

Original article

Detection of differentiation- and activation-linked cell surface antigens on cultured mast cell progenitors

Background: Mast cells (MC) are multifunctional effector cells of the immune system. They derive from uncommitted CD34⁺ hemopoietic progenitor cells (HPC). Depending on the stage of maturation and the environment, MC variably express differentiation- and activation-linked antigens. Little is known, however, about the regulation of expression of such antigens in immature human MC.

Methods: We analyzed expression of CD antigens on human MC grown from cord blood-derived CD34⁺ HPC. The HPC were isolated by magnetic cell sorting (MACS) and FACS to >97% purity, and were cultured in stem cell factor (SCF) and interleukin (IL)-6 with or without additional cytokines (IL-4 or IL-10) in serum-free medium. The cell surface phenotype of MC was determined by monoclonal antibodies and flow cytometry.

Results: Cultured MC progenitors were found to react with antibodies against various CD antigens including CD58, CD63, CD117, CD147, CD151, CD203c, and CD172a, independent of the growth factors used and time-point investigated (days 14–42). CD116 [granulocyte–macrophage colony-stimulating factor receptor α (GM-CSFR α)] and CD123 (IL-3R α) were expressed on MC precursors on day 14, but disappeared thereafter. Cultured MC did not express CD2, CD3, CD5, CD10, CD19, or CD25. Addition of IL-10 to MC cultures showed no effect on expression of CD antigens. However, IL-4 was found to promote expression of CD35 and CD88 on cultured MC without changing expression of other CD antigens.

Conclusions: Most MC antigens may already be expressed at an early stage of mastopoiesis. Whereas IL-3R and GM-CSFRs are lost during differentiation of MC, these cells may acquire complement receptors (CD35, CD88) under the influence of distinct cytokines.

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Mast cells (MC) are multifunctional effector cells of allergic and inflammatory reactions (1, 2). These cells express a number of activation-linked cell surface antigens as well as an array of vasoactive and proinflammatory mediator substances (3, 4). Activation of MC through distinct cell surface molecules is associated with mediator release (3–5). However, recent data suggest that MC are heterogeneous cells in terms of mediator production and expression of cell surface antigens (2–4). Thus, depending on the stage of maturation, the tissue environment, and the type of disease, MC variably express activation-linked and other cell surface antigens (4, 6–10).

In common with other myeloid cells, MC derive from uncommitted (CD34⁺) hemopoietic progenitor cells (HPC) (11–17). These HPC can be detected in the bone marrow as well as in the peripheral blood (11–17). Differentiation and maturation of MC are regulated by

distinct cytokines. The stroma cell-derived cytokine stem cell factor (SCF) is a major regulator of MC development. In fact, this cytokine induces differentiation of MC in bone marrow- and blood-derived HPC (13–17). Other cytokines, like interleukin (IL)-6 or IL-4, may also influence MC differentiation (16–21). Thus, using SCF, IL-6, and other cytokines, various culture systems for the growth and differentiation of human MC have been established (16–21).

During SCF-induced differentiation, MC progenitors acquire cell-specific differentiation antigens such as tryptase, chymase, or Fc ϵ RI (19–23). These antigens appear in an ordered sequential manner during mastopoiesis. Likewise, tryptase expression is already seen at an early stage of MC development, whereas expressions of Fc ϵ RI and chymase have been described as relatively 'late' events (13, 14, 17–21). It has also been reported that IL-4 promotes expression of Fc ϵ RI and chymase in immature

cultured MC (17, 18). In other studies, the CD antigen profile of developing human MC has been examined. In these studies, it was found that cultured immature MC express a CD phenotype similar to mature tissue MC (19–24). However, these immature MC express additional 'myeloid' CD antigens such as CD13 (20–22). In addition, cultured MC have been reported to express cytokine receptors [IL-3R, IL-5R, granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR)] not detectable on mature tissue MC (20, 21). During cytokine-induced MC development, most of these receptors may decrease in expression (20, 24). An exception in this regard is the receptor for SCF (KIT), which is constantly expressed during MC development as well as on mature MC (13, 20–24).

During the past few years, substantial progress has been made in the characterization of the CD phenotype of human MC in health and disease (4, 7–10, 25, 26). In fact, several novel differentiation- and/or activation-linked CD antigens have been detected on the surface of normal and/or neoplastic human MC (4, 25, 26). The aim of the present study was to examine expression of novel CD antigens on immature MC progenitors cultured in serum-free medium in the presence of SCF and other cytokines.

Materials and methods

Monoclonal antibodies and other reagents

A large panel of monoclonal antibody (mAb) was used to characterize the cell surface antigen phenotype of cultured human MC. Most of the antibodies were purchased. A specification of these mAb is provided in Table 1. Other mAb used in this study were MoS39 (CD14), PM-81 (CD15), B4 (CD19), 4B4 (CD29), 153-4D9 (CD84), VMP-55 (CD85), BF-22 (CD126), AM64 (CD130), and 14A2.H1 (CD151). These mAb were obtained from the Vth or Vth International Workshops and Conferences on Human Leukocyte Differentiation Antigens (Boston, 1992; Kobe 1996). The mAb VIT13 (CD2R) and VIM5 (CD87) were kindly provided by Dr O. Majdic (University of Vienna, Vienna, Austria), mAb MEM-M6/1 (CD147) by Dr H. Stockinger (University of Vienna, Vienna, Austria), and the mAb SE5A5 (CD172a) and 97A6 (CD203c) by Dr H.-J. Bühring (University of Tübingen, Tübingen, Germany). Recombinant human (rh) SCF was purchased from R&D (Minneapolis, MN, USA), and IL-4, IL-6, and IL-10 from Peprotech (Rocky Hill, NJ, USA). Ultraculture medium was purchased from Bio-Whittaker (Walkersville, ML, USA), RPMI-1640 medium from PAA laboratories (Linz, Austria), fetal calf serum (FCS), L-glutamine, penicillin, and streptomycin from Gibco Life Technologies (Gaithersburg, MD, USA), and human AB-serum from Sera Lab (Crawley Down, UK).

Purification of cord blood progenitors

Mast cell progenitors were isolated from cord blood obtained from full-term deliveries ($n = 13$) after informed consent was given by mothers. Cord blood mononuclear cells (MNC) were separated by using Ficoll. CD34⁺ cells were purified from cord blood MNC by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch-

Table 1. Specification of commercial mAb

mAb	CD	Ig-class	Source	Obtained from
T11	CD2	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
RPA-2.10	CD2	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
S5.2	CD2	IgG _{2a}	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
6F10.3	CD2	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
39C1.5	CD2	IgG _{2a}	Rat	Beckman-Coulter (Brea, CA, USA)
SK7	CD3	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
SK3	CD4	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
L17F12	CD5	IgG _{2a}	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
SK1	CD8	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
SS2/36	CD10	IgG ₁	Mouse	Dako (Glostrup, Denmark)
MOP9	CD14	IgG _{2b}	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
MMA	CD15	IgM	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
2A3	CD25	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
B1.49.9	CD25	IgG _{2a}	Mouse	Beckman-Coulter (Brea, CA, USA)
581	CD34	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
8G12	CD34	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
E11	CD35	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
S7	CD43	IgG _{2a}	Rat	BD Biosciences Pharmingen (San Diego, CA, USA)
L178	CD44	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
ALB12	CD45	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
84H10	CD54	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
1C3	CD58	IgG _{2a}	Mouse	Beckman-Coulter (Brea, CA, USA)
CLBgran12	CD63	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
L78	CD69	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
W17/1	CD88	IgG ₁	Mouse	Serotec (Oxford, UK)
S5/1	CD88	IgG _{2a}	Mouse	Serotec (Oxford, UK)
S-50	CD116	IgG ₁	Mouse	Santa Cruz (Santa Cruz, CA, USA)
YB5.B8	CD117	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
104D2	CD117	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
9F5	CD123	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
7G3	CD123	IgG _{2a}	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)
R34.34	CD127	IgG ₁	Mouse	Beckman-Coulter (Brea, CA, USA)
S-16	CD131	IgG ₁	Mouse	Serotec (Oxford, UK)
AC133	CD133	IgG ₁	Mouse	Miltenyi Biotec (Bergisch-Gladbach, Germany)
L243	NC	IgG ₁	Mouse	BD Biosciences Pharmingen (San Diego, CA, USA)

NC, not yet clustered; mAb, monoclonal antibody; Ig, immunoglobulin.

Gladbach, Germany) using mAb QBEND/10, and consecutive cell sorting with mAb 8G12 (CD34) and a FACS Vantage SE (BD Biosciences, San Jose, CA, USA). The purity of the isolated CD34⁺ progenitor cells amounted to >97%.

Mast cell differentiation assay

CD34⁺ cord blood-derived progenitors ($0.5 \times 10^6/\text{ml}$) were cultured in the presence of (i) SCF (100 ng/ml) + IL-6 (100 ng/ml), (ii) SCF (100 ng/ml) + IL-6 (100 ng/ml) + IL-4 (100 U/ml), or (iii) SCF (100 ng/ml) + IL-6 (100 ng/ml) + IL-10 (100 ng/ml), or (iv) SCF (100 ng/ml) + IL-6 (100 ng/ml) + IL-4 (100 U/ml) + IL-10 (100 ng/ml). The differentiation assay was performed essentially as described (13–20). All cytokine-combinations were applied in (i) serum-free ultraculture medium containing 1% L-glutamine and antibiotics, and parallel in (ii) RPMI-1640 medium containing 10% FCS, L-glutamine (1%), and antibiotics. In a separate set of experiments, MC progenitors were cultured in the presence of cytokines together with supernatant (30%) of HMC-1 cells, a human MC line (27) kindly provided by Dr J. H. Butterfield (Mayo Clinic, Rochester, MN, USA). All cultures were maintained at 37°C and 5% CO₂. Cells were fed on days 3 and 7, and thereafter in 1–2 week intervals. At various time-points (days 7, 14, 21, 28, 35 and 42), cultured cells were examined for viability (trypan blue exclusion), cell number, morphology (Wright-Giemsa stain), and expression of CD antigens by flow cytometry.

In situ staining experiments

To confirm the identity of MC and their percentage among cultured cells, *in situ* immunocytochemistry staining experiments were conducted using cytospin slides and the antitryptase mAb G3 (Chemicon, Temecula, CA, USA) essentially as described (28, 29). After cytospin preparation, cells were fixed in acetone and incubated with mAb G3 for 60 min at room temperature. Thereafter, slides were washed in Tris-buffered saline (pH 7.3) and incubated with a biotinylated goat-anti-mouse immunoglobulin G (IgG; Biocarta, Hamburg, Germany; 30 min) and then with streptavidin-alkaline phosphatase complex (Biocarta) for another 30 min. Neofuchsin was used as chromogen. Slides were counterstained in Mayer's Haemalaun.

Cell surface staining – flow cytometry

Flow cytometry experiments were performed on cultured MC using antibodies against CD antigens essentially as described previously (7, 28). In all experiments, cells were preincubated in AB serum to prevent nonspecific staining reactions. As 'second-step' reagents, goat F(ab')₂ anti-mouse FITC or goat F(ab')₂ anti-rat FITC (Caltag Laboratories, Burlingame, CA, USA) were used. Expression of CD antigens on MC was analyzed by flow cytometry on a FACScan (Becton Dickinson, San Jose, CA, USA). In all experiments, staining reactions were controlled using isotype-matched antibodies.

Results

Expression of CD antigens on cultured MC on day 42

In cultures containing SCF and IL-6, more than 80% of all cells were MC at day 42 as assessed by Wright-Giemsa staining. Tryptase-staining confirmed the presence and percentage of MC in these cultures. No major differences in cell viability (>90% in all cases), percentages in MC, or expression of CD antigens were seen when comparing serum-free cultures with serum-containing cultures. As assessed by flow cytometry, cultured MC on day 42 were

found to express the pan leukocyte antigen CD45, CD63, and KIT (CD117) as well as the ecto-enzyme E-NPP3 (CD203c) and low amounts of SIRPα (CD172a; Table 2). By contrast, these cells did neither express lymphocyte-related antigens (CD2, CD3, CD4, CD5, CD10, CD19) nor the monocyte-related antigen CD14 (Table 2). This

Table 2. Expression of CD antigens on MC progenitors cultured in the presence of SCF and IL-6

CD	Antigen	Expression on day				
		7	14	21	42	63
CD2	LFA-2	–	–	–	–	–
CD2R	T11-3	ND	ND	–	–	–
CD3	T3	ND	ND	–	–	–
CD4	T4	ND	ND	–	–	–
CD5	T1	ND	ND	–	–	–
CD8	T8	ND	ND	–	–	–
CD10	CALLA	ND	ND	–	–	–
CD14	LPS-R	–	–	–	–	–
CD15	Lewis X	++	++	++	++	++
CD19	B4	ND	ND	ND	ND	–
CD25	IL-2Rα	ND	ND	–	–	–
CD29	β of β ₁ integrin	ND	ND	+	+	+
CD34	HPCA-1	–/+	–/+	–	–	–
CD35	CR1	ND	ND	–	–	–
CD43	Leukosialin	ND	ND	ND	ND	ND
CD44	Pgp-1	ND	ND	++	++	++
CD45	LCA	ND	ND	++	++	++
CD54	ICAM-1	ND	ND	–/+	–/+	+
CD58	LFA-3	ND	ND	+	+	+
CD63	LAMP-3	ND	ND	+	+	+
CD69	AIM	ND	ND	–	–	–
CD84	CD2-like	ND	ND	+	+	+
CD85	LIR-1	ND	ND	–	–	–
CD87	uPAR	ND	ND	–	–	–
CD88	C5aR	ND	ND	–/+	–/+	+
CD116	GM-CSFRα	+	+/-	–/+	–	–
CD117	KIT/SCF-R	++	++	++	++	++
CD123	IL-3Rα	+	+/-	–/+	–	–
CD126	IL-6Rα	ND	ND	–	–	–
CD127	IL-7R	ND	ND	–	–	ND
CD130	gp130	ND	ND	–	–	–
CD131	Common β-chain	–/+	–	–	–	–
CD132	Common γ-chain	ND	ND	–	–	ND
CD147	Basigin	ND	ND	++	++	++
CD151	PETA-3	ND	ND	+	–/+	–
CD172a	SIRPα	–/+	–/+	–/+	–/+	–/+
CD203c	E-NPP3	++	+	+	+/-	–/+
NC	HLA-DR	ND	ND	–	–	–

Cultures were initiated from purified CD34⁺ cord blood progenitor cells using SCF and IL-6 as growth factors. Expression of CD antigens on developing MC progenitors was analyzed by flow cytometry.

NC, not yet clustered; ND, not determined; LFA, leukocyte function-associated antigen; LPS, lipopolysaccharide; IL, interleukin; CR, complement receptor; LCA, leukocyte common antigen; ICAM, intercellular adhesion molecule; AIM, activation inducer molecule; uPA, urokinase plasminogen activator; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor; HLA, human leukocyte antigen; SCF-R, stem cell factor receptor; MC, mast cell.

phenotype corresponds with the cell surface antigen profile of mature human tissue MC.

Expression of cytokine receptors at various stages of MC development

Immature (days 7–14) as well as more mature (days 21–42) MC progenitors expressed KIT (CD117). At an immature stage of their development (days 7 and 14), MC progenitors were also found to express the α -chain of the IL-3R (CD123) and α -chain of the GM-CSFR (CD116; Fig. 1). However, these surface antigens could not be detected at a later stage of MC development (days 35 or 42) in our culture system (Fig. 1). Interestingly, the common β -chain of the IL-3R and GM-CSFR was not detectable on MC in these cultures (neither on day 7 nor at later time-points). Cultured MC were also found to lack the IL-2R α -chain (CD25), IL-6R α -chain (CD126), and gp130 (CD130) at all time-points investigated. Identical results were obtained in cultures maintained with serum-free or serum-containing medium. In addition, no differences in expression of cytokine receptors on MC were observed when comparing progenitor cultures maintained in the presence of various cytokine combinations.

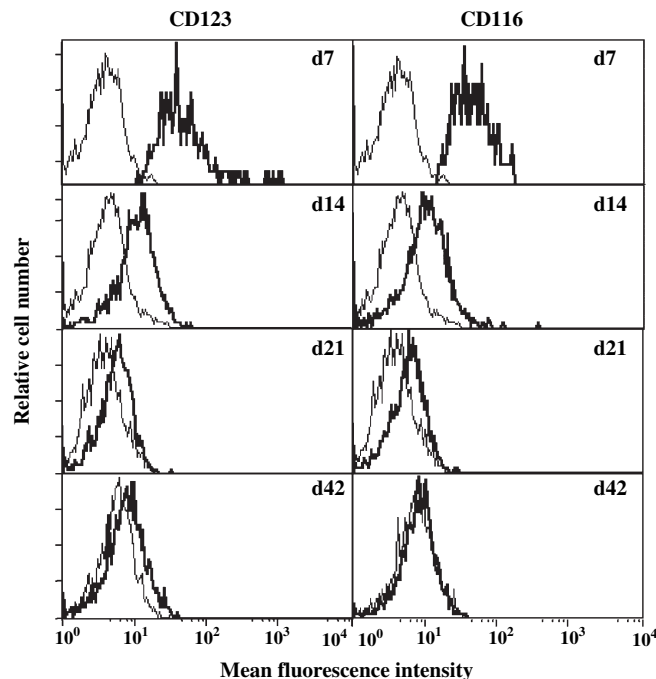


Figure 1. Expression of interleukin (IL)-3- and granulocyte-macrophage colony-stimulating factor (GM-CSF)-receptor on cultured mast cell progenitors. Highly enriched CD34⁺ cord blood progenitors were cultured in stem cell factor (SCF; 100 ng/ml) and IL-6 (100 ng/ml) for various time periods (day = d) as indicated in serum-free culture. Expression of the IL-3R α -chain (CD123) and GM-CSFR α -chain (CD116) was analyzed by flow cytometry. As visible, expression of both CD123 and CD116 decreased during mast cell development.

Expression of adhesion-related antigens on developing MC

Cultured MC were found to express a number of adhesion-related cell surface antigens including the common β -chain of β -1 integrins (CD29), the homing receptor Pgp-1 (CD44), intercellular adhesion molecule-1 (ICAM-1; CD54), leukocyte function antigen-3 (LFA-3; CD58), PETA-3 (CD151), and low amounts of SIRP α (CD172a). These antigens were detected on cultured MC at all time-points investigated (days 7–42). Thus, adhesion-related antigens appear to be expressed already at an early stage of differentiation of human MC. Unexpectedly, immature cultured MC also expressed sialyl Lewis X (CD15). Throughout their development, cultured MC failed to express LFA-2 (CD2), an adhesion molecule that is expressed on neoplastic MC in patients with systemic mastocytosis (4, 9, 10, 28, 30). In fact, none of the CD2 antibodies applied including CD2R produced a positive staining-reaction on immature cultured MC. Identical patterns of adhesion receptors on MC were found in cultures maintained with serum-free or serum-containing medium. In addition, no differences in expression of adhesion molecules on cultured MC were observed when comparing cultures maintained under various cytokine combinations.

Expression of activation-linked CD antigens on cultured human MC

Recent data suggest that mature human tissue MC express a number of activation-linked CD molecules such as CD63 or CD203c. In the current study, we found that most of these antigens are already expressed at an early stage (days 7 and 14) of MC development. In fact, MC cultured from their progenitors in the presence of SCF and IL-6 expressed CD63, CD84, CD88, CD147, and CD203c. Most of these antigens were found to be expressed at a constant level throughout mastopoiesis (days 7–42). An exception appeared to be CD203c. In fact, this antigen was found to be strongly expressed on the surface of cultured MC until day 21, but was only weakly expressed on MC on day 42 (Fig. 2). This is of interest as normal MC express low levels of CD203c (4). The SCF-/IL-6-cultured MC did not express detectable levels of CD2, CD25, and CD35, antigens that are expressed on MC in patients with mastocytosis or MC leukemia (4, 9, 10, 28, 30). In addition, cultured MC failed to express human leukocyte antigen (HLA)-DR. Identical results were obtained for MC cultured in serum-free and FCS-containing cultures.

Influence of IL-4 and IL-10 on expression of activation-linked CD antigens on MC progenitors

Recent data suggest that IL-4 and IL-10 are critical regulators of expression of differentiation antigens in MC. In this study, addition of IL-4 to SCF-/IL-6-cultured

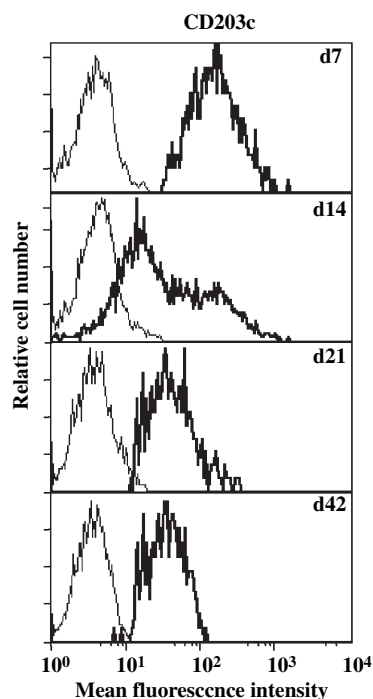


Figure 2. Expression of CD203c on developing human mast cells. Highly purified CD34⁺ cord blood progenitors were cultured in stem cell factor (SCF; 100 ng/ml) and interleukin (IL)-6 (100 ng/ml) in serum-free medium for various time periods (day = d). Expression of CD203c on developing mast cell (MC) was determined by flow cytometry.

MC resulted in a substantial increase in expression of CD88 (C5aR) as well as expression of CD35 (CR1; Fig. 3). This is of particular interest because these complement receptors are 'over-expressed' on MC in some patients with chronic inflammatory disorders as well as in patients with systemic mastocytosis or MC leukemia (4, 30, 31). Therefore, we also examined the effect of supernatant of neoplastic MC (HMC-1) on expression of CD35 and CD88 on cultured MC. Interestingly, this supernatant induced the expression of CD35 on cultured MC in the same way as IL-4 did, whereas no significant effect of HMC-1 supernatant on expression of CD88 was seen (Fig. 3). In contrast to IL-4, no effects of IL-10 on expression of CD35 or CD88 on cultured MC were found. Also, no effects of IL-4 or IL-10 on expression of other CD antigens tested, could be substantiated.

Discussion

Depending on their stage of maturation, the tissue environment, and the underlying disease, MC express variable amounts of differentiation- and activation-linked surface antigens (4, 6–9, 17, 19–20). During the past few years, a number of novel CD antigens have been detected on the surface of normal and/or

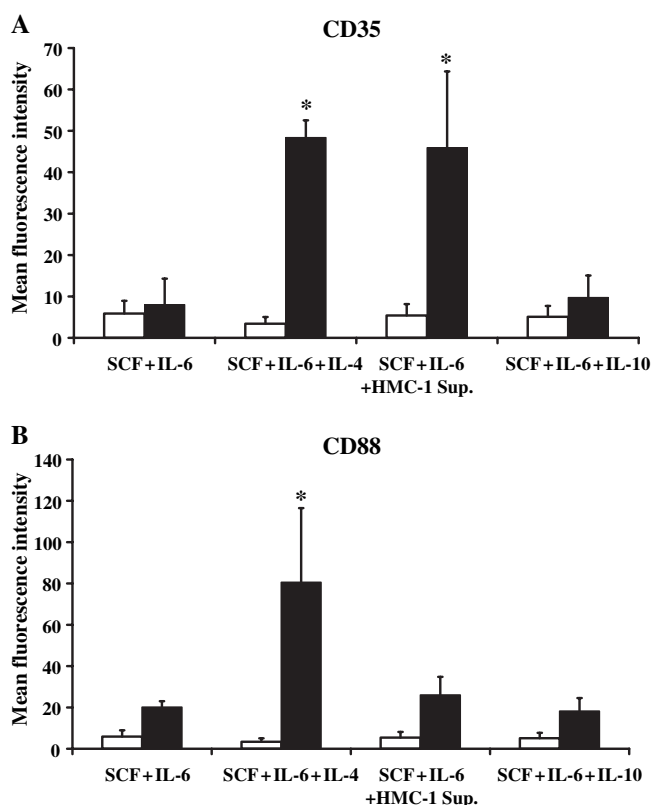


Figure 3. Effects of interleukin (IL)-4 and IL-10 on expression of complement receptors on mast cells. Mast cell progenitors were grown from cord blood-derived CD34⁺ progenitor cells in serum-free medium for 42 days using stem cell factor (SCF; 100 ng/ml) and IL-6 (100 ng/ml) as basic growth factors. On day 7, cultures were split and then maintained in SCF + IL-6 with or without additional cytokines (IL-4, 100 ng/ml or IL-10, 100 ng/ml) or HMC-1 supernatant (Sup; 30%) as indicated. Expression of CD35 (3A) and CD88 (3B) was determined by monoclonal antibodies and flow cytometry. Black bars indicate mean fluorescence intensities obtained with CD35 and CD88 antibodies (mean $\pm$ SD of five independent experiments). The isotype control is also shown (open bars). Significant differences in CD antigen expression under additional stimuli (IL-4, IL-10, HMC-1 Sup) compared with SCF + IL-6 alone, is indicated by an asterisk ($P < 0.05$).

neoplastic MC (4, 25, 26). In the present study, we have examined expression of novel and previously defined CD antigens on cultured human MC at various phases of their development. The results of our study show that cultured MC exhibit novel differentiation- and activation-linked CD antigens, most of them being expressed already at an early phase of mastopoiesis. In addition, our data show that IL-4 promotes expression of the complement receptors CD35 (CR1) and CD88 (C5aR) on immature-cultured MC.

Recent data suggest that immature MC progenitors express various cytokine-binding sites including the IL-3R and GM-CSFR, and that during differentiation and maturation, these cytokine receptors decrease in

expression over time and disappear. Thus, mature human tissue MC lack IL-3R and GM-CSFRs (32, 33). The results of our study confirm the decrease in the IL-3R and GM-CSFR on cultured MC during development from their CD34⁺ progenitor cells. In fact, whereas on day 14, many of these MC progenitors reacted with antibodies against CD116 and CD123, these molecules were not detectable on cultured MC after day 21. These data correspond well with the results of Ochi et al. (20), but are in contrast to the data presented by Dahl et al. (21). These differences may be explained by different levels of sensitivity in the assays used to detect the receptor or by the different growth factors used. Likewise, Dahl et al. (21) used IL-3 (a strong basophil-differentiation factor), whereas in this study, IL-3 was not applied. Moreover, in the paper by Dahl et al. (21), IL-3-binding sites were only detectable in significant amounts on MC by using biotinylated IL-3, but was hardly detectable by immunostaining using CD123 antibody (similar low MFI compared with this study). An unexpected finding in our experiments was that 'day-14' progenitor cells did not express the common β -chain of GM-CSFR and IL-3Rs. This may be due to low-level expression of this antigen or to selective expression of α -chains. All in all, our data suggest that receptors for IL-3 and GM-CSF do not play a major role in the development of MC-committed progenitors. In line with this notion, neither IL-3 nor GM-CSF have been described as potent growth factors for human MC (12–14).

Recent data suggest that neoplastic MC in patients with systemic mastocytosis express abnormal (increased) levels of certain CD antigens including CD2, CD25, CD35, CD88, and CD203c (4, 9, 10, 28–31). Because neoplastic MC are often immature in patients with mastocytosis, we were interested to know whether these antigens are expressed on cultured immature (normal) MC in our cultures. In these experiments, we found that neither CD2 nor CD25 is expressed on cultured immature MC. By contrast, CD203c was clearly expressed on MC progenitors at an early stage of their development, whereas later, during mastopoiesis, the levels of CD203c decreased. Based on this observation, it is tempting to speculate that abnormal expression of CD203c on MC in patients with mastocytosis may be related to their immaturity. Alternatively, however, expression of CD203c on neoplastic MC may be due to abnormal gene regulation.

With regard to CD35 and CD88, expression on MC progenitors appeared to depend on the cytokines used. Thus, both markers were detectable on MC when cultured in IL-4, but not in the absence of this cytokine. As MC produce IL-4 under various conditions, this observation raises the possibility that IL-4 or other autocrine factors are involved in abnormal expression of complement receptors on neoplastic MC. Notably, as mentioned above these antigens are overexpressed on MC in patients with systemic mastocytosis (4, 9, 10, 28–31). In order to discover whether autocrine factors might be

involved in abnormal expression of CD35 and CD88 on neoplastic MC, supernatants of the MC leukemia line HMC-1 were added to cultured progenitors. These supernatants were found to be equally effective in inducing expression of CD35 on cultured MC (compared with IL-4), whereas no effect on expression of CD88 was found. These observations argue against a role of IL-4 as autocrine regulator of expression of CD35 in neoplastic MC. All in all, our data suggest that expression of CD antigens on MC is dependent on multiple factors and mechanisms.

A number of data suggest that MC and monocytic cells share a common progenitor (34, 35). Likewise, MC and monocytes co-express cell surface and cytoplasmic antigens (4). In addition, it has been hypothesized that MC directly derive from a monocytic cell (34). Other data suggest, however, that MC and monocytes represent two different myeloid lineages (14, 25). In this study, we found that cultured immature MC express most MC-related antigens already at an early phase of development. Interestingly, these immature progenitors were also found to co-express CD15, a broadly distributed myelomonocytic antigen. By contrast, MC progenitors failed to express CD14, a monocyte-specific antigen. These data argue against development of MC from (CD14⁺) monocytes in our culture system. On the contrary, MC and monocytes may derive from a common myelomastocytic progenitor. Expression of CD15 on immature MC would be in line with this hypothesis.

The MC and their progenitors express a number of adhesion molecules (6, 8, 14, 19). These antigens are considered to be essential for binding of MC and MC progenitors to other cells and tissue matrix elements. Thus, expression of adhesion-related antigens may be important for homing of MC and MC progenitors. Especially immature (circulating) MC progenitors may require the expression of homing receptors (19). In line with this assumption, most adhesion-related antigens including the β -1 integrin CD29 and the ICAM-1 and LFA-3 antigen, were found to be expressed already at an early phase of MC development in our culture system.

In conclusion, our data show that immature cultured MC express several functionally important cell surface antigens including adhesion receptors, cytokine receptors, and novel lineage-related antigens. Some of these antigens decrease during mastopoiesis. Expression of distinct activation-linked cell surface antigens, i.e. the complement receptors CD35 and CD88 are only expressed on MC-progenitors after exposure to IL-4.

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