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Phenotypic characterization of stem cell factor-dependent human foetal liver-derived mast cells

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SUMMARY

Human foetal liver cells are an enriched source of mast cell progenitors that complete their differentiation and mature in response to stem cell factor, the ligand for Kit, in liquid culture. These mast cells are Kit$^+$, metachromatic with toluidine blue$^+$, tryptase$^+$, histamine$^+$ and show ultrastructure features of mast cells. Using a panel of monoclonal antibodies (mAb) against different cell-surface antigens (33 mAb were used), the cell-surface phenotype of human stem cell factor-dependent foetal liver-derived mast cells was examined by flow cytometry. Consistent with previous reports on tissue-derived mast cells, those derived from foetal liver *in vitro* expressed HLA class I, CD9, CD29, CD33, CD43, CD45 and Kit. Unlike mast cells dispersed from tissue, a high expression of CD13 was found. Also, these *in vitro*-derived mast cells express little, if any, high-affinity IgE receptor. However, small amounts of mRNA for the α-chain in foetal liver-derived mast cells compared to KU812 cells (a human basophil-like cell line) could be detected by Northern blotting. Full expression of FcεRI may require additional growth factor(s).

INTRODUCTION

Recently, recombinant human stem cell factor (rhSCF) (also called Kit ligand, steel factor or mast cell growth factor), a ligand for the c-*kit* proto-oncogene-derived product called Kit, was identified as the first cytokine with major growth factor activity for human mast cells.[1] Kit is expressed on nearly all haematopoietic stem cells,[2,3] which, when occupied with SCF, serves to potentiate the activities of lineage-committing cytokines such as erythropoietin for erythrocytes and granulocyte–macrophage colony-stimulating factor (GM-CSF) for myelocytes. In contrast to the effects of SCF on the differentiation and growth of other haematopoietic cells, SCF is the only exogenous cytokine required to stimulate the growth and differentiation of mast cells from foetal liver, and expression of Kit on the mast cell surface increases as the cells mature. Human foetal liver cells in liquid culture with rhSCF for 5 weeks give rise to a cell population in which at least 75% of the cells are toluidine blue$^+$, tryptase$^+$, histamine$^+$ and Kit$^+$. Transmission electron microscopy shows these cells to exhibit ultrastructure features consistent with a mast cell phenotype. Mast cells derived from foetal liver by culture with rhSCF are predominantly of the MC_T type, i.e. most lack detectable chymase, similar to mast cells derived by co-culture of human foetal liver cells with mouse 3T3 fibroblasts.[4] By contrast, mast cells derived by co-culture of human cord blood mononuclear cells with murine 3T3 fibroblasts exhibit primarily an MC_{TC} phenotype.[5] However, using murine fibroblasts to stimulate human mast cell growth and differentiation yields contamination of mast cells with fibroblasts, a mixture of human and rodent cells and a more complicated microenvironment.

To contrast mast cell development in humans and rodents, major growth factors for rodent mast cells include SCF and interleukin-3 (IL-3); less potent growth factors include IL-4, IL-9, IL-10 and nerve growth factor (reviewed in ref. 6). Except for SCF, each factor appears to be inactive as a mast cell growth factor in humans (reviewed in ref. 6). Receptors for IL-3, for example, are absent from tissue-derived human mast cells.[7] In humans, IL-3 favours the development of basophils and other cell types (such as eosinophils) over mast cells from bone marrow,[8–12] foetal liver[1] and cord blood.[13]

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Abbreviations: MIF, mean intensity of fluorescence; rhSCF, recombinant human stem cell factor.

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To characterize further foetal liver-derived mast cells, cell-surface markers were analysed by flow cytometry using well-characterized monoclonal antibodies. Previous studies of mast cell and basophil surface markers have been performed on cells that had matured *in vivo*, where the tissue microenvironment may influence the surface phenotype. Valent *et al.*[14,15] showed that basophils from the circulation and mast cells from lung both express CD9, CD33, CD43, CD45, major histocompatibility complex (MHC) class I molecules and FcεRI, whereas only mast cells express Kit and only basophils express Bsp-1, CD18, CD11b, CD11c, CD32, CD35 and CD40. In contrast, Columbo *et al.* found small but functional amounts of Kit on peripheral blood basophils.[16] Guo *et al.* examined the surface antigens of mast cells obtained from human uterus,[17] which were identical to those on lung mast cells except for the additional presence of CD11c/CD18 and CD32. Whether these differences are accounted for by differences in methods of analysis or cell dispersal, tissue microenvironments, mast cell subtypes or mast cell activation status are not known. The current study shows that human mast cells derived from foetal liver cells treated with rhSCF exhibit a pattern of cell-surface antigens distinct from those of basophils, and similar, though not identical, to those previously reported for lung and uterine mast cells.

MATERIALS AND METHODS

Cell cultures

Foetal livers were obtained at the time of therapeutic abortions. The protocol was reviewed and approved by the Human Studies Committee at Virginia Commonwealth University. Foetal liver cells were dispersed and the low-density fraction obtained as described previously.[1] Cells were cultured at 1×10^6 cells/ml in tissue culture flasks (Costar, Cambridge, MA). RhSCF[164] (AMGEN, Thousand Oaks, CA) was added to the cultures at 50 ng/ml. In parallel experiments foetal liver cells were cultured in the presence of rhSCF and 50 U/ml of rhIL-4; 1 ng/ml of rhIL-3; or 10% v/v of conditioned media from the human T-cell line called MO.[18] RhIL-3 and rhIL-4 were generous gifts of Dr N. Arai (DNAX Research Institute, Palo Alto, CA). The media were changed weekly and both adherent and non-adherent cells were harvested using 0·1 mM EDTA in phosphate-buffered saline (PBS) and mechanical dispersion to remove the loosely adherent cells from the plastic surface.

Flow cytometry

Cultured cells were harvested after 4–12 weeks of culture, washed in PBS, and examined for cell number and viability by use of a Buerker haemocytometer and trypan blue exclusion. The percentages of tryptase positive cells were determined from cytocentrifuge preparations using the G3 murine monoclonal anti-tryptase antibody as described previously.[19] For flow cytometry, cells ($0{\cdot}1$–$0{\cdot}3 \times 10^6$/tube) were preincubated with RPMI containing 10% human serum to block Fc receptor binding of monoclonal antibodies, and were then washed with PBS containing 1% bovine serum albumin (BSA) and 0·1% sodium azide (washing buffer). The antibodies (see below) were used for analysis of cell-surface antigens. Cells were incubated with primary antibodies for 30 min. After washing three times with washing buffer, the cells were incubated with a fluorescein isothiocyanate (FITC)-labelled $F(ab')_2$ fragment of rabbit anti-mouse Ig (Dako Corp., Carpenteria, CA) for 30 min. All incubations and washes were performed at 4°. Labelled cells were washed as above, resuspended in PBS containing 1% paraformaldehyde, and analysed with a FACScan (Becton Dickinson, Mountain View, CA). The cells were gated according to their forward and side scatter, and were analysed for binding of primary antibodies. The cell line KU812[20] was used as a positive control for anti-FcεRIα monoclonal antibody and peripheral blood basophils (enriched to 40% purity by Percoll density-dependent sedimentation) for the Bsp-1 monoclonal antibody.[21]

Antibodies

Antibodies against CD3 (Genclone 3), CD4 (Genclone 4), CD8 (Genclone 8), 10 (Genclone 10) and 21 (Genclone 21), and anti-HLA-DR (B8.12.2) were obtained from GenTrac Inc. (Plymouth Meeting, PA); against CD9 (ALB 6), 29 (K20), 54 (84H10) and 63 (Clb/Gran/12) from Amac Inc. (Westbrook, ME); against CD11b (904) and CD18 (H52) from Dr S. Fong (Genentech, Inc, South San Francisco, CA); against CD13 (WM-47), CD33 (WM-54), CD43 (DF-T1), CD45RB (PD27/26) and CD45RO (UCHL-1) from Dako Corp.; against CD14 (RMO 52), CD16 (NKP 15), CD19 (4G7), CD25 (2A3), CD34 (MY10), CD45 (HLe-1), CD45RA (L48) and CD56 (MY31) from Becton Dickinson; against HLA-ABC from Serotec (Oxford, U.K.); against CD11c (HB85) and CD35 (HB85.92) from American Type Tissue Culture (Rockville, MD); against CD32 (KUFC79) from Dr T. Mohanakumar (Washington University, St Louis, MO); against CD40 (G28-5) from Dr E. Clark (Seattle, WA); against Kit (YB5.B8);[22] against FcεRIα (22E7)[23] from Dr R. Chizzonite (Hoffman LaRoche, Nutley, NJ); and against a basophil surface antigen (Bsp-1).[21]

Northern blot analysis

To investigate if mRNA for the α-chain of FcεRI was expressed in foetal liver-derived mast cells, total RNA was prepared as described previously[24] from 46-day cultures of foetal liver cells with rhSCF (50 ng/ml). RNA from untreated KU812 cells, which express FcεRI,[25] was used as a control. Poly A RNA was selected using oligo(dT) cellulose (Kabi-Pharmacia, Uppsala, Sweden). Five micrograms of poly A+RNA per lane was subjected to electrophoresis on a 1% agarose gel containing 2·2 M formaldehyde. The gel was blotted onto nitrocellulose-coated nylon filters (Amersham International, Amersham, U.K.) and baked at 80° under vacuum for 2 hr. ^{32}P-labelled probes were prepared by random priming according to the manufacturer's instructions (Amersham International). The following probes were used: for FcεRIα, a 1·4 kilobase (kb) *Eco*RI fragment[26] was a kind gift from Dr L. Hellman (Uppsala University, Sweden); for glyceraldehyde-3-phosphate-dehydrogenase, the plasmid pHcGAP3[27] was provided by Dr R. Wu (Cornell University, NY). Hybridization was carried out under high stringency conditions at 42° overnight in a solution containing 50% formamide, 5 × SSC, 1 × Denhardt's solution, 0·1% sodium dodecyl sulphate, and 100 μg/ml of single-stranded salmon sperm DNA. After high stringency washing at 60°, the filters were exposed to KODAK XAR5 film (Eastman-Kodak, Rochester, NY) for 1–25 days.

Table 1. Comparison of cell-surface antigens on rhSCF-dependent foetal liver-derived human mast cells to those on human lung and uterine mast cells, and to human basophils*

Antigen	Mast cells			Basophils
	Foetal liver	Lung	Uterine	
CD3	–	–	NA	–
CD4	–	–	NA	–
CD8	–	–	NA	–
CD9	+	+	+	+
CD10	–	–	NA	–
CD11b	–	–	–	+
CD11c	–	–	±†	+
CD13	+	–	NA	+
CD14	–	–	–	–
CD16	–	–	–	–
CD18	–	–	+	+
CD19	–	–	NA	–
CD21	–	–	NA	–
CD25	–	–	NA	+
CD29	+	NA	+	NA
CD32	–	–	+	+
CD33	+	+	+	+
CD34	–	–	NA	–
CD35	–	–	NA	+
CD40	–	–	NA	+
CD43	+	+	+	+
CD45	+	+	+	+
CD45RA	–	NA	NA	NA
CD45RB	+	NA	NA	NA
CD45RO	–	NA	NA	NA
CD54	–	+	+	+
CD56	–	NA	NA	NA
CD63	+	NA	NA	+
Bsp-1	–	–	NA	+
HLA-ABC	+	+	+	+
HLA-DR	–	–	NA	–
Kit	+	+	+	–
FcεRI	–	+	+	+

NA, data not available.

* Data for human lung mast cells,[14,15] human uterine mast cells,[17,31] and basophils [14,15,28–30] were obtained from the referenced literature.

† CD11c was detected on uterine mast cells in one study,[17] but not in another.[31]

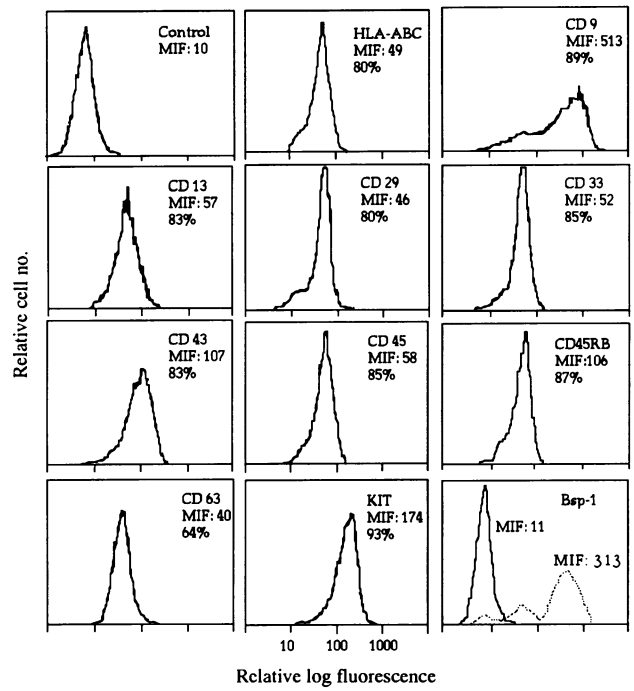

Figure 1. Representative flow cytometric histograms for various surface antigens present on rhSCF-dependent foetal liver-derived mast cells (——). The percentage of positive cells compared to control, and the MIF is given for each histogram. For Bsp-1 the expression on peripheral blood basophils (. . . .) is given as a control.

RESULTS AND DISCUSSION

Analysis of mast cells by flow cytometry

Foetal liver cells cultured with rhSCF (50 ng/ml) for 5 or more weeks consisted of 85% to 95% tryptase$^+$ mast cells. Flow cytometric analysis of the forward and side light scatter pattern of these cells revealed that approximately 80% of the events reside within this area of forward scatter, and previous sorting experiments revealed that cells within this region are predominantly tryptase$^+$ mast cells. Thus, data on surface marker expression by flow cytometry of cells within this region are representative of mast cells.

Surface antigens of foetal liver-derived human mast cells

A summary of the surface marker composition of human mast cells derived *in vitro* from foetal liver cells is shown in Table 1 together with data from the literature for human basophils [14,15,28-30] and mast cells obtained from lung[14,15] and uterus.[17]

The corresponding flow cytometric patterns of selected markers for foetal liver-derived mast cells are illustrated in Fig. 1. Mast cells derived from foetal liver express human class I MHC molecules (HLA-ABC) and the early myeloid markers, CD13 and CD33. By comparison, CD13 is expressed on basophils, but not on mast cells obtained from lung tissue, while CD33 is detected on both basophils and tissue mast cells. These antigens preferentially are expressed early in the development of myelocytes. The immature nature of rhSCF-dependent foetal liver-derived mast cells was previously suggested by ultrastructural analysis,[1] suggesting a rationale to explain the apparent increased expression of these surface antigens in mast cells examined in the current study compared to those in tissues.

Like most mature myeloid cells, foetal liver-derived mast cells expressed the β_1 integrin molecule (CD29) found in the class of adhesion molecules known as very late activation antigens (VLA-n); however, the pattern of α-chain (CD49n) expression was not evaluated in this study. CD49d (VLA-4) and CD49e (VLA-5) are expressed on uterine, lung and skin mast cells,[17,31] whereas CD49b and CD49f are not. Tissue-derived mast cells also express CD51 (α-chain of vitronectin receptor) and CD61 (β-chain of β_3-integrins).[31] Other classes of adhesion molecules, including β_2 integrins (CD11b, CD11c, CD18),[32] ICAM-1 (CD54) and NCAM (CD56), were not detected on foetal liver-derived mast cells. In contrast, ICAM-1 is expressed on uterine[17] and lung[15] mast cells. Expression of ICAM-1 on lung mast cells is up-regulated after addition of IL-4,[33] a

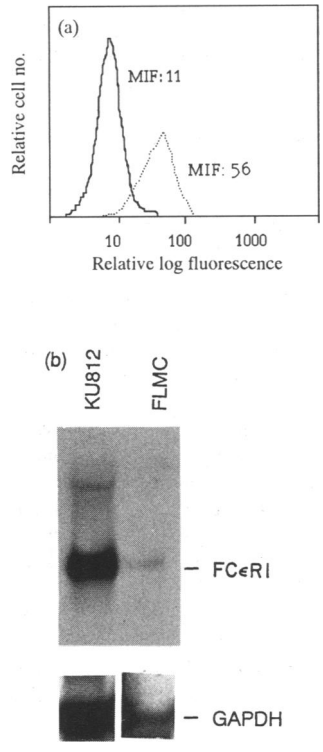

Figure 2. Expression of FcεRI. (a) Comparision of the expression of the FcεRI on KU812 cells (.....) and rhSCF-dependent foetal liver-derived mast cells (————). (b) Northern blot analysis of mRNA (5 μg poly A+RNA) obtained from rhSCF-dependent foetal liver-derived mast cells cultured in the presence of rhSCF (50 ng/ml) for 6 weeks and KU812 cells. Cell RNA was hybridized to a probe for FcεRIα mRNA, and exposed to autoradiographic films for 25 days. After washing the filters, hybridization was performed with a probe for GAPDH and the filter was exposed for 5 days.

cytokine that cells may be exposed to naturally *in vivo*, but not *in vitro*.

The surface antigen giving the highest mean intensity of fluorescence (MIF) is CD9, a gp24 kinase. Also expressed is CD63, an antigen present on the membranes of intracellular granules in basophils[30] and platelets[34] that is transferred to the cell surface during granule exocytosis. Hence, CD63 on the mast cell surface may reflect ongoing degranulation, a possibility raised previously by the ultrastructural appearance of these cells and by the finding of substantial amounts of histamine and tryptase in the media of cultures containing mast cells of high viability.[1] That rhSCF may activate as well as prime mast cells in humans and rodents provides a possible mechanism to explain the activation state of foetal liver-derived human mast cells.[16,35,36]

Two murine monoclonal antibodies that have been used to distinguish mature basophils from mature mast cells in humans are YB5.B8 and Bsp-1. YB5.B8 recognizes Kit, which is strongly expressed on mature mast cells but not on mature basophils,[15,22,37] while Bsp-1 recognizes a membrane glycoprotein of 80,000 MW and a minor band at 45,000 MW, with unknown function, that is expressed on mature basophils, but not on mature mast cells.[15,21] Mast cells derived from foetal liver are strongly labelled with YB5.B8 (MIF = 174), consistent with their designation as mast cells. They are not labelled with Bsp-1 (MIF = 11). This surface antigen profile supports the designation of rhSCF-dependent foetal liver-derived cells as being mast cells.

Foetal liver-derived mast cells did not express surface antigens characteristic of a lymphoid lineage, such as CD3, CD4, CD8, CD10, CD19 and CD21; or of a myeloid lineage, such as CD14, CD16, CD32 and CD35. No expression of the early myeloid marker CD34 could be detected on tryptase$^+$ foetal liver-derived mast cells; whether the progenitors of mast cells in foetal liver express this molecule, a possibility based on data showing CD34$^+$ bone marrow-derived cells give rise to mast cells,[38] was not assessed in the current study.

As reported for lung mast cells, foetal liver-derived mast cells also lack both the IL-2 receptor (CD25), which when expressed on helper T-cells reflects their activation and on eosinophils is involved in chemotaxis to IL-2,[39] and CD40, which when expressed on human B cells and stimulated by its ligand on the surface of T-cells is involved in IgE production.[40] Both leukosialin, a glycosphingolipid known as CD43, and the leucocyte common antigen called CD45, are expressed on foetal liver-derived mast cells. Of the different isoforms of CD45, CD45RB, but not CD45RA or CD45RO, was detected. Expression of different isoforms on mast cells from tissues or basophils has not been described.

Analysis of FcεRI on foetal liver-derived mast cells

The high-affinity receptor for IgE, FcεRI, is selectively expressed *in vivo* on both mature mast cells and basophils (reviewed in refs 41, 42). FcεRI is a tetramer, consisting of one α, one β and two γ subunits, each partially embedded in the plasma membrane. The α-chain binds IgE and is exposed on the outer surface of the plasma membrane, whereas β- and γ-chains are involved in signal transduction and are exposed on the cytoplasmic surface of the plasma membrane. Surprisingly, FcεRIα examined by flow cytometry with non-neutralizing FcεRIα-specific antibody[23] was not detected on rhSCF-dependent foetal liver-derived mast cells, while its presence on KU812 cells was clearly demonstrated (Fig. 2). This was true for mast cells from 4 to 12 weeks of culture. Also, binding of human IgE from the U266 myeloma to basophils, but not to rhSCF-dependent foetal liver-derived mast cells was observed (data not shown). Analysis of expression of FcεRIα mRNA in foetal liver-derived mast cells, revealed a hybridization signal after 25 days of exposure that appeared to be substantially weaker than for KU812 cells (Fig. 2). By comparison, human mast cells derived *in vitro* from cord blood progenitors cultured on mouse 3T3 fibroblasts, express FcεRI, although to a lesser degree than on human lung mast cells.[6] Both for IL-3-dependent bone marrow-derived cells from mice, and for IL-3-stimulated bone marrow cells in humans, transcripts for FcεRIα were detected early during development, even before granulation was noted.[43] For mice expression of FcεRI early in culture was presumed to be in mast cells, because mast cells predominate over basophils at later time-points, but basophil progenitors were not excluded as a source of receptor. For humans expression was presumed to be in basophils, because this is the predominant cell type that develops later in response to IL-3. IL-3 has negligible effects on the growth and development of rhSCF-dependent foetal liver-derived human mast cells,[1] including the expression of FcεRI (data not shown). Neither IL-4 nor conditioned media from a

T-cell line (MO) in combination with rhSCF induced expression of FcεRI detectable by flow cytometry. Thus, the network of cytokines and accessory signals from other cells required for the expression of the high-affinity receptor for IgE on human mast cells is not fully resolved and will be explored in future studies.

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