

Acquisition and alteration of adhesion molecules during cultured human mast cell differentiation

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Background: Mature human mast cells express several types of adhesion molecules on their surface. Interactions between extracellular matrix (ECM) and adhesion molecules may be important for the migration and localization of mast cells and their precursors in tissues. Little is known about the regulation of adhesion molecules on mast cells during their differentiation.

Objectives: To clarify the evolution of adhesion phenotype and function, we examined the expression of adhesion molecules during cultured human mast cell (CHMC) differentiation and tested adhesion of mature CHMCs to various ECM proteins. **Methods:** CHMCs were obtained by culturing human cord blood–derived CD34⁺ cells in the presence of stem cell factor and IL-6. Indirect immunofluorescence and flow cytometry was used to study cell surface expression of adhesion molecules and other markers. Mature CHMCs were tested for adhesion molecule function with immobilized matrix proteins.

Results: At 1 week of culture, cells expressed CD11a, CD18, CD29, CD49d, and CD49e. At 14 weeks of culture, more mature CHMCs expressed CD11b, CD11c, CD29, CD49b, CD49c, CD49d, CD49e, CD51, CD61, and CD54 and weakly expressed CD18 and CD11a. CD11c, CD51, and CD61 appeared de novo by 4 weeks of culture, whereas CD49b and CD49c appeared by 8 weeks. CD29 decreased at 4 weeks but returned to the identical levels of 1-week-old cells by 8 weeks. Compared with levels at week 1, the levels of CD11a, CD18, CD49d, and CD49e at 4 weeks and beyond decreased during culture. Expression of CD49a, CD49f, and α d integrin was never detectable during CHMC differentiation. Fourteen-week-old CHMCs significantly adhered to the leucine-aspartic acid-valine—containing connecting segment 1 fragment of fibronectin, the 120-kd arginine-glycine-aspartic acid—containing fragment of fibronectin, vitronectin, and laminin through specific integrins.

Conclusion: Expression of integrins and CD54 is differentially regulated during CHMC differentiation, and mature CHMCs can adhere to many ECM proteins. These changes may facilitate emigration from the bone marrow into the circulation and ultimately contribute to the tissue homing and localization pattern seen with mature mast cells.

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Key words: Mast cells, adhesion, adhesion molecules, integrins, maturation, differentiation, stem cells, matrix proteins, fibronectin, laminin, vitronectin

Mast cells are known to originate from distinct bone marrow hematopoietic progenitors that leave the bone marrow and circulate before entering tissues and differentiating into mature mast cells.^{1,2} Specific human mast cell (HMC) progenitors have been identified in peripheral blood as CD34⁺ mononuclear cells that differ from monocytes by their surface expression of kit (CD117), the receptor of stem cell factor (SCF).^{3,4} SCF is a potent and specific mast cell growth factor that is constitutively expressed on fibroblast membranes.^{5,6} Indeed, CD34⁺ cells obtained from cord blood, bone marrow, or fetal liver cells, which contain hematopoietic progenitor cells, proliferate into mature human cultured mast cells in the presence of SCF in vitro.⁷⁻¹⁰ However, how mast cell progenitors localize within tissues is not well known.

Adhesion molecules on mast cells are certain to be important in the migration of mast cell progenitors from bone marrow into tissue. Human tissue mast cells, obtained from lung, skin, or uterus, express integrins such as very late activation antigen (VLA) 3, VLA-4, VLA-5, and p150,95.¹¹⁻¹³ In contrast, human stem cells express VLA-4, VLA-5, intercellular adhesion molecule (ICAM) 1, and leukocyte functional antigen (LFA) 1.¹⁴⁻¹⁶ Because mast cell development, tissue localization, and maturation are associated with changes in adhesion molecules, studies of adhesion molecules expressed during their differentiation are relevant to mast cell biology. Several investigators reported that α v β 3 integrins were expressed on cultured HMCs (CHMCs) derived from fetal liver cells or CD34⁺ cells but not on HMC progenitor cells, suggesting that certain β 3 integrins are gained during the differentiation of mast cells in vitro.^{17,18} However, the regulation of adhesion molecules on HMCs has not been well investigated.

When CD34⁺ cells obtained from human cord blood are cultured with SCF and IL-6, most of the cells become mast cells.^{10,19,20} These cells contain tryptase and chymase¹⁰ and have functional Fc ϵ RI, which, when cross-linked, are capable of inducing histamine release, indicating that CHMCs have characteristics typical of mature mast cells. In this study, we used this culture system to examine the change of expression and function of adhesion molecules on CHMC during their differentiation in vitro. Our data demonstrate profound changes in integrin expression at different time points during CHMC differentiation.

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TABLE I. mAbs used in the study and their sources

Antigen	Clone name	Source
β1 Integrins		
CD29 (β1 integrin)	4B4	Coulter-Immunotech, Hialeah, Fla
CD49a (α1 integrin)	TS2/7	T cell Diagnostics, Inc, Cambridge, Mass
CD49b (α2 integrin)	Gi9	Coulter-Immunotech
CD49c (α3 integrin)	PIB5	GIBCO-BRL, Gaithersburg, Md
CD49d (α4 integrin)	HP2/1	Coulter-Immunotech
CD49e (α5 integrin)	SAM1	Coulter-Immunotech
CD49f (α6 integrin)	GoH3	Coulter-Immunotech
β2 Integrins		
CD18 (β2 integrin)	7E4	Coulter-Immunotech
CD11a	25. 3. 1	Coulter-Immunotech
CD11b	44	R&D Systems, Minneapolis, Minn
CD11c	BU15	Coulter-Immunotech
αd Integrin	169A	Dr Pat Hoffman, ICOS Corp, Bothell, Wash
Others		
CD51 (αv integrin)	AMF7	Coulter-Immunotech
CD61 (β3 integrin)	SZ.21	Coulter-Immunotech
ICAM-1	84H10	Coulter-Immunotech
FcεRI	22E7	Dr Richard Chizzonite, Hoffmann-La Roche Inc, Nutley, NJ

Abbreviations used

- CHMC: Cultured human mast cell
- CS-1: Connecting segment 1
- Fn120k: Fibronectin 120-kd fragment
- HMC: Human mast cell
- ICAM: Intercellular adhesion molecule
- IMDM: Iscove's modified Dulbecco's medium
- LFA: Leukocyte functional antigen
- SCF: Stem cell factor
- VLA: Very late activation antigen

METHODS

HMC culture

CHMCs were generated as previously reported.^{10,20} In brief, heparin-treated umbilical cord blood was diluted and layered over Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) at room temperature. Cord blood mononuclear cells were purified by density gradient centrifugation methods followed by immunomagnetic positive selection with mAb against CD34 (Miltenyi Biotec Inc, Auburn, Calif). The purified CD34⁺ cells were cultured in Iscove's modified Dulbecco's medium (IMDM; GIBCO BRL, Gaithersburg, Md) supplemented with 10 μg/mL insulin, 5.5 μg/mL transferrin, 6.7 ng/mL selenium, 5 × 10⁻⁵ mol/L 2-mercaptoethanol and 5% FBS (GIBCO BRL), 100 ng/mL SCF (generously provided by Dr Martha E. Vincent, Amgen, Thousand Oaks, Calif), and 50 ng/mL IL-6 (Biosource, Camarillo, Calif) for up to 15 weeks. The purity of mast cells was determined by staining with May-Grünwald and Giemsa reagents. The purity of mast cells was 99% or greater by 14 to 15 weeks of culture.

Flow cytometric analysis of cell surface integrins and other molecules

The surface phenotype of CHMC was examined by indirect immunofluorescence and flow cytometry as previously described.¹² Cultured cells at 1, 4 to 5, 8 to 9, and 14 to 15 weeks were collected and incubated for 30 minutes on ice in PBS (containing 0.2% BSA and 4 mg/mL human IgG; Sigma Chemical Co, St Louis, Mo)

with saturating concentrations of mAb or an equivalent concentration of irrelevant IgG control mAb (Table I). Cells were then washed and incubated with a 1:100 dilution of R-phycoerythrin-conjugated F(ab')₂ goat antimouse IgG (Biosource) for another 30 minutes on ice. After fixation in 1% paraformaldehyde in PBS, 5000 cells were evaluated with an EPICS Profile II flow cytometer (Coulter Corp, Hialeah, FL). Net mean fluorescence intensity was determined after values for IgG1 controls were subtracted (average, 2.1 ± 0.3).

Mast cell adhesion assays

Adhesion assays were modified from methods in a previous report.¹¹ In brief, 96-well plates (Nunc, Maxi-sorb Immuno-plates; PGC Scientific Corp, Gaithersburg, MD) were coated overnight at 4°C with 100-μL aliquots of optimal concentrations of proteins, including 1.85 mg/mL connecting segment 1 (CS-1) of fibronectin (generously provided by Dr Roy Lobb, Biogen Inc, Cambridge, Mass), 5 mg/mL fibronectin 120-kd fragment (Fn120k, GIBCO-BRL), 5 mg/mL vitronectin (GIBCO-BRL), or 5 mg/mL laminin (Sigma) diluted in PBS containing 1 mmol/L CaCl₂ and MgCl₂. The wells were then blocked with IMDM containing 1% BSA (IMDM-BSA) for 1 hour at room temperature to reduce adherence to plastic. Control adherence was measured in wells coated with PBS alone and blocked with IMDM-BSA. CHMCs that were 14 to 15 weeks old (1 × 10⁴ in 100 μL) were added to the wells in duplicate and incubated for up to 1 hour at 37°C. In the blocking experiments, CHMCs were preincubated with saturating concentrations of blocking mAbs (Table I) before being added to the wells. At the end of the adhesion assay, non-adherent cells were removed by being rinsed twice with 100 μL of IMDM-BSA, and adherent cells were lysed with 1.6% perchloric acid. The liquid from each well was collected and transferred into tubes containing 0.9 mL of saline solution. Each tube was centrifuged, and the supernatants were stored until histamine analysis. CHMCs did not release histamine during the adhesion assay (data not shown). The histamine content of adherent cell lysates was determined with an automated histamine analyzer,²¹ and the percentage of adherence was calculated by comparison of the histamine content of adherent cell lysates with that of separate 100-μL aliquots of cell suspension.

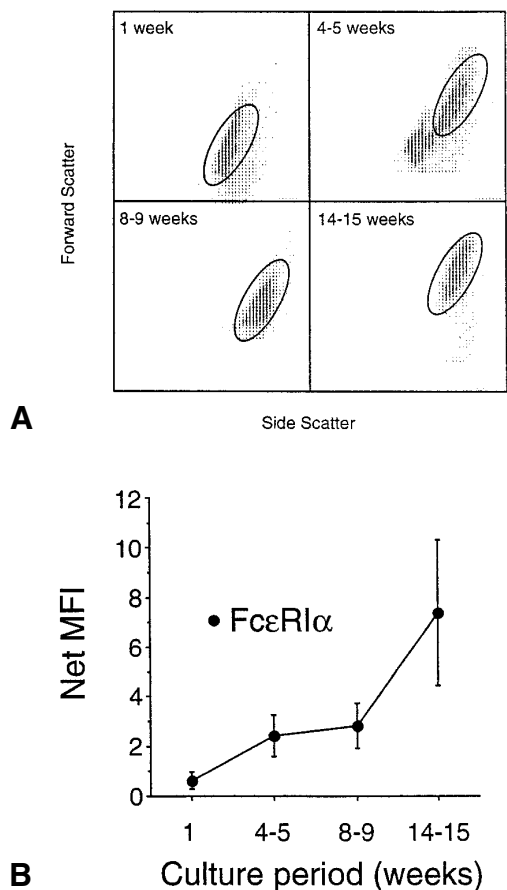


FIG 1. Light scatter characteristics (**A**) and FcεRI α -chain expression (**B**) of cultured cells. **A**, Data are shown from a single batch of CHMCs representative of 3 experiments. **B**, Data are averaged from 3 to 5 separate experiments (mean $\pm$ SEM). MFI, Mean fluorescence intensity.

Effect of adherence to matrix proteins on FcεRI-mediated histamine release and IL-8 production by CHMC

CHMCs (0.5×10^5 to 1×10^5 /mL) were sensitized with 5 mg/mL human myeloma IgE (generously provided by Dr Teruko Ishizaka²²) at 37°C for 2 days in mast cell culture medium. After the incubation, cells were washed twice with IMDM-BSA to remove cytokine and free IgE. CHMCs were then added to the matrix protein-coated wells for 1 hour at 37°C. Polyclonal goat antihuman IgE (1 mg/mL)^{23,24} or control medium was then added, and cells were incubated for another hour at 37°C. All reactions, performed in duplicate, were stopped by centrifugation at 4°C, and the supernatants were collected and stored at -20°C until histamine analysis. In each experiment, perchloric acid at a final concentration of 1.6% was added to duplicate tubes to determine total histamine content. Histamine was assayed as above by an automated analyzer, and the percentage of histamine released was calculated from the ratio of each sample against total histamine after spontaneous release (typically < 5%) was subtracted.

For IL-8 release, IgE-sensitized CHMCs were washed twice and resuspended at 2×10^5 to 5×10^5 cells/mL in IMDM-0.2% BSA

containing 100 ng/mL SCF. CHMCs were incubated on the matrix protein-coated wells for 1 hour and challenged with or without 1 mg/mL goat antihuman IgE at 37°C for another 6 hours. After incubation, the supernatants were collected and stored at -20°C. IL-8 protein levels were measured by ELISA as previously reported.²⁵ The detection limit for IL-8 was 30 pg/mL.

Statistical analyses

All results are shown as mean $\pm$ SEM. Statistical analyses were performed with a paired Student *t* test. The level of significance was set at $P < .05$.

RESULTS

Cytofluorographic characteristics and expression of FcεRI during the differentiation of CHMC

At week 1, cultured cells were homogeneous with respect to light scatter. At weeks 4 to 5, cells separated into 2 populations based on forward and side scatter (Fig 1, A). The lower population at weeks 4 to 5 was identical to the population at week 1. The higher scatter population was similar to that seen in later parts of the culture period, suggesting that these cells were converting to mast cell precursors. However, the lower population may still contain mast cell progenitors.¹⁷ At weeks 8 to 9, cells again became a single population, although by weeks 14 to 15, light scatter continued to increase. These observations were consistent with those of a previous report.¹⁷

In the following experiments, we focused on the population that was gated as shown in Fig 1, A. We first examined expression of FcεRI on cultured cells in the absence of IgE to confirm that we indeed had CHMCs or their precursors. Expression of FcεRI was low and increased at 4 to 5 weeks. During the last month, FcεRI expression became most prominent. Even though FcεRI was weakly expressed on CHMCs, these cells were capable of releasing histamine and producing cytokines after cross-linking of FcεRI.²⁶ Gating on the lower scatter population at weeks 4 to 5 revealed barely detectable levels of FcεRI, similar to those seen at week 1 (data not shown).

Change in adhesion molecule phenotype during differentiation of CHMCs

To clarify the ontogeny of CHMC adhesion molecule phenotype, we examined the levels of adhesion molecules on CHMCs at several times during their differentiation. CD11a and CD18 were brightly expressed on cells at week one and dramatically decreased at 4-5 weeks (Fig 2). By 14-15 weeks, CD11a and CD18 were still weakly expressed. At week 1, CD11b was weakly expressed, and levels did not change during the culture. CD11c expression was not detectable at week 1 but became weakly expressed during weeks 4 to 9. Levels of α d remained undetectable throughout the culture (data not shown). Thus most of the β 2 integrins disappeared during differentiation in vitro. Unlike mature mast cells, which only express low levels of p150,95, if any at all, the cultured cells expressed low levels of CD11a and CD11b integrins.

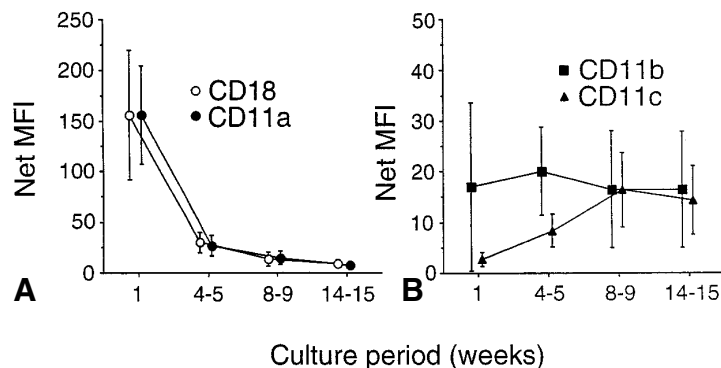


FIG 2. Expression of $\beta 2$ integrins during mast cell differentiation. **A**, Expression of CD18 and CD11a. **B**, Expression of CD11b and CD11c. Data are mean $\pm$ SEM of 3 to 5 separate experiments. *MFI*, Mean fluorescence intensity.

After 1 week in culture, cells brightly expressed CD29 ($\beta 1$ integrin), CD49d ($\alpha 4$ integrin), and CD49e ($\alpha 5$ integrin) (Fig 3). CD29 expression decreased at 4 to 5 weeks but by 8 to 9 weeks returned to the identical levels of the first week, where it remained at weeks 14 to 15 (Fig 3, *A*). Expression of CD49d and CD49e markedly decreased in the first 4 to 5 weeks of culture and remained at these levels for the next 10 weeks (Fig 3, *B*), resembling levels on mature tissue mast cells. At week 1, cells did not express CD49b or CD49c. Both CD49b and CD49c began to appear at 4 to 5 weeks and increased during the next 4 weeks, where levels reached a plateau (Fig 3, *C*). Expression of CD49c resembled that of mature mast cells, whereas the low levels of CD49b differed from levels of mature mast cells, which do not express this integrin. Levels of CD49a, a collagen receptor, and CD49f, a laminin receptor, remained undetectable throughout the culture (data not shown). These data suggest that the decrease in CD29 expression was related to decreases in VLA-4 and VLA-5 and that the increase of CD29 between 4 and 8 weeks was related to the de novo expression of VLA-2 and VLA-3.

CD51/CD61, the vitronectin receptor, was not expressed at week 1 but continuously increased during culture (Fig 4). CD54, or ICAM-1, was expressed at week 1 and increased 2- to 3-fold until 8 to 9 weeks, where it reached a plateau. In summary, as shown in Fig 5, cultured cells at week 1 expressed CD29, CD49d, CD49e, CD11a, CD18, and CD54. Fourteen-week-old CHMCs still expressed CD29, CD49d, CD49e, CD11a, CD18, and CD54, although in most cases levels were quite different (eg, CD49d, CD49e, CD11a, and CD18 decreased; CD54 increased; and CD29 remained unchanged). The 14-week-old CHMCs, unlike the 1-week-old cells, also expressed CD11b, CD11c, CD51/CD61, CD49b, and CD49c. Although the 14-week-old CHMCs' phenotype resembled that of tissue mast cells, expression of CD11a, CD11b, and CD49b on tissue mast cells has not been observed.¹¹⁻¹³

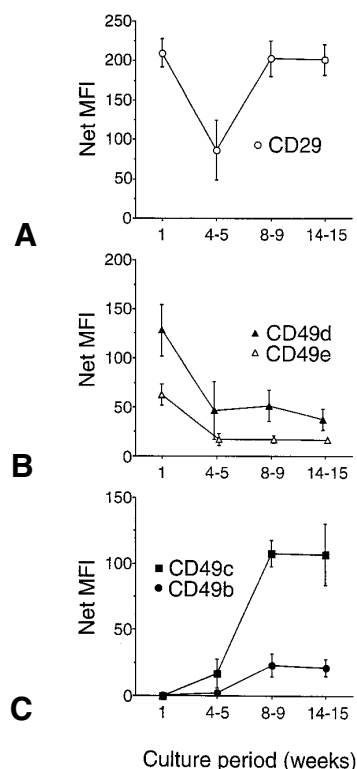


FIG 3. Expression of $\beta 1$ integrins during mast cell differentiation. Expression of CD29 (**A**), CD49d and CD49e (**B**), and CD49c and CD49b (**C**) was examined. Data are mean $\pm$ SEM of 3 to 5 separate experiments.

Adhesion of CHMCs to extracellular matrix proteins and its effect on histamine and IL-8 release

To verify that the integrins expressed on mature CHMCs were functional, adhesion of 14-week-old cells to extracellular matrix proteins was examined. As shown

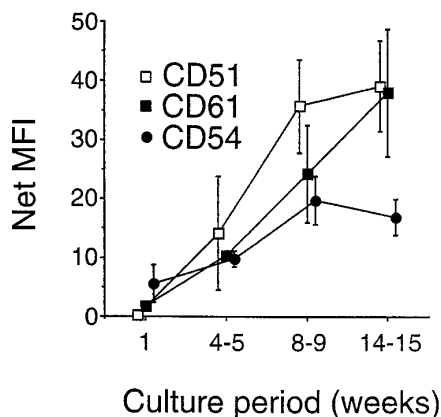


FIG 4. Expression of $\beta 3$ integrins and ICAM-1 during mast cell differentiation. Expression of CD51, CD61, and CD54 (ICAM-1) was examined as indicated. Data are mean $\pm$ SEM of 3 to 5 (CD51 and CD54) or 1 to 4 (CD61) separate experiments.

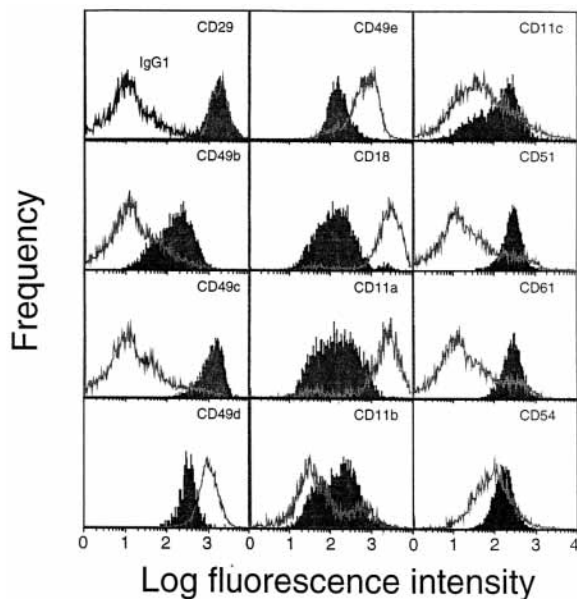


FIG 5. Representative flow cytometric histograms for various adhesion molecules on 1-week-old (gray line) and 14-week-old (black filled) cultured cells. In the upper left panel is labeling with the IgG control mAb, shown for comparison.

in Fig 6, A, significant cell adhesion was observed to each of the matrix proteins. Laminin was the best ligand, whereas vitronectin adhesion was lowest. In addition, CHMC adhesion to all matrix proteins was significantly enhanced by SCF (100 ng/mL, 1 hour), except for laminin. Increased adhesion to BSA was also seen, although this was not statistically significant and was of lesser magnitude than that seen with CS-1, Fn120k, and vitronectin. As shown in Fig 6, B, adhesion to each matrix protein was integrin dependent because specific mAb significantly inhibited adhesion. CHMC adhesion

to CS-1 required $\alpha 4$ (although effects of the $\alpha 4$ mAb did not reach statistical significance), adhesion to Fn120k required $\alpha 5$, adhesion to laminin required $\alpha 3$, and adhesion to vitronectin required both αv and $\alpha 3$.

We also tested whether adhesion to matrix proteins enhanced Fc ϵ RI-mediated histamine release and IL-8 production by CHMCs. In contrast to previous reports using rat basophilic leukemia cells^{27,28} and HMC-1 cells,²⁹ histamine release and IL-8 production by CHMCs were not enhanced by adhesion to any of the extracellular matrix proteins tested (data not shown).

DISCUSSION

The surface phenotype of tissue-derived HMCs and the CD34⁺ stem cell has been explored.^{11,12,14-16,30} Previous studies using mast cells derived from skin, lung, or uterine tissues detected surface expression of VLA-3, VLA-4, VLA-5, $\alpha v\beta 3$, p150/95, and ICAM-1.^{11,12,30} This phenotype differs markedly from that of the CD34⁺ stem cell, which expresses high levels of LFA-1, modest levels of VLA-4, VLA-5, and ICAM-1, and little or no $\beta 3$ integrins. It was clear, therefore, that a number of phenotypic changes occurred during differentiation from stem cells into mature tissue mast cells. The purpose of this study was to determine whether the newly developed methods for generating CHMCs would lead to the generation of mature mast cells with an adhesive phenotype similar to that of mast cells enzymatically isolated from human tissues. A potential advantage of the CHMC system is that it permits an analysis of the kinetics of this adhesive phenotype evolution. In addition, it is likely that some of the phenotypic changes that occur during the generation of mast cell precursors in the bone marrow may facilitate their emigration from the bone marrow into the circulation and from the circulation into local tissue sites, where it is presumed that they complete their differentiation.

In this study, the adhesive phenotype of 1-week-old cultured cells displayed a phenotype similar to that previously reported for CD34⁺ stem cells.¹⁴⁻¹⁶ Expression of CD11a on stem cells almost completely disappeared during culture with SCF and IL-6, with most loss occurring during the first month of culture. During that same period, levels of CD49d and CD49e decreased dramatically, reaching a plateau at levels similar to those seen on tissue mast cells (Fig 3 and References 11, 12, and 30-33). The marked decrease in these integrins was accompanied by a parallel decrease in their associated $\beta 2$ and $\beta 1$ subunits, respectively. This 1-month time point is analogous to the point at which several mast cell-specific characteristics have already appeared, such as Fc ϵ RI, tryptase, chymase, and metachromatic staining (Fig 1 and References 10 and 17). It is interesting, however, that levels of $\beta 1$ integrin rebound during the second month of culture, corresponding to the de novo appearance of CD49b and CD49c (Fig 3). Although ICAM-1 was constitutively present on stem cells, levels rose throughout the 3 months of culture, as did levels of $\alpha v\beta 3$ integrins, the latter appearing de novo. These changes were remarkably unimodal in most cases,

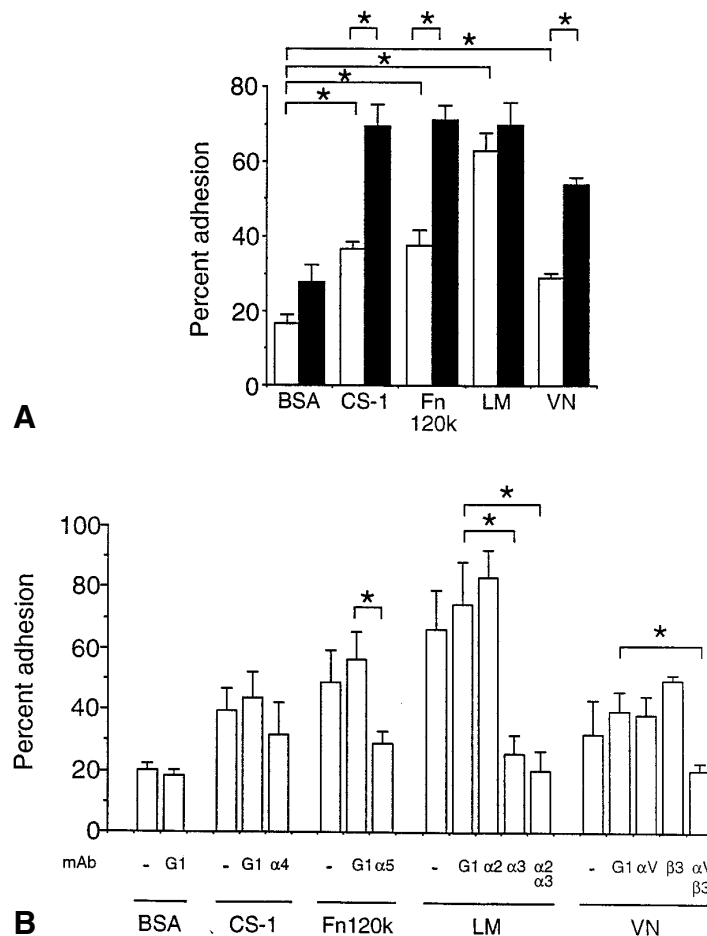


FIG 6. Adhesion of CHMCs to extracellular matrix proteins. **A**, Fourteen-week-old CHMCs were incubated on the extracellular matrix protein-coated wells in the presence (black bar) or absence (white bar) of 100 ng/mL SCF for 1 hour, and the percentage of adhesion was determined. **B**, Fourteen-week-old CHMCs were preincubated with the indicated mAb for 30 minutes before the adhesion assay. Data are mean $\pm$ SEM of 3 separate experiments. * $P < .05$. LM, Laminin; VN, vitronectin.

with the exception of CD11b (Fig 5), suggesting that the gated population was quite consistent in its adhesion molecule phenotype. By the end of the 14-week culture period, these CHMCs resembled human tissue-derived mast cells in both their pattern and overall level of adhesion molecule expression, with the exception that CD11a, CD11b, and CD49b have never been detected on human tissue mast cells, although CD11b has been detected by others on CHMCs and murine mast cells.^{34,35} Finally, it was demonstrated with mature CHMCs that the integrins present on these cells mediate adhesive function for a variety of matrix proteins, with $\alpha 3$ integrin-mediated adhesion to laminin being the most impressive in these assays.

In both mast cell culture systems using cells derived from fetal liver and cord blood, mast cell precursors were reported to express VLA-4, whereas mature cells derived from these precursors expressed VLA-4 along with VLA-5, $\alpha v\beta 3$, and ICAM-1.^{17,18} However, in the culture system using fetal liver cells, precursors also expressed

VLA-3, whereas the mature cells did not express VLA-3. Our results, that expression of $\alpha v\beta 3$ integrin was not detected in progenitors and was highly detectable on mature mast cells, were consistent with results of previous reports.^{17,18}

It is extremely likely that genes for each of the adhesion molecules studied here must be differentially regulated at distinct time points during mast cell differentiation in vitro. For example, signals for activation of CD49b, CD49c, and $\alpha v\beta 3$ genes appeared to be turned on at the same time as for Fc ϵ RI and tryptase, whereas genes for CD11a and CD18 expression are downregulated (Fig 1 and References 10 and 17). Transcriptional regulation of integrin gene expression has been reported with other cells. For example, $\alpha 2$, $\alpha 3$, $\alpha 4$, αv , and $\beta 3$ integrins are regulated by promoter sites in the 5' flanking region.³⁶⁻⁴³ In mice, the 5' flanking region of the αv integrin gene contains Sp1, Ets, and GATA binding sites,^{41,42} and the $\alpha 4$ integrin gene promoter has Ets binding sites.⁴⁴ Ets binding sites have also been identified in the promoters of

CD11b, CD11c, and CD18.⁴⁵ PU.1 is a member of the Ets family and regulates SCF/IL-3/IL-6-induced CD34⁺ cell differentiation in mice,⁴⁶ suggesting that PU.1 might be activated in signaling by SCF, IL-3, and IL-6 receptors. Although Ets family members have been shown to regulate cytokine production in mice,^{47,48} whether these factors regulate integrin expression in HMCs is currently unknown. In addition, effects of membrane-bound SCF on mast cell function are greater than effects of soluble SCF,⁴⁹ and engagement of the SCF receptor (CD117) can alter integrin function⁵⁰; therefore both SCF and integrins can work in concert to modulate mast cell adhesion and localization. Future studies are needed to examine the molecular mechanisms by which integrin gene expression and integrin function are regulated during mast cell differentiation *in vitro*.

In addition to examining the function of adhesion molecules on mature CHMC, we determined whether adhesion of these cells to various matrix proteins altered their mediator release responses. It has previously been shown that histamine release from adherent rat basophilic leukemia cells to fibronectin is enhanced 2- to 3-fold.²⁷ It has also been reported that murine bone marrow-derived mast cells can undergo heterotypic aggregation with activated T cells through ICAM-1 and LFA-1 and that this also induces histamine release.⁵¹ In the present studies, adhesion to various substrates had no effect on FcεRI-dependent histamine release or IL-8 production. Although it is possible that other stimuli, such as cytokines, are necessary to facilitate integrin-mediated signaling and alteration of mast cell mediator release, there is as yet no evidence to suggest that such outside-in signaling events occur with human CHMCs. This may therefore also represent differences among cell types and species. Nevertheless, it is clear that mast cells evolve an integrin phenotype, and the timing of this evolution may play an important role during cell migration from the bone marrow through the circulation into tissue sites.

Recent studies have demonstrated a unique committed mast cell precursor lineage that is distinct from all other cell types.^{3,7,52} What remains unknown, however, is at exactly what stage this committed precursor leaves the bone marrow to enter the circulation. Because it is currently believed that these precursors emigrate into tissues where they undergo terminal differentiation into mast cells, the evolution of the adhesion phenotype of these precursors may be extremely relevant to this process. Unfortunately, it is currently impossible to match the CHMC kinetics of integrin expression *in vitro* to trafficking of mast cell precursors into tissues *in vivo*. Given the fact that by 4 to 5 weeks of culture, the CHMC phenotype begins to display mast cell-specific features, it is tempting to speculate that this may represent the earliest stage for such committed precursors to leave the bone marrow and enter the circulation. It is also possible that the immature circulating mast cell progenitor may be a CD34⁺ cell. The notion that these cells, as well as mast cell-committed progenitors but not more mature mast cells, express β2 integrins, raises the possibility that these adhesion

molecules facilitate homing of immature progenitors to tissues by binding to endothelial cell ligands. Finally, the prominent and *de novo* expression and function of vitronectin and laminin receptors, and persistent but reduced expression of fibronectin receptors, strongly suggest that these matrix proteins play an important role in determining where mature mast cells will ultimately reside within tissues. This is consistent with their prominent localization in perivascular tissue sites rich in these proteins. However, until methods become available to identify and isolate mast cell precursors in human peripheral blood, the exact timing and relevance of the CHMC phenotype to that of the circulating mast cell precursor phenotype *in vivo* remains to be determined.

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REFERENCES

1. Kitamura Y, Matsuda H, Hatanaka K. Clonal nature of mast-cell clusters formed in W/W^v mice after bone marrow transplantation. *Nature* 1979;281:154-5.
2. Kitamura Y, Yokoyama M, Matsuda H, Ohno T, Mori KJ. Spleen colony-forming cell as common precursor for tissue mast cells and granulocytes. *Nature* 1981;291:159-60.
3. Rodewald HR, Dessing M, Dvorak AM, Galli SJ. Identification of a committed precursor for the mast cell lineage. *Science* 1996;271:818-22.
4. Agis H, Willheim M, Sperr WR, Wilfing A, Kromer E, Kabrna E, et al. Monocytes do not make mast cells when cultured in the presence of SCF. Characterization of the circulating mast cell progenitor as a c-kit⁺, CD34⁺, Ly⁻, CD14⁻, CD17⁻, colony-forming cell. *J Immunol* 1993;151:4221-7.
5. Copeland NG, Gilbert DJ, Cho BC, Donovan PJ, Jenkins NA, Cosman D, et al. Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. *Cell* 1990;63:175-83.
6. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, et al. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* 1990;63:213-24.
7. Valent P, Spanblochl E, Sperr WR, Sillaber C, Zsebo KM, Agis H, et al. Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor/kit-ligand in long-term culture. *Blood* 1992;80:2237-45.
8. Irani AM, Nilsson G, Miettinen U, Craig SS, Ashman LK, Ishizaka T, et al. Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. *Blood* 1992;80:3009-21.
9. Mitsui H, Furitsu T, Dvorak AM, Irani AM, Schwartz LB, Inagaki N, et al. Development of human mast cells from umbilical cord blood cells by recombinant human and murine c-kit ligand. *Proc Natl Acad Sci U S A* 1993;90:735-9.
10. Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, et al. Selective growth of human mast cells induced by steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. *J Immunol* 1996;157:343-50.
11. Columbo M, Bochner BS, Marone G. Human skin mast cells express functional β1 integrins that mediate adhesion to extracellular matrix proteins. *J Immunol* 1995;154:6058-64.
12. Guo CB, Kagey-Sobotka A, Lichtenstein LM, Bochner BS. Immunophenotyping and functional analysis of purified human uterine mast cells. *Blood* 1992;79:708-12.
13. 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 human basophils. *Ann Hematol* 1992;65:10-6.
14. Dercksen MW, Gerritsen WR, Rodenhuis S, Dirksen MK, Slaper-Cortenbach IC, Schaasberg WP, et al. Expression of adhesion molecules on

- CD34⁺ cells: CD34⁺ L-selectin⁺ cells predict a rapid platelet recovery after peripheral blood stem cell transplantation. *Blood* 1995;85:3313-9.
15. Saeland S, Duvert V, Caux C, Pandrau D, Favre C, Valle A, et al. Distribution of surface-membrane molecules on bone marrow and cord blood CD34⁺ hematopoietic cells. *Exp Hematol* 1992;20:24-33.
16. Shaw S, Luce GG, Gilks WR, Anderson K, Ault K, Bochner BS, et al. Leukocyte differentiation antigen database. In: Schlossman S, Boumsell L, Gilks W, Harlan J, Kishimoto T, Morimoto C, et al, editors. *Leukocyte typing V: white cell differentiation antigens*. Vol 1. New York: Oxford University Press; 1995. p. 16-198.
17. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated mitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J Exp Med* 1999;190:267-80.
18. Shimizu Y, Irani AM, Brown EJ, Ashman LK, Schwartz LB. Human mast cells derived from fetal liver cells cultured with stem cell factor express a functional CD51/CD61 ($\alpha\text{v}\beta 3$) integrin. *Blood* 1995;86:930-9.
19. Ebisawa M, Shichijo M, Miura K, Tachimoto H, Katsunuma T, Onda T, et al. Human mast cells produce TNF- α , MIP- α and IL-5 following activation through Fc ϵ RI [abstract]. *J Allergy Clin Immunol* 1995;95:219.
20. Tachimoto H, Ebisawa M, Iikura Y, Kimata M, Mori K, Sakaguchi N, et al. Activated human mast cells release factors supporting eosinophil survival in vitro. *Int Arch Allergy Immunol* 1997;113:293-4.
21. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;7:283-90.
22. Ishizaka K, Ishizaka T, Lee EH. Biologic function of the Fc fragments of E myeloma protein. *Immunochemistry* 1970;7:687-702.
23. MacGlashan D Jr, McKenzie-White J, Chichester K, Bochner BS, Davis FM, Schroeder JT, et al. In vitro regulation of Fc ϵ RI α expression on human basophils by IgE antibody. *Blood* 1998;91:1633-43.
24. Adkinson NF Jr. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. 2nd ed. Washington (DC): American Society of Microbiology; 1980.
25. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J Clin Invest* 1995;96:549-57.
26. Tachimoto H, Ebisawa M, Hasegawa T, Kashiwabara T, Ra C, Bochner BS, et al. Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN- γ . *J Allergy Clin Immunol* 2000;106:141-9.
27. Hamawy MM, Oliver C, Mergenhagen SE, Siraganian RP. Adherence of rat basophilic leukemia (RBL-2H3) cells to fibronectin-coated surfaces enhances secretion. *J Immunol* 1992;149:615-21.
28. Hamawy MM, Mergenhagen SE, Siraganian RP. Cell adherence to fibronectin and the aggregation of the high affinity immunoglobulin E receptor synergistically regulate tyrosine phosphorylation of 105-115-kDa proteins. *J Biol Chem* 1993;268:5227-33.
29. Kruger-Krasagakes S, Grutzkau A, Krasagakis K, Hoffmann S, Henz BM. Adhesion of human mast cells to extracellular matrix provides a costimulatory signal for cytokine production. *Immunology* 1999;98:253-7.
30. Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: biochemical and functional characterization. *Adv Immunol* 1992;52:333-423.
31. Valent P, Ashman LK, Hinterberger W, Eckersberger F, Majdic O, Lechner K, et al. Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 1989;73:1778-85.
32. Ghannadan M, Baghestanian M, Wimazal F, Eisenmenger M, Latal D, Kargul G, et al. Phenotypic characterization of human skin mast cells by combined staining with toluidine blue and CD antibodies. *J Invest Dermatol* 1998;111:689-95.
33. Wimazal F, Ghannadan M, Muller 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 techniques. *Tissue Antigens* 1999;54:499-507.
34. Rosenkranz AR, Coxon A, Maurer M, Gurish MF, Austen KF, Friend DS, et al. Impaired mast cell development and innate immunity in Mac-1 (CD11b/CD18, CR3)-deficient mice. *J Immunol* 1998;161:6463-7.
35. Toru H, Kinashi T, Ra C, Nonoyama S, Yata J, Nakahata T. Interleukin-4 induces homotypic aggregation of human mast cells by promoting LFA-1/ICAM-1 adhesion molecules. *Blood* 1997;89:3296-302.
36. Zutter MM, Painter AA, Staats WD, Tsung YL. Regulation of $\alpha 2$ integrin gene expression in cells with megakaryocytic features: a common theme of three necessary elements. *Blood* 1995;86:3006-14.
37. Tsuji T, Han SA, Takeuchi K, Takahashi N, Hakomori S, Irimura T. Characterization of mouse integrin $\alpha 3$ subunit gene. *J Biochem (Tokyo)* 1999;125:1183-8.
38. Kim LT, Wu J, Turnage RH. Integrin αv promoter activity in keratinocytes. *J Surg Res* 1998;76:185-91.
39. Rosen GD, Birkenmeier TM, Dean DC. Characterization of the $\alpha 4$ integrin gene promoter. *Proc Natl Acad Sci U S A* 1991;88:4094-8.
40. Oda N, Abe M, Sato Y. ETS-1 converts endothelial cells to the angiogenic phenotype by inducing the expression of matrix metalloproteinases and integrin $\beta 3$. *J Cell Physiol* 1999;178:121-32.
41. Donahue JP, Sugg N, Hawiger J. The integrin αv gene: identification and characterization of the promoter region. *Biochim Biophys Acta* 1994;1219:228-32.
42. Kambe M, Miyamoto Y, Hayashi M. Characterization of the integrin αv gene promoter in mice. *Biochim Biophys Acta* 1998;1395:209-19.
43. Larouche K, Leclerc S, Giasson M, Guerin SL. Multiple nuclear regulatory proteins bind a single cis-acting promoter element to control basal transcription of the human $\alpha 4$ integrin gene in corneal epithelial cells. *DNA Cell Biol* 1996;15:779-92.
44. Rosen GD, Barks JL, Iademaro MF, Fisher RJ, Dean DC. An intricate arrangement of binding sites for the Ets family of transcription factors regulates activity of the $\alpha 4$ integrin gene promoter. *J Biol Chem* 1994;269:15652-60.
45. Lopez-Rodriguez C, Corbi AL. PU.1 negatively regulates the CD11c integrin gene promoter through recognition of the major transcriptional start site. *Eur J Immunol* 1997;27:1843-7.
46. Anderson KL, Smith KA, Connors K, McKercher SR, Maki RA, Torbett BE. Myeloid development is selectively disrupted in PU.1 null mice. *Blood* 1998;91:3702-10.
47. Henkel G, Brown MA. PU.1 and GATA: components of a mast cell-specific interleukin 4 intronic enhancer. *Proc Natl Acad Sci U S A* 1994;91:7737-41.
48. McKinlay LH, Tymms MJ, Thomas RS, Seth A, Hasthorpe S, Hertzog PJ, et al. The role of Ets-1 in mast cell granulocyte-macrophage colony-stimulating factor expression and activation. *J Immunol* 1998;161:4098-105.
49. Shimizu Y, Ashman LK, Du Z, Schwartz LB. Internalization of Kit together with stem cell factor on human fetal liver-derived mast cells: new protein and RNA synthesis are required for reappearance of Kit. *J Immunol* 1996;156:3443-9.
50. Kovach NL, Lin N, Yednock T, Harlan JM, Broudy VC. Stem cell factor modulates avidity of $\alpha 4\beta 1$ and $\alpha 5\beta 1$ integrins expressed on hematopoietic cell lines. *Blood* 1995;85:159-67.
51. Inamura N, Mekori YA, Bhattacharyya SP, Bianchini PJ, Metcalfe DD. Induction and enhancement of Fc ϵ RI-dependent mast cell degranulation following coculture with activated T cells: dependency on ICAM-1- and leukocyte function-associated antigen (LFA)-1-mediated heterotypic aggregation. *J Immunol* 1998;160:4026-33.
52. Yuan Q, Gurish MF, Friend DS, Austen KF, Boyce JA. Generation of a novel stem cell factor-dependent mast cell progenitor. *J Immunol* 1998;161:5143-6.