

Original article

Detection of novel leukocyte differentiation antigens on basophils and mast cells by HLDA8 antibodies

Background: Basophils (BA) and mast cells (MC) are important effector cells in allergic reactions. Development, growth and effector cell functions are regulated by a network of cytokines, other ligands, and respective cell surface antigens.

Methods: We examined the expression of novel CD antigens on human BA, lung MC, the BA cell line KU-812, and the MC line HMC-1. Expression of surface antigens was analyzed by monoclonal antibodies (mAb) of the HLDA8 workshop and flow cytometry.

Results: Basophils were found to stain positive for CXCR1 (CD181), CCR1 (CD191), CCR2 (CD192), CCR7 (CD197), IL-18R α (CDw218a), IL-18R β (CDw218b), TRAIL-R1 (CD261), TRAIL-R2 (CD262), TACI (CD267), TLR-4 (CD284), LAIR1 (CD305), EMR-2 (CD312), JAM1 (CD321), and JAM2 (CD322). Lung MC were found to react with mAb against EMR-2 (CD312) and JAM1 (CD321). KU-812 cells were found to stain positive for CXCR1 (CD181), TRAIL-R2 (CD262), B7H2 (CD275), TLR-4 (CD284), JAM1 (CD321), and E-Cadherin (CD324). HMC-1 cells were recognized by mAb against TRAIL-R2 (CD262), B7H2 (CD275), LAIR1 (CD305), EMR-2 (CD312), JAM1 (CD321), and Siglec-6 (CDw327).

Conclusions: Extensive phenotyping with antibodies against novel CD antigens provides further evidence that BA and MC represent two separate hematopoietic cell lineages with unique phenotypic properties observed in mature cells as well as malignant immature cells. Further studies are required to define the functional role of these CD antigens expressed in BA and MC.

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Key words: basophils; mast cells; phenotyping; surface antigens.

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Basophils (BA) and mast cells (MC) are effector cells of allergic reactions (1, 2). These cells produce and store a number of mediators of allergy, such as histamine or leukotrienes (1–4). Both cells express high affinity immunoglobulin (Ig)E-binding sites (5, 6). In addition, MC and BA express various adhesion receptors, virus- and complement-binding sites, surface membrane enzymes, and cytokine receptors (7–15). Most of these surface structures are associated with distinct functional properties including proliferation, adhesion, or mediator secretion (7–15).

During the past two decades, the cell surface membrane phenotypes of human BA and MC have been established using antibodies against CD antigens defined in Workshops and Conferences on Human Leukocyte Differentiation Antigens (HLDAs; 7–15). Using such defined CD antibodies, BA and MC were found to represent two separate hematopoietic cell lineages with unique phenotypic properties (7–15). It was also found that BA and MC display several functionally important cell surface

antigens, such as the stem cell factor receptor KIT (CD117), the interleukin (IL)-3 receptor α -chain (CD123), or the activation-linked ectoenzyme E-NPP3 (CD203c; 16–19).

In the present study, expression of novel CD antigens on human MC and BA was examined using monoclonal antibodies (mAb) provided by the 8th International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA8; 20).

Materials and methods

Reagents

A number of mAb were used to determine expression of cell surface antigens on BA and MC. The vast majority of mAb were obtained from the HLDA8 workshop. A specification of the mAb used is provided in Table 1. The phycoerythrin (PE)-conjugated mAb YB5.B8 (CD117) was purchased from Pharmingen (San Diego, CA), the PE-labeled mAb 97A6 (21) from Immunotech (Marseille,

Table 1. Specification of mAbs

Reactive antigen	CD	mAb (clone name)	Ligand(s)	Important functional aspects
(a) Cytokine and chemokine receptors				
LIF-R	CD118	12D3	LIF	LIF-exposed bone marrow fibroblasts support the
LIF-R	CD118	7G7		growth of murine mast cells, differentiation of hematopoietic cells
CXCR1	CD181	42705	IL-8, GCP-2, GRO α	Cell migration (chemotaxis), mediator secretion (basophils)
CXCR2	CD182	48311	Multiple chemokines: IL-8, NAP-2, others	Cell migration and mediator secretion (basophils)
CXCR5	CD185	51505	BLC, chemokines?	Cell migration (lymphoid cells)
CCR1	CD191	53504	MIP-1 α , RANTES, MCP-3, MIP-5	Cell migration and mediator secretion (basophils)
CCR2	CD192	48607	MCP-1 through -5	Mediator secretion and chemotaxis (basophils)
CCR6	CD196	53103	MIP-3 α	Cell migration and cytokine production (various cells)
CCR7	CD197	150503	MIP-3 β , SLC	Cell migration (various cells)
CCR9	CDw199	112509	TECK	Cell migration (lymphocyte homing?)
IL-18R α	CDw218a	B-C41	IL-18	Inflammation, regulation of cytokine production in murine basophils and
IL-18R α	CDw218a	B-D42		mast cells as well as KU-812 cells
IL-18R β	CDw218b	B-E43	IL-18	Inflammation, regulation of cytokine production in murine basophils and
				mast cells as well as KU-812 cells
NGF-R (p75)	CD271	C40-1457	NGF	Differentiation of myeloid progenitors, activation of human basophils
BMPRI α	CD292	87908	BMPs	Bone formation, proliferation, cell migration
BMPRI β	CDw293	88614	BMPs	Bone formation, proliferation, cell migration
Leptin-R	CD295	52273	Leptin	Adipocyte homeostasis
FGFR1	CD331	133105	FGF	Fibrosis, angiogenesis, progenitor cell growth, migration
FGFR2	CD332	987939	FGF	Fibrosis, angiogenesis, progenitor cell growth, migration
FGFR2	CD332	98725		
FGFR2	CD332	98707		
FGFR2	CD332	98726		
FGFR3	CD333	136334	FGF	Fibrosis, angiogenesis, progenitor cell growth, migration
FGFR3	CD333	136312		
FGFR4	CD334	137114	FGF	Fibrosis, angiogenesis, progenitor cell growth, migration
Jagged-1	CD339	188331	Notch	Progenitor cell development (including mast cell progenitors?)
(b) Against death receptors				
TRAIL	CD253	FMU-TRAIL1	TRAIL-ligand, TRAIL-Rs	Regulation of apoptosis
TRANCE	CD254	70513	RANK, OPG	Regulation of apoptosis, osteoclast differentiation, activation of immune cells
APRIL	CD256	FMU-APRIL1	BCMA, TACI	Regulation of apoptosis, lymphocyte development and function
APRIL	CD256	FMU-APRIL2		
BAFF (Blys)	CD257	FMU-Blys1	BCMA, TACI, BAFF-R	Regulation of apoptosis, B-lymphocyte development and function
BAFF (Blys)	CD257	FMU-Blys2		
LIGHT	CD258	FMU-Light1	HVEM	Regulation of apoptosis, lymphocyte development and function
LIGHT	CD258	FMU-Light2		
LIGHT	CD258	FMU-Light3		
LIGHT	CD258	FMU-Light4		
TRAIL R1	CD261	B-K32	TRAIL	Regulation of apoptosis in various cells, regulation of immune cells
TRAIL R2	CD262	B-B42	TRAIL	Regulation of apoptosis in various cells, regulation of immune cells
TRAIL R2	CD262	B-D37		
TRAIL R2	CD262	FMU-DcR5.1		
TRAIL R2	CD262	FMU-DcR5.2		
TRANCE R (RANK)	CD265	80704	TRANCE	Regulation of apoptosis, osteoclast differentiation, activation of immune cells
TWEAK R	CD266	ITEM-1	TWEAK	Regulation of apoptosis, angiogenesis
TACI	CD267	165604	TALL1, BAFF	Regulation of apoptosis, lymphocyte biology, humoral immunity
BCMA	CD269	FMU-BCMA1	TALL1, BAFF	Regulation of apoptosis, lymphocyte biology, regulation of B lymphocytes
(c) Adhesion receptors				
TEM1 (Endosialin)	CD248	B1/473.16	Unknown	Expressed on tumor vessels
DC-SIGN-related	CD299	120604	ICAM3, HIV-1 gp120	Enhances HIV-1 infection of T cells, expressed on liver sinusoid
				endothelium and lymph nodes
CRACC	CD319	162	CRACC	Inhibitory receptor on lymphocytes, B-cell homotypic adhesion
JAM1	CD321	M.Ab.F11	LFA-1	(i) A receptor for reovirus, (ii) a ligand for the integrin LFA1,
				involved in leukocyte transmigration, and (iii) a platelet receptor
JAM2	CD322	H36	JAMs	Localized in the tight junctions, regulation of vascular permeability
JAM2	CD322	d22		and leukocyte adhesion and transmigration
E-Cadherin	CD324	67A4	E-Cadherin	Cell-cell interaction through homotypic adhesion, mediates
				bacterial adhesion to mammalian cells

Table 1. Continued

Reactive antigen	CD	mAb (clone name)	Ligand(s)	Important functional aspects
Ep-CAM	CD326	9C4	Ep-CAM	Homotypic calcium-independent cell adhesion molecule.
Ep-CAM	CD326	FMU-EpCAM1		Target for immunotherapy of human carcinomas
Ep-CAM	CD326	FMU-EpCAM2		
Ep-CAM	CD326	FMU-EpCAM3		
(d) Other novel CD antigens				
SIRPβ	CD172b	B1D5	Unknown	Negative regulation of receptor tyrosine kinase-coupled signaling processes
SIRPβ	CD172b	B4B6		
SIRPγ	CD172g	OX116	CD47	T-cell activation, cell–cell adhesion
CXCR6	CDw186	56811	CXCL16	Expressed on activated T lymphocytes, immunodeficiency virus coreceptor
Aminopeptidase A	CD249	2D3	Unknown	Membrane-bound zinc metallopeptidase
B7H2 (GL50, ICOSL)	CD275	2D3/B7-H2	ICOS	T-cell activation
B7H2 (GL50, ICOSL)	CD275	HIL-131		
ENDO-180 (TEM22)	CD280	E1/183	Unknown	Collagen receptor, remodeling, expressed on fibroblasts
TLR4	CD284	HTA125	LPS, pneumolysin	Pathogen recognition and activation of innate immunity
ART4	CD297	MIMA 52	Unknown	Carrier of the Dombrock blood group system antigens
ART4	CD297	MIMA 53		
BDCA2	CD303	AC144	Unknown	Mediates antigen capture and is a potent inhibitor of interferon α/β induction
BDCA2	CD303	AD5-4B8		
BDCA2	CD303	AD5-13A11		
BDCA4	CD304	AD5-17F6	VEGF and PLGF-2	=neuropilin-1: angiogenesis, cell survival, migration, invasion
LAIR1	CD305	FMU-LAIR1.1	Unknown	Inhibitory functions on antigen-specific effector T cells
LAIR1	CD305	9.1C3		
LAIR1	CD305	NKTA255		
LAIR2	CD306	FMU-LAIR2.1	Unknown	Unknown
LAIR2	CD306	FMU-LAIR2.3		
EMR-2 (EGF-TM7 family)	CD312	2A1	Chondroitin sulfate	Mediates cell attachment
NKG2D	CD314	149810	MIC A/B, ULBPs	Expressed on NK cells, recognition of malignant cells
NKG2D	CD314	ID11		
NKG2D	CD314	ON72		
CDCP1	CD318	CUB-1	Unknown	Overexpressed in colon and lung cancers. Marker for immature hematopoietic progenitor cell subsets
CDCP1	CD318	CUB-2		
CDCP1	CD318	CUB-4		
8D6A	CD320	8D6	Unknown	Follicular dendritic cell stimulation of B-cell growth in germinal centers
8D6A	CD320	3C8		
8D6A	CD320	4G10		
Siglec 6	CDw327	E20-1232	Sialylated glycans, leptin	Expressed in placenta and B cells
Siglec 7	CDw328	F023-420.2	Sialylated glycans	Mainly expressed on NK cells, negative regulation of TCR signaling
Siglec 9	CDw329	E10-286	Sialylated glycans	Can inhibit serotonin release from basophilic leukemia cells, negative regulation of TCR signaling
NKp46	CD335	900	viral HA	Natural cytotoxicity receptor, essential for antitumor activity, involved in natural killer cell suicide
NKp46	CD335	BAB281		
NKp46	CD335	195314		
NKp46	CD335	D2.9A5		
NKp46	CD335	461.G1		
NKp44	CD336	Z231	Viral hemagglutinins	Natural cytotoxicity receptor, essential for antitumor activity, involved in natural killer cell suicide
NKp30	CD337	Z25	Unknown	Natural cytotoxicity receptor: NK cell activation receptor
NKp30	CD337	210845		
NKp30	CD337	210857		
ABCG2 (BCRP1)	CDw338	5D3	Multiple ligands	Multidrug resistance protein, breast cancer resistance protein

LIF, leukemia inhibitory factor; NK, natural killer; TCR, T-cell receptor; IL, interleukin; mAb, monoclonal antibody.

France), IL-4 from Strathmann Biotec (Hannover, Germany), collagenase type II from Worthington (Lakewood, NJ), fetal calf serum (FCS), penicillin, streptomycin, gentamycin, amphotericin B, RPMI 1640 medium, and AB-serum from PAA Laboratories (Pasching, Austria), L-glutamine and Iscove's modified Dulbecco's medium (IMDM) from Gibco Life Technologies (Gaithersburg, MD), fluorescein isothiocyanate (FITC)-goat F(ab')₂ antimouse IgG from Caltag Laboratories (San Francisco, CA). One liter Tyrode's

buffer (Mg/Ca-free) contained 0.2 g KCl, 0.05 g NaH₂PO₄·H₂O, 8.0 g NaCl, and 1 g glucose.

Enrichment and staining of human blood basophils

Basophils were obtained from six healthy volunteers. Cells were enriched by Ficoll separation. Prior to staining with antibodies, BAs

were preincubated in AB serum for 15 min at room temperature. Expression of CD antigens on BAs was determined by two-color flow cytometry using a PE-labeled CD203c mAb and HLDA8 antibodies. In a first-step, cells were incubated with unlabeled WS antibodies, washed, and then incubated with a second-step FITC-labeled goat antimouse F(ab')₂ conjugate. Thereafter, cells were washed and incubated with the PE-conjugated CD203c antibody 97A6. Stained cells were analyzed on a FACSCalibur or a FACScan (Becton Dickinson, San Jose, CA). At least two staining experiments were conducted for each antibody. In case of divergent results after the first two experiments, at least one additional staining experiment was performed. In control experiments, cells were preincubated with mouse immunoglobulin before exposure to PE-labeled mAbs to block nonspecific antibody-binding reactions.

Enrichment and staining of human tissue mast cells

Lung tissue was obtained from surgical specimens from patients with bronchiogenic carcinoma ($n = 3$). All patients gave informed consent. MC were isolated according to published techniques (8, 22). First, 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 180 min (2×90 min) at 37°C. After digestion, cells were recovered, washed, and examined for the presence and percentage of MC by Wright-Giemsa staining. Isolated MC were cultured in RPMI 1640 medium and 10% FCS at 37°C for at least 12 h before being subjected to antibody staining experiments. Expression of CD antigens on MC was determined by two-color flow cytometry using a PE-labeled CD117 (YB5.B8) mAb and HLDA8 antibodies. In a first-step, cells were incubated with AB serum for 15 min at room temperature. Then, unlabeled WS antibodies were added, incubated for a further 15 min. Cells were washed, and then incubated with a second-step FITC-labeled goat antimouse F(ab')₂ conjugate. Thereafter, cells were washed and incubated with the PE-conjugated CD117 mAb YB5.B8. At least two staining experiments were conducted for each antibody. In case of divergent results after the first two experiments, at least one additional staining experiment was performed.

Preparation and culture of cell lines

The BA precursor cell line KU-812 (23) was kindly provided by Dr K. Kishi (Niigata University, Niigata, Japan). KU-812 cells were cultured in RPMI 1640 medium with 10% FCS, L-glutamine, and antibiotics (37°C, 5% CO₂). As control of clonal stability, KU-812 cells were checked periodically for the presence of metachromatic granules and expression of CD123. The human MC line HMC-1 (24) was kindly provided by Dr J. H. Butterfield (Mayo Clinic, Rochester, MN). HMC-1 cells were cultured in IMDM containing 10% FCS, L-glutamine, α -thioglycerol, and antibiotics (37°C, 5% CO₂). HMC-1 cells were re-thawed from an original stock every 4–7 weeks and passaged weekly. As control of 'clonal stability', HMC-1 cells were examined for (i) the presence of metachromatic granules, (ii) expression of CD117, and (iii) downregulation of CD117 by IL-4 (100 U/ml, 48 h; 25). These control experiments were performed prior to each set of experiments, and only HMC-1 cells exhibiting all features of the original clone were used.

Evaluation of expression of CD antigens on HMC-1 cells and KU-812 cells

HMC-1 cells and KU-812 cells were evaluated for cell surface marker expression by single-color flow cytometry as described (15,

21, 24). Cells were first preincubated in AB serum for 15 min. Then, cells were incubated with mAb (15 min, room temperature), washed, and finally exposed to a second-step fluoresceine-labeled goat F(ab')₂ antimouse IgG fragment (15 min, room temperature). After washing, cells were analyzed on a FACScan (Becton Dickinson). At least two staining experiments were conducted for each antibody. In case of divergent results after the first two experiments, at least one additional staining experiment was performed.

Results

Phenotype of human blood basophils

Based on the specificity of CD203c for BAs among white blood cells (18, 19, 21), this antigen was used for BA detection. As assessed by two-color flow cytometry, CD203c-positive blood BAs were found to stain positive for a number of novel CD antigens including the chemokine receptors CXCR1 (CD181), CXCR2 (CD182), CCR1 (CD191), CCR2 (CD192), CCR6 (CD196), and CCR7 (CD197). In addition, BA were found to express several cytokine receptors, such as IL-18R α (CDw218a; Fig. 1), IL-18R β (CDw218b), BMPR-1 β (CDw293), and FGFR2 (CD332; Table 2a). Interestingly, of the four different anti-FGFR2 mAb tested, only one showed a positive staining result, whereas three other mAb did not react with BA (Table 2a).

Basophils were also found to display death receptors and their ligands. In particular, BA were recognized by mAb against TRANCE (CD254), TRAIL-R1 (CD261), TRAIL-R2 (CD262; Fig. 2), and TACI (CD267;

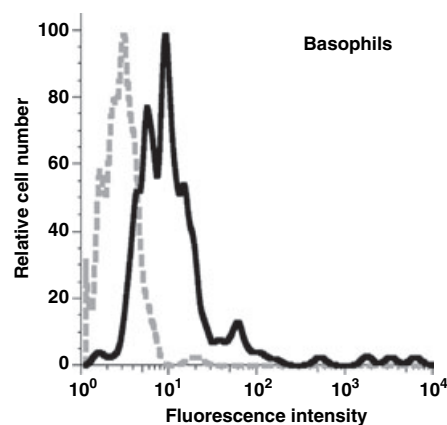


Figure 1. Expression of the α -chain of the interleukin (IL)-18 receptor on human basophils. Basophils were analyzed by two-color flow cytometry using a CD203c antibody for basophil detection. In a first-step, cells were incubated with an unlabeled antibody (clone: B-D42) against the IL-18R α chain (CDw218a), washed, and then incubated with a second-step fluorescein isothiocyanate (FITC)-labeled goat antimouse F(ab')₂ conjugate. Thereafter, cells were washed and incubated with the phycoerythrin (PE)-conjugated CD203c antibody 97A6. As visible, these CD203c-positive basophils express substantial amounts of IL-18R α (black bold line). The antibody-omission control (FITC control) is shown as dashed line.

Table 2. Antibody staining results

Molecule	CD	Ab (name)	Basophils	KU-812	HMC-1	Lung mast cells
(a) Cytokine and chemokine receptors						
LIF-R	CD118	12D3	—	—	—	—
LIF-R	CD118	7G7	—	n.t.	n.t.	n.t.
CXCR1	CD181	42705	+++	+	—	—
CXCR2	CD182	48311	+	—	—	—
CXCR5	CD185	51505	—	—	—	n.t.
CCR1	CD191	53504	++	—	—	—
CCR2	CD192	48607	++	—	—	—
CCR6	CD196	53103	+	—	—	—
CCR7	CD197	150503	++	—	—	—
CCR9	CDw199	112509	—	—	—	—
IL-18R α	CDw218a	B-C41	++	—	—	—
IL-18R α	CDw218a	B-D42	++	—	—	—
IL-18R β	CDw218b	B-E43	+	—	—	n.t.
NGF-R	CD271	C40-1457	—	—	—	—
BMPRI α	CD292	87908	—	—	—	—
BMPRI-1 β	CDw293	88614	+	—	—	—
Leptin R	CD295	52273	—	—	n.t.	n.t.
FGFR1	CD331	133105	—	—	—	—
FGFR2	CD332	987939	—	—	—	—
FGFR2	CD332	98725	—	—	—	—
FGFR2	CD332	98707	++	—	—	—
FGFR2	CD332	98726	—	—	—	—
FGFR3	CD333	136334	—	—	—	—
FGFR3	CD333	136312	—	n.t.	n.t.	n.t.
FGFR4	CD334	137114	—	—	—	—
Jagged-1	CD339	188331	—	—	—	—
(b) Death receptors						
TRAIL	CD253	FMU-TRAIL1	—	n.t.	n.t.	n.t.
TRANCE	CD254	70513	+	—	—	—
APRIL	CD256	FMU-APRIL1	—	n.t.	n.t.	n.t.
APRIL	CD256	FMU-APRIL2	—	n.t.	n.t.	n.t.
BAFF (Blys)	CD257	FMU-Blys1	—	n.t.	n.t.	n.t.
BAFF (Blys)	CD257	FMU-Blys2	—	n.t.	n.t.	n.t.
LIGHT	CD258	FMU-Light1	—	n.t.	n.t.	n.t.
LIGHT	CD258	FMU-Light2	—	n.t.	n.t.	n.t.
LIGHT	CD258	FMU-Light3	—	n.t.	n.t.	n.t.
LIGHT	CD258	FMU-Light4	—	n.t.	n.t.	n.t.
TRAIL R1	D261	B-K32	+++	—	—	—
TRAIL R2	CD262	B-B42	+++	+	++	—
TRAIL R2	CD262	B-D37	+	—	—	—
TRAIL R2	CD262	FMU-DcR5.1	—	n.t.	n.t.	n.t.
TRAIL R2	CD262	FMU-DcR5.2	—	n.t.	n.t.	n.t.
TRANCE R (RANK)	CD265	80704	—	—	—	—
TWEAK R	CD266	ITEM-1	—	—	n.t.	n.t.
TACI	CD267	165604	++	—	—	—
BCMA	CD269	FMU-BCMA1	—	n.t.	n.t.	n.t.
(c) Adhesion receptors						
TEM1 (Endosialin)	CD248	B1/473.16	—	—	—	—
DC-SIGN-related	CD299	120604	+	—	—	—
CRACC	CD319	162	—	n.t.	n.t.	n.t.
JAM1	CD321	M.Ab.F11	+++	++	+++	++
JAM2	CD322	H36	++	n.t.	n.t.	n.t.
JAM2	CD322	d22	—	n.t.	n.t.	n.t.
E-Cadherin	CD324	67A4	—	++	—	—
Ep-CAM	CD326	9C4	—	n.t.	n.t.	n.t.

Table 2. Continued

Molecule	CD	Ab (name)	Basophils	KU-812	HMC-1	Lung mast cells
EpCAM	CD326	FMU-EpCAM1	—	n.t.	n.t.	n.t.
EpCAM	CD326	FMU-EpCAM2	—	n.t.	n.t.	n.t.
EpCAM	CD326	FMU-EpCAM3	—	n.t.	n.t.	n.t.
(d) Other novel CD antigens						
SIRP β	CD172b	B1D5	—	—	—	—
SIRP β	CD172b	B4B6	—	—	—	—
SIRP γ	CD172g	OX116	—	—	—	—
CXCR6	CDw186	56811	—	—	—	—
Aminopeptidase A	CD249	2D3	—	n.t.	n.t.	n.t.
B7H2	CD275	2D3/B7-H2	—	—	—	—
(GL50, ICOSL)						
B7H2	CD275	HIL-131	—	+	++	n.t.
(GL50, ICOSL)						
ENDO-180	CD280	E1/183	—	—	—	—
(TEM22)						
TLR4	CD284	HTA125	++	++	—	—
ART4	CD297	MIMA 52	—	+	—	—
ART4	CD297	MIMA 53	—	—	—	—
BDCA2	CD303	AC144	+	n.t.	n.t.	n.t.
BDCA2	CD303	AD5-4B8	+	n.t.	n.t.	n.t.
BDCA2	CD303	AD5-13A11	+	n.t.	n.t.	n.t.
BDCA4	CD304	AD5-17F6	—	n.t.	n.t.	n.t.
LAIR1	CD305	FMU-LAIR1.1	++	—	+	+
LAIR1	CD305	9.1C3	++	n.t.	n.t.	n.t.
LAIR1	CD305	NKTA255	++	n.t.	n.t.	n.t.
LAIR2	CD306	FMU-LAIR2.1	—	n.t.	n.t.	n.t.
LAIR2	CD306	FMU-LAIR2.3	—	n.t.	n.t.	n.t.
EMR-2	CD312	2A1	+++	—	++	++
NKG2D	CD314	149810	—	—	—	—
NKG2D	CD314	ID11	—	n.t.	—	n.t.
NKG2D	CD314	ON72	—	—	—	—
CDCP1	CD318	CUB-1	—	—	—	—
CDCP1	CD318	CUB-2	—	+	—	—
CDCP1	CD318	CUB-4	—	—	—	—
8D6A	CD320	8D6	—	—	—	—
8D6A	CD320	3C8	—	—	—	—
8D6A	CD320	4G10	—	—	—	—
Siglec 6	CDw327	E20-1232	—	—	+	+
Siglec 7	CDw328	F023-420.2	—	n.t.	n.t.	n.t.
Siglec 9	CDw329	E10-286	+	+	—	—
NKp46	CD335	900	—	—	—	—
NKp46	CD335	BAB281	—	—	—	—
NKp46	CD335	195314	—	—	—	n.t.
NKp46	CD335	D2.9A5	—	—	—	—
NKp46	CD335	461.G1	—	—	—	—
NKp44	CD336	Z231	—	—	—	—
NKp30	D337	Z25	—	—	—	—
NKp30	CD337	210845	—	n.t.	n.t.	n.t.
NKp30	CD337	210857	—	n.t.	n.t.	n.t.
ABCG2 (BCRP1)	CDw338	5D3	—	n.t.	n.t.	n.t.

The ratio between median fluorescence intensity (MFI) Ag 4 of the antigen-specific antibody and the control antibody was calculated and converted according to the following arbitrary score: MFI ratio >8 = +++, MFI ratio 3.01–8 = ++, MFI ratio 1.81–3 = +, MFI ratio ≤ 1.8 = —. In case of divergent results, the most frequent score is shown. Asterisk indicates that the staining result with the respective antibody was not positive in all cases (+) or was not negative in all cases (—) examined.

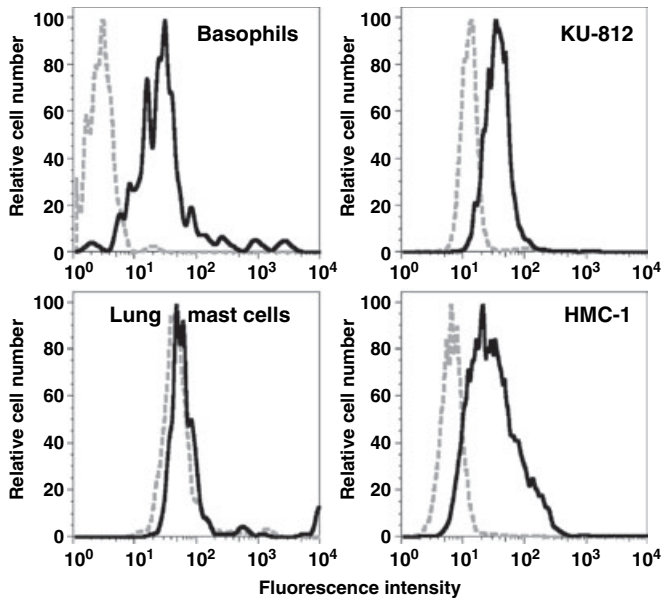


Figure 2. Evaluation of expression of TRAIL-R2 on human basophils and mast cells. Primary cells (blood basophils, isolated lung mast cells) were analyzed by two-color flow cytometry using a phycoerythrin (PE)-labeled CD203c antibody (97A6) for basophil detection, and a PE-labeled CD117 antibody (YB5.B8) for mast cell detection as well as the anti-TRAIL-R2 antibody B-B42 (CD262). The cell lines KU-812 and HMC-1 were examined by single-color flow cytometry using the B-B42 antibody (black bold line). The antibody-omission control [fluorescein isothiocyanate (FITC) control] is also shown (dashed line).

Table 2b). However, not all mAb clustered as CD262 were found to stain BA (Table 2b). Basophils also failed to stain positive for the death receptor ligands TRAIL (CD253), APRIL (CD256), BAFF (CD257), and LIGHT (CD258) as well as for TRANCE receptor (CD265), TWEAK receptor (CD266), and BCMA (CD269). Other important cell surface structures detectable on BA were the adhesion receptors JAM1 (CD321), JAM2 (CD322), and DC-SIGN-related (CD299; Table 2c), the pneumolysin receptor TLR-4 (CD284), as well as BDCA2 (CD303) and LAIR1 (CD305), EMR-2 (CD312; Fig. 3), and Siglec-9 (CDw329; Table 2d).

Phenotype of human tissue mast cells

Human MC were isolated from lung tissue and were identified by a PE-labeled antibody against KIT (CD117). As assessed by two-color flow cytometry, KIT-positive lung MC were found to co-express JAM1 (CD321) (Table 2c) and EMR-2 (CD312; Fig. 3 and Table 2d). By contrast, most other CD antigens including cytokine receptors and death receptors, were not detectable on the surface of human lung MC thereby contrasting BA (Fig. 3 and Table 2a–d). These data confirm that BA and MC represent two separate lineages within the hematopoietic cell family.

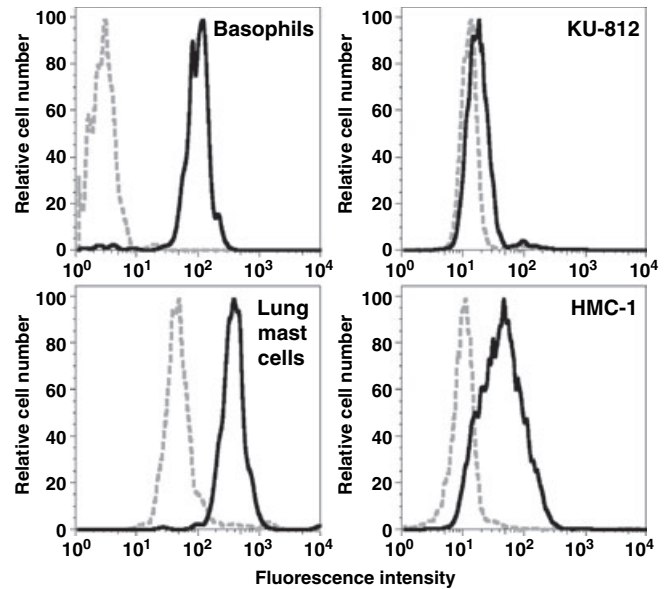


Figure 3. Analysis of human basophils and human mast cells for expression of EMR-2. Primary cells (blood basophils and lung mast cells) were analyzed by two-color flow cytometry using a phycoerythrin (PE)-labeled CD203c antibody (97A6) for basophil detection, and a PE-labeled CD117 antibody (YB5.B8) for mast cell detection as well as the anti-EMR-2 antibody 2A1 (CD312). The cell lines KU-812 and HMC-1 were examined by single-color flow cytometry using the same CD312 antibody. Reactivity of cells with the CD312 antibody is shown as bold black graph, and the antibody-omission control [fluorescein isothiocyanate (FITC) control] is indicated by a dashed line.

Phenotype of HMC-1 cells and KU-812 cells

The human BA cell line KU-812 and the human MC line HMC-1 were examined for CD antigen expression by single-color flow cytometry. In control experiments, KU-812 cells were found to display CD123 and CD203c, and HMC-1 cells were found to express substantial amounts of KIT (CD117), but did not express CD123, confirming previous data (11–19).

Of the cytokine and chemokine receptors tested, KU-812 cells were found to express only CXCR1 (CD181; Table 2a). Interestingly, KU-812 cells did not express CXCR2, CXCR5, CCR1, CCR2, CCR6, or CCR7. Also mAb to IL-18R and FGFRs also failed to bind to BA. The only death receptor detectable on KU-812 was TRAIL-R2 (CD262; Fig. 2 and Table 2b). Interestingly, only a subset of mAb clustered as CD262 were found to recognize KU-812 cells. In contrast to primary BA, KU-812 cells failed to display TRANCE and TACI.

Of adhesion receptors analyzed, KU-812 cells were found to express JAM1 (CD321), which was also found on primary BA. In contrast to BA, KU-812 cells also stained positive for E-Cadherin (CD324; Table 2c). Other cell surface structures detectable on KU-812 cells were

TLR-4 (CD284), ART4 (CD297), CDCP1 (CD318), and Siglec-9 (CDw329; Table 2d). Apart from CDCP1 and ART4, these CD antigens were also found to be expressed on BA.

HMC-1 cells were found to stain positive for the chemokine/cytokine receptor LAIR1 (CD305; Table 2a). Death receptors detectable on HMC-1 cells were TRAIL-R1 and TRAIL-R2 (Fig. 2 and Table 2b). Interestingly, most adhesion-related antigens tested were not detectable on HMC-1 cells. In fact, only one mAb (of two tested) against JAM1 was found to react with HMC-1 cells in our staining experiments. Other antigens detected on HMC-1 cells were EMR-2 (CD312; Fig. 3), Siglec-6 (CDw327), and ICOSL (CD275).

Discussion

During the past two decades, the cell surface membrane phenotypes of human BA and human MC have been established using mAb directed against CD antigens defined in the series of International Workshops and Conferences of Human Leukocyte Differentiation Antigens (HLDA1 through HLDA7; 7–19). Although the sensitivity of the staining techniques applied may differ and may yield divergent results when MC from different organs are examined, these analyses revealed substantial and important new information regarding the expression of distinct surface antigens on BA and MC in each workshop. In the present study, we have extended these important studies by testing a large panel of new mAb provided by HLDA8, and asked whether the respective antigens are expressed on human BA, MC, and in respective cell lines.

A number of previous and more recent data suggest that BA express a large number of functionally active receptors for immunomodulating peptides (chemokines) and cytokines (11, 12, 14, 26–28). In the present study, we were able to confirm that BA express numerous chemokine receptors including CXCR1 (CD181), CXCR2 (CD182), CCR1 (CD191), CCR2 (CD192), CCR6 (CD196), and CCR7 (CD197). In addition, BA were found to display IL-18 receptor α - and β -chain (CDw218a and CDw218b) as well as FGFR2 receptor and several death receptors. With regard to chemokine receptors, these data are confirmatory to previous observations (26–28). However, several data represent new information on the phenotype and on the potential functional repertoire of BAs. Likewise, to the best of our knowledge, this is the first report demonstrating expression of IL-18 receptors on human BAs.

So far, the IL-18 receptor was described to be expressed in cultured mouse BA and cultured mouse MC as well as in the human BA cell line KU-812 (29, 30). Our data suggest that primary human blood BA express IL-18 receptor α - and β -chain (CDw218a and CDw218b). By contrast, we were not able to detect significant amounts

of CDw218a on human MC, contrasting expression of the receptor in murine MC. This discrepancy may have several explanations. First, human MC may express only very low amounts of the receptor, which were not detected by the staining technique applied. In this regard it is noteworthy that flow cytometry-based staining techniques may differ in sensitivity depending on the antibody and fluorochromes used. A second possibility may be species-specific expression of cytokine receptors on MC. Indeed, several different cytokine receptors, such as the IL-3 receptor, are only expressed in significant amounts on murine MC, but not on human MC (11–17, 31, 32). Alternatively, expression of the IL-18 receptor on MC is dependent on cytokine stimulation, e.g. exposure of (murine) MC to IL-3. In addition, baseline cytokine receptor levels may vary from donor to donor, depending on multiple factors, and in case of patients, on the type of disease. Other possible explanations for the failure to detect the IL-18 receptor on MC would be expression at an immature stage of MC development or MC heterogeneity.

So far, the functional significance of expression of IL-18 receptors on human BA remains unknown. In T cells and B lymphocytes, the IL-18 receptor and its ligand may serve as regulators of differentiation and cell activation (33). More recently, it has been described that IL-18 regulates activation (mediator secretion) in murine BA and MC through the IL-18 receptor (29). It has also been described that IL-18 is upregulated in patients with various immunologic disorders including allergic states (33). Based on these observations, it is tempting to speculate that the IL-18 receptor expressed on BAs may be involved in the regulation of allergic reactions (e.g. mediator release). Studies attempting to clarify the functional role of the IL-18 receptor on human BAs are currently under way.

A number of previous and more recent data have shown that BA and MC display unique (nonidentical) CD phenotypes. The results of the present study are in line with this notion. A remarkable aspect in our study was that MC express only a few CD antigens examined, and clearly fewer antigens compared with primary BA or HMC-1 cells. Similar observations have been made with previous sets of WS antibodies (9–17). One possible explanation for this ‘loss of CD antigens’ on mature MC is a decrease in myeloid antigens during differentiation of MC that has been described for several different surface antigens, such as the IL-3 receptor or CD13 (34, 35). Another (related) possibility would be that many of the CD antigens expressed on HMC-1 cells are not specific for MC but reflect an immature (precommitted) stage of myeloid differentiation.

As mentioned above, BA were found to express several death receptors including TRAIL receptor, and death receptor ligands, such as TRANCE. The functional significance of expression of these receptors on BA remains unknown. An interesting aspect of the present

study was that MC did not express any of these death receptors. As regards it is noteworthy that in contrast to BA, MC are extremely long-lived cells that can even survive degranulation. Whether the lack of death receptors facilitates long-term survival and the capacity of MC to regranulate without undergoing rapid apoptosis, remains to be determined.

The clear differences in antigen expression between primary cells (BA and MC) and respective cell lines (KU-812 and HMC-1) is confirmatory to previous studies (11–15) and suggests that all screen results obtained with cell lines have to be reconfirmed for primary cells, especially when considering biologic or clinical aspects in such observations. It should also be pointed out that both cell lines not only exhibit a cell lineage background, but also a disease-specific background with disease-specific onco-

proteins, i.e. BCR/ABL in KU-812 cells, and the mastocytosis-related KIT mutant D816V in HMC-1 cells.

In conclusion, our data provide further evidence that BA and MC express a unique composition of cell surface antigens and thus represent two distinct cell lineages within the hematopoietic cell family. The application of respective antibodies should facilitate the isolation and functional characterization of these cells.

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References

- Valent P, Bettelheim P. The human basophil. *Crit Rev Oncol Hematol* 1990;**10**:327–352.
- Schwartz LB. The mast cell. In: Kaplan AP, editor. *Allergy*. Edinburgh, UK: Churchill Livingstone, 1985:53–92.
- Johnston SL, Holgate ST. Cellular and chemical mediators – their roles in allergic diseases. *Curr Opin Immunol* 1989;**2**:513–524.
- Serafin WE, Austen KF. Mediators of immediate hypersensitivity reactions. *N Engl J Med* 1987;**317**:30–34.
- Ishizaka T, Ishizaka K. Activation of mast cells for mediator release through IgE receptors. *Prog Allergy* 1984;**34**:188–235.
- Metzger H. The receptor with high affinity for IgE. *Immunol Rev* 1992;**125**:37–48.
- Stain C, Stockinger H, Scharf M, Jäger U, Gössinger H, Lechner K et al. Human blood basophils display a unique phenotype including activation linked membrane structures. *Blood* 1987;**70**:1872–1879.
- Valent P, Ashman LK, Hinterberger W, Eckersberger F, Majdic O, Lechner K et al. Mast cell typing: demonstration of a distinct hemopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 1989;**73**:1778–1785.
- Valent P, Majdic O, Maurer D, Bodger M, Muhm M, Bettelheim P. Further characterization of surface membrane structures expressed on human basophils and mast cells. *Int Arch Allergy Appl Immunol* 1990;**91**:198–203.
- Sperr WR, Agis H, Czerwenka K, Klepetko W, Kubista E, Boltz-Nitulescu G et al. Differential expression of cell surface integrins on human mast cells and basophils. *Ann Hematol* 1992;**65**:10–16.
- Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: biochemical and functional characterization. *Adv Immunol* 1992;**52**:333–423.
- Agis H, Füreder W, Bankl HC, Kundi M, Sperr WR, Willheim M et al. Comparative phenotypic analysis of human mast cells, blood monocytes and blood basophils: dissection of three distinct myeloid cell lineages. *Immunology* 1996;**87**:535–543.
- Wimazal F, Ghannadan M, Müller MR, End A, Willheim M, Meidlinger P et al. Expression of homing receptors and related molecules on human mast cells and basophils: a comparative analysis using multi-color flow cytometry and toluidine blue/immunofluorescence staining technique. *Tissue Antigens* 1999;**54**:499–507.
- Füreder W, Agis H, Sperr WR, Lechner K, Valent P. The surface membrane antigen phenotype of human blood basophils. *Allergy* 1994;**49**:861–865.
- Ghannadan M, Hauswirth AW, Scherthaner GH, Müller MR, Klepetko W, Schatzl G et al. Detection of novel CD antigens on the surface of human mast cells and basophils. *Int Arch Allergy Immunol* 2002;**127**:299–307.
- Agis H, Sperr WR, Willheim M, Füreder W, Spanblöchl E, Bankl HC et al. SCF receptor epitope mapping on mast cells using mAb to extracellular domains of c-kit. In: Schlossmann SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Motimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder TF, Todd RF, editors. *Leukocyte typing: V. White cell differentiation antigens*, Vol. 2. Oxford, UK: Oxford University Press, 1995:1920–1921.
- Agis H, Wimazal F, Hagen W, Baghestanian M, Ghannadan M, Lechner K et al. Myeloid blind panel flow cytometric analysis: evaluation of human mast cells and human blood basophils with monoclonal antibodies of the myeloid workshop panel. In: Kishimoto T, Kikutani H, dem Borne AEG, Goyert SM, Mason DY, Miyasaka M et al. editors. *Leucocyte typing VI*, Vol. 6. New York, USA: Garland Publishing, 1998:1046.
- Bühring HJ, Seiffert M, Giesert C, Marxer A, Kanz L, Valent P et al. The basophil activation marker defined by antibody 97A6 is identical with the ectonucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3; CD203c). *Blood* 2001;**97**:3303–3305.
- Bühring HJ, Valent P, Sano K. CD203c cluster report. In: Mason D, Andre P, Bensussan A, Buckley C, Civin C, Clark E et al. editors. *Leucocyte typing VII*, Vol. 8. Oxford, England (UK): Oxford University Press, 2001:377.
- Zola H, Swart B, Nicholson I, Aasted B, Bensussan A, Boumsell L et al. CD molecules 2005: human cell differentiation molecules. *Blood* 2005;**106**:3123–3126.

21. Bühring HJ, Simmons PJ, Pudney M, Müller R, Jarrossay D, van Agthoven A et al. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 1999;**94**:2343–2356.
22. Schulman ES, MacGlashan DW, Peters SP, Schleimer RP, Newball HH, Lichtenstein LM. Human lung mast cells: purification and characterization. *J Immunol* 1982;**129**:2662–2667.
23. Kishi K. A new leukemia cell line with Philadelphia chromosome characterized as basophil precursors. *Leuk Res* 1985;**9**:381–390.
24. Butterfield JH, Weiler D, Dewald G, Gleich GJ. Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* 1988;**12**:345–355.
25. Sillaber C, Strobl H, Bevec D, Ashman LK, Butterfield JH, Lechner K et al. IL-4 regulates c-kit proto-oncogene product expression in human mast and myeloid progenitor cells. *J Immunol* 1991;**147**:4224–4228.
26. Füreder W, Agis H, Semper H, Keil F, Maier U, Muller MR et al. Differential response of human basophils and mast cells to recombinant chemokines. *Ann Hematol* 1995;**70**:251–258.
27. Weber M, Dahinden CA. Basophil and eosinophil activation by CC chemokines. *Int Arch Allergy Immunol* 1995;**107**:148–150.
28. Kaplan AP. Chemokines, chemokine receptors and allergy. *Int Arch Allergy Immunol* 2001;**124**:423–431.
29. Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc Natl Acad Sci U S A* 1999;**96**:13962–13966.
30. Zaitsu M, Yamasaki F, Ishii E, Midoro-Horiuti T, Goldblum RM, Hamasaki Y. Interleukin-18 primes human basophilic KU812 cells for higher leukotriene synthesis. *Prostaglandins Leukot Essent Fatty Acids* 2006;**74**:61–66.
31. Valent P, Besemer J, Sillaber Ch, Maurer D, Butterfield JH, Kishi K et al. Failure to detect interleukin-3 binding sites on human mast cells. *J Immunol* 1990;**145**:3432–3437.
32. Valent P. The riddle of the mast cell: c-kit(CD117)-ligand as the missing link? *Immunol Today* 1994;**15**:111–114.
33. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001;**19**:423–474.
34. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J Exp Med* 1999;**190**:267–280.
35. Scherthaner GH, Hauswirth AW, Baghestanian M, Agis H, Ghannadan M, Worda C et al. Detection of differentiation- and activation-linked cell surface antigens on cultured mast cell progenitors. *Allergy* 2005;**60**:1248–1255.