Increased Adhesion of Erythrocytes to Components of the Extracellular Matrix: Isolation and Characterization of a Red Blood Cell Lipid That Binds Thrombospondin and Laminin

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Red blood cell (RBC) adhesion to the vascular endothelium under physiologic flow is minimal. However, RBC adhesion to the vasculature is increased in several pathologic conditions. For example, erythrocytes infected with Plasmodium falciparum bind to host endothelial cells and sequester in the postcapillary venules of several organs, likely contributing to the fatal complication of cerebral malaria. In sickle cell disease, sickle erythrocytes have increased adhesion to vascular endothelium that may contribute to vaso-occlusive crises. In addition, RBCs obtained from patients with diabetes mellitus show increased adhesion to cultured endothelial cells in vitro. Although the enhanced adhesion of pathologic erythrocytes to endothelial cells has been well described, less is known regarding the mechanism of erythrocytes binding to components of the subendothelial matrix in both physiologic and pathologic conditions.

Adhesive proteins implicated as substrates for RBC binding to the vascular endothelium or subendothelial matrix include thrombospondin (TSP), von Willebrand factor (vWF), fibrinogen, and fibronectin. TSP is present in the subendothelial matrix, plasma, and platelet α storage granules and is released in high local concentration by activated platelets. TSP binds to Plasmodium falciparum-infected RBCs via malarial proteins that are expressed on the surface of the infected RBC and may mediate the adhesion of parasitized RBCs to vascular endothelium. In addition, soluble TSP enhances the adhesion of sickle RBCs to cultured endothelial cells in vitro. Therefore, TSP may be an important adhesive ligand mediating RBC adhesion. vWF is a large, multimeric plasma protein stored in platelets and endothelial cells and released in response to various stimuli. Both in vitro and ex vivo studies support a role for vWF in sickle RBC adhesion to the vascular endothelium. Fibrinogen and fibronectin may also promote sickle RBC-endothelial cell adhesion, but the observed effect has been minimal. The true role of these potential plasma and/or extracellular matrix adhesive ligands in the interaction of erythrocytes with the vessel wall remains unknown.

Potential adhesion molecules on the erythrocyte surface that may mediate interactions with the vessel wall include the integrin αβ, CD36 (Glycoprotein IV), and sulfated glycolipids. The integrin αβ is a receptor for fibronectin and endothelial cell vascular cellular adhesion molecule (VCAM-1), and is present on both normal and sickle reticulocytes. CD36 is a nonintegrin adhesive receptor located on the surface of endothelial cells and platelets and has been recently described on both sickle and normal reticulocytes. CD36 is a receptor for TSP and collagen and is postulated to play a role in the adhesion of sickle erythrocytes to the vascular endothelium via soluble TSP, which may bridge CD36 receptors present on both cell types. Sulfated glycolipids are present in the membranes of RBCs, platelets, brain tissue, and other organ tissues and have adhesive properties. TSP, vWF, and laminin have all been shown to bind sulfated glycolipids. This binding is specific, saturable, and is not because of low affinity ionic interactions since similar binding does not occur to other anionic phospholipids, gangliosides, or cholesterol 3-sulfate.

To gain insight into the potential interactions of erythrocytes with components of the subendothelial matrix, we
sought to identify specific purified extracellular matrix adhesive proteins that preferentially bind RBCs under conditions of controlled flow. Because the enhanced binding of RBCs to the vascular endothelium has been described in sickle cell disease, sickle RBCs served as a pathologic model in this investigation. Both normal and sickle RBCs were found to have increased adhesion to the purified extracellular matrix proteins TSP and laminin. In addition, the adhesion of RBCs to TSP and laminin was inhibited by anionic polysaccharides. Because sulfated glycolipids are known to bind to TSP and laminin, and this interaction is inhibited by anionic polysaccharides, we hypothesized that a RBC membrane lipid(s) contributes to the adhesive properties of RBCs. Therefore, we isolated and characterized an acidic lipid(s) from normal and sickle erythrocytes that binds the adhesive proteins TSP and laminin.

MATERIALS AND METHODS

Reagents. Chondroitin sulfates A and C were purchased from CalBiochem (San Diego, CA). Heparan sulfate (Bovine intestinal mucosa), keratan sulfate (Bovine cornea), dermatan sulfate (Porcine skin), dextran sulfates (average MW 500 kD and MW 5 kD), fucoidan (Fucus vesiculosus), gangliosides (Type II, Bovine brain, 15% N-acetyl-neuraminic acid), cholesterol 3-sulfate, M199 tissue culture medium, azure A, and neuraminidase were obtained from Sigma (St. Louis, MO). Sepharose 4B, heparin Sepharose CL-6B, and DEAE-Sepharose 6B were obtained from Pharmacia (Piscataway, NJ). Human laminin, human fibronectin, human vitronectin, and normal and sickle RBCs served as a pathologic model in this investigation. Both normal and sickle RBCs were found to have increased adhesion to the purified extracellular matrix proteins TSP and laminin. In addition, the adhesion of RBCs to TSP and laminin was inhibited by anionic polysaccharides. Because sulfated glycolipids are known to bind to TSP and laminin, and this interaction is inhibited by anionic polysaccharides, we hypothesized that a RBC membrane lipid(s) contributes to the adhesive properties of RBCs. Therefore, we isolated and characterized an acidic lipid(s) from normal and sickle erythrocytes that binds the adhesive proteins TSP and laminin.

RBC preparation. After obtaining informed consent, blood samples were collected from patients with hemoglobin SS disease, patients with high reticulocyte counts but no hemoglobinopathy, and healthy controls in 3.8% sodium citrate (1:9). The RBCs were separated from platelet rich plasma by centrifugation (180 g, 20 minutes, 22°C), washed three times with a citrate/albumin/saline buffer, and resuspended at a 2% hematocrit in M199 serum-free cell culture medium (SFM) with 0.2% bovine serum albumin (BSA) for flow adhesion studies.

Dynamic flow adhesion assay. RBC-adhesive ligand interactions were assayed using a parallel plate perfusion chamber that was developed in the laboratories of Drs McIntire and Moake at Rice University (Houston, TX).27 The flow experiments were performed at 37°C using an air curtain incubator and conducted on an inverted phase microscope to permit direct visualization during the entire flow study. The purified adhesive protein of interest was coated on the surface of a 35-mm2 tissue culture plate (2 µg/cm2, 60 minutes, 22°C) followed by insertion of a gasket and the flow chamber and secured by a low pressure vacuum around the outer perimeter. An initial rinse period of 3 to 5 minutes with SFM (that contains 0.2% BSA) served to block the plates similar to incubation of plates with a modified SFM containing 2% BSA for 60 minutes, 22°C before the flow adhesion assay (data not shown). After rinsing with SFM, washed RBCs suspended in SFM (hematocrit 2%) were perfused through the flow chamber and interacted with the adhesive surface under the controlled flow forces of 1 dyne/cm2 for 4 minutes. Following a 5 to 10 minute rinse period, the number of adherent RBCs per unit surface area were counted by direct microscopic visualization of the adhesive surface in a previously calibrated grid of known area in 4 to 6 random areas near the center of the flow surface. For inhibition experiments, the inhibitor was added to the red cell suspension for 30 minutes at 37°C before the initiation of the flow experiment.

Lipid preparation. Washed RBC ghosts (100 to 200 mL) were prepared by hypotonic lysis (7.5 mmol/L NaH2PO4, 1 mmol/L EDTA, pH 7.5) of washed RBCs as previously described.28 Contaminating leukocytes and platelets are resistant to lysis and were removed by discarding the small thick white pellet that is underneath the fluffy red cell ghosts after each centrifugation. Washed RBC ghosts were extracted (Ghosts: H2O:MeOH:CHCl3, 1:3:10.5, vol/vol/vol/vol) for 30 minutes at 22°C with stirring. After drying in a rotary evaporator, the crude lipid extract was treated with alkaline methanolysis (0.2 mol/L NaOH in MeOH, 37°C, 30 minutes) to degrade ester linked phospholipids, then neutralized with 0.9 volume of 0.2 mol/L HCl in MeOH and dried to a syrup.30 The extract was desalted by dialysis against distilled H2O, lyophilized, and resuspended in CHCl3:MeOH:H2O (30:60:8). The extract was then fractionated by anion exchange chromatography on a DEA-Sepharose 6B column equilibrated with CHCl3:MeOH:H2O (30:60:8). Neutral lipids, sphingomyelin, and cholesterol do not bind to the column and are recovered in the flow through the acidic lipids were eluted with stepwise increasing concentrations of NH4HCO3 (0.01 mol/L to 0.5 mol/L) in MeOH. Lipids were resolved by thin layer chromatography (TLC) on HPTLC aluminum silica gel 60 sheets (E. Merck) with CHCl3:MeOH:0.2% CaCl2 (60:35:7) and visualized by spraying with 10% H2SO4. Preparative TLC was performed on silica gel 150A (Whatman, Fairfield, NJ). Sulfated glycolipids were quantitated by azure A analysis as previously described.11

Solid phase binding assay. Lipid, in appropriate solvent, was coated on the base of microtiter wells and the solvent evaporated. For laminin binding, all lipids (0.5 µg/well) were coated in microtiter wells with the addition of the carrier lipids phosphatidylycholine (50 ng/well) and cholesterol (50 ng/well). After blocking with Tris-buffered saline containing 1% BSA and 5 mmol/L CaCl2 (Binding buffer), TSP (0.3 µg/well), or laminin (0.5 µg/well) in Binding buffer was added to the cells and incubated at 4°C overnight. After blocking again with Binding buffer, bound protein was quantitated by ELISA using anti-TSP MoAb C6.7 or antilaminin MoAb 4E10 followed by alkaline phosphatase conjugated goat antimouse IgG. Immune complexes were detected using the ELISA amplification system (GIBCO).
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RESULTS

Adhesion of erythrocytes to purified extracellular matrix proteins. To determine specific adhesive proteins that bind RBCs, the adhesion of normal hemoglobin AA red blood cells (AA-RBCs) and hemoglobin SS red blood cells (SS-RBCs) to several purified extracellular matrix proteins was tested under the conditions of controlled flow at a wall shear stress of 1 dyne/cm². These forces are similar to forces found in the postcapillary venule, a proposed site of vaso-occlusion in malaria and sickle cell disease. There was only minimal adhesion of AA-RBCs (2 to 4 RBCs/mm²) to the immobilized adhesive proteins: vWF (Fig 1), fibronectin and vitronectin (data not shown). In addition, the adhesion of SS-RBCs to the immobilized endothelial cell supernatant, containing increased levels of HMW polysaccharides as potential inhibitors. As shown in Fig 1, the glycosaminoglycan chondroitin sulfate A inhibited more than 75% of SS-RBC adhesion to immobilized TSP. However, chondroitin sulfate C, which differs from chondroitin sulfate A only in the position of sulfation of N-acetyl-β-D-galactosamine, caused significantly less inhibition. Dermatan sulfate, which differs from chondroitin sulfate A in the substitution of glucuronic acid with iduronic acid, also had less inhibitory activity compared to chondroitin sulfate A. The glycosaminoglycans keratan sulfate and heparan sulfate, which possess charge densities similar to the chondroitin sulfates, caused only mild to moderate inhibition. In addition, fucoidan and heparin, which have higher charge densities than the chondroitin sulfates, were only moderately inhibitory, even at high concentrations. This suggests that the inhibition is not solely caused by charge interactions. When the anionic polysaccharide, dextran sulfate was added to the sickle RBCs, the HMW form (MW 500 kD) caused TSP or laminin receptors that have been reported on the RBC surface. To examine whether either of these RBC membrane components were involved in mediating cytoadhesion to immobilized TSP, OKM-5, a murine anti-CD36 MoAb that blocks TSP binding to CD36, and the TSP peptide CSVTCG, which blocks the interaction of TSP with CD36, were tested for their ability to inhibit SS-RBC adhesion to immobilized TSP. Both OKM-5 and CSVTCG failed to inhibit the binding of SS-RBCs to immobilized TSP under conditions of controlled flow (Fig 2).

To determine whether sulfated glycolipids were responsible for the adhesion of RBCs to TSP or laminin, we performed SS-RBC adhesion studies using a number of anionic polysaccharides as potential inhibitors. As shown in Fig 3, the glycosaminoglycan chondroitin sulfate A inhibited more than 75% of SS-RBC adhesion to immobilized TSP. However, chondroitin sulfate C, which differs from chondroitin sulfate A only in the position of sulfation of N-acetyl-β-D-galactosamine, caused significantly less inhibition. Dermatan sulfate, which differs from chondroitin sulfate A in the substitution of glucuronic acid with iduronic acid, also had less inhibitory activity compared to chondroitin sulfate A. The glycosaminoglycans keratan sulfate and heparan sulfate, which possess charge densities similar to the chondroitin sulfates, caused only mild to moderate inhibition. In addition, fucoidan and heparin, which have higher charge densities than the chondroitin sulfates, were only moderately inhibitory, even at high concentrations. This suggests that the inhibition is not solely caused by charge interactions. When the anionic polysaccharide, dextran sulfate was added to the sickle RBCs, the HMW form (MW 500 kD) caused

followed by horseradish peroxidase conjugated rabbit antirat mouse IgG. Bound TSP was visualized by chemiluminescence using the ECL detection system (Amersham, Arlington Heights, IL).
Fig 3. Sickle cell adhesion to TSP is inhibited by chondroitin sulfate A (CSA) and high molecular weight (MW) dextran sulfate (DS). Washed SS-RBCs were incubated with control buffer (None, N = 10) or 500 μg/mL of indicated anionic polysaccharide for 30 minutes at 37°C before the flow adhesion assay. Treated RBCs were perfused through flow chambers coated with immobilized TSP as described in Fig 1. CS-A, CSA (N = 10); CS-C, chondroitin sulfate C (N = 4); DrS, dermatan sulfate (N = 5); KS, keratan sulfate (N = 3); HS, heparan sulfate (N = 5); Fuc, fucoidan (N = 7); Hep, heparin (N = 4); DS-Hi, dextran sulfate, MW 500 kD (N = 12); and DS-Lo, dextran sulfate, MW 5 kD (N = 3).

A marked inhibition of the adhesion of sickle RBCs to immobilized TSP. However, the lower molecular weight dextran sulfate (MW 5 kD) did not significantly inhibit adhesion of SS-RBCs to immobilized TSP, even at high concentrations. A dose-response curve for increasing concentrations of high MW dextran sulfate revealed a 50% inhibitory concentration of approximately 0.1 μg/mL (Fig 4A). The dose-response curve for chondroitin sulfate A revealed a 50% inhibitory concentration that was approximately 10-fold higher, in the 5 to 10 μg/mL range (Fig 4B). Thus, the enhanced adhesion of sickle RBCs to immobilized TSP was inhibited, in a dose-dependent manner, by the anionic polysaccharides chondroitin sulfate A and HMW dextran sulfate to a greater degree than other anionic polysaccharides with similar structure and/or charge density.

Because sulfated glycolipids also bind to laminin, the effect of anionic polysaccharides on the adhesion of sickle RBCs to immobilized laminin was tested. The addition of 500 μg/mL HMW dextran sulfate inhibited the adhesion of sickle RBCs to immobilized laminin by 60 ± 5% (mean ± SE, N = 10). The dose response curve with increasing amounts of HMW dextran sulfate revealed a 50% inhibitory concentration that was approximately 50 μg/mL (Fig 4C). This 50% inhibitory concentration for HMW dextran sulfate is approximately 50-fold higher than that required for the same level of inhibition for the adhesion of sickle RBCs to TSP.

Isolation and characterization of a RBC lipid that binds TSP and laminin. Because these data were consistent with RBC membrane sulfated glycolipids mediating binding to TSP and laminin, TSP-binding lipids from normal RBCs and sickle RBCs were isolated and characterized. Lipids were extracted from washed red cell ghosts from either normal healthy controls or patients with sickle cell disease. Follow-
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Concentrations of thrombopsonin on DEAE-Sepharose. Acidic lipids were eluted with increasing concentrations of NH_4HCO_3 in MeOH: 0.01 mol/L (N1), 0.02 mol/L (N2), 0.05 mol/L (N3), 0.1 mol/L (N4), and 0.5 mol/L (N5). The sulfated glycolipid content of each fraction was quantitated by azure A assay. Lipids separated by TLC were assayed for TSP binding activity with bound TSP detected by chemiluminescence as described in Materials and Methods (\( I \), TSP Binding). In both AA-RBC and SS-RBC lipid preparations, TSP-binding lipid fractions were recovered coincident with or just after peak sulfated glycolipid fractions (azure A analysis). The results are depicted as the mean \( \pm \) SE of \( N = 5 \) AA-RBC and \( N = 5 \) SS-RBC preparations.

**Fig 5.** TSP binding and sulfatide content of lipid fractions from DEAE-Sepharose column. Following alkaline methanolysis and desalting by dialysis, lipid extracts from AA-RBC, (A) AA lipid or SS-RBCs (B) SS lipid were fractionated by anion exchange chromatography on DEAE-Sepharose. Acidic lipids were eluted with increasing concentrations of NH_4HCO_3 in MeOH: 0.01 mol/L (N1), 0.02 mol/L (N2), 0.05 mol/L (N3), 0.1 mol/L (N4), and 0.5 mol/L (N5). The sulfated glycolipid content of each fraction was quantitated by azure A analysis (\( \bullet \), Sulfatide content). TSP binding to immobilized lipid was detected by a solid phase binding assay as described in Materials and Methods (\( I \), TSP Binding). In both AA-RBC and SS-RBC lipid preparations, TSP-binding lipid fractions were recovered coincident with or just after peak sulfated glycolipid fractions (azure A analysis). The results are depicted as the mean \( \pm \) SE of \( N = 5 \) AA-RBC and \( N = 5 \) SS-RBC preparations.

These findings were obtained from both normal and sickle RBC lipid preparations. Further biochemical analysis of the purified TSP-binding lipid (Band 2) revealed that TSP binding was stable to base treatment that degrades phosphoglycerolipids, but spares most sulfated glycolipids and gangliosides (Fig 7). TSP binding to the purified lipid was also stable to neuraminidase treatment that destroys sialic acid residues in gangliosides. However, TSP binding was labile to solvolysis with acid that selectively desulfates most sulfated glycolipids, but spares most gangliosides. These properties were similar to the sulfatide control (Fig 7). To determine if TSP binding to the purified lipid was primarily because of charge interactions, TSP binding to other acidic or similarly charged lipids was tested. As shown in Fig 7, TSP did not bind to either purified gangliosides or cholesterol 3-sulfate, which have a similar charge as sulfatides.

To determine if the adhesive protein laminin also bound to the purified RBC lipid, soluble laminin was incubated with the immobilized purified lipid in the solid-phase binding assay. As shown in Fig 8, soluble laminin bound to the purified RBC lipid. In addition, laminin did not bind to the acidic lipids, gangliosides, or to cholesterol 3-sulfate. Therefore, the binding of laminin to the purified RBC lipid was not solely because of charge interactions.

To further characterize the purified lipid, the effect of anionic polysaccharides on TSP binding was tested. TSP binding to purified RBC lipid was inhibited the most by HMW dextran sulfate, similar to previous findings for the inhibition of intact RBCs to immobilized TSP under conditions of flow (Fig 9). The two gangliosides with the highest charge density, fucoidan and heparin, caused approximately 60% inhibition of TSP binding to the purified lipid, suggesting that a charge effect may be important in this more purified system.

**DISCUSSION**

The enhanced adhesion of erythrocytes to purified, immobilized TSP and laminin under conditions of flow (1 dyne/cm²) has not previously been reported. The level of adhesion was further increased in erythrocytes obtained from patients with sickle cell disease but not from patients with high reticulocyte counts but no hemoglobinopathy. This suggests that the increased adhesion of sickle RBCs was not simply because of the increased numbers of reticulocytes found in...
Fig 7. TSP binding to the purified RBC lipid is stable to neuraminidase treatment and acid-labile. Microtiter wells were coated (0.15 μg lipid/well) with sulfatide (Sulfatide), purified RBC lipid (Band 2, Lipid), gangliosides (Gangl), cholesterol 3-sulfate (Ch-3-S), and treated with 0.2 mol/L NaOH in MeOH (NaOH, previously treated during lipid preparation), 0.06 mol/L HCl in MeOH, 4 hours, 22°C (HCl), or 0.2 U/mL neuraminidase in 0.05 mol/L NaAcetate, 0.15 mol/L NaCl, 0.009 mol/L CaCl₂, pH 5.5, 16 hours, 22°C (Neuraminidase). TSP bound to the immobilized treated lipid was detected as described in Fig 5.

sickle cell disease. Sugihara et al⁶ have reported enrichment of sickle reticulocyte adhesion to immobilized, purified TSP under static conditions, but did not study total RBC adhesion nor flow conditions. In contrast, the adhesion of RBCs to other purified adhesive extracellular matrix proteins was minimal, including vWF, fibronectin, and vitronectin. The high level of adhesion of RBCs to purified TSP and laminin suggests that binding of erythrocytes to exposed TSP or laminin in the vasculature may play an important role in the evolution of vascular pathology.

The enhanced adhesion of erythrocytes to TSP is in agreement with previous reports that soluble TSP, but not vWF, vitronectin, fibrinogen, or fibronectin, increased the adhesion of sickle RBCs to cultured endothelial cells in vitro.⁶ This suggests that soluble TSP can participate in the binding of sickle RBCs to intact vascular endothelium. In addition, there is evidence that TSP mediates the adhesion of Plasmodium falciparum-infected RBCs to vascular endothelium in vitro, probably via the expression of parasitic proteins on the infected erythrocyte membrane.⁴⁷,¹⁶,¹³ In several studies, the supernatant derived from stimulated endothelial cell cultures (rich in HMW vWF) increased the adhesion of SS-RBCs to endothelial cells.⁵ In addition, the treatment of ex vivo mesocecum with desmopressin, known to increase circulating levels of vWF, increased the adhesion of SS-RBCs.¹¹ The effect was blocked by anti-vWF antibodies, supporting a role for vWF in SS-RBC adhesion. However, the data in this study do not support a role for erythrocyte adhesion to immobilized purified vWF or endothelial cell supernatant rich in HMW vWF. Because the binding characteristics of adhesive ligands can vary depending on the shear force and whether the ligand is in solution phase versus the solid phase,¹⁹,²⁰ the above discrepancies may be due to the fact that the previous studies entailed soluble vWF and intact endothelium whereas this system used purified immobilized vWF.

Recently, two groups have proposed that CD36 on the surface of sickle reticulocytes plays a role in TSP mediated adhesion of SS-RBCs to endothelium.⁵,⁶ However, in the present study, both the blocking MoAb OKM5 directed against CD36, and the blocking TSP peptide, CSVTCG, failed to inhibit the adhesion of SS-RBCs to immobilized TSP under conditions of flow. This difference in results...
could be because of the fact that the other studies were primarily evaluating RBC adhesion to endothelial cells via soluble TSP, whereas our study involved direct RBC binding to immobilized TSP. However, Sugihara et al. did report that both OKM-5 and CSVTCG inhibited the adhesion of sickle reticulocytes to immobilized TSP under static conditions. However, this discrepancy in results may be caused by the fact that this group limited their data to the reticulocyte subpopulation of RBCs, known to contain CD36, or to the static conditions of their assay system.6 Because CD36 is primarily found on the reticulocyte subpopulation of RBCs, the lack of adhesion of high reticulocyte controls to TSP further argues against the hypothesis that CD36 mediates the enhanced adhesion of sickle RBCs to immobilized TSP at a shear stress of 1 dyne/cm². However, the possibility that reticulocytes in certain disease states, such as sickle cell disease, have an altered or abnormally exposed form of CD36 has not been ruled out by this study. Overall, these data suggest a limited role for CD36 as the etiology of the enhanced adhesion of sickle RBCs to immobilized TSP under the conditions of this study. However, our studies have not ruled out the possibility that CD36 may play a role in erythrocytes binding to soluble TSP, TSP bound to endothelial cells, the reticulocyte subpopulation of RBCs binding to immobilized TSP, or RBC binding that may occur under other types of shear forces.

The data in this study provide evidence that an acidic RBC membrane glycolipid(s) likely participates in erythrocyte adhesion to TSP and laminin. Sickle RBC binding to immobilized TSP was inhibited by HMW dextran sulfate and chondroitin sulfate A whereas sickle RBC binding to immobilized laminin was partially inhibited by HMW dextran sulfate. These findings are similar to previous reports that the binding of TSP and laminin to purified sulfated acid is differentially inhibited by anionic polysaccharides.3,22,25 In agreement, an acidic RBC lipid(s) that binds TSP and laminin was identified and purified from both normal and sickle RBC membranes. This lipid had biochemical properties consistent with a sulfated glycolipid, including stability to base and neuraminidase treatment, acid lability, and detection by azure A analysis. In addition, TSP binding to the immobilized purified lipid was inhibited by HMW dextran sulfate, similar to previous findings for the inhibition of intact sickle RBCs binding to immobilized TSP under the conditions of flow. However, chondroitin sulfate A did not preferentially inhibit the binding of TSP to the immobilized lipid. This may be explained by alterations in conformation of either TSP, because it is soluble instead of being immobilized in this adhesion assay, or the lipid, because it is now purified and no longer in the native RBC lipid bilayer. Alternatively, the purification process may have altered some of the properties of this lipid, such as by inadvertent oxidation or loss of associated membrane components.

The TSP- and laminin-binding lipid purified in this study was found in preparations from both normal and sickle erythrocytes. This implies that this lipid is normally present on RBCs, but may be modified or abnormally exposed on the sickle erythrocyte surface. The sickle red cell membrane has increased levels of oxidative damage3,4,14 that could alter the adhesive properties of this lipid. In addition, in sickle cell disease the typical lipid bilayer is disrupted with loss of the normal phospholipid asymmetry.4 This disruption of the lipid membrane bilayer could expose or alter the conformation of native RBC membrane lipids and proteins, thereby modulating adhesive properties of these membrane components. We propose that the TSP- and laminin-binding RBC lipid purified in this study contributes to the low level binding of normal RBCs to TSP and laminin detected and that this lipid is abnormally exposed or modified on sickle RBCs, thereby participating in the enhanced adhesion of sickle RBCs to TSP and laminin.

In this study, we have shown that erythrocytes have increased adhesion to the purified adhesive molecules TSP and laminin under conditions of controlled flow and that an acidic lipid(s) with biochemical properties consistent with a sulfated glycolipid on the RBC surface likely contributes to this adhesion. One model for erythrocyte adhesion to vascular endothelium is that a multivalent adhesive ligand, such as TSP or laminin, binds to sulfated glycolipids on the RBC surface and links the RBC to the subendothelial matrix or another adhesive molecule on the vascular endothelium. In this way, sulfated glycolipids may be important adhesive molecules that are functionally relevant to RBC adhesion. The enhanced adhesive properties of sickle RBCs may be caused by lipids naturally present in the red cell membrane that have been biochemically altered or abnormally exposed. Further characterization of this lipid(s) will be required to determine whether this lipid(s) is abnormally exposed or modified on RBCs in pathologic states, thereby participating in the enhanced adhesion of RBCs to adhesive proteins and potentially in the pathogenesis of vaso-occlusive diseases.

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