Bidirectional Role of Tumor Necrosis Factor-α in Coronary Microembolization
Progressive Contractile Dysfunction Versus Delayed Protection Against Infarction
Andreas Skyschally, Petra Gres, Simone Hoffmann, Michael Haude, Raimund Erbel, Rainer Schulz, Gerd Heusch

Abstract—In patients with unstable angina, plaque rupture and coronary microembolization (ME) can precede complete coronary artery occlusion and impending infarction. ME-induced microinfarcts initiate an inflammatory reaction with increased tumor necrosis factor-α (TNF-α) expression, resulting in progressive contractile dysfunction. However, TNF-α is not only a negative inotrope but can also protect the myocardium against infarction. In anesthetized pigs, we studied whether ME protects against infarction when TNF-α expression is increased. ME (group 1; n = 7) was induced by intracoronary infusion of microspheres (42 μm; 3000 per mL/min inflow). Controls (group 2; n = 8) received saline. Groups 3 and 4 (n = 4 each) were pretreated with ovine TNF-α antibodies (25 mg/kg body weight) 30 minutes before ME or placebo, respectively. Ischemia (90 minutes) was induced 6 hours after ME when TNF-α was increased (66 ± 21 pg/g wet weight; mean ± SEM) or after placebo (TNF-α, 21 ± 10 pg/g; P < 0.05). Infarct size (percentage area at risk) was determined after 2 hours of reperfusion (triphenyl tetrazolium chloride staining). ME decreased systolic wall thickening progressively over 6 hours (group 1 versus group 2, 65 ± 4% versus 90 ± 1%; percentage of baseline; P < 0.05). TNF-α antibodies attenuated the progressive decrease in systolic wall thickening following ME (group 3, 77 ± 5% of baseline; P < 0.05 versus group 1) with no effect in controls (group 4; 90 ± 8% of baseline). With ME, infarct size was decreased to 18 ± 4% versus 33 ± 4% in group 2 (P < 0.05). The infarct size reduction was abolished by TNF-α antibodies (group 3 versus group 4, 29 ± 3% versus 35 ± 5%). In ME, TNF-α is responsible for both progressive contractile dysfunction and delayed protection against infarction. (Circ Res. 2007;100:140-146.)

Key Words: microembolization ▪ inflammation ▪ myocardial infarction ▪ TNF-α

The rupture of an atherosclerotic plaque in an epicardial coronary artery with subsequent occlusive coronary thrombosis has been established as the decisive event in the pathogenesis of acute myocardial infarction. Milder forms of plaque rupture may result in subsequent embolization of atherosclerotic and thrombotic debris into the coronary microcirculation. The immediate consequences of coronary microembolization are a transient decrease in coronary blood flow with subsequent reactive hyperemia and a moderate reduction in regional myocardial function that recovers partially within minutes. Subsequently, progressive contractile dysfunction develops in the presence of normal or increased blood flow, i.e., there is a perfusion/contraction mismatch. Microinfarcts in the microembolized myocardium initiate an inflammatory response with an increased tissue expression of tumor necrosis factor-α (TNF-α). The major sources of TNF-α in microembolized myocardium are the viable cardiomyocytes surrounding the microinfarcts, and it is this increase in myocardial TNF-α that is causal for the progressive contractile dysfunction.

Although coronary microembolization causes the release of coronary venous adenosine, it neither induces acute preconditioning against acute infarction nor interferes with a classic ischemic preconditioning protocol. We now wondered whether the increased TNF-α expression may not only mediate progressive contractile dysfunction but also induce delayed protection against infarction. In fact, in isolated rat hearts, pretreatment with TNF-α reduces the infarct size after ischemia/reperfusion. This protective effect was confirmed in mouse and rabbit models. Moreover, TNF-α is also involved in the endogenous protection by ischemic preconditioning. In TNF-α knockout mice, acute and delayed ischemic preconditioning are abrogated and TNF-α antibodies inhibit delayed ischemic preconditioning in rats.

Therefore, for the present report, we used our established model of coronary microembolization in pigs to investi-
gate whether 6 hours after coronary microembolization—at a time when TNF-α tissue levels are increased and contractile dysfunction has developed—the myocardium is protected against infarction. This experimental protocol attempts to replicate the clinical scenario of a patient experiencing coronary microembolization caused by fissure of an unstable coronary plaque and subsequent massive plaque rupture and thrombotic occlusion of a large epicardial coronary artery with impending myocardial infarction some hours later.

### Materials and Methods

The experimental protocols were approved by the Bioethical Committee of the district of Düsseldorf, and the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by NIH Publication 85-23, revised 1996.

### Experimental Preparation

Twenty-three Göttinger minipigs (20 to 40 kg) of either sex were initially sedated using ketamine hydrochloride (1 g IM) and then anesthetized with thiopental (Trapanal, 500 mg IV). Through a midline cervical incision the trachea was intubated for connection to a respirator (Dräger, Lübeck, Germany). Anesthesia was then maintained using enflurane (1% to 1.5%) with an oxygen/nitrous oxide mixture (40%-60%). Arterial blood gases were monitored frequently in the initial stages of the preparation until stable and then periodically throughout the study (Radiometer, Copenhagen, Denmark). Rectal temperature was monitored and maintained between 37°C and 38°C by use of a heated surgical table and drapes. The common carotid arteries were cannulated with polyethylene catheters, the first to measure arterial pressure and the other to supply blood to the extracorporeal circuit. The jugular veins were cannulated for volume replacement using warmed 0.9% saline.

A left lateral thoracotomy was performed in the fourth intercostal space and the pericardium opened. A micromanometer (P7; Koningsberg Instruments Inc, Pasadena, Calif) was placed in the left ventricle through the apex, together with a saline-filled polyethylene catheter (used to calibrate the micromanometer in situ). Ultrasonic dimension gauges were implanted in the left ventricular myocardium to measure the thickness of the anterior and posterior (control) wall. The left anterior descending coronary artery was dissected over a distance of 1.5 cm, ligated, cannulated, and perfused from an extracorporeal circuit. Before coronary cannulation, the pigs were anticoagulated with 20 000 IU sodium heparin; additional doses of 10 000 IU were given at hourly intervals. The system included a roller pump, windkessel, and a side port for the injection of microspheres. Coronary arterial pressure was measured from the sidearm of a polyethylene T-connector (Cole-Parmer, Chicago, Ill) used as catheter tip with an external transducer (pvb Medizintechnik, Kirchseeon, Germany). Initially, minimal coronary arterial pressure was held at >70 mm Hg by adjusting the roller pump of the extracorporeal circuit to avoid hypoperfusion before ischemia. Heart rate was controlled throughout the study by left atrial pacing (Hugo Sachs Elektronik Type 215/T, Hugstetten, Germany) slightly above the spontaneous rate.

### Regional Myocardial Blood Flow

Approximately 270 000 radiolabeled microspheres (15 μm in diameter; 141Ce, 51Cr, 103Ru, 95Nb, or 46Sc; NEN-Perkin-Elmer, Boston, Mass) were injected into the coronary perfusion circuit to determine the regional myocardial blood flow and its distribution throughout the left anterior descending coronary artery perfusion bed (model 5912, Gammaszint BF 5300 Packard, Germany). Use of microspheres of this diameter and number does not cause microinfarction in our experience.

### Coronary Microembolization

Coronary microembolization was induced by injecting 3000 white-colored polystyrene microspheres (diameter, 42 μm; Dynospheres, Dyno Particles, Lillestrøm, Norway) per mL·min⁻¹ coronary inflow into the perfusion system. The microspheres were suspended with 0.02% Tween in 1 mL of saline as vehicle. In preliminary experiments, this size and number of injected microspheres best reflected the pattern of microinfarcts that was previously observed in patients with unstable angina who died of sudden cardiac death and were subsequently autopsied.1,4

### TNF-α Tissue Concentration and Neutralization of TNF-α

The tissue concentration of TNF-α was determined in myocardial snap frozen drill biopsies (weight 10 to 20 mg) taken 6 hours after coronary microembolization or placebo, respectively, from the microembolized myocardium and a remote control area. Tissue concentrations of TNF-α were measured using a commercial ELISA assay specific for porcine TNF-α (R&D Systems, Minneapolis, Minn).

To neutralize TNF-α, antibodies raised in a sheep against recombinant murine TNF-α were slowly infused intracoronarily at a total dose of 25 mg/kg body weight. In prior in vitro experiments, the TNF-α antibodies neutralized TNF-α in serum samples from microembolized dogs by more than 80%.8

The infusion of TNF-α antibodies was initiated 30 minutes before coronary microembolization or placebo, respectively. The presence of the TNF-α antibodies in the myocardium was proven in biopsies taken at 6 hours after coronary microembolization or placebo, respectively, by immunofluorescence microscopy using fluorescein isothiocyanate–coupled antibodies directed against sheep IgG (Santa Cruz Biotechnology, Santa Cruz, Calif). Because the TNF-α antibodies were assumed to neutralize TNF-α but not to interfere with its expression, TNF-α was not measured in homogenates from animals receiving TNF-α antibodies.

### Infarct Size

At the end of each study, the heart was removed and sectioned from base to apex into 5 transverse slices in a plane parallel to the atrioventricular groove. The slices were immersed in 0.09 mol·L⁻¹ sodium phosphate buffer (pH 7.4) containing 1.0% triphenyl tetrazolium chloride (TTC) (Sigma–Aldrich Chemie GmbH, Munich, Germany) and 8% dextran (molecular mass, 77 800 Da) for 20 minutes at 37°C to identify infarcted tissue. The amount of infarcted tissue is expressed as percentage of the left ventricular area at risk, as determined by the microspheres technique.1,8

### Experimental Protocols

#### Group 1

Following baseline measurements of systemic hemodynamics and regional myocardial blood flow, coronary microembolization was induced in group 1 (n=7), as described above. During microembolization, coronary inflow was held constant. Thereafter, coronary perfusion pressure was maintained at baseline levels for 6 hours. Measurements of systemic hemodynamics and regional myocardial blood flow were repeated, and myocardial drill biopsies were taken. Ischemia was induced by reduction of coronary inflow to 15% of baseline flow. At 5 minutes of ischemia, measurements were again repeated. After 90 minutes ischemia the myocardium was repertused for 2 hours before infarct size was determined by TTC staining.

#### Group 2

The protocol of this group (n=8) was identical to that of group 1, except that coronary microembolization was replaced by placebo, ie, intracoronary injection of 1 mL of vehicle.

#### Group 3

Following baseline measurements of systemic hemodynamics and regional myocardial blood flow, TNF-α antibodies were slowly infused intracoronarily in group 3 (n=4). Thirty minutes later, coronary microembolization was induced and this protocol was then identical to that of group 1.
**Hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (min⁻¹)</th>
<th>LVP (mm Hg)</th>
<th>dP/dt (mm Hg sec⁻¹)</th>
<th>WTa (%)</th>
<th>CBF (mL min⁻¹)</th>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>93 ± 2</td>
<td>97 ± 2</td>
<td>1474 ± 77</td>
<td>44.8 ± 5.2</td>
<td>27.5 ± 3.5</td>
</tr>
<tr>
<td>ME</td>
<td>94 ± 2</td>
<td>98 ± 2</td>
<td>1502 ± 98</td>
<td>39.6 ± 4.7</td>
<td>41.8 ± 4.8*</td>
</tr>
<tr>
<td>6 hours</td>
<td>95 ± 2</td>
<td>91 ± 2</td>
<td>1189 ± 66*</td>
<td>29.3 ± 3.9*</td>
<td>43.8 ± 3.9*</td>
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<tr>
<td>I5</td>
<td>96 ± 3</td>
<td>78 ± 1*</td>
<td>931 ± 51*</td>
<td>−0.9 ± 0.9*</td>
<td>3.8 ± 0.6*</td>
</tr>
<tr>
<td>Rep</td>
<td>100 ± 6</td>
<td>67 ± 9*</td>
<td>859 ± 143*</td>
<td>−0.2 ± 0.5*</td>
<td>51.8 ± 11.6*</td>
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<td><strong>Group 2</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>98 ± 4</td>
<td>99 ± 2</td>
<td>1451 ± 77</td>
<td>45.6 ± 5.5</td>
<td>27.6 ± 2.2</td>
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<tr>
<td>6 hours</td>
<td>101 ± 3</td>
<td>90 ± 2*</td>
<td>1280 ± 45</td>
<td>41.1 ± 5.0</td>
<td>37.6 ± 3.1</td>
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<td>I5</td>
<td>109 ± 4</td>
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<td>−0.5 ± 1.1*</td>
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<tr>
<td>Rep</td>
<td>105 ± 4</td>
<td>82 ± 2*</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>89 ± 3</td>
<td>93 ± 6</td>
<td>1194 ± 46</td>
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<tr>
<td>ME</td>
<td>87 ± 2</td>
<td>91 ± 6</td>
<td>1178 ± 64</td>
<td>33.1 ± 1.5</td>
<td>54.1 ± 11.5*</td>
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<tr>
<td>6 hours</td>
<td>101 ± 4</td>
<td>89 ± 2</td>
<td>1179 ± 77</td>
<td>28.8 ± 2.2</td>
<td>56.9 ± 13.5*</td>
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<tr>
<td>I5</td>
<td>106 ± 13</td>
<td>81 ± 3</td>
<td>895 ± 54</td>
<td>−1.3 ± 1.8*</td>
<td>4.5 ± 1.0*</td>
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<tr>
<td>Rep</td>
<td>126 ± 13*</td>
<td>70 ± 7*</td>
<td>1133 ± 68</td>
<td>0.2 ± 0.1*</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>87 ± 5</td>
<td>92 ± 3</td>
<td>1377 ± 153</td>
<td>39.2 ± 1.6</td>
<td>26.5 ± 4.9</td>
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<tr>
<td>6 hours</td>
<td>109 ± 6*</td>
<td>93 ± 4</td>
<td>1420 ± 150</td>
<td>34.9 ± 2.3</td>
<td>43.5 ± 12.1</td>
</tr>
<tr>
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<td>112 ± 12*</td>
<td>73 ± 3*</td>
<td>872 ± 69*</td>
<td>−2.2 ± 1.7*</td>
<td>3.0 ± 1.0*</td>
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<tr>
<td>Rep</td>
<td>135 ± 16*</td>
<td>77 ± 1*</td>
<td>1482 ± 371</td>
<td>1.3 ± 0.8*</td>
<td>75.5 ± 5.5*</td>
</tr>
</tbody>
</table>

ME indicates 10 minutes after coronary microembolization; 6 hours, 6 hours after coronary microembolization or placebo, respectively; I5, 5 minutes of ischemia; Rep, 60 minutes of reperfusion; LVP, peak left ventricular pressure; dP/dt, maximum of the first derivative of left ventricular pressure; WTa, anterior systolic wall thickening; CBF, mean LAD coronary inflow. *P < 0.05 vs baseline.

**Group 4**

Except for the replacement of coronary microembolization by intracoronary injection of 1 mL vehicle, the protocol of this group (n = 4) was identical to that of group 3.

To exclude an indirect protective effect of TNF-α through reduced contractile function before ischemia/reperfusion, we performed 4 additional experiments in which the myocardial dysfunction observed after coronary microembolization was matched by graded intracoronary infusion of thiopental over 6 hours. Thereafter, an ischemia/reperfusion protocol identical to that of groups 1 to 4 was performed. The concentration of thiopental in the coronary blood ranged from 5 to 70 µg/mL. The total amount of thiopental infused over 6 hours was 180 ± 55 mg.

**Data Analysis and Statistics**

Data are reported as mean values ± SEM. Systemic hemodynamics, regional myocardial function, subendocardial blood flow at 5 minutes of ischemia, area at risk, infarct size, and TNF-α tissue concentrations were analyzed by 2-way ANOVA (microembolization versus placebo, with or without TNF-α antibodies). To account for interindividual differences of regional myocardial function at baseline, all values were normalized to baseline equal 100% before further analysis. Fisher’s least-significant difference tests were used for post hoc comparisons. Linear regressions between subendocardial blood flow and infarct size in groups 1 and 2 were compared by analysis of covariance (ANCOVA). A probability value less than 0.05 was taken to indicate a significant difference. Because the additional experiments to exclude indirect protection by TNF-α through prolonged myocardial dysfunction were performed as separate controls and not randomized, they were not included in the 2-way ANOVA and statistical comparison.

**Results**

At baseline, systolic wall thickening of the anterior wall was not different among the groups. Coronary microembolization per se decreased systolic wall thickening of the anterior wall. At 10 minutes after microembolization in groups 1 and 3, anterior systolic wall thickening remained slightly decreased and coronary blood flow was increased (Table). At 6 hours after coronary microembolization in group 1, systolic wall thickening of the anterior wall had progressively decreased to 65 ± 4% of baseline (Figure 1) versus 90 ± 1% in placebo controls of group 2 (P < 0.05). Pretreatment with TNF-α antibodies attenuated the progressive dysfunction following coronary microembolization in group 3 (systolic wall thickening of the anterior wall at 6 hours, 77 ± 5% of baseline; P < 0.05 versus group 1). In group 4, the pretreatment with TNF-α antibodies had no influence on the time course of anterior systolic wall thickening (at 6 hours, 90 ± 8% of baseline; Figure 1). With the onset of the sustained ischemia, anterior systolic wall thickening was reduced in all groups (Table).

In group 1, the tissue concentration of TNF-α was increased to 66 ± 21 pg/g wet weight in the microembolized anterior wall compared with 12 ± 11 pg/g wet weight in remote control myocardium (P < 0.05). Such increase of TNF-α was not observed in group 2 (anterior wall: 21 ± 10 pg/g wet weight versus remote area: 7 ± 4 pg/g wet weight).
The area at risk and the subendocardial blood flow at 5 minutes of ischemia were comparable among all groups (Figure 2). The infarct size resulting from 90 minutes of ischemia and 2 hours of reperfusion was reduced to 18% of the area at risk in group 1 with prior coronary microembolization, as compared with 33% in group 2 with prior placebo (Figure 2). The pretreatment with TNF-α antibodies (TNF-α AB) attenuated this microembolization-induced progressive myocardial dysfunction in group 3 but had no effect in group 4.

The TNF-α antibodies per se had no effect on infarct size (group 4: 35±5%). With coronary microembolization, the relationship between infarct size and subendocardial blood flow was shifted downward, reflecting smaller infarct size for any given subendocardial blood flow (P<0.05 ANCOVA; Figure 3).

In 4 additional experiments with gradually increasing thiopental infusion, regional myocardial function was decreased from 38.5±8.6% to 25.0±5.6%, ie, to 65±0.1% of baseline. Subendocardial blood flow during ischemia was 0.032±0.009 mL/min per gram, and infarct size was 31±5% of the area at risk.

**Discussion**

Coronary microembolization has been recognized as a spontaneous event in patients with ischemic heart disease and as...
an iatrogenic complication in patients undergoing percutaneous coronary interventions.29 Spontaneous coronary microembolization may result from release of atheromatous and/or thrombotic material from small plaque fissures. Studies using serial intravascular ultrasound suggest that spontaneous plaque rupture and healing are indeed part of the natural process of atherosclerosis.20 In the present study, we addressed the potential clinical scenario of spontaneous coronary microembolization several hours before massive plaque rupture and thrombotic occlusion of a large coronary artery with impending myocardial infarction.

Apart from an immediate decrease in coronary blood flow with subsequent reactive hyperemia and a transient contractile dysfunction, the major consequence of coronary microembolization is the development of multiple microinfarcts. Although the aggregate amount of infarction is small (<5%) of the microembolized myocardium in our experimental studies in dogs and pigs,8,9,11,21 these microinfarcts initiate a typical inflammatory response, characterized by increased myocardial TNF-α expression and leukocyte infiltration.8,11,21 The increased TNF-α expression is an autocrine/paracrine response of viable cardiomyocytes surrounding the microinfarcts, possibly mediated by local shear stress between contracting and noncontracting, infarcted myocardium.8 The increasing tissue concentration of TNF-α is causal for the resulting progressive myocardial dysfunction.8–11,22 The typical long-term consequences of coronary microembolization, increased levels of TNF-α in the microembolized myocardium and progressive myocardial dysfunction, were confirmed in group 1 of the present study. In the placebo animals of group 2, the myocardial concentration of TNF-α and contractile function remained unchanged. Slightly higher levels of TNF-α were observed in the anterior than in the remote posterior wall, possibly as a result of the extracorpo-
real perfusion of the left anterior descending coronary artery.25

However, TNF-α is not only a negative inotrope, as reflected by the progressive contractile dysfunction. Pretreatment with TNF-α mimics preconditioning in mice14 and isolated rat15,24 and rabbit15 hearts; in TNF-α knockout mice, the endogenous protection against infarction by acute14 or delayed ischemic preconditioning is abrogated. Such bidirectional effects of TNF-α are typically attributed to concentration- or site-specific effects.25,26 Somewhat in contrast, we have now studied the bidirectional (negative inotropic versus cardioprotective) action of one and the same TNF-α expression in the same experimental model, which mimics a realistic scenario of an acute coronary syndrome, and with the same target, ie, cardiomyocytes.

Infarct size after 90 minutes of ischemia and 2 hours of reperfusion was determined by TTC staining and served as the major end point of the present study. Under conditions of some residual blood flow during ischemia, as in the present study, it is a major determinant of final infarct size and must be taken into account.27 Therefore, we analyzed not only infarct size but also the relationship between infarct size and subendocardial blood flow during sustained ischemia as a more specific end point of cardiomyocyte protection. The major finding of the present study is that the myocardium is indeed protected against infarction at 6 hours after coronary microembolization. Infarct size was reduced almost by 50%, although coronary microembolization per se is expected to increase infarct size by approximately 5%.11 The observed protection was a direct effect of TNF-α per se and not an indirect protection secondary to reduced contractile function before ischemia/reperfusion, because thiopental induced almost identical dysfunction but no reduction in infarct size.

The pretreatment with the neutralizing TNF-α antibodies before coronary microembolization attenuated not only the progressive myocardial dysfunction, as already observed in dogs,8 but also abolished the infarct size reduction by coronary microembolization, establishing that increased TNF-α is causal both for contractile dysfunction and protection against infarction. Although this confirms previous observations that TNF-α can protect the myocardium from ischemia/reperfusion injury,13,14 our data are in contrast to other observations from our own and other laboratories. The role of TNF-α for infarct size after ischemia/reperfusion is controversial in mice,16,28 and in rabbits, the neutralization of
TNF-α by TNF-α antibodies was associated with reduced infarct size after acute ischemia/reperfusion. A protective effect was not observed in the present study in the pigs of group 4. The attribution of such discrepancy to potential species differences is not really satisfying. An alternative explanation comes from model differences that result in differences of TNF-α concentrations. In fact, in the mice and rabbit studies, ischemia was induced by complete occlusion of a coronary artery, whereas in our present preparation, residual blood flow provided a continuous washout. Protection by infusion of exogenous TNF-α also requires a mandatory washout phase before the sustained ischemia, and the magnitude of infarct size reduction largely depends on the dose of TNF-α. The protective effect of TNF-α was only observed at a lower dose, and further increasing doses of TNF-α attenuated the infarct size reduction. A similar link between low-dose TNF-α and cardioprotection was recently reported. Hyperoxic exposure of mice induced a mild pulmonary inflammation with an increased TNF-α concentration. Increases in circulating serum TNF-α levels were not detected, possibly because of concentrations below the detection limit. Again, in these mice infarct size after ischemia/reperfusion was reduced, and this effect was abolished in TNF-α knockouts.

Apart from the causal role of TNF-α in progressive myocardial dysfunction and delayed protection against infarction following coronary microembolization, there are further similarities in the signal transduction cascade. Myocardial protection by exogenous TNF-α depends on a sphingolipid signaling pathway. The ceramidase inhibitor N-oleoyl ethanolamine abolishes TNF-α preconditioning, and ceramide or sphingosine 1-phosphate mimic TNF-α protection. Similarly, for the microembolization-induced progressive myocardial dysfunction, we have identified a signal transduction cascade with nitric oxide upstream and sphingosine downstream of TNF-α. N-Oleoyl ethanolamine given before coronary microembolization prevented the progressive myocardial dysfunction, despite an increased concentration of TNF-α in the myocardium.

In a more recent study, Lecour et al also report that free radicals are involved in TNF-α-induced cardioprotection. With TNF-α infusion, the formation of free radicals, as measured by electron spin resonance, was increased and the infarct size reduction by exogenous TNF-α was abolished by the antioxidant N-2-mercaptopropylglycine. With coronary microembolization in pigs, we have recently reported that in microembolized myocardium tropomyosin is oxidatively modified by formation of disulfide cross bridges and that the amount of disulfide cross bridges correlates to the degree of myocardial dysfunction at 6 hours after coronary microembolization. The oxidative modification of contractile proteins was attributed to free radicals because scavenging free radicals with ascorbic acid largely attenuated both the tropomyosin oxidation and myocardial dysfunction. Again, free radicals in the heart act in a dose-dependent bidirectional action. Small amounts of free radicals may serve a signaling function and result in cardioprotection, whereas larger amounts result in myocardial damage. In the scenario of ischemia/reperfusion, small amounts of free radicals are important signaling elements of acute ischemic preconditioning, whereas large amounts of free radicals cause stunning and cell death.

We conclude that although coronary microembolization is a deleterious event that may occur spontaneously in patients with unstable coronary plaques or iatrogenically during percutaneous interventions, it also exerts a beneficial effect. When microembolized myocardium is subjected to ischemia/reperfusion after several hours, it is protected against infarction. This delayed protection by coronary microembolization is mediated by TNF-α.

The observation of a bidirectional role of TNF-α may provide a mechanistic explanation for the divergent clinical data on targeted anti-TNF-α therapy in patients with heart failure. In agreement with the observed improvement in contractile function with TNF-α antibodies in the present study, ventricular function was improved by a soluble TNF-α receptor that binds and inactivates TNF-α in an initial trial in a small group of patients with heart failure. Subsequent studies in more patients either did not confirm an improvement in clinical status or even reported an increased mortality with anti-TNF-α therapy, possibly reflecting the loss of TNF-α-related protection.

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Disclosures
None.

References


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