Critical Factors in Basal Cell Adhesion Molecule/Lutheran-mediated Adhesion to Laminin*

(Received for publication, September 10, 1998, and in revised form, October 17, 1998)

Qin Zen‡§, Maisha Cottman¶, George Truskey¶, Robin Fraser¶, and Marilyn J. Telen‡

From the ‡Division of Hematology and the Duke Comprehensive Sickle Cell Center, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710, the ¶School of Engineering, Duke University, Durham, North Carolina 27710, and the ¶Glasgow and West Scotland Blood Transfusion Service, Glasgow G2 5UA, Scotland

Basal cell adhesion molecule (B-CAM) and Lutheran (LU) are two spliceforms of a single immunoglobulin superfamily protein containing five Ig domains and comprise the sickle (SS) red cell receptor for laminin. We have now analyzed laminin binding to murine erythrocytes transfected with various human B-CAM/LU constructs. B-CAM and LU bound equally well to laminin, indicating that the longer cytoplasmic tail of LU is not required for binding. However, binding of soluble laminin did require the presence of the membrane-proximal fifth immunoglobulin superfamily (IgSF) domain of LU, while deletion of IgSF domains 1, 2, 3, or 4 individually or together did not abrogate laminin binding. Under flow conditions, MEL cells expressing B-CAM, LU, and LU lacking domains 1, 2, 3, or 4 adhered to immobilized laminin with critical shear stresses over 10 dynes/cm². However, MEL cells expressing LU lacking domain 5 bound to laminin poorly (critical shear stress = 2.3 dynes/cm²). Moreover, expression of only IgSF domain 5 of LU was sufficient to mediate MEL cell adhesion to immobilized laminin (critical shear stress >10 dynes/cm²). Finally, Scatchard analysis showed that SS red cells had an average of 67% more B-CAM/LU than normal red cells, and low density red cells from sickle cell disease patients expressed 40–55% more B-CAM/LU than high density SS red cells. B-CAM/LU copy number thus may also play a role in the abnormal adhesion of SS red cells to laminin.

Vaso-occlusion in sickle cell disease patients is a complex process involving not only the mechanical obstruction of vessels by misshapen and nondeformable sickle (SS) red cells but also cellular adhesion and the activation of coagulation (1). In addition, sickle cell disease is characterized by endothelial damage, some of which may result from the effects of a variety of cytokines on endothelial cells, resulting in increased circulating endothelial cells and, potentially, exposure of subendothelial matrix to flowing blood (2, 3).

Among the subendothelial matrix molecules to which SS cells adhere, laminin is the one to which these cells adhere most avidly (4). Laminin is a family of at least 11 heterotrimeric proteins, each containing an α, β, and γ subunit (5). We have reported previously that basal cell adhesion molecule/Lutheran glycoprotein (B-CAM/LU), a member of the immunoglobulin superfamily with two known isomers (B-CAM and LU), is the major laminin receptor on SS red cells and promotes SS but not normal red cell adhesion to immobilized as well as soluble laminin (6). Expression of B-CAM/LU appeared to be increased on SS red cells, as measured by flow cytometry, and the level of B-CAM/LU expression was proportional to laminin binding within red cells from both normal donors and individuals with sickle cell disease (6). However, the exact scale of increased expression was not determined in that study. The role of B-CAM/LU in adhesion to laminin was further supported by the binding of transfected MEL cells expressing recombinant human B-CAM (rB-CAM) to both soluble and immobilized laminin.

B-CAM was first described as an IgSF protein expressed along the basal surface of epithelial cells (7). Later, Lutheran (LU) protein was characterized as a spliceform of B-CAM (8, 9). The extracellular portions of B-CAM and LU are identical and contain five IgSF domains: two variable-type, or V, domains and three constant-type 2, or C2, domains (Fig. 1A). B-CAM differs from LU in that it lacks the last 40 amino acids, including a putative SH3 binding site, of the cytoplasmic tail and thus has a molecular mass of about 78 kDa, compared with a mass of 85 kDa for LU. Both proteins are expressed by normal and SS red cells.

Other IgSF proteins have also been identified as laminin receptors, including members of the integrin subfamily (10–12). However, no consensus motif for laminin binding has been identified, possibly because different laminin receptors bind to different sites and different isoforms of laminin. Previous studies of IgSF domain activity in cell adhesion involving other ligands have identified the N-terminal IgSF domain as the most frequent major binding domain, as in ICAM-1/LFA-1 and ICAM-3/LFA-1 interactions (13, 14). The N-terminal IgSF domain of ALCAM, which has two V and three C2 domains similar to B-CAM/LU, also mediates ALCAM’s interaction with CD6 (15). In some cases, the second IgSF domain plays a less critical but important role (16, 17), such as in E-cadherin/β7 interaction. And in other instances, such as in the interactions of CD4 with major histocompatibility complex II and CD8 with major histocompatibility complex I, multiple IgSF domains are involved (18, 19).

In the present study of B-CAM/LU, we have measured the B-CAM/LU copy number on SS and normal red cells, as well as on density fractionated SS red cells, in order to determine if
increased expression of B-CAM/LU is likely to be a factor in the observed increased adhesion of SS red cells to laminin. We have also used a number of recombinant LU proteins from which various IgSF domains have been deleted to demonstrate that the membrane proximal IgSF domain (domain 5) alone is critical for laminin binding, and that the cytoplasmic tail of LU, which is absent from B-CAM, does not appear to affect laminin binding.

MATERIALS AND METHODS

Cell Line and Antibodies—The MEL cell line was obtained from Dr. B. Haynes, Duke University Medical Center, and maintained under standard tissue culture conditions in RPMI 1640 with 10% fetal calf serum. The anti-Lu8 mAb LM342/767:31 was produced by Dr. Robin Fraser, Glasgow and West Scotland Blood Transfusion Service, UK. MAb 4P2 was generated by immunizing a mouse with MEL cells expressing rb-CAM and rLU and recognizing the fifth IgSF domain of B-CAM/LU. Human anti-Lu8, which recognizes an epitope on the second IgSF domain of B-CAM/LU (29), was provided by the Immunochemistry Laboratory of the Duke University Medical Center Transplant Service. Rabbit anti-human laminin was obtained from Life Technologies, Inc. Horseradish peroxidase-linked second antibodies and FITC-conjugated antibodies were obtained from Jackson ImmunoResearch (West Grove, PA).

Quantitation of B-CAM/LU Sites per Cell—Purified anti-Lu8 mAb was radiolabeled with 1.0 mCi of Na125I (Amersham Pharmacia Biotech) per 1.0 mg of protein, using glass tubes coated with IODO-GEN (Pierce) (21). The iodinated mAb was separated from free Na125I by precipitation with cold 10% trichloroacetic acid.

TABLE I

| Construct  | Deletional oligonucleotide (forward sense) | Resulting protein structure |
|-----------|------------------------------------------|-----------------------------|
| LU1       | Met1–Glu29, Ly144–Cys268                | Met1–Glu29, Ly144–Cys268    |
| LU2       | Met1–Thr140, Leu258–Cys268              | Met1–Thr140, Leu258–Cys268  |
| LU3       | Met1–Thr260, Val258–Cys268              | Met1–Thr260, Val258–Cys268  |
| LU4       | Met1–Pro396, Gln245–Cys268              | Met1–Pro396, Gln245–Cys268  |
| LU5       | Met1–Thr426, Gln245–Cys268              | Met1–Glu29, Gln245–Cys268   |

Construction of Lutheran Proteins with IgSF Domain Deletions

A complete LU cDNA constructed and inserted into the pcDNA3.1(+) expression vector as described above was used as the template for all further PCR reactions to create constructs encoding domain deletion mutants. The cDNA sequences encoding each IgSF domain were deleted individually from the full-length LU cDNA using deletional oligonucleotide primers in overlapping extension PCR (24). The forward primers used to produce the 3′ cDNA fragments of each construct are listed in Table I. Complementary reverse oligonucleotide primers were utilized to generate the 5′ fragments of each construct. A first round of PCR reactions was performed using one of the deletional oligonucleotide primers (forward or reverse sense) and a corresponding vector primer under the following cycling conditions: 1 cycle at 94 °C for 3 min; 4 cycles at 94 °C for 1 min, 42 °C for 1 min, 72 °C for 1.5 min; 30 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min; and finally, 1 cycle at 72 °C for 10 min. The two overlapping cDNA fragments were then used to prime each other for 4 cycles (94 °C for 1 min, 66 °C for 1 min, 72 °C for 1.5 min). The 5′ and 3′ vector primers were then added to the PCR reactions to amplify the entire mutagenized cDNA, and PCR was performed as follows: 30 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min; and 1 more cycle at 72 °C for 10 min. A LU construct containing only domain 5 (LU5) without domains 1–4 was also generated using the same PCR method and primers as listed in Table I. The amino acids expressed by each LU deletion construct are also listed in Table I, and the structure of all constructs used is illustrated in Fig. 2A.

All cDNAs were subcloned into the pcDNA3.1(+) expression vector; restriction mapping and DNA sequencing confirmed that the vectors contained the desired constructs with deletions, and constructs were transfected into MEL cells by electroporation, as described previously (23). The two transfected cell lines were grown in RPMI 1640 containing 10% fetal calf serum and 0.5 mg/ml Geneticin (Life Technologies, Inc.), and expanded to include the desired proteins were selected by sterile sorting using a Becton Dickinson FACStar Plus at Duke University Cancer Center.

Stably transfected MEL cells were assayed for their ability to bind soluble laminin by incubation with 3 μg/ml human laminin (Life Technologies, Inc.) at 37 °C for 1 h, followed by staining with rabbit anti-laminin and FITC-conjugated secondary Ab, and repeated washing. MEL cells expressing LU1 were incubated with human anti-Lu8. MEL/LU5 cells were incubated with mAb 4P2. Transfected expressing high levels of desired proteins were selected by sterile sorting using a Becton Dickinson FACStar Plus at Duke University Cancer Center.

Flow Cytometric Assays and Cell Sorting—MEL cells (106/ml) transfected with LU1, LU3, LU4, or LU5 constructs and grown for >2 weeks in medium containing Geneticin were incubated with anti-Lu8 mAb at 4 °C for 1 h, followed by washing with phosphate-buffered saline, pH 7.4, with 1 g/dl bovine serum albumin, and incubation with FITC-linked secondary Ab, and repeated washing. MEL cells expressing LU31 were incubated with human anti-Lu8. MEL/LU5 cells were incubated with mAb 4P2. Transfected expressing high levels of desired proteins were selected by sterile sorting using a Becton Dickinson FACStar Plus at Duke University Cancer Center.

Stably transfected MEL cells were assayed for their ability to bind soluble laminin by incubation with 3 μg/ml human laminin (Life Technologies, Inc.) at 37 °C for 1 h, followed by staining with rabbit anti-laminin and FITC-conjugated secondary Ab, as described previously (6). Flow cytometric analysis of soluble laminin binding was performed on an Orthocytorox Absolute flow cytometer (Ortho Diagnostic Systems, Raritan, NJ). Each transfected cell line was tested for its ability to bind laminin a minimum of three times, and data shown are representative of the results obtained.

Western Blots—Red cell membrane proteins were prepared as previously described (21, 25, 26). MEL cell transfecants were lysed in Tris-buffered saline with 1% Triton X-100, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (Sigma). All membrane protein lysates were boiled in non-reducing SDS-PAGE sample buffer for 5 min. Five μg of SS red cell membrane proteins and 10 μg of MEL transfected membrane lysates were separated by SDS-PAGE and transferred to nitrocellulose (27). A combination of Mabs 4P2 and anti-Lu8 was used as primary antibody, since no single antibody recognized all the LU deletion mutants expressed. Blots were developed using the ECL system (Amersham Pharmacia Biotech). A complete LU cDNA constructed and inserted into the pcDNA3.1(+) vector as described above was used as the template for all further PCR reactions to create constructs encoding domain deletion mutants. The

2 M. Lacaze, Q. Zen, and M. Telen, unpublished data.
with MEL cell transfectants containing a LU construct or vector only and quantification of cells in each of seven fields along the length of the slide, flow was applied at 20 ml/min for 6 min. After flow, the adherent cells were again counted at the same seven areas of the slide, corresponding to shear stresses ranging from 0.2 to 10 dynes/cm². Results were calculated as the percentage of cells adherent after flow, compared with the number of cells initially seen in each field after the static adherence phase. Critical shear stress is defined as the shear stress at which 50% of the cells become detached. Each transfected cell line was examined at least twice, with concordant results, by this method.

RESULTS

B-CAM/LU Copy Number on the Surface of SS and Normal Red Cells—As previous results have suggested that the B-CAM/LU proteins are overexpressed by SS red cells (6, 28), we measured the direct binding of radiolabeled anti-Lu b mAb to determine by Scatchard analysis the copy number of B-CAM/LU expressed by both SS and normal red cells. Red cells from six randomly selected normal blood donors expressed 1550, 1750, 1800, 1820, 1900, and 1970 molecules of B-CAM/LU per cell, with an average of 1798 ± 6 molecules per cell. The red cells from six patients with sickle cell anemia expressed 2500, 2600, 2700, 2980, 3080, and 4300 molecules of B-CAM/LU per cell, with an average copy number per cell of 3027 ± 662 (p < 0.005 compared with red cells from normal donors). Thus, on average, SS red cells expressed about two-thirds more B-CAM/LU molecules than did normal red cells.

Similar studies using density fractionated red cells were also performed on red cells from four sickle cell disease patients (Table II) selected to represent a range of B-CAM/LU copy numbers per cell. Although the copy number of B-CAM/LU per cell for low density red cells enriched in reticulocytes was about 40–55% more than that seen in the high density fraction, the B-CAM/LU copy number of unfractionated SS red cells was only 2–9% more than that of the high density fraction (Table II), demonstrating that the presence of reticulocytes with higher numbers of B-CAM/LU molecules per cell affected the overall expression of B-CAM/LU by circulating red cells very little. Furthermore, the binding affinities of the radiolabeled anti-Lu b mAb to B-CAM/LU on normal and SS red cells varied only slightly among red cells donors. The range of dissociation constants (Kd) observed was 1.0–5.6 pM. In addition, the antibody also showed similar affinities when reacted with high, low, and unfractionated SS cells (data not shown), suggesting that the conformation of the B-CAM/LU Lu b antigen was similar among cells of different ages.

Role of Lutheran Cytoplasmic Domain on Laminin Binding—In order to determine whether the presence or absence of the cytoplasmic tail lacking from B-CAM affects laminin binding, stably transfected MEL cells expressing B-CAM or LU were tested for their ability to bind soluble laminin by flow cytometric assay (Fig. 1B). Recombinant B-CAM and LU proteins were easily detected by anti-Lu b on the transfectants containing B-CAM or LU cDNAs, but not on cells containing vector alone. Likewise, soluble laminin bound equally well to both B-CAM and LU transfectants but not to the cells transfected with vector alone. This indicates that the cytoplasmic region has little or no effect on laminin binding.

Expression of Lutheran Protein Deletion Mutants—To determine the domain of B-CAM/LU that contributes to laminin binding, we expressed cDNA constructs containing LU cDNA from which had been deleted individual regions encoding each of the five IgSF domains (Fig. 2A). Stably transfected MEL cells expressing these constructs were then examined by immunoblot with anti-Lu b and 4F2 (anti-domain 5) mAbs (Fig. 2B). These studies demonstrated double protein bands arising from expression of all the Lutheran deletion constructs expressed in MEL cells; in each case, the lower band was 62–70 kDa and the
The higher band was 75–85 kDa. We hypothesize that these multiple bands arise from varying degrees of glycosylation in MEL cells. The lower protein bands of the LUΔ1, LUΔ2, LUΔ3, and LUΔ5 mutated proteins were expressed as dominant bands, while the upper ones were expressed weakly; the higher band of MEL/LUΔ5 was barely detectable. However, the two protein bands of LUΔ4 were equally expressed, and the molecular weights of these were lower than that of the other four deletion mutants, consistent with the fact that most of the glycosylation sites are on domain 4 (7, 20). MEL cells transfected with full-length LU cDNA also showed two bands, with molecular mass values of approximately 80 and 95 kDa, suggesting that in MEL cells, LU protein can be more extensively glycosylated than it is in human red cells.

**Binding of Soluble Laminin to Recombinant LU Proteins Lacking Individual IgSF Domains**—Stably transfected MEL cells expressed all the LU domain deletion constructs to a variable but easily detectable degree (MFC = 106.5–165.3, versus MEL/vector MFC = 59.8) when reacted with either anti-Luβ mAb or a human anti-LU8 antibody in flow cytometric assays (Fig. 3A). LU8 recognizes the second IgSF domain of LU (20) and was therefore used for detecting the expression of LU protein lacking IgSF domain 1 (LUΔ1), because anti-Luβ mAb recognizes the first IgSF domain. Binding of LU8 to MEL/vector followed by FITC goat anti-human second Ab resulted in similar “background” fluorescence as that observed with anti-Luβ and FITC goat anti-mouse (data not shown).

The observed ability of LU proteins without IgSF domains 1, 2, 3, or 4 to bind soluble laminin was variable, most likely in large part due to the different levels of protein expression achieved. Nevertheless, MEL cells expressing the recombinant proteins LUΔ1, LUΔ2, LUΔ3, and LUΔ4 all gave distinctive higher fluorescence when reacted with soluble laminin (MFC = 126.6, 100.3, 110.8, and 120.7, respectively) than did MEL/vector (MFC = 84.7) (Fig. 3B). However, the recombinant LU protein without domain 5 (LUΔ5) bound laminin minimally, with MFC of only about 89.6, even though the expression of LUΔ5 protein detectable by anti-Luβ mAb (MFC = 152.4) was much higher than those of LUΔ2 (MFC = 106.5), LUΔ3 (MFC = 117.6), and LUΔ4 (MFC = 139.5). No laminin binding was detected.
when LUΔ5 was expressed at levels similar in terms of MFC to those of MEL/LUΔ2 or MEL/LUΔ3. These data, therefore, suggested that domain 5 of LU protein is critical for laminin binding. In order to demonstrate that Lutheran domain 5 mediates laminin binding, we deleted from LU cDNA sequences encoding the first four IgSF domains. This new construct (LUΔ5) was translated into a 25-kDa protein by MEL cells and was detected by immunoblot using the anti-domain 5 mAb 4F2 (Fig. 2B, lane 9). As shown in Fig. 4, mAb 4F2 bound significantly to MEL/LU5, as did soluble laminin, when examined by flow cytometry.

Adhesion of Transfected MEL Cells Expressing Recombinant LU Proteins to Immobilized Laminin—We also studied the ability of various mutated LU proteins expressed by stably transfected MEL cells to mediate intact cell adhesion to immobilized laminin using a flow chamber assay. The MEL/vector control cell line began to detach at a shear stress of 0.3 dyne/cm², and 50% of cells were detached at a shear stress of 0.7 dyne/cm² (critical shear stress). In contrast, cells expressing LU or LU deletion constructs LUΔ1, LUΔ2, LUΔ3, or LUΔ4 all showed little detachment at shear stress as high as 10 dyne/cm² (Table III). The critical shear stress for LUΔ5 transfected MEL cells, however, was only 2.3 dyne/cm² (Fig. 6), only slightly above that seen with MEL/vector transfectants. In addition, we also tested the adhesion of MEL/LU5 to immobilized laminin under similar flow conditions. As shown in Fig. 5, the critical shear stress of MEL/LU5 attachment to laminin was above 10 dyne/cm², indicating that LU domain 5 alone is sufficient to mediate strong cell adhesion to immobilized laminin.

**DISCUSSION**

Scatchard analysis of 125I-labeled anti-Lub mAb binding to SS and normal red cells allowed us to evaluate red cell expression of B-CAM/LU. Although the expression of B-CAM/LU on SS and normal red cells varied among individuals, on average, SS red cells expressed 67% more molecules of B-CAM/LU than did normal red cells, consistent with our earlier observations using flow cytometric assays (6). The mechanism of B-CAM/LU overexpression on SS cells is not known. While the expression of B-CAM/LU by reticulocytes from sickle cell disease patients was 40–50% higher than that of unfractionated or high density red cells from sickle cell disease patients, expression of B-CAM/LU by dense SS red cells was only minimally different from that of total circulating cells, indicating that overexpression of B-CAM/LU is a characteristic of the total circulating SS red cell population, not only of the reticulocytes. This is also consistent with our previous results using two-color immunostaining and flow cytometry to characterize B-CAM/LU expression on reticulocytes. The affinity of 125I-labeled anti-Lub mAb binding to B-CAM/LU on either unfractionated or fractionated SS cells, as well as normal red cells, did not vary significantly, making it unlikely that the Lub epitope of IgSF domain 1 is activated or altered in sickle cell anemia. However, this does not rule out the possibility of conformational change elsewhere in B-CAM/LU of SS cells, especially at the laminin binding site.

Our previous data had suggested that soluble laminin binding to both normal and SS red cells was proportional to B-CAM/LU copy number (6). However, under conditions of flow, adhesion of SS red cells to immobilized laminin was at least 3-fold stronger than that of normal red cells. Since our current data indicate that the magnitude of the increased expression of B-CAM/LU is less than 2-fold, the increased laminin binding of SS red cells is likely to be due at least in part to factors other than simple overexpression of the laminin receptor. Possible explanations include that increased adhesion might be partially due to an activation process or that increased B-CAM/LU protein expression markedly enhances homotypic aggregation or protein-protein interaction, leading to receptor activation and much stronger cell adhesion.

This study also confirms that both B-CAM and LU proteins can mediate laminin binding. We had previously shown that on Western blot both B-CAM and LU proteins from normal and SS red cells bound soluble laminin and that rB-CAM expressed by MEL cells mediated strong adhesion to immobilized laminin (6). We have now shown that both B-CAM and LU on intact cells mediated both binding of soluble laminin and adhesion to immobilized laminin equally well. Thus, presence or absence of the cytoplasmic tail of LU does not appear to affect laminin binding. The role of the putative SH3 binding domain or of possible serine/threonine phosphorylation of the cytoplasmic domain of LU, as well as the potential functional differences between B-CAM and LU, thus require further study.

*3 M. Udani, Q. Zen, and M. Telen, unpublished data.*

---

**Table III**

| Cell line/construct | Critical shear stress (dyne/cm²) |
|---------------------|--------------------------------|
| MEL/VEC             | 0.7                            |
| MEL/LU              | >10                            |
| MEL/LUΔ1            | >10                            |
| MEL/LUΔ2            | >10                            |
| MEL/LUΔ3            | >10                            |
| MEL/LUΔ4            | >10                            |
| MEL/LUΔ5            | 2.3                            |

**Fig. 4.** Lutheran domain 5 expression and soluble laminin binding by transfected MEL cells (MEL/LU5). A, binding of mAb 4F2 to MEL/LU5 (thick line) and MEL cells expressing vector alone (thin line). B, binding of soluble laminin to MEL/LU5 (thick line) and MEL/vector (thin line).
Expression of LU cDNAs by MEL cells resulted in double bands on Western blot. For the complete LU cDNA, the higher band demonstrated a molecular mass of 95 kDa, even higher than the 85-kDa LU band of red cells. LU deletion mutants also gave two bands. We hypothesize that this phenomenon results from differences in the glycosylation process. Since all five putative N-glycosylation sites are within domains 3 and 4 (8), deletion of which does not affect laminin binding, it is therefore likely that laminin binding to LU is glycosylation-independent.

Studies designed to identify the laminin binding domain of LU by both flow cytometry and flow chamber assays revealed that LU IgSF domain 5 is both critical and sufficient for laminin binding. Only deletion of domain 5 (MEL/LUΔ5) significantly decreased soluble laminin binding to transfected MEL cells, and MEL/LUΔ5 cells detached from immobilized laminin easily at low shear stress (critical shear stress = 2.3 dynes/cm²) under flow conditions. Deletion of all of the other four IgSF domains did not abrogate laminin binding. In addition, MEL/LUΔ5 adhered strongly to immobilized laminin under high shear stress, although, in flow cytometric assays, soluble laminin did not bind to MEL/LUΔ5 as strongly as it bound to MEL/LU. One possibility is that deletion of all first four IgSF domains affected the conformation of the remaining domain 5, altering its ability to bind soluble laminin. A second possibility is that the level of expression of LU5 achieved, which was lower than the level of expression achieved for LU, resulted in lower laminin binding. A third possibility is that additional LU domains may contribute to the localization of laminin to its binding site on domain 5.

So far, structural studies identifying the functional domains of other IgSF proteins, especially IgSF adhesins, have shown the N-terminal IgSF domain(s) as the major heterotypic binding site(s) (13, 14). However, our work shows that a membrane-proximal IgSF domain can also be a major heterotypic binding site. Laminin, a family of heterotrimeric molecules with molecular masses up to 800 kDa, might be imagined to have difficulty accessing such a membrane-proximal domain. Thus, although LU domain 5 alone could bind to both soluble and immobilized laminin directly, it may not be the first contact point for laminin in vivo. A non-critical site might exist elsewhere in B-CAM/LU and act as a preliminary docking site for laminin, thereby facilitating the attachment of laminin to LU domain 5.

With the identification of the active laminin-binding domain of LU, further studies are now feasible to determine the importance of red cell adhesion to laminin in sickle cell vaso-occlusion. We anticipate the generation of inhibitory antibodies or other ligands directed against domain 5, followed by in vivo studies, perhaps in current murine models of sickle cell disease, to determine the physiologic effect of blocking red cell adhesion to laminin. If adhesion to laminin should prove physiologically important in sickle cell anemia, as we suspect, B-CAM/LU may become a new target for therapeutic intervention in this disease. In addition, further investigation into the mechanism of B-CAM/LU overexpression and activation on SS red cells should offer additional opportunities to understand and modulate the vaso-occlusion process.

Acknowledgment—We are indebted to Manisha Udani for technical assistance and many thoughtful suggestions.

REFERENCES
1. Embury, S. H., Hebbel, R. P., Steinberg, M. H., and Mohandas, N. (eds) (1994) in Sickle Cell Disease: Basic Principles and Clinical Practice, pp. 311–328, Raven Press, New York.
2. Sowemimo-Coker, S. O., Meiselman, H. J., and Francis, R. B., Jr. (1989) Am. J. Hematol. 31, 263–265.
3. Hebbel, R., Solovyev, A., Lin, Y., Gui, L., Key, N., and Browne, P. (1997) Sickle Cell Disease in the 21st Century: National Sickle Cell Disease Conference, Washington, D. C., September 15–20, 1997, p. 109.
4. Hillery, C. A., Du, M. C., Montgomery, R. R., and Scott, J. P. (1996) Blood 87, 4879–4886.
5. Engvall, E., and Wewer, U. M. (1996) J. Cell. Biochem. 61, 493–501.
6. Udani, M., Zen, Q., Cottman, M., Leonard, N., Jefferson, S., Daymont, C., Truskey, G., and Telen, M. (1998) J. Clin. Invest. 101, 2550–2558.
7. Campbell, I. G., Foulkes, W. D., Senger, G., Trowsdale, J., Garin-Chesa, P., and Rettig, W. J. (1994) Cancer Res. 54, 5761–5765.
8. Parsons, S. F., Mallinson, G., Holmes, C. H., Houlihan, J. M., Simpson, K. L., Mawby, W. J., Spurr, N. K., Warne, D., Barclay, A. N., and Anstee, D. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5496–5500.
9. Rahuel, C., Le Van Kim, C., Matei, G. M., Cartron, J. P., and Colin, Y. (1996) Blood 88, 1865–1872.
10. Kramer, R. H. (1994) Methods Enzymol. 245, 129–146.
11. Giancotti, F. G. (1996) J. Cell Sci. 109, 1185–1172.
12. Mercurio, A. M., and Shaw, L. M. (1991) Bioessays 13, 169–173.
13. Staunton, D. E., Rustin, M. L., Erickson, H. P., and Springer, T. A. (1990) Cell 61, 243–254.
14. Krickstein, L. B., York, M. R., de Pouverolles, A. R., and Springer, T. A. (1996)
15. Bowen, M. A., Bajorath, J., D'Egidio, M., Whitney, G. G., Palmer, D., Kobarg, J., Atarling, G. C., Siadak, A. W., and Aruffo, A. (1997) Eur. J. Immunol. 27, 1469–1478
16. Karecla, P. I., Green, S. J., Bowden, S., Coadwel, J., and Kilshaw, P. J. (1996) J. Biol. Chem. 271, 30909–30915
17. Newham, P., Craig, S. F., Seddon, G. N., Schofield, N. R., Rees, A., Edwards, R. M., Jones, E. Y., and Humphries, M. J. (1997) J. Biol. Chem. 272, 19429–19440
18. Gao, G. F., Tormo, J., Gerth, U. C., Wyer, J. R., McMichael, A. J., Stuart, D. I., Bell, J. I., Jones, E. Y., and Jakobsen, B. K. (1997) Nature 387, 630–634
19. Moebius, U., Pallai, P., Harrison, S. C., and Reinherz, E. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8259–8263
20. Parsons, S. F., Mallinson, G., Daniels, G. L., Green, C. A., Smythe, J. S., and Anstee, D. J. (1997) Blood 89, 4219–4225
21. Telen, M. J., Palker, T. J., and Haynes, B. F. (1984) Blood 64, 599–606
22. Lowenthal, J. W., Coethesy, P., Touyne, C., Lees, R., MacDonald, H. R., and Nabholtz, M. (1985) J. Immunol. 135, 3988–3994
23. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660–672
24. Ho, S. N., Hunt, H. D., Norton, R. M., Pullen, L. R., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59
25. Laemmli, U. K. (1970) Nature 227, 680–685
26. Dodge, J. T., Mitchell, C., and Hanahan, D. J. (1963) Arch. Biochem. Biophys. 100, 119–130
27. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
28. El Nemer, W., Gane, P., Colin, Y., Bony, V., Rahuel, C., Galacteros, F., Cartron, J. P., and Le Van Kim, C. (1988) J. Biol. Chem. 273, 16686–16693