Reduction of the Extent of Ischemic Myocardial Injury by Neutrophil Depletion in the Dog

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SUMMARY Accumulation of polymorphonuclear neutrophils during the acute inflammatory response may exacerbate tissue injury through the release of activated oxygen products or proteolytic enzymes or both. To assess the role of neutrophils in acute myocardial infarction, circulating neutrophil levels in dogs were reduced by 77 ± 2% (mean ± SEM) by administering rabbit antiserum to dog neutrophils. Acute myocardial infarction was induced in open-chest anesthetized dogs by 90 minutes of left circumflex coronary artery occlusion followed by 6 hours of reperfusion. Dogs treated with neutrophil antiserum (n = 8) developed myocardial infarcts that were an average of 43% smaller than infarcts in dogs treated with nonimmune rabbit serum (n = 7) (27.0 ± 4.5% vs 47.1% ± 7.5% of the area at risk, p < 0.05). In a saline-treated control group (n = 8), infarct size was 48.0 ± 4.7% of the area at risk, a value not significantly different from that of the nonimmune serum group but significantly greater than that in the neutrophil antiserum dogs (p < 0.05). There were no major hemodynamic differences between groups. Histopathologic examination revealed that infarcted myocardium from dogs given saline or treated with nonimmune serum had a substantial neutrophilic infiltrate, which was virtually absent in infarcted tissue from dogs treated with neutrophil antiserum. These observations suggest that neutrophil accumulation in response to myocardial ischemia may be responsible for a substantial portion of the irreversible myocardial injury resulting from temporary coronary artery occlusion.

THE MIGRATION of polymorphonuclear neutrophils into recently infarcted myocardium represents the initial phase of a process that leads to demolition and subsequent organization of injured tissue and culminates in the replacement of necrotic myocardium with fibrous scar.1,2 Infiltration of neutrophils into irreversibly injured myocardium facilitates the breakdown of necrotic myocardium, which promotes resorption or phagocytosis by macrophages.2 After the removal of tissue debris, capillaries and fibroblasts invade the infarcted area and lead to the formation of collagen-rich scar tissue, which replaces the necrotic area.3

During the early acute inflammatory response, polymorphonuclear neutrophils undergo a complex series of functional and biochemical alterations that promote tissue lysis. Although these events are important to the repair process, they may also result in the destruction of potentially viable tissue elements. Stimulated neutrophils release highly reactive and cytotoxic activated oxygen species such as superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen. These activated oxygen radicals degrade extracellular macromolecules, attack membrane phospholipids and, thus, promote cell injury or death.5,4 In addition, activated neutrophils release lysosomal enzymes capable of proteolytic disruption and liquefaction of viable as well as irreversibly injured tissue.5 Finally, stimulated neutrophils trigger membrane phospholipids to release arachidonate, which is converted by specific lipoxygenases to potent chemotactic hydroxy-icosatetraenoic acids (HETEs).6 These chemoattractant substances promote the further recruitment of neutrophils into the acute inflammatory response at the site of tissue injury. In short, the migration and accumulation of neutrophils at the site of tissue injury may exacerbate the extent of tissue destruction by cellular mechanisms also linked to organization and repair.

In an effort to assess the importance of neutrophils in determining the ultimate extent of myocardial injury induced by ischemia, dogs were depleted of polymorphonuclear neutrophils by administration of rabbit antiserum to canine neutrophils. Regional ischemia was then induced by occlusion of the left circumflex coronary artery.

Methods

Induction of Regional Myocardial Ischemia

Male mongrel dogs (12–17 kg) were used in the experimental protocol to assess the effect of neutrophil depletion on the ultimate extent of irreversible myocardial injury. Each dog was anesthetized with i.v. sodium pentobarbital (30 mg/kg) and ventilated with a positive-pressure respirator. A left thoracotomy was performed at the fifth intercostal space. The left circumflex coronary artery (LCx) was isolated near its origin under the left atrium, distal to its atrial branch and proximal to any major ventricular branch.

Each dog had continuous electrocardiographic and hemodynamic monitoring during the course of the experiment. Arterial blood pressure was measured with a Statham P23 pressure transducer attached to a woven Dacron catheter advanced to the abdominal aorta through the right femoral artery. A second Statham P23 pressure transducer was connected to a #7F pigtail catheter, which was inserted into the left carotid artery and advanced past the aortic valves into the left...
ventricle. This catheter permitted continuous recording of peak ventricular pressure and left ventricular end-diastolic pressure. Each pressure transducer was calibrated against a mercury manometer at the start of each experiment.

Basal blood flow in the LCx was with an electromagnetic flow probe affixed to the artery. A critical stenosis was established by partially constricting the LCx with a ligature tied around the artery and a 20-gauge needle. The degree of partial constriction was considered adequate when the hyperemic response to a 10-second occlusion was abolished without altering basal LCx coronary blood flow. The LCx was then completely occluded with a second ligature for 90 minutes, followed by reperfusion with the critical stenosis remaining in place. The critical stenosis limits the reperfusion hyperemia, which reduces the severity of reperfusion arrhythmias, the incidence of hemorrhagic myocardial infarcts and the potential of developing ventricular fibrillation.7,8

Determination of the Extent of Irreversible Myocardial Injury

Six hours after the initiation of reperfusion through the LCx, the heart was fibrillated electrically and rapidly excised for postmortem evaluation of infarct size. The ex vivo dual perfusion technique used to determine area at risk of infarction and the extent of irreversible myocardial injury has been reported in detail.9 Briefly, one cannula was inserted into the aorta above the coronary ostia and another into the LCx at the site of occlusion. At a constant pressure of 100 mm Hg, 0.5% Evans Blue dye was infused into the aorta and 1.5% 2,3,5-triphenyltetrazolium chloride (TTC) was infused into the LCx for 10 minutes. The heart was sliced into six or seven 1-cm-thick sections perpendicular to the apex-base axis. The area at risk of infarction was identified by the lack of Evans Blue stain. The regions of irreversibly injured myocardium within the area at risk were demarcated by the absence of TTC staining. Viable myocardium within the area at risk stained brick red. Fishbein et al.10 reported that TTC clearly differentiates irreversibly injured myocardium from viable tissue as early as 6 hours after the onset of the ischemic insult. The area of left ventricle not dependent on the LCx, the area at risk, and the region of infarcted myocardium were traced carefully onto clear plastic sheets from both sides of each of the transverse sections for analysis of infarct size by planimetry. This ex vivo dual perfusion technique permits the expression of infarct size as a percentage of area at risk as well as a percentage of the total left ventricle.

Depletion of Canine Polymorphonuclear Neutrophils

Antiserum to dog polymorphonuclear neutrophils was prepared by purifying neutrophils from heparinized whole canine blood with a Ficoll-Hypaque discontinuous density gradient according to the methods of English and Anderson.11 Microscopic examination of the purified neutrophil suspension revealed less than 3% contamination with other cells. Rabbits were inoculated by intradermal injection of 1 × 10⁸ dog neutrophils suspended in complete Freund's adjuvant. Ten days later, the rabbits received a secondary challenge of 1 × 10⁷ dog neutrophils in incomplete Freund's adjuvant. The rabbits were bled 20 days after the initial exposure to dog neutrophils (i.e., 10 days after the secondary challenge). The sera were pooled and heat-inactivated. Nonimmune serum administered to the control group of dogs was prepared by bleeding uninoculated rabbits and heat-inactivating the pooled serum.

On the day of the experiment, the dogs were assigned randomly to receive nonimmune serum or neutrophil antiserum. The identity of the serum was known only to one investigator, who was not directly involved in the occlusion/reperfusion protocol or the histologic assessment of myocardial tissue. The treatment group regimens were not revealed until infarct size analysis and histologic assessment had been completed on the first 15 dogs. After analyzing these two groups, we evaluated a saline control group to assess the effects of the nonimmune serum on the variables being measured. Experiments involving the saline control group were conducted in an identical manner to those for the blinded groups, and were performed by the same investigators.

A blood sample was taken immediately before the slow i.v. administration of 4 ml of saline or heat-inactivated serum. Preliminary experiments revealed that 4 ml of neutrophil antiserum resulted in a vasodepressor response that returned to baseline values within 30-45 minutes. Subsequent administration of neutrophil antiserum did not affect hemodynamics. To assure that the identity of the serum remained unknown to the investigators responsible for the occlusion/reperfusion phase of the protocol, no hemodynamic or electrocardiographic recordings were made for 1 hour after the initial administration of the serum or saline.

The dogs were then subjected to complete occlusion of the LCx for 90 minutes. Twenty minutes before the reinstitution of LCx coronary blood flow, an additional 2 ml of i.v. saline or serum was administered. During the 6-hour reperfusion period, 0.75 ml of serum or saline was administered every 30 minutes.

Circulating neutrophil levels were assessed by total peripheral white cell counts in conjunction with differential counts. Blood samples were taken every hour, immediately before the administration of additional saline or serum. Neutrophil antiserum specifically lowered circulating polymorphonuclear neutrophil counts and did not significantly alter lymphocyte or monocyte levels.

Histopathologic Examination of Myocardial Tissue

After completing the tracings to be used to assess infarct size by planimetry, an approximately midventricular myocardial slice from each heart was fixed in 10% formalin for histopathologic examination. Two transmural tissue samples were excised from each slice so as to include the entire area at risk in addition to a small margin of nonischemic (blue-stained) myocardial.
um at either end of the tissue sample. Each tissue sample was then embedded in paraffin, sliced into 5-μ sections and stained with hematoxylin-eosin. Light microscopy was used to assess the general features of infarcted myocardium. An arbitrary, semiquantitative grading scheme was devised to gauge the extent of leukocytic infiltrate associated with irreversibly injured myocardium. A score of ++ + + was assigned to the myocardial sample with the most dense and diffuse leukocytic infiltrate. A score of 0 would theoretically have been given to sections in which no extravascular leukocytes were observed. Intermediate scores were assigned based on the varying intensity and extent of leukocyte infiltrate. Repetitive blind trials of the same tissue samples showed that this grading system was highly reproducible. All sections were coded so that the pathologist was unaware of the treatment given to each dog.

A second midventricular slice was selected from each heart for histologic confirmation of tissue considered, on the basis of TTC staining, to be irreversibly injured. A small cube of tissue was taken from the nonischemic (blue-stained) area, from apparently viable myocardium (red-stained) in the area at risk, and from irreversibly injured (unstained) tissue. As before, tissue samples were embedded in paraffin, sectioned and stained with hematoxylin-eosin.

Statistics

Analysis of variance was used to investigate treatment differences in the variables that were measured once (percentage of area at risk infarcted, percentage of total left ventricle infarcted and percentage of left ventricle at risk). If a difference was detected, multiple, pairwise comparisons with an experimental alpha level of 0.05 (using Bonferroni’s method) were computed.

The hemodynamic variables and the neutrophil counts were initially analyzed using a profile analysis. The time points included in the analysis were the control, 60 minutes into occlusion and 6 hours after reperfusion. For analyses indicating time-treatment interactions, analyses of variance were computed separately for each of the three times. In addition, for variables where there was indication that control values were different between groups, analysis of covariance was used to adjust for these control values. For analyses found to be significant (p < 0.05), pairwise comparisons were completed using the Bonferroni method to control experimental alpha error rates.

Results

Twenty-eight dogs were used to evaluate the effect of neutrophil depletion on the extent of irreversible myocardial injury after 90 minutes of LCx occlusion. Three dogs, one treated with nonimmune serum and two with neutrophil antiserum, died of intractable ventricular fibrillation during the course of the experiment. Criteria were established before the start of the study to ensure that dogs included in data analysis were subjected to a comparable degree of regional ischemia. For a dog to be included in the study, LCx occlusion had to result in marked ST-segment elevation (lead II), a distant zone of epicardial cyanosis greater than 9 cm² that persisted for the duration of the occlusion, and the development of arrhythmias upon reperfusion. Using these criteria, two dogs (nonimmune serum–treated) were excluded during the experiment, before the identity of the serum was revealed. Thus, 23 of the 28 dogs were used in the analysis: seven in the nonimmune serum group, eight in the neutrophil antiserum group and eight in the saline control group.

The administration of rabbit antiserum to dog neutrophils resulted in a marked reduction in circulating neutrophil counts during the experiment (fig. 1). One hour after the first dose of antiserum, circulating neutrophils had decreased to an average of 14 ± 6% of values obtained before serum administration. When calculated over the entire experiment, dogs treated with neutrophil antiserum were depleted of circulating neutrophils by an average of 77 ± 2% (range 70–86%). In contrast, control dogs that received saline or nonimmune rabbit serum manifested a progressive in-

![Figure 1. Effect of neutrophil antiserum on circulating neutrophil counts. Neutrophils were counted manually in blood samples taken before (control) and every hour after the start of serum or saline administration. The large increase in neutrophil counts in dogs treated with nonimmune serum or saline is attributed to surgical trauma and regional myocardial ischemia. A profile analysis of the control, occlusion and 6-hour postreperfusion time points revealed a significant time-treatment interaction. Analyses of variance and covariance revealed significant differences between the two control groups and the neutrophil antiserum group at occlusion and 6 hours after reperfusion, although the control groups did not differ from each other. The values at the control time were not statistically different.](image-url)
crease in neutrophil counts during the experiment. After 6 hours of LCx reperfusion, dogs treated with saline and those treated with nonimmune serum had average circulating neutrophil counts of $153 \pm 12 \times 10^9$ and $162 \pm 17 \times 10^9$ per ml, respectively. A profile analysis for neutrophil counts showed a time-treatment interaction. An analysis of variance for the control time showed no significant difference ($p = 0.14$); however, the means appeared sufficiently different to warrant analysis of covariance using the control values as the covariate. Both the analyses of covariance and analyses of variance showed differences at the two follow-up times, i.e., 1 hour into occlusion and 6 hours after reperfusion. In both analyses, and for both times, the control groups (nonimmune serum and saline) had significantly higher counts than the antiserum group, but these control groups were not different from each other.

Hemodynamic data for the three groups are presented in Table 1. In the dogs given nonimmune serum or saline, the heart rate increased progressively from basal values of $152 \pm 10$ and $162 \pm 6$ beats/min to $192 \pm 7$ and $196 \pm 13$ beats/min, respectively. The mean arterial blood pressure declined from $115 \pm 5$ to $105 \pm 9$ mm Hg in the nonimmune serum group and from $118 \pm 6$ to $97 \pm 5$ mm Hg in the saline group after 6 hours of reperfusion. Rate-pressure products (calculated from heart rates and peak systolic arterial pressure) and peak left ventricular pressure changed less than 15% relative to basal measurements. The profile analysis for the five hemodynamic variables revealed no interactions ($p > 0.05$) or group differences for heart rate ($p = 0.56$), mean arterial pressure ($p = 0.60$), rate-pressure product ($p = 0.99$) or peak left ventricular systolic pressure ($p = 0.95$). An interaction ($p < 0.05$) with time was found for left ventricular end-diastolic pressure, so analyses of variance were performed for each time. The only "difference" was at 6 hours ($\alpha = 0.10$, $p = 0.08$). In dogs that received saline or nonimmune serum, left ventricular end-diastolic pressure increased from basal values of 3 $\pm$ 1 and 2 $\pm$ 0.4 mm Hg to 6 $\pm$ 1 and 9 $\pm$ 1 mm Hg after 6 hours of reperfusion, respectively. Dogs that received neutrophil antiserum had an average basal left ventricular end-diastolic pressure of 2 $\pm$ 0.4 mm Hg, which increased to 5 $\pm$ 1 mm Hg after 6 hours of reperfusion. No differences were found at the control period ($p = 0.33$) or 60 minutes into occlusion ($p = 0.13$).

Histopathologic examination of myocardium sampled at different sites in the 1-cm midventricular tissue section established the presence of infarcted myocardial tissue in regions failing to react with TTC, thereby providing verification for the assessment of infarct size by the ex vivo dual perfusion technique. Tissue samples from myocardial regions stained blue were histologically normal. Sections assumed to be viable on the basis of brick-red staining were confirmed as being viable by histologic examination. In a few instances, however, small foci of necrotic fibers interdigitated with viable, histologically normal myocardium. This observation reflects the difficulty of excising only re-stained tissue due to the irregularity of the infarct border. Nonstained myocardium in the area at risk had undergone extensive necrosis, although a subendocardial rim of viable tissue two or three cells thick was usually present. Myocardial fibers in the necrotic area

**Table 1. Mean Hemodynamic Values for All Groups**

<table>
<thead>
<tr>
<th>Saline control (n = 8)</th>
<th>Control</th>
<th>After saline</th>
<th>Occlusion</th>
<th>2 hr</th>
<th>4 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>162 $\pm$ 6</td>
<td>160 $\pm$ 6</td>
<td>171 $\pm$ 6</td>
<td>170 $\pm$ 8</td>
<td>190 $\pm$ 14</td>
<td>196 $\pm$ 13</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>118 $\pm$ 6</td>
<td>113 $\pm$ 4</td>
<td>96 $\pm$ 5</td>
<td>95 $\pm$ 6</td>
<td>105 $\pm$ 7</td>
<td>97 $\pm$ 5</td>
</tr>
<tr>
<td>Rate $\times$ pressure ($\times 10^3$)</td>
<td>21.3 $\pm$ 1.2</td>
<td>20.2 $\pm$ 0.9</td>
<td>18.2 $\pm$ 1.5</td>
<td>18.8 $\pm$ 2.0</td>
<td>23.6 $\pm$ 2.9</td>
<td>23.1 $\pm$ 3.0</td>
</tr>
<tr>
<td>LV peak pressure (mm Hg)</td>
<td>124 $\pm$ 9</td>
<td>122 $\pm$ 7</td>
<td>110 $\pm$ 7</td>
<td>106 $\pm$ 7</td>
<td>115 $\pm$ 8</td>
<td>110 $\pm$ 6</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>3 $\pm$ 1</td>
<td>3 $\pm$ 1</td>
<td>6 $\pm$ 1</td>
<td>7 $\pm$ 1</td>
<td>5 $\pm$ 1</td>
<td>6 $\pm$ 1</td>
</tr>
</tbody>
</table>

| Nonimmune serum (n = 7) | | | | | | |
|-------------------------| | | | | | |
| Heart rate (beats/min) | 155 $\pm$ 6 | 164 $\pm$ 9 | 166 $\pm$ 9 | 175 $\pm$ 9 | 171 $\pm$ 10 | 177 $\pm$ 9 |
| MAP (mm Hg) | 115 $\pm$ 5 | 120 $\pm$ 6 | 116 $\pm$ 5 | 104 $\pm$ 8 | 101 $\pm$ 6 | 105 $\pm$ 9 |
| Rate $\times$ pressure ($\times 10^3$) | 20.5 $\pm$ 0.9 | 22.8 $\pm$ 1.2 | 21.8 $\pm$ 0.9 | 21.4 $\pm$ 1.2 | 21.1 $\pm$ 1.0 | 22.2 $\pm$ 1.1 |
| LV peak pressure (mm Hg) | 116 $\pm$ 3 | 121 $\pm$ 6 | 118 $\pm$ 3 | 108 $\pm$ 7 | 106 $\pm$ 4 | 111 $\pm$ 7 |
| LV end-diastolic pressure (mm Hg) | 2 $\pm$ 0.4 | 2 $\pm$ 0.5 | 5 $\pm$ 1 | 8 $\pm$ 1 | 8 $\pm$ 1 | 9 $\pm$ 1 |

| Neutrophil antiserum (n = 8) | | | | | | |
|-----------------------------| | | | | | |
| Heart rate (beats/min) | 152 $\pm$ 10 | 152 $\pm$ 10 | 169 $\pm$ 10 | 178 $\pm$ 8 | 185 $\pm$ 7 | 192 $\pm$ 7 |
| MAP (mm Hg) | 122 $\pm$ 5 | 113 $\pm$ 8 | 116 $\pm$ 8 | 114 $\pm$ 7 | 105 $\pm$ 7 | 97 $\pm$ 8 |
| Rate $\times$ pressure ($\times 10^3$) | 20.9 $\pm$ 1.7 | 20.2 $\pm$ 2.3 | 22.6 $\pm$ 2.0 | 23.3 $\pm$ 1.5 | 23.0 $\pm$ 1.3 | 22.9 $\pm$ 2.1 |
| LV peak pressure (mm Hg) | 122 $\pm$ 6 | 120 $\pm$ 8 | 124 $\pm$ 6 | 116 $\pm$ 7 | 109 $\pm$ 7 | 102 $\pm$ 7 |
| LV end-diastolic pressure (mm Hg) | 2 $\pm$ 0.4 | 1 $\pm$ 0.4 | 4 $\pm$ 1 | 5 $\pm$ 1 | 5 $\pm$ 1 | 5 $\pm$ 1 |

Values are mean $\pm$ SEM.

Abbreviations: MAP = mean arterial pressure; LV = left ventricular.
showed increased cytoplasmic eosinophilia, variable homogenization and loss of myofibrillar detail, and patchy foci of contraction bands alternating with flocculent cytoplasm. Many nuclei within these altered muscle cells showed early karyolysis, and a few were pyknotic. Modest numbers of extravasated erythrocytes were occasionally scattered within the necrotic areas.

Examples of the histologic appearance of infarcted myocardium from a dog receiving nonimmune serum and from a dog treated with neutrophil antiserum are shown in figure 2. The histologic appearance of the necrotic myocardium per se and the extent of erythrocyte extravasation did not differ in the two groups. Dense leukocyte infiltrate, composed primarily of neutrophils, was present in infarcted myocardium from a dog treated with nonimmune serum (fig. 2A). In marked contrast, irreversibly injured myocardium from a dog that received neutrophil antiserum (fig. 2B) is almost devoid of leukocytic infiltrate. Histologic examination of tissue from hearts of the saline-treated group revealed features identical to those in the hearts from the dogs treated with nonimmune serum. Thus, the nonimmune serum did not alter the inflammatory response associated with ischemic myocardial injury.

A summary of results from the semiquantitative histologic assessment of the extent of leukocytic infiltration in infarcted myocardium in neutrophil antiserum— and nonimmune—serum treated animals is presented in figure 3. The semiquantitative score is presented for each dog in the order in which the coded tissue sections were examined. When the identity of each tissue section was revealed at the end of the first series of experiments, it became evident that depletion of circulating neutrophils was associated with a marked restriction in the extent of leukocytic mobilization into infarcted myocardium. Tissue samples from dogs treated with neutrophil antiserum contained only rare interstitial neutrophils or mononuclear cells. Thus, every dog received a score of +/−, indicating virtual absence of leukocytic infiltrate. In marked contrast, tissue samples from every dog receiving nonimmune serum contained significant cellular infiltrates, composed almost

![Image](image_url)

**Figure 2.** (A) Myocardium from a control dog treated with nonimmune serum. None of the myocardium in this field appears viable. Many fibers, particularly in the upper right quadrant, appear dark because of increased cytoplasmic eosinophilia. Contraction bands are evident in the lower right and upper midportions of the field. Karyolysis and pyknosis, although present, are difficult to discern at this magnification. An interstitial leukocytic infiltrate is obvious. Hematoxylin-eosin stain; original magnification × 188. (B) Myocardium from a neutrophil-depleted dog. Some viable myocardium remains in the left upper quadrant of the field. The necrotic fibers, particularly at the extreme right, appear dark because of increased eosinophilia and homogenization of cytoplasm. Contraction bands are also prominent, especially in the right lower quadrant. In contrast to panel A, there is virtually no leukocytic infiltrate. Hematoxylin-eosin stain; original magnification × 188.

![Image](image_url)

**Figure 3.** Results of the histopathologic assessment of leukocyte infiltrate into infarcted myocardium for dogs given nonimmune serum and those given neutrophil antiserum. Each coded tissues specimen was ranked for the extent of leukocytic infiltrate associated with infarcted myocardium on a scale ranging from zero (no white cells) to + + + + (assigned to the specimen with the most extensive accumulation of leukocytes). The score for each dog is presented in the order in which the coded samples were evaluated. Each dog treated with neutrophil antiserum received a score of +/−, representing the presence of rare interstitial leukocytes. Dogs treated with nonimmune serum had scores ranging from + to + + + +, indicating substantial neutrophil accumulation in the infarcted myocardium. Samples from dogs given saline exhibited leukocytic infiltration comparable to that in the nonimmune serum group (data not shown).
entirely of neutrophils. Individual leukocyte infiltrate scores in this group ranged from + to +++++.

The effect of neutrophil depletion on the ultimate extent of ischemia-induced myocardial injury is summarized in table 2. Although the extent of left ventricle at risk of infarction as a result of 90 minutes of LCx occlusion was not significantly different between the three treatment groups (p > 0.05 by analysis of variance), the extent of irreversible injury expressed as a percentage of the area at risk or as a percentage of the left ventricle was significantly less in the dogs treated with neutrophil antiserum. When the extent of irreversible injury is expressed as a percent of the area at risk of infarction, neutrophil depletion resulted in a 43% reduction in infarct size compared with nonimmune serum treatment (from 47.1 ± 7.5% in the dogs receiving nonimmune serum to 27.0 ± 4.5% in the neutrophil antiserum group, p < 0.05). Compared with saline-treated dogs, the neutrophil-depleted dogs showed a 44% reduction in infarct size (27.0 ± 4.5% vs 48.0 ± 4.7% of the area at risk, p < 0.05). If the average infarct size is expressed as a percentage of the left ventricle, neutrophil depletion resulted in a 52% reduction in the extent of irreversible myocardial injury compared with the nonimmune serum group (9.6 ± 1.6% vs 20.1 ± 3.5% of the left ventricle, respectively, p < 0.05), and a 53% decrease relative to the saline group (9.6 ± 1.6% vs 20.3 ± 1.9%, respectively, p < 0.05). Thus, neutrophil depletion produced by the antiserum is associated with a substantial reduction in the ultimate extent of irreversible myocardial injury.

### Discussion

Observation of the release of cytotoxic products by stimulated neutrophils has led to the suggestion that neutrophil infiltration into infarcted myocardium may exacerbate ischemic injury by the destruction of otherwise viable tissue. The results of the experiments described in the present report are in accord with that suggestion and emphasize the importance of the acute inflammatory response in the pathophysiology of myocardial infarction.

The administration of rabbit antiserum to dog neutrophils effectively depleted circulating neutrophils by an average of 77 ± 2% (fig. 1). The major advantages of inducing neutropenia by using neutrophil antiserum as opposed to chemical means such as mecloethamine hydrochloride or hydroxyurea include specificity of cell depletion and minimal side effects. The most noticeable side effect associated with the administration of neutrophil antiserum was a transient vasodepressor response, but mean arterial blood pressure returned to baseline values within 30–45 minutes.

In contrast to the marked reduction in circulating neutrophils in dogs that received neutrophil antiserum, dogs given nonimmune serum or saline had substantially higher neutrophil levels (fig. 1). The elevation in circulating neutrophil counts was not attributable to nonimmune serum, since similar increases in neutrophil counts were observed in the saline-treated group. Furthermore, the extent of irreversible myocardial injury averaged 47.1 ± 7.5% of the area at risk in the group treated with nonimmune rabbit serum, compared with 48.0 ± 4.7% in the saline group. The increase in circulating neutrophil counts was probably related to surgical trauma.

Reduction of circulating neutrophil counts was associated with a reduction in the extent of irreversible myocardial injury when expressed as a percentage of either the area at risk or the left ventricle (table 2). The hemodynamic data (table 1) indicate that no substantial differences were seen between dogs treated with saline, nonimmune serum or neutrophil antiserum. The only noticeable difference was a reduction in the average left ventricular end-diastolic pressure after 6 hours of reperfusion, probably a result of the smaller amount of infarcted myocardium observed in the neutrophil antiserum dogs. Thus, the significant reduction in the extent of irreversible myocardial injury in the dogs treated with neutrophil antiserum cannot be attributed to an alteration in hemodynamics that reduced myocardial oxygen consumption during regional ischemia.

Any explanation of the mechanism by which an intervention could reduce infarct size must consider possible alterations in the distribution of myocardial blood flow and myocardial contractility. Whereas we did not study regional myocardial blood flow or assess regional myocardial contractility, such changes, if they did occur, would have to be attributed to the presence of the antibody, because the nonimmune serum dogs and the saline-treated dogs had similar degrees of irreversible myocardial injury. In future studies of antineutrophil serum, possible alterations in regional coronary blood flow and myocardial contractility should be considered a possible mechanism by which the depletion of circulating neutrophils reduces the extent of ischemic myocardial cell injury. It has been reported that leukocytes, because of their ability to adhere to impaired vascular endothelium, lead to capillary obstruction, which may further impair reperfusion to the jeopardized myocardial region. Plasma proteins, erythrocytes, and neutrophil granulocytes have been observed in capillaries and venules as well as in the extracellular space, as well as adhering to damaged myocardial cells. Neutrophil depletion
might prevent the influence of such a mechanism upon reperfusion, resulting in the salvage of jeopardized myocardial tissue.

Histopathologic examination of myocardial tissue revealed a striking difference in the extent of leukocytic infiltrate in infarcted myocardium from dogs treated with neutrophil antisemur compared with tissue from dogs receiving nonimmune serum (fig. 2). Although changes in myocardial cells characteristic of irreversible injury were present in all groups of dogs, neutrophilic infiltrate was virtually absent in infarcted myocardium from dogs receiving neutrophil antisemur. The summary of the semiquantitative assessment of leukocyte infiltrate in figure 3 clearly demonstrates the pronounced differences between the two groups of dogs receiving rabbit serum. The dogs receiving neutrophil antisemur were consistently found to have virtually no neutrophilic infiltrate, whereas dogs treated with nonimmune serum had a significant degree of neutrophil accumulation in infarcted tissue. In short, the two treatment groups did not overlap with respect to the semiquantitative assessment of leukocyte infiltrate. Although these data do not prove that neutrophil depletion spares ischemic myocardium, there is certainly a strong association between the lack of neutrophilic infiltrate in infarcted myocardium and the reduction in the extent of ischemia-induced myocardial injury.

Recent efforts to protect ischemic myocardium pharmacologically have resulted in reports of the beneficial actions of certain nonsteroidal antiinflammatory agents in experimental models of myocardial ischemia. Although the mechanism by which these agents exert their cardioprotective effects is not known, the antiinflammatory effects of these agents may be of central importance in the mechanism of salvaging ischemic myocardium. In particular, a study in our laboratory has recently shown a substantial reduction in the accumulation of In-labeled leukocytes in infarcted myocardium in dogs treated with ibuprofen (12.5 mg/kg, i.v., every 4 hours for 24 hours). A reduction in the extent of irreversible myocardial injury by 40% of the area at risk was associated with the suppression of leukocyte infiltration into infarcted myocardium. Thus, further evaluation of the impact of the nonsteroidal antiinflammatory agents and other compounds capable of altering the inflammatory process on leukocyte function and migration in infarcted tissue is necessary to understand the cardioprotective actions of these drugs.

Polymorphonuclear neutrophils play an important role in the demolition and repair of infarcted myocardium. Clearly, the inflammatory response is necessary to the ultimate replacement of nonfunctional necrotic myocardium with fibrous scar tissue of considerable tensile strength. These considerations reveal a drawback of attempts to protect ischemic myocardium pharmacologically by altering the inflammatory response. Although the use of glucocorticoids to salvage ischemic myocardium is controversial, Klener et al. demonstrated that high doses of methylprednisolone inhibit the inflammatory response to myocardial injury and slow the removal of necrotic myocytes, resulting in impaired healing in an experimental model of myocardial infarction. However, the potential deleterious effects of methylprednisolone might be more directly related to the inhibitory effects of glucocorticoids on protein synthesis and tissue healing, events that are independent of neutrophil function.

The findings from the present study are supported by reported findings implicating the participation in the complement system of inflammatory proteins in the pathogenesis of myocardial tissue injury in response to regional myocardial ischemia. Hill and Ward conducted experimental studies in the rat and noted that ablation of the third component of complement (C3) in the serum by a C3 inactivator prevented the accumulation of neutrophils in the infarcted tissue. Maroko et al. demonstrated that cobra venom factor, known to deplete serum C3, reduced the ultimate extent of myocardial ischemic injury in response to coronary artery ligation. Somewhat related studies in the baboon confirmed the efficacy of cobra venom factor in protecting the ischemic myocardium and demonstrated that there is a significant accumulation of C3 in ischemic myocardium, which was decreased in the complement-depleted animal.

One can only speculate on the mechanism by which the complement-mediated injury is brought about in the ischemic myocardium. The subject has been reviewed recently by Fantone and Ward. Thus, injured myocardial tissue is believed to release a protease capable of cleaving C3 into leukotactic fragments resulting in the accumulation of neutrophils in the injured myocardial tissue and the regions bordering the ischemic and jeopardized heart muscle. It is possible that the local production of oxygen metabolites (O₂, OH, and H₂O₂) by the sequestered leukocytes leads to vascular endothelial and myocardial cell injury. Furthermore, the leukocyte-generated free radicals may augment the inflammatory responses by the enhanced production of a chemotactic lipid resulting from the metabolism of arachidonic acid by the lipoygenase pathway. Therefore, the leukocyte-derived oxygen metabolites may serve as a positive feedback mechanism that potentiates the local inflammatory response through the generation of chemotactic factors. Our recent demonstration that continuous infusion of superoxide dismutase and catalase to the anesthetized dog could reduce the extent of regional myocardial ischemic injury in response to occlusion/reperfusion of the LCx supports this concept.

Based on the present observations, we suggest that neutrophil accumulation in ischemic myocardial tissue is an important determinant of the ultimate extent of tissue necrosis due to myocardial ischemic injury. The length of the present studies were intentionally limited and do not provide any information about the potential long-term benefits of reducing the circulating neutrophil count. It would be valuable to know whether a return of the neutrophil count, after its initial reduction, could result in a resumption of the cellular de-
structive processes and the ultimate extension of the infarct or whether neutrophil depletion only delays the development of cellular changes leading to necrosis. These questions are extremely important and should be of interest for those who seek to limit infarct size experimentally or clinically. The present observations are even more significant in view of two recent reports describing the infarct size–sparring effects of BW755C,24,25 a dual inhibitor of lipoxygenase and cyclooxygenase. The beneficial effects of the drug may be related to an effect on leukocytes, either to prevent their migration or to inhibit their function by inhibiting the lipoxygenase enzymes. The production by leukocytes of products of arachidonic acid metabolism, which are known chemotactic agents,26 and the leukotrienes, which can cause coronary artery vasoconstriction,27 along with free radical formation,22 can enhance the degree of myocardial cell injury. Thus, myocardial cell damage in response to ischemia may, in part, be mediated by the cells that migrate into the ischemic region and the immediate surrounding regions (area of extension). Therefore, the proposed role of the leukocyte as a mediator of myocardial cell injury offers an opportunity to examine pharmacologic interventions that act to prevent or modify the actions of the leukocytes in response to tissue injury. Additional studies are needed to define more completely the role of the leukocyte and modification of its function in attempts to limit ischemic myocardial injury.

The results described in the present report provide insight into the importance of neutrophilic infiltration in determining the extent of ischemia-induced myocardial injury. While efforts to salvage ischemic myocardium pharmacologically should be directed toward the prevention of inappropriate destruction of viable myocardium by neutrophils, the potential benefits of reducing myocardial injury must be balanced against the possible untoward effects of impaired healing.

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