MICROVASCULAR HEMODYNAMICS AND IN VIVO EVIDENCE FOR THE ROLE OF INTERCELLULAR ADHESION MOLECULE-1 IN THE SEQUESTRATION OF INFECTED RED BLOOD CELLS IN A MOUSE MODEL OF LETHAL MALARIA

DHANANJAYA K. KAUL, XIAO-DU LIU, RONALD L. NAGEL, AND HANNAH L. SHEAR

Division of Hematology, Albert Einstein College of Medicine, and Montefiore Medical Center, Bronx, New York

Abstract. The cytoadherence of infected red blood cells (IRBCs) to the vascular endothelium is the major cause of IRBC sequestration and vessel blockage in the cerebral form of human malaria. Among the rodent models of malaria, Plasmodium yoelii 17XL-infected mice show many similarities with the human cerebral malaria caused by P. falciparum. In both, the sequestration of the IRBCs in the brain vessels is secondary to the cytoadherence of IRBCs to the vascular endothelium. Similar to P. falciparum infection in the human but in contrast to P. berghei ANKA infection in mice, P. yoelii 17XL results in little, if any, accumulation of monocytes in the brain. In vivo microcirculatory studies reported here were designed to further understand the hemodynamic aspects and mechanisms underlying cytoadherence of IRBCs in the P. yoelii model using the easily accessible cremaster muscle vasculature. The results show significant decreases in arteriovenous red blood cell velocities (Vrbc) and wall shear rates in the microcirculation of P. yoelii-infected mice, with a maximal decrease occurring in small-diameter postcapillary venules, the main sites of cytoadherence. This reflects contributions from IRBC cytoadherence as well as from increased rigidity of parasitized red blood cells. No cytoadherence is observed in arterioles of the infected mice despite decreased wall shear rates, indicating that endothelial receptors for cytoadherence are restricted to venules. Infusion of a monoclonal antibody (MAB) against the intercellular adhesion molecule-1 (ICAM-1) resulted in significant increases in both arteriolar and venular Vrbc and wall shear rates, accompanied by detachment of adhered IRBCs at some venular sites. The peripheral blood smears taken after the MAB infusion showed a distinct increase in the percentage of schizonts, again indicating detachment and/or prevention of cytoadherence. An MAB against the vascular cell adhesion molecule-1 (VCAM-1) as well as an irrelevant control antibody had no effect on these parameters. These results provide the first in vivo microcirculatory evidence indicating involvement of ICAM-1, but not of VCAM-1, in the sequestration of IRBCs in a rodent model of cerebral malaria.

The lethal complications of Plasmodium falciparum malaria in humans, known as cerebral malaria, are associated with the sequestration of infected red blood cells (IRBCs) in the brain microcirculation. The sequestration of IRBCs is due to cytoadherence of these cells to the endothelial cells of postcapillary venules. Only red blood cells containing mature parasite stages (i.e., trophozoites and schizonts) can interact with the vascular endothelium via parasite-generated red blood cell membrane knobs, as well as form aggregates (rosette formation) with uninfected red blood cells. Both these properties contribute to the microvascular obstruction by IRBCs. Other investigators have suggested that changes in cytokine and nitric oxide levels rather than sequestration play a role in cerebral malaria.

We have recently demonstrated in vivo cytoadherence and microvascular sequestration of IRBCs in mice infected with the lethal strain of the rodent malaria parasite P. yoelii 17XL. This murine model of cerebral malaria, originally described by Yoeli and Hargreaves, shows cytoadherence of IRBCs to the endothelium of postcapillary venules in the brain and in the cremaster muscle. Thus, the easily accessible mouse cremaster preparation can be used for direct monitoring of the IRBC cytoadherence. The endothelium-IRBC interaction in the mouse does not require the presence of red blood cell membrane knobs. In this regard, the behavior of P. yoelii-IRBCs resembles a knobless clone of P. falciparum-IRBCs. Furthermore, this model resembles human cerebral malaria more closely than P. berghei ANKA infection in mice because it shows little, if any, accumulation of monocytes or macrophages in the brain.

Cytoadherence of IRBCs may involve multiple adhesion receptors on the endothelium and parasite-induced ligands on IRBCs. Endothelial receptors proposed as participants in adhesion of P. falciparum-IRBCs are intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), glycoprotein IV (CD36), thrombospondin (TSP), and chondroitin sulfate A (CSA). The cytoadherence properties of IRBCs may vary with strains of P. falciparum (knobby versus knobless strains), as well as with the cell-line (cultured endothelial cells, melanoma, or transfected cells) and the assay system (in vitro, ex vivo, or in vivo system) used. For example, cultured human umbilical vein endothelial cells and C32 melanoma cell line may express different concentrations of receptor molecules (i.e., ICAM-1, CD36, and TSP), while TSP appears to be the predominant endothelial receptor for IRBCs in an ex vivo microvascular preparation. On the other hand, a transfected cell line would express only a selected receptor molecule. Accordingly the number and density of receptors that are being expressed on a given cell line contribute to the variability in results. Therefore, the information derived from in vitro studies has to be considered tentative until in vivo studies confirm their relevance. Furthermore, in some rodent malarias such as P. berghei, different developmental stages of the parasite accumulate in different organs.

So far no studies have been performed to determine the relative roles of adhesion molecules under in vivo microvascular flow conditions. In view of the similarities between human cerebral malaria and P. yoelii 17XL infection in the mouse, it is important to investigate whether cytoadherence in the mouse model involves similar mechanisms. The pres-
ent study was designed to compare the roles of ICAM-1 and VCAM-1, members of the immunoglobulin superfamily, in the cytoadherence of IRBC in the *P. yoelii* infection. In cerebral malaria, elevated TNF-α levels may result in up-regulation of these molecules. In addition, microrheologic consequences of the infection have not been explored on both arteriolar and venular sides of the microcirculation. The objectives of the present study were as follows: 1) to determine arteriovenous red blood cell velocity (Vrbc) and wall shear rate profiles in infected mice and 2) to ascertain the relative roles of the antibodies to ICAM-1 and VCAM-1 in cytoadherence by monitoring changes in arteriovenous microrheologic parameters, as well as in the peripheral blood smear.

**Materials and Methods**

**Animals and infection with parasites.** Swiss mice, 8–10 weeks old, were obtained from Taconic Farms (Germantown, NY). *Plasmodium yoelii* 17XL (lethal), stored in liquid nitrogen, were used to infect source mice. Experimental mice were infected by intraperitoneal inoculation of 5 × 10⁸ infected erythrocytes obtained from a source mouse. Parasitemia was monitored by making blood smears from the tail vein and counting 50 Giemsa-stained fields or at least 300 RBCs under oil immersion (1,000×). Mice had patent parasitemia (less than 1% infected RBCs) by day 2. The parasitemia increased quickly thereafter and reached 60–80% after one week of infection. The infection was lethal by days 7–9.

**Antibodies.** Monoclonal antibody (MAb) anti-mouse ICAM-1 was purified from the YN/1.7.4rat hybridoma line obtained from the American Type Culture Collection (ATCC), Rockville, MD. Briefly, cell supernatants were concentrated, precipitated with NH₄SO₄, and purified on a Bio-Rad (Hercules, CA) Affi-Gel Protein A agarose column according to the manufacturer’s instructions. The protein concentration of the purified antibody was determined using the Bio-Rad protein assay. Anti-murine VCAM-1 was purified similarly from M/K-2.7 (ATCC). Control (irrelevant) MAb was a rat MAB antibody of the same isotype as anti-ICAM-1, 10-2.16 (ATCC) purified in the same way.

**In vivo microcirculatory studies in the cremaster muscle preparation.** Male mice weighing approximately 30–35 g were used. The level of parasitemia in the infected mice varied between ~5% and 15%. In *in vivo* microcirculatory observations were made in the cremaster muscle microvasculature. Mice were anesthetized intraperitoneally with 10% urethane and 2% a-chloralose in saline (6 ml/kg). The animals were tracheostomized and the right jugular vein was cannulated for antibody infusion. The open cremaster muscle was prepared according to the method of Baez. The suffusion and maintenance of the mouse open cremaster muscle for intravitral observations was done as described by Kaul and others. Briefly, the preparation was suffused with a bicarbonate Ringer’s solution of the following composition: 135 mM NaCl, 5.0 mM KCl, 27.0 mM NaHCO₃, 0.64 mM MgCl₂, and 11.6 mM glucose. The pH was adjusted to 7.35–7.4 by continuous bubbling with 94.6% N₂ and 5.6% CO₂. The osmolality of the solution, as measured by a Micros-mette (Precision Systems, Sudbury, MA), was 320 mOsm, which is similar to that reported for the mouse plasma. The temperature of the suffusion solution (flow rate = 5–6 ml/min) bathing the cremaster was maintained at 34.5–35°C and monitored by a telethermometer (YSI Inc., Yellowsprings, OH) during the entire experiment. The oxygen tension (pO₂) of the suffusion solution bathing the cremaster was 15–20 mm of Hg, as determined using a microoxygen electrode (model MI-730; Microelectrodes Inc., Bedford, NH). The preparation was allowed to stabilize for 30 min before the initiation of the experiment.

The microvascular branch orders in the mouse cremaster have been described recently. Diameter and Vrbc measurements were made in A2 arterioles and V4, V3, and V2 venules of infected mice and appropriate controls. In the infected mice, following the baseline microcirculatory measurements, a given antibody (anti-ICAM-1, anti-VCAM-1, or a control antibody) was infused (each 100 μg per mouse) via the jugular vein, and the microcirculatory measurements resumed after an interval of 30 min.

Vessel luminal diameter was measured on-line by image-shearing using an image shearing device (model 907; Instruments for Physiology and Medicine, San Diego, CA). Red blood cell was measured along the vessel centerline using the dual-slit photodiode technique of Wayland and Johnson. The dual-slit photo detector was placed over the projected image of the vessel and on-line analysis of the optical signals was performed using a cross-correlator as described by Tompkins and others (model 102 BF; Instruments for Physiology and Medicine). The Vrbc measurements were made using a water immersion 40× objective and a 6.3× eyepiece. The wall shear rates were calculated from the mean Vrbc (Vmean) and vessel diameter. The centerline velocity was converted to the mean red blood cell velocity across the vessel diameter using a conversion factor of 1.6 (Vrbc/Vmean = 1.6) originally described by Baker and Wayland. Seki and Lipowsky have confirmed the validity of 1.6 ratio of Vrbc/Vmean for transilluminated vessels of the cremaster tissue. Shear rates along the wall of microvessel of a given internal diameter (D) were calculated using the relationship wall shear rate = 8 Vmean/D. In separate experiments, blood smears were made before and 1 hr after the infusion of a given antibody to monitor percent parasitemia and parasite stages (i.e., ring stage, trophozoite, schizont, and gamocytes).

**Statistical analysis.** Data were analyzed using paired the t-test or unpaired Student’s test as indicated. For comparisons among groups, the Newman-Keuls multiple comparison was used. The statistical analysis was performed using Statgraphics Plus (version 2.1) program for Windows (Manugistics Inc., Rockville, MD) and a 486 computer.

**Results**

**In vivo microcirculatory observations in control and infected mice.** Direct microscopic observations and videotaping of the microcirculatory flow revealed cytoadherence of red blood cells to the endothelium of mainly slow-flowing, smaller-diameter postcapillary venules (V4 and V3), which was mostly absent in larger venules (V2) of infected mice. No cytoadherence was evident in arterioles. The venular specificity of cytoadherence in *P. yoelii*-infected mice...
is in accordance with our previous studies. In addition, in the infected mice, there was an apparent increase in weak adhesion of the leukocytes to endothelium leading to leukocyte rolling along the vascular endothelial surface.

On-line measurements of microvascular diameters and centerline Vrbc in V2, V3, and V4 arterioles and in V2 arterioles were made under conditions of postcapillary venules (V2–V4) (P < 0.01–0.001). There is greater than a 50% decrease in Vrbc in any given vessel order, with maximal decrease (~70%) occurring in V4 and V3 venules, the main sites of cytoadherence. In the infected mice, there was essentially the same pattern as noted for Vrbc. Thus, in the arterioles and venular branches of the infected mice compared with uninfected controls (P < 0.01–0.001). The break in the x-axis indicates that on the arteriolar side microcirculatory comparisons are made only for A2 arterioles and not in the lower arteriolar branch orders (i.e., A3, A4). Bars at data points represent ± SD. *P < 0.01–0.001 compared with infected mice.

**Figure 1.** Arteriovenous red blood cell velocity (Vrbc) and wall shear rate profiles in the cremaster microcirculation of uninfected control mice. (a), Vrbc (mm/sec) is significantly higher in uninfected control mice in both A2 arterioles and various orders of postcapillary venules (V2–V4) (P < 0.01–0.001). There is greater than a 50% decrease in Vrbc in any given vessel order, with maximal decrease (~70%) occurring in V4 and V3 venules, the main sites of cytoadherence. (b), wall shear rates (sec^-1), calculated from Vrbc and diameters in the same vessels, show similar extent of decreases in the microcirculation of the infected mice compared with uninfected controls (P < 0.01–0.001). The numbers in parentheses represent the number of vessels. The break in the x-axis indicates that on the arteriolar side microcirculatory comparisons are made only for A2 arterioles and not in the lower arteriolar branch orders (i.e., A3, A4). Bars at data points represent ± SD. *P < 0.01–0.001 compared with infected mice.

**Effect of anti-ICAM-1 and anti-VCAM-1 antibodies on microcirculatory parameters.** Infected mice were divided into three groups to receive either anti-ICAM-1, or anti-VCAM-1, or a control irrelevant antibody (100 μg per mouse) via the jugular vein, and the microcirculatory observations were made in the same set of vessels before and after an interval of 30 min. The microvascular diameters showed no significant change following the administration of any given antibody including control irrelevant antibody. The infusion of anti-ICAM-1 antibody resulted in an increase in both Vrbc and wall shear rates on both arteriolar and venular sides (Table 1 and Figure 2a and b). This was accompanied by reversal of cytoadherence at some sites in the venules that were being monitored for the flow parameters (Figure 3). Anti-ICAM-1 antibody also appeared to decrease leukocyte rolling in the infected mice.

In the anti-ICAM-1 antibody group, A2 arterioles showed 28% and 34% increases in Vrbc and wall shear rates, respectively (P < 0.02, by paired t-test) (Table 1 and Figure 2a and b). Maximal increases in Vrbc and wall shear rates (~85% and ~90%, respectively) were recorded in V4 venules (P < 0.02 and P < 0.01, respectively) while V2 venules showed 45–48% increases in these parameters (P < 0.02) (Table 1 and Figure 2a and b). On the other hand, there was no significant effect of either an anti-VCAM antibody (Table 1 and Figure 2c and d) or the control irrelevant antibody (Table 1) on the microcirculatory parameters.

**Effect of anti-ICAM-1 and anti-VCAM-1 antibodies on parasitemia and parasite stages.** Because there was a distinct increase in both Vrbc and wall shear rates as well as evidence of reversal of cytoadherence in some venules following the administration of anti-ICAM-1 antibody, we determined, in separate experiments, the % parasitemia and differential counts of parasite stages before and after the infusion of these antibodies. Parasitemia levels in the mice prior to antibody infusions, with one exception (31%), ranged between 4.1% and 13.2%. Table 2 and Figure 4 give the percent changes in the counts along with the mean ± SD. In three infected mice, anti-ICAM-1 antibody resulted in a 29–62% increase in total parasitized red blood cells in the pe-
### Table 1

<table>
<thead>
<tr>
<th>Vessel order</th>
<th>Antibody</th>
<th>Pre-Ab (Vrbc, mm/sec)</th>
<th>Post-Ab (Vrbc, mm/sec)</th>
<th>Wsr, sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>Anti-ICAM-1 (n = 5)³</td>
<td>6.37 ± 1.6 (5)</td>
<td>3.4 ± 0.7 (5)</td>
<td>0.71 ± 0.67 (5)</td>
</tr>
<tr>
<td></td>
<td>Anti-VCAM-1 (n = 4)</td>
<td>8.17 ± 1.74 (3)</td>
<td>6.22 ± 1.06 (3)</td>
<td>0.72 ± 0.38 (4)</td>
</tr>
<tr>
<td>A3</td>
<td>Control Ab (n = 3)</td>
<td>9.5 (1)</td>
<td>8.5 (1)</td>
<td>0.8 ± 0.19 (2)</td>
</tr>
</tbody>
</table>

* Values are the mean ± SD. Values in parentheses are the number of vessels. For any given vessel order, Vrbc and wsr were determined in the same set of vessels before and 30 min after the infusion of a given antibody.

**Values of the mean ± SD. Values in parentheses are the number of vessels. For any given vessel order, Vrbc and wsr were determined in the same set of vessels before and 30 min after the infusion of a given antibody.

³ Number of mice. ² Number of mice. ³ , 0.01–0.02 compared with the respective pre-antibody values (paired t-test).

### DISCUSSION

The studies presented here make two major points. First, despite significant reductions in arteriovenous Vrbc and wall shear rates in the microcirculation of *P. yoelii* 17XL-infected
mice (Figure 1), the cytoadherence of IRBCs is restricted to the venules. Second, the infusion of an anti-ICAM-1 antibody, but not anti-VCAM-1 antibody, results in a distinct improvement in the microrheologic parameters, as well as in an increase in % IRBCs and schizonts in the peripheral circulation; the latter indicates desequestration of IRBCs.

The maximal decrease (~65–70%) in Vrbc and wall shear rates is recorded in smaller-diameter postcapillary venules (V4 and V3), the main sites of cytoadherence. These results are in overall agreement with our previous study12 that depicted cytoadherence associated with significant reductions in these microrheologic parameters in venules < 40 mm in diameter. Although in that study, vessels were not arranged according to the branch order, venules < 40 mm in diameter

TABLE 2

<table>
<thead>
<tr>
<th>Antibody* (Ab)</th>
<th>Mouse no.</th>
<th>Paracitemia</th>
<th>% change</th>
<th>Rings</th>
<th>Trophozoites</th>
<th>Schizonts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-ICAM-1</td>
<td>1</td>
<td>34.6</td>
<td>−38.7</td>
<td>62.1</td>
<td>225.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>62.1</td>
<td>−53.6</td>
<td>95.2</td>
<td>217.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29.0</td>
<td>−56.3</td>
<td>−18.0</td>
<td>53.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>41.9 ± 17.7</td>
<td>−49.5 ± 9.5†</td>
<td>46.4 ± 58.2</td>
<td>165.0 ± 97.1†</td>
<td></td>
</tr>
<tr>
<td>Anti-VCAM-1</td>
<td>1</td>
<td>20.5</td>
<td>−10.0</td>
<td>−25.0</td>
<td>−20.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.0</td>
<td>−12.2</td>
<td>13.6</td>
<td>−7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.7</td>
<td>−11.9</td>
<td>−28.5</td>
<td>−46.2</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>25.4 ± 12.9</td>
<td>−3.2 ± 13.4†</td>
<td>−13.3 ± 23.4</td>
<td>−24.6 ± 19.7†</td>
<td></td>
</tr>
<tr>
<td>Control Ab</td>
<td>1</td>
<td>13.0</td>
<td>−1.2</td>
<td>0</td>
<td>−22.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.2</td>
<td>−11.9</td>
<td>−17.5</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−2.0</td>
<td>−5.9</td>
<td>1.7</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>7.7 ± 8.4</td>
<td>−6.3 ± 5.4†</td>
<td>−5.3 ± 10.6</td>
<td>13.4 ± 34.5†</td>
<td></td>
</tr>
</tbody>
</table>

* A bolus of a given antibody (100 μg per mouse) was infused intravenously. The blood smears were made before and 1 hr after the infusion of the antibody.
† The anti-ICAM-1 group showed a significant difference compared with the anti-VCAM-1 and control groups. (P < 0.05, by Newman-Keuls multiple comparison). There was no significant difference between the anti-VCAM-1 and control groups.
ROLE OF ICAM-1 IN MALARIA CYTOADHERENCE

The infusion of anti-ICAM-1 antibody caused a significant increase in arteriovenous Vrbc and wall shear rates in small-diameter venules and arterioles. In particular, the increase in schizonts in the anti-ICAM-1 group indicates that detachment of adherent IRBCs in small-diameter venules (Figure 3) could result in the improved microvascular flow. Although the increase in Vrbc and wall shear rates was significant in arterioles (A2), as well as in V2 and V4 venules, it must be reiterated that the overall arteriovenous Vrbc and wall shear rates in the mice treated with anti-ICAM-1 antibody are still significantly lower compared with control values due to the likely reduced deformability of IRBCs.

The second point pertains to the involvement of adhesive proteins in IRBC cytoadherence. The experiments designed to compare the effect of MAb against ICAM-1 and VCAM-1 show a distinct increase in arteriovenous Vrbc and wall shear rates following the infusion of anti-ICAM-1 antibody but no effect of anti-VCAM-1 or a control antibody. The overall improvement in the flow parameters in mice treated with anti-ICAM-1 antibody could be the result of a combination of factors that include detachment of adherent IRBC at some venular sites (Figure 3) and/or prevention of new cytoadherence.

Both ICAM-1 and VCAM-1 are expressed on activated endothelium and participate in leukocyte-endothelial interactions. Besides E- and P-selectins, VCAM-1 can also mediate rolling (transient adhesion) of leukocytes by binding to leukocyte molecules such as $\alpha_\beta$.

The direct evidence of detachment and/or prevention of cytoadherence of IRBC comes from differential counts of IRBCs for parasite stages in the peripheral blood smears. In P. yoelii-infected mice, unlike human P. falciparum malaria, the peripheral blood smear shows the presence of schizonts. This indicates that in the mouse model, not all IRBC containing mature stages are sequestered. However, in each of the three infected mice (Table 2), the MAb against ICAM-1 caused a 53–225% increase in schizonts in the peripheral blood, accompanied by an increase in percent parasitemia. In contrast, there was no apparent effect of an anti-VCAM-1 antibody or a control antibody on the differential counts. In particular, the increase in IRBCs containing schizonts in the anti-ICAM-1 group indicates that detachment of adherent IRBCs is due to a consequence of increased TNF-$\alpha$ levels in these mice, as also reported for human malaria.

Most relevant to this discussion is a similar up-regulation of ICAM-1 and VCAM-1 in the cerebral vessels of P. falciparum cerebral malaria, lipopolysaccharides (LPS) can induce up-regulation of ICAM-1 in small-diameter brain vessels, while VCAM-1 expression is limited to larger vessels.

In addition, leukocyte rolling may affect microvascular Vrbc and wall shear rates. In the cremaster venules of P. yoelii-infected mice, there appears to be an increase in the numbers of rolling leukocytes compared with control mice but there is no adhesion and extravasation of these cells. Interestingly, the rolling is largely abolished in the infected mice treated with anti-ICAM-1 but not with anti-VCAM-1 antibody. There is histologic evidence of leukocyte accumulation in the brain microvasculature in the P. yoelii infection.
induced rolling of leukocytes in P-selectin deficient mice is
totally abolished with concomitant deficiency of ICAM-1,48
again indicating a role of ICAM-1 in this phenomenon.
However, in contrast to the observed rolling of P. falcipa-
rum-IRBCs on the endothelial monolayer in flow system29
as well as in venules of the ex vivo mesocirculation
preparation (Kaul DK, unpublished data), P. yoelii-IRBCs
exhibit no such behavior.

Previous studies have suggested that malaria binding site
of ICAM-1 is different from the site involved in binding to
LFA-1 (CD11a/CD18). It has been shown that the mouse
ICAM-1 differs from human ICAM-1 in having two
immunoglobulin domains rather than five.50 Nevertheless,
the MAb against mouse ICAM-1 (i.e., YN1/7.4) used in the
present studies has been previously shown to block the in-
terraction of mouse LFA-1 to ICAM-116 as well as to block
the adhesion of human IRBCs in the SCID mouse.17 This
may indicate a close proximity of a malaria binding site
of ICAM-1 to its LFA-1 binding site.

In summary, the present studies demonstrate that in the
P. yoelii model of cerebral malaria, IRBCs cause a signifi-
cant decrease in the arteriovenous Vrbc and wall shear rates.
Importantly, the results show that ICAM-1, but not VCAM-
1, is involved in IRBC cytoadherence in the mouse model.
The overall improvement in the rheologic parameters
in infected mice treated with anti-ICAM-1 antibody could be
the result of a combination of factors, i.e., 1) detachment
of adherent IRBC at some adhesion sites (Figure 3), 2) pre-
vention of new cytadherence, and 3) an apparent decrease
in leukocyte rolling. Future work will be needed to delineate
the role of other adhesion molecules (e.g., CD36, TSP, CSA)
in this model. Based on the present studies, P. yoelii 17XL
mouse model of cerebral malaria presents several features
common with human P. falciparum malaria and could be
useful in testing therapeutic approaches for human cerebral
malaria.

Financial support: This work was supported in part by grants AI-
34064 (Hannah L. Shear) and HL-45931 (Dhananjaya K. Kaul) from
the National Institutes of Health.

Authors’ addresses: Dhananjaya K. Kaul, Xiao-Du Liu, and Ronald
L. Nagel, Division of Hematology, Albert Einstein College of Med-
icine, 1300 Morris Park Avenue, Bronx, NY 10461. Hannah L.
Shear, Division of Hematology, Montefiore Medical Center, 111 East
210th Street, Bronx, NY 10467.

Reprint requests: Dhananjaya K. Kaul, Department of Medicine,
Room U-917, Albert Einstein College of Medicine, 1300 Morris
Park Avenue, Bronx, NY 10461.

REFERENCES


47–102.

3. Macpherson GG, Warrell MJ, White NJ, Looareesuwan S, War-
rell DA, 1985. Human cerebral malaria. A quantitative ultra-
structural analysis of parasitized erythrocyte sequestration.


5. Luse SA, Miller LH, 1971. Plasmodium falciparum malaria:
ultrastructures of parasitized erythrocytes in cardiac vessels.

Membrane knobs are required for the microcirculatory ob-
struction induced by Plasmodium falciparum-infected eryth-

role of membrane knobs in microvascular obstruction induced
by Plasmodium falciparum-infected erythrocytes. Trans As-
soc Am Physiol 294: 987–994.

8. David PH, Handunnetti SM, Leech JH, Gamage P, Mendi KN,
1988. Rosetting: a new cytoadherence property of malaria-

9. Udomsangpetch R, Wahlin B, Carlson J, Berzins K, Torii M,
Aikawa M, Perlmann P, Wahlgren M, 1989. Plasmodium fal-
ciparum-infected erythrocytes form spontaneous erythrocyte

10. Kaul DK, Roth EF Jr, Nagel RL, Howard RJ, Handunnetti SM,
1991. Rosetting of Plasmodium falciparum-infected red blood
cells enhances microvascular obstruction under flow condi-

cytokines, nitric oxide, and human malaria. Parasitol Today
7: 205–207.

in mice: demonstration of cytoadherence of infected red cells
and microhemorheologic correlates. Am J Trop Med Hyg 50:
512–521.

duced by a virulent strain of rodent malaria. Science 184:
572–574.

14. Udomsangpetch R, Aikawa M, Berzins K, Wahlgren M, Perl-
mann P, 1989. Cytoadherence of knobless Plasmodium falcipar-
um-infected erythrocytes and its inhibition by a human

P, 1987. Tumor necrosis factor (cachectin) as an essential me-

16. Brendt AR, Simmons DL, Tansey J, Newbold CI and Marsh K,
1989. Intercellular adhesion molecule 1 is an endothelial re-

sequestration of Plasmodium falciparum-infected human ery-
throcytes: a severe combined immunodeficiency mouse

18. Ockenhouse CF, Tegeosi T, Maeno Y, Benjamin C, Ho M, Kan
vascular endothelial adhesion receptors for Plasmodium fal-
ciparum-infected erythrocytes: roles for endothelial leukocyte

functions in vitro as a receptor for a cytoadherence ligand on
Plasmodium falciparum-infected erythrocytes. J Clin Invest
84: 765–772.

20. Ockenhouse CF, Tandon NN, Magowan C, Jameison GA, Chu-
lay JD, 1989. Identification of a platelet membrane glycopro-
cin as a falciparum malaria sequestration receptor. Science
243: 1469–1471.

Thrombospondin binds falciparum malaria parasitized eryth-

22. Rock ER, Roth EF Jr, Rojas-Corona RR, Sherwood JA, Nagel
the cytoadherence of P. falciparum infected red cells to vas-

Multiple ligands for cytoadherence can be present simultane-
ously on the surface of Plasmodium falciparum-infected eryth-

Chondroitin sulfate A is a cell surface receptor for Plasmo-