

Original article

Human mast cells express receptors for IL-3, IL-5 and GM-CSF; a partial map of receptors on human mast cells cultured *in vitro*

Background: Mast cells have long been recognized as the principal cell type that initiates the inflammatory response characteristic of acute allergic type 1 reactions. Our goal has been to further characterize maturation of progenitors to mast cells.

Methods: Mast cells were cultured from human cord blood derived CD133⁺ progenitors. Mast cell function was tested using histamine release. During differentiation mast cells surface marker expression was monitored by flow cytometry.

Results: CD133⁺ progenitors expressed the early haematopoietic and myeloid lineage markers CD34, CD117, CD13 and CD33. Mature mast cells expressed CD117, CD13 and CD33, and expression of the high affinity immunoglobulin E receptor FcεRI increased during culture. Cytokine receptors interleukin (IL)-5R, IL-3R, granulocyte-macrophage-colony stimulating factor (GM-CSF)R and IL-18R were expressed at high levels during maturation. Chemokine receptors CXCR4 and CXCR2 were highly expressed on both newly purified CD133⁺ cells and mature cells.

Conclusion: Human mast cells can be cultured from a CD34⁺/CD117⁺/CD13⁺/CD33⁺ progenitor cell population in cord blood that is tryptase and chymase negative. Developing and mature mast cells express a wide range of chemokine and cytokine receptors. We found high levels of expression of CD123, IL-5R and GM-CSF receptors, also found on eosinophils and basophils, and high levels of expression of the receptor for the inflammatory cytokine IL-18.

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Key words: human mast cells; cell culture; flow cytometry; surface marker expression; CD117; FcεRI; interleukin-3R; interleukin-5R; GM-CSFR.

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Mast cells have diverse effector functions that are firmly implicated in the pathophysiology of acute allergic responses (1, 2). Moreover, hyperplasia of mast cells within lesional tissue is a recognized histopathological feature of rheumatoid arthritis (3), and fibrotic diseases (4) and reflects a likely role for mast cells in chronic inflammatory diseases.

The differentiation process of human mast cells is not yet well described. It is established that they are bone marrow derived, but their exact lineage relationship to other haematopoietic cells and the phenotype of committed, specific mast cell precursors remain obscure (5, 6). Immature CD133⁺ progenitors circulate in blood and home to extravascular tissues where they mature into mast cells (7, 8).

Mechanisms underlying the influx of mast cell progenitors into tissue with subsequent differentiation are largely unknown (9–11). We have developed a culture system in which functional mature human mast cells can be obtained from cord blood derived CD133⁺ progenitor cells (12). To better characterize the developmental process, we have mapped the expression of selected surface markers.

During the 12-week culture time it takes to mature mast cells from CD133⁺ progenitor cells, they were examined by flow cytometry at weekly intervals.

We investigated early haematopoietic and myeloid lineage markers as well as adhesion molecules, CD45 and human leucocyte antigens. Furthermore immunoglobulin (Ig), cytokine and chemokine receptors were determined (Table 1).

Materials and methods

Antibodies and reagents

Ficoll–Paque cell separation solution was purchased from Amersham Pharmacia Biotech (Hillerød, Denmark). The AC133 cell isolation kit and LS+ cell separation columns were purchased from Miltenyi Biotech (Auburn, CA). Foetal calf serum (FCS) and penicillin/streptomycin were purchased from GIBCO BRL (Grand Island, NY). Human recombinant stem cell factor (rh-SCF), human rh-interleukin (IL)6 and human rh-IL3 and fluorokine rh IL-3 biotin kit were purchased from R&D systems (Abingdon, UK). Serum free medium StemSpan was purchased from Stem Cell technologies (Vancouver, Canada). Table 1 shows mAb used for flow cytometry and their sources.

Table 1. Antibodies used in the study and their conjugates, clones and sources

Antigen	Conjugation	Clone	Source
Early haematopoietic cells			
CD34	FITC	BIRMA-K3	Dako
CD38	FITC/PE	AT13/5	Serotec
CD117 (C-kit R)	FITC	104D2	Serotec
Myeloid lineage markers			
CD33	PE	WM53	Serotec
CD13	PECY5	WM47	Dako
Common leukocyte antigens			
CD45	PECY5	H130	Serotec
Human leukocyte antigen			
HLA-DR	FITC	B-F1	Serotec
Cytokine and chemokine receptors			
CXCR4 (SDF-1 α R)	PE	44717.111	R&D Systems
CXCR3	FITC	49801.111	R&D Systems
CXCR2 (IL-8R)	PE	48311.211	R&D Systems
CXCR1 (IL-8R)	FITC	42705.111	R&D Systems
CCR5	FITC	45523.111	R&D Systems
CCR3	PE	61828.111	R&D Systems
IL-18R	PE	70625.111	R&D Systems
IL-5R α	PE	A14	BD Biosciences
IL-5R α	FITC	Sf21-derived	R&D Systems
GM-CSFR α	FITC	M5D12	BD Biosciences
GM-CSFR α	FITC	Sf21-derived	R&D Systems
CD123 (IL-3R α)	PE	9F5	BD Biosciences
Immunoglobulin receptors			
FC ϵ RI	FITC	CRA-1	Kyokuto Pharm
CD23 (FC ϵ RII)	PE	MHM6	Dako
CD64 (FC γ RI)	FITC	10.1	Serotec
CD32 (FC γ RII)	FITC	AT10	Serotec
CD16 (FC γ RIII)	FITC	LNK16	Serotec
CD89 (FC α R)	PE	MIP-8a	Serotec
Adhesion molecules			
CD29	PE	3S3	Serotec
CD51/CD61	FITC	23C6	Serotec
CD54	FITC	15.2	Serotec

Culture conditions

Purification and culture of progenitor cells from cord blood was done as described recently (12). Mononuclear cells were purified from fresh umbilical cords, and CD133⁺ cells were purified by MACS. The cells were suspended in a StemSpan serum free based medium supplemented with SCF and IL-6. During the first 2 weeks, IL-3 was added, and thereafter IL-3 was removed from the cell cultures. From week 8 on, 10% FCS was added.

Flow cytometry

Surface marker expression was assessed by flow cytometry using a fluorescent activated cell sorter (FACS) Calibur. Flow cytometry was performed every week for 12 weeks. Analyses were performed 4 days after medium change. Human cultured mast cells were washed two times with phosphate-buffered saline containing 0.1% bovine serum albumin before labelling with antibody at a dilution of 1 : 100. Cells were incubated at 4°C for 30 min. with directly conjugated monoclonal receptor antibodies or with appropriate control antibodies. For monitoring expression of FC ϵ RI, cells were stained with CRA-1 (Cosmo Bio Co., Tokyo, Japan) and with a secondary fluorescent isothio-cyanate (FITC)-conjugated anti-

mouse IgG2b antibody (Serotec, Oslo, Norway). The human IL-3 receptor density and percentage of positive cells were also determined using a human IL-3 fluorokine kit according to manufactures instructions. Surface expression of receptors for GM-CSF and IL-5 was confirmed with two polyclonal antibodies (Table 1). Propidium iodide (PI) was added (final concentration 10 μ g/ml) to a cell sample to identify and exclude dead cells, typically < 1%. Overton Subtraction (13) was used to calculate the net percentage of positive cells.

Immunocytochemical staining for tryptase and chymase

Immunostaining for tryptase and chymase was performed using a modified method described by Craig et al. (14). Mast cells were centrifuged onto a glass slide using a cytocentrifuge (Cytospin 3, Shandon). The smears were air dried, fixed in methanol/acetone solution, and were incubated overnight at 4°C with primary antibody against tryptase or chymase. They were stained using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method using the APAAP kit from Dako (Glostrup, Denmark). Briefly, the cells were incubated with the secondary antibody (a rabbit anti-mouse immunoglobulin) for 30 min at room temperature. After being washed, the smears were incubated with the alkaline phosphatase mouse anti-alkaline phosphatase for 30 min. Negative controls were performed using isotype matched mouse IgG1 instead of the primary antibody.

Histamine release from cultured human mast cells

For determination of histamine content and histamine release a microfibre-based method was used. The method is based on the binding of histamine to ground glass microfibres and measures histamine by fluorescence of a histamine-o-phthaldehyde complex (15). Briefly, cultured human mast cells were incubated with human myeloma IgE 2 μ g/ml (a generous gift from Lars K. Poulsen, National State Hospital, Copenhagen) for 24 h. They were washed in StemSpan with SCF 100 ng/ml and IL-6 50 ng/ml and stimulated with anti-IgE in the same medium for 30 min in 5% CO₂, 37°C. Unstimulated cells were used as controls.

Statistics

Results are presented as mean $\pm$ SD of the indicated number of experiments. Statistical analyses were performed with a paired Student *t*-test. The level of significance was set at *P* < 0.05.

Results

Proliferation and phenotype of cultured human mast cells

The number of cells in culture increased progressively from $2.8 \pm 1.1 \times 10^6$ CD133⁺ cells at seeding to $29 \pm 5.3 \times 10^6$ after 8 weeks (*n* = 6). At week 4 the purity of the cultured cells accessed by metachromatic staining was $91.3 \pm 2.9\%$, and after week 7, and up to week 12, $100 \pm 0\%$ were pure mast cells.

Only very few cells stained positive for tryptase and chymase after 2 weeks of culture. After 12 weeks $97 \pm 2\%$ stained positive for tryptase and $25 \pm 0.7\%$ stained positive for chymase. The histamine content of the cells increased over the 12-week culture period

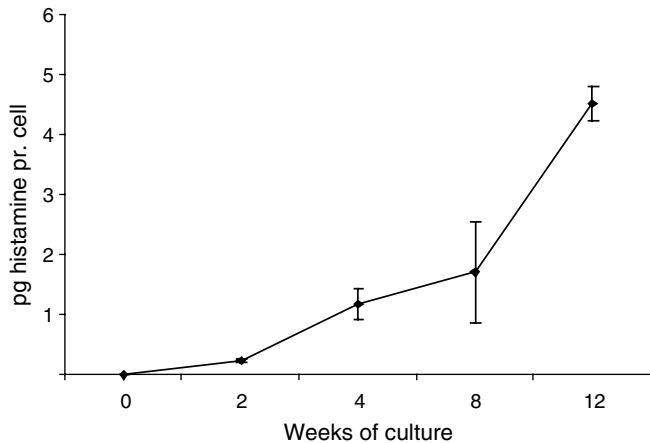


Figure 1. Total histamine content per cell during cell maturation and differentiation.

(Fig. 1). Newly purified cord blood CD133⁺ cells did not contain histamine but after 12 weeks of culture each cell contained 4.5 ± 0.28 pg histamine per cell. IgE dependent histamine release was performed week 2, 4 and 12. At week 2 no histamine was released, at week 4 the cells released up to 40% of their histamine and at week 12 up to 45% of the histamine was released ($n = 3$ for each experiment).

The cultured mature mast cells had homogeneous light scatter suggesting that all cells in culture responded evenly to the maturation conditions. Flow cytometry performed every week for 12 weeks revealed an extraordinary reproducibility between different cultures of forward scatter (related to cell size) and side scatter (related to cell granularity) (data not shown).

Early haematopoietic, myeloid and leucocyte lineage markers

We first examined the expression of the relevant early haematopoietic (CD34, CD117 and CD38) and myeloid markers (CD13 and CD33) (Fig. 2). CD117 and CD34 were co expressed on $86 \pm 8.9\%$ of the seeded CD133⁺ cells (not shown). CD34 expression decreased rapidly in the presence of IL-3, and had disappeared after week 4. At seeding purified CD133⁺ cells were $88.2 \pm 8.7\%$ positive for CD117. After 12 weeks in culture between 98% and 100% of cells were CD117⁺. A dramatic increase in receptor density of CD117 was seen after week 2, when IL-3 was removed from the culture medium. CD38 was expressed on $22.8 \pm 12.3\%$ of the CD34⁺ cells but after the second week of cell culture the expression of this marker was $<5\%$. CD38 mean fluorescent intensity (MFI) was low throughout culture and was absent after 8 weeks (data not shown). CD38 was assessed by both an FITC and a PE conjugated antibody of the same clone. CD13 was coexpressed on $69.5 \pm 14.7\%$ of CD34⁺ cells. The expression of CD13 increased during cell development and was $83 \pm 1.7\%$ at

week 12. The mean fluorescent intensity (MFI) increased significantly from week 3 and remained at a steady level after the addition of serum at week 8 ($P < 0.001$). The expression pattern of CD13 resembled very much the expression pattern of CD117. The monocyte/myeloid lineage cell marker CD33 was coexpressed on $58 \pm 14.6\%$ of CD34⁺ cells. The addition of serum at week 8 reduced the percentage of CD33⁺ cells from $96.3 \pm 1.2\%$ at week 8 to $72.6 \pm 12.3\%$.

Expression of the common leucocyte antigen CD45 increased steadily during culture and was expressed by $60.7 \pm 2.8\%$ of mature human mast cells.

MHC class II expression

In Fig. 3A,B data on expression of HLA-DR and CD45 are presented. A large proportion of CD133⁺ cells and mature mast cells expressed the MHC-II molecule HLA-DR. The increase in MFI between week 0 and 2 correlated with the appearance of larger vacuolated cells during this period resembling monocytes/macrophages. This disappeared when IL-3 was removed from the medium. After 12 weeks of culture the percentage of HLA-DR positive cells was 86%.

Cytokine and chemokine receptors

A number of cytokines and chemokines have been identified as regulators of human mast cell function (Table 1). CXCR4, the SDF-1 receptor, was found to be expressed on $90.1 \pm 3.1\%$ of seeded CD133⁺ cells and the expression was retained on nearly all cells ($94.3 \pm 1.4\%$) after 12 weeks of culture. Throughout the culture period the amount of CXCR2 positive cells remained high; as $85.5 \pm 2.1\%$ of the purified CD133⁺ cells were positive at week 0 and at 12 weeks $78.1 \pm 6.7\%$ of the cells were positive (Fig. 4). CXCR1 was expressed on $34.6 \pm 0.3\%$ of the purified CD133⁺ cells but disappeared after the second week of culture, when IL-3 was removed (data not shown). CXCR3, CCR3 and CCR5 were not expressed by CD133⁺ cells or human cultured mast cells in any experiment ($n = 3$ each; data not shown).

The IL-3, IL-5 and GM-CSF receptors were expressed by both the newly purified CD133⁺ cells, the maturing and mature cultured human mast cells (Fig. 4). During culture in IL-3, IL-3R expression increased, whereas IL-5R and GM-CSFR expression decreased. Addition of serum decreased IL-3R expression. More than 90% of CD133⁺ cells and $>80\%$ of 12-week-old mature mast cells expressed the IL-5 and GM-CSF receptors. Expression of the receptors for IL-5 and GM-CSF was confirmed with a polyclonal antibody towards the two receptors on 12 weeks old mast cells (Fig. 5). Using a flourokine kit the IL-3 receptor was detected on $62.5 \pm 3.2\%$ of 12 weeks old mast cells (Fig. 5).

The IL-18 receptor has been described on a wide range of cell lines but has never before been described on

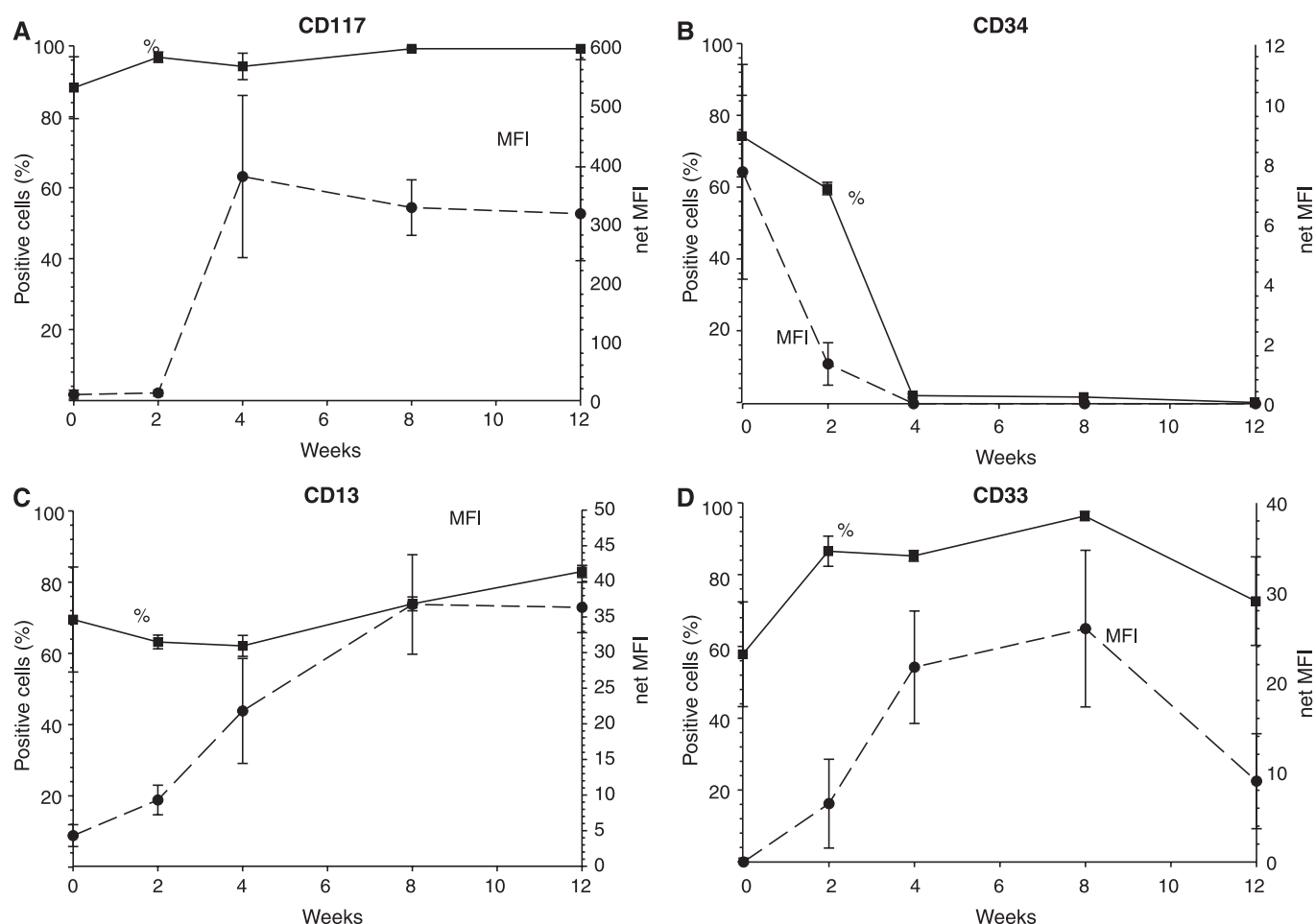


Figure 2. The FACS analysis of differentiating mast cells on early haematopoietic and myeloid lineage markers. Expression of (A) CD117, (B) CD34, (C) CD13 and (D) CD33 are shown. Data on newly purified CD133⁺ cells and on 2, 4, 8 and 12-week-old cells are presented as MFI (---) and percentage of positive cells (—) calculated by Overton Subtraction. Note the different scales on the right Y-axis (MFI). Data points shown mean $\pm$ SD values from four independent experiments.

human mast cells or their progenitors. Expression decreased during the first 2 weeks of culture with IL-3, and remained constant until the cells had matured. At week 12, $72 \pm 3.7\%$ were IL-18 receptor positive (Fig. 4).

Immunoglobulin receptors

The CD133⁺ purified cells and the cultured human mast cells were analysed for their expression of IgG receptors Fc γ RI, Fc γ RII and Fc γ RIII, IgE receptors Fc ϵ RI and Fc ϵ RII and the IgA receptor Fc α RI. Selected data are presented in Fig. 6.

The expression of Fc ϵ RI was examined on CD133⁺ purified cells and during cell culture in the absence of IgE. There was an increase in Fc ϵ RI expression throughout the culture period, significantly so after addition of FCS. At week 12, $48.4 \pm 7.3\%$ of the mature cultured human mast cells expressed the high affinity IgE receptor.

Immunofluorescence microscopy was performed to confirm Fc ϵ RI expression on 12-week-old mast cells (Fig. 7). The low affinity IgE receptor, Fc ϵ RII, was only expressed on $<10\%$ of the cells after 4 weeks of culture and markedly decreased after IL-3 was withdrawn from the medium.

At week 8, $62.5 \pm 14.1\%$ of the human cultured mast cells expressed CD32 and $64.9 \pm 8.4\%$ expressed CD16. CD32 increased in the presence of IL-3, and decreased after the removal of IL-3 from the medium while both receptors were suppressed by addition of serum at week 8. Approximately 20% of the mature mast cells expressed CD16 and CD32. Fc γ RI (CD64) was found on $14.4 \pm 0.5\%$ of CD133⁺ cells, but this increased to 20% of the mature mast cells.

The IgA receptor Fc α RI (CD89) was expressed on $<10\%$ of CD133⁺ cells and $11 \pm 4.2\%$ of 12-week-old mast cells.

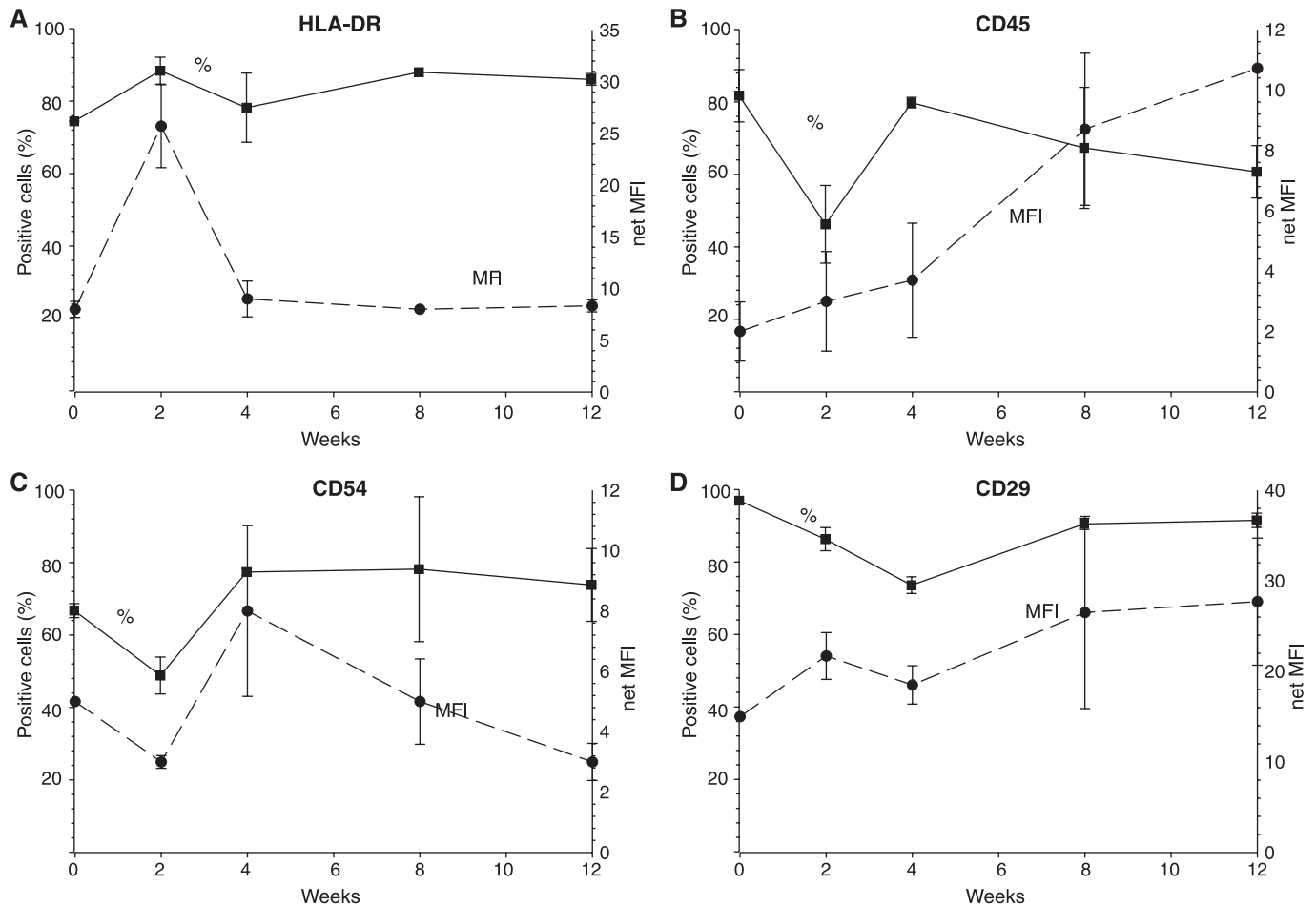


Figure 3. The FACS analysis of differentiating human mast cells. (A) HLA-DR and (B) common leucocyte antigen CD45 and two of its isoforms CD45RA and CD45RO were examined. CD45RA and CD45RO data are not shown. (C) Adhesion molecules ICAM-1 (CD54), (D) β_1 -integrin (CD29) and vitronectin (CD51/CD61). Data on vitronectin (CD51/CD61) not shown. Data on newly purified CD133⁺ cells and on 2, 4, 8 and 12-week-old cells are presented as MFI (---) and percentage of positive cells (—) calculated by Overton Subtraction. Note the different scales on the right Y-axis (MFI). Data points shown mean $\pm$ SD values from four independent experiments.

Adhesion molecules

Intercellular adhesion molecule-1 (ICAM-1, CD54) was detected already on the newly purified CD133⁺ cells and after week 4 remained expressed on a high percentage of cells as $73.8 \pm 5.2\%$ of mature mast cells expressed the receptor.

The vitronectin receptor (CD51/CD61) was not detected on the newly purified CD133⁺ cells but the amount of positive cells increased during cell differentiation and was $41 \pm 6.7\%$ after 12 weeks. CD51/CD61 receptor density seemed to decrease after serum supplementation.

The B_1 -integrin (CD29) was also expressed on newly purified cells and remained highly expressed: $86 \pm 2.6\%$ at week 0 and $92 \pm 1.5\%$ at week 12 with an increasing MFI (Fig. 3C, D.)

Discussion

In the current study we have further characterized the maturation of human cord blood derived mast cells from CD133⁺ cord blood precursors. The advantage of the method we have developed previously (12) is the large number of functionally mature cells accessible and the culture conditions have been shown to produce extremely reproducible cells in regard to cell size and granularity, histamine content and content of tryptase and chymase.

It is of interest to note that 60–80% of our CD133⁺ cells are CD34⁺ but already after 2–4 weeks CD34 disappears. The study of early haematopoietic and myeloid lineage markers showed expression of CD117/CD34/CD33 and CD13 to a large extent while CD38 was not detected. This is in partial agreement with Kirchen-

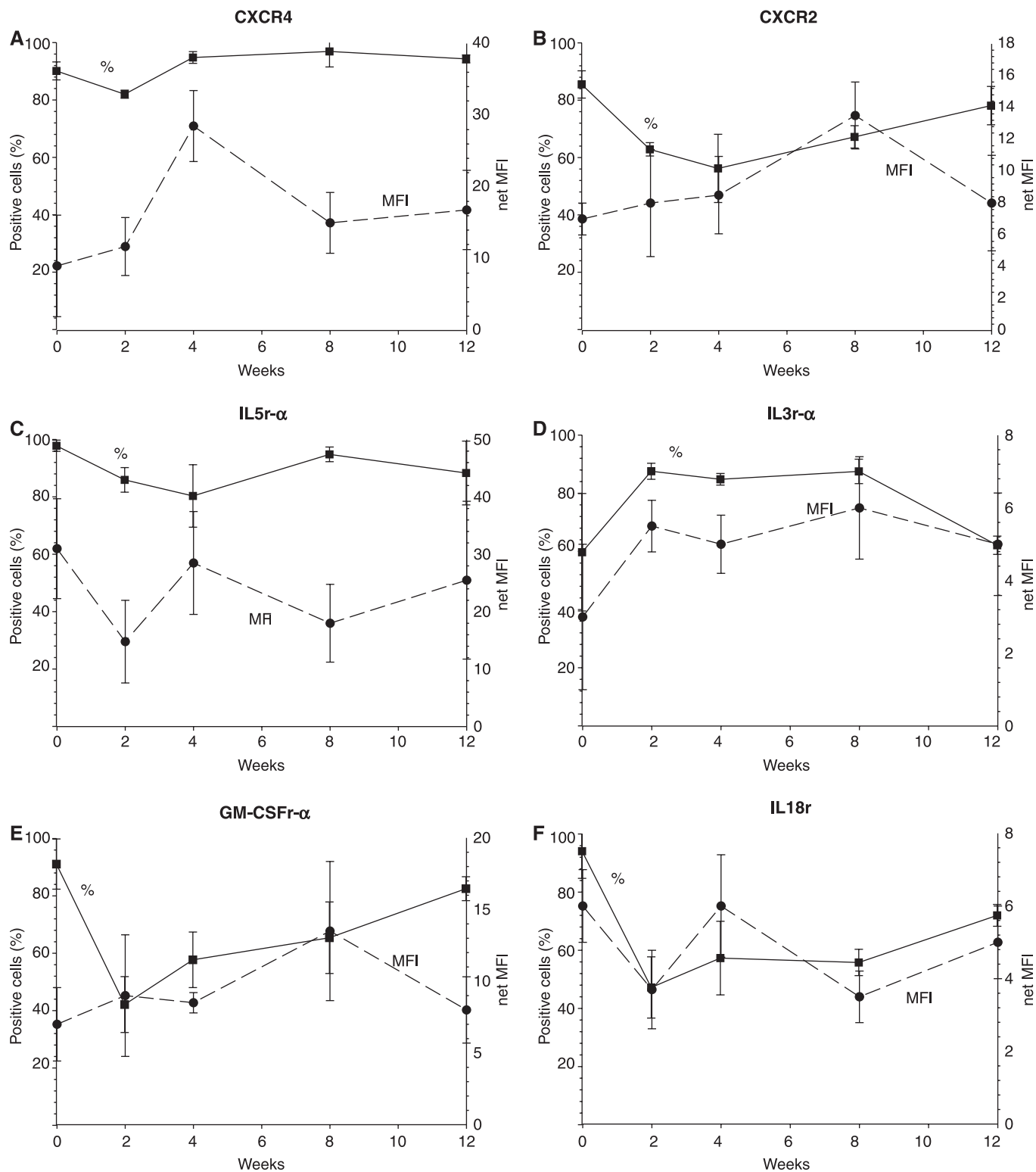


Figure 4. The FACS analysis of seven different chemokine and cytokine receptors on maturing human cultured mast cells. Data on CXCR1, CXCR3, CCR3 and CCR5 are not shown. Data on newly purified AC133⁺ cells and on 2, 4, 8 and 12-week-old cells are presented as MFI (---) and percentage of positive cells (—) calculated by Overton Subtraction. Note the different scales on the right Y-axis (MFI). Data points shown mean $\pm$ SD values from four independent experiments.

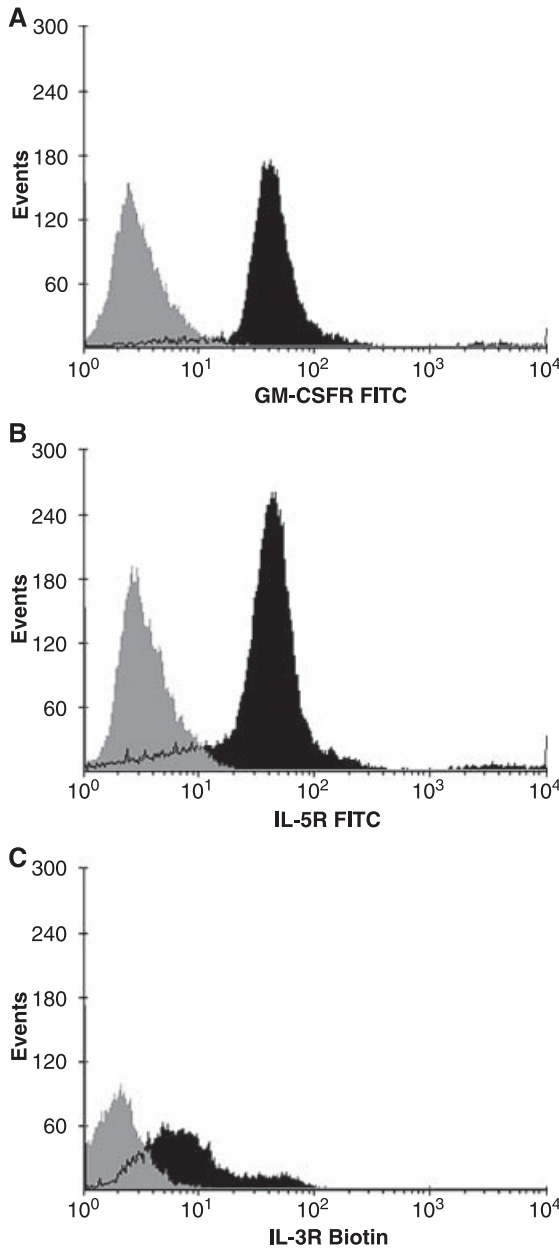


Figure 5. Expression of (A) GM-CSFR α , (B) IL5R α and (C) IL-3R α . (A), (B) and (C) shows histograms on 12 weeks old mast cells with alternative reagents. The interleukin (IL)-3 receptor (c) was shown by binding biotinylated IL-3 to the receptor and secondary detecting the avidin-fluorescein fluorescence, filled curve. The grey curve represents the isotype control. Representative examples of three separate experiments.

baum et al. (8) who finds that mast cells are CD34⁺/CD117⁺/CD13⁺ and express CD38. CD13 is also found expressed on human mast cells cultured from human foetal liver cells (16). The presence of CD38⁺ mast cell precursors could not be demonstrated in our system and from the mature mast cells, we are in agreement with Valent et al. and Agis et al. in that mature mast cells lack CD38 (17, 18).

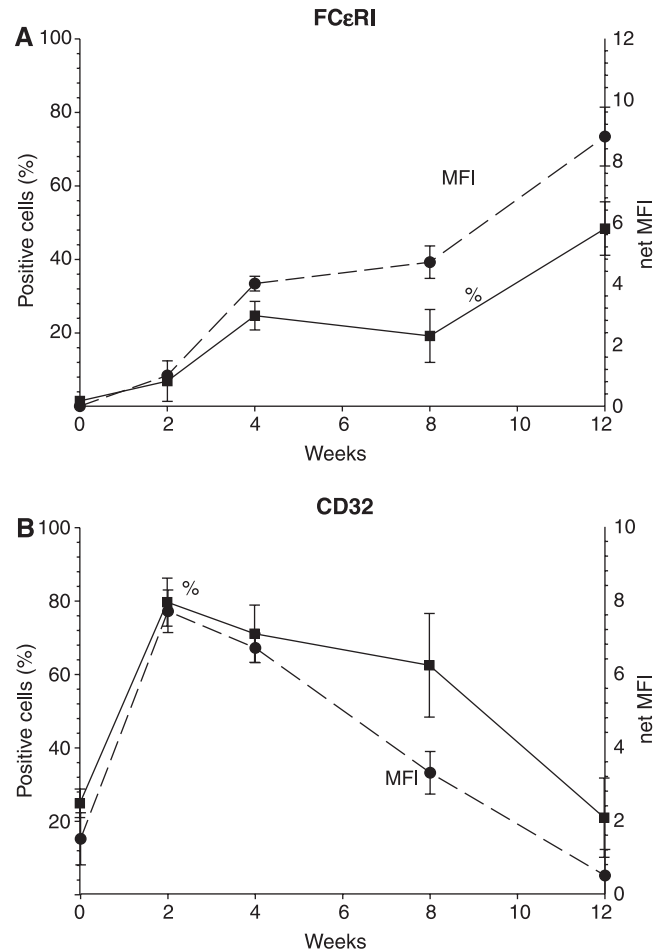


Figure 6. The FACS analysis of immunoglobulin (Ig) receptors. The IgE (Fc ϵ RI), IgG subclasses (Fc γ RII, Fc γ RI, Fc γ RIII) and IgA (Fc α RI) were examined. Data for Fc γ RI, Fc γ RIII and Fc α RI are not shown. Data on newly purified CD133⁺ cells and on 2-, 4-, 8- and 12-week-old cells are presented as MFI (---) and percentage of positive cells (—) calculated by Overton Subtraction. Note the different scales on the right Y-axis (MFI). Data points shown mean $\pm$ SD values from four independent experiments.

After 12 weeks 100% of the cells stained methachromatic with alcian blue. 96.7% of the cells were tryptase positive and 32% of the cells stained positive with for chymase.

Each cell had an average histamine content of 4.5 pg/cell and the cells released around 45% of their histamine upon stimulation with anti-IgE. It is not possible to determine whether all cells released 45% of their histamine, or whether 45% of the cells released all their histamine. Most haematopoietic cells lose CD117 expression during differentiation. Mast cells retain a high expression of this receptor also when resident in the tissues as mature mast cells. Between 98 and 100% of 12-week-old cells were CD117⁺.

The present study suggested a detectable constitutive expression of the chemokine receptors CXCR2 and

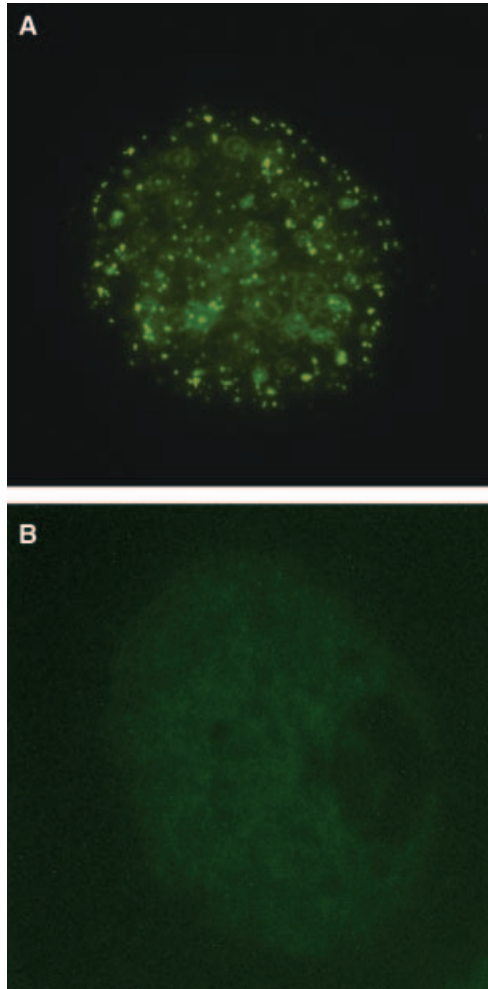


Figure 7. Immunofluorescence appearance of cytoplasm of 12-week-old human mast cell. Cells were incubated with CRA-1 (A) primary monoclonal antibody against the high affinity immunoglobulin (Ig)E-receptor for 30 min at 4°C. After this cells were washed in phosphate-buffered saline containing 0.1% bovine serum albumin and incubated for 30 min. at 4°C with secondary FITC labelled antibody. ITC (B) was performed using an isotype matched IgG2b instead of CRA-1.

CXCR4 on the surface of cord blood derived cultured human mast cells. The ligand for CXCR2, IL-8, has been shown to be chemotactic for mast cells (19) as has Stromal-derived factor-1, the ligand for CXCR4 (20). The expression of these chemokine receptors on human mast cells and their progenitors may provide a mechanism through which these cells might be selectively recruited into the tissues.

Our cord blood derived mast cells failed to express CCR3, a receptor previously found on human mast cells (21, 22). In a study performed by Paulis et al. (23) it was shown that CCR3 was preferentially expressed by mast cells present in connective tissue rather than in mucosal mast cells. Tryptase is present in the vast majority of human mast cells while chymase is predominantly associated with connective tissue mast cells (24). Our mast

cells predominantly expressed tryptase and to a lesser degree chymase. Ochi et al. cultured human mast cells from cord blood in the presence of SCF, IL-6 and IL-10 (22). The inclusion of IL-10 in their culture conditions may account for the high chymase immunoreactivity seen in their cultured human mast cells (100% in 9-week-old cells) as mouse IL-10 induces the expression of chymase in bone marrow derived mouse mast cells (25, 26).

The difference in the findings of expression of CCR3 may be explained by the different culture conditions giving rise to mast cells resembling different phenotypes.

We confirmed our previous finding of HLA-DR on CD133⁺ precursors. In addition the common leucocyte antigen CD45 was detected. Both maintained weak but definite expression during mast cell maturation.

Regarding immunoglobulin receptors it is interesting that the high affinity IgE receptor FcεRI gradually increases during the 12-week culture period while the IgG receptors FcγRII and FcγRIII seem to develop and persist until FCS is added to the media. It is well known that IgE can upregulate the expression of FcεRI (27, 28) just as it has been demonstrated that FcγRI is upregulated on mast cells by interferon (IFN)γ (29) where the FcγRI expression increased from ~2 to 44% by IFNγ exposure. The expression of FcγRI in our culture system was around 20% on mature mast cells and this was not markedly affected by the addition of FCS. An interaction of IgE and IgG receptors has been demonstrated in a murine model and in human blood basophils (30, 31). Further studies may reveal an interaction between these receptors in our human mast cell system.

For the first time the expression of high levels of the three receptors for IL-3, IL-5 and GM-CSF on mast cell precursors and mature mast cells has been demonstrated. Welker et al. (32) showed that the density of the GM-CSF receptor decreased on HMC-1 cells and similar results using cord blood derived mast cells after the addition of FCS. We also found a decrease in the GM-CSF receptor density after addition of serum at week 8 while the percentage of receptor positive cells remained high suggesting an internalization of the receptor.

We found a continuous expression of the IL-3 receptor on approximately 62% of our 12-week-old human cultured mast cells. The MFI was low suggesting few receptors, but binding of the biotinylated ligand IL-3 to the receptor on the 12-week-old mast cells confirmed the presence of the IL-3 receptor on mast cells. This is in contrast to Ochi et al. (22) who found that the IL-3 receptor had disappeared after 9 weeks in culture. In their culturing system 10% serum was added from the beginning of the culture period. In contrast we had serum free culturing conditions until week 8. After addition of serum we found a decrease in both the percentage of IL-3 receptor positive cells as well as the MFI of the receptor. These differences could be explained by the fact that components of serum decrease expression of the IL-3 receptor, which is known to be the case for a range of other

surface proteins as shown in this paper. Alternatively, the mast cells of Ochi et al. may have a different phenotype to ours. Gebhardt et al. (33) found that human mast cells isolated from intestinal mucosa expressed the IL-3 receptor and also demonstrated the functional activity of this receptor. Human intestinal mucosal mast cells have previously been shown to be about 81% tryptase positive cells and only 19% of the cells expressing both tryptase and chymase (24), very much resembling the phenotype of our cultured mast cells, but in contrast to the mast cells cultured in the presence of IL-10 by Ochi et al. that were mostly double positive for both tryptase and chymase.

The IL-18 receptor was expressed by a large percentage of the human mast cells. This receptor is very similar to the IL-1 receptor (34). Basophils and mast cells derived from mouse bone marrow have been shown to express the IL-18R α and upon stimulation with IL-18 the cells were able to release IL-13 and IL-4. Both of these cytokines induce IgE switching and IgE synthesis by human B-cells (35). The IL-18 might be critically involved in induction of the allergic inflammation and it would be interesting to elucidate the pathophysiological relevance of IL-18 stimulation of mast cells.

It seems important to thoroughly characterize the *in vitro* cultured human mast cells as differences in culturing conditions give rise to mast cells of different phenotypes, expressing different characteristics. The addition of different growth factors and serum affects the expression of surface proteins on the cultured cells. To correlate findings using *in vitro* cultured human mast cells to *in vivo* conditions, it seems vital that the cells are well described.

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