

Cell Surface Membrane Antigen Phenotype of Human Gastrointestinal Mast Cells

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Key Words

Mast cells · Intestinal inflammation ·
Mast cell heterogeneity

Abstract

Background: Mast cells (MC) are important effector cells of allergic and inflammatory reactions in diverse organs. These cells interact with a number of other immune cells and structural cells in the tissues as well as with proinflammatory mediators and cytokines. The various interactions are considered to be mediated through distinct cell surface membrane receptors on MC. **Methods:** In the present study, we have established the cell surface membrane phenotype of human gastrointestinal MC (HGMC) using a panel of monoclonal antibodies and indirect immunofluorescence staining techniques. **Results:** HGMC were found to react with antibodies against CD29, CD33, CD44, CD45, CD47, CD54, CD55, CD58, CD63, CD117, CD147, CD151, CD172a, and CD203c. By contrast, HGMC did not express detectable amounts of CD1, CD2, CD4, CD5, CD14, CD15, CD16, CD22, CD24, CD25, CD26, CD27, CD28, CD31, CD32, CD34, CD35, CD88, or CD116. The

α -chain of the IL-3 receptor (CD123) was detectable neither in resting HGMC nor in HGMC exposed to stem cell factor and interleukin-4. **Conclusions:** HGMC express a unique profile of surface antigens including the receptor for mast cell growth factor, adhesion-related molecules, and activation-linked membrane antigens.

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Introduction

Mast cells (MC) are highly specialized proinflammatory effector cells of the immune system [1, 2]. These cells produce an array of inflammatory and vasoactive mediators [1–5]. In response to IgE receptor cross-linking or other stimuli, MC release their products into the extracellular space and thereby can participate in allergic and inflammatory reactions [1–6]. Differentiation, migration, and activation of MC are regulated by a complex network of cytokines, other mediators, and cell surface membrane antigens [6–12]. These molecules regulate the distribution and accumulation of MC in normal and inflamed tissues as well as mediator release [6–10, 13–15]. The most potent cytokine regulating differentiation and mediator secretion in human MC appears to be the KIT ligand, stem cell factor (SCF) [8–10, 13–15].

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The phenotype and functional properties of MC vary depending on the stage of differentiation, organ site, type of disease, activation by cytokines, and other factors [16–22]. Likewise, MC express variable amounts of adhesion receptors and recognition molecules as well as receptors for complement products [20–22].

The gastrointestinal (GI) tract is an important target of acute and chronic inflammatory diseases including allergic reactions, autoimmune processes, or infectious diseases [23–25]. Various types of leukocytes including eosinophils, basophils, and lymphocytes are considered to contribute to the pathogenesis of GI disorders [26–28]. MC may also be critically involved in allergic and infectious diseases of the GI tract [15, 25, 29–31]. This notion is supported by the increase in tissue MC that is often seen in intestinal inflammation, and by the observation that MC-derived mediators (histamine, TNF- α , and others) play an important role in the pathogenesis of GI tract disorders [28–31]. However, only limited information about the cell surface phenotype of the human intestinal MC has become available to date [32, 33]. In the present study, we have established the cell surface membrane phenotype of human GI MC (HGMC), and compared it with the well-established cell membrane phenotype of human lung and skin MC [20, 32, 34–37].

Materials and Methods

Monoclonal Antibodies

A number of monoclonal antibodies (mAbs) were used to determine the immunophenotype of HGMC. Part of mAbs (unconjugated) were obtained from the 4th, 5th, or 6th International Workshop and Conferences on Human Leukocyte Differentiation Antigens (HLDAs) (table 1). The following mAbs were purchased: E-124.2.8 (anti-IgE), FITC-labeled CLB-gran12 (CD63), FITC-84H10 (CD54), FITC-1C3 (CD58), and PE-97A6 (CD203c) from Immunotech (Marseilles, France), PE-RPA210 (CD2), PE-SK3 (CD4), FITC-L17F12 (CD5), ICRF44 (CD11b), FITC-M Φ P9 (CD14), FITC-MMA (CD15), FITC-NKP15 (CD16), PE-P67.6 (CD33), FITC-581 (CD34), E11 (CD35), L178 (CD44), PerCP-labeled 2D1 (CD45), B6H12 (CD47), IA10 (CD55), FITC-M5D12 (CD116), PE-YB5.B8 (CD117), and PE-7G3 (CD123) from Becton Dickinson/Pharmingen (San Jose, Calif., USA), and FITC-AT10 (CD32) and W17/1 (CD88) from Serotec (Oxford, UK). The mAb SE5A5 (CD172a) was kindly provided by Dr. Hans-Jörg Bühring (University of Tübingen, Germany), and MEM-M6 (CD147) kindly provided by Dr. H. Stockinger (Medical University of Vienna, Austria). A specification of mAbs used in this study is shown in table 1.

Culture Reagents and Buffers

Collagenase type II was purchased from Sebak (Suben, Austria), fetal calf serum (FCS) from Hyclone (Logan, Utah), penicillin,

streptomycin, gentamicin, and fungizone from Gibco Life Technologies (Gaithersburg, Md., USA), and *L*-glutamine-containing RPMI-1640 medium from PAA Laboratories (Linz, Austria). Recombinant human (rh) SCF, rh interleukin-3 (IL-3), and rhIL-4 were from Strathmann Biotech (Hannover, Germany). One liter Mg/Ca-free Tyrode's buffer contained 0.2 g KCl, 0.05 g NaH₂PO₄·H₂O, 8.0 g NaCl and 1 g glucose.

Preparation and Culture of HGMC

GI tissue was obtained from surgical specimens of 18 patients (12 females, 6 males; median age: 60 years; range 51–70 years) who underwent (hemi)colectomy for GI cancer or chronic inflammation (colitis, Crohn's disease). Informed consent was obtained from patients before surgery in each case. MC were isolated from GI specimens according to published techniques [32, 33, 38]. In brief, tissue was cut into small pieces and washed extensively in Tyrode's buffer. Tissue fragments were then incubated in collagenase type II (2 mg/ml) for 120 min at 37°C. Then, cells were recovered, washed, and examined for the presence and percentage of MC by Wright-Giemsa staining. Isolated HGMC were cultured in RPMI-1640 medium containing *L*-glutamine, 10% FCS, and antibiotics at 37°C for at least 12 h before analysis. In selected experiments, HGMC were cultured in the presence or absence of rhSCF (100 ng/ml) and/or rhIL-4 (20 ng/ml) at 37°C and 5% CO₂ for 14 days. After 3 and 14 days, cells were recovered and analyzed for expression of CD45, CD63, CD116, CD123, and CD203c.

Indirect Immunofluorescence Staining Techniques

Expression of cell surface antigens on HGMC was analyzed by two different staining techniques, i.e. (1) by combined toluidine blue (TB)/immunofluorescence (IF) staining (TB/IF) and (2) by a multicolor IF staining technique.

Combined TB/IF staining was performed as described previously [32, 35–37]. In brief, cells were incubated with mAbs for 30 min at 4°C, washed in phosphate-buffered saline (PBS), exposed to a FITC-conjugated 'second-step' goat F(ab')₂ IgG anti-mouse antibody, and washed in PBS. Cells were then fixed in glutaraldehyde (0.025%) for 1 min, washed, and stained with freshly dissolved TB (0.0125%) for 8–10 min at room temperature. After washing, cells were examined with a fluorescence microscope (Olympus, Vienna, Austria) under bright-field and fluorescence light.

Multicolor flow cytometry was performed according to an established protocol [37, 39] using an APC-labeled CD117 mAb, PerCP-CD45 mAb, and additional PE-labeled and/or FITC-conjugated mAbs. Mast cells were defined as CD45+/CD117+ cells.

All antibody-staining reactions were controlled using isotype-matched control antibodies. In each experiment, cells were exposed to AB serum (room temperature, 15 min) before exposure to mAbs.

Histamine Release Experiments

In two donors, isolated HGMC were incubated with histamine release buffer (Immunotech) in the presence or absence of rhIL-3 (100 ng/ml) at 37°C for 30 min. Then, cells were split and incubated in triplicates with either control buffer, IL-3 (100 ng/ml), or with an anti-IgE antibody (10 μ g/ml) for another 30 min. After incubation, cell suspensions and cell-free supernatants were recovered and examined for the levels of histamine by radioimmunoassay (Immunotech). Histamine release was expressed as percentage of total histamine (= cellular + released histamine in suspension).

Table 1. Specification of mAbs

mAb	CD	Reactive antigen	Ig class	Source	Obtained from
L544	CD1a	T6	IgG1	m	5th WS on HLDA
L161	CD1c	T6	IgG1	m	5th WS on HLDA
RPA210	CD2	LFA-2	IgG1	m	BD, Pharmingen
SK3	CD4	T4	IgG1	m	BD, Pharmingen
L17F12	CD5	Tp67	IgG2a	m	BD, Pharmingen
ICRF44	CD11b	C3b1R	IgG1	m	BD, Pharmingen
MΦP9	CD14	LPSR	IgG2b	m	BD, Pharmingen
MMA	CD15	3-FAL	IgM	m	BD, Pharmingen
NKP15	CD16	FcγRIII	IgG1	m	BD, Pharmingen
HD39	CD22	BL-CAM	IgG1	m	5th WS on HLDA
HB8	CD24	BA-1	IgG	m	5th WS on HLDA
HIL-2R-M1	CD25	IL2Rα	IgG1	m	5th WS on HLDA
BA5	CD26	DPPIV	IgG2a	m	4th WS on HLDA
MT271	CD27	T14	IgG1	m	5th WS on HLDA
Kolt2	CD28	Tp44	IgG1	m	5th WS on HLDA
K20	CD29	β1-β chain	IgG2a	m	5th WS on HLDA
5A2-G5	CD31	PECAM-1	IgG1	m	5th WS on HLDA
AT10	CD32	FcγRII	IgG1	m	Serotec
P67.6	CD33	Siglec-3	IgG1	m	BD, Pharmingen
581	CD34	HPCA-1	IgG1	m	BD, Pharmingen
E11	CD35	CR1	IgG1	m	BD, Pharmingen
L178	CD44	Pgp-1	IgG1	m	BD, Pharmingen
2D1	CD45	LCA	IgG1	m	BD, Pharmingen
B6H12	CD47	IAP	IgG1	m	BD, Pharmingen
84H10	CD54	ICAM-1	IgG1	m	Immunotech
IA10	CD55	DAF	IgG2a	m	BD, Pharmingen
1C3	CD58	LFA-3	IgG2a	m	Immunotech
CLB-gran12	CD63	LIMP	IgG1	m	Immunotech
W17/1	CD88	C5aR	IgG1	m	Serotec
M5D12	CD116	GM-CSFRα	IgM	m	BD, Pharmingen
YB5.B8	CD117	KIT	IgG1	m	BD, Pharmingen
7G3	CD123	IL-3Rα	IgG2a	m	BD, Pharmingen
MEM-M6	CD147	Basigin	IgG1	m	H. Stockinger
14A2H1	CD151	PETA-3	IgG1	m	6th WS on HLDA
SE5A5	CD172a	SIRPα	IgG1	m	H.-J. Bühring
97A6	CD203c	E-NPP3	IgG1	m	Immunotech

WS on HLDA = International Workshops and Conferences on Human Leukocyte Differentiation Antigens; BD = Becton Dickinson; m = mouse.

Results

Phenotypic Characterization of HGMC

In a first step, we established the cell surface membrane phenotype of HGMC by applying mAbs against pan-hematopoietic and lineage-associated antigens. As visible in table 2, HGMC were found to express the leukocyte common antigen, LCA (CD45). In addition, HGMC were found to express the myeloid surface antigen Siglec-3 (CD33), the tetraspan antigen CD63 (an activation-linked myeloid antigen), and the signal regulatory protein (SIRP)-α (CD172a). Figure 1 shows expres-

sion of CD33, CD63, and CD172a on human intestinal MC. HGMC were also found to react with an antibody against the ectoenzyme CD203c (E-NPP3) that is also expressed on basophils (fig. 1, table 2). Interestingly, the levels of cell surface CD203c were found to be slightly higher on HGMC cultured in the presence of IL-4 (with or without SCF) compared to HGMC cultured in SCF alone or in control medium (fig. 2). HGMC did not express detectable levels of CD1, CD5, CD11b, CD22, CD24, CD26, CD27, CD28, CD32, CD34, or CD35 (table 2).

Table 2. Expression of cell surface antigens on HGMC: comparison with other MC types

CD	Antigen	Antigen expressed on			
		HGMC	LMC ¹	SMC ¹	HMC-1 ¹
CD1a	T6	–	–	–	–
CD1c	T6	–	–	–	–
CD2	LFA-2	–	–	–	+
CD4	T4	–	–	–	–
CD5	Tp67	–	–	–	–
CD11b	C3biR	–	–	–	–
CD14	LPSR	–	–	–	–
CD15	3-FAL	–	–	–	–
CD16	FcγRIII	–	–	–	–
CD22	BL-CAM	–	–	n.k.	+/-
CD24	BA-1	–	–	–	–
CD25	IL2Rα	–	–	–	+/-
CD26	DPPIV	–	–	n.k.	–
CD27	T14	–	–	–	–
CD28	Tp44	–	–	–	–
CD29	β1-β-chain	+	+	+	+
CD31	PECAM-1	–	–	–	–
CD32	FcγRII	–	–	+	+
CD33	Siglec-3	+	+	+	+
CD34	HPCA-1	–	–	–	–
CD35	CR1	–	–	–	–
CD44	Pgp-1	+	+	+	+
CD45	LCA	+	+	+	+
CD47	IAP	+	+	+	+
CD54	ICAM-1	+	+	+	+
CD55	DAF	+	+	+	+
CD58	LFA-3	+	+	+	+
CD63	LIMP	+	+	+	+
CD88	C5aR	–	–	+	+
CD116	GM-CSFRα	–	–	–	+
CD117	KIT	+	+	+	+
CD123	IL-3Rα	–	–	–	–
CD147	Basigin	+	+	+	+
CD151	PETA-3	+	+	+	+
CD172a	SIRPα	+	+	+	+
CD203c	E-NPP3	+	+	+	+

n.k. = Not known.

¹ Data refer to previously published results [11, 12, 14, 20–22, 32, 34–37]. LMC = Lung MC; SMC = skin MC.

Expression of Cytokine Receptors on HGMC

Confirming previous data obtained with human MC from other organs, we found that unstimulated HGMC express substantial amounts of c-kit = SCF receptor (CD117) on their surface. By contrast, resting isolated HGMC did not react with mAbs against IL-2Rα (CD25), IL-3Rα (CD123), or GM-CSFRα (CD116) (table 2). The α-chain of the IL-3 receptor (CD123) was detectable nei-

ther in resting HGMC nor in HGMC exposed to SCF and IL-4 (fig. 2). In line with this result, IL-3 did not induce histamine-release in HGMC and failed to promote the anti-IgE-induced release of histamine in these cells (fig. 3).

Expression of Adhesion-Related Antigens on HGMC

The distribution and function of various cells in the tissues are associated with expression of distinct adhesion receptors. In the present study, the adhesion receptor profile of HGMC was established. We found that HGMC express a number of cell-to-cell and cell-to-matrix type adhesion receptors. Notably, HGMC were found to react with mAbs against the common β-chain of β1 integrins (CD29), the homing receptor Pgp-1 (CD44), the SIRP ligand CD47, ICAM-1 (CD54), SIRPα (CD172a), and LFA-3 (CD58), the natural ligand of CD2 (table 2). HGMC did not express detectable levels of LFA-2 (CD2) or PECAM-1 (CD31) (table 2).

Phenotypic Comparison between HGMC and Other Human MC

A number of previous and more recent data suggest that human MC are heterogeneous in terms of expression of distinct CD surface antigens, like CD32 or CD88. In the present study, we found that HGMC express most CD antigens expressed on human lung or skin MC, but do not express CD32 or CD88 (table 2). Thus, the phenotype of HGMC is identical to the phenotype of human lung MC, but differs from that of human foreskin MC which are known to express CD32 and CD88 [20, 35]. Table 2 shows a comparison between HGMC and the phenotype reported for other types of human MC.

Discussion

MC represent an integral component of the microvasculature of the GI tract. These cells have recently been implicated in immunologic reactions and host defense in various intestinal disorders [26–31]. A number of previous and more recent data suggest that certain cell surface antigens on human MC play a pivotal role in their function in various tissues [20–22, 33, 39]. The cell surface antigen phenotype of human MC derived from various organs has been defined during the past decade [20–22, 34–37]. However, no extensive cell typing on intestinal human MC has been presented so far. We now have established the phenotype of HGMC using a large panel of antibodies directed against CD antigens.

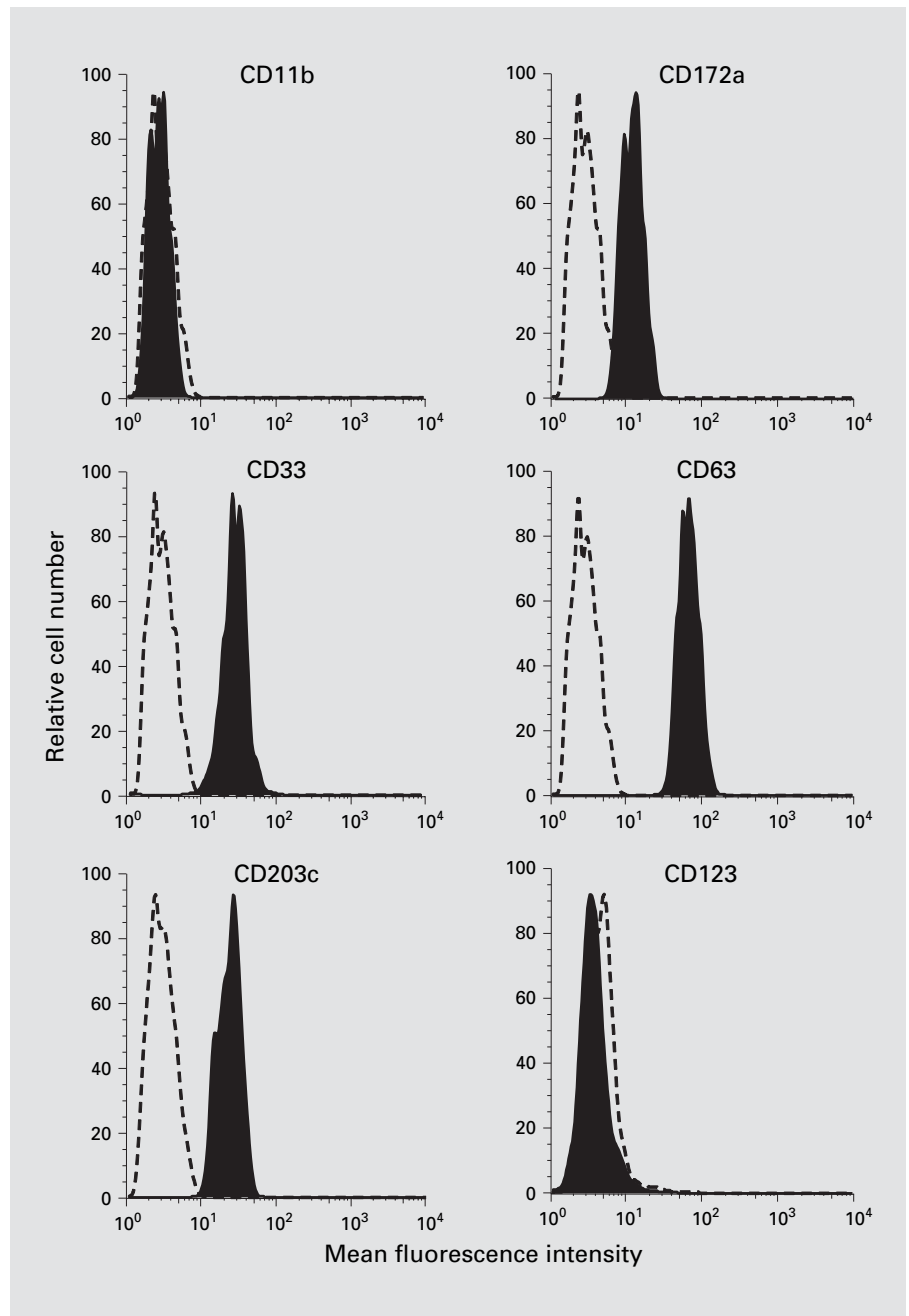
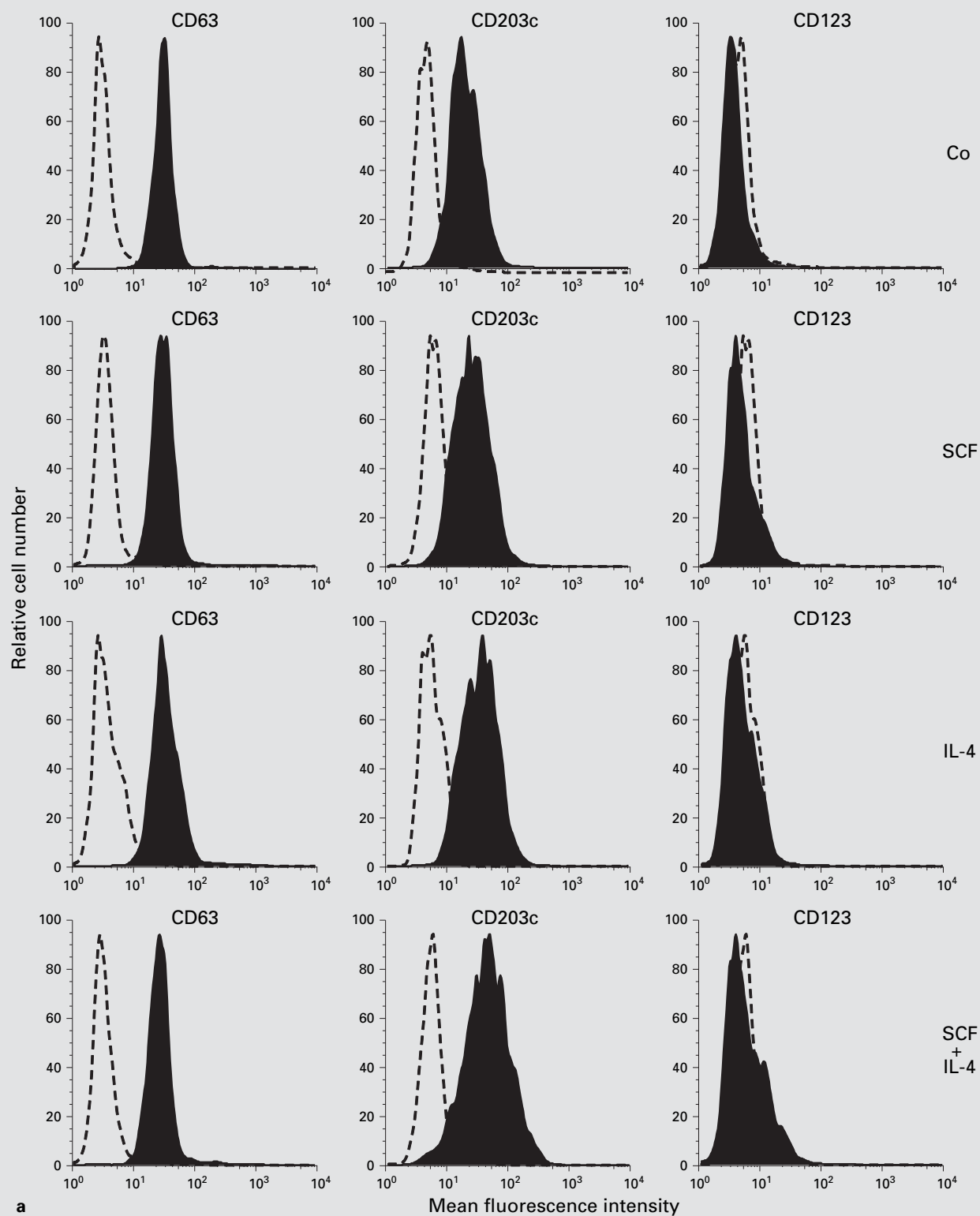


Fig. 1. Evaluation of expression of C3biR (CD11b), SIRP α (CD172a), Siglec-3 (CD33), CD63, E-NPP3 (CD203c), and IL-3 receptor α -chain (CD123) on freshly isolated HGMC. Expression of CD antigens on HGMC was examined by multicolor flow cytometry using PE-labeled CD117 mAb YB5.B8. The graphs show the reactivity of HGMC with these mAbs (black) as well as their reactivity with isotype-matched control antibodies (dashed lines). Unstimulated HGMC express CD33, CD63, CD172a, and CD203c, but do not express CD11b or CD123 on their surface.

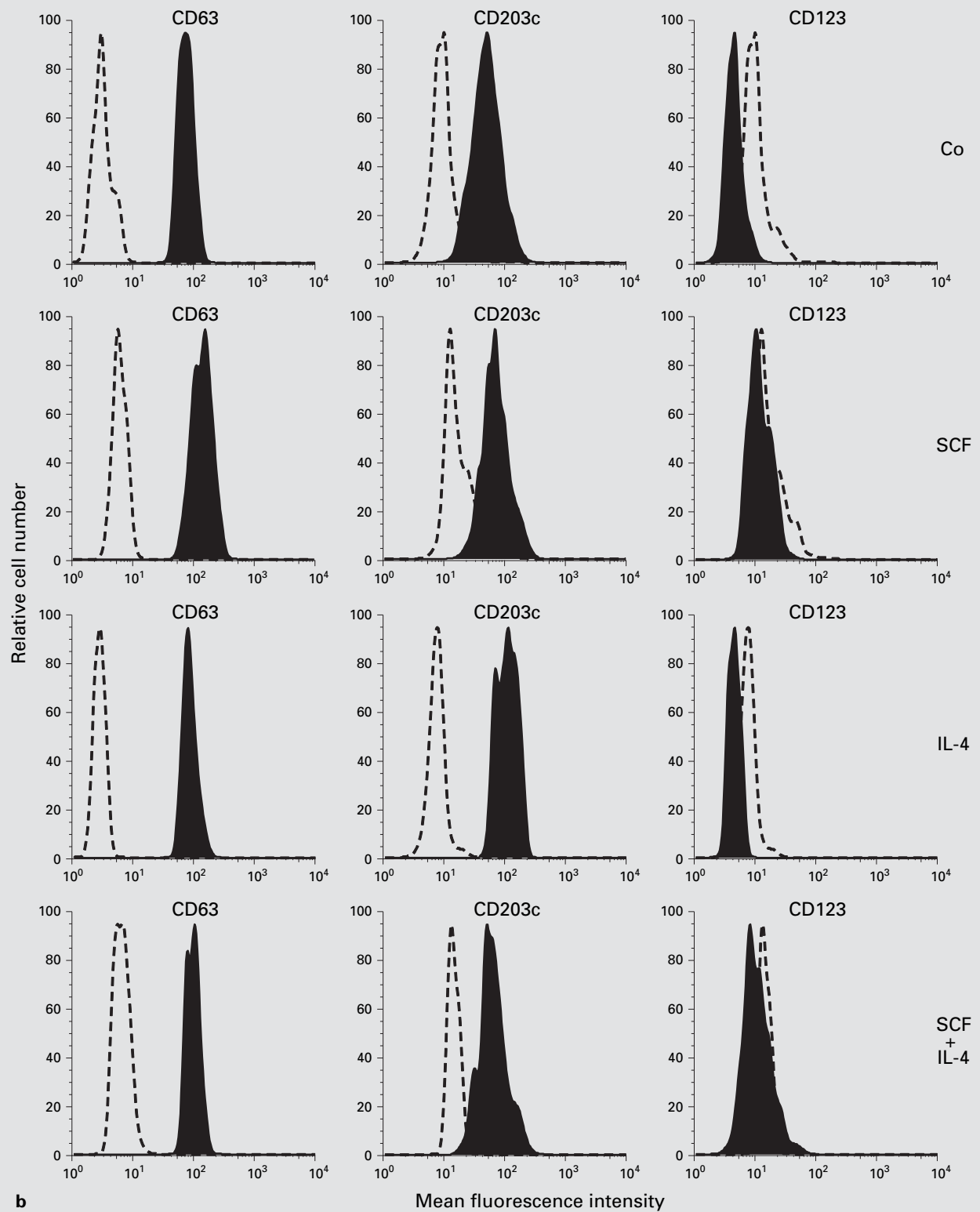
A number of previous and more recent data suggest that MC are heterogeneous cells in terms of expression of cell surface antigens [20–22]. Especially skin MC may differ in cell surface marker expression compared with other types of MC. Likewise, human foreskin MC reportedly express the C5a receptor (CD88) and are responsive against C5a, whereas MC derived from lung or uterine tissue do not express C5a receptors [20, 35]. Moreover,

in contrast to other MC types, human skin MC express the low affinity Fc γ receptor CD32 [35]. In the present study, HGMC were found to express various CD antigens that are also expressed on human skin and lung MC, but were found to lack CD32 and CD88. This observation provides further evidence for MC heterogeneity and suggests that the phenotype of HGMC is identical to that of human lung MC, but differs from that of skin MC.



2

(For legend to fig. 2 see page 118.)



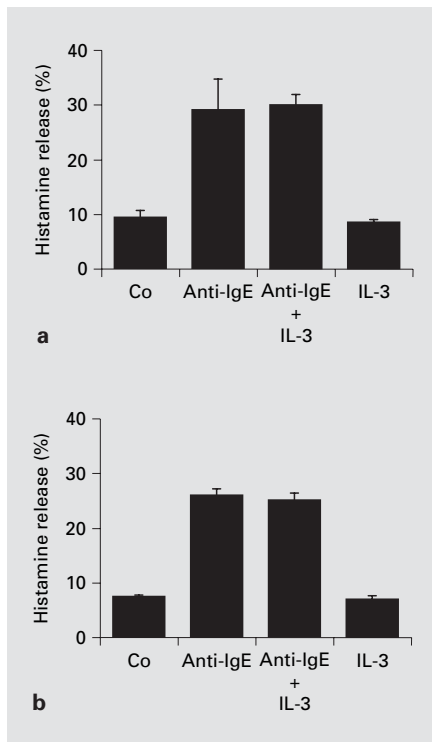


Fig. 3. Histamine release in HGMC. HGMC derived from two donors (**a**, **b**) were incubated in control buffer in the presence or absence of rhIL-3 (100 ng/ml) at 37°C for 30 min. Then, cells were split. Buffer-pretreated cells were incubated in either control buffer (Co) or an anti-IgE antibody (10 µg/ml) for another 30 min (anti-IgE), and IL-3-pretreated cells were incubated with either IL-3 (100 ng/ml) (IL-3) or with an anti-IgE antibody (10 µg/ml) for another 30 min (anti-IgE+IL-3). Results are given as percent of total histamine and represent the mean $\pm$ SD of triplicates. Exposure of HGMC to anti-IgE resulted in release of histamine, whereas exposure to IL-3 did not induce histamine secretion in HGMC. Moreover, IL-3 failed to promote the anti-IgE-induced release of histamine in HGMC.

Fig. 2. Effect of SCF and IL-4 on expression of CD123 on HGMC. HGMC were cultured in the presence of control medium (Co), SCF (100 ng/ml), IL-4 (20 ng/ml), and SCF + IL-4, for 3 days (**a**) or 14 days (**b**). After culture, cells were harvested and stained with mAbs against CD117 and CD45 for MC detection as well as mAbs against CD63, CD203c, and CD123. Expression of CD antigens on HGMC was analyzed by multicolor flow cytometry. The graphs show the reactivity of HGMC with CD mAbs (black) as well as their reactivity with isotype-matched control antibodies (dashed lines). Exposure to SCF or IL-4 alone or SCF + IL-4, did not result in expression of CD123. A slight increase in expression of CD203c on HGMC was found after exposure to IL-4.

A number of different cytokines including SCF and IL-6 are involved in the regulation of growth, survival and activation of human MC [8–10, 13–15, 33]. These cytokines reportedly act on MC through specific receptors [8, 13, 14]. In the present study, we were able to show that resting HGMC express receptors for SCF (KIT, CD117). This observation confirms previous analyses on other MC types [8, 13, 14]. By contrast, we were not able to detect the GM-CSF receptor α -chain (CD116), IL-3 receptor α -chain (CD123), or other cytokine receptors by the staining techniques applied. These data are also in line with previous studies suggesting that human lung and skin MC lack IL-3- and GM-CSF binding sites [34, 36, 40].

Recently, Gebhardt et al. [33] have reported that HGMC express low levels of CD123, and that activation of HGMC with SCF and IL-4 results in an increase in expression of CD123. By contrast, in the present study, we were not able to detect the IL-3 receptor α -chain (CD123) on resting or cytokine-stimulated HGMC. The reason for these discrepant results is not known. One possibility could be that the levels of IL-3 binding sites on HGMC are very low, so that these receptors were not detected by the staining method applied. An alternative possibility would be that in previous analyses, expression of IL-3 binding sites on HGMC was induced by additional cytokines present in culture media. In fact, Ochi et al. [41] have shown that IL-3 receptors can be induced in cultured human MC by application of multiple cytokines.

A number of adhesion-related cell surface antigens are expressed on MC and are considered to be responsible for the distribution and accumulation of these cells in the tissues [12, 21, 34–37]. We therefore tried to find out whether HGMC express adhesion-related antigens. The results of our study show that HGMC indeed express a number of different cell surface adhesion receptors including CD29, Pgp-1 (CD44), CD47, ICAM-1 (CD54), LFA-3 (CD58), and SIRP α (CD172a). These data confirm previous analyses performed on other subtypes of human MC [34–37].

Recent data have shown that neoplastic MC in the bone marrow of patients with systemic mastocytosis express CD2 and CD25, whereas normal MC do not express these CD antigens [22, 39, 42]. Moreover, MC in patients with systemic mastocytosis express increased levels of CD35 and CD88 [43]. In the present study, HGMC did not express detectable levels of CD2, CD25, CD35, or CD88. Whether HGMC in patients with systemic mastocytosis express CD2 or CD25 is currently under investigation.

The ectoenzyme CD203c (E-NPP3) has recently been identified as an activation-linked cell surface antigen on human basophils [44, 45]. Human lung and skin MC were also found to express CD203c [37]. Confirming these data, we found that HGMC express substantial amounts of CD203c in the present study. An interesting aspect was that HGMC express even higher levels of CD203c in culture when exposed to IL-4, whereas no effect of SCF on expression of CD203c was seen. The functional role of CD203c on HGMC and its increase in response to IL-4 remains at present unknown.

In summary, our data show that HGMC express a cell surface antigen profile that is identical to that of human

lung MC. In contrast to skin MC, HGMC do not express the low affinity Fc γ receptor CD32 and C5aR (CD88). Knowledge about the phenotype of HGMC may facilitate their characterization and purification from normal and pathologic intestinal tissues.

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