Expression of Integrins and Examination of Their Adhesive Function in Normal and Leukemic Hematopoietic Cells

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Adhesion of hematopoietic progenitor cells to marrow-derived adherent cells has been noted for erythroid, myeloid, and lymphoid precursors. In this report, we have characterized very late antigen (VLA) integrin expression on normal CD34+ marrow progenitors, on leukemic cell lines, and on blasts from patients with acute myelogenous or monocytic leukemias. CD34+ progenitor cells expressed the integrin β1 chain (CD29), VLA-4α (CD49d), and VLA-5α (CD49e). The myeloid lines KG1 and KG1a also expressed CD49d and CD49e as did the Mo7e megakaryoblastic line. CD29, CD18, and CD11a were also present on each of these cell lines. Only the Mo7e line expressed the cytocomponents GPIbα or GPIb. Binding of KG1a to marrow stroma was partially inhibited by antibodies to CD49d and its ligand, vascular cell adhesion molecule (VCAM-1). The majority of leukemic blasts studied expressed CD49d and CD49e as well. Blasts from patients with acute myelomonocytic leukemia consistently bound to stroma at levels greater than 20%, and adhesion to stroma could in some cases be partly inhibited by anti-CD49d. No role for glycosylphosphatidylinositol (GPI)-linked structures was demonstrated in these binding assays because the adhesion of leukemic blasts to stroma was not diminished after treatment with phosphatidylinositol-specific phospholipase C (PI-PLC). These studies indicate that CD34+ myeloid progenitors, myeloid leukemic cell lines, and leukemic blasts possess a similar array of VLA integrins. Their functional importance individually or in combination with other mediators of attachment, transendothelial migration, and differentiation has yet to be fully elucidated. © 1993 by The American Society of Hematology.
MATERIALS AND METHODS

Cell Separation and Culture

Marrow CD34+ myeloid precursors. A sterile two-step flow cytometric technique used to isolate CD34+ marrow cells has been described previously. BM aspirates were obtained from normal volunteer donors in accordance with institutional guidelines of the Research Subjects Review Board of the University of Rochester. Light-density marrow cells per sample were stained with a 1:20 dilution of fluorescein isothiocyanate (FITC)-conjugated HPCA-1, an antibody that recognizes the CD34 antigen (Becton Dickinson, Mountain View, CA), and with phycoerythrin (PE)-conjugated anti-CD10 (CAL/IO monoclonal antibody; Coulter, Hialeah, FL). One to 5% of cells were positive for CD34 and negative for CD10. Cells were then sorted aspetically using an EPICS C flow cytometer (Coulter). An initial sort performed at high speed resulted in a population that was 60% to 70% CD34+. A second slow-speed sort resulted in a 96% to 99% pure CD34+ population. These cells, which were 95% viable at the conclusion of the second sort, constituted the purified CD34+ myeloid precursors subsequently used in binding assays and indirect immunofluorescence phenotyping studies. At the completion of the sort, centrifugation was performed to remove sorting sheath fluid, and the cells were resuspended in RPMI medium with 20% fetal bovine serum (FBS) for overnight storage at 37°C if further studies could not be conducted soon after sorting conclusion.

In certain experiments, CD34+ cells were isolated by an avidin-biotin column adsorption technique (CellPro, Inc, Bothell, WA). Cells lines. The leukemic cell lines KG1, KG1a, and M07e were obtained from Dr D.W. Golde (Memorial Sloan Kettering, Rye, NY). KG1 and KG1a were maintained in RPMI-1640 (GIBCO, Grand Island, NY) culture medium with 10% FBS (HyClone, Logan, UT). M07e is an interleukin-3 (IL-3)-dependent cell line and was maintained in Iscove Modified Dulbecco Medium (IMDM) (GIBCO) with 20% FBS and 50 pmol/L Idox (kindly provided by Dr. S. Clark, Genetics Institute, Cambridge, MA).

Leukemic patient samples. Marrow or blood obtained with informed consent was layered over Ficoll-Hypaque (specific gravity 1.077; Pharmacia, Piscataway, NJ). Light-density interface cells were washed twice and resuspended in RPMI with 10% FBS. Samples were used in binding assays only if they had greater than 80% blasts.

Stromal cell layers. Marrow stromal cell layers were established from BM aspirates from normal volunteers and were cultured and passaged as previously described. Briefly, light-density BM cells were cultured in McCoy's SA culture medium (GIBCO) supplemented with 12.5% FBS, 12.5% horse serum (Hyclone), and 1 μg/mL hydrocortisone (Sigma Chemical Co, St. Louis, MO). These adherent cell layers were used in binding assays only after passage of more than three times. At final passage, the adherent layers were seeded into either Falcon 24-well plates (Becton Dickinson, Lincoln Park, NJ) for cell line chromium-binding studies or into 35-mm tissue culture plates (Corning, Corning, NY) for CD34+ progenitor cell adhesion assays. The adherent layers were allowed 5 to 7 days of incubation after the final passage to ensure adequate confluent matrix formation for cell-binding assays.

Antibodies and Reagents

Antibodies and additional reagents were obtained from Becton Dickinson Immunocytometry Systems, Mountain View, CA; (HPCA-1 [CD34, IgG1] and FITC-conjugated goat antirabbit isotype control for IgG1), Coulter Immunology, Hialeah, FL; (CD29 [VLA-6 chain, IgG1], CD10PE, and goat antianti mouse isotype controls for IgG1, IgG2a, and IgG3), Fisher Scientific, Orangeburg, NJ; (FITC-conjugated isotype specific goat antimouse IgG1, PE-conjugated isotype specific goat antimouse IgG2b and IgG3), Telios Pharmaceuticals, San Diego, CA; (CD49b [VLA-2 chain, IgG1], CD49c [VLA-3 chain, IgG1], and CD49e [VLA-5 chain, IgG3]), AMAC, Westbrook, ME; (CD49d [VLA-4 chain, IgG1], CD49f [VLA-6 chain, IgG2a], CD61 [GPIIIa or β3, IgG1], and CD34 FITC conjugate), Tago, Burlingame, CA; (FITC-conjugated F(ab')2 goat anti-mouse Ig, and PE-conjugated F(ab')2 goat anti-mouse Ig). Antibodies L1 (anti-LFA-1, α chain, CD1 Ia), 44 alpha (anti-Mo10-chain, CD11b), L29 (anti-p150,95 α chain, CD11c), and 10F12 (anti-β2-chain, CD18) were gifts of Dr. A. Arnaout (Massachusetts General Hospital, Boston, MA). Antibody 12.8, an IgM molecule recognizing CD34, was a gift of Dr. R. Berenson, Seattle, WA. Anti-VCAM (4B9, IgG1) was a gift from Dr J. Harlan (Harborview Medical Center, Seattle, WA). For diagnostic immunophenotyping of leukemic blasts, anti-CD4, anti-CD19, anti-CD33, and anti-CD34 were obtained from Coulter. Antibodies AP1 (anti-GPi), AP2 (anti-GPiIIla), AP4 (anti-GPiIIb), and AP5 (anti-GPiIa) were gifts of Dr T.J. Kunicki (The Blood Center of Southeastern Wisconsin, Milwaukee). Phosphatidylinositol-specific phospholipase C (PI-PLC) was purchased from ICN Biomedicals (Costa Mesa, CA). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 were gifts of Dr S. Clark.

Adhesion Protein Expression

Cell lines were stained for indirect immunofluorescence by incubating with antibody to the adhesion protein at 4°C for 30 minutes followed by three washes in PBS. Cells were then incubated at 4°C for 30 minutes with FITC-conjugated goat anti-mouse Ig. After the final wash, cells were resuspended in 1% paraformaldehyde in PBS at 4°C for flow cytometry analysis. Stained cells were analyzed on an EPICS C flow cytometer. The mean log green fluorescence channel of the cell lines was determined directly from a single parameter histogram. The mean log fluorescence channel was converted to linear equivalents as previously described. In order to estimate the fluorescence intensity specifically due to the test adhesion protein antibody, the mean fluorescence intensity of cells stained with isotype-specific control antibody was subtracted from that of cells stained with the adhesion protein antibody to obtain the final mean fluorescence intensity measurement. To ensure that only specific receptor expression was evaluated, only fluorescence measurements ≥ 5 were defined as positive after subtraction of background staining.

For phenotyping of adhesion proteins on normal CD34+ myeloid progenitors, two-color immunofluorescence was used with light-density marrow cells stained with an adhesion marker versus CD34. 2 × 10⁹-light-density bone marrow cells were incubated with 12.8 anti-CD34 and unconjugated test antibody for 30 minutes at 4°C in PBS plus 20% human AB serum and then washed twice with cold PBS. The cells were then incubated with FITC-conjugated goat anti-mouse IgG specific for the isotype of the unconjugated "test" antibody used and with goat antimouse IgM PE. After staining, cells were resuspended in 1% paraformaldehyde in PBS and kept at 4°C for flow cytometry analysis. Stained cells were analyzed on an EPICS Profile flow cytometer (Coulter) using standard light scatter gates for mononuclear cells to obtain a two-parameter histogram of log green fluorescence (FITC) versus log red fluorescence (PE).

Binding Assays

Chromium binding assay. Binding experiments with cell lines were performed as previously described with minor modifications. KG1, KG1a, or M07e cells (5 × 10⁶) were labeled with 100 μCi ⁵¹Cr (Amersham, Arlington Heights, IL) for 30 minutes at room temperature. Cells were then washed three times in more than 10 vol of PBS. Cells were then resuspended in RPMI-1640 medium with 10% FBS at a concentration of 1.0 × 10⁶ cells/mL. One-half milliliter (5 × 10⁵ cells) were plated over the stromal layer or plastic-coated 24-well tissue culture plate for 2 hours at 37°C. The medium and non-
adherent cells were aspirated and wells washed twice with PBS. The initial aspirated medium and washes were pooled. The adherent layers or plastic wells with bound cells were then treated with 0.05% trypsin–0.5 mmol/L EDTA, (GIBCO) for 20 minutes. The entire adherent contents were then aspirated and counted in a gamma scintillation counter (Packard, Meriden, CT). The entire adherent layer cell counts were calculated as a percentage of total recovered counts and represented the percentage of plated cells that bound to the surface (stromal layer or plastic) under study.

 Colony-forming unit (CFU) assay. Binding experiments using purified CD34+ cells from normal marrow were performed as previously described10 with minor modification. Briefly, CD34+ cells (5 × 103) in 1 mL RPMI-1640 medium with 10% FBS were plated over stromal cell-coated or plastic-coated 35-mm tissue culture plates. After a 2-hour incubation at 37°C, the medium and nonadherent cells were aspirated and the plates washed twice with PBS. The plates were then overlaid with 1 mL of a 0.5% agar mixture containing Iscove’s medium (GIBCO), 10% bovine Serum albumin (fraction V; Sigma Chemical Co), 30% FBS, and 10% Mo cell-conditioned medium (CM) and 5% GCT-CM as sources of hematopoietic growth factors. Granulocyte-macrophage colony-forming unit (CFU-GM) colonies (>20 cells) were scored after incubation for 14 days at 37°C in 5% CO₂.

RESULTS

Expression of VLA Integrin Molecules by the Myeloblastic Cell Lines KG1/KG1a and the Megakaryoblastic Cell Line Mo7e

The expression of integrins on myeloblastic cell lines was examined using immunofluorescence and flow cytometric analysis. The fluorescence intensity of integrin expression on the cell lines KG1, KG1a, and Mo7e is shown in Fig 1. Qualitatively, the expression of the VLA integrins was similar in all three cell lines examined. The common VLA β1 chain (CD29) and the VLA-4 α chain (CD49d) were expressed in all three cell lines, as were to a lesser degree the VLA-5 and VLA-6 α chains (CD49e and CD49f). Expression of the leukocyte integrin subfamily molecules (CAMs) was also similar in the three cell lines (Table 1). LFA-1 (CD11a) and the common β2 chain (CD18) were expressed in all three cell lines with no significant expression of Mo1 (CD11b) and p150,95 (CD11c) on KG1 or KG1a. Mo7e expressed a small amount of CD11c. Expression of GP Ib and GPlIbIIa of the cytoadhesive integrin subfamily was seen on Mo7e, a megakaryoblastic cell line. KG1 and KG1a cell lines expressed none of the cytoadhesive molecules, with the exception of low-intensity GPlb on KG1a (Table 1).

Expression of VLA Integrins by Normal Human Myeloid Progenitors and by Myeloblasts From Leukemic Patient Samples

To further investigate whether differences of integrin expression between normal human myeloid progenitors and malignant myeloid lineage precursors might be evident, the expression of the VLA integrins was examined on CD34+ progenitors from normal BM as well as on myeloblasts obtained from leukemic patient BM. Using two-color immunofluorescence, CD34+ cells derived from normal BM were found to express the common VLA β1 chain (CD29) and the VLA-4 and VLA-5 α chains (Fig 2). No significant VLA-6 α chain or CD61 (integrin β3) was expressed (Fig 2).

Myeloblasts from leukemic patient samples showed a pattern of VLA integrin expression qualitatively similar to that found on normal myeloid progenitors. Eleven patient samples were examined for VLA integrin expression (Table 2): 7 of 7 patients tested expressed the common β1 chain, 10 of 11 expressed VLA-4 α, and 10 of 11 expressed VLA-5 α. The intensity of expression of VLA-4 α and VLA-5 α was quite variable (Table 2). VLA-2, VLA-3, and VLA-6 α expression was minimal in the myeloblasts, similar to the expression of these α chains seen in normal myeloid precursors. Qualitatively, the only difference apparent in VLA integrin expression between normal myeloid precursors and leukemic blasts was a relatively higher expression of the VLA-5 α chain in some patient leukemic samples as compared to normal myeloid precursors. This degree of VLA-5 α expression was also rel-

Table 1. LEU-CAM and Cytoadhesion Expression of Cell Lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>KG1</th>
<th>KG1a</th>
<th>Mo7e</th>
</tr>
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<tr>
<td>LEU-CAM</td>
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<td></td>
</tr>
<tr>
<td>CD11α</td>
<td>17.1 ± 4.2</td>
<td>15.0 ± 3.2</td>
<td>18.7 ± 4.2</td>
</tr>
<tr>
<td>CD11b</td>
<td>3.0 ± 1.7</td>
<td>2.0 ± 1.7</td>
<td>4.7 ± 1.7</td>
</tr>
<tr>
<td>CD11c</td>
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</tr>
<tr>
<td>CD18</td>
<td>10.3 ± 1.7</td>
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<td>14.3 ± 4.5</td>
</tr>
<tr>
<td>Cytoadhesion</td>
<td>GPlb</td>
<td>GPlIbIIa</td>
<td>GPlIbIIa</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 0.6</td>
<td>8.0 ± 1.8</td>
<td>18.0 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>25.0 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>8.5 ± 0.5</td>
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<tr>
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<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>6.2 ± 3.6</td>
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Values shown represent mean ± SEM fluorescence intensity obtained by flow cytometry and staining with a particular antibody (n = 3). Intensity is expressed in arbitrary linear units as described.
Fig 2. Two-parameter flow cytometry histograms of light-density BM cells gated for forward angle light scatter and 90° light scatter; 50,000 cells were counted. Cells were stained with CD34 (or irrelevant IgM) plus the test antibody (or irrelevant isotype-specific control antibody), followed by PE-conjugated goat anti-mouse IgM (y-axis; log red fluorescence) and FITC-conjugated goat anti-mouse CD61 US"). IgG3 (VLA-4) and g(VLA-5) show a significant population of dually labeled cells.

Adhesion of Freshly Isolated Leukemia Myeloblasts to Marrow Stromal Layers

Using a 5Cr-labeling adhesion assay like that used previously to demonstrate the ability of normal CD34+ myeloid progenitors and myeloid leukemic cell lines to bind to marrow stromal layers, the adhesion of myeloblasts from 16 freshly obtained pretreatment marrow samples from leukemic patients was similarly examined (Table 3). In all 16 cases, ≥20% (range 20% to 65%) of blasts bound to stromal layers, and in all cases, binding to stromal layers was greater than to tissue culture-grade plastic (mean 29% vs 6% of cells bound to stroma vs plastic). All cases studied had features of acute myelomonocytic leukemia with the exception of case no. 14, a case of acute promyelocytic leukemia. The degree of CD34 or CD33 positivity of the myeloblasts did not correlate

Table 2. VLA Integrin Expression of Patient Leukemic Blasts

<table>
<thead>
<tr>
<th>Patient</th>
<th>VLA-β1</th>
<th>VLA-2α</th>
<th>VLA-3α</th>
<th>VLA-4α</th>
<th>VLA-5α</th>
<th>VLA-6α</th>
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<td>8</td>
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<td>74</td>
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<td>1</td>
<td>0</td>
<td>49</td>
<td>55</td>
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<td>1</td>
<td>2</td>
<td>7</td>
<td>18</td>
<td>2</td>
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</table>

Shown is the mean linear fluorescence intensity noted with FACS analysis with the indicated antibodies.

Abbreviations: ND = not done; VLA-β1 = CD29; VLA-2α through VLA-6α are CD49a through CD49f, respectively.

Table 3. Adhesion of Leukemic Blasts to Marrow Stromal Layers

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percent Blasts Positive For</th>
<th>Percent Binding To</th>
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<tr>
<td></td>
<td>CD34</td>
<td>CD33</td>
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<tr>
<td>1</td>
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<tr>
<td>16</td>
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<td>ND</td>
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</table>

Shown is a partial blast antigenic phenotype for individual leukemia cases and the percentage of each which adhered to stromal layers or to plastic. Blasts from case 16 had Auer rods and this was therefore considered a case of myelogenous leukemia.

Abbreviation: ND = not done.
with the degree of binding to stroma nor did possession of CD14, a monocytic marker, correlate with degree of binding to tissue culture plastic. In five cases, the effect of PI-PLC on leukemic blast binding to stroma was investigated. Cells were treated with 120 mU PI-PLC for 30 minutes at 37°C, washed once, and plated in the previously described adherence assay. As shown in Fig 3, in only two cases did mean binding decrease in presence of PI-PLC; by 21% in sample 3 and by 27% in sample 5.

**Inhibition of Adhesion by Blocking Antibodies**

To study which of the expressed adhesion proteins might be involved with adhesion of myeloid progenitors to the BM stroma, we used an in vitro model of stromal adhesion to conduct inhibition studies with blocking antibodies to the integrins of interest.

Each of the adhesion receptor antibodies used in the inhibition experiments blocks adhesion at the concentration used. CD29 and CD49d antibodies inhibited adhesion of a B-cell line, NALM-6, to BM fibroblasts by 57% and 52%, respectively. The CD49e and anti-VCAM antibodies were used at a concentration previously demonstrated to show blocking of VLA-5-dependent and VCAM-dependent adhesion, respectively.

**CD34** cell lines and leukemic myeloblasts. The effect of various blocking antibodies on adhesion of the myeloblastic cell lines KG1, KG1a, and Mo7e to bone marrow stroma is shown in Fig 4. Binding of the KG1a, KG1, and Mo7e cells to normal bone marrow stroma was not significantly decreased by anti-CD49e (anti-VLA-5α) or CD29 (anti-β1). Anti-CD49d (anti-VLA-4α), and anti-VCAM had no effect on the adhesion of KG1 or Mo7e to marrow stromal layers. As shown in the case of Mo7e, the combination of anti-VLA-4α and anti-CD29 did not block adhesion either. In contrast, KG1a binding to marrow stroma was partially inhibited by both anti-VLA-4α and anti-VCAM (P < .05 by paired t-test) (Fig 4A). The combination of these two antibodies did not result in greater inhibition than either used alone. No difference in the degree of inhibition was observed between addition of the test antibody at the time of target cell incubation over stromal layers versus preincubation of antibody with ligand-containing cells for 30 minutes at 4°C.

In three cases of myeloid leukemia where sufficient blasts were obtained to study effects of antibodies to VLA integrins on stromal adhesion, anti-VLA-4α inhibited blast binding by a mean of 18% ± 4.9% (range 10% to 27%). In two cases studied, anti-VLA-5α did not result in significant inhibition of blast binding.

**Normal CD34** progenitors. We have previously shown that normal CD34 progenitors attach to stromal layers using a binding assay in which the readout is numbers of CFU-GM colonies. Using this same assay, inhibition of adhesion of normal CD34 progenitors to BM stroma was examined. When the binding assay was performed in the presence of anti-CD29 (β1) (1:100 dilution of 4B4 antibody), inhibition of adhesion of normal progenitors to marrow stroma was 53% (±4.2%, n = 3). Data from these three experiments are shown in Fig 5A. Addition of anti-VLA-4α chain antibody did not enhance the inhibition seen with anti-β1 alone.

In addition, to assess effects of these blocking antibodies upon the entire population of CD34 cells, 51Cr labeling of column-adsorbed CD34 cells was performed followed by the standard adherence assay. As shown in Fig 5B, anti-CD29 (VLA-β1 chain) gave significant binding inhibition in this population as well (P < .01 by paired t-testing), whereas anti-VLA-4α, anti-VCAM, and anti-VLA-5α did not.

**Effects of cytokines on binding of leukemic cell lines.** To assess whether incubation of progenitor or leukemic cells with growth factors would enhance adhesion as has been previously reported for transplanted marrow in murine models or to examine whether exposure of the stromal layers to inflammatory/immune modulators would influence adhesion, adhesion assays were performed after incubation with appropriate cytokines. As shown in Table 4, neither IL-3 nor
INTEGRINS OF HEMATOPOIETIC CELLS

Fig 4. Shown is the mean ± SEM percentage of cells adherent to marrow stroma in the presence of the indicated antibodies for (A) KG1a (n = 3), (B) KG1 (n = 3), and (C) Mo7e (n = 3-5). Antibodies were used at dilutions indicated in Materials and Methods. In (A), anti-IgG1a represents paired control experiments for anti-VCAM, anti-lgG1b for VIA-4 and 484, and anti-lgG3 for anti-VIA-5. In (B), anti-lgG1a represents paired control experiments for anti-VCAM, anti-lgG1b for VIA-4 and 484, and anti-lgG3 for anti-VIA-5. In (C), anti-lgG1a represents controlled paired experiments for anti-VCAM, anti-lgG1b for VIA-4 and 484, and the combination of anti-VIA-4 and 484, and anti-lgG3 for anti-VIA-5. VIA-4 = CD49d, VIA-5 = CD49e, and VIA-6 = CD29.

GM-CSF at concentrations of 50 U/mL and 100 U/mL, respectively, affected binding of KG1a or KG1 to marrow stromal layers. Also, pretreatment of stromal layers with 10⁻⁸ mol/L PMA (phorbol myristate acetate) or 10 U/mL IL-1α did not alter the degree of adhesion of the KG1a or Mo7e cell lines nor of normal CD34⁺ myeloid progenitors to such layers (Table 5).

DISCUSSION

The β₁ (VLA) integrins are generally associated with adhesion to extracellular matrix components. In this study, it has been documented that early myeloid cells, including normal CD34⁺ progenitors, early leukemic cell lines, and acute leukemia myeloblasts all possess a similar array of VLA integrins. Most prominently expressed are CD29 (the common β₁ chain); CD49e (VLA-5α), a receptor for the RGDS cell-binding site of fibronectin; and CD49d (VLA-4α), which can bind to fibronectin domains as well as to a cell-associated ligand, VCAM-1.3 CD49b and CD49c were minimally expressed on all of these cell types. VLA-6, a receptor for laminin, was present only on established leukemic cell lines, and most prominently on Mo7e, a line with some megakaryoblastic properties. We have shown previously that normal CD34⁺ myeloid progenitors possess CD18,11 and here it is shown that immature cell lines (CD34⁺) also possess CD18 and CD11a. No significant expression of cytoadhesins was found on the three cell lines tested except on Mo7e, which has megakaryocytic properties. Normal CD34⁺ progenitors did not possess the β₃ chain (CD61, vitronectin β receptor/ gpIIla).32

The spectrum of β-integrin antigen expression documented here is in keeping with that noted by others using different assay methods. Saeland et al33 have reported a similar array of integrin antigen expression on CD34⁺ cells from marrow and cord blood, and a repertoire of integrin expression during erythroid maturation has also been described.34 Soligo et al,17 using an immunohistochemical detection method, also found VLA-4 presence on immature hematopoietic cells and restriction of β₅ cytoadhesion molecules to megakaryocytes and platelets when unfractionated BM was studied. They also noted only a minor role for β₂ integrins in early hematopoiesis and found variability of β₃ integrin expression on acute myelogenous leukemia specimens, whereas VLA-2, VLA-3, and VLA-6 were absent on nonlymphoid progenitors.17

In the study presented here, VLA-5α (CD49e) was seen on all early myeloid cell types (normal CD34⁺ progenitors, leukemic cell lines, and acute leukemia myeloblasts) with a relative increase seen in some cases of acute myelocytic leukemia (AML), probably reflecting the cell-maturation spectrum involved in leukemogenesis.17 In addition to VLA-5α and the common β₁ chain (CD29), only VLA-4α (CD49d) was prominently expressed on normal early myeloid cells. Within the acute leukemia samples studied, VLA-4α expression did not seem to correlate with maturation as even an
Control
lgG1
Anti-CD29
Incubation Condition
Anti-lgG3
0.0
Anti-VLA-5
1.0
C0
Anti-lgG1
-1
m
5
Anti-VCAM
-1
Antl-VLA4

Fig 5. (A) Shown are the mean ± SEM percentage of CFU-GM bound to marrow stroma in the presence of anti-CD29 (anti-β1) or in the presence of an IgG1 control antibody as compared to binding to stroma without antibody present (100%); n = 3 for all conditions. (B) The percentage of CD34+ cells bound to marrow stroma in the presence of the indicated antibodies is shown with all data obtained in a parallel fashion with the appropriate isotype specific control antibody; n = 4 for all IgG1-paired conditions and n = 3 for VLA-5/IgG3. VLA-4 = CD49d and VLA-5 = CD49e.

M3 specimen with no CD34+ blasts expressed significant levels of VLA-4α. VLA-4 has been found on cultured T lymphocytes to function as a receptor for the heparin-II and IIICS domains of fibronectin. VLA-4α has also been found to be a ligand for VCAM-1, an adhesion molecule of the immunoglobulin gene superfamily found on endothelial cells stimulated with IL-1α, lipopolysaccharide, or tumor necrosis factor α. It is also constitutively expressed on dendritic cells, renal tubular epithelial cells, and tissue macrophages. VCAM has also been found on marrow stromal cell layers such as those used here.

To what extent CD49d (VLA-4α) or CD49e (VLA-5α) antigens participate in homing phenomena or in attachment of myeloid progenitors to marrow microenvironmental components remains unknown. The data presented here show no role for VLA-5α in adhesion of CD34+ cells to stroma as measured in a specific adhesion assay. This is in keeping with previous studies that show no role for RGD sequences in such adhesion processes. In contrast, RGD sequences have been found to have a role in adhesion of erythroid progenitors to marrow.

Antibody inhibition data shown here suggest a role for CD29 (β1 integrin) in adherence of CD34+ CFU-GM-forming cells to marrow stroma and participation of VLA-4α (CD49d) in adhesion of some myeloid progenitors (KG1a cell line and three samples from leukemia patients) to stroma. Data shown here with the KG1a cell line would suggest that VCAM-1 on human marrow stromal cells may participate in leukemic blast adhesion as the ligand for VLA-4. Such an interaction has also been noted for binding of lymphocytes or lymphocyte progenitors to endothelial cells, to murine marrow stromal cells or to human marrow fibroblasts expressing V-CAM-1, and, more recently, for adhesion of myeloid and erythroid progenitors to marrow stromal cells.

In no instance did antibodies to VCAM-1, VLA-4α (CD49d), β1 (CD29), or other VLA integrins completely inhibit myeloid progenitor adhesion to marrow stroma, and, in most cases, inhibition of adhesion noted was minimal. This might suggest a role for other classes of integrins or other types of adhesion receptors in the interaction of leukemic blasts with stroma.

Table 4. Effect of GM-CSF and IL-3 on GK1a and KG1 Adhesion

<table>
<thead>
<tr>
<th></th>
<th>KG1a</th>
<th>KG1a + GM-CSF</th>
<th>KG1a</th>
<th>KG1a + IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroma</td>
<td>65 ± 3.0</td>
<td>63 ± 6.1</td>
<td>63 ± 11</td>
<td>68 ± 9.0</td>
</tr>
<tr>
<td>Plastic</td>
<td>1.3 ± 0.6</td>
<td>2.0 ± 1.0</td>
<td>3.8 ± 1.2</td>
<td>4.0 ± 0.7</td>
</tr>
</tbody>
</table>

Data represent the mean ± standard deviation percentage of the indicated cell line which adhered to marrow stromal layers or plastic in the presence or absence of GM-CSF (100 U/mL) or IL-3 (50 U/mL). Experiments were performed in a paired fashion with n = 3 for all conditions except for KG1a + IL-3 experiments and their controls for which n = 5. Abbreviation: ND = not done.

Table 5. Effect of IL-1α and Phorbol Myristate Acetate (PMA) on Cell Adhesion

<table>
<thead>
<tr>
<th>CD34+ Progenitors</th>
<th>KG1a</th>
<th>Mo7e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroma</td>
<td>14 ± 3.2 (n = 3)</td>
<td>14 ± 3.2 (n = 3)</td>
</tr>
<tr>
<td>Stroma + IL-1α</td>
<td>23 ± 9.5 (n = 4)</td>
<td>23 ± 9.5 (n = 4)</td>
</tr>
<tr>
<td>Stroma + PMA</td>
<td>43 ± 9 (n = 3)</td>
<td>43 ± 9 (n = 3)</td>
</tr>
</tbody>
</table>

Data shown are mean ± SEM percentage of the indicated cell type bound to stromal layers grown in the presence or absence of 10 U/mL IL-1α or 10 μg/mL PMA for 24 hours. Experiments were performed in a paired fashion, n = number of experiments.
hematopoietic cells with marrow. These might include heparin-binding receptors, proteoglycans, L-selectin and its mucin-like ligand, CD44-hyaluronan receptors, thrombospondin, other collagen receptors, or other fibronectin receptors. A role for the 33/66-Kd carboxy-terminal heparin-binding cell adhesion domains of fibronectin in interactions between primitive marrow progenitors and intact irradiated marrow stroma has been found. This interaction appeared to involve the FN-C/H site. Also, other anchored growth factors such as membrane-bound basic fibroblast growth factor, macrophage colony-stimulating factor (M-CSF, CSF-1), or c-kit ligand (stem cell factor) may participate in this adhesion process. Figure 6 illustrates schematically some of the possible receptor-ligand interactions that might contribute to precursor (CD34+) attachment to marrow stroma.

The lack of inhibition seen with anti-CD29 in the case of myeloid/megakaryoblastic cell lines does not necessarily rule out a role for $\beta_6$ integrins in their adhesion to stroma. Changes in conformation of some integrin receptors after antibody binding can “activate” or alter the affinity of ligand recognition. This has been reported for another monoclonal antibody to CD29 (8A2) that stimulated the binding of U937 cells to CHO cells transfected with VCAM-1 complementary DNA (cDNA) and for glycoprotein IbIIa, aIIa$\beta_5$. Whether such a phenomenon might be operational in the adhesion of myeloid leukemic progenitors to stroma can only be speculated on. It is also possible that it does not reflect the role of the CD49d(VLA-4$\alpha_3$/VCAM complex in mediating progenitor adhesion to the microenvironment, one may have to maximize the stromal expression of VCAM-1 by cytokine combinations, as recently shown by Simmons et al.

In this work, the presence of a similar array of VLA integrins on freshly isolated leukemia blasts as on tissue culture-adapted cell lines and normal CD34+ progenitors was identified. Leukemic marrow blasts demonstrated, on average, a comparable degree of binding to marrow stromal layers as did these other cell types. Although the number of samples reported upon here is small, the presence of CD49d and CD49e was consistently noted, and the degree of blast adhesion to stromal layers was independent of subtype; ie, myelogenous versus monocytic versus promyelocytic. In those cases where adhesion inhibition was studied with antibody to CD49d, a small degree of inhibition was noted.

This ability of leukemic blasts to adhere to marrow stromal layers is surprising given the common presence of blood blasts in these cases even when marrow cellularity does not suggest a crowding effect. Because VLA integrin expression appears similar to that of normal progenitors, it is possible that blast egress occurs because of altered function or affinity of these receptors for their respective ligands. It is also possible that these blasts lack adhesive receptors of other uncharacterized classes as has been described for progenitors in chronic myelogenous leukemia (CML). Unlike the situation in CML, the acute myelomonocytic blast adhesion to stroma was not consistently PI-PLC linked. Another explanation for blast exit from the marrow would be altered receptor affinity or changes in integrin phenotype on egress. Such possibilities could be explored by comparing the adhesive properties of circulating blood blasts to marrow blasts. Hematopoietic growth factors such as GM-CSF and IL-3 have been reported to influence adhesion or aggregation of mature granulocytes and monocytes, but their presence did not influence binding of early myeloid cell lines to marrow stromal layers in our hands.

As cells of the granulocyte lineage mature, they lose VLA-4$\alpha_3$, whereas monocytes and eosinophils retain this protein. Changes in integrin phenotype with blast differentiation may partially explain the propensity of monoblasts for tissue invasion. Other factors regulating acute myelogenous leukemia blast traffic may relate to their responses to chemotactic factors or their ability to elaborate collagenases or enzymes...
such as the human heparin-binding elastase homologue (CAP37, azurocidin) that mediates reversible fibroblast and endothelial cell contractility,27 thereby facilitating cell egress into extravascular spaces. Further studies will be required to assess the role of the VLA integrins in leukemia blast egress from the marrow and any role that they might play in stem cell homing during transplantation.28

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Expression of integrins and examination of their adhesive function in normal and leukemic hematopoietic cells

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