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

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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 red blood cells is due 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.1-3 The sequestration of IRBCs is due to cytoadherence of these cells to the endothelial cells of postcapillary venules.4-6 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,7-9 as well as form aggregates (rosette formation) with uninfected red blood cells.7-9 Both these properties contribute to the microvascular obstruction by IRBCs.7, 10 Other investigators have suggested that changes in cytokine and nitric oxide levels rather than sequestration play a role in cerebral malaria.11

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.12 This murine model of cerebral malaria, originally described by Yoeli and Hargreaves,13 shows cytoadherence of IRBCs to the endothelium of postcapillary venules in the brain and in the cremaster muscle.12 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.14 Furthermore, this model resembles human cerebral malaria more closely than P. berghei ANKA infection in mice15 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),16, 17 vascular cell adhesion molecule-1 (VCAM-1),18 glycoprotein IV (CD36),19, 20 thrombospondin (TSP),21, 22 and chondroitin sulfate A (CSA).23-25 The probable ligands on the membrane surface of IRBC include proteins such as P. falciparum erythrocyte membrane protein 1 (PfEMP1) that are encoded by the family of var genes.26-28 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 transplanted 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),29 while TSP appears to be the predominant endothelial receptor for IRBCs in an ex vivo microvascular preparation.30 On the other hand, a transfected cell line would express only a selected receptor molecule.30 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 malaria such as P. berghei, different developmental stages of the parasite accumulate in different organs.31

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 upregulation of these molecules. In addition, microcircroscopic 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 microcircrologic 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 × 10⁷ 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 1 week of infection. The infection was lethal by days 7–9.

**Antibodies.** Monoclonal antibody (MAb) anti-mouse ICAM-1 was purified from the YN/1.7.4 rat 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 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 tapped 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

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 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 photodetector 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 the 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.<ref> 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 gametocytes).

**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 V_{rbc} and in A2 arterioles and various orders of postcapillary venules (V2–V4) (pO2 of suffusion solution, 15–20 mm of Hg) (see Materials and Methods). From these parameters, wall shear rates were calculated. Microcirculatory data from all infected (pre-antibody) mice were pooled and compared with uninfected controls. The microvascular diameters (μm) in controls and infected mice, respectively, were as follows (mean ± SD) and showed no significant differences: A2, 45.6 ± 4.9 and 49.5 ± 6.8; V2, 55.6 ± 15 and 57.8 ± 15.7; V3, 32.4 ± 3.4 and 33.9 ± 7.2; V4, 20.4 ± 3.3 and 16.7 ± 3.4.

Figure 1 shows microvascular V_{rbc} and wall shear rate profiles for control and infected mice. In both controls and infected mice, the highest V_{rbc} was recorded on the arteriolar (A2) side while the lowest values were obtained in V4 postcapillary venules (Figure 1a). Thereafter, a gradual increase in V_{rbc} was noted in the higher orders of venules (V3 and V2). However, in the infected mice, V_{rbc} showed greater than 50% decrease in any given vessel order with maximal decreases (~70%) occurring in the immediate postcapillary venules (i.e., V4 and V3), the main sites of cytoadherence (V_{rbc}, mm/sec: A2 control - 15.75 ± 6.82, A2 infected - 7.48 ± 1.77; V4 control - 2.24 ± 0.84, V4 infected - 0.69 ± 0.53; V3 control - 3.99 ± 1.45, V3 infected - 1.12 ± 0.80; V2 control - 7.26 ± 1.96, V2 - infected 3.40 ± 2.82; P < 0.01–0.001). Also, arteriovenous wall shear rates (8 V_{mean}/D) in control and infected mice (Figure 1b) showed essentially the same pattern as noted for V_{rbc}. Thus, in the infected mice, there was >50% reduction in the wall shear rate in any given vessel order with maximal decreases (i.e., ~65–70%) occurring in V4 and V3 venules (wall shear rate/sec ± SD: A2 control - 1,691 ± 584, A2 infected - 734 ± 181; V4 control - 539 ± 147, V4 infected - 191 ± 116; V3 control - 593 ± 247, V3 infected - 164 ± 114; V2 control - 684 ± 255, V2 infected - 301 ± 206; P < 0.01–0.001).

**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 V_{rbc} 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 V_{rbc} and wall shear rates, respectively (P < 0.02, by paired t-test) (Table 1 and Figure 2a and b). Maximal increases in V_{rbc} 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 V_{rbc} and wall shear rates as well as evidence of reversal of cytoadherence in some venules following the treatment with 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-

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**Figure 1.** Arteriovenous red blood cell velocity (V_{rbc}) and wall shear rate profiles in the cremaster microcirculation of uninfected control (■■■) and Plasmodium yoelii-infected (●●●) mice. (a), V_{rbc} (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 V_{rbc} 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 V_{rbc} 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-intercellular adhesion molecule-1 (ICAM-1) and anti-vascular cell adhesion molecule-1 (VCAM-1) antibodies on red cell blood velocities (Vrbc) and wall shear rates (wsr) in the cremaster microcirculation of Plasmodium yoelii-infected mice*

<table>
<thead>
<tr>
<th>Vessel order</th>
<th>Antibody</th>
<th>A2</th>
<th>V4</th>
<th>V3</th>
<th>V2</th>
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<tr>
<td>A2</td>
<td>Anti-ICAM-1 (n = 5)</td>
<td>6.67 ± 1.61</td>
<td>50.7 ± 1.75</td>
<td>11.3 ± 0.54</td>
<td>2.79 ± 0.29</td>
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<tr>
<td>V4</td>
<td>9.32 ± 2.24</td>
<td>63.3 ± 2.42</td>
<td>31.4 ± 1.26</td>
<td>11.4 ± 0.55</td>
<td>2.79 ± 0.29</td>
</tr>
<tr>
<td>V3</td>
<td>9.02 ± 2.24</td>
<td>49.7 ± 1.75</td>
<td>11.3 ± 0.54</td>
<td>2.79 ± 0.29</td>
<td>2.79 ± 0.29</td>
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<tr>
<td>V2</td>
<td>9.32 ± 2.24</td>
<td>63.3 ± 2.42</td>
<td>31.4 ± 1.26</td>
<td>11.4 ± 0.55</td>
<td>2.79 ± 0.29</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.

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
Figure 3. Videomicrographs showing the effect of anti–intercellular adhesion molecule-1 antibody on in vivo cytoadherence of infected red blood cells (IRBCs) in postcapillary venules in the cremaster muscle microcirculation of Plasmodium yoelii–infected mice. In each case, the large arrow indicates the flow direction. A, cytoadherence of IRBCs (small arrows) to the endothelium of a postcapillary venule before the antibody infusion. The arrow-head indicates a rolling leukocyte that is distinguished from adherent IRBCs by its larger diameter. B, the same venules 30 min after the antibody infusion. Note the absence of most of the adhered IRBCs. A single IRBC is seen adhering at the venular junction (small arrow). C, cytoadherence of IRBCs (small arrows) at a downstream location in the same postcapillary venule before the antibody infusion. The arrowhead indicates a rolling leukocyte. D, the same vessel 30 min after the antibody infusion. Note the absence of the adherent IRBCs and leukocytes. Bar = 15 μm.

Table 2

<table>
<thead>
<tr>
<th>Antibody * (Ab)</th>
<th>Mouse no.</th>
<th>Parasitemia</th>
<th>% change</th>
<th>Rings</th>
<th>Trophozoites</th>
<th>Schizonts</th>
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<tr>
<td>Anti-ICAM-1</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>62.1</td>
<td>225.0</td>
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<tr>
<td>2</td>
<td>62.1</td>
<td>-53.6</td>
<td>95.2</td>
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<td>29.0</td>
<td>-56.3</td>
<td>-18.0</td>
<td>53.0</td>
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<tr>
<td>Mean ± SD</td>
<td>41.9 ± 17.7</td>
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<td>46.4 ± 58.2</td>
<td>165.0 ± 97.1⁺</td>
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<tr>
<td>Anti-VCAM-1</td>
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<tr>
<td>3</td>
<td>15.7</td>
<td>11.9</td>
<td>-28.5</td>
<td>-46.2</td>
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</tr>
<tr>
<td>Mean ± SD</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>
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<tr>
<td>Control Ab</td>
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<tr>
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<tr>
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<td>-2.0</td>
<td>-5.9</td>
<td>1.7</td>
<td>15.8</td>
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<tr>
<td>Mean ± SD</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>
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</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.
would include virtually all V3 and V4 venules. Furthermore, the present study demonstrates that despite > 50% reductions in Vrbc and wall shear rates in A2 arterioles in the infected mice (Figure 1), no cytoadherence is observed in arteriolar vessels. This observation indicates that endothelial receptors for IRBC cytoadherence are localized in venules, and probably not in arterioles. Thus, wall shear rates are not the only determining factor in the vascular preference and in the occurrence of IRBC cytoadherence. In addition, there may be differences in endothelial ligands in arteriolar and venular segments.

The overall decrease in arteriovenous Vrbc and wall shear rates in the infected mice is probably a consequence of both cytoadherence and likely rheologic abnormalities of IRBCs. Red blood cells infected with malaria parasite such as P. falciparum show a significantly higher bulk viscosity and a decreased deformability compared with uninfected red blood cells.\(^6,7,41\) Also, infusion of P. yoelii-IRBCs results in higher peripheral resistance in an ex vivo preparation, indicating rheologic abnormalities of these cells.\(^12\)

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.\(^42,43\) Besides E- and P-selectins, VCAM-1 can also mediate rolling (transient adhesion) of leukocytes by binding to leukocyte molecules such as \(\alpha_4\beta_2\).\(^44-46\) On the other hand, ICAM-1 is mainly involved in firm adhesion of these cells via lymphocyte function-associated antigen (LFA or CD11a/CD18) and/or Mac-1 (\(\alpha_m\beta_2\) integrin) (CD11b/CD18), expressed on leukocytes.\(^47\) Immunohistochemical studies of exteriorized cremaster muscle of mice have revealed that surgical trauma induces ICAM-1 expression in all postcapillary venules, but only partially in arterioles.\(^48\) In fact, most arterioles remain negative for ICAM-1.\(^48\) In a severe combined immunodeficiency (SCID) mouse model for human 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.\(^17\)

Most relevant to this discussion is a similar up-regulation of ICAM-1 and VCAM-1 in the cerebral vessels of P. yoelii 17XL-infected mice (Shear HL and others, unpublished data), which is probably a consequence of increased TNF-\(\alpha\) levels in these mice, as also reported for human malaria.\(^32\) More importantly, in P. yoelii17XL-infected mice, as also reported in the SCID mouse model for human cerebral malaria,\(^17\) the cytoadherence and sequestration of IRBCs in small-diameter brain vessels accompanies increased expression of ICAM-1. However, in contrast to P. berghei-infected mice,\(^19\) there is no histologic evidence of leukocyte accumulation in the brain microvasculature in the P. yoelii infection.\(^12\)

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 P. yoelii-infected mice, as also reported for human malaria,\(^32\) is probably a consequence of increased TNF-\(\alpha\) levels in these mice, as also reported for human malaria.\(^32\)
induced rolling of leukocytes in P-selectin deficient mice is totally abolished with concomitant deficiency of ICAM-1, again indicating a role of ICAM-1 in this phenomenon. However, in contrast to the observed rolling of *P. falciparum*-IRBCs on the endothelial monolayer in flow system in venules of the ex vivo mesocirculatory 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. Nevertheless, the MAAb against mouse ICAM-1 (i.e., YN1/7.4) used in the present studies has been previously shown to block the interaction of mouse LFA-1 to ICAM-1 as well as to block the adhesion of human IRBCs in the SCID mouse. 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 significant 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 microrheologic 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) prevention 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.

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REFERENCES

24. Rogerson SJ, Chaiyaroj SC, Ng K, Reeder JC, Brown GV, 1995. Chondroitin sulfate A is a cell surface receptor for *Plasmo-
**ROLE OF ICAM-1 IN MALARIA CYTOADHERENCE**

247


