

# Postischemic Administration of an Anti-Mac-1 Antibody Reduces Ischemic Cell Damage After Transient Middle Cerebral Artery Occlusion in Rats

Michael Chopp, PhD; Rui Lan Zhang, MD; Hua Chen, MD; Yi Li, MD; Ning Jiang, MD; James R. Rusche, PhD

**Background and Purpose** Postischemic cerebral inflammation may contribute to ischemic cell damage. The CD11b/18 (Mac-1) integrin mediates stimulated neutrophil binding to endothelia. We therefore investigated the effect of administration of an anti-Mac-1 monoclonal antibody on cerebral ischemic cell damage in the rat.

**Methods** Rats (n=10) were subjected to 2 hours of middle cerebral artery occlusion; the anti-Mac-1 antibody was administered at a dose of 2 mg/kg IV at 1 hour of reperfusion and 1 mg/kg IV at 22 hours of reperfusion or an isotype-matched control antibody (n=10) was administered using the same experimental protocol. Rats were killed at 46 hours of reperfusion, and brain sections were stained with hematoxylin and eosin for histological evaluation. In a separate population of rats given either vehicle (n=8) or anti-Mac-1 antibodies

(n=9), intraparenchymal neutrophils were measured by means of a myeloperoxidase assay.

**Results** The lesion volume was significantly smaller (28%) in the anti-Mac-1 antibody group compared with the vehicle control group ( $P<.01$ ). Numbers of intraparenchymal polymorphonuclear cells were significantly reduced ( $P<.05$ ) in the cortex of the anti-Mac-1 antibody group compared with the vehicle control group.

**Conclusions** Our data demonstrate that administration of anti-Mac-1 antibody 1 hour after onset of reperfusion results in significant reductions of ischemic cell damage and intraparenchymal neutrophils after transient (2-hour) focal cerebral ischemia in the rat. (*Stroke*. 1994;25:869-876.)

**Key Words** • cerebral ischemia • leukocytes • rats • neutrophils

The interaction between molecules expressed on the surface of leukocytes (receptors) and molecules on the surface of potential target cells (ligand), such as endothelia, is important in the development and maintenance of inflammatory and immune responses. Although many adhesion proteins have been identified on leukocytes, there is increasing experimental evidence in vivo that the  $\beta$ -2 integrins on leukocytes may be important in the pathogenesis of inflammatory diseases.<sup>1,2</sup> There are three leukocyte integrins (LFA-1, Mac-1, p150, 95) that have a common  $\beta$ -chain, CD18, and three different  $\alpha$ -chains (CD11a, CD11b, and CD11c, respectively).<sup>2-6</sup> CD11b/18 (Mac-1) primarily mediates stimulated neutrophil binding to endothelia.

Mac-1 (CD11b/18) participates in reperfusion ischemia injury, such as myocardial ischemia<sup>7,8</sup> and liver ischemia.<sup>9</sup> Reperfusion after ischemia may exacerbate tissue injury and cause a peripheral inflammatory response in many organ systems.<sup>10-12</sup> Ischemic brain injury evokes not only endogenous brain parenchymal cell damage but also an exogenous inflammatory response, which includes infiltration and accumulation of poly-

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morphonuclear leukocytes (PMNs) and monocytes/macrophages, as well as microvascular proliferation.<sup>13-17</sup> Neutrophils are initially the predominant leukocytes at inflammation sites, followed by infiltration of mononuclear phagocytes.<sup>18,19</sup> The migration and accumulation of neutrophils into the ischemic tissue after reperfusion ischemia is not only associated with tissue repair processes but also may result in injury to potentially viable tissue.<sup>10,20</sup> The migration of neutrophils into the injured tissue, together with red blood cells and plasma proteins, may cause capillary plugging and a reduction of microvascular blood flow.<sup>21</sup> Stimulated neutrophils release oxygen free radicals and proteinase that may promote cell death.<sup>12,18</sup>

Reduction of the numbers of peripheral neutrophils, using antibodies to neutrophils, diminishes cell damage after myocardial ischemia,<sup>22-24</sup> lung ischemia,<sup>25,26</sup> and liver ischemia<sup>11</sup> and reduces ischemic injury<sup>27</sup> and physiological dysfunction<sup>28</sup> in the central nervous system. Neutropenia improves cortical somatosensory-evoked response after cerebral ischemia in the dog induced by air embolism,<sup>29</sup> reduces cerebral infarct volume after transient middle cerebral artery (MCA) occlusion in the rat,<sup>30</sup> and improves cortical electrical activity after incomplete forebrain ischemia in the rat.<sup>31</sup> Depletion of neutrophils in a rabbit model of thromboembolic stroke reduces ischemic injury and increases cerebral blood flow.<sup>27</sup> However, the induction of neutropenia has a general toxic effect of

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From the Department of Neurology, Henry Ford Hospital, Detroit, Mich (M.C., R.L.Z., H.C., Y.L., N.J.), the Department of Physics, Oakland University, Rochester, Mich (M.C.), and the Repligen Corporation, Cambridge, Mass (J.R.R.).

Correspondence to Michael Chopp, PhD, Department of Neurology, Henry Ford Hospital, 2799 W Grand Blvd, Detroit, MI 48202.

depressing the immune system and causes a significant and prolonged reduction of total peripheral white blood cells. As opposed to using neutropenia to investigate the contribution of the neutrophil to ischemic cell damage, we investigated the role of the Mac-1 integrin that specifically participates in the process of neutrophil infiltration into the ischemic brain tissue. Mac-1 mediates the binding of activated leukocytes to vascular endothelial cells,<sup>2,32</sup> and anti-Mac-1 antibodies have been shown to prevent migration of leukocytes into tissue and to reduce ischemic cell damage.<sup>9,33,34</sup>

If ischemic cell damage is exacerbated by a delayed process of leukocyte presence in the affected tissue, it is reasonable to assume that postischemic interference with this process of inflammation may reduce the detrimental consequences of leukocyte involvement with ischemic cell damage. To our knowledge, there have been no studies in which an anti-integrin antibody has been administered after onset of reperfusion. Therefore, in the present experiment we investigated the effect on ischemic cell damage of administering the anti-Mac-1 antibody to rats subjected to 2 hours of middle cerebral artery occlusion and 1 hour of cerebral reperfusion. Our data indicate that delayed administration of this antibody significantly reduces ischemic cell damage.

### Materials and Methods

Male Wistar rats (270 to 300 g;  $n=20$ ) were used in the experiment. Transient MCA occlusion was induced by advancing a 4-0 surgical nylon suture into the internal carotid artery (ICA) to block the origin of the MCA.<sup>14,35,36</sup> Briefly, animals were anesthetized with 3.5% halothane, and anesthesia was maintained with 1.0% to 2.0% halothane in 70% N<sub>2</sub>O and 30% O<sub>2</sub> using a face mask. Rectal temperature was maintained at 37°C throughout the surgical procedure using a feedback-regulated water heating system. The right femoral artery and vein were cannulated for measuring blood gases (pH, PO<sub>2</sub>, PCO<sub>2</sub>) before ischemia, for collecting blood samples, and for drug administration, respectively. A length of 18.5- to 19.5-mm 4-0 surgical nylon suture, determined by the animal weight, was advanced from the external carotid artery (ECA) into the lumen of the ICA until it blocked the origin of the MCA. Two hours after MCA occlusion, animals were reanesthetized with halothane, and reperfusion was performed by withdrawal of the suture until the tip cleared the ICA lumen.

The anti-Mac-1 antibody that we used in our studies acts against the CD11b epitope. This antibody, obtained from Repligen Corporation, is a mouse anti-rat antibody (35, clone 1B6c). The endotoxin level of the anti-Mac-1 antibody is  $<0.1$  EU/mg.

Rats were in a fasting condition overnight before surgery but had free access to water. Animals were randomly divided into two groups: 1B6c group ( $n=10$ ) and vehicle control group ( $n=10$ ). The 1B6c group was subjected to MCA occlusion, and the anti-Mac-1 antibody was infused intravenously over a 3-minute interval at doses of 2 mg/kg at 1 hour and 1 mg/kg at 22 hours after reperfusion. The vehicle control group was subjected to MCA occlusion and was administered an isotype-matched control antibody at 1 hour and 22 hours of reperfusion, using the same volume dose as the 1B6c group. Ischemic and vehicle control rats were weighed before fasting and at 22 hours and 46 hours after reperfusion.

Peripheral blood samples were obtained in rats before and 15 minutes after each antibody administration, and at 46 hours after the first infusion. Measurements of peripheral white blood cell (WBC) counts and differentials were performed manually using a hemocytometer and by blood smears stained

with Wright-Giemsa stain, respectively. One hundred cells were counted for each of the differentials. The percentage of differentials was multiplied by the WBC counts to obtain the absolute number per milliliter of blood.

Rats were anesthetized with ketamine (44 mg/kg IM) and xylazine (13 mg/kg IM) at 46 hours of reperfusion. Rats were transcardially perfused with heparinized saline and 10% buffered formalin, and brains were removed. To confirm that the inserted suture passed the origin of the MCA, animals were injected intravenously with 1 mL of 2% Evans blue dye 30 minutes before perfusion. The Evans blue dye stains the vascular wall along the path of the suture. Each brain was cut into 2-mm-thick coronal blocks, for a total of 7 blocks per animal, using a rat brain matrix. The brain tissue was processed, embedded, and 6- $\mu$ m-thick paraffin sections from each block were cut and stained with hematoxylin and eosin for histopathological evaluation.

Tissue volume was measured using a GLOBAL LAB IMAGE image analysis program (Data Translation). Each hematoxylin-eosin section was evaluated at  $\times 2.5$  magnification. The lesion area and the ipsilateral hemispheric area (in square millimeters) were calculated by tracing the areas on the computer screen, and the volumes (in cubic millimeters) were determined by multiplying the appropriate area by the section interval thickness.<sup>37</sup> To avoid errors associated with processing of the tissue for histological analysis, the lesion volume size was also presented as the percentage of lesion to the ipsilateral hemisphere.

Numbers of PMNs within brain were quantified using a myeloperoxidase (MPO) activity assay described by Barone et al<sup>38</sup> and modified in our laboratory. Measurements were performed on vehicle control rats ( $n=8$ ) and on rats given anti-Mac-1 antibody ( $n=9$ ) killed 48 hours after MCA occlusion.

PMNs were also collected from normal rats ( $n=8$ ) and evaluated for MPO activity to establish a quantitative relation for units of MPO activity per PMN. Whole blood was collected by cardiac puncture in syringes containing heparin (Eli Lilly & Co; approximately 20 U/mL blood). The samples were centrifuged at 150g for 45 minutes (25°C). The leukocyte buffy coat was harvested. The separation of PMNs from mononuclear leukocytes and red blood cells using the buffy coat was accomplished by centrifugation through discontinuous gradients of Ficoll (Histopaque-1077, Sigma Diagnostics), after which the PMNs were isolated from residual red blood cells by dextran sedimentation (Dextran T 500, Pharmacia LKB).

For MPO analysis in ischemic brain tissue, rats were subjected to 2 hours of MCA occlusion, and 46 hours later they were anesthetized with ketamine and xylazine and perfused transcardially with 200 mL heparinized saline solution (25°C at a pressure of 100 mm Hg) before brain removal to flush all blood components from the vasculature. Forebrain tissue was obtained from interaural 12 mm to interaural 2 mm.<sup>39</sup> Cortical tissue and the subcortical portion of the hemisphere were dissected. Each specimen was sectioned into cortical and subcortical tissue from both the ipsilateral and contralateral hemispheres. The four forebrain segments were immediately frozen on dry ice and transferred at  $-80^{\circ}\text{C}$  for MPO analysis.

For the biochemical assay of MPO, tissue segments were thawed on ice, and wet weight in grams was rapidly measured. The tissue was homogenized using a glass homogenizer in 4 mL of 10 mmol/L tris(hydroxymethyl)aminomethane hydrochloride (pH 7.4, 4°C). A standardized 100-mg wet weight for the MPO activity assay was rehomogenized in 20 mL of 5 mmol/L phosphate buffer (pH 6.0, 4°C) using a Tissumizer homogenizer (Tekmar Co; on/off cycles at 5-second intervals) and centrifuged at 30 000g for 30 minutes at 4°C. The supernatant was discarded, and the pellet was washed again as described above. After decanting the supernatant, the pellet (or  $0.1$  to  $4.0 \times 10^6$  PMN) was extracted by suspending the material in 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Co) in 50 mmol/L potassium phosphate buffer

**TABLE 1. Peripheral White Blood Cell and Differential Values Before and After Antibody Administration in Rats Treated With Control Vehicle and Anti-Mac-1 1B6c**

Group	Time	White Blood Cells, ×1000/mm <sup>3</sup>	Lymphocytes, ×1000/mm <sup>3</sup>	Neutrophils, ×1000/mm <sup>3</sup>	Monocytes, ×1000/mm <sup>3</sup>
Vehicle (n=4)	Before	7.5±0.4	4.9±1.0	2.1±1.1	0.2±0.1
	15 min	7.3±0.6	3.9±0.9*	3.3±1.0*	0.2±0.2
	22 h	7.1±0.5	4.2±0.8	2.5±0.6	0.2±0.1
	22 h 15 min	7.3±0.4	4.6±0.9	2.4±0.7	0.1±0.1
	46 h	7.1±0.8	4.9±1.3	2.4±1.0	0.1±0.1*
1B6c (n=4)	Before	7.4±0.4	5.7±0.9	1.4±0.6	0.2±0.2
	15 min	7.3±0.8	4.6±0.5*	2.2±0.4*	0.2±0.1
	22 h	6.9±0.7	6.1±0.8*	0.6±0.4*	0.2±0.2
	22 h 15 min	6.7±0.9	5.9±0.9	0.7±0.2*	0.1±0.1
	46 h	6.9±0.8	5.8±0.9	0.8±0.4*	0.3±0.2
Normal range†		6.0-17.0	3.9-14.5	0.5-5.8	0-0.9

Values are mean±SD.

\**P*<.05 compared with the value before administration.

†Taken from Reference 42.

(1:10), pH 6.0, at 25°C. The specimens were frozen on dry ice and subjected to three freeze-thaw cycles, after which sonication was repeated between cycles. After the last sonication, the samples were then centrifuged at 12 000g for 15 minutes at 4°C, and the MPO activity in the supernatant was assayed as described earlier by Bradley et al.<sup>40</sup> The rate at which a colored product formed during the MPO-dependent reaction of *o*-dianisidine dihydrochloride (0.167 mg/mL; Sigma Chemical Co) was measured. The change in absorbance at 460 nm was recorded at 1-minute intervals over 2 minutes, using a UV160U recording spectrophotometer (Shimadzu). One unit of MPO activity was defined as that degrading 1 micromole of peroxide per minute at 25°C.<sup>41</sup> Units of MPO activity for each forebrain segment were normalized on the basis of grams of wet-weight tissue.

### Statistics

Paired *t* tests were performed on the peripheral WBC and differential counts and on animal weights to detect differences in values before and after antibody administration and ischemia within each group, respectively. Two sample *t* tests with Bonferroni correction were performed to detect differences in animal weights between two groups at each time point and in ischemic lesion area, volume, and tissue PMN numbers between the two groups. All data are presented as mean±SD.

### Results

Evans blue staining at 46 hours of reperfusion confirmed the successful placement of the suture past the MCA in all rats. Both groups of animals exhibited an ischemic lesion within the MCA territory. Blood gases were within normal physiological range in both groups of rats (data not shown).

Table 1 shows the peripheral WBC and differential counts in the two ischemia groups. No difference was detected in WBC counts after antibody administration in both groups. After the first antibody injection, an increase of PMNs and a corresponding decrease of lymphocytes were observed in both groups. A decrease of neutrophils was detected in the 1B6c group (*P*<.05) at day 1 and at day 2 after antibody injection compared with pre-antibody-administration values. Likewise, an

increase of lymphocytes was detected in the 1B6c group before and after antibody administration at 22 hours of reperfusion compared with preantibody administration value (*P*<.05). However, all the WBC and differential values were within normal physiological range.<sup>42</sup>

Linear interpolation of the MPO data from eight rats indicated that  $1 \times 10^6$  PMNs provided  $0.49 \pm 0.04$  U MPO. MPO recovered from  $0.1$  to  $4.0 \times 10^6$  neutrophils was  $4.84 \pm 0.07 \times 10^{-7}$  U per neutrophil and was proportional to the number of neutrophils subjected to extraction (*P*<.001; *r*=.979).

Fig 1 shows representative hematoxylin-eosin-stained coronal sections from the rats treated with anti-Mac-1 and control vehicle and illustrates the significant reduction of the ischemic lesion in the group treated with anti-Mac-1 compared with the vehicle-treated group. Reduction of the lesion as a result of anti-Mac-1 administration appeared to be most prominent in the cortex.

Fig 2 shows the percentage of area of infarction in each of the seven coronal sections for both the vehicle control group and the anti-Mac-1 group. In sections 2, 3, and 4, the rats treated with anti-Mac-1 exhibited a significantly reduced area of infarction compared with the homologous sections in the vehicle-treated animals.

Table 2 summarizes the absolute values of the ipsilateral hemisphere and the lesion volumes, the percent volume of the lesion, and the PMN numbers within the lesion in the cortical and subcortical tissue in the anti-Mac-1 and vehicle MCA occlusion groups. Rats treated with anti-Mac-1 exhibited a significantly smaller lesion volume (*P*<.01) and cortical tissue PMN numbers (*P*<.05) than the vehicle group.

Within each group, a significant decline of weight was detected at 22 hours ( $230 \pm 11.4$  g vehicle,  $245 \pm 14.2$  g treated) and at 46 hours ( $218 \pm 9.9$  g vehicle,  $246 \pm 21.2$  g treated) of reperfusion when compared with values before MCA occlusion ( $260 \pm 4.8$  g vehicle,  $261 \pm 3.9$  g treated) in both groups of animals (*P*<.001). Rats from the vehicle group continued to lose weight at 46 hours of reperfusion. At both 22 and 46 hours of reperfusion, the

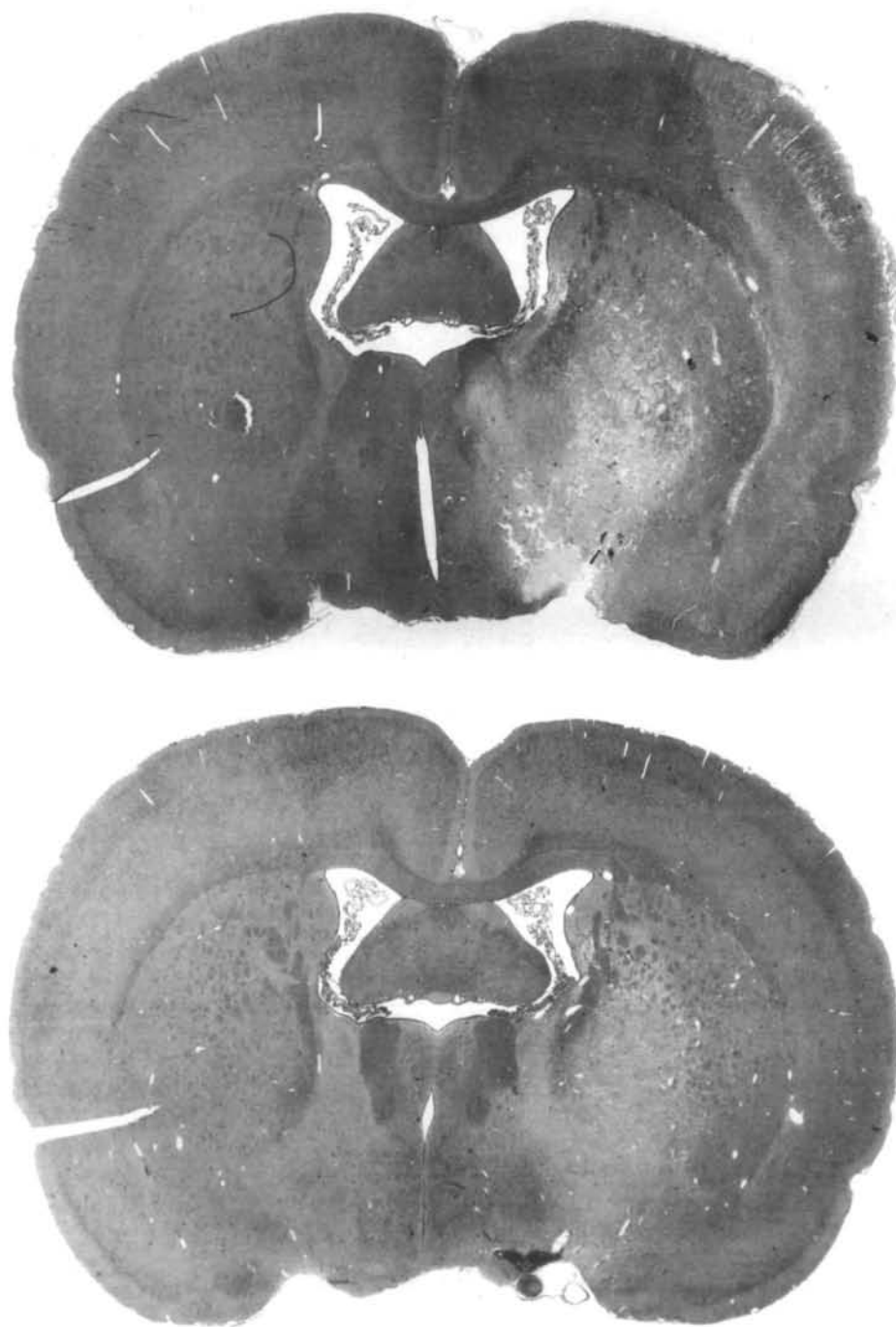


Fig 1. Photomicrographs demonstrate hematoxylin and eosin staining of 6- $\mu$ m-thick paraffin-embedded sections from representative rats from the control group (top) and the 1B6c group (bottom).

1B6c group of rats exhibited significantly higher weight than the vehicle group of rats ( $P < .05$ ).

### Discussion

We have demonstrated that administration of an anti-Mac-1 antibody (1B6c) to rats 1 hour after a 2-hour occlusion of the MCA is effective in significantly reducing ischemic cell damage and improving physiological outcome as indicated by a reduction of postischemic weight loss. Our data therefore support the role of inflammation as a contributing factor to ischemic cell damage and interference with this process as an effective means of therapeutic intervention for the rat subjected to transient focal ischemia.

We have recently reported that administration of anti-Mac-1 to rats immediately on reperfusion after 2 hours of MCA occlusion causes a reduction of approximately 43% in lesion volume.<sup>43</sup> The present study indicates that although the reduction of the lesion is somewhat diminished (approximately 28%) when the antibody is given 1 hour after onset of reperfusion, delayed administration of the antibody is a promising method to reduce the size of a focal ischemic lesion.

Administration of the anti-Mac-1 antibody caused a decline in the number of peripheral neutrophils. Although the neutrophil numbers were within normal physiological range, we cannot exclude the possibility that the reduction in number of neutrophils may have

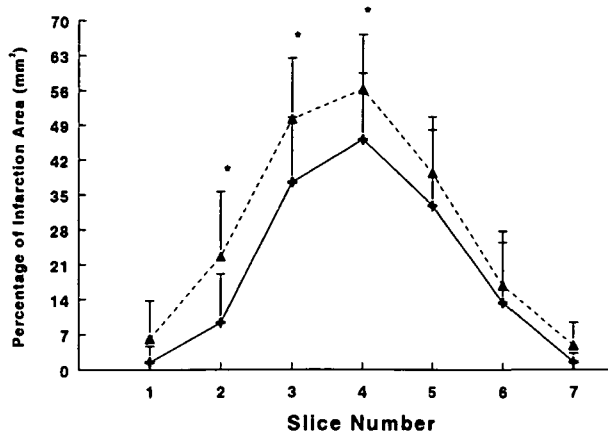


FIG 2. Graph shows mean $\pm$ SD of the percent of area of infarction (y axis) to the area of the ipsilateral hemisphere in each of the seven forebrain coronal sections (x axis).  $\blacktriangle$  indicates rats treated with control vehicle (n=10); +, rats treated with anti-Mac-1 (n=10). \* $P < .05$  indicates a significant difference in area of infarction within a particular tissue slice between rats treated with control vehicle and those treated with anti-Mac-1.

contributed to the reduction of the lesion. However, near-complete depletion of systemic neutrophils in the same model of MCA occlusion<sup>30</sup> resulted in a similar reduction in the volume of infarct (29%) as in the present study. In addition, administration of the anti-Mac-1 antibody immediately on reperfusion causes a nearly identical decline in peripheral blood neutrophil values, yet the reduction of the lesion volume exceeds that in the present study.<sup>43</sup> Thus, it is unlikely that reduction of number of peripheral neutrophils is solely responsible for the reduction of the lesion. The reduction of lesion size is associated with a reduction of the number of neutrophils within the lesion, lending support that the neutrophils contribute to ischemic cell damage. We note that reduction of parenchymal neutrophils was significantly different only in the cortical areas of the lesion, and this reduction of tissue neutrophils is consistent with the observation that the primary reduction of the lesion attributed to anti-Mac-1 treatment was located in the cortex.

The mechanisms by which the anti-Mac-1 antibody reduces ischemic cell damage are not certain. Reduction in lung injury after intestinal occlusion occurs without a reduction in sequestration of neutrophils.<sup>44</sup> There is strong evidence that the Mac-1 is needed for oxidative bursts, and that administration of an antibody to block this integrin may inhibit neutrophil-induced free radical damage.<sup>44</sup> Thus, the reduction of neutro-

phils within the tissue may not be a necessary condition for reduction of the neutrophil-mediated ischemic cell damage.

Our data also demonstrate an improvement of physiological response after the administration of the antibody. The rats appeared to lose less weight and/or regain weight more rapidly after administration of the anti-Mac-1 antibody than without the antibody.

We restricted the present study to the role of neutrophils in contributing to ischemic cell damage, although other inflammatory cells enter the ischemic tissue and express the Mac-1 adhesion molecule. The rationale for focusing our efforts on the neutrophil is that the neutrophil is the primary leukocyte involved in the acute phase of injury<sup>18,19</sup> and is the predominant cell type seen in histological sections of this<sup>37,45</sup> and other models of cerebral ischemia.<sup>46</sup> We cannot rule out the possibility that other blood cells are involved in contributing to ischemic cell damage and may be affected by administration of an anti-Mac-1 antibody.

There are a number of additional cautionary notes and limitations that are of concern in the present study. The anti-Mac-1 antibody, such as the one we are using in the present study (1B6), may act to reduce ischemic cell damage in a nonspecific and as yet undetermined way. In addition, each anti-Mac-1 antibody likely reacts with a different epitope on the antibody, and different antibodies to the same integrin may have different antiischemic properties. The reduction in ischemic cell damage has been tested in a specific model of 2 hours of MCA occlusion in the Wistar rat. It is necessary to test whether different durations of ischemia in different models of cerebral ischemia are provided with the benefits of the anti-Mac-1 therapy. In addition, other adhesion molecules also mediate neutrophil migration into the affected tissue (for review, see Reference 47), and the anti-Mac-1 antibody will not completely inhibit neutrophil entry into the tissue; possibly, a combination of anti-integrin and ligand antibodies would be more effective in reducing ischemic cell damage.

We cannot exclude the possibility that administration of the anti-Mac-1 antibody simply delays the maturation of the lesion and that if the animals were killed at a later time point, eg, 1 week, the lesion might not differ between treated and untreated groups. Further studies are required to test this issue. However, our present data clearly demonstrate the beneficial effect of administering anti-Mac-1 antibody in reducing lesion size and improving physiological function as reflected in animal weight at 2 days after the ischemic event.

TABLE 2. Absolute Hemisphere and Lesion Volumes, Percent Lesion Volume to the Ipsilateral Hemisphere, and Tissue PMN Numbers in the Two MCA Groups

	Hemisphere, mm <sup>3</sup>	Lesion, mm <sup>3</sup>	% Lesion Volume	PMNs/g wet weight ( $\times 10^6$ )	
				Cortex (n=8)	Subcortex
Vehicle (n=10)	484.7 $\pm$ 44.4	154.2 $\pm$ 31.3	31.7 $\pm$ 4.8	0.69 $\pm$ 0.21	0.43 $\pm$ 0.18
1B6c (n=10)	480.9 $\pm$ 35.0	110.6 $\pm$ 34.6*	22.9 $\pm$ 6.8*	0.44 $\pm$ 0.30†	0.37 $\pm$ 0.20

PMN indicates polymorphonuclear leukocyte; MCA, middle cerebral artery. Values are mean $\pm$ SD.

\* $P < .01$  compared with the vehicle group.

† $P < .05$  compared with the vehicle group.

In summary, we have demonstrated that an intravenous administration of an appropriate dose of a monoclonal antibody against the leukocyte adhesion molecule Mac-1 1 hour after transient (2 hours) focal cerebral ischemia significantly inhibits postischemic weight loss, reduces ischemic lesion volume, and inhibits the infiltration of PMNs into the cortical ischemic tissue. These data support the hypothesis that the leukocyte contributes to ischemic cell damage<sup>15,17,27,28,31,48</sup> in the central nervous system and that specifically blocking leukocyte adhesion molecules may in the future provide a directed and effective therapeutic intervention to reduce ischemic cell damage and be of benefit when used in conjunction with thrombolytic therapies.

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## Editorial Comment

In this article, Chopp and colleagues present the first evidence to suggest that even relatively late infusion of an antibody against neutrophil integrins serves to reduce cerebral parenchymal tissue damage during reperfusion after ischemia. This is an encouraging finding. If confirmed in other species and tested in humans, the finding may have therapeutic potential as an adjunct to thrombolytic therapy and possibly to other perfusion deficiencies with neutrophil-endothelium interaction. Expression of integrins on the neutrophil plasma membrane is a requirement for firm adhesion and spreading of neutrophils on vascular endothelium. Immunoprotection serves to prevent adhesion and to block signal transmission in endothelial cells after contact with leukocytes as well as block migration of leukocytes into the interstitium.<sup>1</sup> The motivation for the study comes from recent evidence indicating that neutrophils accumulate in local regions of the microcirculation not only in classic forms of inflammation but also in regions of low flow and during reperfusion after low-flow states.

The antibody used in this study acts against a CD11b epitope of the Mac-1 integrin (CD11b/CD18).<sup>2</sup> Although previous reports have served to demonstrate that infusion of an antibody against the Mac-1 receptor improves microvascular perfusion and reduces obstruction of capillaries in the brain during reperfusion,<sup>3-12</sup> the antibody was administered either before or just minutes after the reperfusion period. Direct intravital observations of the events in ischemia and reperfusion of peripheral tissues suggest that accumulation of leukocytes during short periods of ischemia occurs predominantly during the reperfusion period. During reperfusion, the transport of leukocytes into ischemic tissue is enhanced by the increased flow rates, and oxygen free radicals are produced by xanthine oxidase in endothelial cells. Microvascular shear rates return to or even exceed control values during reperfusion, and the adhesive stress of the leukocyte membrane is increased as well by rapid translocation of P-selectin from the endothelial Weibel-Palade bodies and ICAM-1 (the counter receptor to Mac-1) expression on the endothelial cells. During reperfusion, major accumulation of leukocytes can be observed in the microcirculation, and damage is inflicted on innocent bystander parenchymal cells, particularly those with low levels of antioxidants.<sup>13</sup> Al-

though blockade of leukocyte adhesion with an integrin antibody interferes with the accumulation of leukocytes on the endothelium, endothelial cell-derived free radicals can still be produced. Thus, the greater the contribution of leukocytes to tissue injury the better the protection rendered by the proposed intervention.

One of the potential limitations of antibodies against adhesion proteins in the general circulation is that their administration may lead to recruitment of marginating leukocytes back into the general circulation, ie, acute neutrophilia. Such neutrophilia can be counterproductive if recruited leukocytes are activated. During neutrophilia reperfusion is accompanied by an enhanced traffic of activated leukocytes into ischemic tissue and may lead to accumulation of leukocytes due to alternative mechanisms of adhesion to microvascular endothelium. The current results provide no evidence that significant numbers of leukocytes were recruited into the active circulation, thereby optimizing the benefit of the intervention against leukocyte adhesion.

An important question is raised by the current study: Under what conditions and to what extent are adhesion molecules expressed on circulating cells and on cerebral microvascular endothelium? A detailed picture in this regard will serve to identify optimal interventions against leukocyte infiltration and will permit to explore other ischemic or inflammatory conditions which could potentially be subject to a similar treatment. It will also provide a better understanding for the success in the current study. Late administration of the antibody (1 hr after initiation of reperfusion) may have occurred prior to maximal expression of ICAM-1 on the ischemic endothelial cells,<sup>14</sup> and thereby may serve to block the most injurious process in this ischemic model. In any case, since there is evidence that patients with cerebral ischemia have expression of CD18 on their neutrophils,<sup>15</sup> the use of immunoprotection against leukocyte adhesion molecules deserves to be explored as a therapeutic approach.

**Geert W. Schmid-Schönbein, PhD, Guest Editor**  
*Institute for Biomedical Engineering*  
*University of California, San Diego*  
*San Diego, Calif*