Identification of Novel K562 Membrane Proteins That Adhere to Bone Marrow Fibroblasts

By Linda J. Bendall, Kenneth F. Bradstock, and David J. Gottlieb

Adhesion of myeloid leukemia cells to the bone marrow (BM) microenvironment is mediated in part by \( \beta 1 \) and \( \beta 2 \) integrins. Cells of the erythroleukemia line K562, derived from a patient with chronic myeloid leukemia, bind to BM fibroblasts (BMFs) but the adhesion cannot be accounted for by integrins or other known adhesion proteins including CD44 or members of the Ig or selectin families. Membrane fragments from K562 cells were radioiodinated and allowed to adhere to BMF monolayers. Adherent proteins were solubilized together with the fibroblasts, analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and visualized by autoradiography. Four adherent proteins were consistently observed. Two of these, with reduced molecular weights of 52 kD and 35 to 37 kD, were prominent. Addition of soluble thrombospondin and heparin but not fibronectin inhibited binding of K562 membrane proteins to adherent BMFs and immobilized thrombospondin- and heparin-bound K562 proteins. The 52-kD protein has a multimeric structure nonreduced and has characteristics of a glycoprotein. Its adhesion to fibroblasts is divalent cation and temperature sensitive. The 35- to 37-kD protein, whose function is divalent cation but not temperature sensitive, is phosphoinositol-linked and has characteristics identical to an adherent 35- to 37-kD protein identified on murine progenitor cells. Membrane preparations from two cases of acute myeloid leukemia showed an adherent 35- to 37-kD protein and in one case an adherent 52-kD protein without other adherent bands. A K562 subclone with reduced adherence to BMFs showed reduced amounts of adherent 52-kD and 35- to 37-kD proteins. These proteins may be responsible for the adhesion of malignant and normal hematopoietic progenitor cells to the BM microenvironment.

\( \text{© 1996 by The American Society of Hematology.} \)
binding hematopoietic progenitors have been identified. The physiologic role of these remains unclear.

This study was devised to identify molecules on human leukemic cells that mediate binding to BMF monolayers from normal donors. The K562 cell line, derived from a patient with chronic myeloid leukemia, was chosen for initial experiments because although it binds strongly to BMF monolayers, it does not express VLA-4 and its adhesion cannot be reduced by antibodies to \( \beta_1 \) and \( \beta_2 \) integrin chains used alone or in combination, or by antibodies to CD44 (data not shown). Therefore, it binds reproducibly to BM stroma by a currently unidentified mechanism. Our results show four membrane components from K562 cells capable of adhering to BMFs. One of these has characteristics similar to the 37-kD protein described above in the murine system and has been found on the surface of leukemic cells freshly explanted from patients with AML.

**MATERIALS AND METHODS**

**Cells.** The following cell lines were maintained in suspension culture at a cell density of 0.5 to 1.0 \( \times 10^6/\text{mL} \) in RPMI-1640 (GIBCO, Grand Island, NY) containing 10% fetal calf serum (FCS) (PABCO Biologicals, Sydney Australia) in a humidified atmosphere containing 5% CO\(_2\) in air at 37°C for at least 1 week before use: the myelomonocytic cell line HL60, the erythroleukemia cell line K562, the myeloid cell line U937, the T-cell line Molt-4, the pre-B cell line Nalm-6, and the B-cell line Raji. Leukemic blasts were obtained from peripheral blood (PB) samples collected with informed consent and institutional ethics committee approval. The K562 cell line, derived from a patient with chronic myeloid leukemia, was chosen for initial studies. One of these has characteristics similar to the 37-kD protein described above in the murine system and has been found on the surface of leukemic cells freshly explanted from patients with AML.

**Enzyme treatments.** Cells, \( 5 \times 10^7 \), were washed three times in PBS before enzyme treatment. Trypsin (Boehringer Mannheim, Mannheim, Germany) was used at 1 mg/mL for 10 minutes, neuraminidase from *Vibrio cholerae* (Calbiochem, La Jolla, CA) at 0.3 U/mL for 15 minutes, and PI-PLC (Immunootech, Marseilles, France) at 0.1 U/mL for 30 minutes. All incubations were at 37°C. Cells were washed twice in PBS after enzyme treatment before isolation of cell membranes. None of the enzyme treatments had any effect on cell viability as assessed by trypan blue exclusion.

**Cell membrane preparation.** Membranes were isolated by a modification of the method published by Shiota et al. Briefly, \( 5 \times 10^7 \) cells were washed three times in PBS and resuspended in 4 mL of 10 mmol/L Tris(hydroxymethyl)aminomethane (Tris) containing 2 mmol/L MgCl\(_2\) and protease inhibitors leupeptin, 5 \( \mu \)g/mL, phenylmethylsulfonyl fluoride (both from Sigma, St Louis, MO), 1.25 mmol/L, and diisopropylfluorophosphate (Calbiochem, Alexandria, Australia), 12.5 \( \mu \)mol/L. Protease inhibitors were maintained at these concentrations throughout all subsequent procedures. The cells were incubated on ice for 20 minutes before disruption by \( \approx 50 \) strokes in a Dounce homogenizer (Crown Scientific, Sydney, Australia). All subsequent steps were performed on ice or at 4°C. Homogenization was monitored by light microscopy and considered complete when \( \approx 90\% \) of cells were ruptured. Four milliliters of 0.5 mol/L sucrose was added and the suspension layered over 2 \( \times 4-\text{mL} \) aliquots of 1.5 mol/L sucrose and centrifuged at 138,000g for 1 hour. Plasma membranes were collected from the interface with intact cells, nuclei, and mitochondria being found in the pellet. The plasma membranes were washed once in 10 mmol/L Tris by centrifugation at 138,000g for 40 minutes and the purity checked by electron microscopy. Purified membranes were solubilized in 1% (vol/vol) Triton X-100 in PBS on ice for 20 minutes with occasional mixing. Triton insoluble material was removed by microfuge centrifugation at 14,000g for 15 minutes and samples stored at \(-80^\circ\text{C}\) until required. Isolated membranes consisted primarily of plasma membrane vesicles with occasional ribosomes being observed.

**Iodination of membranes.** Two hundred to four hundred micrograms of solubilized membrane protein were labeled with 19 GBq of Na\(^{125}\)I (Australian Radioisotopes, Menai, Australia) using the lactoperoxidase (Boehringer Mannheim, Mannheim, Germany) method. Free \(^{125}\)I was separated from the labeled proteins on a 10-mL Sephadex G-25 column (Pharmacia Biotech, Uppsala, Sweden). Peak protein fractions were used in binding studies.

**Binding of labeled proteins to BMFs.** One hundred micrograms of labeled proteins were diluted into 5 mL of RPMI containing 5% FCS. The dilution of cell membranes in RPMI was determined by analyzing the concentration of Triton to which BMFs could be exposed without undergoing permeabilization. BMF monolayers were exposed to serial dilutions of the column fractions and examined for trypan blue exclusion. A 1 in 50 dilution of the labeled membranes was required to prevent permeabilization. This dilution gives an estimated final Triton concentration of 0.01%, allowing for a 1 in 2 dilution of Triton during labeling. The possibility of proteins being solubilized out of the BMF plasma membrane without permeabilization was examined by exposing BMF monolayers to serial dilutions of a 24-well plate (Costar, Cambridge, MA) containing confluent BMF monolayers. After 1 hour at room temperature, unless otherwise indicated, nonadherent proteins were removed and the monolayers washed three times with RPMI containing 5% FCS. The BMF monolayers were then solubilized in 1 mL of 1% Triton X-100 in PBS. In experiments using red blood cells, cells were washed by centrifugation in the same total volume of PBS.
NEW ADHERENT K562 PROTEINS

**RESULTS**

*K562 membrane proteins adhere to BMFs.* The binding of Triton-soluble, labeled membrane proteins from K562 cells to BMF monolayers was examined. Four proteins were consistently present in five independent membrane preparations analyzed on separate occasions (Fig 1A). The most prominent band has a molecular weight of 52 kD under reducing conditions but only barely enters the stacking gel when nonreduced, suggesting that it may exist as a multimer. The next most prominent band has a molecular weight of 35 to 37 kD when nonreduced and appears as a more diffuse band at around 37 kD before reduction. The other two proteins appear as bands of 57 and 45 kD when analyzed on reducing gels. All four bands resolved as a single spot on 2D gel electrophoresis where proteins were separated according to their isoelectric point in the first dimension and by reduced SDS-PAGE in the second dimension, suggesting that each band represents a single protein (data not shown).

The binding of these proteins appears to be specific because they do not bind to plastic wells and could not be detected in the medium from the final wash of the BMFs (Fig 1A). An excess of unlabelled K562 membranes was able to completely inhibit the adhesion of 52- and 35- to 37-kD proteins to BMF (Fig 1B). The ability of these proteins to bind nonspecifically to any cell type was also examined. Labeled membrane proteins were incubated with 1.25 × 10⁶ trypsinized BMFs or 12.5 × 10⁶ sheep red blood cells (Fig 2). The 35- to 37- and 45-kD proteins failed to bind to either trypsinized BMFs or sheep red blood cells. Binding of the
52- and 57-kD proteins to sheep red blood cells was barely detected. Binding of the 52-kD protein to trypsinized BMFs was observed at approximately 50% of control whereas that of the 57-kD protein was little affected.

Characterization of membrane proteins. Before the isolation of plasma membranes K562 cells were treated with trypsin, neuraminidase, or PI-PLC. The protein content of each membrane preparation and that of an untreated preparation was determined using the BCA reagent kit. Two hundred forty micrograms of protein from each condition was labeled and analyzed for their ability to bind to BMF monolayers (Fig 3). Specific activities for labeled membranes from all conditions were similar with a mean of 2,871 ± 128 dpm/µg of protein. Densitometry scans of the autoradiograph were performed to compare conditions. The 52-kD protein is trypsin sensitive and requires sialic acid residues for binding. It also appears that it could be anchored via phosphoinositol although PI-PLC treatment failed to completely eliminate binding. The 35- to 37-kD protein was not affected by trypsin treatment but was almost completely removed by PI-PLC, indicating a phosphoinositol linkage. Sialic acid residues appear to be involved in binding, but their removal by neuraminidase only partially inhibited adhesion of this protein. The 57-kD protein is resistant to treatment with PI-PLC or trypsin but is sensitive to neuraminidase treatment, whereas the 45-kD protein is only resistant to PI-PLC. Adhesion of each of the four membrane proteins described can thus be ablated by prior enzyme treatment of whole K562 cells which had no effect on cell viability. Nevertheless, we also considered the possibility that these proteins could be whole or proteolytic fragments of cytoskeletal proteins contaminating K562 membrane preparations. In particular, actin that has a molecular weight of 42 kD was considered a potential contaminant. However, Western blots of whole K562 lysates and K562 membrane preparations with the anti-actin antibody C4 showed only a single band at 42 kD, whereas blotting of BMF-adherent K562 membranes showed no evidence of bands at 52 or 35 to 37 kD (data not shown).

Temperature and divalent cation dependence. Divalent cations are required for the function of adhesion molecules such as cadherins, integrins, and selectins. The effect of the removal of divalent cations was investigated. The presence of 5 mmol/L EDTA during the adhesion step inhibited the binding of the 52-, 45-, and 35- to 37-kD proteins. The binding of the 57-kD protein was independent of the presence of divalent cations (Fig 3). Performing the adhesion step at 4°C dramatically reduced the binding of 57-, 52-, and 45-kD proteins but had no effect on the binding of the 35- to 37-kD protein (Fig 4).

Binding to thrombospondin and heparin. The presence of 100 µg/mL of soluble thrombospondin almost completely inhibited the binding of 35- to 37-, 45-, and 52-kD proteins.

Figure 2. (A) Analysis of 125I-labeled K562 membrane proteins that adhere to BMFs (lane 1) or sheep red blood cells (lane 2) by SDS-PAGE under reducing conditions. (B) As for (A), except lane 2 shows binding to trypsinized BMFs.

52- and 57-kD proteins to sheep red blood cells was barely detected. Binding of the 52-kD protein to trypsinized BMFs was observed at approximately 50% of control whereas that of the 57-kD protein was little affected.

Characterization of membrane proteins. Before the isolation of plasma membranes K562 cells were treated with trypsin, neuraminidase, or PI-PLC. The protein content of each membrane preparation and that of an untreated preparation was determined using the BCA reagent kit. Two hundred forty micrograms of protein from each condition was labeled and analyzed for their ability to bind to BMF monolayers (Fig 3). Specific activities for labeled membranes from all conditions were similar with a mean of 2,871 ± 128 dpm/µg of protein. Densitometry scans of the autoradiograph were performed to compare conditions. The 52-kD protein is trypsin sensitive and requires sialic acid residues for binding. It also appears that it could be anchored via phosphoinositol although PI-PLC treatment failed to completely eliminate binding. The 35- to 37-kD protein was not affected by trypsin treatment but was almost completely removed by PI-PLC, indicating a phosphoinositol linkage. Sialic acid residues appear to be involved in binding, but their removal by neuraminidase only partially inhibited adhesion of this protein. The 57-kD protein is resistant to treatment with PI-PLC or trypsin but is sensitive to neuraminidase treatment, whereas the 45-kD protein is only resistant to PI-PLC. Adhesion of each of the four membrane proteins described can thus be ablated by prior enzyme treatment of whole K562 cells which had no effect on cell viability. Nevertheless, we also considered the possibility that these proteins could be whole or proteolytic fragments of cytoskeletal proteins contaminating K562 membrane preparations. In particular, actin that has a molecular weight of 42 kD was considered a potential contaminant. However, Western blots of whole K562 lysates and K562 membrane preparations with the anti-actin antibody C4 showed only a single band at 42 kD, whereas blotting of BMF-adherent K562 membranes showed no evidence of bands at 52 or 35 to 37 kD (data not shown).

Temperature and divalent cation dependence. Divalent cations are required for the function of adhesion molecules such as cadherins, integrins, and selectins. The effect of the removal of divalent cations was investigated. The presence of 5 mmol/L EDTA during the adhesion step inhibited the binding of the 52-, 45-, and 35- to 37-kD proteins. The binding of the 57-kD protein was independent of the presence of divalent cations (Fig 3). Performing the adhesion step at 4°C dramatically reduced the binding of 57-, 52-, and 45-kD proteins but had no effect on the binding of the 35- to 37-kD protein (Fig 4).

Binding to thrombospondin and heparin. The presence of 100 µg/mL of soluble thrombospondin almost completely inhibited the binding of 35- to 37-, 45-, and 52-kD proteins.
Fig 3. Effect of enzymes or divalent cation chelation on adhesion of K562 membrane proteins to BMFs. Membranes were isolated from K562 cells treated with trypsin (A), PI-PLC (B), or neuraminidase (C), and adsorbed to BMFs in the presence (D) or absence (A through C) of 5 mmol/L EDTA. Adsorbed proteins were analyzed by SDS-PAGE and autoradiographs obtained. Densitometry tracings of autoradiographs are shown. Solid lines indicate control membranes obtained from sham-treated K562 adsorbed in the absence of EDTA, and the dashed line indicates the experimental condition.

and markedly inhibited the binding of the 57-kD protein (Fig 5A). In contrast, addition of soluble fibronectin at the same concentration had no effect on binding of any of the K562 proteins. Immobilized thrombospondin was able to bind the 35- to 37-, 45-, and, to a lesser extent, the 52-kD protein (Fig 5B). Addition of heparin at a final concentration of 10 μg/mL also inhibited protein binding of 45-, 52-, and, to a lesser extent, 57-kD proteins but not of the 35- to 37-kD protein (Fig 5C). At a one-log higher concentration, heparin also inhibited binding of the 35- to 37-kD protein (data not shown). The same concentration (10 μg/mL) of another negatively charged glycosaminoglycan, fucoidan, had no effect on binding whereas chondroitin sulfate only marginally inhibited binding of the 52- and 57-kD proteins. When an equal volume of heparin-Sepharose and K562 membrane preparations were combined all K562 membrane proteins were removed (Fig 5D).

Absence of chondroitin sulfate side-chains on adherent proteins. Chondroitin sulfate can inhibit the binding of AML blasts to BMFs (unpublished observations, April 1993) and binding of normal hematopoietic progenitors to stromal layers can be inhibited by treating the progenitors with chondroitinase. Digestion of solubilized adherent proteins with chondroitinase ABC had no effect on the molecular weight of the 52- or 35- to 37-kD proteins (Fig 6). Activity of the enzyme under these experimental conditions is apparent from the alteration in the molecular weight of solubilized BMF proteins present in the preparation which could be seen by Coomassie Blue staining.

Distribution of proteins on other cell lines. Membranes were isolated from two other myeloid cell lines, HL60 and U937, the pre-B cell line Nalm-6, the mature B-cell line Raji, and the T-cell line Molt-4. The 57-kD protein was found only on Nalm-6 and faintly on another B-cell line Raji, whereas the 52-kD protein was widely expressed, although at lower levels, on all cell lines tested with the exception of Raji. The 35- to 37-kD protein was only clearly observed on U937. However, there was a faint band present on Nalm-6. The 45-kD protein was not observed on any other cell line tested (Fig 7A).

Distribution of proteins on normal hematopoietic cells. Solubilized membrane proteins from lymphocytes and granulocytes obtained from normal volunteers were assessed for the presence of these proteins (Fig 7B). The 57-kD and 52-kD proteins were observed in both lymphocytes and granulocytes, but the level of expression of the 52-kD protein was significantly less than that observed on K562. The 35- to 37-kD protein was observed in the granulocyte membranes with the 37-kD form dominating, but a minor band visible at 35 kD. In the lymphocyte membranes this band was barely detectable. Densitometry showed a 16-fold greater intensity...
of the 35- to 37-kD band in the granulocyte lane compared with the lymphocyte lane. Because the lymphocyte preparation contained 5% granulocytes and 4% monocytes the possibility that the 35- to 37-kD band observed in the lymphocyte membranes was derived from contaminating myeloid cells cannot be excluded. The 45-kD protein was not observed in granulocyte or lymphocyte membranes.

**Distribution of proteins on fresh leukemic blasts.** Peripheral blood from two patients with AML, one M2 and one M5, was obtained and the blasts purified on Ficoll Hypaque. The percentage blasts in the purified samples were 92% and 88%, respectively. Membranes were prepared and the solubilized labeled proteins adsorbed to BMF monolayers for cell lines. The 35- to 37-kD protein was observed in both cases although the apparent molecular weight was 35 kD in the M2 case and 37 kD in the M5 case. The 52-kD protein was only observed in the M5 case and the other two proteins were not observed (Fig 7C).

**Reduced expression of the 35- to 37- and 52-kD proteins on a subadherent line of K562.** A subclone of K562 with reduced adhesion to BMFs was isolated by cloning K562 cells repeatedly selected for their failure to bind BMFs. This subclone displayed only 25% of the adhesion of the parent cells. Expression of VLA-5 and lymphocyte function associated antigen-1 were identical to that of the parent. Membranes isolated from the subline showed reduced levels of both the 52-kD and 35- to 37-kD proteins and, to a lesser extent, of the 45-kD protein (Fig 8).

**DISCUSSION**

Although K562 cells bind well to BMFs, the adhesion cannot be accounted for by β1 or β2 integrins, by CD44, or by other known adhesion ligands including CD54, CD58, CD2, or selectin family members (data not shown). Adhesion of human AML cells to the BM microenvironment has a similar pattern. Its mechanism is totally unexplained in 50% of cases and is only partially accounted for in the remainder.18 We have identified four membrane proteins of 57, 52, 45 and 35 to 37 kD from K562 cells that consistently bind to human BMFs and that may be responsible, at least in part, for K562 adhesion to the BM stroma. Proteins of 52 and 35 to 37 kD were most prominent. We are confident that our assays detect surface rather than cytoplasmic membrane structures since short exposure of whole cells to at least one of the enzymes trypsin, neuraminidase or PIPLC before cell rupture and membrane isolation, was able to prevent adhesion of each of the four identified components. Our experimental method used the adhesion of radiiodinated solubilized cell membranes to adherent BMF monolayers followed by solubilization of fibroblasts and adherent molecules with detection by autoradiography. In early experiments we showed that the concentration of Triton present in the solution of membrane proteins plated onto adherent monolayers was not able to permeabilize BMFs, excluding the possibility that K562 membrane proteins bind intracellular BMF ligands. Adsorption of proteins present in culture media onto cell membranes is unlikely to explain our findings, given the absence of adherent 52- or 35- to 37-kD proteins in solubilized membranes from lymphoid cell lines. No protein adhesion was seen when iodinated membranes were allowed to adhere to plastic instead of cellular layers and there was no adhesion of the 35- to 37- or 45-kD proteins and very marked reduction in adhesion of the 52- and 57-kD proteins when K562 membranes were allowed to adhere to sheep red blood cells. A similar result was observed for K562 membranes binding trypsinized BMFs, although binding of 52- and 57-kD proteins at lower levels was observed.

Heparin and soluble thrombospondin but not fibronectin inhibited the adhesion of 52- and 35- to 37-kD proteins to BMFs. Thrombospondin is a homotrimeric glycoprotein of approximately 450 kD that is a component of the BM stroma extracellular matrix. It contains at least four functional domains that might serve in receptor recognition. A CSVTCG sequence mediates the high-affinity interaction of thrombospondin with CD36, a known thrombospondin receptor with a molecular weight of 90 kD. An amino terminal heparin binding domain interacts with heparin and other proteoglycans whereas an RGDA sequence is probably responsible for binding to integrins of the αvβ3 and αIβ3 class. Of particular relevance to our findings is the recent description of the attachment of peptides containing the sequence VVM from the carboxy-terminal of thrombospondin to 52- and 37-kD proteins on the surface of K562 cells. Similar 52- and 35- to 37-kD proteins capable of binding thrombospondin peptides were present on a wide variety of human, bovine and murine tissues. Characteristics of the 52-kD protein described in that study (including heparin binding) are very similar to those described here, raising the possibility that 52- and 35- to 37-kD K562 proteins adherent to BMFs are cellular thrombospondin receptors. In support of this idea, we found that 35- to 37-, 45-, and 52-kD proteins from
K562 cells were able to bind to thrombospondin. Normal hematopoietic progenitors are also known to bind immobilized thrombospondin, although the cellular receptor used has not been identified. Our data, together with the finding of Gao and Frazier that 37- and 52-kD proteins have a wide tissue distribution, suggest that receptors to carboxy-terminal thrombospondin sequences may be important in stromal adhesion of normal and malignant hematopoietic progenitors. All four K562 membrane proteins bound heparin, suggesting that binding to glycosaminoglycans is a common characteristic. Similar concentrations of the other strongly negatively charged molecules fucoidan and chondroitin sulfate were effective in blocking binding of K562 proteins to immobilized thrombospondin.
droitin sulfate were not effective at inhibiting adhesion. Binding to heparin or heparan sulfate is a feature of primitive human and murine hematopoietic progenitors.\textsuperscript{36-38} We have observed that heparin inhibits binding of whole K562 cells to BMFs (data not shown), supporting the concept that heparin-binding proteins are functionally important in cellular adhesion to BM stroma.

The molecular weights of 52- and 35- to 37-kD and the apparently multimeric configuration of the 52-kD protein fail to conform to recognized adhesion ligands of either the integrin or selectin group or to any member of the Ig superfamily including CD58 (lymphocyte function associated molecule-3, 55 to 70 kD),\textsuperscript{39,40} CD54 (intercellular adhesion molecule-1, 85 to 110 kD),\textsuperscript{41,42} or CD31 (platelet endothelial cell adhesion molecule-1, 130 kD).\textsuperscript{43} Although intercellular adhesion molecule-2 has a reduced molecular weight of 55 to 65 kD,\textsuperscript{44} LFA-1, its only described ligand, is not present on BMFs (data not shown). Furthermore, Western blot of solubilized proteins from K562 membranes using an anti-intercellular adhesion molecule-2 antibody did not show a 57-kD size band. Membrane bound growth factors such as stem cell factor, interleukin-3, granulocyte-macrophage colony-stimulating factor, or interleukin-1\textalpha\ or \textbeta, are all of smaller molecular weight.\textsuperscript{45-47} None of the varying molecular weight species of macrophage colony-stimulating factor\textsuperscript{50-53} conforms to the characteristics of the BMF-adherent proteins observed, whereas cytokine receptors such as c-kit (stem cell factor receptor, 145 kD), c-fms (macrophage colony-stimulating factor receptor, 150 kD) and others that could bind transmembrane growth factors or growth factors within the fibroblast-associated matrix are of larger molecular weight.\textsuperscript{54-61} Western blotting of membrane preparations using a polyclonal antifibronectin antiserum to detect fibronectin proteolytic fragments binding VLA-4 or \alpha 4\beta 7 on BMFs\textsuperscript{1,62} failed to detect bands at or around 52 or 35 to 37 kD. Other extracellular matrix proteins also have higher molecular weights, eg, laminin (200 to 400 kD),\textsuperscript{63,64} vitronectin (65 to 75 kD),\textsuperscript{65} tenascin (190 to 220 kD),\textsuperscript{66} and collagen,\textsuperscript{67,68} although membrane-associated fragments of these cannot be completely excluded. The ubiquitously expressed CD44 molecule that mediates adhesion to hyaluronate and fibronectin has a molecular weight of 90 to 240 kD and does not account for our findings.\textsuperscript{69,70} Anti-CD44 antibodies also fail to inhibit K562 binding to BMFs in adhesion assays.

The binding of the 35- to 37-kD protein was divalent cation sensitive and independent of temperature. The protein itself was trypsin resistant and phosphoinositol-linked. These characteristics are similar to a 37-kD membrane protein described recently in a murine system by Shiota et al.\textsuperscript{21} In that study, a 37-kD (p37) protein (with a 35-kD doublet) was selectively adsorbed from a cloned murine stromal cell line onto FDCP-1 cells. The p37 was also present on FDCP-1
and FDCP-Mix cells and could be absorbed onto murine but not human erythrocytes. In our experiments a doublet was observed in the adherent 35- to 37-kD protein from human AML samples and normal granulocytes but not from membranes of B- or T-lymphoid cell lines and only faintly in membranes from human peripheral blood lymphocytes, possibly due to neutrophil contamination. However, an adherent band at 37 kD was detected from membranes of the monocytic cell line U937, raising the possibility that in the human hematopoietic system the 35- to 37-kD protein is myeloid restricted. In contrast, the 52-kD component was widely distributed on all cells analyzed, with the exception of the B-lymphocyte–derived Raji cell line. It was protease and neuraminidase sensitive, suggesting that it was likely to be a glycoprotein. Its divalent cation and temperature sensitivity together with an apparent multimeric structure may indicate that it acts as a ligand for an integrin or a receptor with similar binding requirements.

Several other lines of evidence strongly support the concept that the membrane components we have identified, and in particular the 35- to 37- and 52-kD components, are relevant in the binding of fresh whole human AML cells to adherent BM layers. Membrane fractions from a subadherent K562 cell line in culture in our laboratory showed reduced amounts of both 35- to 37- and 52-kD components, suggesting that these components are functionally relevant in adhesion. Most importantly, solubilized membranes of leukemic cells from two patients with AML produced bands of 35- to 37-kD after adhesion to BMFs. One of these also yielded an adherent band of 52 kD. Protein purification and

![Fig 7. Distribution of BMF-adherent membrane proteins on cell lines, normal lymphocytes, and granulocytes and myeloid leukemic blasts. Solubilized 125I-labeled membrane proteins were adsorbed to BMF monolayers and analyzed by SDS-PAGE and autoradiography. (A) Results from the cell line Raji (lane 1), Nalm-6 (lane 2), U937 (lane 3), HL60 (lane 4), or Molt-4 (lane 5); (B) normal lymphocytes (lane 1), and granulocytes (lane 2); and (C) blasts from two cases of AML (M2, lane 1 and M5, lane 2).]


56. Roussel MF, Sherr CJ: Mouse NIH 3T3 cells expressing human colony-stimulating factor 1 (CSF-1) receptors overgrow in serum-free medium containing human CSF-1 as their only growth factor. Proc Natl Acad Sci USA 86:7924, 1989
62. Chen BMC, Elices MJ, Murphy E, Hemler ME: Adhesion to vascular cell adhesion molecule 1 and fibronectin. Comparison of
α4β1 (VLA-4) and α4β7 on the human B cell line JY. J Biol Chem 267:8366, 1992
Identification of novel K562 membrane proteins that adhere to bone marrow fibroblasts

LJ Bendall, KF Bradstock and DJ Gottlieb