Immunology 1996 **88** 308–314

Stem cell factor-dependent human cord blood derived mast cells express α- and β-tryptase, heparin and chondroitin sulphate

G. NILSSON,* T. BLOM,† I. HARVIMA,‡ M. KUSCHE-GULLBERG,§ K. NILSSON* & L. HELLMAN† *Departments of Pathology, †Immunology and §Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden, and ¶Department of Dermatology, University of Kuopio, Kuopio, Finland*

SUMMARY

The present study sought to determine the expression of α- and β-tryptase in *in vitro* differentiated human cord blood derived mast cells. We also analysed the glycosaminoglycan composition and the phenotype of the cells. The major protease in human mast cells is tryptase, and cDNAs for two different human tryptases have been characterized, the so-called α- and β-tryptase. By reverse transcriptase–polymerase chain reaction (RT-PCR) we could show that stem cell factor (SCF)-dependent cord blood derived mast cells express both α- and β-tryptase. Furthermore, the cells were stained with a monoclonal antibody (mAb) against tryptase, and the tryptase was enzymatically active cleaving the substrate Z-Gly-Pro-Arg- methoxy-2-naphthylamide (MNA). The majority of the cord blood derived mast cells could also be stained with mAbs against chymase, cathepsin G and CD68. They also expressed Kit/SCFR (CD117), CD13, CD29 and CD45 on the cell surface. The proteoglycan-derived polysaccharide composition of the cells was estimated to be 25–35% of heparin origin and 65–75% of chondroitin sulphate origin. Hence, the cord blood derived mast cells exhibit a phenotype in common with the so-called MC_{TC} type of human mast cells.

INTRODUCTION

Mast cells are frequently found at the interface of the internal and external environments, such as skin, conjunctiva, gut, and respiratory mucosal surfaces. At these locations, mast cells can be activated, e.g. through cross-linking of the high-affinity immunoglobulin E (IgE) receptor, upon which the cells release their content of inflammatory mediators (i.e. histamine, proteinases, proteoglycans, lipid mediators and cytokines). Two distinct types of mast cells have been described in humans based on the neutral protease content of their granules.[1] One contains tryptase and is usually referred to as MC_T, while the other contains tryptase, chymase, carboxypetidase A[2] and a cathepsin G-like proteinase[3] and is usually referred to as MC_{TC}.

Tryptase is the principal enzyme accounting for the trypsin-like activity detected in human mast cells.[4] Substantial amounts of tryptase are present in MC_{TC} cells (35 pg/cell) and in MC_T cells (10 pg/ml).[5] Catalytically active tryptase exhibits a tetrameric structure that is stabilized in this form by heparin.[6,7]

Received 14 November 1995; revised 22 January 1996; accepted 24 January 1996.

Abbreviations: CBMC, cord blood derived mast cells; mAb, monoclonal antibody; RT-PCR, reverse transcriptase–polymerase chain reaction; SCF, stem cell factor.

Correspondence: Dr Gunnar Nilsson, Department of Pathology, University of Uppsala, S-751 85 Uppsala, Sweden.

Five different, but highly homologous tryptase cDNAs have been cloned from human skin and lung mast cell populations.[8–10] These tryptases can be divided into two groups, α- and β-tryptase. The four β-tryptase cDNA molecules are 90% similar to α-tryptase and 99% identical to one another. Recently, we showed the expression of α- and β-tryptase in different human cell lines. The β-tryptase was found to be expressed in the basophilic cell line KU812,[11] the mast cell line HMC-1[12] and the monocytic cell line U-937.[13] In contrast, the monocytic cell line Mono Mac 6 expressed α-tryptase only.[13] The β-tryptase in HMC-1 was enzymatically active, indicating that the expression of both forms of tryptase is not a prerequisite for the tryptase to be enzymatically active.

The most important growth factor for human mast cells has been shown to be stem cell factor (SCF), also referred to as mast cell growth factor or Kit-ligand. SCF induces the development of mast cells from fetal liver,[14] bone marrow and peripheral blood,[15] and cord blood.[16] The *in vitro* developed fetal liver derived mast cells share several characteriztics with lung mast cells, e.g. expression of tryptase but not chymase.[14,17] In this study, we took advantage of the technique to differentiate human mast cells from cord blood mononuclear cells in order to analyse the RNA expression of α- and β-tryptase. The *in vitro* developed SCF-dependent cord blood derived mast cells (CBMC) were also analysed with respect to their phenotype, expression of tryptase protein, enzymatic activity of the tryptase, and the content of glycosaminoglycans.

MATERIALS AND METHODS

Cell cultures

Mononuclear cells were separated from heparinized umbilical cord blood by Ficoll-Paque (Pharmacia AB, Uppsala, Sweden) gradient centrifugation. Cells were washed in phosphate-buffered saline (PBS) and suspended in RPMI-1640 medium, supplemented with 10% fetal calf serum (Life Technologies, Renfrewshire, UK), 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 0·1 mM non-essential amino acids, 100 IU/ml of penicillin and 50 μg/ml of streptomycin (Sigma, St Louis, MO). The cell suspensions were seeded at a density of 1×10^6 cells per ml in 10 ml plastic flasks (Costar, Cambridge, MA). Recombinant human stem cell factor, kindly provided by Immunex Inc. Seattle, WA, was added at 50 ng/ml. The medium was changed weekly.

Enzyme cytochemistry and immunocytochemical staining

Cells were spun onto glass slides using a cytocentrifuge. The cells were stained with antibodies using the peroxidase–antiperoxidase (PAP) technique or the avidin–biotin system (Dako, Glostrup, Denmark). Monoclonal antibodies (mAb) against tryptase (G3) and chymase (B7)[18] were kind gifts from Dr L. B. Schwartz, Medical College of Virginia, Richmond, VA. Antibodies against cathepsin G and elastase were purchased from Serotec (Cambridge, UK); against myeloperoxidase and CD68 from Dako, and against eosinophil cationic protein (EG 1) from Pharmacia Diagnostics (Uppsala). Cytochemical methods were used for the demonstration of peroxidase, naphthol AS-D acetate esterase (NASDAE) and naphthol AS-D chloroacetate esterase (NASDCAE) as described previously.[19] Granulocytes, bone marrow smears and the KU812 cell line,[20] a human basophilic-like cell line which express FceRI [21], were used as controls.

Protease activity in CBMC, HMC-1, Mono Mac 6 and skin were demonstrated with different synthetic substrates which measure tryptic and chymotryptic enzyme activity. The cytospin slides were first fixed in cold acetone for 10–15 min. The following substrates were used: Bz-Arg-4-methoxy-2-naphthylamide (Bz-Arg-MNA), Z-Gly-Pro-Arg-MNA, Suc-Val-Pro-Phe-MNA (Bachem, Bubendorf, Switzerland), Z-Gly-Arg-MNA, Z-Lys-Arg-MNA and Z-Pro-Arg-MNA (Enzyme Systems Products, Livermore, CA). The substrate solution was prepared freshly and consisted of 1 mM substrate (20 mM stock solution dissolved in dimethylformamide), and 0·5 mg/ml Fast black K salt (Sigma) as the chromogen dissolved in 0·1 M Tris–HCl, pH 7·5. The substrate solutions were then subjected to a short centrifugation at 10 000 *g*. Aliquots (200 μl) of each substrate solution were applied on cytospin preparations. The reaction was stopped after 30 min incubation in a humidified chamber at room temperature by short rinsing with deionized water. The formed dark blue to violet azo dye was stabilized by immersing the slides into 2% $CuSO_4$ for 5–10 min. As a control for the protease activity we used skin sections which were stained in parallel with the CBMC cytocentrifuge preparations.

Flow cytometry analysis

The cell surface antigen expression on CBMC was analysed by indirect immunofluorescence using a fluorescence-activated cell sorter (FACScan) (Becton Dickinson, Mountain View, CA). The cells ($0{\cdot}5 \times 10^5$) were washed with PBS + 0·5% bovine serum albumin (BSA) + 0·1% sodium azide (a washing buffer) and incubated with primary antibodies for 30 min in cold. After washing three times with the washing buffer, the cells were incubated with a fluorescein isothiocyanate (FITC)-labelled $F(ab')_2$ fragment of rabbit-anti mouse immunoglobulin (Ig) (Dako) for 30 min. Labelled cells were washed as above, and resuspended in PBS containing 1% paraformaldehyde. Monoclonal antibodies against the following cell surface markers were used: CD13 (WM-15) and CD45 (75–5D3) from the 4th International Workshop of Human Leukocyte Differentiation Antigens, CD29 (K20) and CD63 (Clb/Gran/12) (Immunotech International, Marseille, France); and against CD33 (WM-54) (Dako). The mAb against the α-chain of the FcεRI receptor (29C6)[22] was kindly provided by Drs R. Chizzonite and F. Riske (Hoffmann-La Roche, Nutley, NJ); the antibody against Kit (YB5.B8)[23] was a kind gift from Dr L. Ashman (Hanson Centre for Cancer Research, Adelaide, Australia). The Bsp-1 monoclonal antibody (mAb) which recognizes an antigen expressed on human basophils[24] was a kind gift from Dr M. Bodger (Christchurch, New Zealand).

Reverse transcription and polymerase chain reaction (PCR) amplification

Total RNA was prepared from CBMC, the mast cell line HMC-1,[25] the basophilic cell line KU 812[20] and the monocytic cell line Mono Mac 6[26] and dissolved in DEPC-treated water as previously described.[27] The reverse transcription reactions consisted of the following: 1 μg RNA, 7·5 mM $MgCl_2$, 0·2 mM of each deoxynucleotide, 1 × Mg-free Taq polymerase reaction buffer (Promega Inc., Madison, WI), 1 μg downstream oligonucleotide primer (see below), 2·5 U RNAguard (Pharmacia) and 200 U AMV reverse transcriptase (Promega). The total volume was 20 μl for each reaction. The reactions were incubated at 42° for 30 min and 99° for 5 min. All of the resulting products were used for the PCR reaction.

The two oligonucleotide primers for the tryptase PCR reaction were directed against a region where the α- and β-tryptase are identical in sequence. The product amplified is expected to be 432 nt long. The sequence of the oligonucleotide primers were as follows: (a) the 5′ primer, **CTGAATTCTGGGACCGGACGTCAAGGATCT**, (b) the 3′ (downstream) primer, **GTAAGCTTTTCCCGGCACACAGCATGTCGT**. For the FcεRI α-chain reverse transcription and PCR reactions, the primers used were: (a) the 5′ primer, **GGAATTCAGCACAGTAAGCACCAGGAGTCCATG**, (b) the 3′ (downstream) primer, **GAAGCTTTCCTTGAGCACAGACGTTTCTATG**. The PCR product contains the entire coding region. PCR amplification was carried out in 40 cycles of denaturation (94°, 40 seconds), annealing (65°, 50 seconds) and polymerization (72°, 1 min 30 seconds). The final $MgCl_2$ concentration was 2·5 mM and 1 μg of each primer was used. As a control of PCR disequilibrium and hybridization efficiency of the α- and β-tryptase probes, PCR templates of the same size and with known concentration derived from α- and β-tryptase were used in different ratios (see Fig. 1).

Five microlitres out of 100 μl of each tryptase PCR product was loaded on a 1·0% agarose gel in duplicates. Care was taken to load the same amount of DNA in the two duplicate lanes. The gel was blotted after electrophoresis on Hybond N+ membranes (Amersham, Buckinghamshire, England) that were cut in halves after the blotting. Blotting was done for 3 hr with a

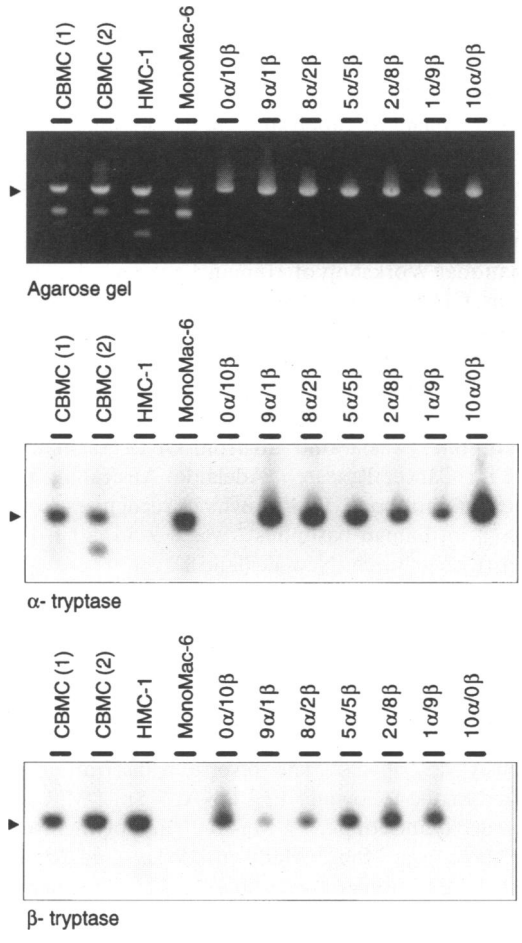

Figure 1. Expression of α- and β-tryptase in CBMC, HMC-1 and Mono Mac 6. The figure shows PCR products using the tryptase primers on CBMC, HMC-1 and Mono Mac 6 total RNA, blotted to two filters and hybridized with either α- or β-tryptase oligonucleotide probes. The origin of the PCR material is indicated above each lane. As a control, α- and β-tryptase templates were mixed in different ratios and used as PCR templates, indicated as 'parts α/parts β' in the figure above the corresponding lanes. The probe used is stated directly below the autoradiograms. The agarose gel shows the appearance of the bands on the electrophoresis gel before blotting to filters. CBMC(1) and CBMC(2) corresponds to two different preparations of cord blood derived mast cells.

'VacuBlot' unit (Pharmacia) and cross-linked by u.v. irradiation. Two oligonucleotide probes, specific for α- or β-tryptase, respectively, were used for analysing the specific signal for α- or β-tryptase.[13] The oligonucleotides were radioactively phosphorylated using γ^{32}P-ATP (Amersham) and T4 polynucleotide kinase (Pharmacia). The filters were hybridized in a solution containing 6 × (SSC), 1 × Denhardts solution, 2 mM ethylene diamine tetraacetic acid (EDTA), 100 μg/ml yeast RNA and 0·1% sodium dodecyl sulphate (SDS) at 65° overnight. The filters were washed at low stringency (6 × SSC, 0·1% SDS, 1 hr at 65°). The filters were scanned in a BioRad GS-250 Molecular Imager™, and the density ratios between α- and β-tryptase were calculated.

The FcεRI α-chain PCR products were loaded on a 1% agarose gel containing ethidium bromide. After the electrophoresis a photo was taken. The gel was then blotted over to a filter in an identical fashion as with the tryptase products, and hybridized with a cDNA probe for the FcεRI α-chain. The MegaPrime kit (Amersham) was used to label the probe. The filters were washed in 2 × SSC, 0·1% SDS, at 65° for 1 hr.

Analysis of glycosaminoglycan chains

CBMC, HMC-1 and Mono Mac 6 were incubated for 5 hr in fresh medium containing 0·2 mCi/ml of inorganic [^{35}S]sulphate (carrier free) (Amersham). The cell suspensions were collected and centrifuged at 1000 r.p.m. (250 ***g***) for 5 min. The cell pellets were solubilized in 3 ml of 1% Triton X-100, 0·05 M Tris–HCl pH 7·4 at +4° for 20 min. After centrifugation (13 000 ***g***, 5 min) the supernatants were used for purification of the labelled polysaccharides. The samples were adjusted to 0·15 M NaCl and applied to a 300 μl diethylaminoethyl (DEAE)-Sephacel column (Pharmacia) equilibrated in 0·15 M NaCl Tris–HCl pH 7·4, 0·1% Triton X-100. The column was washed with the equilibration buffer, followed by 0·15 M NaCl, 0·05 M acetate buffer pH 4·0, 0·1% Triton X-100. The labelled macromolecules were eluted with 1·5 M NaCl, 0·05 M acetate buffer pH 4·0, 0·1% Triton X-100. The polysaccharide chains were released from the peptide core of the protein by alkaline treatment as described previously.[12] After the addition of 0·5 mg of pig mucosa heparin as carrier, the labelled material was dialysed against water. Treatment with nitrous acid at pH 1·5 was performed according to Stigson and Kjellén.[28] Digestion with chondroitinase ABC was done as described previously.[12] Polysaccharides treated with nitrous acid or chondroitinase ABC were subjected to analyses by a gel filtration column (1 × 200 cm) of Sephadex G 25 superfine (Pharmacia), eluted in 0·2 M NH_4HCO_3 at a flow rate of 5 ml/hr. Fractions of 25 min were collected and analysed for radioactivity.

RESULTS

Phenotypic characterization of CBMC

The phenotype of CBMC developed in this study was analysed with immunohistochemistry and flow cytometry. The expression of markers for the mast cell lineage, as well as for markers expressed on neutrophils, eosinophils, basophils and monocytes, was analysed. The CBMC was stained with mAbs directed against tryptase, chymase, cathepsin G and CD68 (Table 1). The cells were negative for proteins commonly found in granulocytes: ECP, Myeloperoxidase and Elastase. CBMC contained NASDCAE-esterase activity, but neither NASDAE-esterase activity nor peroxidase. CBMC expressed Kit, CD13, CD29 and CD45 on its surface (Table 2). A low expression of CD63 could be detected on a subpopulation of the cells. No binding of the mAb Bsp-1 (basophil-specific) on CBMC could be detected.

One of the classical markers on mast cells and basophils is the high-affinity IgE receptor. Analysis of the CBMC for presence of the α-chain of the FcεRI-complex revealed no expression, neither at the protein level detected with the mAb (Tables 1 and 2) nor at the RNA level detected by reverse transcriptase–polymerase chain reaction (RT–PCR) (data not shown). When the RT–PCR product was hybridized with a probe for the FcεRI α-chain, only the product from KU812 gave a signal (data not shown).

Table 1. Cytochemical properties of CBMC analysed after 10 weeks in culture in the presence of SCF. Similar results were obtained in three independent cord blood derived mast cell preparations

Marker	Percentage positive cells
Tryptase	92%
Chymase	80%
Cathepsin G	97%
FceRI-α-chain	neg
CD68	98%
ECP	neg
Elastase	neg
Myeloperoxidase	neg
Peroxidase	neg
NASDAE	neg
NASDCAE	90%

neg, no staining could be detected.

Expression of α- and β-tryptase in CBMC

The reverse transcriptase and the polymerase chain reaction was performed in two consecutive steps as described in the 'Materials and Methods' section. The PCR product was separated and transferred to two identical filters. The filters were either hybridized with a oligonucleotide probe specific for α-tryptase, or with a β-tryptase specific oligonucleotide. As shown in Fig. 1 the PCR products derived from Mono Mac 6 and HMC-1 showed hybridization only to α- or β-tryptase, respectively. However, the PCR product of CBMC hybridized with both the α- and the β-tryptase probes. When the ratio between α- and β-tryptase expressed in CBMC was calculated, the α- and β-tryptase were found to be expressed in about equal amounts.

Protease activity in mast cells

The principal substrate for demonstrating tryptic activity in mast cells is Bz-Arg-MNA that showed only weak staining in this as well as in the previous study (Table 3).[29] Addition of Gly to the P2 position (Gly-Arg) diminished the staining reaction, and Lys (Lys-Arg) could not give any beneficial effect. However, Pro in the P2 position (Pro-Arg) increased greatly the staining intensity in both CBMC and HMC-1 mast cells. Elongation of the peptide length to Gly-Pro-Arg even increased the deposition of the reaction products. The enzyme activity is really trypsin-like, since replacing Arg in the Pro-Arg by Phe or Val resulted in complete disappearance of staining reaction. Mono Mac 6 cells with α-tryptase could not exhibit any positive staining with any of the substrates. No chymotryptic activity could be detected in any of the cell lines analysed.

Table 2. Flow cytometry analysis of cell surface antigen expression on CBMC cultured for 12 weeks in the presence of SCF. More than 90% of the cell population analysed was tryptase positive. The percentage of positive cells are given in the table. The percentage of cells stained with irrelevant mAbs was about 6%. The analyses were done on three different CBMC cultures

Cell surface marker	Percentage positive cells
Kit	80
FcεRI-α-chain	6
CD13	90
CD29	50
CD45	92
CD63	16
mAb Bsp-1	8

Table 3. Protease activity in CBMC, HMC-1, Mono Mac 6 and skin mast cells demonstrated with different synthetic substrates which measure tryptic and chymotryptic enzyme activity. The experiment was repeated twice on two different CBMC-cultures

Substrate (1 mM)	Enzyme activity	CBMC	HMC-1	Mono Mac 6	Skin*
Bz-Arg-MNA	Tryptic	±	+	−	±
Z-Gly-Arg-MNA	Tryptic	−	−	−	−
Z-Lys-Arg-MNA	Tryptic	±	−	−	+
Z-Pro-Arg-MNA	Tryptic	+ +	+ +	−	+ +
Z-Gly-Pro-Arg-MNA	Tryptic	+ + +	+ + +	−	+ + +
Suc-Val-Pro-Phe-MNA	Chymo-tryptic	−	−	−	+ +

* adopted from [29].

−, no apparent staining; +, weak staining; + +, moderate staining; + + +, strong staining.

Expression of glycosaminoglycan chains in CBMC

After incubation of CBMC with ^{35}S-sulphate, proteoglycans were isolated by DEAE ion-exchange chromatography. The polysaccharide composition of the labelled proteoglycans was determined by gel chromatography after specific degradation with either nitrous acid or chondroitinase ABC, which results in the depolymerization of heparin-like glycosaminoglycans and chondroitin sulphate, respectively (Fig. 2). The heterogeneous appearance of the disaccharide peak in CBMC is probably due to the fact that heparin disaccharides may contain one or two *o*-sulphate groups which will affect the elution profile. The amount of heparin was calculated as the sum of ^{35}S-radioactivity recovered as di- and tetrasaccharides divided by total radioactivity eluted from the column. Heparin amounts to approximately 24% (21·7% disaccharides plus 2% tetrasaccharides) of the glycosaminoglycans in the CBMC. Chondroitinase ABC is specific for *N*-acetyl-galactosamine containing galactosaminoglycans and will not degrade heparin. The first peak, eluting in the void volume, represents material resistant to the eliminase and amounts to about 25% of total radioactivity (Fig. 2). This peak represents most probably heparin, which is in good agreement with the results obtained with nitrous acid degradation. The second peak contains disaccharides generated in the eliminase reaction, representing chondroitin sulphate and amounts to approximately 75% of the glycosaminoglycans in the CBMC. When repeated on two

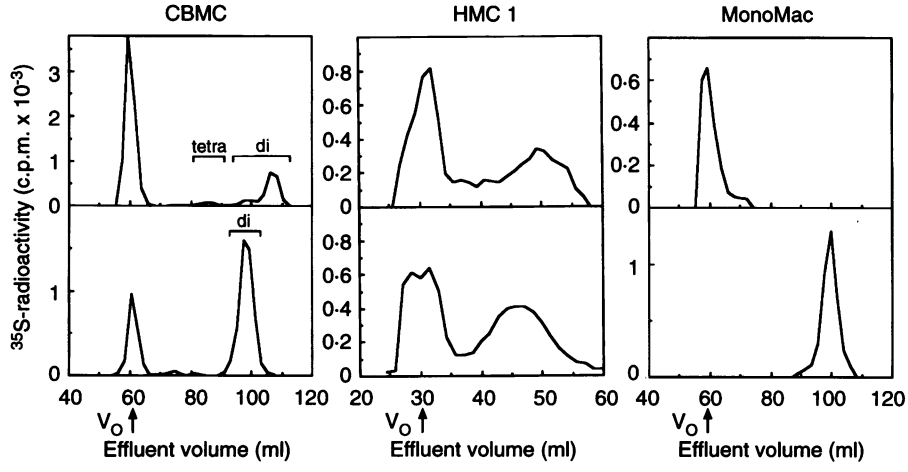

Figure 2. Glycosaminoglycan composition of CBMC (91% tryptase positive), HMC-1 and Mono Mac 6. Gel chromatography on Sephadex G 25 of 35-S-labelled polysaccharide chains (20×10^3 c.p.m.) after degradation with nitrous acid (upper panel) or chondroitinase ABC (lower panel). Untreated polysaccharide chains eluted in the void volume. Blue dextran was added as a marker of the void volume (V_0). The digestion and nitrous acid depolymerization reactions were performed two times each with two different cell cultures. di, disaccharides; tetra, tetrasaccharides. Results for HMC-1 adopted from reference 12.

different cultures, similar patterns were obtained and the content of heparin and chondroitin sulphate was calculated to be 25–35% and 65–75%, respectively in CBMC.

HMC-1 cells expressed heparin and chondroitin sulphate in approximately equal amounts, while Mono Mac 6 expressed only chondroitin sulphate (Fig. 2).

DISCUSSION

Studies of human mast cells have for long been hampered by the poor response of such cells to inducers of differentiation *in vitro*. Recently it was shown that SCF is the major growth factor for human mast cells.[14–16] Using this model for *in vitro* differentiation of human mast cells it has been possible to further study different aspects of human mast cell biology, e.g. differentiation,[30–33] and migration.[34].

This report shows that SCF-dependent cord blood derived mast cells express α- and β-tryptase. mRNA for both α- and β-tryptase as well as tryptase protein could be detected in these cells. This is in agreement with a recent report describing the expression of α- and β-tryptase in human lung and skin mast cells.[35] Furthermore, by the use of synthetic substrates we were able to show that the tryptase is active. The tryptase in both CBMC and the β-tryptase in HMC-1 mast cells showed a preference for Pro-Arg sequence over Gly-Arg and Lys-Arg, which is in good agreement with previous findings on mast cells in normal, mastocytoma and psoriatic skin sections.[29,36] This similarity in the substrate preference of tryptic activity in CBMC and HMC-1 mast cells as in mast cells in normal and diseased skin strongly suggests that the tryptases responsible for the substrate cleavage in each cell type have similar catalytic properties.

The α-tryptase expressed in Mono Mac 6 did not show any enzymatic activity. This is in contrast to the tryptase expressed in CBMC and the β-tryptase in HMC-1 cells and could have several, alternative explanations. Firstly the α-tryptase may be enzymatically near inactive. Secondly it may have a different enzymatic specificity. Thirdly Mono Mac 6 cells may not be able to assemble an active tetrameric tryptase, and, finally, since the Mono Mac 6 cells contain chondroitin sulphate but not heparin the tryptase may be unstable.[7,37]

Both heparin and chondroitin sulphate was detectable in CBMC developed in vitro which is in accordance with what has been found in human tissue mast cells.[38,39] Unlike mast cells in rodents, where connective-tissue mast cells are the principal producers of heparin while mucosal mast cells synthesise chondroitin sulphate, all human mast cells produce heparin and chondroitin sulphate E.[40]

The phenotypic characterization of CBMC showed that the majority of these cells express both tryptase, chymase, cathepsin G and CD68. This indicates that CBMC represent the MC_{TC} type of human mast cells. The lack of chymotryptic activity in CBMC could be explained by the presence of protease inhibitors in the serum of the culture medium, since tissue sections treated in the same way as the cytocentrifuge preparations showed chymotryptic activity. Furthermore, proteinase inhibitors in plasma with the ability to inactivate chymase have been described by Schechter *et al.*[41] In addition, chymase immunoreactive mast cells lacking chymase activity have been described in psoriatic lesions.[42] In that study the inhibitors were found to be localized in the mast cells. In addition to the expression of the proteinases, CBMC also showed strong staining with a mAb against CD68, a transmembrane glycoprotein belonging to a family of haematopoietic mucin-like molecules.[43] CD68 has been found to be highly expressed in human monocytes and tissue macrophages.[44] Normal and neoplastic human mast cells, and HMC-1 cells, also exhibit a strong CD68 staining, predominantly in the cytoplasmic granules.[12,45] A similar cytoplasmic staining was seen in CBMC. Taken together these results indicate that CD68 cannot be used as a marker to discriminate between monocytes/macrophages and mast cells.

The CBMC differ in phenotype to some extent from SCF-dependent fetal liver-derived cells. In contrast to CBMC, the majority of the mast cells derived from fetal liver do not express chymase, thereby representing a MC_T phenotype.[14] However,

the expression of different cell surface markers, e.g. Kit, CD13, CD29, CD45, CD63, is similar on CBMC and fetal liver-derived mast cells.[17] The high-affinity IgE-receptor complex, FcεRI, consists of the extracellular IgE-binding α-chain, the transmembrane β-chain, and two intracellular γ-chains. We could not detect any expression of the FcεRI-α-chain in CBMC, either with RT–PCR or with a mAb against the α-chain. Similar results have been shown in human SCF-dependent fetal liver-derived mast cells,[17] in human SCF-dependent bone-marrow derived mast cells,[46] and in the human mast cell line HMC-1.[12,25] However, the results appear to be at variance with the findings of Mitsui *et al.* who reported binding of IgE to mast cells developed from cord blood.[16] It would be of interest to determine the reasons for the differences in the results obtained on expression of FcεRI. In a recent paper Li *et al.* described how conditioned media from a cell strain derived from a patient with mastocytosis induced the expression of FcεRI as well as of mast cell granule proteases in *in vitro* differentiated SCF-dependent bone marrow-derived mast cells.[46] However, they were not able to characterize the factor(s) responsible for the effect. The regulation of FcεRI expression during mast cell differentiation will be explored as part of future studies.

ACKNOWLEDGMENTS

The skilful technical assistance of Viveka Svensson and Annelie Kraft is gratefully acknowledged. This work was supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council, Ollie and Elof Ericssons Foundation, Söderbergs Foundation, and from King Gustav V's 80-years Foundation.

REFERENCES

1. Irani A.A., Schechter N.M., Craig S.S., DeBlois G. & Schwartz L.B. (1986) Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci USA* **83,** 4464.
2. Irani A.A., Goldstein S.M., Wintroub B.U., Bradford T. & Schwartz L.B. (1991) Human mast cell carboxypeptidase. Selective localization to MCTC cells. *J Immunol* **147,** 247.
3. Schechter N.M., Irani A.M., Sprows J.L., Abernethy J., Wintroub B. & Schwartz L.B. (1990) Identification of a cathepsin G-like proteinase in the MCTC type of human mast cell. *J Immunol* **145,** 2652.
4. Schwartz L.B., Lewis R.A. & Austen K.F. (1981) Tryptase from human pulmonary mast cells: Purification and characterization. *J Biol Chem* **256,** 11939.
5. Schwartz L.B., Irani A.M., Roller K., Castells M.C. & Schechter N.M. (1987) Quantitation of histamine, tryptase and chymase in dispersed human T and TC mast cells. *J Immunol* **138,** 2611.
6. Schwartz L.B. & Bradford T.R. (1986) Regulation of tryptase from human lung mast cells by heparin. Stabilization of the active tetramer. *J Biol Chem* **261,** 7372.
7. Alter S.C., Metcalfe D.D., Bradford T.R. & Schwartz L.B. (1987) Regulation of human mast cell tryptase. Effects of enzyme concentration, ionic strength and the structure and negative charge density of polysaccharides. *Biochem J* **248,** 821.
8. Miller J.S., Westin E.H. & Schwartz L.B. (1989) Cloning and characterization of complementary DNA for human tryptase. *J Clin Invest* **84,** 1188.
9. Miller J.S., Moxley G. & Schwartz L.B. (1990) Cloning and characterization of a second complementary DNA for human tryptase. *J Clin Invest* **86,** 864.
10. Vanderslice P., Ballinger S.M., Tam E.K., Goldstein S.M., Craik C.S. & Caughey G.H. (1990) Human mast cell tryptase: Multiple cDNAs and genes reveal a multigene serine protease family. *Proc Natl Acad Sci USA* **87,** 3811.
11. Blom T. & Hellman L. (1993) Characterisation of a tryptase mRNA expressed in the human basophil cell line KU812. *Scand J Immunol* **37,** 203.
12. Nilsson G., Blom T., Kusche G.M. *et al.* (1994) Phenotypic characterization of the human mast cell line HMC-1. *Scand J Immunol* **39,** 489.
13. Huang R., Åbrink M., Gobl A.E. *et al.* (1993) Expression of a mast cell tryptase mRNA in the human monocytic cell lines U-937 and Mono Mac 6. *Scand J Immunol* **38,** 359.
14. Irani A.A., Nilsson G., Miettinen U. *et al.* (1992) Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. *Blood* **80,** 3009.
15. Valent P., Spanblöchl E., Sperr W.R. *et al.* (1992) Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor/kit-ligand in long-term culture. *Blood* **80,** 2237.
16. Mitsui H., Furitsu T., Dvorak A.M. *et al.* (1993) Development of human mast cells from umbilical cord blood cells by recombinant human and murine C-kit ligand. *Proc Natl Acad Sci USA* **90,** 735.
17. Nilsson G., Forsberg K., Bodger M.P. *et al.* (1993) Phenotypic characterization of stem cell factor dependent human fetal liver derived mast cells. *Immunology* **79,** 325.
18. Irani A.A., Bradford T.R., Kepley C.L., Schechter N.M. & Schwartz L.B. (1989) Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* **37,** 1509.
19. Sundström C. & Nilsson K. (1977) Cytochemical profile of human haematopoietic biopsy cells and derived cell lines. *Br J Haematol* **37,** 489.
20. Kishi K. (1985) A new leukemia cell line with Philadelphia chromosome characterized as basophil precursors. *Leuk Res* **9,** 381.
21. Blom T., Huang R., Aveskogh M., Nilsson K. & Hellman L. (1992) Phenotypic characterization of KU812, a cell line identified as an immature human basophilic leukocyte. *Eur J Immunol* **22,** 2025.
22. Riske F., Hakimi J., Mallamaci M. *et al.* (1991) High affinity human IgE receptor (FcepsilonRI). Analysis of functional domains of the alpha-subunit with monoclonal antibodies. *J Biol Chem* **266,** 11245.
23. Lerner N.B., Nocka K.H., Cole S.R. *et al.* (1991) Monoclonal antibody YB5.B8 identifies the human c-kit protein product. *Blood* **77,** 1876.
24. Bodger M.P., Monsey G.L. & Fitzgerald P.H. (1987) A monoclonal antibody reacting with human basophils. *Blood* **69,** 1414.
25. Butterfield J.H., Weiler D., Dewald G. & Gleich G.J. (1988) Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* **12,** 345.
26. Ziegler-Heitbrock H.W.L., Thiel E., Fütterer A., Herzog V., Wirtz A. & Riethmüller G. (1988) Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. *Int J Cancer* **41,** 456.
27. Chomczynski P. & Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162,** 156.
28. Stigson M. & Kjellén L. (1991) Large disulfide-stabilized proteoglycan complexes are synthesized by the epidermis of axolot embryos. *Arch Biochem Biophys* **290,** 391.
29. Harvima I.T., Naukkarinen A., Harvima R.J. & Fräki J.E. (1988) Immunoperoxidase and enzyme-histochemical demonstration of human skin tryptase in cutaneous mast cells in normal and mastocytoma skin. *Arch Dermatol Res* **280,** 363.

30. Agis H., Willheim M., Sperr W.R. *et al.* (1993) Monocytes do not make mast cells when cultured in the presence of SCF—characterization of the circulating mast cell progenitor as a c-kit+, CD34+, Ly−, CD14−, CD17−, colony-forming cell. *J Immunol* **151,** 4221.
31. Nilsson G., Miettinen U., Ishizaka T., Irani A.M. & Schwartz L.B. (1994) IL-4 inhibits the expression of Kit and tryptase during the stem cell factor-dependent development of human mast cells from fetal liver cells. *Blood* **84,** 1519.
32. Rottem M., Okada T., Goff J.P. & Metcalfe D.D. (1994) Mast cells cultured from the peripheral blood of normal donors and patients with mastocytosis originate from a CD34+ Fc(epsilon)-RI(−) cell population. *Blood* **84,** 2489.
33. Nilsson G. & Nilsson K. (1995) Effects of IL-13 on immediate-early response gene expression, phenotype and differentiation of human mast cells. Comparision with IL-4. *Eur J Immunol* **25,** 870.
34. Nilsson G., Butterfield J.H., Nilsson K. & Siegbahn A. (1994) Stem cell factor is a chemotactic factor for human mast cells. *J Immunol* **153,** 3717.
35. Xia H.Z., Kepley C.L., Sakai K., Chelliah L., Irani A. & Schwartz L.B. (1995) Quantitation of tryptase, chymase, Fc epsilon RI alpha and Fc epsilon RI gamma mRNAs in human mast cells and basophils by competitive reverse transcription-polymerase chain reaction. *J Immunol* **154,** 5472.
36. Harvima I.T., Naukkarinen A., Harvima R.J. & Horsmanheimo M. (1988) Enzyme- and immunohistochemical localization of mast cell tryptase in psoriatic skin. *Arch Dermatol Res* **281,** 387.
37. Harvima I.T., Schechter N.M., Harvima R.J. & Fräki J.E. (1988) Human skin tryptase: purification, partial characterization, and comparision with human lung tryptase. *Biochim Biophys Acta* **957,** 71.
38. Stevens R.L., Fox C.C., Lichtenstein L.M. & Austen K.F. (1988) Identification of chondroitin sulfate E proteoglycan and heparin proteoglycans in the secretory granules of human lung mast cells. *Proc Natl Acad Sci USA* **85,** 2284.
39. Thompson H.L., Schulman E.S. & Metcalfe D.D. (1988) Identification of chondroitin sulfate E in human lung mast cells. *J Immunol* **140,** 2708.
40. Craig S.S., Irani A.A., Metcalfe D.D. & Schwartz L.B. (1993) Ultrastructural localization of heparin to human mast cells of the MCT and MCTC types by labelling with antithrombin III-gold. *Lab Invest* **69,** 552.
41. Schechter N.M., Sprows J.L., Schoenberger O.L., Lazarus G.S., Cooperman B.S. & Rubin H. (1989) Reaction of human skin chymotrypsin-like proteinase chymase with plasma proteinase inhibitors. *J Biol Chem* **264,** 21308.
42. Harvima I.T., Naukkarinen A., Paukkonen K. *et al.* (1993) Mast cell tryptase and chymase in developing and mature psoriatic lesions. *Arch Dermatol Res* **285,** 184.
43. Holness C.L. & Simmons D.L. (1993) Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins. *Blood* **81,** 1607.
44. Parwaresh M.R., Radzun H.J., Kriepe H., Hansmann M.L. & Barth J. (1986) Monocyte/macrophage reactive monoclonal antibody Ki-M6 recognizes an intracytoplasmic antigen. *Am J Pathol* **125,** 141.
45. Horny H.P., Schaumburg L.G., Bolz S., Geerts M.L. & Kaiserling E. (1990) Use of monoclonal antibody KP1 for identifying normal and neoplastic human mast cells. *J Clin Pathol* **43,** 719.
46. Li L., Macpherson J.J., Adelstein S. *et al.* (1995) Conditioned media from a cell strain derived from a patient with mastocytosis induces preferential development of cells that possess high affinity IgE receptors and the granule proteases phenotype of mature cutaneous mast cells. *J Biol Chem* **270,** 2258.