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Differential response of human basophils and mast cells to recombinant chemokines

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Abstract Chemokines are proinflammatory peptides regulating the functions of various hematopoietic cells. We have analyzed the effects of seven recombinant human (rh) chemokines (MCAF, RANTES, MIP-1α, MIP-1β, IL-8, GRO, and IP-10) on the growth and function of human basophils and mast cells. We found that MCAF, but not RANTES, MIP-1α, MIP-1β, IL-8, GRO, or IP-10, causes direct and dose-dependent histamine release from basophils (MCAF, 5 μg/ml: 26.9 ± 3.4%; other chemokines: < 5% of total histamine). An increased (2.1 to 3.5-fold) response to MCAF was obtained when basophils were preincubated with rh interleukin-3 (100 units/ml). Moreover, IL-3-primed basophils became responsive to physiologic concentrations (< 1 μg/ml) of MCAF, IL-8, and RANTES. None of the chemokines tested was able to induce histamine secretion in mast cells obtained from lung ($n = 2$), skin ($n = 1$), uterus ($n = 3$), or tonsils ($n = 3$), even when cells had been preincubated with the mast cell agonist SCF. The chemokines also failed to modulate the expression of activation antigens (CD11b/C3biR, CD25/IL-2Rβ, CD63, IL-3Rα, CD117/c-kit) on the mast cell line HMC-1 or the basophil cell line KU-812 and were unable to induce differentiation of basophils or mast cells in culture. Together, our results show that basophils respond to rhIL-8, rhMCAF, and rhRANTES and that, unlike human basophils, human mast cells are unresponsive to recombinant chemokines.

Key words Human basophils · Mast cells · Recombinant chemokines

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Introduction

Basophils and mast cells are multifunctional secretory immune cells. Both cells originate from hematopoietic progenitors and share functional and biochemical properties [38]. They store vasoactive and immunomodulating mediator substances which are released into the extracellular space upon cell activation through the IgE receptor or other cell surface membrane receptors [40]. Despite similarities in morphology and mediator expression, basophils and mast cells are two distinct cell lineages within the hematopoietic system. In contrast to blood basophils, mast cells are extravascular cells and usually cannot be detected in the blood stream [19]. Basophils and mast cells also differ from each other in cell surface antigen expression and response to various stimuli and growth factors [42].

Recently, several hematopoietic cytokines and peptides have been identified as regulators of human basophils or mast cells. Basophils are responsive to multiple cytokines including IL-3 [34, 44, 45], IL-5 [21], GM-CSF [20, 44], or NGF [8]. In contrast, human mast cells cannot be activated by these cytokines [42]. However, stem-cell factor (SCF), the ligand of c-kit, promotes mast cell differentiation as well as mediator secretion in mature human mast cells

[7, 16, 32, 41, 46]. Other studies have shown that substance P (SP) and vasoactive intestinal peptide (VIP) cause histamine liberation from human skin mast cells [6, 29]. Interestingly, mast cells in other organs, as well as blood basophils, are not responsive to these peptides.

Chemokines are structurally related peptide mediators which interact with various immune cells through specific receptors [22, 25, 31]. Based on the spacing of the first two cysteine residues, two types of chemokines (CC and CXC) can be distinguished. A number of studies have shown that both CC and CXC chemokines induce mediator secretion and/or chemotaxis in various leukocytes, including macrophages, eosinophils, and lymphocytes [3, 5, 35]. Basophils have also been described as being responsive to chemokines such as IL-8 [25], RANTES [10], or MCAF [2, 9, 35]. More recently, Alam et al. reported that mouse peritoneal mast cells release histamine when incubated with MIP-1α [1, 4]. In contrast, studies of human mast cells with regard to chemokine activation have so far not been presented.

The aim of this study was to analyze and compare the effects of recombinant human CC and CXC chemokines on mediator secretion from human basophils and from mast cells obtained from human lung, skin, uterus, and tonsils.

Materials and methods

Reagents and cytokines

Recombinant human (rh) monocyte chemotactic and activating factor (MCAF), rhRANTES (regulated on activation, normal T-cell expressed, and secreted), rhMIP-1α (macrophage inflammatory protein 1α), rhMIP-1β (macrophage inflammatory protein 1β), rhIP-10 (γ-interferon inducible protein-10), and rhGRO (= MGSA, melanoma growth-stimulating activity) were purchased from Peprotech (Rocky Hill, NJ). Rh interleukin-8 (rhIL-8) and rhIL-3 were provided by Sandoz, Vienna. RhSCF was purchased from Genzyme (Cambridge, MA). One liter of Tyrode's buffer contained 0.2 g KCl, 0.05 g $NaH_2PO_4 \cdot H_2O$, 0.8 g NaCl, and 1 g glucose; 1 l phosphate buffered saline (PBS) contained 1 g $Na_2HPO_4 \cdot 2H_2O$, 0.137 g KH_2PO_4, and 4.092 g NaCl. KCl, $NaH_2PO_4 \cdot H_2O$, $Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4, and glucose, as well as glutaraldehyde, were purchased from Merck (Darmstadt, Germany), collagenase (type II) from Sebak Co. (Suben, Austria). Toluidine blue and EDTA were from Sigma Chemical Co. (St. Louis, MO) and Ficoll and dextran T70 from Pharmacia (Uppsala, Sweden). Iscove's modified Dulbecco's medium, glutamine, penicillin, and streptomycin were purchased from Gibco Life Technologies (Gaithersburg, MD), and RPMI 1640 medium, gentamycin, amphotericin B, and fetal calf serum (FCS) from Sera Lab (Crawley Down, UK).

Antibodies

The monoclonal antibodies (mAbs) E-124-2-8 (anti-IgE), IOM1 (CD11b), and IOP63 (CD63) were purchased from Immunotech (Marseille, France); the anti-IL-3R alpha chain mAB 9F5 was from Pharmingen (San Diego, CA), the anti-IL-3Rβ chain mAb S16 from Santa Cruz Biotechnology (Santa Cruz, CA), and mAb MEM-74 (CD17) from Monosan (Uden, Netherlands). The mAbs 3G10 (CD25) and 4E3 (CD25) were kindly provided by O. Majdic and W. Knapp, Inst. of Immunology, University of Vienna, Austria. The anti c-kit mAb YB5.B8 was a kind gift from L.K. Ashman (University of Adelaide, Australia). The CD128 (anti-IL-8 receptor) antibody B-G20 was obtained from Fifth International Workshop on Human Leukocyte Differentiation Antigens, Boston 1993. Myeloma IgE (myeloma cell line U266) was purchased from Chemicon (Temecula, CA). The fluorescence-labeled second-step anti-mouse antibody (IgG plus IgM) was from An-Der-Grub company (Kaumberg, Austria).

Preparation of blood basophils

Peripheral blood cells of seven nonallergic donors were fractionated by incubation in 1.1% dextran T70 and 0.008 mol/l EDTA for 90 min at room temperature. Cells of the granulocyte-rich upper layer were then centrifuged (180 g at room temperature for 8 min) and washed twice in 0.9% NaCl. Granulocytes were resuspended in histamine release buffer (Immunotech, Marseille, France) and used immediately for histamine release experiments. For combined immunofluorescence/toluidine blue staining experiments, basophils were isolated together with mononuclear blood cells (MNC) by Ficoll density centrifugation [18].

Purification of primary mast cells

Lung tissue (after lobectomy) were obtained from two patients suffering from bronchiogenic carcinoma, after informed consent was given. Lung tissue was prepared following the method described by Schulman et al. [37]. Uterus mast cells were obtained from three donors (after informed consent had been given) suffering from uterine myomata, according to the method described by Massey et al. [30]. Tonsillar mast cells [15] were dispersed from surgical specimens removed by tonsillectomy in three patients suffering from chronic tonsilitis (after informed consent was obtained). Skin mast cells were obtained from juvenile foreskin ($n = 1$) after circumcision (after informed consent was given) as described [6, 29].

In brief, tissue was placed in Tyrode's buffer immediately after resection, chopped into small fragments, and washed extensively in Mg/Ca-free Tyrode's buffer. Then tissue fragments were incubated with collagenase (30 units/ml) at 37°C for 1–3 h. Thereafter, dispersed cells were recovered by filtration through Nytex cloth, washed three times in NaCl, and examined for the percentage of mast cells by toluidine blue staining. Tissue mast cells were cultured in RPMI 1640 medium supplemented with 10% FCS, glutamine, and antibiotics (penicillin, streptomycin, gentamycin, and amphotericin B) at 37°C. Isolated mast cells were kept in culture for at least 24 h before being activated with chemokines.

Cell lines

The human mast cell line HMC-1 [14] was kindly provided by J.H. Butterfield (Mayo Clinic, Rochester, MN). HMC-1 cells were cultured in Iscove's modified Dulbecco's medium supplemented with 10% FCS, glutamine, and antibiotics at 37°C and 5% CO_2. The basophil-precursor cell line KU-812 [23] was kindly provided by K. Kishi (Nijagata University, Nijagata, Japan). KU-812 cells were cultured in RPMI 1640 medium with 10% FCS, glutamine, and antibiotics (37°C, 5% CO_2).

Histamine release assay

Histamine release was performed as described previously [45]. In brief, mast cells (after at least 1 day in culture) were washed twice in NaCl and, in case of IgE activation, were incubated with myeloma IgE for 3 h at 4°C. Cells were then washed twice and used for histamine release experiments. Primary cell suspensions containing mast cells and basophils were adjusted to final cell concentrations of 0.5×10^6/ml and 1.7×10^6/ml, respectively. After incubation with cytokines (basophils with rhIL-3, 100 units/ml for 15 min at 37°C, mast cells with rhSCF, 1 ng/ml for 15 min at 37°C) or control medium, histamine release was performed using various concentrations (0.1–5 μg/ml) of rh chemokines or anti-IgE. Histamine was measured in cell-free supernatants after centrifugation (350 g for 8 min at 4°C) using a commercial radioimmunoassay (Immunotech). In each experiment, a monoclonal anti-IgE antibody at appropriate concentrations (0.01–10 μg/ml) was used as positive control. Nonspecific histamine release was quantified using control medium instead of specific stimuli. Total histamine in cell suspensions were quantified after cell lysis. Histamine release was expressed as percentage of total histamine.

Determination of cell surface membrane antigens

Primary basophils ($n = 3$) and uterus or lung mast cells ($n = 4$) were analyzed by combined toluidine blue/immunofluorescence staining, as described elsewhere [18, 43]. In brief, cells were incubated with mAbs at 4°C for 30 min, washed in phosphate-buffered saline (PBS), exposed to a fluoresceine-conjugated second-step antibody (IgG + IgM), and washed in PBS (4°C). Then, cells were fixed in glutaraldehyde (0.025%) for 1 min, washed in PBS, and stained with toluidine-blue (0.0125%) for 8 min at room temperature. Cells were examined with a fluorescence microscope (Olympus) under bright field and immunofluorescence light by two independent observers.

To analyze the possible effect of rh chemokines on expression of basophil and mast cell activation antigens, flow cytometry was performed using the human basophil cell line KU-812 and the human mast cell line HMC-1. Briefly, cells were preincubated with rh chemokines (1 μg/ml), rhSCF (1 ng/ml), rhIL-3 (100 units/ml), or control (RPMI 1640) medium for 1 h and 4 h. Thereafter, cells were washed twice in PBS and incubated with mAbs for 30 min at 4°C. They were washed again and exposed to a fluoresceine-labeled sheep $F(ab')_2$ anti-mouse IgG + IgM antibody for 30 min. After washing, cells were subjected to FACS analysis using a FACScan (Becton-Dickinson).

Differentiation assay

Basophil differentiation assay. Basophil differentiation was analyzed in a bone marrow culture system as described by Valent et al. [44]. Bone marrow was obtained from 11 healthy subjects (bone marrow transplant donors, giving informed consent). Heparinized bone marrow cells were fractionated using Ficoll. MNC (0.5×10^6/ml) were cultured in 24-well plates (Costar, Cambridge, MA) at 5% CO_2, 37°C in RPMI 1640 medium containing 10% FCS and antibiotics. Cells were exposed to rh chemokines (100 ng/ml) in the presence or absence of rhIL-3 (100 units/ml) or were kept in control medium. After 14 days of culture, cells were analyzed for total cell numbers by a hematocytometer (Coulter Electronics, Luton, UK), percentage of basophils (by Giemsa staining), and mediator content (histamine RIA).

Mast cell differentiation assay – long-term culture. Growth and differentiation of mast cells in vitro was analyzed in long-term suspension culture as described previously [46]. In brief, peripheral blood MNC ($n = 3$, healthy volunteers) were cultured with rh chemokines (100 ng/ml) alone or with rhSCF (100 ng/ml). As a control, cells were incubated with rhSCF or control medium (RPMI 1640). Cells were cultured in 24-well microculture plates (Costar) at 5% CO_2, 37°C. Cells were fed every 2 weeks by carefully removing the supernatants (50% of volume) and replacing the volume with fresh medium containing rh chemokines (10 ng/ml) and/or rhSCF (10 ng/ml). After 42 days of culture, cells were harvested and analyzed for the percentage and absolute numbers of mast cells and for mediator content (tryptase). Tryptase was measured using a commercial radioimmunoassay (Pharmacia Biochemical Inc., Milwaukee, WI) as described.

Results

Effects of rh chemokines on histamine release from human blood basophils

Of the seven rh chemokines tested, rhMCAF, at high concentrations (5 μg/ml) was able to induce histamine release from normal human blood basophils. Six of 7 donors (responders) were found to respond to MCAF at 5 μg/ml (Table 1, Fig. 1a). After preincubation of basophils with rhIL-3 (100 units/ml, 15 min, 37°C) the amount of histamine liberated in response to MCAF (in responsive individuals) increased from 26.9 ± 3.4% to 73.6 ± 9.8% ($p < 0.05$). Furthermore, these IL-3-primed basophils became responsive to lower concentrations (0.5–1.0 μg/ml) of MCAF (Fig. 1). Also, IL-3-primed basophils responded to RANTES and IL-8 (0.5–5.0 μg/ml) in two of three basophil donors (Table 1). Nonspecific histamine release (incubation with control medium instead of cytokines) was always less than 5%, and histamine release in basophils incubated with IL-3 amounted to 5.8 ± 2.7%. Incubation of basophils with rhSCF showed neither an effect on spontaneous release nor a priming effect for subsequent stimulation with chemokines (not shown). The effects of MCAF, RANTES, and IL-8 on histamine secretion of human basophils were dose dependent (Fig. 1). No histamine release was obtained with MIP-1α, MIP-1β, GRO, or IP-10, even when high concentrations of the chemokines were used (5 μg/ml) or the cells had been primed with IL-3 (Table 1, Fig. 1b).

Effects of rh chemokines on histamine release from human tissue mast cells

In order to evaluate whether rh chemokines cause mediator secretion from human tissue mast cells, histamine-release experiments were performed with a number of human mast cell types including lung, skin, uterus and tonsillar mast cells. In control experiments, a prolonged exposure (60–90 min) to rhSCF (100 ng/ml) induced mediator secretion (lung mast cells: 16–34%; skin mast cells: 10–19%; tonsillar mast cells: 11–29%; uterus mast cells: 35–89%) in all mast cell types. In contrast, none of the chemokines tested (MCAF, RANTES, MIP-1α, MIP-1β, GRO, IP-10,

Table 1 Effects of rh chemokines on histamine release from human blood basophils and mast cells (*nt* not tested)

Priming	% Histamine release upon stimulation with							
	MCAF	RANTES	MIP1α	MIP1β	GRO	IP-10	IL-8	MIX
Blood basophils ($n = 7$)								
Co	26.9 ± 3.4[a]	2.6 ± 1.9	< 0.1	< 0.1	0.3 ± 0.2	1.2 ± 0.8	4.3 ± 2.2	nt
IL-3	73.6 ± 9.8	39.2 ± 5.8	2.1 ± 1.2	0.4 ± 0.2	< 0.1	< 0.1	22.0 ± 11.0	nt
Lung mast cells ($n = 2$)								
Co	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.9 ± 0.7	1.4 ± 0.1	0.9 ± 0.1	0.7 ± 0.5	0.8 ± 0.6
SCF	1.3 ± 0.9	2.2 ± 0.5	1.7 ± 0.9	1.7 ± 0.9	2.1 ± 1.5	1.6 ± 1.1	0.3 ± 0.2	2.0 ± 1.4
Uterus mast cells ($n = 3$)								
Co	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1 ± 0.1	< 0.1	< 0.1
IL-3	< 0.1	< 0.1	< 0.1	< 0.1	0.2 ± 0.1	0.7 ± 0.5	< 0.1	nt
SCF	< 0.1	< 0.1	0.6 ± 0.4	0.3 ± 0.2	< 0.1	< 0.1	< 0.1	< 0.1
Skin mast cells ($n = 1$)								
Co	0.1	< 0.1	< 0.1	< 0.1	0.2	2.3	< 0.1	< 0.1
SCF	< 0.1	0.5	0.7	< 0.1	0.6	4.0	0.1	1.2
Tonsillar mast cells ($n = 3$)								
Co	0.5 ± 0.3	0.1 ± 0.1	0.3 ± 0.3	< 0.1	0.6	1.2	3.9	1.0
IL-3	1.4 ± 0.8	0.8 ± 06	1.2 ± 0.9	0.5 ± 0.3	nt	nt	nt	nt
SCF	< 0.1	0.5	2.9	5.4	0.2	2.9	< 0.1	2.0

[a] Results are expressed as % of total histamine after subtraction of nonspecific or cytokine-induced histamine release. In human basophils, nonspecific release was 2.1 ± 0.1%, and the IL-3-induced histamine release 5.8 ± 2.7%. Chemokines were used at a concentration of 5 μg/ml, except for the chemokine mixture (MIX) containing 1.0 μg/ml of each peptide.

IL-8) was able to induce histamine release from human tissue mast cells over the dose range (0.1–5 μg/ml) tested ($p > 0.05$) (Fig. 2 and Table 1). Short-term exposure (priming) of mast cells to rhSCF (1 ng/ml, 15 min, 37°C) resulted in an increase (1.4- to 1.7-fold at 1 μg/ml of anti-IgE) of the IgE-dependent histamine release, but was not followed by a response to rh chemokines (Table 1). To determine whether combinations of peptides would cause mediator secretion from mast cells, we prepared chemokine mixtures containing equal amounts of the seven chemokines (final concentration of each chemokine: mixture 1: 0.1 μg/ml; mixture 2: 1.0 μg/ml). However, in our experiments the mixtures failed to induce histamine release from human tissue mast cells (Table 1).

Effects of recombinant human chemokines on basophil differentiation

The effects of the rh chemokines on growth and differentiation of basophils were analyzed in a bone marrow culture system [44]. Incubation with rhIL-3 for 14 days resulted in formation of basophils (control: 8.3 ± 6.3 × 10^3/ml versus rhIL-3, 100 units/ml: 58.0 ± 21.8) and in a significant rise of the total cellular histamine values (control: 7.2 ± 5.1 versus rhIL-3, 100 units/ml: 123.3 ± 71.8 ng/ml; $p < 0.05$). In contrast, the chemokines tested in this study showed no significant effect on basophil differentiation and histamine synthesis in vitro ($p > 0.05$), although a slight effect of MIP-1α on histamine synthesis was observed in two of 11 bone marrow donors. None of the seven rh chemokines was able to promote IL-3-dependent in vitro differentiation of basophils or the IL-3 dependent rise of cellular histamine in our culture system (not shown). The cellular histamine concentrations (pg histamine per basophil) ranged between 0.2 and 2.7 pg and did not significantly vary between cultures maintained in the presence vs absence of rh chemokines.

Effects of recombinant human chemokines on mast cell differentiation

To analyze the effects of the chemokines on growth/differentiation of human mast cells, MNC of three healthy volunteers were exposed to (a) rhSCF, (b) rh chemokines, (c) rhSCF + rh chemokines, or (d) control medium in long-term (day 42) suspension culture [46]. In the absence of any factor (spontaneous mast cell growth), cellular tryptase levels were below 2 ng/ml. RhSCF induced formation of mast cells and formation of measurable amounts of tryptase (17.4 ± 5.1 ng/ml) in long-term culture. However, the rh chemokines tested failed to promote SCF-induced formation of mast cells or tryptase in these cultures and in some cases even caused decreases in mast cell and/or tryptase formation (not shown). None of the seven rh chemokines tested was able to induce formation of mast cells or to raise tryptase levels above control values in the absence of rhSCF.

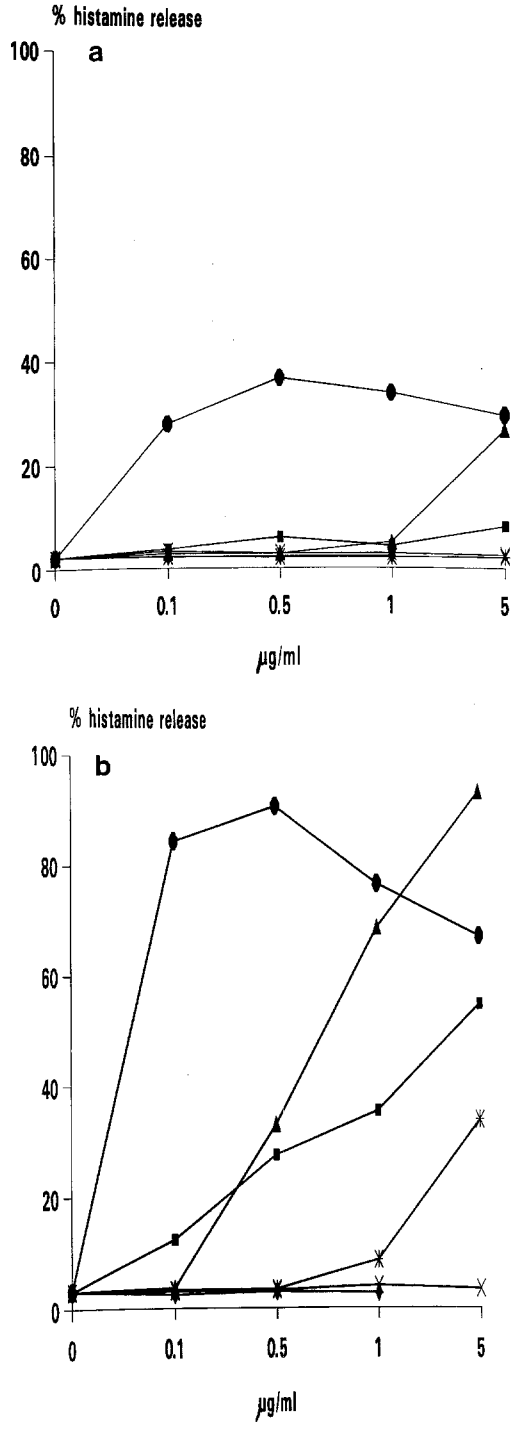

Fig. 1a, b Response of human basophils to recombinant chemokines. Basophils were isolated and exposed to various agonists, as described in the text. Blood basophils were preincubated in either control medium (**a**) or rhIL-3, 100 units/ml, (**b**) at 37°C for 10 min and then exposed to various concentrations of chemokines, as indicated. The following rh chemokines were used: MCAF (▲), RANTES (*), MIP-1α (×), MIP-1β (◆), and IL-8 (■). As positive control, cells were incubated with anti-IgE (●). Histamine release is expressed as percentage of total histamine

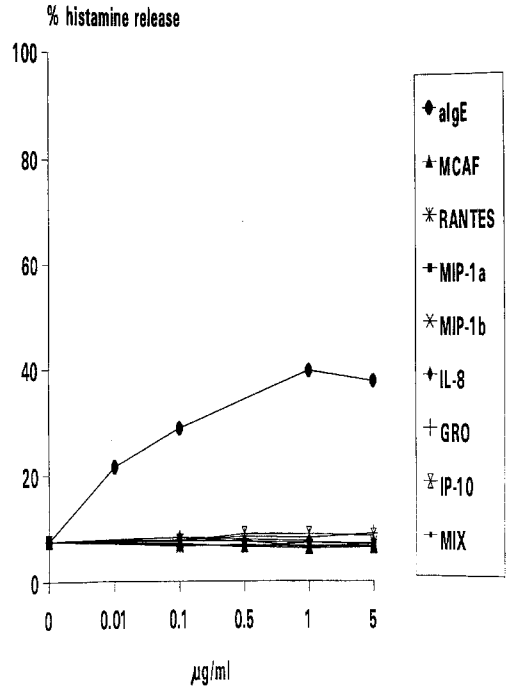

Fig. 2 Effects of rh chemokines on histamine secretion in mast cells. Mast cells were obtained from human lung, as described in the text. Cells were exposed to various concentrations of rh chemokines, as indicated. Histamine release is expressed as percentage of total histamine. Values from one experiment are shown; almost identical results were obtained in mast cells from eight other donors

Effects of rh chemokines on expression of cell surface antigens on KU-812 and HMC-1 cells

A number of cell surface molecules expressed on mast cells and/or basophils are associated with cell activation and mediator secretion. In order to determine whether the rh chemokines could influence the expression of (activation-linked) cell surface antigens on the human mast cell line HMC-1 (CD11b, CD63, CD117) or on the human basophil precursor cell line KU-812 (CD11b, CD25, CD63, IL-3R), cells were incubated with rh chemokines (MCAF, RANTES, MIP-1α, MIP-1β, GRO, IP-10, IL-8; 1 µg/ml each) for 1 or 4 h at 37°C. Then, expression of activation antigens on respective cells was analyzed by flow cytometry (FACScan, Becton-Dickinson). However, no significant changes in expression of the cell surface antigens (CD11b, CD25, CD63, CD117, IL-3Rα) upon stimulation with rh chemokines were substantiated.

Expression of IL-8 receptor on human basophils and mast cells

Recent studies using radiolabeled IL-8 suggest that human basophils express IL-8 binding sites [25, 42]. The IL-8 receptor has been clustered as CD128. In this

Table 2 Expression of cytokine receptors on human basophils and mast cells as assessed by combined toluidine blue/immunofluorescence staining[a] (*nc* not yet clustered)

mAb	Cytokine R	CD	Reactivity of mAbs with	
			Blood basophils	Tissue mast cells
3G10	IL-2Rβ	25	+[b]	−
9F5	IL-3Rα	123	+	−
S16	IL-3Rβ	nc	+	−
B-G20	IL-8R	128	+/−	−
YB5.B8	SCFR/c-kit	117	−	+

[a] Cells were incubated with mAbs at 4°C for 30 min, washed in PBS, exposed to a fluoresceine-conjugated second-step antibody (IgG + IgM), and washed in PBS (4°C). Then cells were fixed in glutaraldehyde (0.025%) for 1 min, washed again in PBS, and stained with toluidine blue (0.0125%) for 8 min at room temperature. Cells were examined under bright field and immunofluorescence light by two independent observers.

[b] Score reactivity: +, more than 90% of basophils/mast cells reactive; +/−, 50–80% of basophils/mast cells reactive; −, less than 10% of basophils/mast cells reactive.

study an antibody against CD128 was used to analyze expression of IL-8 receptors on human basophils and mast cells, employing a combined toluidine blue/immunofluorescence staining technique. Normal human blood basophils were found to express low amounts (50–80% reactive cells) of IL-8 receptor, whereas we were unable to detect the IL-8 receptor CD128 on the surface of human lung or uterus mast cells (Table 2). Confirming previous studies, basophils were found to express IL-3 receptor (both the alpha and beta chain), and mast cells were found to express significant amounts of c-kit (SCF receptor) on their surface (Table 2).

Discussion

Chemokines are structurally related, proinflammatory mediator peptides regulating the functions and growth of various hematopoietic cells, including granulocytes, macrophages, or lymphocytes [3, 5, 35, 36]. In the present study the effects of seven recombinant human chemokines on growth and function of human basophils and mast cells (derived from lung, skin, uterus, and tonsils) were analyzed and compared. The results of this study show that (a) basophils are responsive to MCAF, RANTES, and IL-8, (b) both CC and CXC chemokines fail to induce histamine release in lung, skin, uterus, or tonsillar mast cells, and (c) chemokines do not induce or promote basophil or mast cell differentiation in vitro. A number of chemokines have recently been identified as modulators of secretion and/or chemotaxis of various leukocytes. Therefore, it was tempting to speculate that these mediator peptides would also modulate the secretory functions of mast cells or basophils. Human blood basophils have been variously described as being responsive to IL-8, MCAF, RANTES, or MIP-1α [27, 28]. In most cases, relatively high concentrations of chemokines were required to elicit a direct response. However, priming of basophils with IL-3 (or IL-5 or GM-CSF) resulted in (increased) responsiveness of basophils to at least three chemokines, MCAF, RANTES, and IL-8 [9, 17, 26–28]. In the present study similar results were obtained, in that MCAF (in contrast to the other chemokines tested) directly induced mediator secretion in basophils at high concentrations (5 μg/ml), and priming with rhIL-3 was associated with increased releasability and a measurable response to rhRANTES and rhIL-8. Whether chemokine-induced activation of basophils is indeed relevant for pathologic conditions in vivo (such as allergic or inflammatory reactions) remains to be elucidated.

A number of recent observations suggest that mast cells are involved in various inflammatory processes and probably also in chemokine-dependent reactions. For example, mast cells are a potential source of IL-8 [33] and MCAF [39]. In addition, recent data from Alam et al. [1, 4] have shown that mouse peritoneal mast cells release histamine in response to MIP-1α. In the present study, however, human mast cells did not respond at all to these chemokines, and the chemokines showed no effect on mast cells even when used in combination or when mast cells had been preincubated with the c-kit ligand SCF.

Recently, mast cell heterogeneity with regard to peptide activation has been described. Thus, human skin mast cells, unlike mast cells derived from all other organs, are responsive to substance P and VIP. Therefore, in the present study, examinations were extended to mast cells isolated from various organs. However, no effects of rh chemokines were found in lung, skin, uterus, or tonsillar mast cells.

The reason for the differential response of basophils and mast cells to recombinant MCAF, RANTES, and IL-8 remains unknown at present. One possibility could be that mast cells, unlike basophils, do not express substantial amounts of chemokine-binding sites [22] on their surface. It is noteworthy that human blood basophils reportedly express IL-8 binding sites [25] and in this study reacted with an antibody against the IL-8R/CD128, whereas human tissue mast cells were not recognized by anti-IL-8 receptor antibody. Whether human basophils or mast cells express or lack specific MCAF receptors remains to be determined.

In many cases, cell activation processes are linked to expression of distinct cell surface antigens. Basophil activation antigens are CD11b [11, 12], CD63 [24], IgER, and IL-3R. Mast cell activation antigens include CD63, CD117/c-kit, and IgER. In this study the effects of recombinant human chemokines on expression of activation-linked cell surface antigens were analyzed using a basophil cell line (KU-812) and a human mast

cell line (HMC-1). However, none of the chemokines tested was found to alter expression of CD11b, CD25, CD63, or IL-3R on KU-812 basophils, or expression of CD11b, CD63, and CD117 on HMC-1 cells.

Recent observations suggest that chemokines not only induce or promote cell functions in mature hematopoietic effector cells, but also are able to modulate growth/differentiation of respective precursor cells [13]. In the present study, however, no effect of the chemokines tested on in vitro growth of human basophil or mast cells was found.

In conclusion, our study shows that MCAF, RANTES, and IL-8 are basophil agonists, and that human mast cells, unlike many other hematopoietic cells, are unresponsive to both CC and CXC chemokines.

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