Prevention of cerebral vasospasm by a humanized anti-CD11/CD18 monoclonal antibody administered after experimental subarachnoid hemorrhage in nonhuman primates

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Object. Leukocyte–endothelial cell interactions occurring in the first hours after subarachnoid hemorrhage (SAH) initiate changes in the endothelium and vessel wall that lead to an influx of leukocytes and the development of chronic vasospasm days later. Upregulation of intercellular adhesion molecule–1 (ICAM-1), also called CD54, appears to be a crucial step in this process. There is increasing experimental evidence that blocking the interaction between ICAM-1, which is expressed on endothelium, and integrins such as lymphocyte function–associated antigen–1 (CD11a/CD18) and macrophage antigen–1 (complement receptor 3, CD11b/CD18), which are expressed on the surface of leukocytes, prevents not only inflammation of vessel walls but also chronic vasospasm. The authors extend their previous work with monoclonal antibody (mAb) blockade of leukocyte migration to a nonhuman primate model of chronic, posthemorrhagic cerebral vasospasm.

Methods. Before surgery was performed, six young adult male cynomolgus monkeys underwent baseline selective biplane common carotid and vertebrobasilar artery cerebral angiography via a transfemoral route. On Day 0, a right frontosphenoidal craniectomy was performed with arachnoid microdissection and placement of 2 to 3 ml of clotted autologous blood in the ipsilateral basal cisterns. The animals were given daily intravenous infusions of 2 mg/kg of either a humanized anti-CD11/CD18 or a placebo mAb beginning 30 to 60 minutes postoperatively. The monkeys were killed on Day 7 after a repeated selective cerebral angiogram was obtained. The area of contrast-containing vessels observed in each hemisphere on anteroposterior angiographic views was calculated for the angiograms obtained on Day 7 and expressed as a percentage of the area on baseline angiograms (percent control areal fraction). Review of flow cytometry and enzyme immunoassay data confirmed the presence of the anti-CD11/CD18 antibody in the serum and bound to leukocytes in the peripheral blood of treated animals. Comparisons of the groups revealed 53 ± 4.8% control vascular areal fraction in the placebo group (two animals) and 95.8 ± 9.4% in the anti-CD11/CD18–treated group (three animals), a statistically significant difference (p = 0.043, t-test).

Conclusions. These results show that blockade of leukocyte migration into the subarachnoid space by an anti-CD11/CD18 mAb is effective in preventing experimental cerebral vasospasm in nonhuman primates, despite the unaltered presence of hemoglobin in the subarachnoid space. These experimental data support the hypothesis that inflammation plays a role in cerebral vasospasm after SAH.

Key Words • subarachnoid hemorrhage • vasospasm • leukocyte • anti-CD11/CD18 monoclonal antibody • intercellular adhesion molecule–1 • cynomolgus monkey

Expression of cell adhesion molecules occurring in the first hours after SAH initiates changes in the endothelium and vessel wall that lead to an influx of leukocytes and the development of chronic vasospasm days later.\textsuperscript{28,50,41} Upregulation of ICAM-1 (also called CD54) appears to be a crucial step in this process. The upregulation of ICAM-1 occurs within the first 3 hours after periadventitial exposure of the rat FA to blood.\textsuperscript{29} and ICAM-1 is also upregulated in intracranial models of SAH.\textsuperscript{29}

There is increasing experimental evidence that blocking the interaction of ICAM-1, which is expressed on endothelium, and integrins such as LFA-1 (CD11a/CD18) and Mac-1 (complement receptor 3, CD11b/CD18), which are expressed on the surface of leukocytes, prevents not only inflammation of vessel walls but also chronic posthemorrhagic vasospasm.\textsuperscript{7,12,28,44} In the FA model of chronic vasospasm in rats, intraperitoneal administration of an anti–ICAM-1 mAb can inhibit vasospasm after periadventitial exposure of vessels to blood.\textsuperscript{28} There is also experimental evidence that blocking the integrins LFA-1 and

Abbreviations used in this paper: ACA = anterior cerebral artery; FA = femoral artery; ICA = internal carotid artery; ICAM-1 = intercellular adhesion molecule–1; Ig = immunoglobulin; LFA-1 = lymphocyte function–associated antigen–1; mAb = monoclonal antibody; Mac-1 = macrophage antigen–1; MCA = middle cerebral artery; SAH = subarachnoid hemorrhage; SEM = standard error of the mean.
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Mac-1 on the surface of neutrophils and macrophages, respectively, prevents the development of chronic, posthemorrhagic vasospasm in the FA in rats. The efficacy of LFA-1 blockade has also been proven in an intracranial model of chronic, posthemorrhagic vasospasm in rabbits. The perivascular release of ibuprofen, a nonselective cyclooxygenase inhibitor that also downregulates ICAM-1, is also effective in preventing experimental posthemorrhagic vasospasm, provided it is delivered within the first 24 hours after exposure to blood.

Although small animals have been used as models of posthemorrhagic vasospasm, none faithfully reproduces the time course and anatomy of chronic vasospasm that are seen in cerebral vessels after aneurysmal SAH in humans. Although the rat FA model reproduces the time course of cerebral vasospasm in a vessel similar to its histological features to human cerebral vessels, it is not an intracranial artery. In the rabbit basilar artery model, vasospasm develops on a more rapid time course than that seen in humans, and the canine model requires multiple injections of blood into the subarachnoid space to generate vasospasm. For these reasons, we have extended our previous work with mAb blockade of leukocyte migration to the well-established nonhuman primate model of chronic, posthemorrhagic cerebral vasospasm.

Materials and Methods

Nonhuman Primates

Six young adult male cynomolgus monkeys weighing 2 to 5 kg were acquired from BRF/Charles River Laboratories (Houston, TX). The animals were housed and cared for under the guidelines of the Johns Hopkins Animal Care and Use Committee and the US Department of Agriculture. Food intake was restricted for 12 hours prior to all procedures; however, the animals had unlimited access to water. The monkeys received acetylsalicylic acid (15 mg/kg) rectally after all procedures.

Study Design

The animals underwent baseline cerebral angiography before surgery. On Day 0, surgery was performed as described later to generate an experimental SAH. The monkeys were examined twice daily and a neurological grade was assigned as previously described.

The animals also underwent phlebotomy daily after ketamine sedation (10–20 mg/kg) was induced intramuscularly. On selected occasions, 1.5 ml of blood was removed from the saphenous or femoral vein for flow cytometry binding site saturation determinations and for enzyme immunoassay analysis of antibody levels. All animals received intravenous injections of 2 mg/kg of humanized anti-CD11/CD18 mAb or placebo following surgery, and then daily thereafter. The monkeys were randomly assigned to one of the two groups and treatment was given in a blinded fashion. The initial treatment dose was administered 30 to 60 minutes after clot deposition. The animals also received daily intramuscular injections of ceftriaxone (25 mg/kg). The monkeys were killed on Day 7 after a repeated cerebral angiogram was obtained. At the time of preoperative angiography and on the day of death, 2 ml of blood was also withdrawn for complete blood counts and an electrolyte panel. All procedures in this study were approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine and conform to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996).

Humanized Anti-CD11/CD18 mAb (Hu23F2G)

The humanized anti-CD11/CD18 mAb Hu23F2G (ICOS Corp., Bothell, WA) is a chimeric mAb created using a complementary DNA construct containing the variable region from a mouse mAb specific for the combination of CD11 and CD18 (constituents of integrins LFA-1 and Mac-1) and the human G4 constant region. This humanized mAb retains the ability to recognize CD11/CD18 but does not fix complement, has low Fc receptor binding, and usually does not elicit an immune response in humans.

Angiographic Studies

The monkeys underwent selective, biplane cerebral angiography before, Day 0 and on Day 7. According to the following procedure: animals were sedated with intramuscularly delivered ketamine (10–20 mg/kg) and atropine (0.05 mg/kg). Intravenous access was obtained with a 24-gauge angiocatheter implanted in the saphenous or brachial vein, and the animals were anesthetized with intravenously administered pentobarbital (12.5 mg/kg). Additional doses of Nembutal were given as necessary to keep the animals anesthetized. The heart rate and oxygen saturation were monitored throughout the procedure. An intravenous infusion of lactated Ringer solution was maintained at a rate of approximately 10 to 15 ml/kg/hr. The groin was prepared and draped in a sterile fashion. A No. 4 French micro puncture set was used to obtain percutaneous access to the FA, followed by placement of a No. 4 French sheath. A 0.035-in guidewire was then used to pass a No. 4 French pigtail catheter into the left ventricle. The anterolateral and posterior vessels of the circle of Willis were injected by hand. Images were acquired with a biplane fluoroscope (Integris; Phillips Medical Systems, Best, The Netherlands) at three frames per second. The anteroposterior and lateral distances for all images were 102 and 106 cm, respectively. Some vessels were selectively catheterized using a No. 4 French Alexander Iron-Duke catheter. After the procedure the FA sheath was removed and pressure was placed on the groin for 20 minutes. The animals were allowed to recover and then returned to their cages. After angiograms were obtained on Day 7, anesthesia was maintained until the monkeys were killed.

Surgical Procedure

The animals underwent surgery on Day 0: anesthesia was induced with intramuscularly delivered ketamine (10–20 mg/kg), the animals were subsequently intubated, and anesthesia was maintained with halothane (0.7–1.5 vol %) and nitrous oxide (0.5 L/minute). Intravenous access was obtained as described earlier. Before surgery, the animals had received cefazolin (25 mg/kg), dexamethasone (0.1 mg/kg), Lasix (0.5 mg/kg), and mannitol (0.5 mg/kg), all delivered intravenously. Approximately 3 ml of peripheral blood was then removed and placed in a Vacutainer gel and clot activator tube. Heart rate, temperature, respiratory rate, and oxygen saturation were continuously monitored. Lactated Ringer solution was infused at a rate of 10 ml/hour.

Each animal’s head was shaved, prepared with povidone–iodine and ethanol, and draped in a sterile fashion. A trapdoor incision was made on the right side with the base of the skin flap along the zygomatic bone, and the flap was elevated. Electrocautery was used to elevate the temporalis muscle separately with its base along the posterior skin incision by separating it from the skull along its insertions at the linea temporalis, orbital rim, and zygoma. A high-speed drill was then used to make a small defect near the pterion. Rongeurs and Kerrison punches were used to make a 2.5 × 3-cm craniectomy defect centered on the sphenoid wing, and a C-shaped dural incision was made anteriorly with its base along the sphenoid bone by using a No. 11 scalpel blade and microscissors. The operating microscope was focussed at the field. Under high magnification, the common carotid artery, containing the ICA, MCA origin, and the ACA origin were opened and cerebrospinal fluid was drained using a 45° angled microdissector and an arachnoid knife. Autologous clot was then placed around the exposed vessels in the basal and Sylvian cisterns. The dura was closed primarily with an interrupted 6-0 monofilament suture and covered with temporalis fascia and Gelfoam. The temporalis muscle was sutured down to tissue cuffs remaining on the skull, orbital rim, and zygoma by using a 3-0 braided absorbable suture. The skin was approximated in two layers and the same suture was used, with buried, interrupted, and cutaneous running stitches. Approximately 30 to 60 minutes after placement of the clot and...
TABLE 1

Results of neurological examinations in six monkeys with SAH*

<table>
<thead>
<tr>
<th>Exam Interval</th>
<th>Anti-CD11/CD18 Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>975</td>
<td>69-101</td>
</tr>
<tr>
<td>baseline</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
<tr>
<td>Day 1</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
<tr>
<td>Day 2</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
<tr>
<td>Day 3</td>
<td>1 1 1 1 1 1</td>
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<tr>
<td>Day 4</td>
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</tr>
<tr>
<td>Day 5</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
<tr>
<td>Day 6</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
<tr>
<td>Day 7</td>
<td>1 1 1 1 1 1</td>
<td>1 1 1 1 1 1</td>
</tr>
</tbody>
</table>

* Numbers directly over columns are the individual monkey’s identifying number.
† Neurological grading scale scores were as follows: 1, normal; 2, decreased activity but no focal deficit; 3, lethargic, focal deficit but responds to stimulation; 4, obtunded, focal deficit and poor response to stimulation; and 5, moribund. (The scale was developed by Weir and colleagues).

once daily thereafter, each animal was treated with either 2 mg/kg of anti-CD11/CD18 mAb or a placebo mAb. Fifteen minutes after the initial treatment, 1.5 ml of blood was withdrawn for flow cytometry and enzyme immunoassay determination of posttreatment CD11/CD18 receptor binding site saturation and serum antibody levels. The animals were allowed to recover and then returned to their cages. Neurological examinations were conducted on the night of surgery and then twice daily.

Flow Cytometry

Flow cytometry was performed on peripheral leukocytes to determine CD11/CD16 receptor binding site saturation by using standard methods. Briefly, whole blood was collected in ethylenediamine tetraacetic acid Vacutainer tubes and shipped to the University of Washington Hematopathology Laboratory (Seattle, WA) for analysis. Specimens were fixed with 0.4% formaldehyde and 0.2% glucose in phosphate-buffered saline (1:1 ratio). Erythrocytes were lysed using an ammonium chloride buffer, and leukocytes were stained with a murine mAb specific for human IgG4 and conjugated with fluorescein isothiocyanate. This antibody specifically detects the bound anti-CD11/CD18 mAb used in these experiments. An isotype-matched murine mAb conjugated to fluorescein isothiocyanate was used as a control. Total CD11/CD18 levels were assessed by analyzing leukocytes incubated with 10 µg/ml of the anti-CD11/CD18 mAb (saturating dose). Stained cells were analyzed using an EPICS-XL flow cytometer and Systems II software, version 3.0 (both from Beckman/Coulter, Fullerton, CA). The percentage of saturation of CD11/CD18 receptors was calculated as previously reported.

Enzyme Immunoassay

Blood was collected in red Vacutainer tubes, and serum was isolated from red blood cells in a microcentrifuge at 14,000 rpm. The plasma was stored at −70°C prior to analysis. Diluted serum samples or anti-CD11/CD18 mAb standards were added to microtiter wells coated with a complex of Fe-specific goat anti-mouse Ig and the mAb 58G12A (specific for the complementarity-determining regions of the anti-CD11/CD18 mAb). Bound anti-CD11/CD18 mAb was detected with Fe-specific goat anti-human Ig linked to horseradish peroxidase. Tetramethylbenzidine was used as the peroxidase substrate.

Evaluation of Angiographic Data

Angiographic images were obtained and digitized. All digital images were printed at similar magnifications. Measurements were made in a blinded fashion, as follows: a point-counting grid was placed over each image, and the number of points that fell on contrast-containing vascular profiles was counted, generating a vascular areal fraction for each hemisphere. Only anterior circulation vascular profiles were included; the ACA distal to the anterior communicating artery (azygous in these primates) was excluded. The vascular areal fraction of the right (experimental) hemisphere was then

TABLE 2

Flow cytometry data: percent receptor binding site saturation for CD11/CD18 in six monkeys*

<table>
<thead>
<tr>
<th>Exam Interval</th>
<th>Anti-CD11/CD18 Group</th>
<th>Placebo Group</th>
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</thead>
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<tr>
<td></td>
<td>Lymphocyte</td>
<td>Monocyte</td>
</tr>
<tr>
<td>baseline</td>
<td>0.4 ± 0.3</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>post 1st dose</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>predose</td>
<td>102.8 ± 1.1</td>
<td>93.6 ± 3.5</td>
</tr>
<tr>
<td>Day 1</td>
<td>88.4 ± 5.4</td>
<td>90.0 ± 3.8</td>
</tr>
<tr>
<td>Day 2</td>
<td>97.8 ± 9.4</td>
<td>100.2 ± 2.9</td>
</tr>
<tr>
<td>Day 3</td>
<td>87.1 ± 6.6</td>
<td>90.3 ± 2.0</td>
</tr>
<tr>
<td>Day 4</td>
<td>96.6 ± 0.5</td>
<td>100.9 ± 0.2</td>
</tr>
<tr>
<td>Day 5</td>
<td>87.7 ± 0.4</td>
<td>96.2 ± 1.6</td>
</tr>
<tr>
<td>Day 6</td>
<td>82.9 ± 15.0</td>
<td>94.1 ± 10.0</td>
</tr>
<tr>
<td>Day 7</td>
<td>86.7 ± 0.5</td>
<td>84.6 ± 6.9</td>
</tr>
<tr>
<td>mean DPS†</td>
<td>92.5 ± 1.1</td>
<td>84.6 ± 6.9</td>
</tr>
</tbody>
</table>

* Values are given as the means ± the SEM. Abbreviation: DPS = daily predose saturation.
† p < 0.001.

TABLE 3

Enzyme immunoassay: anti-CD11/CD18 mAb levels (µg/ml) in two monkeys*

<table>
<thead>
<tr>
<th>Exam Interval</th>
<th>Anti-CD11/CD18 Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>post 1st dose</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>47.35</td>
</tr>
<tr>
<td>Day 1</td>
<td>11.36</td>
<td>40.32</td>
</tr>
<tr>
<td>Day 2</td>
<td>44.06</td>
<td>43.64</td>
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<tr>
<td>Day 3</td>
<td>50.76</td>
<td>58.66</td>
</tr>
<tr>
<td>Day 4</td>
<td>68.83</td>
<td>68.83</td>
</tr>
</tbody>
</table>

* Numbers directly over columns are the individual monkey’s identifying number.
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expressed as a percentage of the left (control) hemisphere. Areal fractions were normalized to preoperative angiograms. The vascular areal fraction was chosen as the method of measurement because it takes into consideration global hemispheric vascular status, reflecting the caliber of proximal intracranial vessels as well as the more distal anterior circulation vasculature.

Makign accurate diameter measurements of the proximal intracranial vasculature in these animals is difficult even with the latest angiographic equipment, given the small caliber of these vessels and the inherently high resolution of digital subtraction angiograms. Nevertheless, measurements of diameter were taken in the proximal intracranial vessels, the supraclinoid ICA, the ACA, and the MCA. All measurements were made to the nearest millimeter and taken either immediately in front of or just beyond the bifurcation.

Statistical Analysis

All data are presented as the mean ± the SEM. Angiographically determined percent control vascular areal fractions, proximal vessel diameters, and physiological parameters were compared using a t-test because all data sets passed normality and equal variance tests.

Results

Physiological Data

The mean laboratory values (± SEM) for baseline and Day 7 electrolyte and hematological parameters were compared. Student t-tests were conducted to compare pre- and posttreatment values within each group. Statistically significant differences were found for some of the electrolyte parameters; however, these values all fell within physiological ranges for cynomolgus monkeys. Although there is a large apparent difference in the baseline and posttreatment white blood cell counts (8.2 ± 0.8 and 62.9 ± 20.3 × 10^9/mm^3, respectively) in the animals receiving the anti-CD18 mAb, as would be expected, statistical significance was not reached (p = 0.054) because of the wide variability in the values. This probability value represents a trend, a near statistical significance with one value outside the physiological range. In the placebo group, the baseline and posttreatment white blood cell counts were 8.9 ± 2.8 and 11.8 ± 1.4 × 10^9/mm^3, respectively (p = 0.408). Two animals in the treatment group exhibited decreased activity over several days after the initiation of treatment but had no focal deficits; no animal in the placebo group displayed similar behavior (the relevance of this observation is unclear). Table 1 presents the baseline and subsequent neurological grades of animals in both groups throughout the course of treatment.

Flow Cytometry

The mean percentage of CD11/CD18 receptor binding site saturation was calculated for lymphocytes, monocytes, and granulocytes in the two groups before treatment, immediately after the initial dose, and on selected Days 1 to 7 before each day’s dosing (Table 2). Pretreatment saturation levels in all animals for all leukocyte subsets were less than 1.1% (data not shown). Saturation levels 15 minutes after the initial dose ranged from 87.7 to 105.1% in animals receiving the anti-CD11/CD18 mAb and 0 to 2.1% in animals receiving placebo (data not shown). The mean daily pre- and posttreatment saturation level for all leukocytes was 92.5% in anti-CD11/CD18-treated animals and 2.1% in placebo-treated animals (p < 0.001, t-test; Table 2). The highest single saturation level seen in any placebo-treated control animal was 18.6% (data not shown).

Enzyme Immunoassay

Levels of anti-CD11/CD18 mAbs were quantified for four animals (two in the treatment group and two in the placebo group) in the study before treatment, immediately after the initial dose, and then daily before dosing (Table 3). Levels were undetectable before treatment (baseline) in all animals and ranged from 0.5 to 68.8 μg/ml in treated animals.

Angiographic Measurements

Comparison of the groups revealed a 53 ± 4.8% control vascular areal fraction in the placebo group (two animals) and a 95.8 ± 9.4% vascular areal fraction in the anti-CD11/CD18 treatment group (three animals), a statistically significant difference (p = 0.043, t-test; Figs. 1 and 2). Measurements of proximal vessel diameters revealed a consistent trend toward increased diameter in the anti-CD11/CD18 treatment group (ICA: 75.7 ± 0.1% compared with 53.5 ± 0.1%, p = 0.324; MCA: 74.3 ± 0.1% compared with 50 ± 0.2%, p = 0.222; ACA: 89 ± 0.2% compared with 50 ± 0%, p = 0.071). One animal in the placebo group was excluded from analysis as a result of technical problems that made their Day 7 angiogram unavailable for digitization and analysis.

Discussion

There is evolving evidence to support a role for inflammation in the pathophysiological mechanisms of chronic cerebral vasospasm following aneurysmal SAH.29–32,34,36,42 We have postulated that molecular events occurring in the first hours after vasospasm initiate upregulation of cell adhesion molecules on the endothelium of vessel walls, which leads to an influx of leukocytes and the development of chronic vasospasm.28,39,41 In this hypothesis we predict that blockade of the interaction between ICAM-1 on the vascular endothelium and integrins on the surface of leukocytes can prevent chronic cerebral vasospasm by interrupting the necessary cascade of molecular inflammatory events. In this preliminary study we demonstrate that it is generally possible in a primate model to maintain 80% or greater saturation of CD11/CD18 receptors on leukocytes for 7 days and that this prevents neuroimaging-confirmed cerebral vasospasm at 1 week after experimental SAH.

These results support the idea that leukocyte–endothelial interactions play an important role in the development of chronic cerebral vasospasm. In this model, as well as in the others in which leukocyte blockade has been shown to prevent experimental vasospasm,7,12,28 blood remains in the periadventitial space. From this we infer that although the presence of periadventitial blood is necessary for the development of vasospasm, its presence alone is not sufficient. The only other major change in cerebral vessels that is visible at the cellular level after SAH is the presence of leukocytes in the vessel wall and subarachnoid space. In these models, prevention of leukocyte influx averts the development of vasospasm.7,12,28 Taken together, these facts indicate that molecular events resulting from the presence of leukocytes in the vessel wall and subarachnoid space are the primary factor that determines the cellular processes behind vasospasm. The hemorrhage itself may be responsible only
for the initial molecular signals leading to the subsequent inflammatory state.

Leukocytes are known to produce endothelins, which are potent constrictors of vascular smooth muscle that have been implicated in cerebral vasospasm. In the rat FA model, exposure of the abluminal vessel wall to endothelin alone is capable of initiating and maintaining changes and luminal narrowing consistent with chronic posthemorrhagic vasospasm. Leukocytes also release free radicals in the form of reactive oxygen moieties that can interact with and destroy endogenous nitric oxide, a crucial molecule in endothelium-dependent relaxation. Leukocytes migrating through the vessel wall are ideally positioned in proximity to the endothelium and the vascular media. This is crucial to creating and disrupting molecular messages that act over short distances and involve highly reactive, short-lived molecules such as nitric oxide.

The humanized anti-CD11/CD18 mAb used in this investigation has been studied in multiple clinical trials. Results of two of these trials have been published, one in patients with multiple sclerosis and the other in patients with acute myocardial infarction who underwent angioplasty. Although both were Phase I studies and not designed to evaluate the agent’s efficacy, both included clinical variables that could be assessed for an effect of the antibody. In the multiple sclerosis study neither the patients’ neurological status nor their magnetic resonance findings appeared to improve with treatment, and in the myocardial infarction study single-photon emission computerized tomography scanning failed to demonstrate a decrease in the size of the left ventricular defect, despite the fact that both investigations demonstrated high saturation (> 80%) of CD11/CD18 receptors, and in both cases similar animal models in nonhuman primates demonstrated protective effects associated with this antibody. Further work is needed to understand the discrepancies that appear when generalizing the observed effects from animals to humans.

The vascular areal fraction measurements demonstrated...
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Fig. 2. Histogram showing the percent control vascular areal fraction in the placebo- and anti-CD11/CD18–treated groups. The difference is statistically significant according to t-tests (p = 0.043).

near-complete reversal of cerebral vasospasm, whereas the effects on the diameter measurements of the proximal cerebral vasculature were not as dramatic. The latter showed a clear trend toward reversal of vessel narrowing; however, the results are not statistically significant given the power of the experiment. Preliminary analysis of histological measurements of vessel cross-sectional area revealed even greater variability in proximal vessel caliber. One possible explanation of this discrepancy is that the effect of leukocyte blockade in this model of cerebral vasospasm is more prominent in the distal cerebral vasculature than in the larger proximal conducting vessels. Further experiments will be necessary to understand this observation and its significance.

In this study, animals received the anti-CD11/18 mAb within 1 hour of the deposition of autologous clot in the subarachnoid space. More experiments will be needed to assess the time window over which this treatment can be initiated and remain effective. Previous work has indicated that antiinflammatory treatment designed to disrupt leukocyte–endothelial interactions exhibits efficacy in preventing chronic vasospasm only when initiated within 24 hours of periadventitial blood exposure.41 Many patients who present for treatment after acute SAH are diagnosed within this 24-hour window of opportunity, making an antiinflammatory treatment strategy feasible.

Conclusions

In this study we have demonstrated the feasibility of using a humanized anti-CD11/18 mAb in the treatment of chronic cerebral vasospasm in nonhuman primates after experimental SAH. In addition, we found a statistically significant decrease in angiographically confirmed vasospasm in the anti-CD11/CD18 mAb group despite the low power of the experiment. Further experiments are necessary to confirm these findings in a larger number of animals and at various times after the onset of hemorrhage before considering human clinical trials of this agent for cerebral vasospasm.

Acknowledgment

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Disclosure

Drs. Ogata and Dietsch and Mr. Gebremariam are employees of ICOS Corporation but the company did not fund the study.

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