An Anti-CD18 Antibody Limits Infarct Size and Preserves Left Ventricular Function in Dogs With Ischemia and 48-Hour Reperfusion

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Objectives. This study investigated whether an antibody against neutrophil adhesion protein CD18 could limit myocardial infarct size and preserve left ventricular function after prolonged reperfusion in a canine model.

Background. Myocardial reperfusion injury is mediated in part by accumulation of activated neutrophils. Although antibodies against CD18 have been shown to reduce neutrophil influx and infarct size after ischemia and 3 to 4 h of reperfusion, it is unknown whether protection is sustained beyond this time or whether there is meaningful preservation of ventricular function.

Methods. Dogs undergoing 90-min circumflex coronary artery occlusion and 48-h reperfusion were randomized to receive 1 mg/kg bodyweight of R15.7 (an anti-CD18 antibody, n = 12) or saline (control, n = 12) 10 min before reperfusion. Contrast left ventriculography was used to measure left ventricular ejection fraction and regional chord shortening at baseline, during occlusion and at 48 h. Microspheres injected during occlusion were used to measure collateral flow and risk region size. Postmortem infarct size was measured with triphenyltetrazolium chloride.

Neutrophils are believed to be causally related to reperfusion injury of various organs, including the heart, intestine and lung (1–3). Inhibition of neutrophils by drugs (4) or removal by filtration (5) significantly reduces reperfusion injury of the heart. Adherence of neutrophils to vascular endothelium is believed to represent an essential first step in the process of neutrophil transmigration and influx into injured myocardium (6). Although initial attachment of neutrophils to vascular endothelium is thought to be mediated by molecules belonging to the selectin family, the CD11/CD18 integrins are primarily responsible for tight cellular adhesion, diapedesis out of the vascular lumen, chemotaxis, phagocytosis and adherence-related oxidant production (6,7–11).

Results. In the dose administered, R15.7 bound to neutrophils in vivo, with >85% saturation of CD18 for >24 h, with sustained antibody excess in the plasma. R15.7 significantly reduced infarct size after adjusting for the effect of collateral flow (p = 0.0002, analysis of covariance). In a subgroup of dogs with collateral flow <30% of nonischemic flow, infarct size was reduced from 34.6 ± 3.9% (mean ± SE) of the region at risk in the control group to 19.5 ± 3.3% in the antibody group (p = 0.008). Ejection fraction and regional chord shortening did not differ between the two groups at baseline or during occlusion, but after 48-h reperfusion, ejection fraction and inferior wall regional cord shortening (representing the infarct zone) were both higher in the R15.7 group than the control group (43.6 ± 2.9% vs. 28.5 ± 1.8%, p < 0.01; 2.55 ± 0.29% vs. 1.06 ± 0.18%, p < 0.05).

Conclusions. A single injection of an anti-CD18 antibody given before reperfusion can limit myocardial infarct size by nearly 50% and preserve global and regional left ventricular function after 48 h of reperfusion.

Anti-CD18 monoclonal antibodies have been reported to reduce organ injury and improve survival from hemorrhagic shock (12) and prevent reperfusion-induced lung (13) and intestinal vascular injury (2). Anti-CD18 antibodies have also been shown to reduce short-term myocardial reperfusion injury in some studies (14–17), although not all have reported efficacy (18). There are important gaps in the current body of knowledge concerning anti-CD18 antibodies and reperfusion injury. Most previous studies have used short-term reperfusion lasting only a few hours. It is therefore possible that these antibodies merely delay the appearance of lethal myocardial injury during reperfusion rather than produce a true reduction in infarct size. In addition, no study has examined the effect of antibody treatment on left ventricular function. It is therefore unknown whether the reduction in infarct size observed in previous studies would be translated into a meaningful improvement in cardiac function.

Our study was done to determine the maximal benefit that could be achieved with an anti-CD18 antibody in a canine model of myocardial ischemia and prolonged reperfusion. An anti-CD18 antibody, R15.7, was selected that had been shown (9,10) to effectively inhibit adherence-related functions of canine neutrophils in vitro. A dose was chosen that resulted in
Methods

Experimental animals. Adult mongrel male and female dogs weighing 16 to 27 kg were used for these studies. All experiments were performed in accordance with the guiding principles of the American Physiological Society. All animals were vaccinated for common viral infections, dewormed and observed for 2 weeks to rule out acute illness. All had negative test results for heartworm infection.

Surgical preparation. Dogs were anesthetized with 30 mg/kg body weight of sodium pentobarbital and mechanically ventilated with room air. All surgical procedures were performed aseptically, including aseptic preparation of the skin and use of sterile surgical drapes and instruments and wearing of surgical masks and sterile gloves. After left thoracotomy was performed aseptically, including aseptic preparation of the skin and use of sterile surgical drapes and instruments and wearing of surgical masks and sterile gloves. After left thoracotomy was performed, the left chest was opened and the heart excised. After excision of the heart, tissue samples were obtained and counted in a scintillation counter, along with reference blood samples. Neutrophil isolation was accomplished by erythrocyte lysis using whole-blood lysing reagent (Coulter Immunology) and the cells were fixed in 1% paraformaldehyde. Antibody binding was then determined by flow cytometry (Coulter EPICS ELITE, Coulter Immunology), and fluorescence intensity was used as a quantitative measure of binding. In addition, to assess the saturation of R15.7 binding sites on circulating neutrophils, a second aliquot of whole blood at each time point was incubated with a saturating concentration of R15.7 (20 μg/ml) for 30 min at 4°C, and the difference in fluorescence intensity was recorded.

Regional blood flow measurement. Radioactive microspheres (DuPont), 15 μm in diameter and labeled with gadolinium-153, tin-113, ruthenium-103, niobium-95 or scandium-46, were used to measure myocardial blood flow (19). Microspheres (2 × 10⁶) were injected into the left ventricle through the pigtail catheter, and a simultaneous reference blood sample was withdrawn from the arterial sheath. After excision of the heart, tissue samples were obtained and counted in a scintillation counter, along with radioactive standards and reference blood samples. Regional myocardial blood flow was calculated by standard methods (19). Mean flows (ml/min per g) to the inner two-fifths, middle one-fifth and outer two-fifths of the left ventricular wall within the ischemic-reperfused region and to the opposite nonischemic region were determined at each time point. Samples located at the border between ischemic and nonischemic areas were not included in this analysis.

Assessment of infarct, risk and no-reflow areas. The left ventricle was sectioned into five slices parallel to the atrioventricular ring. After excision of the right ventricle, each slice was weighed, incubated in a 2% solution of triphenyltetrazolium chloride (TTC) for 30 min at 37°C to visualize the infarct area and photographed. Area at risk was assessed by the regional blood flow data according to the following method. Contiguous
strips, 3 to 6 mm wide and 2 to 3 mm deep, were excised from the basal surface of each left ventricular slice, extending from the endocardial to the epicardial surface and encompassing the entire ischemic circumflex region (8 to 11 strips were usually sufficient). One strip was taken from the nonischemic region in each left ventricular slice. Each strip was then divided into five equal samples (0.05 to 0.2 g), and each was weighed and analyzed for regional flow. Flow to nonischemic myocardium was calculated by averaging all the nonischemic flow values over all the left ventricular slices at each time point. Samples from the circumflex artery territory were considered “ischemic” (and therefore within the region at risk) if the flow during coronary artery occlusion was <50% of the nonischemic value. Samples were classified as having “no reflow” if the flow at 48 h of reperfusion was <50% of nonischemic flow. These criteria have been defined in previous studies from our laboratory (20,21).

The location of each myocardial strip was marked by a scalpel incision on each left ventricular slice before photography. Subsequently, the photographic transparencies were projected, and the epicardial and endocardial edges, the infarct area (TTC negative) and the locations of the tissue samples were traced. Each sample that met the definition of “ischemic” or “no-reflow,” or both, was color coded. The areas of the ischemic risk region, no-reflow region and the infarcted myocardium were determined by computerized planimetry of the tracings. Infarct and no-reflow region sizes were then calculated by summing the values for each left ventricular slice (the no-flow region was included in the infarct region) and expressed as a fraction of the risk region or left ventricular for each heart. The amount of infarct hemorrhage was judged from the color transparencies for each left ventricular slice using a four-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe), without knowledge of group assignment. A score was calculated for each dog by averaging the scores of the five left ventricular slices.

Left ventriculography. Left ventriculography was performed at baseline, during coronary occlusion and 10 min and 48 h after reperfusion. Omnipaque 350 (Iohexol, Sanofi Winthrop) was injected into the left ventricle at 7 ml/s for 4 s, and 20° right anterior oblique images were recorded at 30 frames/s, and a calibration grid was filmed at heart level immediately afterward. Ventriculograms were technically adequate for analysis in 10 of 12 control dogs and in all 12 antibody-treated dogs. The developed cine films were processed for analysis with the system, the end-diastolic and end-systolic images were selected. These images were digitized and analyzed with an ImageComm System equipped with a 512 × 512 high resolution monitor. Using software available with the system, the end-diastolic and end-systolic outlines of the left ventricle were drawn manually, excluding the papillary muscles. Ejection fraction was calculated by the area-length method, assuming a prolate spheroid shape of the left ventricle (22). The end-diastolic volume was determined using the area-length method (22). Using the centerline method, 100 chords were automatically generated around the perimeter, and the shortening of each chord was expressed as a fraction of the perimeter length (23). The left ventricle was divided into five regions of 20 chords each (anterobasal, midanterior, apex, midinferior, inferobasal), and the shortening of each of these regions was expressed by the shortening of the center chord within that region. Regional anterior wall motion represented the average of the central chord shortening for the anterobasal and midanterior regions, whereas inferior wall motion represented the average of the inferobasal and midinferior regions.

Myeloperoxidase assay. Transmural myocardial samples weighing 0.5 to 1.0 g were taken from both the central portion of the area at risk and the normal area of a midventricular slice. The samples were frozen immediately after counting microsphere activity and stored at -20°C. The activity of myeloperoxidase, an index of neutrophil accumulation, was measured as previously described (24). Results were expressed as units of myeloperoxidase/100 mg wet tissue weight.

Statistical analysis. Hematologic and hemodynamic variables during ischemia and reperfusion in control and R15.7 groups were compared by repeated measures analysis of variance. Two-way analysis of variance, combined with Dunnett’s post hoc test for multiple comparisons, was used to test for an overall difference between groups in each variable, and for differences at each time point within and between groups. Comparisons of infarct size and tissue myeloperoxidase activity were made by the Student t test. The relation between infarct size (percent of risk region) and collateral blood flow was determined by linear regression analysis, and analysis of covariance was used to determine the significance of the treatment effect and the relation between infarct size and collateral blood flow. All results are reported as mean value ± SE.

Results

Forty-one dogs survived until the time of randomization to R15.7 or saline (10 min before reperfusion). Of these, five dogs in the control group and four in the R15.7 antibody group died before completion of the experiment. Eight additional dogs were eliminated because of a technical failure of the coronary occlusive device, whereby occlusion or release did not occur properly. This group included three dogs with a mechanical snare occluder (two control group, one R15.7 group) that failed to reperfuse on the basis of microsphere flow measurements. In two of these dogs, flow did not increase at all from occlusion to 10 min of reperfusion, whereas in the third, it increased only 25% and then fell to 40% below occlusion values at 3.5 h of reperfusion. Two dogs (one control group, one R15.7 group) were ischemic at baseline (flow <20% of the nonischemic value), apparently related to premature closure of the occluder. Three dogs (all control group) with a balloon occluder were eliminated because of balloon rupture and unsuccessful coronary artery occlusion. The remaining 24 animals (12 control group, 12 antibody group) form the basis of this report.
Antibody binding to circulating neutrophils. Blood samples from four dogs in the antibody group were analyzed by indirect flow cytometry. Mean channel fluorescence for neutrophils isolated before R15.7 injection was 3.2 ± 0.4 and increased to 205.7 ± 7.6 at 10 min after injection, indicating significant R15.7 binding to neutrophils. Saturation of R15.7 binding sites averaged 86.6 ± 7.6% at the time of reperfusion (10 min after injection) and 88.4 ± 11.7%, 95.2 ± 4.8%, 92.2 ± 1.8% and 62.6 ± 30.3% at 1, 3.5, 24 and 48 h of reperfusion, respectively.

Hemodynamic variables. During coronary artery occlusion, systolic, diastolic and mean blood pressures decreased in both control and antibody groups but recovered to baseline by 3.5 h of reperfusion. Heart rate did not change significantly. Overall, the antibody and control groups did not differ significantly in any hemodynamic variable. Diastolic and mean blood pressures were slightly lower in the antibody group than the control group during occlusion (62 ± 4 vs. 71 ± 2 mm Hg, 72 ± 4 vs. 82 ± 2 mm Hg, respectively, at 70 min of occlusion, both p < 0.05), but otherwise there were no significant differences between the two groups. Antibody injection produced no measurable hemodynamic effect.

Hematologic variables. Overall, significant differences occurred between antibody and control groups in leucocyte count (p < 0.0001), neutrophil count (p = 0.0006) and hematocrit (p = 0.0065). During coronary occlusion and the first 3.5 h of reperfusion, the following changes were recorded. Leucocyte and neutrophil counts were increased in the control group at 3.5 h of reperfusion; hematocrit was increased slightly during occlusion and at 10 min of reperfusion in the antibody group; and platelet count increased in both groups during occlusion and the first hour of reperfusion. There were no significant differences between the two groups during the first 3.5 h of reperfusion. After 3.5 h of reperfusion, leucocyte and neutrophil counts increased at 24 and 48 h of reperfusion in both groups, but significantly more so in the antibody group at 3.5 h of reperfusion; hematocrit was increased slightly during occlusion and at 10 min of reperfusion in the antibody group; and platelet count increased in both groups during occlusion and the first hour of reperfusion. There were no significant differences between the two groups during the first 3.5 h of reperfusion. After 3.5 h of reperfusion, leucocyte and neutrophil counts increased at 24 and 48 h of reperfusion in both groups, but significantly more so in the antibody group at the 24-h time point (36,700 ± 900 vs. 25,300 ± 2,300 leucocytes/μl; 32,600 ± 3,100 vs. 23,300 ± 2,200 neutrophils/μl, both p < 0.05). Hematocrit and platelet count were reduced below baseline levels at 48 h of reperfusion in both groups, but there were no significant differences between groups.

Regional myocardial blood flow. Myocardial blood flow to the inner two-fifths, mid one-fifth, and outer two-fifths of the left ventricular wall within the ischemic–reperfused region is shown in Table 1. Blood flow decreased in both groups during coronary artery occlusion to ~6% of nonischemic flow in the endocardium and 30% in the epicardium. At 10 min of reperfusion, hyperemia occurred in all layers, although the increase in the endocardium tended to be less than the other layers. At 3.5 h of reperfusion, flow decreased and was below baseline levels in the endocardium at both 3.5 and 48 h of reperfusion (p = NS). There were no significant overall differences between the control and antibody groups, nor were there any significant differences in any of the left ventricular layers at any time point during the experiment. Similarly, flow in the nonischemic region did not change significantly during the experiment and did not differ between the two groups.

Infarct size. Figure 1 shows a representative mid-left ventricular slice from a dog in the control group and a comparative dog in the antibody group. The two dogs were selected to have a similar-sized area at risk (27.4% vs. 26.2% of the left ventricle, respectively) and similar ischemic severity (transmural collateral flow 0.11 ml/min per g in both). Infarct size was 52% of the area at risk in the control dog but only 17.2% of the area at risk in the dog treated with R15.7.

Infarct size was inversely related to collateral blood flow in both groups (Fig. 2), and the regression line for the antibody group (y = 29.6 - 0.67x, r = 0.67) was displaced downward in a parallel manner compared with that for the control group (y = 49.4 - 0.90x, r = 0.88). The contribution of antibody treatment and collateral flow to infarct size were determined by analysis of covariance using a dummy variable for the treatment group. The effect of treatment was significant (p = 0.0002), after adjusting for flow. A significant protective effect of antibody treatment was also observed when epicardial or endocardial collateral flow rather than transmural flow was used (p = 0.0024 for epicardial flow, 0.016 for endocardial flow).

Including all dogs, and without adjusting for flow, mean infarct size, expressed as a percent of the area of risk, was reduced in the antibody group (18.4 ± 3.2%) compared with that in the control group (28.3 ± 4.5%), but the difference was of borderline statistical significance (p < 0.09). Excluding dogs with collateral flow >30% of nonischemic flow allowed better matching of the two groups for collateral flow. In this subgroup (9 control group, 11 antibody group dogs), infarct size was 19.5 ± 3.3% of the risk region in the antibody group compared with 24.6 ± 3.9% in the control group (p = 0.008) (Fig. 3). Transmural collateral flow was 14.2 ± 2.0% of normal versus 15.7 ± 1.8%, and risk region was 23.1 ± 2.7% of the left
ventricle versus 23.4 ± 3.1%, respectively, in this subgroup (neither difference statistically significant). The no-reflow area tended to be smaller in the antibody group, but the difference was not statistically significant (p = 0.16) (Fig. 3). This finding is consistent with the observation that antibody treatment did not attenuate the blood flow reduction at 48 h after reperfusion (Table 1). The infarct hemorrhage scores did not differ significantly between the antibody and control groups (1.3 ± 0.3 vs. 1.2 ± 0.2, respectively).

**Left ventricular function.** Left ventricular ejection fraction in the antibody group was similar to that in the control group at baseline, during occlusion and at 10 min of reperfusion; but at 48 h of reperfusion, ejection fraction was significantly higher in the antibody group than the control group (28.5 ± 1.8% vs. 43.6 ± 2.9%, p < 0.01) (Fig. 4). Overall, ejection fraction differed significantly between the two groups by two-way analysis of variance (p = 0.012). End-diastolic volumes at 48 h of reperfusion were similar in the control and antibody groups (60 ± 5 vs. 57 ± 2 ml, respectively). Concomitant with preservation of global left ventricular ejection fraction, regional wall motion in the inferior portion of the left ventricle (infarct zone) at 48 h of reperfusion was significantly higher in the antibody group than the control group (fractional shortening 1.06 ± 0.18% vs. 2.55 ± 0.29%, p < 0.05) (Fig. 5). Non-ischemic anterior wall motion was similar in the two groups.

**Myocardial myeloperoxidase activity.** Myeloperoxidase activity in transmural myocardial samples was measured to assess neutrophil accumulation at 48 h of reperfusion. Myeloperoxidase activity was higher in ischemic-reperfused myocardium than in nonischemic myocardium in both groups (control group: 2.88 ± 1.0 vs. 0.15 ± 0.09 U/100 mg, p < 0.05; antibody group: 1.23 ± 0.28 vs. 0.11 ± 0.07 U/100 mg, p < 0.01). Myeloperoxidase activity in the ischemic-reperfused region tended to be lower in the antibody group, but the difference was not statistically significant (p = 0.095).

**Figure 3.** Comparison of area at risk as a percent of left ventricle (LV) and infarct and no-reflow areas as a percent of area at risk. Dogs with collateral flow >30% of normal were excluded from this analysis to provide better matching of collateral flow between the two groups. Bars = mean value ± SE.
Discussion

Although coronary reperfusion can salvage ischemic myocardium and limit the extent of infarction, there is increasing evidence that reperfusion can also result in lethal injury of myocardial injury. Neutrophils enter the reperfused myocardium, release high concentrations of oxidants and other injurious mediators, or aggregate in the microvasculature to produce further ischemia. Adherence of neutrophils to endothelial cells and matrix proteins is essential for these processes and is dependent on surface expression and activation of the CD11/CD18 integrin complex. Our study demonstrates that a single injection of an anti-CD18 monoclonal antibody before reperfusion, after 90 min of coronary artery occlusion, results in almost a 50% decrease in myocardial infarct size and a significant preservation of global and regional left ventricular function measured 48 h later. To our knowledge, ours is the first study to document sustained myocardial protection in conjunction with a meaningful preservation of left ventricular function after treatment with an anti-CD18 antibody.

Previous results with anti-CD18 antibodies. Several previous studies have examined the ability of anti-CD11/CD18 antibodies to limit myocardial infarct size in animal models of ischemia-reperfusion. Myocardial salvage has been reported after treatment with anti-CD18 antibodies before or during coronary artery occlusion in several small animal models (rabbits, ferrets and rats) reperfused for 5 to 48 h (15,16,25). When the antibody was given only before reperfusion, a considerably smaller effect was seen (16). Ma et al. (14) reported that R15.7, the same antibody used in our study, produced a profound reduction in myocardial infarct size and preservation of endothelium-dependent vasoreactivity when given before reperfusion in cats with 1.5 h of ischemia followed by 4.5 h of reperfusion. However, collateral flow was not measured, making it difficult to be certain that the severity of ischemia in the treated and control groups was comparable. In contrast, Tanaka et al. (18) reported that F(ab')\(^2\) fragments of the anti-CD18 antibody IB4, given both before coronary occlusion and after reperfusion, failed to modify infarct size in dogs with 90 min of ischemia followed by 3 h of reperfusion, despite a considerable reduction of neutrophil accumulation in the infarct region. Studies from our own laboratory (17) showed that both R15.7 and MHM.23, another anti-CD18 antibody, can reduce infarct size in dogs with 90 min of ischemia followed by 3.5 h of reperfusion, but that MHM.23 was ineffective in animals with the most severe ischemia (collateral flow \\
< 0.1 ml/min per g). None of these studies examining anti-CD18 antibodies measured the effect of treatment on left ventricular function. Other approaches producing putative neutrophil suppression, including F(ab')\(^2\) fragments of an anti-CD11b antibody (26), perfluorochemical (27) and adenosine (28), have resulted in sustained limitation of infarct size or preservation of left ventricular function, or both, in canine models.
Properties of R15.7. Our study was done to determine the maximal sustained benefit that could be achieved in our experimental model using an anti-CD18 antibody to block adherence-related neutrophil functions. The antibody selected has been shown in vitro to effectively block adherence of canine neutrophils to cultured canine endothelial cells and maximally inhibit H2O2 release from canine neutrophils stimulated by platelet-activating factor (9,17). In addition, 1 mg/kg of R15.7 was shown (17) in vivo to produce antibody binding to >95% of circulating neutrophils, with >85% saturation of CD18 molecules, and neutrophil accumulation in infarcted myocardium was reduced by 64%. In the present study, we demonstrated nearly complete saturation of CD18 receptors and sustained excess of circulating antibody for at least 24 h.

Our data indicate that R15.7 is directed against a functionally important epitope on CD18 and that it binds to this epitope with high affinity. Although R15.7 was raised against canine peritoneal macrophages and screened against human CD18, it also cross-reacts and is functional in the cat (14), cow (29), rabbit (30) and human (9), in addition to the dog, suggesting that the epitope for R15.7 is highly conserved among species. Amino acids 96 to 389 and those in the cytoplasmic domain are known to be 95% identical among human, murine and bovine CD18, although canine CD18 has not yet been sequenced (31). Other monoclonal antibodies raised against CD18 may not bind with as high an affinity, requiring higher or more frequent in vivo dosing, or both to saturate available CD18 binding sites, or may not bind to functionally important epitopes. Antibodies raised against porcine CD18 have been found to cross-react with antigens expressed on rat, mouse, hamster, rabbit, dog, cow and human leukocytes, supporting the notion that CD18 contains several highly conserved epitopes (32).

Mechanisms of action of anti-CD18 antibodies. Although anti-CD18 antibodies can provide protection against myocardial reperfusion injury, it is not clear which of the several CD18-dependent neutrophil functions must be neutralized. Adherence of neutrophils to endothelial cells and matrix proteins, as well as other neutrophils, can be blocked by anti-CD18 antibodies in vitro. In vivo, these effects are thought to translate into reduced transmigration of activated neutrophils into the reperfused myocardium, as well as improved microvascular perfusion. Perhaps even more important, impaired adhesion can result in reduced release of oxygen radicals and other oxidants from stimulated neutrophils, potentially preventing oxidant-mediated myocyte death (9,17). Of course, anti-CD18 antibodies cannot inhibit those mechanisms of cell death that are independent of CD18, such as direct complement-mediated injury (through the membrane attack complex) or myocyte apoptosis.

Potential adverse effects of anti-CD18 antibodies. Inhibition of neutrophil function by anti-CD18 antibodies could theoretically have adverse consequences because it represents a “sledgehammer” approach that interferes with all CD18-dependent cellular functions throughout the body. Even a single injection of a holoantibody like R15.7 would presumably inhibit these functions for up to 48 h. This inhibition could lead to increased susceptibility to infection and possibly to delayed healing of the infarct, with aneurysm formation (33). Although no apparent infections occurred in the present study, patients with leukocyte adhesion deficiency related to mutations in the CD18 protein are subject to recurrent infections, often fatal in childhood, and their neutrophils are unable to bind to and cross the endothelium at sites of infection (11). Pretreatment of mice with an anti-CD11b antibody was reported to markedly increase mortality after inoculation with Listeria monocytogenes (34), but other investigators have not found increased mortality or infectious complications in other models (35).

Other approaches. Other approaches to preventing neutrophil-mediated reperfusion injury that might more selectively inhibit those neutrophils targeting or being activated within the reperfused myocardium are currently being explored. These include antibodies to intercellular adhesion molecule-1 (ICAM-1), the endothelial ligand for CD18 (26,36); antibodies or oligosaccharides to block P-selectin, a protein responsible for initial loose adhesion of neutrophils to reperfused endothelium (37); and inhibitors of neutrophil activation, including inhibitors of complement (38), leukotriene B4 (4) and platelet-activating factor (39). Although these approaches are of interest, it is unknown whether they will prove to be as efficacious as anti-CD18 antibodies, given the remarkable redundancy of mechanisms that exist for activating and targeting neutrophils in vivo.

We gratefully acknowledge the valuable assistance of Cynthia Siu, PhD in the statistical analyses and Christine G. Borkowski in the preparation of the manuscript.

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