those of PMC (Fig. 1a, c, e). For example, basic bone marrow proteins BB1–BB6 corresponded in molecular weights (MW) and isoelectric points (pI) to intestinal mucosal basic proteins IB1–IB6. A similar relationship was seen between BB12 and IB8 (both 24 000 MW), the dominant protein spots in BMMC and IMMC, respectively. There were also some differences between the basic proteins of BMMC and IMMC. For example, BB8–BB11 (24–27 000 MW and approximately

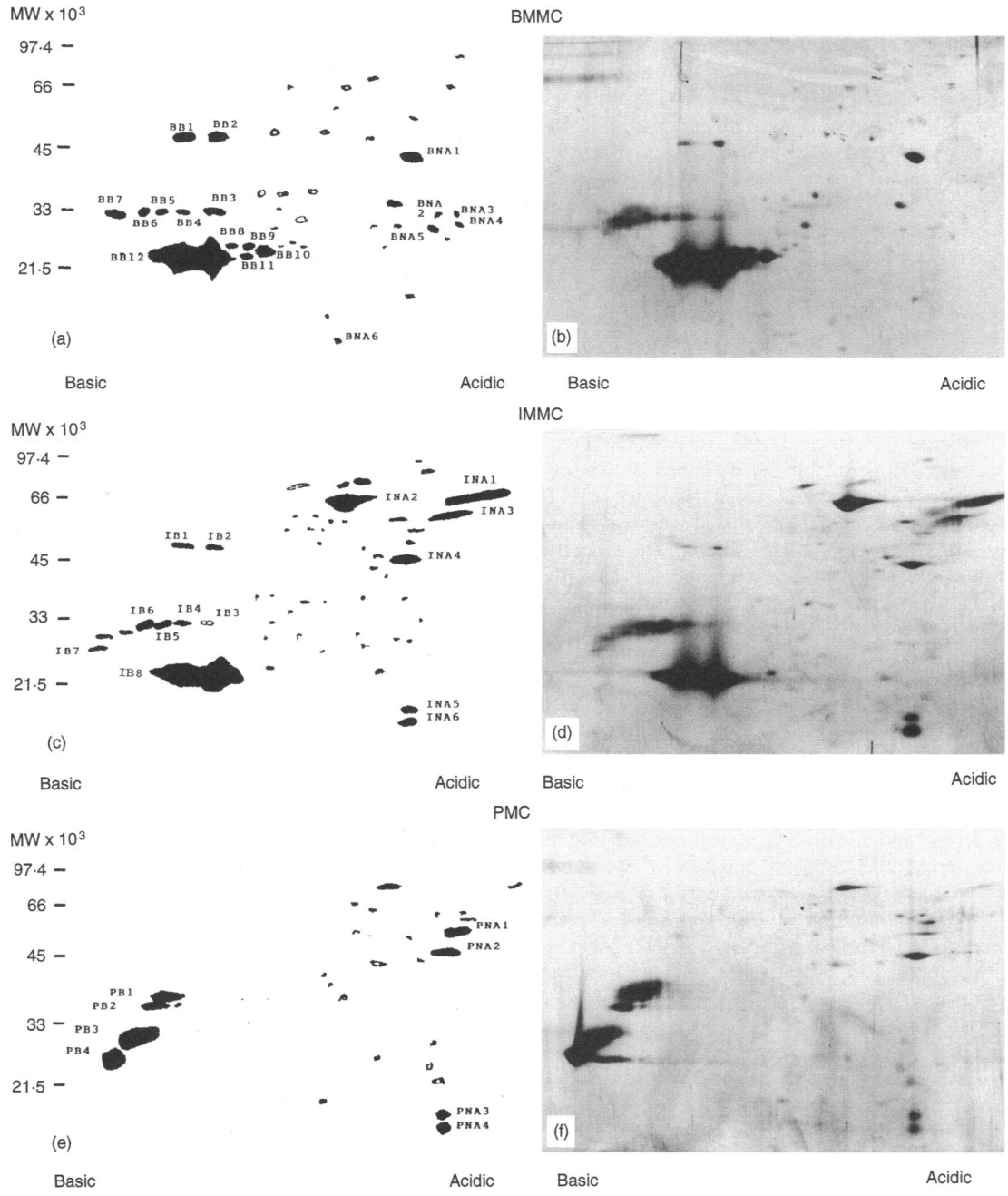

Figure 1. Spectrum of silver-stained proteins from rat BMMC, IMMC and PMC separated by two-dimensional gel electrophoresis using the NEPHGE system. (b), (d) and (f) are representative gels of BMMC, IMMC and PMC, respectively, and (a), (c) and (e) are tracings of the same gels with major spots labelled which consistently appeared in all eight BMMC, six IMMC and four PMC gels, respectively. B, BMMC; I, IMMC; P, PMC; BB, BMMC basic; BNA, BMMC neutral–acidic.

neutral pI) were not seen consistently in IMMC. Similarly, IB7 had no counterpart in BMMC gels.

The patterns of neutral–acidic proteins in all three types of MC were more variable from gel to gel, probably because of their much lower abundance in comparison to the basic proteins. In general, proteins with sizes of 45–95 000 MW predominated. All three MC types shared a spot with molecular mass of 45 000 and pI of ≈6–6·5 (BNA1, INA4 and PNA2). There were also differences in neutral–acidic proteins among the MC types. For example, IMMC and PMC shared spots of molecular masses 16 000 and 14 000 and a pI of ≈6·0 (INA5, INA6 and PNA3, PNA4) which were sometimes, but not always, found in BMMC. BMMC also contained some acidic proteins (BNA2–BNA5) which were not found in either IMMC or PMC.

Proteinase expression by BMMC and IMMC

On Western blot analysis (Fig. 2), both BMMC and IMMC expressed RMCP-2, which was positive at spots corresponding to BB12 and IB8 (24 000 MW), respectively. In some immunoblots, RMCP-2 positivity was also detected at BB1, BB2, IB1

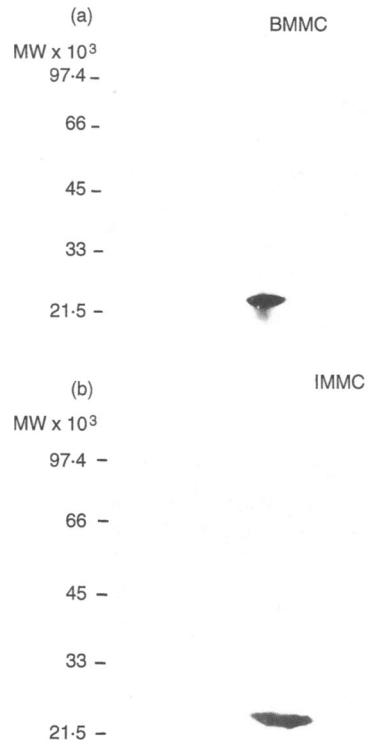

Figure 2. Immunoblot analysis of BMMC and IMMC proteins separated by two-dimensional gel electrophoresis and probed with anti-RMCP-2 antibodies. RMCP-1, MCP-5 and CPA were undetectable.

and IB2 (48 000 MW) (data not shown) in a manner similar to previously published results using IMMC.[17] Neither BMMC nor IMMC expressed the CTMC-associated proteinases RMCP-1, MCP-5 or CPA (negative blots not shown). Consistent with previous results, PMC did not express RMCP-2.[6,7]

Preformed and newly synthesized TNF-α in BMMC and IMMC

With the exception of supernatants from unstimulated MC after 6 hr of culture (Fig. 3), there were no significant differences between the absolute amounts of cell-associated TNF-α (i.e. in the cell pellet) in unstimulated MC or 6 hr after treatment with anti-IgE. However, compared with IMMC, BMMC showed a greater increase in TNF-α generation, both released and cell associated, following stimulation with anti-IgE.

Cytotoxicity of BMMC, IMMC and PMC for WEHI-164 cells

There were no significant differences between the three types of MC tested in the TNF-α-dependent cytotoxicity assay after 16 hr of incubation (Fig. 4). BMMC, IMMC and PMC showed comparable cytotoxicity for the tumour cell target.

Effect of rrIFN-γ on anti-IgE-mediated release of β-hexosaminidase by BMMC and PMC

Incubation of PMC with rrIFN-γ for 20 hr resulted in a dose-dependent inhibition of β-hex release following stimulation

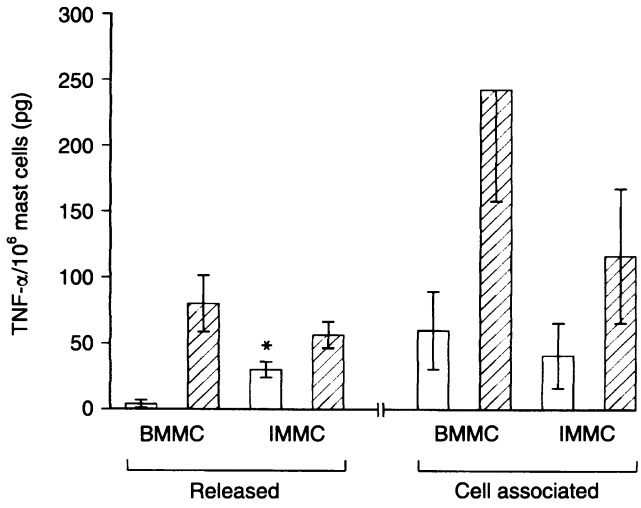

Figure 3. Comparison of secreted and cell-associated TNF-α in rat BMMC and IMMC after incubation for 6 hr with either buffer (□) or mouse monoclonal antibody to rat IgE (▨); data represents the mean ± SD of three experiments (i.e. three separate BMMC cultures and IMMC isolations) with triplicates in each experiment. There were no significant differences between BMMC and IMMC, with the exception of supernatants from unstimulated MC (BMMC = 4·0 ± 3·0; IMMC = 30·1 ± 6·1 pg TNF-α/10^6 MC; $*P < 0{\cdot}05$, $n = 3$, Student's *t*-test for paired data).

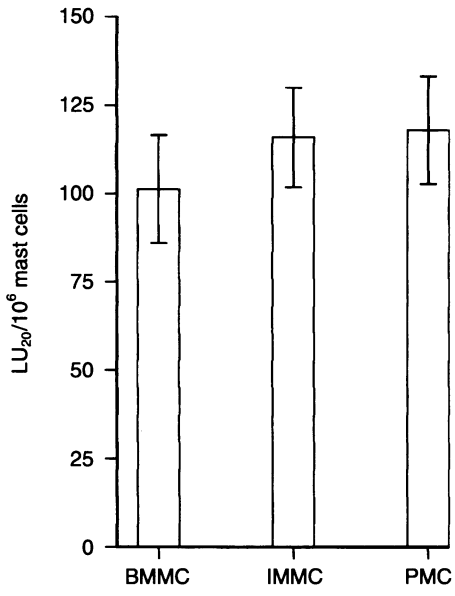

Figure 4. Comparison of the cytotoxic activities of rat BMMC, IMMC and PMC against TNF-α-sensitive WEHI-164 cells; data represents the mean ± SD of three experiments (i.e. three separate BMMC cultures and IMMC and PMC isolations) with triplicates in each experiment. Cytotoxic activity is expressed in lytic units for 20% toxicity (LU_{20}) per 10^6 MC; there were no significant differences between the three MC types.

with anti-IgE, which was significant ($P < 0{\cdot}025$) at 400 and 800 U/ml IFN-γ (Fig. 5). Using IFN-γ at 800 U/ml, the inhibition of β-hex release was ≈40%. By contrast, IFN-γ had no significant effect on β-hex release from anti-IgE-treated BMMC. Using a high concentration of IFN-γ (800 U/ml),

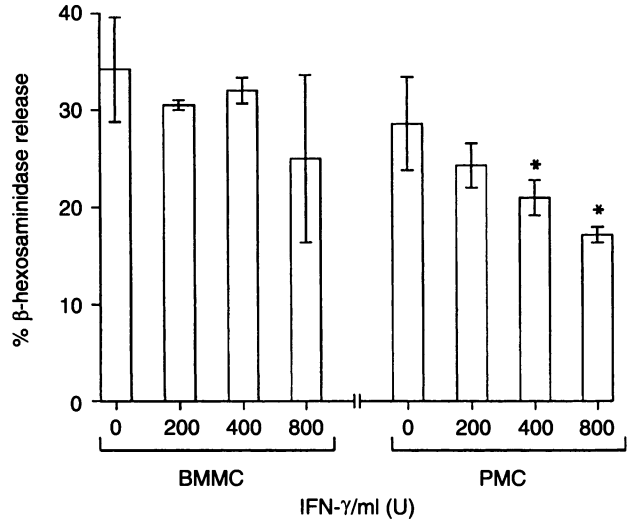

Figure 5. Effect of recombinant rat IFN-γ (rrIFN-γ) on release of β-hexosaminidase (β-hex) from rat BMMC and PMC. MC were preincubated with a range of concentrations of rrIFN-γ for 20 hr, washed and treated with mouse anti-rat IgE for 15 min. Spontaneous release in the absence of anti-IgE was less than 5%. Each bar represents the mean ± SD of three experiments (i.e. three separate BMMC cultures and PMC isolations) with triplicates in each experiment; $*P < 0.025$; Student's *t*-test with multiple comparisons).

there was a slight, although not statistically significant, reduction in β-hex released by BMMC. Previous studies have shown that like BMMC, IMMC are unresponsive to IFN-γ under these experimental conditions, in terms of histamine release.[11]

DISCUSSION

Cultured rat BMMC were compared biochemically and functionally in parallel assays with their putative *in vivo* counterparts, IMMC, to assess their appropriateness as *in vitro*-derived 'mucosal MC'.

The results of our two-dimensional gel and immunoblotting analysis confirm previous comparisons of protein profiles and proteinase phenotype of IMMC and PMC[7,17] and extend these findings to define cultured rat BMMC as highly analogous to IMMC. The electrophoretic pattern of BMMC proteins was not, however, entirely homologous with that of IMMC components. Silver-stained spots BB8–BB11 (Fig. 1a) were prominent in BMMC, but not in IMMC (even when IMMC purity was 93%). This region stains with anti-RMCP-2 antibodies, although resolution of the individual spots is not always possible. *In vitro* translation of mRNA from IMMC[22] yielded a 27 000 MW putative RMCP-2 precursor, which is consistent in molecular mass and approximate pI with BB8 and/or BB9. Comparison of the relative abundance of BB3–BB7 and BB8–BB11 in BMMC and IMMC could facilitate identification of these protein species by microsequencing. The BMMC at the time of harvesting (days 21, 25 and 28 of culture in the present study) may be developmentally more immature than the IMMC, and actively synthesizing RMCP-2 in its precursor forms. There were no obvious differences in the protein profiles of 21-, 25- or 28-day-old BMMC, although we did not examine BMMC towards the end of their lifespan in cultures of 35–42 days. The use of BMMC will facilitate the isolation of proteins which are in low abundance in IMMC.

Rat BMMC were shown, for the first time, to store TNF-α and to synthesize this potent proinflammatory cytokine following IgE-mediated activation. Like mouse BMMC,[23] rat BMMC stored considerably less TNF-α than was previously reported for PMC from mice[23] and rats.[11] The elevated TNF-α in supernatants of unstimulated IMMC, compared with BMMC (Fig. 3), may reflect the isolation procedure used for IMMC, although no significant difference was observed following stimulation with anti-IgE. In the present study, BMMC were more akin to IMMC than PMC in terms of their TNF-α content and synthesis.

Unstimulated BMMC, IMMC and PMC showed comparable cytotoxicity for the TNF-α-dependent cell line, WEHI-164, notwithstanding the higher spontaneous release of TNF-α by IMMC, compared with BMMC (Fig. 3), and that reported for PMC relative to IMMC.[11] This may reflect a threshold level of TNF-α above which no further increase in cytotoxicity is attainable.

The IgE-mediated release of β-hex, an enzyme whose release is highly correlated with that of histamine from rat BMMC (A.J. MacDonald, unpublished data), was not significantly affected by prior incubation in a range of concentrations of IFN-γ (Fig. 5). In this respect, BMMC function like IMMC which, unlike PMC, are unresponsive to IFN-γ in terms of histamine release.[11] Interestingly, IFN-γ has recently been reported to augment histamine release from cultured human MC;[24] however, the conditions under which the human MC were grown were considerably different to those inducing rat BMMC growth.

As we used different strains of rats as our sources of IMMC and BMMC, we were initially concerned that this might result in functional and biochemical disparities between the two MC types. However, our results revealed a convergence of phenotype, rather than a strain-related divergence, between IMMC and BMMC. This is consistent with previous observations where no appreciable strain-associated differences were noted among either IMMC[10,12] or BMMC[8,9,14,15] obtained from different rat strains in terms of their mediator contents and responses to secretagogues.

Mouse BMMC are a widely used cultured MC type which express an immature MMC-like phenotype,[4] a mixture of CTMC- and MMC-associated proteinases[25] and considerable phenotypic plasticity.[26,27] Rat BMMC are much more homogeneous in their proteinase content[28] and are more committed to the MMC phenotype.[29,30] As such, we propose that rat BMMC may be more relevant models of mature MMC than mouse BMMC.

In summary, rat BMMC are strikingly similar to IMMC in terms of:

(1) their protein profiles on two-dimensional gels;
(2) proteinase expression;
(3) TNF-α content;
(4) cytotoxicity for a TNF-α-sensitive target cell line; and
(5) response to IFN-γ.

These findings identify rat BMMC as highly appropriate *in vitro* models for mucosal MC.

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