Human Vascular Endothelial Cell Adhesion Receptors for *Plasmodium falciparum*-infected Erythrocytes: Roles for Endothelial Leukocyte Adhesion Molecule 1 and Vascular Cell Adhesion Molecule 1

By Christian F. Ockenhouse,* Tatsuya Tegoshi,‡ Yoshimasa Maeno,‡ Christopher Benjamin,§ May Ho,‖ Khin Ei Kan,¶ Ye Thway,** Kyan Win,**Masamichi Aikawa,‡ and Roy R. Lobb§

*From the *Immunology Branch, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington DC 20307; the ‡Institute of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106; §Biogen, Inc., Cambridge, Massachusetts 02142; the ‖Hospital for Tropical Diseases, Mahidol University, 10400 Bangkok, Thailand and the Department of Microbiology and Infectious Diseases, University of Calgary, Calgary T2N 4N1 Canada; the ¶Department of Medical Research, Yangoon, Myanmar; and **No. 2., Military Hospital, Yangoon, Myanmar*

Summary

The clinical complications associated with severe and cerebral malaria occur as a result of the intravascular mechanical obstruction of erythrocytes infected with the asexual stages of the parasite, *Plasmodium falciparum*. We now report that a primary *P. falciparum*-infected erythrocyte (parasitized red blood cell [PRBC]) isolate from a patient with severe complicated malaria binds to cytokine-induced human vascular endothelial cells, and that this adhesion is in part mediated by endothelial leukocyte adhesion molecule 1 (ELAM-1) and vascular cell adhesion molecule 1 (VCAM-1). PRBC binding to tumor necrosis factor α (TNF-α)-activated human vascular endothelial cells is partially inhibited by antibodies to ELAM-1 and ICAM-1 and the inhibitory effects of these antibodies is additive. PRBCs selected in vitro by sequential panning on purified adhesion molecules bind concurrently to recombinant soluble ELAM-1 and VCAM-1, and to two previously identified endothelial cell receptors for PRBCs, ICAM-1, and CD36. Post-mortem brain tissue from patients who died from cerebral malaria expressed multiple cell adhesion molecules including ELAM-1 and VCAM-1 on cerebral microvascular endothelium not expressed in brains of individuals who died from other causes. These results ascribe novel pathological functions for both ELAM-1 and VCAM-1 and may help delineate alternative adhesion pathways PRBCs use to modify malaria pathology.

Cell adhesion molecules (CAM)[1] on postcapillary human endothelium play important roles in the recruitment of white blood cells to tissue sites of acute and chronic inflammation (for review see reference 1). Endothelial CAMs mediate the initial phase of white cell adhesion preceding diapedesis and migration of white blood cells to sites of tissue injury and immunologic challenge. Cytokines such as TNF-α and IL-1 regulate and induce the expression of distinct endothelial CAMs that bind specific counter receptors on human neutrophils, monocytes, lymphocytes, eosinophils, and basophils. Endothelial CAMs belong to two distinct gene families. The Ig-like supergene family of proteins includes three molecules expressed on endothelium, intercellular adhesion molecule (ICAM)-1 (2, 3), ICAM-2 (4) and vascular cell adhesion molecule (VCAM)-1 (5). Endothelial CAMs ELAM-1 (also designated E-selectin) (6, 7) and GMP140 (8) are included in a family of molecules called selectins. Selectins are structurally related proteins with homology to mammalian lectins, epidermal growth factor, and complement regulatory proteins. In addition to their function in normal physiologic cell–cell immune recognition and embryogenesis, CAMs appear to play important roles in various pathological processes including rhinovirus infection mediated by ICAM-1 (9–11) and ICAM-1–dependent adhesion to erythrocytes infected with *Plasmodium falciparum* (12–15).

[1] *Abbreviations used in this paper:* CAM, cell adhesion molecule; ELAM-1, endothelial leukocyte adhesion molecule 1; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule 1; PRBC, parasitized red blood cell; TSP, thrombospondin; VCAM-1, vascular cell adhesion molecule 1.

Unlike the response of white blood cells to inflammatory stimuli, human erythrocytes under normal physiological conditions do not bind to postcapillary endothelium. However, intraerythrocytic parasitization by *P. falciparum* malaria parasites is characterized by the adhesion of infected erythrocytes (parasitized red blood cells [PRBCs]) to endothelium in specific tissues and organs such as the brain precipitating cerebral malaria and other complicated forms of the disease. The sequestration of PRBCs within capillaries and postcapillary venules of specific host tissues is dependent upon specific receptor–ligand interactions between molecules expressed on the endothelial cell surface and parasitized red cell counter receptors. Two cell surface molecules CD36 (16–18) and ICAM-1 (12–15) and the extracellular matrix protein thrombospondin (TSP) (19) bind PRBCs from naturally acquired infections and are immunocytochemically detected on microvascular endothelium of brain tissue from patients who have died from cerebral malaria (20).

Pathological functions of VCAM-1, a member of the Ig gene superfamily, and members of the selectin gene family have not been previously described. In this report, we describe the adhesion of malaria-infected erythrocytes to two additional CAMs, ELAM-1, and VCAM-1, and discuss clinical implications ensuing from multifunctional PRBC binding to divergent receptor molecules.

Materials and Methods

Proteins and Antibodies. Recombinant soluble (rs)ELAM-1 and rsVCAM-1 lacking the transmembrane and cytoplasmic domains, were constructed and stably expressed in CHO cells as previously described (21, 22). rsELAM-1 and rsVCAM-1 were purified by immunoaffinity chromatography and were fully functional when immobilized onto plastic tissue culture plates. CD36 was purified from the membranes of human platelets (23), and ICAM-1 was purified from the detergent-solubilized membranes of Reed-Sternberg cells and purified by immunoaffinity chromatography with ICAM-1 mAb 84H10 as previously described (24).

Polyclonal rabbit antisera to ELAM-1 (R347) was obtained by immunization of rabbits with purified rsELAM-1 using conventional methods. mAb BB11 (anti-ELAM-1) was prepared as previously described (25). mAbs 84H10 (anti-ICAM-1) and 4B9 (anti-VCAM-1) were gifts from Drs. S. Shaw (National Cancer Institute, National Institutes of Health) and J. Harlan (University of Washington) respectively.

Endothelial Cells. Human umbilical vein endothelial cells (HUVECs) were isolated and cultured as described (26). HUVECs (10^5/well) were cultured and grown to confluence in 24-well tissue culture plates before adhesion assays. TNF-α (200 U/ml) (Advanced Biotechnologies, Inc., Columbia, MD) was added to cultures 4 or 24 h before adhesion assays.

Parasites. Four wild-type primary parasite isolates were obtained from patients infected with *P. falciparum* from the western province of Kanchanaburi, Thailand and adapted to continuous culture in vitro. A strategy to enrich for a subpopulation of parasitized erythrocytes which bind to multiple adhesion receptors by sequential panning was developed from one such patient (CY36) and is illustrated in Fig. 1. Purified rsELAM-1 (10 μg/ml), rsVCAM-1 (10 μg/ml), ICAM-1 (70 ng/ml), and CD36 (1 μg/ml) were adsorbed overnight at 4°C to tissue culture dishes (model 1007; Falcon Labware, Oxnard, CA). To reduce nonspecific binding, the protein-coated plates were blocked for 30 min with PBS/1% BSA before the adhesion assay. Synchronized PRBCs in continuous culture were enriched for the tropozoite/schizont stage of development by sedimentation on gelatin. The CY36 PRBCs were washed, adjusted to 1% hematocrit in RPMI 1640 medium, and added to the ELAM-1–coated plate. After 60 min incubation, the unattached erythrocytes were removed by aspiration. Plates were rinsed gently three times with RPMI 1640, and the PRBCs remaining attached to the ELAM-1–coated plate were vigorously pipetted several times with tissue culture medium to detach the ELAM-1–binding PRBCs. The PRBCs were concentrated by centrifugation and added to a second plate coated with rsVCAM-1. This method of PRBC binding, washing, and detachment of bound PRBCs was repeated with ICAM-1– and CD36–coated plates. The sequential panning on plates coated with ELAM-1, VCAM-1, ICAM-1, and CD36 enriched for a subpopulation of PRBCs (CY36.1B4) that were subsequently cloned, expanded, and propagated in vitro by continuous culture.

Adhesion Assay. PRBC binding to HUVEC monolayers and to purified proteins was done as described previously (16, 27). Briefly, mature developmental stage PRBCs enriched by gelatin sedimentation were resuspended to 1% hematocrit and added to 24-well tissue culture plates (400 μl/well) containing HUVECs. In some experiments, polyclonal ELAM-1 (R347, 1/200 dilution) antibodies and ELAM-1 (BB11), VCAM-1 (4B9), and ICAM-1 (84H10) mAbs (50 μg/ml) were incubated with HUVECs for 30 min at room temperature before adding PRBCs. After 60 min, unattached erythrocytes were aspirated and cells were fixed with 2% glutaraldehyde and stained with Giemsa.

In binding assays using purified proteins, CAMs were adsorbed to plastic tissue culture plates overnight at 4°C, blocked with PBS/1% BSA and PRBCs (2% hematocrit) added for 60 min at room temperature. In inhibition assays, antibodies were incubated on protein-coated plates 30 min before adding PRBCs. Unattached erythrocytes were removed and the plates were rinsed in RPMI 1640, fixed with 2% glutaraldehyde, and stained with Giemsa. Results were expressed as the number of PRBCs bound per 100 HUVECs or PRBCs bound per mm^2 surface area.

Immunofluorescence Detection of ELAM-1 and VCAM-1. Postmortem brain tissues from patients who died of complications from cerebral malaria in Myanmar (Burma) were fixed in periodate-lysine-paraformaldehyde (PLP) (28), embedded in OCT compound (Lab-Tek, Miles Laboratories, Inc., Naperville, IL) and snap-frozen in iso-pentane cooled in liquid nitrogen. Sections (7-μm thickness) were air-dried and fixed with acetone at 4°C for 10 min. Control samples of brain tissue from post-mortem autopsies were from individuals who died from causes other than malaria. An indirect immunofluorescence assay was used to detect ELAM-1, VCAM-1, ICAM-1, CD36, and TSP expression on the surface of microvascular endothelium. Nonspecific binding was reduced by incubating sections for 1 h with 10% normal goat serum in 0.05 M Tris-buffered saline (pH 7.6). All incubations were performed in an humidified chamber at room temperature. Tissue sections preincubated with the primary antibody (ELAM-1, polyclonal rabbit; VCAM-1, Mab 4B9; ICAM-1, Mab CL203; CD36, polyclonal rabbit; and TSP, polyclonal rabbit) for 1 h were rinsed with Tris-buffered saline and incubated for 1 h with FITC-conjugated anti-rabbit IgG or anti-mouse IgG (Cappel Laboratories, Malvern, PA) diluted 1:200. Sections were rinsed and mounted and examined under UV fluorescent microscope. Negative controls included normal rabbit sera or mouse sera applied as the primary antibody. Additional controls included sections in which the primary antibody was omitted.

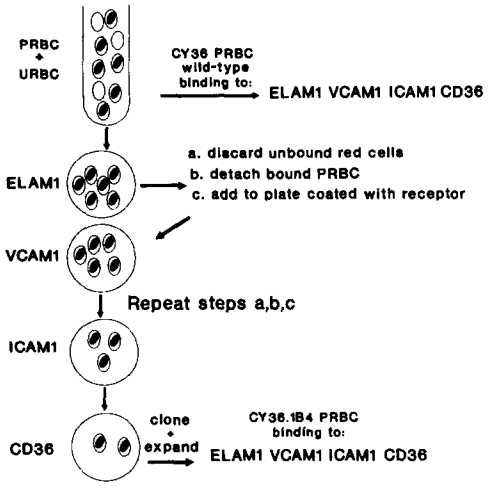

Figure 1. Flow diagram illustrating the sequential panning of malaria-infected erythrocytes on ELAM-1, VCAM-1, ICAM-1, and CD36.

Results and Discussion

Inducible CAMs on HUVECs are variably expressed and regulated by cytokines (for review see reference 29). The expression of ELAM-1 on TNF-α–activated HUVECs is transient with maximal expression at 4–6 h, whereas the expression of VCAM-1 and ICAM-1 is strongly induced and sustained for 48–72 h after TNF-α activation. Adhesion of a primary wild-type PRBC isolate (CY36) to unstimulated HUVECs is augmented as early as 4 h after TNF-α treatment and is additionally increased at 24 h incubation (Fig. 2 *A*). Since HUVECs express low amounts of CD36 (30), CD36 did not appear to be responsible for the binding of PRBCs to the cytokine-activated HUVECs. In an effort to uncover additional endothelial receptors for *P. falciparum*-infected erythrocytes, the PRBC binding properties to TNF-activated HUVECs using blocking antibodies was examined.

Antibodies to CAMs that block important cell–cell adhesion pathways were evaluated for their inhibitory effect on PRBC binding. No inhibition of PRBC binding was observed to TNF-α–activated HUVECs incubated with either anti–ELAM-1 mAb BB11 or anti–VCAM-1 mAb 4B9 (Fig. 2). Anti–ELAM-1 mAb BB11 (25) blocks the lectin-like recognition binding site on ELAM-1 for the sialylated Lewis^x tetrasaccharide on human neutrophils (31), whereas mAb 4B9 blocks VCAM-1 binding to the lymphocyte and eosinophil integrin VLA-4 (32, 33). Despite the apparent lack of blocking effect demonstrated by these two mAbs, a role for ELAM-1 and VCAM-1 in the adhesion of PRBCs to activated HUVECs could not be discounted. These antibodies had been selected originally to block leukocyte-mediated endothelial cell adhesion, and such epitope-specific mAbs would not necessarily block malaria-parasitized erythrocyte binding to spatially distinct blocking sites located on the same molecule. In fact, we and others (14, 15) have recently confirmed that malaria PRBCs bind to ICAM-1 (an alternative endothelial receptor) at a site distinct from the binding site for the leukocyte integrin LFA-1 recognized by mAbs RR1/1 and R6.5.

Rabbit polyclonal anti–ELAM-1 antisera nevertheless inhibited PRBC binding to HUVECs activated for 4 but not 24 h with TNF-α. The transient expression of ELAM-1 on 4-h TNF-activated HUVECs accounted for the difference in adhesion. The degree of inhibition of PRBC binding on 4-h activated HUVECs by polyclonal anti–ELAM-1 did not exceed 50% (Fig. 2 *B*). mAbs to ICAM-1 (84H10) partially blocked PRBC binding on both 4-h and 24-h activated HUVECs, and the effects were at least additive when combined with anti–ELAM-1 polyclonal antisera (Fig. 2). The lack of complete inhibition suggests either that PRBCs bind alternative endothelial receptors or that they recognize binding sites on adhesion receptors partially occupied by the antibodies used in these studies.

Since PRBC binding to dominant or high affinity receptors on endothelial cells may mask the function of alternative CAMs which also contribute to PRBC adhesion, we investigated PRBC binding to purified receptors. Malaria-infected erythrocytes from a naturally acquired infection (CY36) bound to recombinant soluble ELAM-1 and VCAM-1, and to membrane-purified ICAM-1 and CD36 proteins adsorbed onto a solid substrate (Fig. 3). PRBCs did not bind to tissue culture plates coated with the irrelevant proteins GPIb, fibronectin, or BSA. The low level of PRBC binding did not indicate a lack of specificity of the interaction with ELAM-1 and VCAM-1, rather it reflected high sensitivity in the detection of a small subpopulation of malaria-infected erythrocytes that

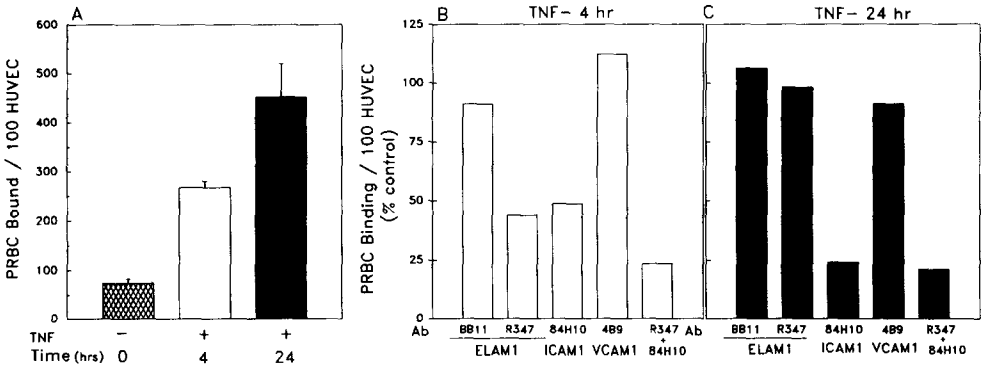

Figure 2. Malaria-infected erythrocyte adhesion to cytokine-activated endothelial cells. (*A*) PRBCs were added to wells containing HUVECs pretreated with TNF-α for 4 or 24 h. Results represent the mean ± SD of three samples. Inhibition of PRBC adhesion to HUVECS incubated with TNF-α for 4 h (*B*) or 24 hours (*C*) by anti-VCAM-1 (4B9), anti-ELAM-1 (*BB11*, *R347*), and anti-ICAM-1 (*84H10*) antibodies. Results represent the mean per cent binding of duplicate samples compared with samples incubated in RPMI 1640 alone.

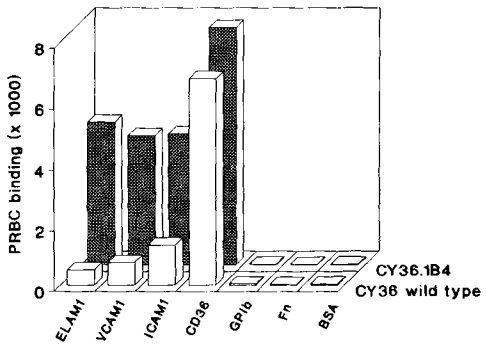

Figure 3. PRBC binding to cell adhesion molecules. Primary isolates of *P. falciparum*-infected red cells (CY36) or PRBCs selected by sequential priming on adhesion molecules (CY36.1B4) as described in Materials and Methods were assayed for binding to plates coated with CAMs and control proteins. Results represent the mean PRBCs bound per mm^2 surface area of triplicate samples.

Table 1. *Immunofluorescence Detection of Cell Adhesion Molecules on Cerebral Vascular Endothelium*

Patient	CD36	TSP	ICAM-1	ELAM-1	VCAM-1
Cerebral malaria					
01/90	−	+	+	+	+
02/90	+	+	+	+	+
03/90	−	+	+	+	+
04/90	+	+	+	+	+
05/90	−	−	−	−	−
06/90	−	+	+	+	+
07/90	−	+	−	−	+
08/90	+	+	+	−	+
09/90	−	+	−	+	+
10/90	+	+	+	+	+
01/91	−	−	−	+	−
02/91	+	+	+	+	+
03/91	+	+	−	+	−
Control brain					
91-434	−	−	−	−	−
91-481	−	−	−	−	−
91-492	−	−	−	−	−
92-15	−	−	−	−	−
92-22	−	−	−	−	−

bind multiple CAMs. This was demonstrated by the selection and propagation of a subpopulation of PRBCs by the sequential panning of PRBCs on plates coated with ELAM-1, VCAM-1, ICAM-1, and CD36 (Fig. 1). A cloned population of PRBCs, CY36.1B4, showed increased binding to all CAMs compared with the parental wild-type isolate (Fig. 3). This is the first demonstration that a single cloned population of parasitized erythrocytes possess multiple counter receptors for at least four endothelial cell receptors.

PRBC binding to rsELAM-1 and rsVCAM-1 was directly proportional to protein concentration with maximal binding at ~10 μg/ml (Fig. 4 *A*). Rabbit polyclonal anti-ELAM-1 antisera blocked PRBC binding to rsELAM-1, but not to rsVCAM-1 demonstrating the specificity of the interaction (Fig. 4 *B*). mAb BB11 which blocks neutrophil-mediated adhesion to ELAM-1 had no effect on PRBC binding to rsELAM-1 (data not shown). The binding of PRBCs to rsVCAM-1 was partially blocked by anti-VCAM-1 mAb 4B9 (Fig. 4 *C*). Despite increasing concentrations of mAb 4B9, PRBC inhibition to VCAM-1–coated plates never exceeded 60%. Whereas no data is available pertaining to the functional binding sites on VCAM-1 for PRBCs, the binding site is probably close to but distinct from the binding site for the integrin receptor VLA-4 (34), since adhesion of lymphocytes and eosinophils to VCAM-1 is completely blocked by mAb 4B9 (32, 33). The above results illustrate that PRBC binding to ELAM-1 and VCAM-1 is specific and independent of other CAMs and implies the existence of distinct binding domains on one or more counter receptors located at the parasitized red cell surface.

The multi-functional binding sites on the parasitized red cell surface which recognize structurally unrelated CAMs ELAM-1, VCAM-1, ICAM-1, CD36, and the extracellular matrix protein TSP (35), demonstrates the versatile adhesive properties of malaria-infected erythrocytes. Adhesion of primary wild-type isolates of *P. falciparum* PRBCs to ELAM-1 and VCAM-1 may be clinically relevant in vivo since we have detected low but significant binding to these CAMs from three of four isolates examined to date (data not shown). A large retrospective study which will investigate whether any clinical correlation occurs between the severity of clinical disease

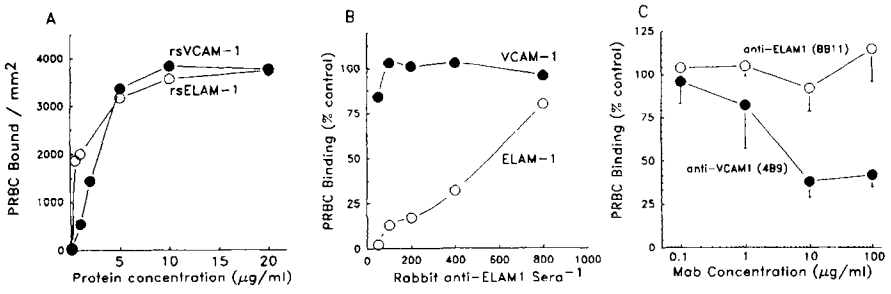

Figure 4. Malaria-infected erythrocyte adherence to ELAM-1 and VCAM-1–coated surfaces. (*A*) PRBC (CY36.1B4) binding to increasing concentrations of ELAM-1 and VCAM-1. (*B*) Rabbit polyclonal anti-ELAM-1 antisera blocks adhesion of CY36.1B4 PRBCs to rsELAM-1–coated (10 μg/ml), but not rsVCAM-1–coated (10 μg/ml) plates. (*C*) Inhibition of PRBC binding to rsVCAM-1 by anti-VCAM-1 mAb 4B9.

(uncomplicated infections versus cerebral malaria) and the in vitro PRBC binding to functional CAMs may help clarify the role of specific CAMs in severe and complicated malaria.

Previously, it had been established that although the severity of falciparum malaria did not necessarily correlate with adhesion to purified CD36 or ICAM-1 (13), the clinical course of the disease did vary with serum TNF-α levels (36, 37). It is tempting to speculate that cytokine-activated cerebral vascular endothelium induces the transient or sustained expression of multiple CAMs including ELAM-1 and VCAM-1 in organs associated with malaria parasite sequestration. By indirect immunofluorescence, we have demonstrated the expression of ELAM-1 and VCAM-1 on brain microvascular endothelium from 10 of 13 (77%) patients with cerebral malaria, but not in brain tissue from patients who had died from causes other than malaria (Table 1 and Fig. 5). These observations support the hypothesis that circulating TNF-α and IFN-γ (38) modulate the expression of multiple CAMs both in vitro and in patients with cerebral malaria. Whereas no single CAM was associated with cerebral malaria as assessed by immunohistochemistry, the coexpression of multiple CAMs may direct PRBC migration to where adhesion is the strongest, resulting in vascular occlusion, malaria rosette formation, and the initiation of endothelial cell pathologic responses. Nevertheless, since the binding of a subpopulation of PRBC (CY36.1B4) to multiple purified receptors is specific and not crossreactive, inhibiting parasite sequestration to deep vascular beds by soluble receptor-based analogues may be problematic if compensatory binding to alternative receptors occur.

It is interesting that the binding of naturally occurring malaria-infected red cells is in part mediated by adhesion molecules belonging to three distinct gene families, the Ig-like supergene family, the selectin gene family, and the newly de-

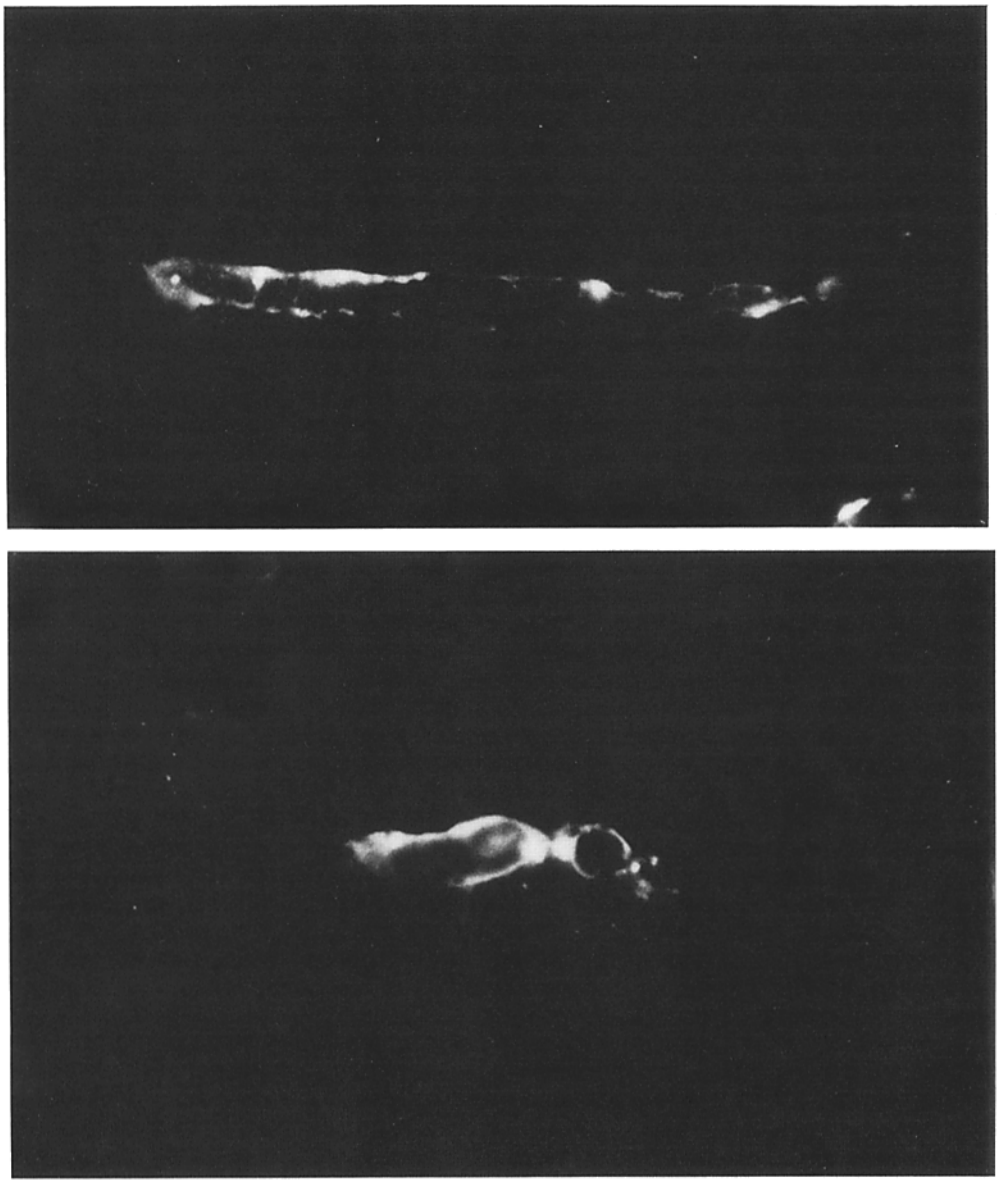

Figure 5. Detection of ELAM-1 (*a*) and VCAM-1 (*b*) by immunofluorescence on cerebral vascular endothelium from post-mortem brain tissue of patients with cerebral malaria. ×280.

scribed CD36/LIMPII gene family (39). VCAM-1, like ICAM-1 belongs to the Ig-like supergene family and is comprised of six extracellular domains (5). The adhesion of PRBCs to VCAM-1 and ICAM-1 is not typical of other Ig-like proteins since PRBCs do not bind cells transfected with cDNAs coding for mouse ICAM-1 or human ICAM-2 (14). PRBCs bind to the first Ig-like domain of human ICAM-1 at a region distinct from the LFA-1 and rhinovirus binding sites. Whether PRBCs also bind an analogous site on the first NH_2-terminal domain of VCAM-1 remains to be determined.

This study also demonstrated that PRBCs bind ELAM-1, which structurally comprises an amino terminal lectin-like domain, followed by an epidermal growth factor domain, and several tandem domains with homology to complement regulatory proteins (6). The amino terminal lectin-like domain recognizes carbohydrate structures common to both Sialyl Le^a (sialylated lacto-*N*-fucopentaose II) and Sialyl Le^x (sialylated lacto-*N*-fucopentaose III) on human neutrophils (40). The interaction between ELAM-1 and neutrophils is inhibited by mAb BB11, which has no effect on PRBC adhesion, indicating that the primary binding site on ELAM-1 for PRBCs (like that on VCAM-1 for PRBCs) is dissimilar from the adhesion receptor's recognition domain responsible for binding to its natural counter receptor. Although no functional activities have as yet been attributed to the epidermal growth factor-like or complement regulatory protein-like domains on ELAM-1, it would be interesting if the subversion by erythrocytes infected with *P. falciparum* of ELAM-1 occurs at one of these sites.

We thank Dr. Graham Jamieson, of the American Red Cross for his gift of human platelet-derived CD36, and Dr. Steven Shaw of the National Cancer Institute for his gift of ICAM-1. We acknowledge the excellent technical assistance of Gloria Chi-Rosso and Irene Dougas.

This work was supported in part by grant from the U. S. Agency for International Development (DPE-0453-A-009-141); UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases; the U. S. Public Health Service (AI-10645); and the U. S. Army R&D Command (Contract DAMD 17-90C-0010).

Address correspondence to Dr. Christian F. Ockenhouse, MAJ, MC, USA, Immunology Branch, Walter Reed Army Institute of Research, Washington, DC 20307.

Received for publication 9 March 1992 and in revised form 4 June 1992.

References

1. Springer, T.A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425.
2. Staunton, D.E., S.D. Marlin, C. Stratowa, M.L. Dustin, and T.A. Springer. 1988. Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell.* 52:925.
3. Simmons, D., M.W. Makgoba, and B. Seed. 1988. ICAM, an adhesion ligand for LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature (Lond.).* 331:624.
4. Staunton, D.E., M.L. Dustin, and T.A. Springer. 1989. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature (Lond.).* 339:61.
5. Osborn, L., C. Howard, R. Tizard, C. Vassallo, S. Luhowskyj, G. Chi-Russo, and R. Lobb. 1989. Direct expression cloning of vascular cell adhesion molecule-1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 59:1203.
6. Bevilacqua, M.P., S. Stengelin, M.A. Gimbrone Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160.
7. Hession, C., L. Osborn, D. Goff, G. Chi-Rosso, C. Vassallo, M. Pasek, C. Pittack, R. Tizard, S. Goelz, K. McCarthy et al. 1990. Endothelial leukocyte adhesion molecule 1: direct expression cloning and functional interactions. *Proc. Natl. Acad. Sci. USA.* 87:1673.
8. Johnston, G.I., R.G. Cook, and R.P. McEver. 1989. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell.* 56:1033.
9. Greve, J.M., G. Davis, A.M. Meyer, C.P. Forte, S. Connolly Yost, C.W. Marlor, M.E. Kamarck, and A. McClelland. 1989. The major human rhinovirus receptor is ICAM-1. *Cell.* 56:839.
10. Staunton, D.E., V.J. Merluzzi, R. Rothlein, R. Barton, S.D. Marlin, and T.A. Springer. 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell.* 56:849.
11. Marlin, S.D., D.E. Staunton, T.A. Springer, C. Stratowa, W. Sommergruber, and V. Merluzzi. 1990. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature (Lond.).* 344:70.
12. Berendt, A.R., D.L. Simmons, J. Tansey, C.I. Newbold, and K. Marsh. 1989. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. *Nature (Lond.).* 341:57.
13. Ockenhouse, C.F., M. Ho, N.N. Tandon, G.A. VanSeventer, S. Shaw, N. White, G.A. Jamieson, J.D. Chulay, and H.K. Webster. 1991. Molecular basis of sequestration in severe and uncomplicated *Plasmodium falciparum* malaria: differential adhe-

sion of infected erythrocytes to CD36 and ICAM-1. *J. Infect. Dis.* 164:163.
14. Ockenhouse, C.F., R. Betageri, T.A. Springer, and D.E. Staunton. 1992. Plasmodium falciparum-infected erythrocytes bind ICAM-1 at a site distinct from LFA-1, Mac-1, and human rhinovirus. *Cell.* 68:63.
15. Berendt, A.R., A. McDowall, A.G. Craig, P.A. Bates, M.J.E. Sternberg, K. Marsh, C.I. Newbold, and N. Hogg. 1992. The binding site in ICAM-1 for *Plasmodium falciparum*-infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. *Cell.* 68:71.
16. Ockenhouse, C.F., N.N. Tandon, C. Magowan, G.A. Jamieson, and J.D. Chulay. 1989. Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. *Science (Wash. DC).* 243:1469.
17. Barnwell, J.W., A.S. Asch, R.L. Nachman, M. Yamaya, M. Aikawa, and P. Ingravallo. 1989. A human 88 kD membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on *Plasmodium falciparum*-infected erythrocytes. *J. Clin. Invest.* 84:765.
18. Oquendo, P., E. Hundt, J. Lawler, and B. Seed. 1989. CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. *Cell.* 58:95.
19. Sherwood, J.A., D.D. Roberts, K. Marsh, E.B. Harvey, S.L. Spitalnik, L.H. Miller, and R.J. Howard. 1987. Thrombospondin binding by parasitized erythrocyte isolates in falciparum malaria. *Am. J. Trop. Med. Hyg.* 36:228.
20. Aikawa, M., M. Iseki, J.W. Barnwell, D. Taylor, M.M. Oo, and R.J. Howard. 1990. The pathology of human cerebral malaria. *Am. J. Trop. Med. Hyg.* 43:30.
21. Lobb, R.R., G. Chi-Rosso, D.R. Leone, M.D. Rosa, S. Bixler, B.M. Newman, S. Luhowskyj, C.D. Benjamin, I.G. Douglas, S.E. Goelz et al. 1991. Expression and functional characterization of a soluble form of endothelial-leukocyte adhesion molecule 1. *J. Immunol.* 147:124.
22. Lobb, R., G. Chi-Rosso, D. Leone, M. Rosa, B. Newman, S. Luhowskyj, L. Osborn, S. Schiffer, C. Benjamin, Irene Douglas et al. 1991. Expression and functional characterization of a soluble form of vascular cell adhesion molecule 1. *Biochem. Biophys. Res. Commun.* 178:1498.
23. Tandon, N.N., R.H. Lipsky, W.H. Burgess, and G.A. Jamieson. 1989. Isolation and characterization of platelet glycoprotein IV (CD36). *J. Biol. Chem.* 264:7570.
24. Van Seventer, G.A., Y. Shimizu, K.J. Horgan, and S. Shaw. 1990. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *J. Immunol.* 144:4579.
25. Benjamin, C., I. Dougas, G. Chi-Rosso, S. Luhowskylj, M. Rosa, B. Newman, L. Osborn, C. Vassallo, C. Hession, S. Goelz, et al. 1990. A blocking monoclonal antibody to endothelial-leukocyte adhesion molecule-1 (ELAM-1). *Biochem. Biophys. Res. Commun.* 171:348.
26. Udeinya, I.J., C. Magowan, and J.D. Chulay. 1989. Long-term cultured human vascular endothelial cells (EC-FP5) bind *Plasmodium falciparum* infected erythrocytes. *Am. J. Trop. Med. Hyg.* 41:400.
27. Udeinya, I.J., L.H. Miller, I.A. McGregor, and J.B. Jensen. 1983. *Plasmodium falciparum* strain-specific antibody blocks binding of infected erythrocytes to amelanotic melanoma cells. *Nature (Lond.).* 303:429.
28. Aikawa, M., A.E. Brown, C.D. Smith, T. Tegoshi, R.J. Howard, T.H. Hasler, Y. Ito, C. Perry, W.E. Collins, and H.K. Webster. 1992. A primate model for human cerebral malaria: *P. coatneyi*-infected rhesus monkeys. *Am. J. Trop. Med. Hyg.* 46:391.
29. Osborn, L. 1990. Leukocyte adhesion to endothelium in inflammation. *Cell.* 62:3.
30. Swerlick, R.A., K.H. Lee, T.M. Wick, and T.J. Lawley. 1992. Human dermal, microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. *J. Immunol.* 148:78.
31. Lowe, J.B., L.M. Stoolman, R.P. Nair, R.D. Larsen, T.L. Berhend, and R.M. Marks. 1990. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell.* 63:475.
32. Carlos, T.M., B.R. Schwartz, N.L. Kovach, E. Yee, M. Rosa, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J.M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood.* 76:965.
33. Weller, P.F., T.H. Rand, S.E. Goelz, G. Chi-Rosso, and R.R. Lobb. 1991. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci. USA.* 88:7430.
34. Elices, M.J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M.E. Hemler, and R.R. Lobb. 1990. VCAM-1 on activated endothelium interacts with with leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577.
35. Roberts, D.D., J.A. Sherwood, S.L. Spitalnik, L.J. Panton, R.J. Howard, V.M. Dixit, W.A. Frazier, L.H. Miller, and V. Ginsburg. Thrombospondin binds falciparum malaria parasitized erythrocytes and may mediate cytoadherence. *Nature (Lond.).* 1985. 318:64.
36. Grau, G.E., T.E. Taylor, M.E. Molyneux, J.J. Mirima, P. Vassalli, M. Hommel, and P.H. Lambert. 1989. Tumor necrosis factor and disease severity in children with falciparum malaria. *N. Engl. J. Med.* 320:1586.
37. Shaffer, N., G.E. Grau, K. Hedberg, F. Davachi, B. Lyamba, A.W. Hightower, J.G. Breman, and P. Nguyen-Dinh. 1991. Tumor necrosis factor and severe malaria. *J. Infect. Dis.* 163:96.
38. Wick, T.M., J.K. Johnson, R.A. Swerlick, K. Grady, and P. Millet. 1992. Cytoadherence of *Plasmodium falciparum* parasitized red blood cells (PRBC) to human microvascular endothelial cell (MEC) CD36 and ICAM-1 is strain-specific and regulatable. *FASEB (Fed. Am. Soc. Exp. Biol.).* 6:A1892.
39. Vega, M.A., B. Segui-Real, J.A. Garcia, C. Calies, F. Rodriguez, J. Vanderkerckhove, and I.V. Sandoval. 1991. Cloning, sequencing, and expression of a cDNA encoding rat LIMP II, a novel 74 kDa lysosomal membrane protein related to the surface adhesion protein, CD36. *J. Biol. Chem.* 266:16818.
40. Berg, E.L., M.K. Robinson, O. Mansson, E.C. Butcher, and J.L. Magnani. 1991. A carbohydrate domain common to both Sialyl Le^a and Sialyl Le^x is recognized by the endothelial cell leukocyte adhesion molecule ELAM-1. *J. Biol. Chem.* 266:14869.