Effect of Anti-CD18 Antibody on Myocardial Neutrophil Accumulation and Infarct Size After Ischemia and Reperfusion in Dogs

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**Background.** Polymorphonuclear neutrophils (PMNs) accumulate in postischemic myocardium and may cause injury to myocardium or to vessels by production of oxygen free radicals or by release of proteases and lipases. PMN accumulation is dependent on adherence to endothelium, which is mediated by a family of glycoproteins on the PMN surface, each of which has a common β-subunit (CD18). The purpose of this study was to determine whether an antibody (IB4) against the CD18 protein could attenuate PMN accumulation and limit myocardial infarct size.

**Methods and Results.** F(ab′)_2 fragments of a mouse monoclonal antibody to human adherence-promoting leukocyte glycoprotein (CD18) were used. Infarct size after 90 minutes of ischemia and 3 hours of reperfusion was compared in dogs with (n=8) and without (n=8) the anti-CD18 treatment. Myocardial PMN accumulation was assessed with ^111In-labeled autologous PMNs. Anti-CD18 treatment significantly reduced the number of PMNs in the ischemic region (19,123±5,352/mg versus 5,204±927/mg in the control and treated groups, respectively; p<0.05). In addition, the ratio of myocardial blood flow (ischemic/nonischemic wall) at 45 minutes into reperfusion was higher in the treated than in the control group (1.18±0.18 versus 0.69±0.09; p<0.05). Nevertheless, infarct size was similar between the control and treated groups (40.5±7.4% versus 48.5±4.4% of the area at risk; p=NS). Transmural mean collateral blood flow to the ischemic myocardium was similar between the two groups, and the inverse relation between infarct size and collateral blood flow was not shifted by anti-CD18 therapy.

**Conclusions.** Although PMN accumulation contributed to reduced postischemic microvascular perfusion, it caused insufficient additional myocardial cell death to measurably affect infarct size in this model.

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**Key Words** • leukocytes, polymorphonuclear • glycoproteins, adherence • reperfusion injury

Polymorphonuclear neutrophils (PMNs) are known to begin accumulating in irreversibly injured myocardium within the first 60 minutes after reperfusion after as little as 40 minutes of preceding ischemia. These accumulating PMNs play an important role in the digestion of necrotic myocytes and participate actively in the healing process, through which the infarct is replaced by scar. However, PMNs also have the potential to cause injury to viable myocytes ("lethal reperfusion injury"). Proposed mechanisms of PMN-mediated injury include the development of recurrent ischemia caused by microvascular plugging or microvascular damage secondary to the release of proteolytic or lipolytic enzymes (e.g., elastase, collagenase, acid hydrolases, and phospholipase A2) and production of free oxygen radicals or hypochlorous acid. Theoretically, any of these mechanisms could increase myocardial infarct size by killing myocytes during the phase of reperfusion.

A number of ways have been devised to prevent the postulated detrimental role of PMNs in myocardium after ischemia and reperfusion. These include 1) systemic depletion of circulating PMNs using an anti-PMN antibody or extracorporeal filtration; 2) interference with neutrophil functions using various anti-inflammatory agents such as BW 755C and prostacyclin; nafazatrom, iloprost, and ibuprofen; and 3) prevention of PMN accumulation in injured tissue by use of antibodies directed against PMN surface glycoproteins that are responsible for PMN adhesion to endothelial cells.

Leukocyte–endothelial cell interactions involve at least two classes of adhesion molecules that are expressed on the surface of neutrophils. These include L-selectin, which is constitutively functional on nonactivated leukocytes, and the β2-integrins (CD11/CD18 family of glycoproteins), which are upregulated when neutrophils are activated. L-Selectin has low-affinity binding properties that are thought to cause neutrophil rolling. Firm adhesion is mediated by β2-integrins.
These consist of a family of heterodimers, each of which has a common β-subunit designated CD18, and one of three known α-subunits designated CD11a (LFA-1), CD11b (Mo1 or Mac-1), and CD11c (gp150). Monoclonal antibodies have been produced against one or more of the α-subunits (e.g., anti-Mo1) and against the common CD18 subunit (e.g., MoAb 60.3 and IBc).

The purpose of the present study was to determine whether treatment of dogs with a mouse monoclonal antibody (IBc) against human PMN CD18 would suppress PMN accumulation in injured myocardium after ischemia and reperfusion, prevent the microvascular leaking to plasma proteins, improve myocardial perfusion after arterial reperfusion, and/or limit myocardial infarct size.

**Methods**

Animal selection, surgical preparation, and postmortem analysis were performed according to the criteria defined in the multicenter Animal Models for Protecting Ischemic Myocardium (AMPIM) study.

**Animal Selection**

Twenty-six adult mongrel dogs of either sex weighing 13.2–23.6 kg were used. Dogs with a hematocrit <35, circulating filariae, or obvious clinical infection were not accepted into the study.

**Isolation and 111In-Labeling of PMNs**

The protocol for isolation and labeling of PMNs was that of Richard et al. with several modifications. These modifications have resulted in improved PMN survival upon readministration of the labeled cells to the dogs, as indicated by a higher cell-associated/total blood-indium ratio, and a longer circulating half-life of the labeled cells compared with our earlier studies. Details are illustrated in Figure 1.

**Surgical Preparations**

After 30–40 mg/kg of intravenous sodium pentobarbital, dogs were intubated and ventilated using a Harvard animal respirator (model 607) at 200 ml/kg per minute of room air supplemented with a low-flow oxygen. The right femoral artery and vein were catheterized to monitor blood pressure, obtain blood samples, and inject the labeled cells, fluids, and additional anesthetic. The chest was opened in the fourth intercostal space, and the heart was suspended in a pericardial cradle. The left circumflex coronary artery was isolated distal to the atrial branch but proximal to the first large marginal branch. A strip of umbilical tape was passed around it for later occlusion. Two cannulas were placed in the appendage of the left atrium to monitor left atrial pressure and to inject microspheres. Throughout the experiment, lead II of the standard ECG, arterial pressure, and left atrial pressure were monitored. Dogs were allowed at least 20 minutes after the surgery to reach a hemodynamic steady state before the injection of labeled PMNs.

**Anti-CD18 Antibody**

In preliminary experiments in our laboratory (unreported data) and elsewhere (K.-E.A., Pharmacia, personal communication), the complete mouse antibody to human CD18 often caused transient allergic response in dogs manifest by a transient episode of hypotension. To avoid this reaction, the F(ab')2 fragment of the antibody (generously provided to us by Pharmacia) was prepared for use in this study. The stock preparations were suspended in saline (1.0 mg/ml) and kept frozen until use. A dose of 0.33 mg protein per kilogram was selected because one of the authors (K.-E.A., unpublished observations) has observed that this dose completely prevents accumulation of PMNs in skin lesions in rabbits; that titration of antibody concentration necessary to prevent PMN–endothelial cell adhesion in vitro assays has revealed equivalent potency across several species including rabbit, pig, dog, horse, and baboon; and that the selected dose produced substantial antibody excess in dogs. The circulating half-life of the complete antibody has been shown to be 11.5 hours in rabbits, and the half-life of the Fab fragments is 4–6 hours (K.-E.A., unpublished observations). Thus, the same dose (0.33 mg protein per kilogram) was administered twice in each treated animal: once before coronary occlusion and a second time during reperfusion (see Figure 2) to assure continued antibody excess throughout the experiment.

**Regional Blood Flow Measurement**

The myocardial blood flow was measured using 10±1-μm microspheres, as described elsewhere. At each measurement time, 2–3 million spheres labeled with 141Ce or 46Sc were injected via the left atrial...
The slices for isotope counting were viewed under ultraviolet light and divided into nonfluorescent (ischemic) and fluorescent (nonischemic) myocardium. To avoid contamination, myocardium on either side of the ischemic–nonischemic interface was eliminated. The ischemic and nonischemic regions were then subdivided into subendocardial, midmyocardial, and subepicardial thirds. Tissue and blood samples were counted immediately using a Packard 5912 gamma counter with corrections made for overlap of isotope spectra.

The slices for the analysis of infarct size were inspected under ultraviolet light, and the border between fluorescent and nonfluorescent regions was drawn directly on the myocardium with India ink. The slices then were incubated for 20 minutes in 0.09 M sodium phosphate buffer (pH 7.4) containing 1% triphenyl tetrazolium chloride (TTC, Sigma) and 8% dextran (MW 77800) for the determination of TTC-positive (viable) and TTC-negative (infarct) regions. After being fixed in 10% phosphate-buffered formalin, basal and apical sides of each slice were photographed on color transparency film. These transparencies (four from each heart) were projected at ×10 magnification, and the images, including boundaries of the area at risk and infarct, were traced. The ischemic and nonischemic areas and infarct area were quantitated using a digitizing tablet interfaced to an IBM-compatible personal computer. Areas from the four surfaces then were summed, and infarct size was calculated both as a percentage of left ventricle and ischemic area at risk.

In addition, microscopic slides were prepared from the apical side of the central ischemic region of the second and fourth myocardial slices and were stained with hematoxylin and eosin and Heidenhain’s variant of Mallory’s connective tissue stain. These slides were examined by light microscopy to assess the inflammatory response and to estimate the transmural extent of infarct. The latter was characterized primarily by contraction band necrosis, and its extent was estimated visually to the nearest 5-10% of the thickness of the subendocardial, midmyocardial, and subepicardial layers. The ischemic–nonischemic interface previously described was established by light microscopy to assess the inflammatory response and to estimate the transmural extent of infarct. The latter was characterized primarily by contraction band necrosis, and its extent was estimated visually to the nearest 5-10% of the thickness of the subendocardial, midmyocardial, and subepicardial layers.

**Calculation of Regional Myocardial Blood Flow, Albumin Space, and PMN Accumulation**

Myocardial blood flow was calculated as tissue isotope counts times reference flow divided by reference isotope counts and was expressed as milliliters per minute per gram.24

Myocardial albumin space was measured as an index of the volume of tissue accessible to plasma proteins. The methods have been described previously94 and are summarized briefly as follows: 5.0 μCi of radioiodinated (I-125) serum albumin (RISA) (Mallinkrodt, St. Louis, Mo.) was injected simultaneously with the labeled PMNs and tissue albumin space ([tissue I-125 counts]/[I-125 counts per milliliter of plasma at time of heart excision]) was measured to correct for 114In associated with plasma protein in the tissue.
TABLE 1. Ventricular Fibrillation and Survival

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Occlusion</th>
<th>VF</th>
<th>Reflow</th>
<th>VF</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>4 (0*)</td>
<td>1 (1*)</td>
<td>8 (67%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CD18</td>
<td>10</td>
<td>3 (2*)</td>
<td>3 (2*)</td>
<td>8 (80%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation.
*Survived.

The number of PMNs in each tissue sample was calculated as described previously. First, net cell-associated tissue $^{111}$In activity was obtained by subtracting $^{111}$In activity associated with plasma proteins from the total $^{111}$In activity. PMN specific activity (\(^{111}\)In counts per PMN) was calculated in each blood sample collected during coronary occlusion and reperfusion and was averaged (mean $^{111}$In counts per PMN). Tissue PMN uptake then was calculated using the formula PMNs per milligram of tissue = (cell-associated $^{111}$In counts per milligram of tissue)/(mean $^{111}$In counts per PMN).

**Statistical Analysis**

Data are expressed as group mean±SEM. Student’s t test (nonpaired) was used for statistical comparisons of mean data between groups. A paired t test was used to compare PMN accumulation and albumin space between ischemic and nonischemic regions within groups. To establish groups with equivalently severe ischemia, dogs with subendocardial collateral blood flow exceeding 0.15 ml/min · g\(^{-1}\) (three dogs in this study) were excluded from group comparison of PMN accumulation, albumin space, infarct size, area at risk, and hemodynamics. In addition, to control for variability in infarct size caused by differences in collateral blood flow, the regression of infarct size versus collateral blood flow was evaluated using data from all dogs, using ANCOVA with collateral flow as an independent variable and infarct size as a dependent variable. A value of $p<0.05$ was considered statistically significant.

**Results**

Mortality and Exclusion

Twenty-six dogs were used for this study. Three were excluded either because of a low PMN yield or a low labeling efficiency. Of the 23 dogs with good PMN isolates that initially were entered into this study (control, $n=13$; treated, $n=10$), one dog from the control group developed minimal ischemia after coronary occlusion and was excluded. The incidence and time of ventricular fibrillation and survival rate are summarized in Table 1. Seven dogs (four control and three treated) developed ventricular fibrillation within 15 minutes after coronary occlusion. Of these, two treated dogs were defibrillated successfully. Ventricular fibrillation during the first 30 minutes of reperfusion occurred in four dogs (one control and three treated); all of these except one treated dog were successfully defibrillated. Thus, overall survival was eight of 12 (67%) in the control group and eight of 10 (80%) in the treated group. Three of these 16 survivors (two control and one treated) had relatively mild ischemia and were excluded from group comparisons but were included when ANCOVA incorporating flow was done (see “Methods”).

**Yield, Purity, and Labeling Efficiency of the PMN Isolates**

PMN preparations of the completed experiments ($n=16$) exhibited the following characteristics: The yield expressed as the percentage of the total number of PMNs present in the original blood was 52.5±4.6%; the average purity expressed as the percentage of PMNs among the total number of isolated cells was 89.±1.4%. Non-PMNs in these isolates were other leukocytes, primarily lymphocytes and monocytes; no red cell contamination was observed. The average labeling efficiency, expressed as the percentage of $^{111}$In incorporated into cells, was 73.6±2.8%. No difference in these characteristics was observed between the isolates used for the two experimental groups.

$^{111}$In-Labeled PMN Kinetics in the Circulation

Figure 3 shows the circulating $^{111}$In activity as a function of time after injection of the labeled PMNs. It is clear that $^{111}$In activity was present in the plasma component as well as in the cellular components of blood, as represented by the difference between whole blood and cell-associated activity. Plasma $^{111}$In was relatively constant over time. Cell-associated activity showed a rapid peak at 10 minutes followed by a decline within the first 60 minutes after cell injection. After 60 minutes, cell-associated activity was relatively stable for the duration of the experiment. At the end of the experiment, an average of 85.0±3.6% of the whole blood counts were cell associated.

**Albumin Space in Myocardium**

Figure 4 shows the albumin space in the postischemic and nonischemic myocardium. There was a significant increase in the albumin space of all three mural layers of the ischemic regions of both groups. This postischemic increase in albumin space is indicative of enhanced microvascular leakage of plasma proteins, but the etiology is unknown. The transmural mean albumin space in the nonischemic and ischemic myocardium averaged 8.4±0.6 and 28.2±1.7 ml/100 g wet wt in the control group and 8.2±0.5 and 28.4±1.1 ml/100 g wet wt in the
PMN Accumulation

The PMN accumulation in the inner, middle, and outer thirds of ischemic and nonischemic myocardium in both groups is shown in Figure 5. In the nonischemic myocardium, the numbers of PMNs averaged 2,258±594 per milligram and 2,676±608 per milligram in the control and treated groups, respectively (p=NS), and these PMNs were distributed uniformly transmurally. In the postischemic myocardium, both groups showed a significant increase in PMN accumulation (p<0.01 in all layers) compared with nonischemic myocardium; this increase was greatest in the subendocardial zone. However, much less PMN accumulation was found in all layers of postischemic myocardium of the treated group compared with controls (6,972±1,203 per milligram versus 22,024±5,137 per milligram myocardium in the subendocardium, 5,676±1,711 per milligram versus 20,624±6,300 per milligram in the midmyocardium, and 3,218±756 per milligram versus 14,745±5,126 per milligram in subepicardium; p<0.01 in all layers). Thus, anti-CD18 treatment markedly reduced the PMN accumulation in the ischemic reperfused myocardium.

Microscopic evaluation of reperfused myocardium from dogs in the control group revealed widespread contraction band necrosis, a moderate degree of interstitial hemorrhage, and a marked inflammatory response primarily composed of PMNs. The latter was present throughout the areas of necrosis. In the CD18-treated hearts, contraction band necrosis and interstitial hemorrhage were present, although the degree of hemorrhage seemed reduced in the treated versus the control group. The PMN response was minimal, and most PMNs observed appeared to be either within vascular lumens or passive components of the interstitial hemorrhage.

Baseline Predictors of Infarct Size

Hemodynamic data from both groups are shown in Figure 6. No hemodynamic effect of the anti-CD18 preparation was observed in any dog. There was no significant difference in the measured hemodynamic parameters between the treated and control groups at any time. The areas at risk (Figure 7) were similar between the two groups, averaging 40.7±1.5% and 44.0±0.5% of the left ventricle in the control and treated groups, respectively (p=NS). The transmural mean collateral blood flow to the ischemic myocardium also was similar between the two groups, averaging 0.09±0.02 and 0.06±0.01 ml/min · g⁻¹ in the control and treated groups. Thus, the two groups were comparable
regarding the major determinants of myocardial infarct size.

**Infarct Size**

Infarct size (Figure 7) was similar in the two groups, averaging 40.5±7.4% and 48.5±4.4% of the occluded vascular bed (area at risk) in the control and treated groups, respectively (p=NS). Thus, by simple group comparison, anti-CD18 treatment had no effect on infarct size estimated by the loss of TTC staining.

To control for the variation of infarct size caused by baseline variation in collateral blood flow, the relation of infarct size to collateral blood flow was analyzed (Figure 8). There was an inverse relation between these parameters in the control group. Anti-CD18 treatment did not shift this relation (confirmed by ANCOVA; F=0.0001, p=NS). Thus, no difference in infarct size was observed between the two groups even when the variation caused by collateral blood flow was controlled.

**Comparison of TTC-Based Infarct Size Versus Microscopic Evaluation of Necrosis**

The relation between TTC-based infarct size and the microscopic extent of necrosis in the central ischemic core is shown in Figure 9. In general, the microscopic estimates were larger than the TTC-based estimates of infarct size in both experimental groups. This difference was expected because the TTC-based estimate was obtained from the entire area at risk in the gross slice of ventricle, whereas the microscopic estimate was based on a microscopic slide containing only the central region of the infarct, where necrosis is most extensive. Because the infarcts were insufficiently developed at 3 hours of reperfusion to permit precise quantitation at low magnification, the precision of the microscopic estimates was expected to be limited. Despite these technical limitations, the observed strong correlation between TTC and microscopic estimates of infarct size suggests that TTC macrochemistry resulted in a valid measurement of infarct size.

**Regional Myocardial Blood Flow in Reperfused Myocardium**

The ratio of posts ischemic myocardial blood flow to nonischemic myocardial blood flow at 45 minutes of reperfusion is shown in Figure 10. This ratio was 0.79±0.14, 0.56±0.10, and 0.66±0.09 in the inner, middle, and outer thirds, respectively, in the control group versus 1.00±0.20, 1.32±0.26, and 1.24±0.16 in the treated group. Thus, treated dogs had significantly higher blood flow in the middle and outer third of the reperfused myocardium than did the control dogs (p<0.05).

**Discussion**

The results of the present study showed that treatment with F(ab')2 fragments of a mouse monoclonal antibody (Ib4) to the CD18 subunit of PMN adherence-promoting glycoprotein markedly reduced the number of PMNs that accumulated in posts ischemic myocardium.
during the first 3 hours of reperfusion after a 90-minute episode of coronary occlusion. This reduction in accumulating PMNs was associated with an increase in postischemic myocardial blood flow. Despite these positive effects, anti-CD18 treatment did not limit infarct size nor did it attenuate microvascular injury, as evidenced by the leakage of plasma proteins into the postischemic tissue.

**Effect of Anti-CD18 Antibody on PMN Accumulation**

Although treatment with anti-CD18 antibody substantially reduced the number of PMNs that accumulated in the first 3 hours of reperfusion, the concentration of PMNs still was significantly greater in the reperfused region compared with the nonischemic control regions of the same hearts. The explanation for this residual PMN accumulation is not entirely clear. Hypothetical possibilities include 1) involvement of other non-CD18-related adhesion molecules, 2) incomplete binding of the CD18 glycoproteins by the dose of antibody used, or 3) accumulation in the tissue for reasons not involving adhesion-promoting proteins, e.g., passive obstruction to PMN passage because of reduced capillary area or passive escape into the tissue from severely disrupted vessels.

The possibility that endothelial-leukocyte interactions are promoted in damaged myocardium via proteins other than the CD11/CD18 family of glycoproteins cannot be excluded; however, the CD11/CD18 family of adhesion-promoting proteins are currently thought to be the major effectors of adhesion of activated neutrophils to endothelial cells. It also should be noted that an antibody against the common CD18 subunit as used in this study provides broader coverage against the CD11/CD18 family of adhesion proteins than does an antibody against one or another of the variable CD11 subunits, as studied previously.

The dose of antibody (0.33 mg protein per kilogram times two injections) used in this study was selected on the basis of pilot studies, as described in “Methods” (K.-E.A., unpublished data). Plasma samples were collected to confirm whether antibody excess was achieved throughout the experiment in each dog; unfortunately, these samples were lost during shipment for analysis. Nevertheless, the dose should have produced a substantial antibody excess.

Thus, the most likely explanation for the residual accumulation of PMNs in the anti-CD18--treated animals is that PMNs were trapped in the tissue by mechanisms not involving adhesion-promoting glycoproteins. For example, passive plugging of capillaries in reperfused myocardium could be enhanced either because of increased PMN rigidity or because of reduced capillary luminal diameter caused by endothelial and myocyte swelling and/or contracture rigor in irreversibly injured myocytes. Microscopic evaluation of reperfused myocardium in our study revealed some PMNs within capillaries in the anti-CD18--treated hearts as well as in the control hearts.

In addition, microscopic evaluation of the tissue sections revealed interstitial hemorrhage indicative of microvascular disruption in the areas of necrosis in both control and antibody-treated hearts. Some PMNs were associated with this interstitial hemorrhage in both groups and probably escaped into the interstitial tissue by passive means.

**Effect of Anti-CD18 Antibody on Myocardial Infarct Size**

Our results regarding myocardial infarct size are in concordance with the results of Chatelain et al27 and Richardson et al.28 The former reported that depletion of circulating PMNs using an anti-PMN antibody failed to limit myocardial infarct size produced in dogs by 3 hours of ischemia followed by 21 hours of reperfusion. The latter reported that a monoclonal antibody (MAB MHM.23) directed against the β-subunit of the PMN adhesion complex failed to limit myocardial infarct size in dogs after 90 minutes of ischemia followed by 3.5 hours of reperfusion. On the other hand, several other investigators have reported that depletion of circulating PMNs, using an anti-PMN antibody29-34 or extracorporeal filtration,35 or inactivation by antibody binding of specific PMN adhesion proteins (e.g., anti-Mo1 antibody31) improved functional recovery and/or limited infarct size. In addition, positive effects on infarct size have been reported using a variety of anti-inflammatory agents such as BW 755C,5,7 prostacyclin,6 nafazatrom,9,10 iloprost,4,11 and ibuprofen,12 although results of other studies using ibuprofen also have been negative.18 The explanation for the differences in results among these studies is unknown.

Among the aforementioned studies, the studies by Simpson et al13,14 will be considered in detail because conclusions contrary to ours were drawn even though all three studies were done using the same animal model and a similar experimental protocol. Major similarities and differences between these two studies are listed in Table 2. All three studies used an anesthetized, open-chest canine model with 90 minutes of left circumflex occlusion and reperfusion. Antibody against a component of PMN adhesion glycoprotein was given, and tissue PMN accumulation and infarct size (based on TTC macrochemistry) were evaluated.

In the studies of Simpson et al,13,14 an antibody to one (CD11b or Mo1) of the three α-subunits of the PMN adhesion glycoproteins was used. This antibody does not block the other two adhesion subtypes (CD11a,
CD11c), and its administration actually failed to attenuate the accumulation of PMNs to the reperfused myocardium in one of their studies.14 In our study, to block all the three subtypes of the adherence-promoting protein, an antibody (IBα) to the common β-subunit (CD18) was used, and an attenuation of PMN accumulation was confirmed at the end of the 3-hour reperfusion period. Thus, it is paradoxical that minimal attenuation of PMN accumulation by antibody to only one subtype limited infarct size, whereas substantial attenuation of PMN accumulation by antibody to the common CD18 subunit was ineffective. Our study also had a second potential therapeutic advantage in that we administered the first dose of antibody before coronary occlusion to assure that all PMNs were exposed to antibody before they could be activated by the initiation of ischemia. Simpson et al administered the first treatment midway through the period of occlusion.

The duration of reperfusion was 3 hours in the present study versus 6 and 72 hours in the studies of Simpson et al. It is possible that the longer reperfusion periods in the Simpson protocols resulted in positive results if infarcts in the control group progressively enlarged because of continued PMN-mediated necrosis between 3 and 72 hours of reperfusion. This possibility seems unlikely because we have shown recently that PMN influx after reperfusion primarily occurs during the first 3 hours and that no additional influx is detectable after 21 hours.32 Moreover, infarct size in untreated control dogs was not different when measured at 3, 6, or 24 hours of reperfusion.32 In another study, we found no difference between infarcts reperfused for 4 hours versus 4 days.33 In addition, we have found that treatment with polyethylene glycol–conjugated superoxide dismutase to achieve sustained anti–free radical treatment (and thereby prevent possible late reperfusion injury) did not limit myocardial infarct size after 90 minutes of ischemia.34

In the present study, infarct size was measured by macrochemistry (TTC) after 3 hours of reperfusion and was based on two transverse slices of left ventricle. This method differs from that used in most of our previously reported studies of myocardial infarct size.21,22,33,35 In previous studies, we measured infarct size from detailed microscopic evaluation of five cross-sectional slices obtained 4 days after reperfusion, when the infarcts were fully developed. The use of TTC in the present study was necessitated by the fact that our primary goal was to evaluate the effect of anti-CD18 on early PMN accumulation and that microscopic infarct sizing was not feasible at this early time. Likewise, the smaller number of cross-sectional slices was mandated by the need to use some fresh tissue for immediate assessment of "TTC" activity. Despite these technical limitations in the present study, mean infarct size in the control group (40.5±7.4% of the area at risk and 16.6±3.2% of the left ventricle) was similar to infarct size in our previous 4-day studies, and the regression lines of infarct size versus collateral blood flow were superimposable.22,34 Moreover, our semiquantitative microscopic evaluation of contraction band necrosis in each heart (Figure 9) suggests that the TTC-based measurement of infarct size was a valid reflection of myocardial necrosis in both experimental groups. In any case, the use of TTC macrochemistry in the present study is not a likely explanation for the different conclusions versus Simpson et al because TTC macrochemistry was used in all three studies.

In studies using a canine model of myocardial infarction, it always is essential to control for the variation in myocardial collateral blood flow because this varies markedly among dogs and is a major predictor of infarct size. Collateral flow was measured in our study and in one14 but not the other13 study of Simpson et al. Whether this omission resulted in a false-positive conclusion because of undetected baseline differences between groups is unknown.

Finally, the manner of reperfusion differed among the three studies. Whereas Simpson et al used a critical stenosis to prevent reactive hyperemia, we reperfused abruptly, allowing full reactive hyperemia to develop. However, we have observed previously that the number of PMNs accumulated in the reperfused myocardium as well as infarct size was not affected by the presence or absence of a critical stenosis at the onset of reperfusion.19

**Table 2.** Comparison of Studies of the Effect of Antibodies to PMN Adhesion Glycoproteins on Myocardial Infarct Size

<table>
<thead>
<tr>
<th>Species</th>
<th>Current study</th>
<th>Simpson et al,13 1988</th>
<th>Simpson et al,14 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of occlusion</td>
<td>Dog</td>
<td>Dog</td>
<td>Dog</td>
</tr>
<tr>
<td>90 Minutes</td>
<td>90 Minutes</td>
<td>90 Minutes</td>
<td></td>
</tr>
<tr>
<td>Duration of reperfusion</td>
<td>3 Hours</td>
<td>6 Hours</td>
<td>72 Hours</td>
</tr>
<tr>
<td>Antibody</td>
<td>F(ab')2 of anti-CD18</td>
<td>Anti-CD11b</td>
<td>F(ab')2 of anti-CD11b</td>
</tr>
<tr>
<td>Therapeutic regimen</td>
<td>Preocclusion and postreperfusion</td>
<td>Midocclusion</td>
<td>Midocclusion and postreperfusion</td>
</tr>
<tr>
<td>Measurement of collateral blood flow?</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Method of infarct sizing</td>
<td>TTC</td>
<td>TTC</td>
<td>TTC</td>
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<tr>
<td>Method of tissue PMN evaluation</td>
<td>111In PMNs</td>
<td>Microscopic grading</td>
<td>Microscopic grading and myeloperoxidase</td>
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<tr>
<td>Effect on tissue PMNs</td>
<td>Reduced</td>
<td>Reduced</td>
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</tr>
<tr>
<td>Critical stenosis used?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Limitation of infarct size?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

PMN, polymorphonuclear neutrophil; TTC, triphenyl tetrazolium chloride.
We do not know which if any of the aforementioned experimental differences account for the opposing results of infarct size limitation of this versus Simpson's studies.

**Effect of Anti-CD18 Antibody on Myocardial Edema**

Myocardial edema and an increase in tissue plasma proteins have been reported previously after either reversible or irreversible ischemic injury of myocytes\(^1\)\(^{13,16}\) and as observed previously, were transmural even though the myocyte necrosis was primarily in the subendocardial half of the ischemic region. These changes presumably indicate functional and/or anatomic changes in the microvasculature, but the precise mechanism of such microvascular injury is unknown. Its development transmurally suggests that it may be independent of the metabolic and collateral blood flow gradients that contribute to the transmural progression of lethal myocyte injury. The failure of the anti-CD18 treatment to prevent the transmural leakage of plasma proteins suggests that binding of the PMN adhesion glycoproteins to the endothelial receptor (ICAM-1) also is not necessary to cause the underlying microvascular lesion. This conclusion is indirectly supported by the report by Stein et al\(^{17}\) that 15 minutes of coronary occlusion followed by 15 minutes of reperfusion caused a 10-fold increase in the rate of leakage of 70,000 MW dextran (61A) from the coronary microvasculature.\(^{37}\) This period of injury causes no myocyte necrosis and in our studies using a similar duration of ischemia (12 minutes) resulted in a reduced myocardial PMN content.\(^1\)

**Effect of Anti-CD18 Antibody on Myocardial Blood Flow After Reperfusion**

It is well known that after 90 minutes of ischemia followed by reperfusion, microvascular perfusion may be impeded.\(^{38,39}\) Possible mechanisms of this no-reflow phenomenon include vascular endothelial swelling, vascular compression (e.g., by contracture of myocytes or interstitial edema), and leukocyte plugging. In the present study, to evaluate the role of PMN adherence proteins and consequent PMN accumulation on microvascular perfusion, regional myocardial blood flow was measured 45 minutes after reperfusion. There was a trend toward better restoration of flow to the reperfused myocardium in the treated group than in the control group (1.08±0.25 versus 0.87±0.16 ml/min·g\(^{-1}\), \(p=NS\)). Moreover, when variation in myocardial blood flow among individual animals was controlled by expressing reperfusion blood flow as a ratio to flow in the corresponding nonischemic region, flow in the midepicardium and subepicardium of the anti-CD18–treated dogs was significantly higher than in controls, indicating better microvascular reperfusion in the treated group. Since the amounts of necrotic myocardium and tissue edema (as reflected by the myocardial albumin space) were similar in the two groups, the improved blood flow in the treated animals most likely is explained by the attenuation of PMN accumulation and consequent reduction in microvascular plugging. These conclusions are consistent with previous studies that have shown improved blood flow when myocardium was reperfused with blood devoid of leukocytes.\(^{27,40}\)

**Conclusions**

The administration of F(ab\(^\prime\))\(_2\) fragments of the monoclonal antibody (IB\(_4\)) directed against the common \(\beta\)-subunit of the PMN adherence–promoting glycoproteins significantly attenuated the accumulation of PMNs and improved microvascular perfusion of the reperfused myocardium in dogs. However, these potentially beneficial effects were not associated with limitation of myocardial infarct size. These results suggest that PMN accumulation contributes to reduced postischemic microvascular perfusion but causes insufficient additional myocardial cell death to measurably affect infarct size in this model.

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Tanaka et al

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