Tumor Necrosis Factor and Interleukin-6 Are Not Elevated in Venous Blood from Ischemic Canine Myocardium (43775)

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Abstract. **To test the hypothesis that cytokines play a role in ischemic or reperfusion injury, we measured tumor necrosis factor (TNF) and interleukin-6 (IL-6) in pentobarbital-anesthetized dogs before, during, and after coronary occlusion lasting 60 min. Epicardial venous samples from the ischemic (IS) area were compared with nonischemic (NIS) and systemic (SYS) samples. Baseline IS TNF levels were low (2.2 ± 1.2 U/ml) and not significantly different from NIS and SYS levels. After 50 min of coronary occlusion and at 40 min postreperfusion, IS, NIS, and SYS TNF levels were unchanged. At baseline, IS IL-6 levels were also relatively low (806 ± 255 U/ml) and not significantly different from NIS and SYS IL-6. Although IS IL-6 increased significantly during coronary occlusion (5682 ± 1495 U/ml) and reperfusion (10309 ± 3708 U/ml), NIS and SYS levels were also elevated and did not differ significantly from IS values. The data indicate that TNF and IL-6 are not uniquely elevated in blood from ischemic or reperfused myocardium.** [P.S.E.B.M. 1994, Vol 206]

The goal of this study was to evaluate the potential role of the cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in acute myocardial ischemia and reperfusion injury. TNF-α or cachectin, while historically described as a mediator of tumor necrosis and cachexia, has since been implicated as a pivotal mediator of the immune response and inflammation including the acute phase response (1–4). Interleukin-6 has functions which widely overlap those of TNF and has been shown to be a primary regulator of the acute phase response (1, 3, 5–9). Based on the rationale that myocardial ischemia, like other types of tissue injury, leads to the development of an inflammatory response (10), we hypothesized that ischemia could stimulate release of TNF and IL-6. If these cytokines are released from ischemic/reperfused tissue, they may contribute to development of additional injury by their effects on cardiac myocytes and endothelial cells, by the recruitment and activation of inflammatory cells, by activation of complement pathway, or by the induction of other cytokines.

Accordingly, our specific objective was to determine if acutely ischemic and/or reperfused myocardium represented a significant source of TNF and IL-6. Initial support for this possibility was provided by Maury and Teppo (4) who reported data indicating that TNF levels were elevated in patients with myocardial infarction. Other researchers obtained similar findings in experimental models in which myocardial damage was produced (11, 12), but in these studies, the sources of TNF and other cytokines documented in systemic plasma samples during ischemic periods were not precisely identified. Recent work by Youker *et al.* (13) suggests that the myocardium itself is a source of IL-6 during ischemia/reperfusion injury. In the present experiments, serial blood samples were

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Received October 12, 1993. [P.S.E.B.M. 1994, Vol 206]
Accepted March 10, 1994.

0037-9727/94/2064-0384$10.50/0

obtained from epicardial veins draining ischemic/reperfused or nonischemic myocardium in dogs to determine if ischemic/reperfused myocardium represented a unique source of TNF and IL-6.

Materials and Methods

Experimental Preparation. The experiments were conducted in adult mongrel dogs of either sex weighing 15–25 kg obtained from a licensed commercial vendor. Animal husbandry and all experimental procedures conformed to the guidelines established by the "Guide for the Care and Use of Laboratory Animals" (DHHS Publication [NIH] No. 86–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205) and were approved by the University of Michigan Unit For Laboratory Animal Medicine's Vertebrate Animal Care and Use of Committee.

The dogs were fasted overnight prior to surgery. Sodium pentobarbital (30–40 mg/kg) was administered intravenously to induce and to maintain anesthesia. The dogs were intubated, placed on a surgical table equipped with a circulating hot water heating pad, and mechanically ventilated with a Harvard respirator. Arterial blood gases and pH were measured periodically to verify that they were within normal limits. Both femoral arteries were cannulated with Tygon catheters to enable continuous recording of arterial blood pressure and to enable withdrawal of reference blood samples for calculation of myocardial blood flow using tracer-labeled microspheres. Blood samples used to measure systemic (SYS) TNF and IL-6 were obtained from one of the arterial catheters. A high-fidelity micromanometer (Millar Instruments Model SPC-350, Houston, TX) was advanced through the left carotid artery into the left ventricle for continuous measurement of left ventricular pressure.

A left thoracotomy was performed through the fifth intercostal space. After exposing the heart, catheters were placed in the left atrium for continuous measurement of left atrial pressure and injection of microspheres. The left anterior descending (LAD) coronary artery was exposed below the first diagonal branch and a pulsed Doppler flowprobe was placed around it to monitor coronary blood flow velocity. A plastic screw occluder was placed distal to the flowprobe to produce partial or complete occlusion of the artery.

To monitor regional myocardial function, sonomicrometers were arrayed to measure wall thickness (14–16) in the area of the left ventricle that would be rendered ischemic by occlusion of the LAD artery. The inner crystal of each pair was inserted tangentially through the myocardium to the endocardium. The outer crystal, attached to a Dacron patch, was secured on the epicardium with sutures while monitoring the signals with an oscilloscope. The transducers were coupled to a Triton Technology sonomicrometer unit (Model 120, Triton Technology, San Diego, CA).

To obtain regional venous blood samples, epicardial venous catheters were placed into veins draining the ischemic (IS) and non ischemic (NIS) myocardium for collection of blood samples used to measure TNF and IL-6. Catheters were oriented upstream in the great coronary vein below the level of the mechanical occluder on the LAD (IS site) and in the middle cardiac vein (NIS site).

Experimental Protocol. Two dogs were used as positive control sources of blood to verify that we could detect TNF and IL-6 with the experimental preparation and assays utilized in the study. In these two dogs, a well-established stimulant of TNF and IL-6 release, lipopolysaccharide (LPS, 10 μg/kg) (17), was infused and blood samples were obtained over several hours for measurement of cytokine levels. Instrumentation in these dogs was restricted to arterial and atrial catheters, coronary occlusion was not produced, and only systemic blood samples were obtained.

Coronary occlusion was produced in eight fully instrumented dogs. In four of the dogs, the occlusion was preceded by 10 min of coronary inflow restricted to approximately 50% of baseline levels as part of the preliminary evaluation of another protocol. Since there were no differences during baseline conditions, coronary occlusion, or after reperfusion in terms of hemodynamics, wall thickness, myocardial blood flow, or cytokine levels between the dogs with and without restricted inflow episodes, the data from all of the dogs were pooled.

While obtaining baseline hemodynamic and wall thickness data, an injection of microspheres was performed. Thereafter, the LAD artery was occluded for 60 min. Microspheres were injected at 10 min and 50 min of occlusion to determine the extent of residual or collateral perfusion during coronary occlusion. Three minutes before release of the occlusion, 50 mg of lidocaine were administered. When the hour-long occlusion period was completed, the occlusion was released gradually over 5 min to further minimize the likelihood of reperfusion induced ventricular fibrillation. A fourth injection of microspheres was made 60 min after initial release of the occluder to verify that reperfusion had been achieved in the ischemic area. Thirty minutes later (90 min postreperfusion), KCl was infused intravenously to sacrifice the animals. The hearts were removed, rinsed, and prepared for injection of dyes to delineate the size of risk areas and development of infarction. Hemodynamic and wall thickness recordings, and blood samples for TNF and

IL-6 levels were obtained during baseline conditions, at 10 and 50 min of coronary occlusion, and at 10, 40, and 80 min of reperfusion.

Area at Risk and Infarct Size Assessment. As in previous studies, area at risk and infarct size were determined with a gross technique involving dual perfusion of the heart with Evans blue and triphenyl tetrazolium chloride (TTC) and computer analysis using the Image 1.22m program (18–21).

Myocardial Blood Flow Measurements. Regional myocardial blood flow was measured and calculated using the reference withdrawal technique employing radiolabeled microspheres (15, 18, 22).

TNF and IL-6 Bioassays. As in previous studies, the WEHI 164 subclone 13 bioassay (Dr. A. Waage, University of Trondheim, Norway) was utilized to detect TNF activity in plasma samples (17, 23). This bioassay is extremely sensitive, being capable of detecting TNF in the picogram range (23). TNF standards were prepared with each assay using purified recombinant human TNF (rhTNF, Cetus, Emeryville, California). To detect plasma IL-6 activity, the IL-6 dependent B-9 hybridoma cell line was utilized using human recombinant IL-6 (rhIL-6, 8 U/ml, provided by Dr. Gordon Wong, Genetics Institute) as standards (17, 24). All assay samples were run in duplicate (IL-6) or triplicate (TNF). Both TNF and IL-6 bioassays have been shown to provide an effective measure of cytokine activity in plasma from dogs (17).

Data Analysis. Hemodynamic and wall thickness data were continuously recorded during each experiment (Gould Model 2800S, Cleveland, Ohio). The data were derived manually from the analog recordings and the average of 10 cardiac cycles were used for analysis of each time point in the protocol. Hemodynamic variables analyzed were left ventricular peak systolic pressure (LV PSP), mean arterial blood pressure (MABP, measured at the level of the midabdominal aorta), mean left atrial pressure (MLAP), and heart rate (HR). Wall thickness variables analyzed were end-diastolic wall thickness (EDWT, defined as the point before the systolic upstroke of left ventricular pressure), end-systolic wall thickness (ESWT, defined at 20 msec before peak negative dP/dt), systolic wall thickening (dWT, the difference between end systolic and end diastolic wall thickening), and percentage wall thickening (% dWT, dWT/EDWT × 100).

Hemodynamic, wall thickness, and myocardial blood flow data were evaluated with repeated measures ANOVA. When significant F values were detected, comparisons between time periods were made with Scheffe tests. The TNF and IL-6 data in each category (SYS, IS, NIS) were analyzed across time periods in the same manner. At each time period, the TNF or IL-6 data were compared among SYS, IS, and NIS categories with single factor ANOVA and Scheffe tests when appropriate.

Results

Positive Control Group. In two dogs, serial blood samples were obtained to serve as "positive controls" so that we could detect TNF and IL-6 with the assays and experimental preparation at our disposal if a standard stimulus was used. Accordingly, LPS was administered to these animals and systemic samples were obtained at intervals corresponding approximately to the times when samples were obtained in the occlusion protocol. As summarized in Figure 1, TNF and IL-6 levels increased dramatically after LPS administration in both animals. TNF increased from baseline levels of <7 U/ml to peak levels of 400 and 800 U/ml respectively, while IL-6 increased from baseline levels of <4000 U/ml to peak levels of 1.17×10^6 and 6.48×10^6 U/ml, respectively. These results demonstrate that the experimental preparation and assays were appropriate to detect changes in TNF and IL-6 levels.

Effects of Coronary Occlusion. Hemodynamic and wall thickness data from the eight dogs with coronary occlusion are summarized in Table I. Consistent with other studies involving coronary occlusion in open-chest, anesthetized dogs, heart rate and left atrial pressure did not change significantly over the course of the experiment but left ventricular and mean arterial

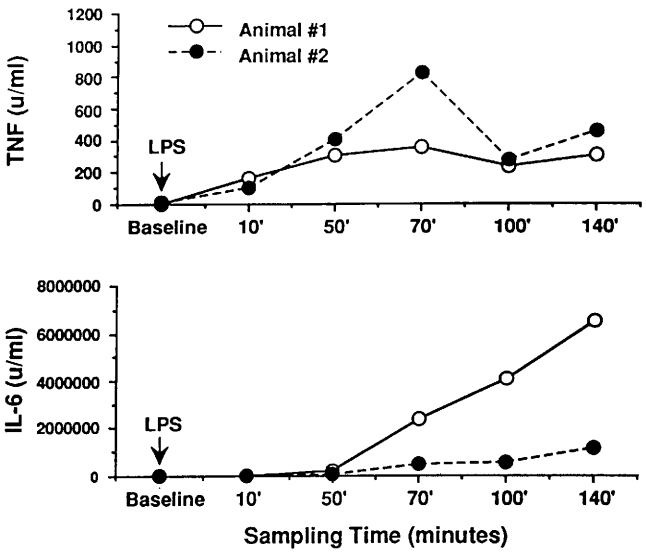

Figure 1. Tumor necrosis factor (TNF) and interleukin-6 (IL-6) plasma levels in dogs injected with lipopolysaccharide (LPS). TNF (upper graph) and IL-6 (lower graph) levels increased progressively and rapidly, by orders of magnitude, following intravenous injection with 10 μg/kg LPS. TNF levels rose by 10 min postinjection, while IL-6 levels increased between 10 and 50 min postinjection. Levels of both cytokines remained elevated above baseline values for 140 min post-LPS injection, at which time the animals were euthanized.

Table I. Hemodynamic and Wall Thickness Data

	BASE	10′ CO	50′ CO	10′ REP	40′ REP	80′ REP
LV PSP	123 ± 7	111 ± 10	103 ± 10[a]	92 ± 8[a]	96 ± 8[a]	85 ± 12[a]
MABP	108 ± 6	95 ± 11	87 ± 12	76 ± 10[a]	79 ± 11[a]	72 ± 14[a]
MLAP	6.9 ± 0.5	8.1 ± 0.7	7.4 ± 1.1	7.7 ± 1.5	6.7 ± 1.2	6.6 ± 1.4
HR	124 ± 10	127 ± 10	122 ± 9	129 ± 13	117 ± 8	116 ± 10
EDWT	10.3 ± 0.6	9.6 ± 0.6	9.9 ± 0.6	12.4 ± 0.9	12.8 ± 1.2	13.4 ± 1.3[a]
ESWT	12.1 ± 0.51	9.1 ± 0.7[a]	19.2 ± 0.8[a]	111.4 ± 1.01	11.9 ± 1.21	12.6 ± 1.1
dWT	1.8 ± 0.3	−0.5 ± 0.2[a]	−0.6 ± 0.2[a]	−0.9 ± 0.2[a]	−1.0 ± 0.2[a]	−0.9 ± 0.2[a]
%dWT	17.7 ± 2.9	−5.7 ± 2.1[a]	−7.1 ± 2.4[a]	−7.9 ± 2.0[a]	−7.6 ± 1.1[a]	−6.5 ± 1.0[a]

Note. BASE = baseline conditions before ischemia; 10′ CO = 10 min of coronary (left anterior descending artery) occlusion; 50′ CO = 50 min of coronary occlusion; 10′ REP = 10 min of reperfusion; 40′ REP = 40 min of reperfusion; 80′ REP = 80 min of reperfusion; LV PSP = left ventricular peak systolic pressure (mm Hg); MABP = mean arterial blood pressure (mm Hg); MLAP = mean left atrial blood pressure (mm Hg); HR = heart rate (beats/min); EDWT = end-diastolic wall thickness (mm); ESWT = end-systolic wall thicknes (mm); dWT = ESWT-EDWT (mm); %dWT = (dWT/EDWT) 100. [a]$P < 0.05$ compared with BASE (Scheffe test after repeated measures ANOVA); data presented as mean ± SEM.

blood pressure were reduced significantly from baseline values after occlusion (1, 19, 25). Systolic wall thickening was replaced by wall thinning during occlusion and there was no recovery in regional function after reperfusion, indicating that coronary occlusion had produced severe ischemia.

Myocardial blood flow data are summarized in Table II. The level of collateral perfusion during LAD occlusion was relatively high, representing approximately 17%, 23%, and 38% of baseline perfusion levels at 50 min of occlusion in the subendocardial, midmyocardial, and subepicardial layers, respectively. Mean transmural blood flow was reduced to approximately 24% of baseline late in the occlusion period (Table II). While the regional wall thickening data indicate that the ischemia was severe enough to produce consistently marked regional dysfunction, collateral perfusion in this group of dogs was evidently high enough to limit development of irreversible injury. The region at risk averaged 19.7 ± 1.9% of the area supplied by the LAD. Expressed as a percentage of the region at risk, infarct size was small (7%). While there was variation in infarct size (range of 0%–21%), there was no correlation between infarct size or region at risk and cytokine levels from any site or at any time point. At one hour after reperfusion, elevated blood flow was observed, demonstrating that reflow had been achieved. Nearly all of the values, including those from nonischemic tissue, were significantly below baseline values because of depressed cardiac function late in the experimental period (Table I) and the frequent occurrence of arrhythmic episodes.

TNF Release During and After Coronary Occlusion. TNF data are summarized in Figure 2. In contrast to our expectations and the effects of LPS administration (Fig. 1), there were no significant differences in TNF levels among the three categories of blood samples (IS, NIS, SYS) at any time point or across all time periods. At baseline, TNF levels averaged 2.2 ± 1.2, 0.3 ± 0.1, and 1.5 ± 1.4 U/ml in IS, NIS, and SYS

Table II. Myocardial Blood Flow Data

	BASELINE	10 min CO	50 min CO	REPERFUSION
Subendocardial MBF				
Nonisch	0.97 ± 0.07	1.00 ± 0.10[b]	0.87 ± 0.11[b]	0.70 ± 0.12[a]
Ischemic	0.86 ± 0.07	0.09 ± 0.05[a]	0.15 ± 0.07[a]	0.86 ± 0.20
Midmyocardial MBF				
Nonisch	0.79 ± 0.06	0.85 ± 0.10[b]	0.74 ± 0.09[b]	0.58 ± 0.10[a]
Ischemic	0.75 ± 0.04	0.12 ± 0.06[a]	0.17 ± 0.07[a]	0.41 ± 0.07[a]
Subepicardial MBF				
Nonisch	0.77 ± 0.05	0.82 ± 0.07[b]	0.73 ± 0.06[b]	0.56 ± 0.06[a]
Ischemic	0.72 ± 0.04	0.25 ± 0.08[a]	0.27 ± 0.07[a]	0.47 ± 0.05[a]
Mean transmural MBF				
Nonisch	0.84 ± 0.06	0.89 ± 0.09[b]	0.78 ± 0.08[b]	0.61 ± 0.09[a]
Ischemic	0.78 ± 0.04	0.16 ± 0.06[a]	0.19 ± 0.07[a]	0.58 ± 0.10

Note. BASELINE = preischemic conditions; 10 min CO = 10 min of coronary occlusion; 50 min CO = 50 min of coronary occlusion; REPERFUSION = 60 min after releasing the coronary occlusion; MBF = myocardial blood flow (in units of ml/min/g); Nonisch = nonischemic myocardium supplied by the left circumflex artery; Ischemic = myocardium supplied by left anterior descending artery, which underwent coronary occlusion. [a]$P < 0.05$ compared with BASELINE; [b]$P < 0.05$, Nonisch versus Ischemic values; data reported as mean ± SEM.

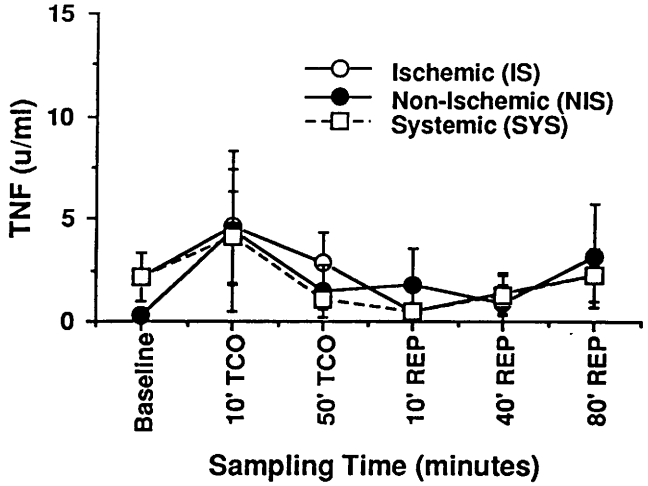

Figure 2. Tumor necrosis factor (TNF) plasma levels from dogs (n = 8) before, during, and after 60 min of coronary occlusion. TNF levels from the ischemic (IS), nonischemic (NIS), and systemic (SYS) sample sites demonstrated no significant differences at any time point or across all time periods. Baseline TNF levels from all sample sites were low and in the same range as baseline levels in the positive control dogs. During and after coronary occlusion, TNF levels, from all sample sites, did not deviate significantly from baseline values and remained orders of magnitude below those observed after LPS administration.

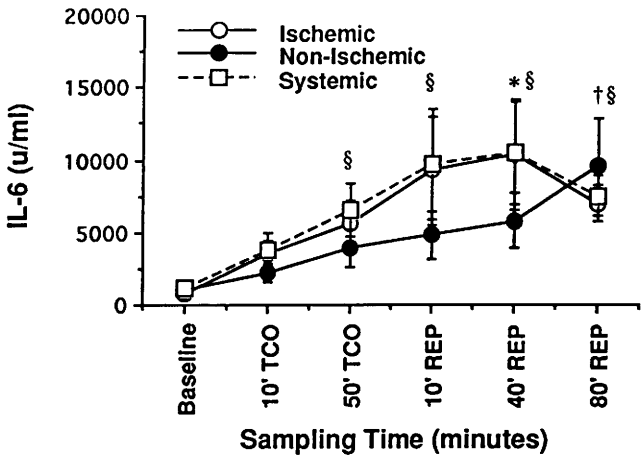

Figure 3. Interleukin-6 (IL-6) plasma levels from dogs (n = 8) before, during, and after 60 min of coronary occlusion. IL-6 levels from the ischemic (IS), nonischemic (NIS), and systemic (SYS) sites increased progressively over time, but demonstrated relatively few statistically significant changes from baseline values. Notably, there were no significant differences among the three types of samples at any time point. Peak IL-6 levels, from all three sample sites, were similar to baseline values observed in animals administered LPS. *$P < 0.05$ compared with Baseline ischemic samples. †$P < 0.05$ compared with Baseline nonischemic samples. §$P < 0.05$ compared with Baseline systemic samples.

samples, respectively, in the same range as baseline levels in the positive control dogs (Fig. 1). During and after coronary occlusion, TNF levels, including those from the ischemic area, did not deviate significantly from baseline values and remained orders of magnitude below those observed after LPS administration.

IL-6 Release During and After Coronary Occlusion. IL-6 data are summarized in Figure 3. Similar to the TNF data, baseline IL-6 levels were not significantly different among IS, NIS, and SYS samples, averaging approximately 800–1100 U/ml. After coronary occlusion, IL-6 levels tended to increase but displayed a great deal of variability from animal to animal. Consequently, relatively few statistically significant changes were detected (Fig. 3). Most notable, however, is the observation that there were no significant differences among the three types of samples, suggesting that elevated IL-6 levels were not unique to venous blood from ischemic or reperfused myocardium.

Discussion

The overall goal of this study was to assess the potential role of the cytokines TNF-α and IL-6 during the early phase of ischemia/reperfusion injury to the myocardium. Our specific objective was to test the hypothesis that these two cytokines are released from acutely injured myocardium as a prelude to investigations focused on modifying their release or effects of their release. Since TNF and IL-6 are important inflammatory mediators, we had postulated that they may contribute significantly to development of reperfusion injury. TNF has been demonstrated, for example, to activate endothelial cells and cardiac myocytes (2, 4, 13) and to recruit, activate, and enhance neutrophil function (2, 26). It also stimulates IL-6 production in vascular smooth muscle cells and fibroblasts (5, 6), and induces production of IL-1α, eicosanoids, platelet activating factor, interleukin-8, and other cytokines (1, 2, 26–28). IL-6, formerly known as B cell-stimulating factor (BSF-2), β-interferon-2, or hepatocyte stimulating factor (HSF), is another pleiotropic cytokine with diverse regulatory functions involving the hematopoietic and immune systems including regulation of TNF expression (1, 5, 7, 28).

The rationale for the study was based on Maury and Teppo's (4) clinical results demonstrating that elevated systemic levels of TNF-α were present in patients with myocardial infarction. Experimental results by Lefer *et al.* (12) also suggested that cytokines were elevated after myocardial infarction. They subjected rats to 10 min of coronary occlusion followed by 24 h of reperfusion and observed elevated systemic levels of TNF. Hegewisch *et al.* (11) observed a TNF-induced cardiomyopathy as a sequelae to chemotherapy for metastatic renal cell carcinoma of the lungs, providing support for the view that significant myocardial damage could result from elevated TNF levels. Although these reports documented systemic elevations of TNF and associated myocardial damage, they did not specifically identify the heart as the source of cytokines nor did they address the potential role of cytokines such as TNF or IL-6 during the ischemic period and the period immediately after restoration of blood flow when reperfusion injury may be occurring.

Therefore, in the present study, we measured plasma levels of TNF and IL-6 collected before, during, and after 60 min of LAD coronary artery occlusion followed immediately by 90 min of reperfusion in anesthetized, acutely instrumented animals. Samples were collected from an epicardial vein draining the region of the heart rendered ischemic, an epicardial vein draining the region of the heart that was nonischemic and from a systemic site, the midabdominal aorta. We hypothesized that if the acutely ischemic or acutely reperfused myocardium represents a significant source of TNF and/or IL-6, the ischemic samples would have higher levels of these cytokines than the nonischemic or systemic samples. In addition, if these cytokines were important in the acute phase of reperfusion injury, we postulated that their levels would increase dramatically after reperfusion in the samples obtained from the vein draining the previously ischemic area.

In contrast to our expectations, TNF and IL-6 levels were not uniquely elevated in venous blood from the ischemic/reperfused area. TNF levels obtained from coronary ischemic, coronary nonischemic and systemic sample sites did not differ significantly from baseline values or from each other over the course of the experiment. The IL-6 levels were augmented in all three types of samples, increasing progressively over the course of the experiment, but at no time point was there a significant difference among ischemic, nonischemic, and systemic values.

The present data appear to contrast with those recently reported by Youker *et al.* (13). They sampled cardiac lymph from conscious, chronically instrumented dogs subjected to 60 min of occlusion of the circumflex coronary artery followed by 72 hr of reperfusion. Cardiac lymph samples, from the ischemic period onwards, were found to stimulate *in vitro* adhesiveness of isolated canine cardiac myocytes to ZAS activated canine neutrophils (peak adhesiveness occurred with samples taken at 90 min of reperfusion). These effects, known to be induced by TNF-α and interleukin-1 (IL-1), were also induced in this study by human recombinant IL-6 (rIL-6). The ability of the postischemic lymph and the rIL-6 to stimulate myocyte adhesiveness was inhibited by polyclonal neutralizing antibodies to rIL-6. Based upon the assumption of equipotency of the anti-rIL-6 to block rIL-6 and canine IL-6 from lymph, Youker *et al.* (13) estimated the IL-6 concentration in the cardiac lymph to range from 0.035 to 0.14 U/ml. These findings suggest that ischemic/reperfused myocardium is a significant source of IL-6; however, the levels appear to be quite low compared with those obtained in both the present study and in other work (6, 17, 30–32).

There are a several possible explanations for the differences between our results and the findings of Youker *et al.* (13). Our experiments were conducted in anesthetized, acutely instrumented, open-chest dogs and cytokine levels were measured in blood samples. Youker *et al.* (13) conducted studies in conscious, previously instrumented dogs and measured proinflammatory activity in lymph samples. If it is true that the activity they observed was largely due to IL-6, their observations could be explained by the generalized increase in IL-6 we observed in ischemic, nonischemic, and systemic samples. Indeed, their work does not evaluate, and therefore can not rule out, a systemic source for increased IL-6 activity.

Another possibility relates to the overall intensity and duration of the ischemic insult achieved in each study. Mean transmural blood flow in our study was reduced by 75% or more from baseline during occlusion. Regional systolic wall thickening was replaced by systolic thinning in the occluded area, documenting that the ischemia was severe enough to produce marked regional wall dysfunction. Infarct sizes, however, were highly variable suggesting that the intensity of ischemia, on the average, was not severe enough to produce extensive, irreversible damage. Consequently, the intensity of the ischemic insult may have been of insufficient magnitude to elicit acute release of cytokines. Additional investigation will be required to determine if different patterns of cytokine release would be evident given longer, more severe ischemic episodes. In this regard, it is notable that Maury and Teppo (4) did not find elevated serum concentrations of TNF in patients unless they had relatively large myocardial infarctions. Similarly, the report by Hegewisch (11) demonstrating production of a large myocardial infarction was associated with high doses of exogenous TNF.

In the present study, we focused on the ischemic and immediate postischemic time periods in acutely instrumented animals. Depending on the severity of ischemia and myocardial damage, release of significant quantities of cytokines may require several hours or days to develop. In earlier clinical and experimental work demonstrating elevated cytokine levels, measurements were made 6–24 hr after ischemic episodes (4, 12). Since the cell types primarily responsible for TNF-α production are monocyte-macrophages (1, 2), differences in TNF release from ischemic and nonischemic areas might not have been detectable unless the resident macrophage population of the heart was activated to produce TNF within the time frame of our experiments. Likewise, recruitment to the myocardium and subsequent activation of circulating monocytes may have required more time than we allowed. The relative insensitivity of less differentiated peripheral blood monocytes versus more differentiated mononuclear phagocytes, such as alveolar macrophages, to express and release TNF, has been de-

scribed previously (29). Interleukin-6 production occurs in a more diverse cell population, including monocyte-macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts (1, 2, 6), but similar time requirements may also exist.

On the other hand, pharmacokinetic data suggest that in the presence of an adequate stimulus the induction of TNF and IL-6 occurs within 30 to 60 min (2, 17, 30, 31). This rapid induction is clearly reflected in our positive control data (Fig. 1) and the report by Youker *et al.* (13). Additionally, it is possible that in the present study, high baseline levels of IL-6 may have obscured changes caused by ischemia/reperfusion. The acute effects of surgery may have induced an increase in IL-6 (30–32), making it difficult to distinguish the ischemic myocardium as a more prominent source.

A potential limitation of our study relates to the assumption that cytokine release is a true measure of increased production. Kriegler *et al.* (33) described the *in vitro* induction from activated monocytes of a novel, cell-associated, cytotoxic, transmembrane form of TNF which, upon cleavage from the cell, serves as the locally released, serum form of the cytokine normally detected by most of the currently utilized bioassays. Pulse chase experiments demonstrated a rapid, early induction in cell-associated TNF, with depletion of this form as serum TNF levels increased (33), essentially following typical TNF kinetics (17, 27, 34). The proposed model suggested that activated monocytes-macrophages would initially synthesize the cell-associated form of TNF which could be cytotoxic by direct cell to cell contact. Upon cleavage, the released form of TNF, which is also cytotoxic, could act locally or systemically if widespread monocyte activation occurs (33). Thus, either form of TNF could mediate local cytotoxicity and other local effects previously attributed to TNF, but would be essentially undetectable by the assay methods used in our study that required release into venous blood (27, 33). Whether or not poorly detectable, cell-associated TNF plays a role in myocardial ischemia/reperfusion injury will require additional investigation to clarify.

In summary, we evaluated the hypothesis that the acutely ischemic and reperfused myocardium could act as a significant source for the release of TNF and IL-6. We observed that 60 min of ischemia followed by 90 min of reperfusion of the canine myocardium was not associated with the unique release of either cytokine from the ischemic area. Tumor necrosis factor-α levels did not change from baseline in any samples, including those from the ischemic area. Interleukin-6 levels, however, increased progressively over the course of the experiments but did so in samples obtained from nonischemic and systemic, as well as ischemic sites. While we cannot discount the interpretation that IL-6 was released from the ischemic myocardial area, high systemic levels of IL-6 suggest that its release was a generalized phenomenon, perhaps related to the open-chest, anesthetized experimental preparation. Additional investigation will be required to determine if a more severe ischemic insult than that produced in the present study may be needed to induce significant release of TNF and IL-6 from acutely ischemic and reperfused myocardium.

This work was supported in part by National Institues of Health Grant HL32043. The authors express their gratitude to Dr. Deborah S. Storm for her support and helpful assistance.

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