ADHESION OF MAST CELLS to components of connective tissue is likely to be important in the recruitment of mast cell progenitors from the circulation into tissue and the development, distribution, retention, and survival of mast cells at such sites. Murine and rat mast cells are known to adhere to extracellular matrix components such as laminin (LM),1,3 fibronectin (FN),4,5 and vitronectin (VN).6 Activation of murine mast cells with phorbol myristate acetate (PMA) or FelR1-mediated activation is required for the adhesion or migration of these mast cells to LM-, FN-, and matrigel-coated surfaces.7,8 Activation is not required for murine bone marrow (BM)-derived mast cells to bind VN-conjugated wells. Moreover, stem cell factor (SCF) promotes the MCP5/L mast cell line and murine interleukin-3 (IL-3)—dependent BM-derived mast cells to adhere to FN.2 Rodent mast cells synthesize mRNA encoding the α (CD49d) and β (CD29 and β7) integrin chains; their expression is modulated by IL-3 and SCF.10

Mature human mast cells have also been characterized phenotypically, and integrin molecules CD29, CD49d, CD49e, CD51, and CD61 are expressed on the surface of mast cells dispersed from lung and uterus. Those dispersed from skin express CD49d and CD49e; information on CD29, CD51, and CD61 is not yet available.11,12 However, the functional status of adhesion molecules expressed in human mast cells was not addressed in these studies. As reported previously, when dispersed fetal liver cells are cultured with recombinant human SCF (rhSCF), most of the cells remaining at 4 weeks become mast cells,13 analogous to what happens to cord blood mononuclear cells,14 BM cells, and peripheral blood cells cultured with rhSCF.15 Phenotypic characterization of fetal liver–derived mast cells16 showed that CD18 integrins (CD18/CD11b and CD18/CD11c) and their ligand (CD54) were not expressed after 4 weeks of culture, but expression and functionality of other integrins were not assessed. Because mast cell development and recruitment into tissues may be associated with one another, studies of adhesion molecules expressed during the development of mast cells are of potential biologic importance. In the current study, the expression and functionality of selected integrins on mast cells derived in vitro from fetal liver cells cultured with rhSCF were studied; the CD51/CD61 (αvβ3) VN receptor (VNR) was the predominant integrin expressed and mediated adhesion to VN.

MATERIALS AND METHODS

Chemicals. Histopaque, Dulbecco’s modified Eagle’s medium (DMEM), L-glutamine, nonessential amino acids, HEPES, PIPES, Iscove’s modified Dulbecco’s medium (IMDM), 2-[N-morpholino]ethane-sulfonic acid (MES), controlled process serum replacement-3 (CPSR-3), antibiotic-antimycotic solution, gentamicin, trypsin/EDTA, propidium iodide, 3-hydroxy-2-naphthoic acid 2,4-dimethylbenzene diazonium chloride hemi(zinc chloride) phosphate (fast blue RR), toluidine blue, Arg-Gly-Asp-Ser (RGDS), Arg-Gly-Glu-Ser (RGES), levanosiole, urea, human AB serum, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co (St Louis, MO). RhSCF was a gift from Amgen (Thousand Oaks, CA). VN and FN were a gift from Becton Dickinson Labware (Bedford, MA); LM, from Calbiochem-Novabiochem Corp (La Jolla, CA); heparin-agarose, from Gibco BRL (Gaithersburg, MD); and Sepharose 4B, from Pharmacia Fine Chemicals (Uppsala, Sweden). VN also was purified from human plasma according to the procedure of Yatohgo et al.17

Antibodies. Murine IgG monoclonal antibodies (MoAbs) B5 (anti-CD18, LFA1 β chain), K20 (anti-CD29, VLA β1 integrin), M-KID 2 (anti-CD49e, VLA-3), HP2/1 (anti-CD49d, VLA-4), and 84H10 (anti-CD54, intercellular adhesion molecule [ICAM]) were purchased from AMAC Inc (Westbrook, MA). Murine IgG MoAbs

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HUMAN MAST CELLS EXPRESS CD51/CD61

ClB-thromb/4 (anti-CDw49b, VLA-2), 15A8 (anti-CDw49d, VLA-4), SAM-1 (anti-CDw49e, VLA-5), CLB-M9 (anti-CD51, VNR-a), and ClB-thromb/L17 (anti-CD61, β3 integrin)18 and rat IgG MoAb G0H3 (anti-CD49f, VLA-5) was obtained from Calbiochem-Novabiochem Corp (La Jolla, CA). FITC-conjugated F(ab')2, rabbit-antimouse IgG was purchased from Research Diagnostics Inc (Flanders, NJ). 4B4 (anti-CD29, β1 integrin)MoAb GOH3 (anti-CD49f, VLA-6) were purchased from Research.

FITC-conjugated rabbit-antirat IgG were obtained from Sigma. FITC-conjugated rabbit-antimouse IgG was prepared as described previously.15,19,20 as was the combinatorial P1F6 neutralizing antibody against αβ5.21 Murine antitryptase MoAb (G3) conjugated to alkaline phosphatase was prepared as described previously.22

Cells. Human fetal liver cells, at 17 to 21 weeks of gestational age, were obtained at therapeutic abortions. The experimental protocol was reviewed and approved by the human studies committee at the Medical College of Virginia. Dispersed fetal liver cells were cultured essentially as reported previously.19 Each fetal liver was washed three times with sterile phosphate-buffered saline (PBS) and miniced. Miniced liver cells were filtered over a sterile no. 80 mesh stainless-steel sieve. After centrifugation at 1,000g for 25 minutes over histopaque, dispersed fetal liver cells at the interface were collected, washed in sterile PBS, and resuspended at 2 × 10^7 cells/mL in complete DMEM supplemented with 10% FCS, 2× antibiotic-antimycotic solution, 2 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, 10 mmol/L HEPES, and 50 mmol/L 2-mercaptoethanol. Cell viability after dispersion always exceeded 98%. The cells were plated into 24-well tissue culture plates (Costar, Cambridge, MA) and cultured overnight. Half of the culture medium was replaced with complete DMEM containing 1× antibiotic-antimycotic solution and rhSCF (final concentration, 100 ng/mL). Cells were cultured in a Napco incubator (Napco Inc, Tualatin, OR) at 37°C in 6% CO2. Half of the culture medium was replaced weekly with complete DMEM with the same concentration of rhSCF. The cells used in the following studies were between 23 and 34 days old. Total cell number and viability, respectively, were examined with a hemocytometer and by trypan blue exclusion. Cell viabilities ranged from 64% to 91%.

K562 cells, which express CD29/CD49e, but not CD51/CD61, were provided by Dr Eric Brown. These cells were grown in IMDM containing 10% (vol/vol) FCS, 50 µg/mL of gentamicin, 4 mmol/L L-glutamine, 0.1 mmol/L nonessential amino acids, and 10 mmol/L HEPES. Culture media was changed three times per week.

Fluorescein-activated cell sorting (FACS) analysis. Expression of surface antigens was examined by flow cytometry. Fetal liver cells were first incubated in DMEM containing 10% human AB serum for 30 minutes at 4°C to block nonspecific Fc receptor binding of MoAb. After centrifugation, cells were incubated with each primary antibody for 30 minutes at 4°C. These cells were washed three times with PBS containing 1% (vol/vol) BSA and 0.1% (vol/vol) sodium azide (wash buffer), and incubated with FITC-conjugated F(ab')2 rabbit-antimouse IgG or FITC-conjugated rabbit-antirat IgG (for G0H3 primary antibody) for 30 minutes at 4°C. Labeled cells were washed as above, resuspended in PBS containing 1 mmol/L EDTA and 10 mmol/L lithium chloride and subjected to flow cytometry with a FACScan (Becton Dickinson, San Jose, CA). Before the analysis, propidium iodide was added to the cell suspension to determine the number of dead cells and exclude them from the analysis. Histograms showing FITC fluorescence intensity and relative cell number were constructed with the CyCLOPS program (Cytomation Inc, Fort Collins, CO).

Adhesion assays. Adhesion assays were performed in Cowaline microtiter wells (Nunc, Naperville, IL). Wells were first activated with Bis(sulfosuccinimidyl) suberate (BS3) in PBS for 30 minutes at room temperature, washed with PBS, and incubated with either BSA (30 mg/mL) as a negative control, or VN (0.05, 0.5, 5, and 50 µg/mL), FN (5 µg/mL), or LM (5 µg/mL), each in 50 µL PBS for 2 hours at 37°C. Wells were washed and blocked with 3% (wt/vol) BSA in PBS at 37°C for 40 minutes. Each well was washed three times with serum-free PIPES buffer (25 mmol/L PIPES, 130 mmol/L NaCl, 5 mmol/L KCl, 5.6 mmol/L glucose, 1 mmol/L CaCl2, and 0.1% [wt/vol] BSA, pH 7.4), and 100 µL of the same buffer was added. Fetal liver-derived cells were washed three times with PBS and resuspended in PIPES buffer at 1 × 10^6 cells/mL. A 100-µL aliquot of this suspension was added to each well and incubated for various time intervals at 37°C. Wells were washed three times with Hanks' balanced salt solution to remove nonadherent cells followed by the addition of 100 µL of trypsin (5 mg/mL), 5.4 mmol/L EDTA for 10 minutes at 37°C to recover adherent cells. Adherent cells were washed twice with PBS and subjected either to flow cytometry or cytocentrifugation. The numbers of both adherent and nonadherent cells were determined using a Sysmex microcell counter (Tokyo, Japan). The percentage of adherent cells was determined as follows:

Net % Adhesion =  

\[
\frac{\text{VN Adherent} - \text{BSA Adherent}}{\text{VN Adherent} + \text{VN Nonadherent} - \text{BSA Adherent}} \times 100.
\]

Inhibition of adhesion. Various compounds were assessed for their ability to inhibit adhesion. Dependence on the RGD amino acid sequence motif was assessed by incubation of fetal liver cells in the presence of 0 to 1,000 µg/mL (PIPES buffer) of the synthetic peptides RGDS or RGES in VN- and BSA-conjugated wells for 40 minutes at 37°C. To examine the effects of Ca^2+ and Mg^2+, cell incubations were performed in PIPES buffer (100 µL) containing various concentrations of either Ca^2+ or Mg^2+. Negative control wells were conjugated with BSA. Other control wells were incubated with 10 mmol/L EDTA or EGTA in the absence of exogenous divalent cations.

Rabbit polyclonal IgG anti-CD51/CD61 was tested at 1:10 to 1:1,000 dilutions by incubation with fetal liver cells for 30 minutes at 37°C. A concentration known to inhibit the adhesion of B lymphocytes to VN and FN.23 As a control, irrelevant rabbit IgG was tested, but net % adhesion was not significantly different than buffer alone. In other studies using neutralizing or combinatorial antibodies, fetal liver cells were preincubated with antibodies for 30 minutes at 37°C, then added into VN-, FN-, LM- or BSA-conjugated wells. Controls were incubated with isotype-matched nonimmune mouse IgG. The concentrations of the antibodies used in the inhibition studies were at least 10 times as high as those used for FACS analysis after titration: 4B4 (anti-CD29, 1:40 dilution), HP2/1 (anti-CD49d, 20 µg/mL), SAM-1 (anti-CD49e, 20 µg/mL), CLB-M9 (anti-CD51, 20 µg/mL), CLB-thromb/1 (anti-CD61, 20 µg/mL), and P1F6 (anti-55/ CD51, 40 µg/mL). 4B4,24-26 HP2/1,27 SAM-1,28 CLB-M9,4 CLB- thromb/1,3 and P1F6 have been shown to have neutralizing activity. The percent inhibition of adhesion was calculated as follows:

\[
\% \text{ Inhibition of Adhesion} = \frac{\text{Net % Adhesion With Receptor Antibody} - \text{Net % Adhesion With Irrelevant Antibody}}{\text{Net % Adhesion With Irrelevant Antibody}} \times 100.
\]

For the adhesion assay using K562 cells, the cells were washed and
three times with IMDM supplemented with 10 mmol/L HEPES and 0.1% BSA, activated with 10 ng/mL of PMA for 10 minutes at 37°C, and incubated either without antibody or with irrelevant rabbit IgG (20 μg/mL), irrelevant mouse IgG (20 μg/mL), rabbit anti-CD51/CD61 antibody (1:40 dilution), or mouse anti-CD29 antibody (1:40 dilution) for 30 minutes.

To examine whether the adhesion of mast cells was selectively affected, wells were washed to remove nonadherent cells from either FN- or LM-conjugated wells, and microtiter plates were centrifuged at 400g for 1 minute followed by the fixation of the adherent cells with 100 μL of methanol for 10 minutes. G3-alkaline phosphatase (40 μL, 1 μg/mL) was added to each well, and the adherent cells were incubated overnight at 4°C. Wells were washed three times with TRIS-buffered saline containing 0.05% (vol/vol) Tween 20, pH 7.4 (TTBS), and incubated with 1 mg/mL of fast blue RR containing levamisole (0.24 mg/mL) and naphthol AS-MX phosphate acid. At least 100 cells were counted in each well, and the percentages of tryptase-positive cells were calculated.

Immunocytochemistry. To determine the portion of fetal liver cells that were mast cells, cytocentrifuge preparations were subjected to immunohistochemistry as described previously, using G3-alkaline phosphatase. Cytocentrifuge preparations were also stained with 0.5% (wt/vol) toluidine blue in 0.5 N HCl overnight to detect cells with metachromatically staining granules. Approximately 70% of fetal liver cells were labeled with G3 and stained metachromatically with toluidine blue after 4 weeks of culture with rhSCF.

Adhesion-mediated mast cell degranulation. To determine whether adhesion of mast cells to VN causes degranulation, the amount of released and retained tryptase was measured by enzyme-linked immunosorbent assay (ELISA) as reported previously. After performing the adhesion assay on VN- and BSA-conjugated wells, nonadherent cells were collected and centrifuged at 200g for 200 minutes at 4°C and the supernatant was removed immediately. Nonadherent and adherent cells were sonicated separately in 0.01 moll L MES (pH 6.5) containing 1 mol/L NaCl. Cells were sonicated with 10 pulses, 50% pulse cycle, power 4 with a sonicator (Ultrasonics, Inc, Plainview, NY). No adherent cells remained intact after sonication by inverted microscope. % release of tryptase was calculated as follows:

% Tryptase Release =

\[
\frac{\text{Supernatant Tryptase}}{\text{Pellet Tryptase} + \text{Supernatant Tryptase}} \times 100.
\]

RESULTS

Surface antigen expression of fetal liver cells during the development of mast cells. To determine whether developing mast cells express integrins, fetal liver cells cultured with rhSCF were examined by flow cytometry on days 0 and 28 for expression of CD29, CD18, CD61 (β3), and β5 integrins along with kit. Although no mast cells were detected on day 0, by day 28, 67% of the cells were tryptase-positive and 68% were metachromatic when stained with toluidine blue, these being two criteria used to identify mast cells. As shown in Fig 1, each histogram includes an isotype-matched negative control. At day 0, less than 20% of the cells were positive for kit and CD49c, and less than 1% of the cells showed detectable levels of the CD61 integrin composed of CD51 (αv) and CD61, which is the classical (though not the only) VN receptor. In contrast, at 4 weeks, 73% of the cells were strongly positive for CD51, 72% for CD61, and 75% for kit. The expression of CD51 was first detected on day 17 (data not shown), at about the same time that tryptase-positive mast cells appear. The β5 integrin, CD51/β5, was negative at day 0, and weakly expressed in less than 20% of the cells by day 28. CD29, the β subunit of β1 integrins, was positive in nearly all cells at day 0, presumably associated mostly with the α subunits CD49d, which also was positive in nearly all cells, and CD49c, which was weakly expressed in about 45% of the cells. By day 28, both CD49d and CD49e were strongly detected in essentially all cells, whereas CD49b, CD49c, and CD49f were nearly negative. Although CD18 (β2) and CD54 (ICAM-1), respectively, were detected in 59% and 50% of the cells at day 0, and less than 10% at day 28, the mean fluorescence intensities were minimally elevated above controls. Because expression of CD51/CD61 appeared to parallel the development of mast cells, further experiments focused on this receptor along with CD29/CD49d, CD29/CD49e, and CD51/β5.

Adhesion of fetal liver–derived mast cells to VN. To determine whether the CD51/CD61 integrin on fetal liver–derived mast cells was functional, adhesion of these cells to VN was investigated. As shown in Fig 2A, net percent adhesion was relatively constant over this range of cell concentrations. Further experiments were performed at 5 × 104 cells/mL. In Fig 2B, the optimal concentration of VN for conjugation to Covalink wells, determined based on adhesion was examined over a concentration range from 0.05 μg/mL to 50 μg/mL. Maximal net percent adhesion was reached at a VN concentration of 5 μg/mL, the concentration chosen for further experiments. The time course of percent adhesion of fetal liver cells cultured with rhSCF for 4 weeks to VN- or BSA-conjugated wells is shown in Fig 2C. The percent adhesion increased rapidly during the first 30 minutes of incubation, and gradually thereafter. However, nonspecific adhesion to BSA began to increase after 60 minutes. Therefore, an incubation period of 40 to 60 minutes was chosen for most adhesion assays to limit nonspecific adhesion. No adhesion to VN occurred when fetal liver cells at 4 weeks of culture with rhSCF were incubated in VN-conjugated wells at 4°C. Moreover, all of the adherent cells could be removed after 2-hour incubation at 4°C (data not shown). VN-adherent fetal liver–derived cells, observed by phase contrast microscopy, appear to spread, whereas those few cells observed in association with BSA were rounded (data not shown).

Selective adhesion of fetal liver–derived mast cells to VN. To evaluate what portion of adherent fetal liver cells were mast cells, adherent cells were procured and analyzed as shown in Fig 3. The percentage of tryptase-positive cells in cytocentrifuge preparations also increased. In three separate experiments, mast cell percentages increased from 63, 66, and 68 to 96, 97, and 99, respectively. The predominantly uniphasic patterns seen in Fig 3 for these surface markers suggest that nearly all of these cells express them, but exhibit some overlap with negative controls. Thus, mast cells are the predominant cell type that adhere to VN in the 4-week-old fetal liver cells under study. The percentage of these
cells expressing surface CD51, CD61, and kit increased from a range of 72% to 75% before adhesion to 83% to 92% after adhesion. Whether all of the mast cells were capable of adhesion was examined by performing sequential adhesion experiments. Nonadherent cells were sequentially removed and added into unused VN-conjugated wells three times. In three separate experiments, the percentages on nonadherent cells that were tryptase-positive were 3%, 3%, and 17%. Thus, a small portion of these mast cells may not possess VN receptors in a functional state. It is possible that activation of these cells might also activate these receptors.

Characteristics of fetal liver-derived mast cell adhesion to VN. The dependence of adhesion on Ca\textsuperscript{2+} and Mg\textsuperscript{2+} was assessed. As shown in Fig 4, both calcium and magnesium facilitated specific adhesion of fetal liver-derived mast cells to VN, values being slightly higher at 5 and 10 mmol/L than at 1 and 2 mmol/L. Not shown in Fig 4 is an increase in percent adhesion (nonspecific) to BSA-conjugated wells with Mg\textsuperscript{2+} (9.3% at 10 mmol/L MgCl\textsubscript{2}), but not Ca\textsuperscript{2+} (0% at 10 mmol/L CaCl\textsubscript{2}). In the presence of 10 mmol/L EDTA and 10 mmol/L EGTA, respectively, without added calcium or magnesium, net percent adhesion to VN was negligible, indicating that specific cell adhesion to VN is dependent on divalent cations.

Regions of FN, collagen, angiogenin, fibrinogen, and von Willebrand factor containing the Arg-Gly-Asp (RGD) sequence are recognized by a variety of cellular adhesion molecules. To examine whether the adhesion of fetal liver-derived mast cells to VN was RGD-dependent, inhibition of adhesion to VN by the synthetic peptides, RGDS and RGES, was evaluated over a concentration range of 0 to 1,000 µg/mL. As shown in Fig 5, RGDS inhibited net percent adhesion in a dose-dependent manner, whereas RGES, in which glutamic acid is substituted for aspartic acid, failed to inhibit adhesion. Adhesion was reduced from 25% to 5% by addition of 1,000 µg/mL of RGDS. In contrast, neither soluble VN nor rabbit IgG antihuman VN antibody inhibited adhesion of the cells to VN-conjugated wells (data not shown). Thus, an RGD-sequence in immobilized VN that apparently was not blocked by the anti-VN antibody used is involved in the binding of fetal liver cells to VN.

To directly determine whether the adhesion was mediated
for 30 minutes at room temperature and added into FN-conjugated wells. Adhesion was not inhibited in the presence of either irrelevant rabbit IgG, irrelevant mouse IgG, or rabbit anti-CD51/CD61, whereas complete inhibition occurred with mouse anti-CD29 antibody, suggesting that rabbit anti-CD51/CD61 antibody does not cross react with CD29/CD49e. Whether this antibody might cross react with a receptor that has not been recognized in the current study cannot be excluded.

Several other antibodies with reported blocking activities for CD29, CD49d, CD49e (these three antibodies were used alone and in combinations of α- and β-specific antibodies), and CD51/β5 (the antibody recognizes a combinatorial epitope) did not block fetal liver–derived mast cell adhesion to VN-conjugated wells (Fig 6B). In some cases, with antibodies against β1 integrins, adhesion appeared to increase slightly, suggesting that CD29, CD49d, and CD49e do not directly mediate adhesion, but when complexed with antibody, may augment adhesion to VN. Thus, the CD51/CD61 integrin appears to be the major molecule responsible for adhesion of fetal liver–derived mast cells to VN.

Comparison of adhesion of fetal liver–derived mast cells to VN, FN, and LM. It was reported previously that adherence of murine mast cells to laminin and fibronectin was not a constitutive property of these cells, but required them to

through the CD51/CD61 receptor, inhibition of adhesion by polyclonal rabbit IgG antihuman CD51/CD61 antibody was assessed. As seen in Fig 6A, the adhesion of fetal liver–derived mast cells to VN was reduced by the rabbit-antihuman CD51/CD61 antibody from 30% to 12% at an antibody dilution of 1:100 (mean percent inhibition of adhesion, 60 ± 1), suggesting that most of the adhesion occurs via CD51/CD61. Irrelevant rabbit IgG did not block the adhesion of fetal liver–derived mast cells to VN-conjugated wells. Interestingly, mouse MoAbs against CD51 and CD61, either alone or together, did not inhibit adhesion (Fig 6B). To confirm that the polyclonal rabbit IgG antihuman CD51/CD61 antibody does not block CD29/CD49e, adhesion of PMA-activated K562 cells to FN was examined. K562 cells, which bind FN through CD29/CD49e, were incubated either without antibodies or with irrelevant rabbit IgG, irrelevant mouse IgG, rabbit anti-CD51/CD61, or mouse anti-CD29

![Fig 2](http://www.bloodjournal.org/content/fig/2.png) Adhesion of fetal liver cells to VN after 4 weeks in culture with rhSCF. (A) Fetal liver cells cultured with rhSCF were washed, resuspended in PIPES buffer, and added into VN (5 μg/mL)- and BSA (30 mg/mL)-conjugated wells at final concentrations of 5 x 10⁴ to 1 x 10⁵ cells/mL (1 x 10⁴ to 2 x 10⁵ cells/well). Cells were incubated for 60 minutes at 37°C, and net percent adhesion was calculated as described in Materials and Methods (n = 3). (B) Wells were conjugated with 50 μL of from 0.05 to 50 μg/mL of VN and BSA. Net percent adhesion of fetal liver cells obtained after 4 weeks of culture with rhSCF was then evaluated (n = 3). Cells (1 x 10⁵/well) were incubated for 60 minutes at 37°C. The percent adhesion of 4-week-old fetal liver cells obtained as above to VN (5 μg/mL) compared with BSA (30 mg/mL) is shown over a 2-hour time course in C. (A through C) Each data point shows the mean and SD.

![Fig 3](http://www.bloodjournal.org/content/fig/3.png) Mast cells are the major cell type in 4-week-old fetal liver cells that adhere to VN. Aliquots of fetal liver cells that had been cultured with rhSCF for 4 weeks were placed into six VN-conjugated microtiter wells at 1 x 10⁵ cells/well and incubated for 40 minutes. Adherent cells were removed by incubation with trypsin/EDTA and washed with PBS. Total cells and adherent cells were analyzed for surface expression of CD51, CD61, or kit by flow cytometry as shown in the upper, middle, and lower panels, respectively. Data shown are representative of two independent experiments.
HUMAN MAST CELLS EXPRESS CD51/CD61

Evaluation of adhesion-dependent degranulation of fetal liver–derived mast cells.

Whether adhesion of mast cells to VN alone causes degranulation was determined. After permitting cells to adhere to VN- or BSA-conjugated wells, nonadherent cells were removed and centrifuged at 4°C to separate supernatant from nonadherent cells. Tryptase in adherent cells and nonadherent cells was extracted by sonication in high salt, and measured in the cell extracts and supernatants by ELISA. The net percent adhesion (mean ± SD) was 21 ± 1, and the cells were 68% ± 13% (mean ± SD, n = 3) viable. The percent tryptase release from VN-adherent fetal liver–derived mast cells was 7.8% ± 7.8% (mean ± SD), and from BSA-adherent cells was 6.8% ± 5.1%. No significant increase in percent release of tryptase from VN-adherent cells compared with controls was observed. Thus, adhesion of fetal liver–derived mast cells to VN does not appear to cause degranulation.

DISCUSSION

Adhesion molecules in developing mast cells are likely to play important roles as these cells migrate from the circulation to peripheral tissues, where they complete their maturation and establish residence. This study shows that rhSCF-dependent human mast cells derived in vitro from fetal liver spontaneously adhere to VN through the CD51/CD61 integrin. Expression of integrins on the cell surface of tissue-derived mast cells, and fetal liver–derived mast cells...
Fig 6. Receptor-mediated adhesion of fetal liver–derived mast cells to VN-conjugated wells. Cell suspension was incubated with serial concentrations of antibody for 30 minutes at 37°C before the assay. Polyclonal rabbit IgG anti-VNR inhibited the adhesion in a dose-dependent manner (A). However, other neutralizing antibodies against CD29 (4B4), CD49d (HP2/1), CD49e (SAM-1), CD51, and CD61 and combinations of α and β chain antibodies did not inhibit net percent adhesion (B). The combinatorial antibody against CD51/β5 did not inhibit net percent adhesion either. Error bars show the range for two experiments, each performed in duplicate.

cultured with rhSCF has previously been investigated by flow cytometry alone. Tissue-derived mast cells, like those derived in vitro from fetal liver, express CD29 integrins CD29/CD49d and CD29/CD49e and the CD61 integrin CD51/CD61 (VNR). The present data show that CD29 and CD49d are strongly expressed on the fetal liver cells at the time of cell dispersion, whereas CD49e, CD51, and CD61 expression increase over time while the cells are cultured with rhSCF. By 4 weeks, ~70% of the cells are mast cells, and essentially all of the mast cells express CD51 and CD61 (Figs 1 and 3).

Because expression of integrins alone does not indicate they are functional, adhesion of fetal liver–derived human mast cells to VN, FN, and LM was assessed. Previous studies with murine mast cells showed that activation of the cells was required for adhesion to either LM or FN, but not for adhesion to VN. Our data showed that the capacity of human fetal liver–derived mast cells to adhere to VN, FN, and LM was constitutive (Figs 2, 6, and 7), occurring in part through the CD51/CD61 receptor, and did not require the cells to be additionally activated. Microvascular endothelial cells were previously reported to attach to laminin through both CD29 and CD61 receptors. CD51/CD61 reportedly mediates adhesion of lymphocytes to vitronectin, fibronectin, and laminin, whereas purified CD51/CD61 binds fibronectin directly. The complicated asymmetric cross-structure of laminin facilitates its recognition through a diversity of receptors. The extended adhesion specificity observed for CD51/CD61 on human mast cells may differ from the more selective specificity observed for the corresponding receptor on rodent mast cells. It also is unlikely that the CD51/CD29 receptor accounts for the adhesion of human fetal liver–derived mast cells to VN, because binding of small cell lung cancer cells to FN has been reported for this integrin far better than to LM and VN. Adhesion of the cells to FN was not observed on day 0 even though CD29/CD49d, which is recognized as a FN receptor, was expressed. Moreover, adhesion of fetal liver–derived mast cells to VN, FN, and
LM was not inhibited by antibodies against CD29/CD49d and CD29/CD49e, suggesting that another stimulus might be required for these receptors to be fully functional.

SCF, necessary for human mast cells to survive in culture, may enhance the activity of certain integrins, a process somewhat analogous to the ability of SCF to induce murine mast cells to adhere to FN.5,29 SCF also may modulate integrin receptor expression; in mouse BM–derived mast cells, SCF increases CD49d mRNA, decreases β1 mRNA, and leaves CD29 mRNA levels unaltered.60 Binding of mouse hematopoietic stem cells to stromal cell–derived extracellular matrix components may be dependent on CD29/CD49d, because neutralizing this receptor with antibody blocks hematopoiesis in vivo.41 Of potential biologic interest in the present study was the observation that the adhesion of fetal liver–derived mast cells to VN (but not to LM and FN) appeared to increase when the cells were incubated with anti-CD49d antibody, which previously was shown to have neutralizing activity.29 These results suggest that cross-linking CD49d stimulates adherence by other adhesion molecules, perhaps by CD51/CD61.

As anticipated, adhesion of fetal liver–derived human mast cells to VN was dependent on divalent cations (Fig 4) and an RGD motif (Figure 5). The CD51 α chain of the VN receptor is known to recognize an RGD region in both VN and FN.30,31,34 Also, the α subunits of integrin receptors in general contain three or four calcium binding regions.42 The critical role of calcium for the VN receptor was shown previously by showing that calcium was required for the receptor to associate with the RGD peptide, and that reconstitution of EDTA-treated receptor activity occurred with calcium, but not with magnesium.43 In the current study, magnesium increased nonspecific adhesion, and therefore was not used in our standard adhesion assay.

 VN is an α1-glycoprotein synthesized in the liver and distributed to tissues from the blood where it was first recognized as a serum spreading factor.44,45 Unlike fibrinogen and FN, VN is not involved in blood clotting.46,47 Current data indicate that fetal liver–derived mast cells do not interact with soluble VN, suggesting that VN in the circulation should not interfere with the capacity of developing mast cells to adhere to VN in tissues. VN is deposited in the loose connective tissue of embryonal lung, smooth and skeletal muscle, and kidney and is associated with dermal elastic fibers in skin,48 renal tissue,48 and the vascular wall.49 The distribution of VN at tissue sites that are both rich (dermis) and poor (kidney) in mast cells suggests that VN is not the only factor involved in the tissue distribution of this cell type. For example, the availability of SCF in tissues appears to be necessary for mast cell survival in primates50 and rodents,31,52 for mast cell development at tissue sites53 and possibly for the chemotaxis54,55 and retention56 of mast cells.

CD61 integrins appear to be phosphorylated when host cells are treated with PMA (osteosarcoma cells or fibroblasts)27 and when CD29/CD49d is cross-linked with either MoAb or natural ligands (VCAM-1 and FN) (pre-B or mature B cells).58 Thus, anti-CD49d antibody may have increased adhesion to VN in the current study by activating CD51/CD61 receptors that had not yet become functional. Moreover, adhesion of fetal liver–derived mast cells to VN did not activate these mast cells to degranulate, as measured by the release of tryptase. That adhesion to VN might modulate the mast cell response to IgE–mediated activation, a process analogous to what happens with rodent mast cells bound to FN,55,59 is still a consideration, but cannot be tested with the fetal liver–derived human mast cells that lack high-affinity IgE receptors.

In conclusion, human mast cells express functional CD51/CD61 integrin receptors during SCF–mediated development, permitting adherence to VN and other components of connective tissue. This may affect the recruitment, distribution, and retention of mast cells at specific tissue sites.

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Human mast cells derived from fetal liver cells cultured with stem cell factor express a functional CD51/CD61 (alpha v beta 3) integrin

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