Differential Expression of Secretory Granule Proteases in Mouse Mast Cells Exposed to Interleukin 3 and \textit{c-kit} Ligand

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Summary

It is now established that the subclasses of mast cells (MC) that reside in mucosal and serosal environments can be distinguished from one another in terms of their expression of specific secretory granule-localized proteases and proteoglycans. Further, the hematopoietic- and connective tissue–derived cytokines that regulate expression of the genes that encode these constituents of the granule can now be identified using recently developed gene-specific probes and recombinant cytokines. When bone marrow–derived MC (BMMC) were developed with recombinant interleukin 3 (rIL-3) and maintained with this cytokine in the absence or presence of recombinant \textit{c-kit} ligand (rKL), they remained safranin−, produced almost no $^{35}$S-labeled heparin proteoglycans, and contained greater levels of mouse MC protease (MMCP) -5 mRNA and mast cell carboxypeptidase A (MC-CPA) mRNA than MMCP-6 mRNA. They did not contain MMCP-4 or -2 mRNA, genes expressed late in the differentiation of progenitor cells into serosal and mucosal MCs, respectively. In contrast, BMMC developed with rKL alone or by sequential culture in medium containing rIL-3 followed by rKL expressed high levels of MMCP-4 and -6 mRNA, as well as the transcripts that encode MMCP-5 and MC-CPA. Although rKL-developed BMMC were safranin+ and produced substantial amounts of $^{35}$S-labeled heparin proteoglycans, they contained only minimal amounts of histamine and MC-CPA enzymatic activity relative to serosal MC. These are the first studies to characterize the transcriptional granule phenotype of a population of BMMC derived using any recombinant cytokine, to demonstrate a dissociation between histochemical staining and granule maturation, and to demonstrate antagonistic regulation of late expressed protease genes by a cytokine.

**Abbreviations used in this paper:** BMMC, bone marrow–derived MC; KL, \textit{c-kit} ligand; MC, mast cell; MC-CPA, MC carboxypeptidase A; MMCP, mouse MC protease; SG-PG, secretory granule proteoglycan peptide core; and TSG, 0.1 M Tris-HCl, 0.1 M sodium sulfate, and 4 M GnHCl.

Microenvironments (10–14). Mouse bone marrow cells cultured in medium containing IL-3 differentiate into morphologically immature MC that express the high affinity IgE receptor FcεRI (15–21). When this receptor is crosslinked with antigen, lipid mediators are generated and released (22–24), cytokines are transcribed and translated (25–28), and preformed mediators are released from the secretory granules (2, 22, 29, 30). These in vitro differentiated mouse bone marrow–derived MC (BMMC) are alcian blue+/safranin− when stained, and they preferentially synthesize chondroitin sulfate E proteoglycans, rather than the heparin proteoglycans that predominate in mouse serosal MC (1). BMMC are poorly granulated (31, 32) and contain low amounts of histamine (16, 20), secretory granule serine proteases (29), and MC-CPA (2). The granule proteases expressed by BMMC derived using rIL-3 (termed BMMCIL-3) have not been determined, but BMMC differentiated in the presence of WEHI-3 cell–conditioned medium as a source of IL-3 (termed BMMCw) ex-
press the transcripts that encode MC-CPA (4), MMCP-5 (7), and -6 (8), but not MMCP-2 (5) or -4 (6). The latter two are late expressed protease genes of mucosal and serosal MC, respectively.

During 4 wk of coculture of BMMC with mouse 3T3 fibroblasts in the presence of WEHI-3 cell-conditioned medium, the resulting cells, termed BMMC-W, become safranin- and preferentially synthesize heparin proteoglycans rather than chondroitin sulfate proteoglycans (12). Their secretory granules also become more electron dense (32), possessing amounts of histamine (12, 33) and MC-CPA activity (2, 33) comparable to in vivo differentiated serosal MC. Both WBB6F1-W/W v mice which contain an altered (34) plasma membrane-localized tyrosine kinase designated c-kit (35, 36), and WCB6F1-SI/SI1, which do not produce a membrane form of the fibroblast-derived cytokine designated c-kit ligand (KL), also known as stem cell factor or MC growth factor (35, 36), are MC-CPA deficient (45, 46). Recombinant KL functions as a pluripotent cytokine (47-49) that elicits proliferation of mouse MC in vitro (41, 48). It also induces the appearance of safranin- MC when injected subcutaneously into SI/SI1 mice (44), but it is not known which granule proteases are expressed by MC exposed to rKL.

In this study, we have examined the transcriptional phenotypes of mouse MC that have been derived in vitro directly from bone marrow cells using either rKL or rIL-3, and sequentially using BMMC-W conditioned with rKL, rIL-3, or both together. We show that even though rIL-3 and rKL both induce the proliferation and differentiation of hematopoietic progenitor cells into MC, the resulting MC can be distinguished by their expression of certain constituents of their secretory granules. In contrast to rIL-3, rKL not only induces the expression of heparin proteoglycans, but also the transcription of a late expressed gene that encodes one of the novel secretory granule proteases (MMCP-4) that is preferentially expressed by mouse serosal MC. rIL-3 has an antagonistic effect on these rKL-induced phenotypic changes. These findings demonstrate cytokine regulation of transcription of secretory granule neutral protease genes, and specifically illustrate the sequential and competitive interaction of IL-3 and KL in determining the eventual protease phenotype of BMMC.

Materials and Methods

Culture of MC. Mouse BMMC were obtained by culturing bone marrow cells from the femurs and tibias of 6-16-wk-old BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, MA) for 1-3 wk in enriched medium (RPMI 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml gentamicin, 2 mM l-glutamine, 0.1 mM nonessential amino acids, 50 μM 2-ME, and 10% FCS [Gibco Laboratories, Grand Island, NY]) containing either 200 ng/ml rKL (Immunex Corporation) or 40 U/ml rIL-3 (Genzyme Corp., Boston, MA). Alternatively, mouse bone marrow cultures were cultured for 3 wk in 50% enriched medium and 50% WEHI-3 cell (line TIB-68; American Type Culture Collection, Rockville, MD) -conditioned medium (20). In some instances, these latter BMMC-W were washed twice with 100% enriched medium, and were then cultured for 1-2 wk at an initial density of 1-3 × 10⁶ cells/ml in enriched medium supplemented with 20-100 U/ml rIL-3, 200 ng/ml rKL, or a combination of both cytokines to study their action on an elicited immature MC population. rKL and rIL-3 were both expressed in yeast. Previous reports had shown that 20 U/ml of IL-3 purified from WEHI-3 cell-conditioned medium was sufficient to induce the proliferation and differentiation of BMMC (20), and that a maximum proliferation of MC-9 cells occurred when this transformed MC line was exposed to 100 ng/ml of rKL (48). Every 7 d, the nonadherent cells in all of the cultures were transferred into fresh culture medium containing the appropriate cytokine and adjusted to a density of 1-3 × 10⁶ cells/ml. Cytocentrifuge preparations of the cultured MC were stained with toluidine or with alcian blue followed by safranin (50, 51).

RNA Blot Analysis. Total cellular RNA was isolated by the method of Chomczynski and Sacchi (52), and was quantified by measurement of its OD at 260 nm. Approximately equal amounts of total RNA were applied to individual lanes of 1.3% formaldehyde-agarose gels, and electrophoresis was carried out for 17-24 h. The separated RNAs were transferred to nylon membranes (Cuno Inc., Meriden, CT) (53), and the resulting blots were probed with radiolabeled cDNAs that encode either mouse actin (34), MC-CPA (4), MMCP-2 (5), -4 (-6), MMCP-5 (7), -6 (8), mouse secretory granule proteoglycan peptide core (SG-PG) (55), or the c~ chain of FceRI (28, 56). All hybridizations were performed with α-32P-dCTP (>3,000 Ci/mmol; DuPont/New England Nuclear, Boston, MA) random-primed (Boehringer Mannheim, Indianapolis, IN) cDNA probes at 43°C for 24 h in 50% formamide, 0.75 M NaCl, 75 mM sodium citrate, 0.1% SDS, 5 mM EDTA, 50 mM sodium phosphate, 2× Denhardt's buffer, and 100 μg/ml denatured, single-stranded herring sperm DNA (Sigma Chemical Co., St. Louis, MO). The RNA blots were washed under conditions of high stringency (55°C; 30 mM NaCl, 3 mM sodium citrate, 0.1% SDS, 1 mM EDTA, and 10 mM sodium phosphate, pH 7.0), and autoradiography was performed with Kodak XAR-5 film, generally for 48 h. The 32P-labeled probe was removed from each blot by a 1-h incubation at 65°C in 0.2 M EDTA, 0.5% pyrophosphate, 0.1× Denhardt's buffer, 5 mM Tris-HCl, pH 8.0. The same blot was then assessed for the presence of other transcripts. The presence of MMCP-4 mRNA in rKL-treated cultures was confirmed using a RNase protection assay kit from Ambion, Inc. (Austin, TX).

Proteoglycan, Histamine, and MC-CPA Analyses. BMMC-W were cultured for 1 or 2 wk in enriched medium supplemented with rIL-3, rKL, or both cytokines, and then were radiolabeled for 3.5 h at 37°C at a density of 5 × 10⁶ cells/ml in fresh enriched medium containing the appropriate cytokine and 50 μCi/ml of [35S]sulfate (10 Ci/mmol; DuPont). The supernatants were removed, analyzed for the presence of 35S-labeled macromolecules (1), and then discarded. The pellet 35S-labeled MC were lysed by suspending the cells in 150 μl of 1% Zwittergent 3-12 (Calbiochem-Behring Corp., San Diego, CA) containing protease inhibitors (57) for ~30 s at room temperature, and then in 1,350 μl of TSG buffer (0.1 M Tris-HCl, 0.1 M sodium sulfate, 4 M GuanHCl, pH 7.0). After cell sonication, 200 μg heparin (Sigma Chemical Co.) and 200 μg chondroitin sulfate C (ICN Biochemicals, Lisle, IL) were added as non-radiolabeled glycosaminoglycan carriers to each supernatant and cell lysate. To determine the incorporation of [35S]sulfate into proteoglycan, a 5% portion of each sample was chromatographed on separate Sephadex G-25/FP-10 (Pharmacia Fine Chemicals, Piscataway, NJ) gel filtration columns that had been equilibrated in TSG buffer. Solid CsCl was added to the remainder of the cell extract samples to achieve final densities of 1.4 g/ml, and these samples were centrifuged at 100,000 g for >42 h at 20°C (58, 59). After ultracentrifugation, each density gradient was divided into two approximately equal fractions. The 35S-labeled macromolecules

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present in samples of both the low and high density fractions were quantified by Sephadex G-25 chromatography. The high density fractions were then dialyzed against distilled water for 1 h at 4°C, against 0.5 M sodium acetate for ~7 h, and finally against 0.1 M ammonium bicarbonate for 48 h. After lyophilization, the samples were resuspended in 1 ml distilled water and stored at ~20°C. To determine the hydrodynamic sizes of the 35S-labeled macromolecules, portions of the purified 35S-labeled macromolecules were made 4 M in GdnHCl, and were applied to a 1 x 85-cm Sepharose CL-4B (Pharmacia Fine Chemicals) column that had been equilibrated at room temperature under dissociative conditions with TSG buffer. The radioactivity present in each collected 0.5-ml fraction was quantified by β-scintillation counting.

To quantitate the amount of 35S-labeled heparin and/or heparan sulfate glycosaminoglycans in the cell lysates, 25-μl samples of the density gradient-purified 35S-labeled macromolecules were incubated for 1 h at 0°C with 75 μl of a fresh preparation of 0.25 N nitrous acid, pH 1.5 (60). The reactions were terminated by the addition of 150 μl of 1 M sodium carbonate followed by 200 μl of TSG buffer. The amount of 35S-labeled chondroitin sulfate in each preparation of 35S-labeled proteoglycans was determined by assessing the susceptibility of each preparation to digestion by chondroitinase ABC (ICN Biochemicals) (61, 62). The nitrous acid-hippuryl-t-phenylalanine.

of enzyme needed to produce 1 nmole of phenylalanine/min from each preparation of 35S-labeled proteoglycans was determined by cubated for 1 h with 75 μl of a fresh preparation of 0.25 N TSG buffer. The amount of 35S-labeled chondroitin sulfate in each preparation of 35S-labeled proteoglycans was determined by assessing the susceptibility of each preparation to digestion by chondroitinase ABC (ICN Biochemicals) (61, 62). The nitrous acid-hippuryl-t-phenylalanine.

Results

Histochemical and Transcriptional Phenotype of Mouse MC Derived Directly by Culturing Mouse Bone Marrow Cells in Medium Containing either rIL-3 or rKL. As assessed by toluidine blue staining, >99% of the cells derived by culturing bone marrow cells in the presence of rIL-3 (BMMCw/IL-3) (Fig. 1 a) or WEHI-3 cell–conditioned medium (BMMCw) (data not shown) for 3 wk were MC. When stained with alcin blue followed by safranin, the granules of all of the MC in these two culture systems were alcin blue+/safranin+ (Fig. 1 c). In contrast, <30% of the cells in the rKL-treated bone marrow cultures were MC (BMMCw/KL) by vital staining of their granules (Fig. 1 b), but almost all of these BMMCw/KL had granules that were alcin blue+/safranin+ (Fig. 1 d).

RNA blot analyses indicated that starting mouse bone marrow cells contained abundant amounts of SG-PG mRNA, but no detectable levels of those transcripts that encode MC-CPA, MMCP-2, -4, -5, -6, or FceRIα. During culture of bone marrow cells for 1–3 wk in the presence of 200 ng/ml rKL, there was a progressive increase in the mRNA levels that encode MC-CPA, MMCP-4, -5, -6, and FceRIα in the nonadherent population of cells. The MMCP-2 transcript that is preferentially expressed by mucosal MC (5) was not detected in any of the cultures. No MMCP-4 or -2 mRNA was present in BMMCw/IL-3 or BMMCw derived by culturing bone marrow cells for 3 wk in enriched medium containing 40 U/ml rIL-3, or 50% WEHI-3 cell–conditioned medium respectively. (Fig. 2). Nevertheless, these two populations of MC contained abundant amounts of those mRNAs that encode MC-CPA, MMCP-5, SG-PG, and FceRIα. Although the MMCP-6 gene was transcribed in BMMCw/IL-3, the amount of this transcript per MC was less than in BMMCw/KL or BMMCw.

Histochemical and Transcriptional Phenotype of BMMCw Subsequently Exposed to rIL-3, rKL, or both Cytokines. When BMMCw were exposed for 1 wk to 20–40 U/ml rIL-3, 200 ng/ml rKL, or these same concentrations of both cytokines together, the number of cells in the cultures increased 1.3 ± 0.3, 6.0 ± 4.3, and 10.5 ± 1.9–fold (mean ± SD, n = 5), respectively. All of the cells in the 1- and 2-wk cultures remained toluidine blue+ (data not shown). All of the granules in the 1- (data not shown) and 2-wk (Fig. 1 e) BMMCw/IL-3 (BMMCw cultured for 1 and 2 wk in enriched medium containing 20 U/ml [n = 2], 40 U/ml [n = 1], or 100 U/ml [n = 3] rIL-3) remained safranin− when stained. Only a few of the cells in the 1-wk BMMCw/IL-3 cultures (BMMCw cultured for 1 and 2 wk in enriched medium containing 200 ng/ml rKL [n = 6]) contained safranin+ granules (data not shown). However, most of the 2-wk BMMCw/KL had at least one safranin+ granule, and in many instances, 100% of the granules in individual MC were safranin+ (Fig. 1 f). No safranin+ MC were detected in the 1-wk (data not shown) or 2-wk (Fig. 1 g) cultures of BMMCw/IL-3, KL (BMMCw cultured for 1 and 2 wk in enriched medium containing a combination of 20–100 U/ml rIL-3 and 200 ng/ml rKL [n = 6]).

Analysis of the transcription phenotype of these different populations revealed that BMMCw/IL-3 (Fig. 3) were similar to BMMCw (Fig. 2) in that they continued to express those transcripts that encode MC-CPA, MMCP-5 and -6, SG-PG, and FceRIα. All but MMCP-6 mRNA remained abundant and apparently unchanged. In contrast, BMMCw/KL expressed detectable levels of MMCP-4 mRNA at 1 wk, and high levels at 2 wk, in addition to those transcripts that encode MC-CPA, MMCP-5 and -6, SG-PG, and FceRIα. (Fig. 3). However, there was no expression of the MMCP-4 gene in BMMCw/IL-3, KL, and the amount of MMCP-6 mRNA was also reduced (Fig. 3). No MMCP-2 mRNA was detected in any of these populations of BMMC.

Proteoglycan, Histamine, and MC-CPA Analyses. BMMCw were exposed to rIL-3, rKL, or both cytokines for 1 wk (n = 3) or for 2 wk (n = 2), and were then radiolabeled with [35S]sulfate for 3.5 h. In each instance, >98% of the 35S-labeled macromolecules produced by the different populations of MC remained cell associated, as determined by Sephadex G-25 chromatography. After density-gradient centrifugation of the high salt/detergent extracts of the individual cell pellets, >84% of the radiolabeled macromolecules were recovered in the bottom fraction, which is consistent with the preferential incorporation of this radioisotope into granule-associated proteoglycans. Although the hydrodynamic sizes of the 35S-labeled proteoglycans produced by BMMCw/IL-3, BMMCw/KL, and BMMCw/IL-3, KL did not differ (Fig. 4),
the types of 35S-labeled glycosaminoglycans bound to these peptide cores differed considerably. When analyzed for their susceptibility to nitrous acid, 9 ± 4, 56 ± 2, and 2 ± 2% (mean ± 50% range, n = 2) of the purified 35S-labeled proteoglycans produced by 2-wk BMMCw/Ir-3, BMMCw/IL-3, and BMMCw/Ir-3.KL, respectively, contained heparin-like glycosaminoglycans (Table 1). 85 ± 1, 40 ± 2, and 95 ± 2% of replicate samples of these same purified 35S-labeled proteoglycans were susceptible to degradation by chondroitinase ABC, indicating the relative amount of chondroitin sulfate glycosaminoglycans. Because the relative amount of 35S-labeled heparin proteoglycans produced by 1-wk BMMCw/KL was not significantly different from that produced by 1-wk BMMCw/IL-3 (n = 3), the rKL-induced switch to biosynthesis of heparin proteoglycans required more

Figure 2. RNA blot analyses of mouse bone marrow cells before (0 wk) and after 1, 2, and 3 wk of culture in enriched medium containing 200 ng/ml of rKL, after 3 wk of culture in enriched medium containing 40 U/ml rIL-3, and after 3 wk of culture in 50% WEHI-3 cell-conditioned medium (WCM)/50% enriched medium. Blots were probed with cDNAs that encode MC-CPA, MMCP-2, -4, -5, -6, SG-PG, FceRIα, and actin.

Figure 3. RNA blot analyses of BMMCw cultured for 1 or 2 wk in enriched medium containing 200 ng/ml rKL, 40 U/ml rIL-3, or the same concentrations of both cytokines. RNA blots were probed with cDNAs that encode MC-CPA, MMCP-2, -4, -5, -6, SG-PG, FceRIα, and actin. A similar transcription phenotype was obtained in two other experiments of BMMCw exposed to 20 U/ml rIL-3, and three other experiments of BMMCw exposed to 100 U/ml of rIL-3 in the absence or presence of 200 ng/ml rKL.

Figure 1. Histochemistry of MC derived directly from bone marrow (a–d) or from an elicited immature MC population (e–g). Bone marrow cells were cultured for 3 wk in enriched medium containing rIL-3 (a and c) or rKL (b and d), and then were stained with toluidine blue (a and b) or alcian blue followed by safranin (c and d). Because the cell population is not pure in b, arrows indicate the BMMCw. BMMCw of >99% purity were cultured for 2 wk more in the presence of 20 U/ml rIL-3 (e), 200 ng/ml rKL (f), or both cytokines (g), and then were stained with alcian blue followed by safranin.
than 1 wk, and was associated with the histochemical detection of safranin + granules.

1-wk BMMCw/IL-3, BMMCw/KL, and BMMCw/IL-3,KL contained 180 ± 18, 570 ± 240, and 520 ± 360-ng histamine (mean ± SD/10^6 cells, n = 3), respectively, and 1.5 ± 0.3, 2.5 ± 0.8, and 0.5 ± 0.3 mU MC-CPA enzymatic activity, respectively. When cultured for 2 wk in enriched medium containing the same respective combinations of cytokines, their respective histamine contents were 80 ± 80, 600 ± 280, and 110 ± 60 ng (mean ± SD/10^6 cells, n = 3), and their respective MC-CPA activities were 2.7 ± 2.4, 6.8 ± 2.8, and 0.6 ± 0.5 mU (Table 1).

Discussion

This study is the first to delineate the transcription phenotypes of two populations of MC derived by culturing bone marrow hematopoietic progenitor cells in the presence of different recombinant cytokines. By comparing the transcription phenotype of the cells derived with either mouse rIL-3 or rKL, we have identified early and late expressed genes that encode the various constituents of the MC's secretory granule. Further, whereas the sequential action of rIL-3 followed by rKL results in the induction of the full complement of early and late expressed protease genes of serosal MC, exposure of BMMC to both cytokines simultaneously allows rIL-3 to prevail, thereby preventing the changes in both the proteoglycan and neutral protease phenotypes that occur in cells cultured with rKL alone.

Although rIL-3 and rKL are both capable of inducing proliferation and differentiation of bone marrow progenitor cells into MC, the resulting BMMCw/IL-3 and BMMCw/KL differed in their histochemistry and in their proportion of the total cells present in the culture. <30% of the cells in cultures of mouse bone marrow cells exposed to rKL for 3 wk were toluidine blue+/alcian blue+/safranin + MC (Fig. 1). BMMCw/KL contained high levels of mRNAs that encode MC-CPA, MMCP-4, -5, -6, and FceRIa, but not MMCP-2 (Fig. 2). This transcriptional phenotype is indistinguishable from that of in vivo differentiated mouse serosal MC, the prototype of the connective tissue MC subclass (Table 2). RNA blot analyses of the starting mouse bone marrow cells, and the 1-, 2- and 3-wk BMMCw/KL revealed that the SG-PG gene was efficiently transcribed in the starting cells, and that the level of this transcript increased in the subsequent weeks of the culture (Fig. 2). In BMMCw/KL, the SG-PG gene was expressed before any of the protease genes, most likely so that these enzymes could be efficiently packaged in the secretory granules.

Table 1. MC Histochemistry, and Content of 35S-labeled Heparin, Histamine, and MC-CPA

| Constituent                  | BMMCw | BMMCw/IL-3 | BMMCw/KL | BMMCw/IL-3,KL | BMMCw/KL | Serosal MC |
|-----------------------------|-------|------------|----------|---------------|----------|------------|
| Predominant histochemistry  | Safranin + | Safranin + | Safranin + | Safranin + | Safranin + | Safranin + |
| [35S]Heparin (percent total PG) | <10%  | 9%         | 56%      | 2%            | ~40%     | >95%       |
| [35S]ChS (percent total PG)  | >90%  | 85%        | 40%      | 95%           | ~55%     | <5%        |
| Histamine (ng/10^6 cells)   | <500  | 80         | 600      | 110           | ~3,000   | >20,000    |
| MC-CPA (mU/10^6 cells)      | ~3    | 2.7        | 6.8      | 0.6           | ~100     | 2,300      |

The histochemistry of the different populations of mouse BMMC (as well as their amounts of 35S-labeled heparin proteoglycan, 35S-labeled chondroitin sulfate [ChS] proteoglycan, histamine, and MC-CPA) were assessed after 2 wk of culture. The data for BMMCw, BMMCw/IL-3,KL, and serosal MC have been previously reported (1, 2, 12, 15, 16, 21, 28, 30, 33).
Table 2. Transcriptional Phenotype of Mouse MC Differentiated In Vivo and In Vitro

| Constituent | Serosal MC | Mucosal MC | BMMC_w | BMMC_IL-3 | BMMC_KL |
|-------------|------------|------------|--------|-----------|---------|
| MMCP-2      | -          | +          | -      | -         | -       |
| MMCP-4      | +          | ND         | -      | -         | +       |
| MMCP-5      | +          | -          | +      | -         | +       |
| MMCP-6      | +          | -          | -      | +         | +       |
| MC-CPA      | +          | +/-        | +      | -         | +       |
| SG-PG       | +          | +          | +      | +         | +       |
| FceRI       | +          | +          | +      | +         | +       |

* BMMC_IL-3 contain MMCP-6 mRNA, but the amount per cell is less than in BMMC_w or BMMC_KL.

The transcription phenotypes of serosal MC and mucosal MC have been previously reported (3-5, 7, 8, 21).

granules (2, 30). MC-CPA, MMCP-5 and -6 were expressed next, followed by MMCP-4.

All of the BMMC derived by culturing bone marrow cells in the presence of either rIL-3 (Fig. 1) or WEHI-3 cell-conditioned medium were toluidine blue+/alcan blue+/ safranin− MC. BMMC_IL-3 and BMMC_w contained high levels of those mRNAs that encode MC-CPA, MMCP-5, and FceRI+, but lacked MMCP-4 mRNA, as well as MMCP-2 mRNA (Fig. 2). The MMCP-6 gene was expressed in BMMC_IL-3, but the amount of this transcript was less than in BMMC_w or BMMC_KL. Although rIL-3 and WEHI-3 cell-conditioned media are more effective than rKL in their ability to induce a virtually pure population of BMMC, only rKL elicits the transcript, MMCP-4. MMCP-4 is a late-expressed gene observed only when progenitor cells differentiate into serosal MC (6).

As BMMC are the only cells identified in the cultures of bone marrow cells that have been exposed for 3 wk to either rIL-3 or WEHI-3 cell-conditioned medium, it was possible to directly assess the effects of rKL and rIL-3 on the expression of MMCP-4 and other phenotypic markers in an immature population of MC. BMMC_w cultured for 2 wk in medium containing rIL-3 remained safranin− (Fig. 1) and continued to produce predominantly 35S-labeled chondroitin sulfate proteoglycans (Table 1) and secretory granule protease mRNAs similar to the starting population of MC except for a reduction in MMCP-6 mRNA (Figs. 2 and 3). BMMC_w cultured in the presence of rKL became safranin+ and produced more 35S-labeled chondroitin sulfate proteoglycans than 35S-labeled chondroitin sulfate proteoglycans (Table 1), as previously observed for BMMC derived with Con A-stimulated splenocyte-conditioned medium and then cultured in the presence of rat rKL (64). The current focus on transcripts that encode secretory granule proteases revealed that mouse rKL induced expression of abundant levels of MMCP-4 mRNA, in addition to those transcripts that encode MC-CPA, MMCP-5 and -6, SG-PG, and FceRI+ (Fig. 3). In contrast, MMCP-2, a late-appearing phenotypic marker of mucosal MC (5) (Table 2), was not detected in BMMC_KL (Fig. 2) or in BMMC_w/KL (Fig. 3). Thus, during the differentiation of mucosal MC in the intestines of helminth-infected mice, there must be other factors that suppress transcription of MMCP-5 and -6 and elicit transcription of MMCP-2. Stimulation of BMMC_IL-3 with rIL-10 elicits the late expressed gene MMCP-2 (65).

KL is produced by fibroblasts (37, 40, 41) and is a cytokine that can induce BMMC_w to preferentially express those protease transcripts and proteoglycan species (Figs. 2-4 and Table 1) present in serosal MC (2-4, 6-8). Thus, KL was a candidate for the fibroblast-derived activity that induced BMMC to increase their histamine and MC-CPA contents 35-100-fold during fibroblast coculture (2, 12, 33). The MC-CPA enzymatic activity in a 2-wk BMMC_w/KL was only ~0.3% of that of a mouse serosal MC, and only ~5% of that of a 2-wk BMMC_w/F (Table 1). Likewise, the amount of histamine in a 2-wk BMMC_w/KL was only ~3% of that of a serosal MC and only 20% of that of a BMMC_w/F (Table 1). Thus, except for heparin biosynthesis, rKL is a relatively ineffective granule-maturation factor. It is possible that the bioactivity of fibroblast-derived KL and purified yeast rKL are different because of altered posttranslational modification of the cytokine or varied presentation (e.g., soluble versus membrane bound) of the cytokine. Alternatively, a second fibroblast-derived factor such as nerve growth factor (66) might act in concert with rKL to achieve granule maturation during fibroblast coculture.

Proliferation was at its highest level when BMMC_w were cultured for 2 wk in medium containing both rIL-3 and rKL, but the rKL-induced differentiation process was suppressed. BMMC_w/KL remained safranin−, produced predominately 35S-labeled chondroitin sulfate proteoglycans rather than 35S-labeled heparin proteoglycans (Table 1 and Fig. 4), did not express MMCP-4 mRNA, and contained less MMCP-6 mRNA than the starting BMMC_w. This is the first demonstration of an antagonistic effect of rIL-3 on MC differentiation. The inability of human rIL-3 to induce human hematopoietic progenitor cells to differentiate into MC (67, 68), and the inability to obtain mature Kirsten sarcoma-immortalized MC lines in the presence of mouse IL-3 (69) may both have been a consequence of this antagonistic effect.
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