Examination of Several Potential Mechanisms for the Negative Outcome in a Clinical Stroke Trial of Enlimomab, a Murine Anti-Human Intercellular Adhesion Molecule-1 Antibody

A Bedside-to-Bench Study

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**Background and Purpose**—Enlimomab, a murine monoclonal anti-human intercellular adhesion molecule (ICAM)-1 antibody, had a negative outcome in a multicenter acute-stroke trial. We did a bedside-to-bench study in standardized rat stroke models to explore mechanisms for these untoward results.

**Methods**—After focal brain ischemia in Wistar rats and spontaneously hypertensive rats (SHR), we administered murine anti-rat ICAM-1 antibody (1A29), subclass-matched murine immunoglobulin (IgG1), or vehicle intravenously. To examine whether rat anti-mouse antibodies were generated against the mouse protein and whether these were deleterious, we sensitized Wistar rats with 1A29 or vehicle 7 days before surgery. Infarct volume, tissue myeloperoxidase activity, neutrophil CD11b expression, and microvascular E-selectin, P-selectin, and ICAM-1 expression were examined 48 hours after surgery. Complement activation was serially assessed for 2 hours after a single injection of either 1A29 or vehicle.

**Results**—1A29 treatment did not significantly reduce infarct size in either strain. 1A29 sensitization augmented infarct size and generated rat anti-mouse antibodies. Although 1A29 inhibited neutrophil trafficking shown by reduction in brain myeloperoxidase activity, circulating neutrophils were activated and displayed CD11b upregulation. Complement was activated in 1A29-sensitized Wistar rats and 1A29-treated SHR. E-selectin (SHR), endothelial P-selectin (Wistar and SHR), and ICAM-1 (SHR) were upregulated in animals treated with 1A29.

**Conclusions**—Administration to rats of a murine antibody preparation against ICAM-1, 1A29, elicits the production of host antibodies against the protein, activation of circulating neutrophils, complement activation, and sustained microvascular activation. These observations provide several possible mechanisms for central nervous system–related clinical deterioration that occurred when Enlimomab was given in acute ischemic stroke. (*Stroke. 2001;32:2665-2674.*)

**Key Words:** antibodies ▪ cell adhesion molecules ▪ cerebral ischemia ▪ clinical trials ▪ leukocytes ▪ rats

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Leukocyte adhesion and transendothelial migration participates in the progression of cellular injury during acute brain ischemia.1–6 Highly specific receptor-ligand interactions with endothelium and extracellular matrix are involved in this process.7–9 Amelioration of postischemic brain damage by blocking these receptor-ligand interactions with monoclonal antibody (MoAb) has succeeded in some preclinical studies.10–14 These results form the backdrop for clinical testing of anti–intercellular adhesion molecule (ICAM)-1 MoAb therapy in acute stroke.

Enlimomab is a murine IgG2a MoAb directed against extracellular domain 2 of human ICAM-1.15 Therapy with this MoAb did not benefit patients with acute ischemic stroke. The Enlimomab Acute Stroke Trial16 enrolled 625 stroke patients. Enlimomab-treated patients did significantly worse on clinical rating scales of neurological function and suffered...
a significantly higher mortality than did placebo control patients. Also, infarct volumes were slightly larger in the Enlimomab group. The discrepancy between the findings in the Enlimomab trial and preclinical studies supporting an anti-ICAM-1 strategy in stroke has added to questions about the capacity of animal experiments to accurately predict outcomes in clinical acute stroke trials. We hypothesized that the deleterious effect of anti-ICAM-1 antibody in the Enlimomab trial that overwhelmed any potential benefit from inhibition of leukocyte accumulation was due to the augmentation of ischemic brain injury by the immunogenicity of Enlimomab. To test this hypothesis, we used established preclinical stroke models to examine several potential effects of a heterologous murine anti-rat ICAM-1 antibody preparation in a bedside-to-bench fashion.

**Materials and Methods**

**Monoclonal Antibodies**

1A29 (specific for rat ICAM-1) and shown to block neutrophil-mediated injury in vivo (ref 10-22) and a control subclass-matched (IgG1) antibody directed against the rat cytoplasmic domain of L-selectin were provided by Boehringer Ingelheim Pharmaceuticals, Inc (Ridgefield, Conn). These 2 purified antibodies and their vehicle included 0.5 endotoxin U/mL (negative). 1A29 is similar to Enlimomab as a murine MoAb raised against ICAM-1 but differs by not been epitope-mapped.

**Surgical Procedure**

Initially, rats were intubated transorally, anesthetized with a 4% halothane in 30% O2/70% N2O gas mixture, and maintained on a mechanical ventilator (Harvard Rodent Ventilator Model 683, Harvard Instrument Co). The right femoral artery was cannulated (PE-50, Clay-Adams) for blood pressure monitoring and serial blood gas sampling. The right external jugular vein was cannulated for administration of 1A29, control antibody, or vehicle. The left common carotid artery (CCA) and its bifurcation were exposed, with preservation of both the vagus nerve and the sympathetic nerve trunk. Transient partial focal cerebral ischemia was induced as described previously. Briefly, the left CCA was occluded with a metal clip (Microaneurysm clip, 85-g pressure, 0.75 mm/0.5 endotoxin U/mL (negative). 1A29 is similar to Enlimomab as a murine MoAb raised against ICAM-1 but differs by subclass (IgG1 versus IgG2a) and target (rat versus human ICAM-1). Whether its binding domain differs is uncertain, inasmuch as it has not been epitope-mapped.

**Experiment 1**

Wistar rats were exposed to 2 hours of tandem left CCA and MCA occlusion (CCAO/MCAO), followed by reopening of the left CCA to permit reperfusion. These rats were divided into 3 groups as previously defined. In the anti-ICAM-1 antibody (1A29)-treated group (1A29 IV, n=9), rats were administered 1A29 (2 mg/kg) on reperfusion and the same dose at 22 hours after reperfusion. In the control antibody–treated group (IgG1 IV, n=7), rats were injected with IgG1, an isotype-matched antibody, on the same schedule with the same volume dose as the 1A29-treated group. In the vehicle-treated group (PBS IV, n=8), rats were injected with the same volume of PBS (pH 7.4) on the same schedule.

**Experiment 2 (Sensitized Group)**

Wistar rats were sensitized with 1A29 (200 μg/kg IP, n=6) or the same volume of PBS (n=5) 7 days before surgery to test the hypothesis that the host response to this foreign protein would include generation of rat anti-mouse antibodies (RAMAs) and deleterious effects on brain ischemia. The animals were divided into 2 groups: (1) 1A29/1A29 animals received 1A29 sensitization, followed by CCAO/MCAO 7 days later and 1A29 IV treatment. (2) PBS/1A29 animals were injected with PBS IP (sensitization control), followed by CCAO/MCAO 7 days later and 1A29 IV treatment. The protocol except for sensitization was the same as in experiment 1.

**Experiment 3**

SHR were exposed to 1 hour of CCAO/MCAO, after which the left CCA was reopened to permit reperfusion. During the 1-hour CCAO, animals were maintained under mechanical ventilation. In a pilot experiment, 2 hours of tandem left CCAO/MCAO in SHR permitted very little reperfusion; thus, the tandem occlusion time was reduced to 1 hour (data not shown). The experimental design was otherwise the same as for experiment 1. There were 3 groups, in which animals received 1A29 IV (n=12), IgG1 IV (n=8), or PBS IV (n=10). Physiological Variables

Mean arterial blood pressure was monitored by using a TA 4000 transducer amplifier (Gould Inc). PaO2, PaCO2, pH, and hematocrit were measured with a Corning pH/Blood Gas Analyzer (model 288, Corning Medical). The rectal and temporalis muscle temperatures were measured and maintained at 37±0.5°C with a heating blanket (K-20, American Pharmaseal Co) during all surgical procedures and during recovery from anesthesia (ie, until normal locomotor activity was observed). Daily body weight was also monitored. Body weight before surgery was taken as 100%, and subsequent daily weight was expressed relative to this value.

**Assessment of the Infarct**

Forty-eight hours after the induction of ischemia, the animals were anesthetized and transcardially perfused with 4% paraformaldehyde. Perfusion-fixed brains were cut into 20-μm coronal sections in a cryostat at 12 predefined serial levels (bregma from 3.70 to −7.30 mm24) and stained with cresyl violet. Infarct areas were observed. Noninfarct areas were traced, and the volumes were calculated by integration of the areas by means of a digitized imaging system (NIH image, version 1.62), followed by correction for brain swelling as previously described. The investigator who analyzed the sections (H.T.) was blinded to the treatment group in all experimental protocols.

**MPO Assay**

Sensitized Wistar rats (3 groups, including PBS/PBS; n=8 per group) and SHR (2 groups, 1A29 and PBS IV; n=5 per group) were included in the present study. Brain myeloperoxidase (MPO) activity was determined 48 hours after the induction of ischemia, as described previously, with minor modifications. PBS-perfused forebrain was sectioned into the ipsilateral and contralateral cortices. Each sample was analyzed in duplicate, and the values were averaged. One unit of MPO activity was defined as that which could degrade 1 μmol/L of peroxide per minute at 25°C.

**Flow Cytometry and Differential Leukocyte Counts**

Sensitized Wistar rats and SHR, including naïve animals, were examined (n=4 per group). Whole blood was collected and mixed with sodium citrate. One hundred microliters of this blood was incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat CD11b (2.5 μL, IgA, Pharmingen) for 20 minutes in the dark. Subclass-matched nonspecific antibody served as a control. After the erythrocytes were lysed, the forward and side scatter characteristics of granulocytes were analyzed by flow cytometer (EPICS XL-MCL, Coulter). The percentage of CD11b-positive cells and their mean fluorescence intensity (linear conversion of log10 fluorescence) were determined. Manual leukocyte counts were performed with a hemocytometer before ischemia and 48 hours later.
Determination of C3a des-Arginine
Sensitized Wistar rats and SHR were anesthetized and cannulated via the right femoral vein, followed by administration of a single dose (2 mg/kg) of 1A29 or the same volume of PBS. No surgical intervention other than venous cannulation was performed. Separate sets of animals (n = 4, each group) were sampled and euthanized at 30 minutes, 1 hour, and 2 hours. At the time of euthanasia, 5 mL of whole blood was withdrawn from the right ventricle into a prechilled EDTA tube containing 6-amidino-2-naphthyl p-guanidinobenzoate dimethanesulfonate (Futhan, Banyu Pharmaceuticals) to inhibit ex vivo complement activation. Plasma levels of the C3a des-arginine (C3a desArg), the inactive and stable form of the anaphylatoxin, were determined by ELISA (Bachem AG). Each sample was analyzed in duplicate, and raw values were averaged. Naïve animals of each strain (n = 4) were used to determine baseline C3a desArg levels.

Immunohistochemistry
Histology was performed on animals subjected to the flow cytometric analysis. Perfused brains were frozen, sectioned coronally at 16 μm, and fixed in acetone (4°C) for 15 minutes for staining RAMAs, E-selectin, and endothelial P-selectin. The striatum and the anterior and posterior hippocampus (bregma 0.70, −1.80, and −2.80 mm, respectively) were examined.

RAMAs
RAMA staining was performed in sensitized Wistar rats. Biotinylated murine monoclonal anti-human L-selectin antibody (Dreg 200, IgG1, Boehringer Ingelheim) at 2 μg/mL was the primary antibody for detection of RAMAs. Dreg 200 does not recognize rat L-selectin and does not bind specifically to any rat brain epitope. However, it can be bound specifically by the RAMA generated when the rats were sensitized with 1A29. The principal of the assay involves the use of a subclass-matched IgG1 mouse immunoglobulin, Dreg 200, which can bind to the free variable region of a RAMA that is bound with its other variable region to 1A29, which is itself bound to ICAM-1 at the site of the lesion (1A29 is an IgG1 mouse immunoglobulin as is Dreg 200; ie, these are isotype subclass-matched). Sites of RAMA binding to Dreg 200 were visualized with indocarbocyanine-conjugated streptavidin. Double immunostaining with antibody against astrocytes (rabbit anti-cow glial fibrillar acidic protein-antibody, 1:500, DAKO) and FITC-conjugated biotin from Griffonia simplicifolia against microglia (10 μg/mL, Sigma Chemical Co) was performed. An FITC-conjugated F(ab')2 fragment of goat anti-rabbit antibody (1:400) was applied as a secondary antibody to detect glial fibrillary acidic protein. The α-d-galactosyl-specific B4 isoelectric derived from Griffonia simplicifolia seeds has been shown to bind selectively to rat microglial cells in normal as well as in pathologically altered brain. Negative controls were naïve, and PBS/PBS animals and PBS/1A29 and 1A29/1A29 animals with the primary antibody (Dreg 200) were omitted.

E- and P-Selectins and ICAM-1
Sensitized Wistar rats and SHR were used. E-selectin, endothelial P-selectin, and ICAM-1 were stained as described by Okada et al. with minor modifications. Five percent normal donkey serum was applied for blocking, followed by incubation overnight at 4°C with an antibody against mouse IgG (Fab fragment goat anti-mouse IgG, Jackson Immuno Research Laboratories, Inc) in a concentration of 1:10 to block detection of the treatment antibody, 1A29. Monoclonal anti-rat E-selectin and P-selectin antibodies (10 μg/mL) provided by Protein Design Labs or monoclonal anti-rat ICAM-1 (clone TLD4C9, 6 μg/mL, Accurate Chemical & Scientific Corp) was then applied. Biotinylated F(ab')2 fragment of donkey anti-mouse IgG was used as the secondary antibody. Antibody-bound peroxidase was detected with the chromogen substrate 3-amino-9-ethylcarbazole for 15 minutes for staining RAMAs, E- and P-Selectins and ICAM-1 negative controls were naïve, and PBS/PBS animals and PBS/1A29 and 1A29/1A29 animals with the primary antibody (Dreg 200) were omitted.

Results

Daily Body Weight Change
In all experimental groups, the animals lost weight during the 48 hours after surgery. There were no significant intergroup differences in the percentage of baseline weight in Wistar rats (for experiment 1, PBS IV 92.5 ± 2.3%, IgG1 IV 93.2 ± 2.6%, and 1A29 IV 92.3 ± 3.6%; for experiment 2, PBS/1A29 90.7 ± 2.3% and 1A29/1A29 90.1 ± 2.4%). However, the percentage of baseline weight in 1A29 IV SHR was significantly lower than that in PBS IV and IgG1 IV SHR (for experiment 3, PBS IV 93.8 ± 2.7%, IgG1 IV 93.5 ± 1.9, and 1A29 IV 89.0 ± 2.7; P < 0.05). Other physiological variables remained in the normal range.

Laser-Doppler Flowmetry
In Wistar rats, local cerebral blood flow in the peri-infarct area decreased to 30% of baseline at its minimum after MCAO. After reopening the CCA, local cerebral blood flow...
Brain MPO Activity

MPO activity of the ipsilateral hemisphere in 1A29/1A29 and PBS/1A29 was significantly lower than in PBS/PBS Wistar rats (0.78±0.41, 1.20±0.86, and 8.55±8.11 U/g wet weight per minute, respectively; *P<0.05; Figure 2, top). Corresponding MPO activities of the contralateral hemispheres were very low and were not affected by any of the treatments (Figure 2, bottom). A similar pattern of MPO activity was noted in SHR (for ipsilateral side, 2.64±2.11 U/g wet weight per minute for 1A29 IV and 5.18±2.05 U/g wet weight per minute for PBS IV, *P<0.05; for the contralateral side, 0.17±0.26 and 0.12±0.15 U/g wet weight per minute, respectively; Figure 2, bottom).

**Flow Cytometry and Differential Leukocyte Counts**

Differential leukocyte counts revealed a significant granulocytosis 48 hours after MCAO in both strains treated with 1A29 (Table). 1A29/1A29 Wistar rats and 1A29 IV SHR showed significant lymphocytopenia compared with PBS/PBS Wistar rats and PBS IV SHR. The percentages of CD11b immunoreactive granulocytes of naïve and sensitized Wistar rats were significantly higher in the 1A29/1A29 and PBS/1A29 samples than in the naïve and PBS/PBS samples (P<0.05, Figure 3A). 1A29 IV SHR showed a higher percentage of CD11b⁺ granulocytes, but the differences did not reach significance (Figure 3C). Mean fluorescence intensity (linear conversion of log₁₀ fluorescence) of CD11b expression was as follows (for sensitized Wistar rats, naïve 46.0±6.8, PBS/PBS 58.0±8.7, PBS/1A29 117.2±27.3, and 1A29/1A29 116.2±19.6; for SHR, naïve 43.2±8.3, PBS IV 62.5±16.3, and 1A29 IV 94.7±25.3; Figure 3B and 3D, respectively). 1A29/1A29 and PBS/1A29 showed significantly higher mean fluorescence intensities of CD11b compared with naïve and PBS/PBS (P<0.01). 1A29 IV SHR showed significantly higher intensities of CD11b as well (P<0.01).

**Determination of Plasma C3a desArg**

Naïve SHR showed significantly higher levels of C3a desArg than did naïve Wistar rats (649.1±262.3 and 253.0±111.7 ng/mL, respectively; *P<0.01). In sensitized Wistar rats, 1A29/1A29 showed significantly higher levels of C3a desArg compared with PBS/PBS and PBS/1A29 (P<0.05; Figure 4, top), which was independent of time. C3a desArg in 1A29 IV SHR was significantly higher than in PBS IV (P<0.00005; Figure 4, bottom). Differences over time (30 minutes versus 1 hour versus 2 hours) were also significant (P<0.05). C3a desArg levels were significantly higher at 30 minutes than at either 1 hour or 2 hours, and values at the latter 2 time points did not differ significantly. Activation of complement by 1A29 did not decomplement the plasma, inasmuch as measured levels of C3a desArg were approximately an order of magnitude lower than maximum levels in zymosan-activated rat serum measured at BACHEM Bioscience, Inc, during development of their assay (10 to 20 µg/mL).

**Immunohistochemistry**

Evidence of RAMAs in sensitized Wistar rats was seen in some populations of astrocytes and microglia within the infarct core and peri-infarct areas in the 1A29/1A29 group (Figure 5). RAMAs were not evident in the contralateral hemisphere. RAMAs were also absent in the PBS/1A29 group throughout all areas examined. A Leitz Laborlux microscope at a magnification of ×100 was used for quanti-
Differential Counts of Leukocytes

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Values are mean±SD.

*P<0.05 compared with PBS/PBS at 48 h after MCAO (1-way ANOVA followed by Sheffe’s comparison); †P<0.01 compared with PBS IV at 48 h after MCAO (1-way ANOVA followed by Sheffe’s comparison).

Tative analysis of E-selectin, endothelial P-selectin, and ICAM-1 expression. Ten high-power fields (HPFs) were examined in each lesioned brain, and the number of vessels expressing immunoreactivity per HPF was counted. Immunoreactive E-selectin in SHR microvascular endothelium (mean±SD per HPF) was 177±30 and 93±35 in 1A29 compared with PBS animals, respectively (n per group=4 or 5; P=0.006). Corresponding values per HPF in Wistar rats were 126±90, 105±118, and 48±32 in 1A29/1A29, PBS/1A29, and PBS/PBS groups, respectively (n per group=4; P=NS). Immunoreactive endothelial P-selectin per HPF in SHR was 353±166 and 82±37 in 1A29 compared with PBS.
Figure 3. A through D, The percentages of CD11b-positive cells and mean fluorescence intensity (linear conversion of log_{10} fluorescence) of CD11b on granulocytes in sensitized Wistar rats (A and B) and SHR (C and D). E, A representative flow cytometric tracing of sensitized Wistar rats. F, A representative flow cytometric tracing of SHR. In panels A and B, the number of CD11b-positive granulocytes and CD11b intensities significantly increased in 1A29-treated Wistar rats (PBS/1A29 and 1A29/1A29) compared with naive or PBS/PBS rats (*P<0.05 for the number; **P<0.01 for the intensities, 1-way ANOVA followed by Sheffé comparison). In panel C, there was a trend toward an increased number of CD11b-positive granulocytes in 1A29 IV SHR, but the differences did not reach significance. In panel D, granulocyte CD11b intensities in 1A29 IV SHR did significantly increase compared with naive rats (**P<0.01, 1-way ANOVA followed by Sheffé comparison).
Discussion

The present study demonstrates that a heterologous MoAb directed against ICAM-1 can sensitize Wistar rats and increase the volume of infarction after standardized focal brain ischemia. In addition, this foreign immunoglobulin can generate RAMAs and activate complement, circulating leukocytes (CD11b upregulation), and endothelium (E-selectin, endothelial P-selectin, and ICAM-1 upregulation). Leukocyte trafficking through the injured tissue as measured by the MPO assay was reduced by anti–ICAM-1, but infarct volume was not reduced significantly in these models.

Monoclonal anti–ICAM-1 antibody therapy has only benefited reperfusion injury in preclinical stroke models. The stroke models used in the present study had relatively poor reperfusion compared with the intraluminal thread model. However, incomplete reperfusion is probably relevant to the flow state experienced by patients enrolled in the Enlimomab trial, who relied on spontaneous reperfusion. The development of host antibodies against passively administered immunoglobulin with possible neutralization of the administered immunoglobulin and anaphylactic or other immune reactions has been viewed as a potential major complication to MoAb therapy. The generation of an anti-immunoglobulin response is dependent on many factors. These include the dose, form, number of injections, immunogenicity of the target antibody, and immunocompetence of the recipient. MoAb treatment generally requires multiple infusions, as in the Enlimomab acute stroke trial. Healthy humans have a 100% antibody response to murine MoAb. A lower frequency of response in cancer patients has been attributed to immunosuppression associated with the neoplastic disease as well as to prior immunosuppressive therapy. These include the dose, form, number of injections, immunogenicity of the target antibody, and immunocompetence of the recipient. MoAb treatment generally requires multiple infusions, as in the Enlimomab acute stroke trial. Healthy humans have a 100% antibody response to murine MoAb. A lower frequency of response in cancer patients has been attributed to immunosuppression associated with the neoplastic disease as well as to prior immunosuppressive therapy. Despite premorbid risk factors in patients with stroke, 100% of the evaluate patients had IgG human anti-mouse antibody responses to Enlimomab, and 93% of the patients had detectable IgM human anti-mouse antibody responses. Because the immunologic relationship between rats and mice is closer than between humans and mice, the finding that sensitization with 1A29 followed by 1A29 treatment caused augmentation of infarct size is significant. This finding may be pertinent to the 5-day period of antibody administration in the Enlimomab trial. RAMAs, rat antibodies against a murine protein presumably directed at 1A29 in the present study (the only mouse antigen in these rats), were detected on astrocytes and microglia in 1A29-sensitized Wistar rats.

Determination of C3a desArg is one index of complement activation. The Fcγ portion of IgG initiates activation of the complement pathway via C1q, which sequentially generates anaphylatoxins C3a and C5a. This cascade elicits an inflammatory response. MoAbs of the IgG2a subclass are able to activate human complement, while IgG1 subclass are able to activate human complement. 1A29 is in the IgG1 subclass and activated complement in sensitized Wistar rats (1A29/1A29) and 1A29 IV SHR in the present study. RAMAs, rat antibodies against a murine protein presumably directed at 1A29 in the present study (the only mouse antigen in these rats), were detected on astrocytes and microglia in 1A29-sensitized Wistar rats.

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CD11b is inducible by activated complement components and cytokines, such as tumor necrosis factor-α. Moreover, CD11b upregulation correlates with the severity of sepsis, granulocytopenia during hemodialysis, and the occurrence of organ failure. Human neutrophil activation was not elicited by a murine anti-human ICAM-1 MoAb of the IgG1 subclass in vitro. Nevertheless, we show that 1A29, a member of the IgG1 subclass, caused rat granulocyte activation, as demonstrated by increased CD11b expression. Because complement contributes to CD11b upregulation, the
complement activation observed in the present study may partly explain the CD11b upregulation.

Rats injected with 1A29 displayed lymphocytopenia and significant granulocytosis in the present study. In humans, the disappearance of lymphocytes from peripheral blood induced by murine IgG1 antibodies requires Fcγ receptor interaction. Both granulocytopenia and granulocytosis that might relate to the Fcγ receptor were also reported.

Increased vascular immunoreactivity of E-selectin, P-selectin, and ICAM-1 indicates activation of endothelium and supports leukocyte–endothelial cell and leukocyte-platelet adhesive interactions. 1A29 upregulated immunoreactive E-selectin (SHR), P-selectin (Wistar and SHR), and ICAM-1 (SHR) expression on segments of brain vascular endothelium 48 hours after MCAO. Because the selectins cause the rolling of leukocytes within the intravascular space rather than their transmigration into tissue and because the ICAM-1 adhesion sites were blocked, this was not reflected in increased MPO activity in whole-brain homogenates from 1A29-treated animals.

In conclusion, our results indicate that the administration of the murine anti-rat ICAM-1 antibody, 1A29, induces an inflammatory state in preclinical models of ischemic stroke. Features of this state include activation of complement, granulocytes, and endothelium. Serial administration of the antibody sensitizes rats to produce anti-mouse antibodies and to develop larger infarctions after MCAO compared with no 1A29 administration. These effects overwhelmed any poten-

Figure 5. Staining of RAMAs (original magnification ×630). RAMAs were detected as described in Materials and Methods in Wistar rats sensitized by 1A29, followed by 1A29 injection (A and C, arrowheads). RAMAs colocalized with glial fibrillary acidic protein (FITC; B; arrowheads indicate astrocyte) and with FITC-conjugated isolecitin B4 from Grifonia simplicifolia (D; arrowheads indicate microglia). Bar scale 20 μm.

Figure 6. Immunoreactive E-selectin and P-selectin in SHR (×200). MCAO elicited minimal expression of E-selectin and P-selectin. 1A29 treatment caused upregulation of both of these adhesion molecules. Naïve SHR were negative for immunoreactive E-selectin and P-selectin. A, E-selectin 1A29 group; B, E-selectin PBS group; C, P-selectin 1A29 group; and D, P-selectin PBS group. Bar scale 50 μm.
tial benefit from inhibition of leukocyte accumulation in the injury zone. Similar responses to the murine anti-human ICAM-1 MoAb (Enlimomab) may have contributed to the adverse outcome of the Enlimomab Acute Stroke Trial. The results of the present study also provide support for the preclinical conduct of “devil’s advocate studies” in experimental models designed to detect harmful or negative properties of an innovation that would not be picked up in a routine toxicology screen. A caveat is that results from novel preclinical models that have not been validated should be interpreted with caution. Results from such models should be used as a guide to expand and intensify monitoring during clinical trials or to modify clinical trial design rather than to deter the trials.

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References


Examination of Several Potential Mechanisms for the Negative Outcome in a Clinical Stroke Trial of Enlimomab, a Murine Anti-Human Intercellular Adhesion Molecule-1 Antibody: A Bedside-to-Bench Study


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