New insights into the pathological role of TNF-α in early cardiac dysfunction and subsequent heart failure after infarction in rats

C. Berthonneche, T. Sulpice, F. Boucher, L. Gouraud, J. de Leiris, S. E. O’Connor, J.-M. Herbert, and P. Janiak. New insights into the pathological role of TNF-α in early cardiac dysfunction and subsequent heart failure after infarction in rats. Am J Physiol Heart Circ Physiol 287: H340–H350, 2004; 10.1152/ajpheart.01210.2003.—A marked increase in plasma TNF-α has been described in patients with chronic heart failure (CHF). Nevertheless, little is known about the direct role of this cytokine early after myocardial infarction (MI) and its possible effects on the subsequent development of CHF. Wistar rats were subjected to permanent in vivo coronary artery ligation. At 5, 7, and 9 days after MI, cardiac function, passive compliance of the left ventricle (LV), and cardiac geometry were evaluated. The same model was used to perform pharmacological studies 7 days and 10 wk after MI in rats treated with monomeric recombinant human soluble TNF-α receptor type II (sTNF-RII, 40 μg/kg iv) or a placebo on day 3. Maximal alterations of cardiac function and geometry occurred 7 days after MI, which correlated chronologically with a peak of cardiac and serum TNF-α, as shown by immunohistochemistry and ELISA, respectively. sTNF-RII improved LV end-diastolic pressure under basal conditions and after volume overload 7 days and 10 wk after MI. Moreover, a significant leftward shift of the pressure-volume curve in the sTNF-RII-treated group 7 days after MI indicated a preservation of LV volume. Infarct expansion index was also significantly improved by sTNF-RII 7 days after MI (P < 0.01). Nevertheless, 10 wk after MI, geometric indexes and passive pressure-volume curves were not significantly improved by the treatment. In conclusion, TNF-α plays a major role in cardiac alterations 7 days after MI in rats and contributes to hemodynamic derangement, but not to cardiac remodeling, in subsequent CHF.

LARGE TRANSMURAL myocardial infarction (MI) initiates a cascade of progressive structural and geometric changes in the myocardium that is commonly referred to as remodeling and may constitute a basis for severe ventricular dysfunction.

Postinfarct left ventricular (LV) dysfunction and heart remodeling are recognized as the primary causes of chronic heart failure (CHF) in industrialized countries.

Since the original report of elevated levels of TNF-α in patients with CHF (18), there has been increasing speculation that TNF-α may contribute to the development of this pathology. For example, it has been shown that pathophysiologically relevant concentrations of TNF-α are sufficient to mimic certain aspects of the CHF phenotype, including LV dysfunction and dilation (2). Moreover, the pathogenic role of TNF-α in CHF is supported by the study of Bryant et al. (4), who reported that transgenic mice with cardiac overexpression of TNF-α develop cardiac hypertrophy, fibrosis, and dilated cardiomyopathy.

The proinflammatory cytokine TNF-α is known to modulate cardiovascular function by a variety of mechanisms. It has been shown to depress myocardial contractility by uncoupling β-adrenergic signaling (6), increasing cardiac nitric oxide and peroxynitrite (5, 7), or altering intracellular calcium homeostasis (38). TNF-α may also induce structural changes in the failing myocardium, such as cardiomyocyte hypertrophy (36), interstitial fibrosis (32), and dilation (4, 16). Additionally, TNF-α may promote cardiomyocyte apoptosis (15); it may also activate metalloproteinases (19, 32) and impair the expression of their inhibitors, possibly contributing to cardiac remodeling.

Ono et al. (24) showed that, in the infarcted region of the myocardium, TNF-α and IL-1β gene expression peaks 1 wk after infarction in rats and rapidly decreases thereafter. These data were extended by Irwin et al. (10), who showed that TNF-α mRNA and protein are persistently expressed by myocytes in the noninfarcted regions of the myocardium from 1 day to 5 wk after coronary ligation. Furthermore, a burst of cardiomyocyte apoptosis has also been demonstrated 1 wk after infarction (21).

Nevertheless, it has not been clearly elucidated whether TNF-α production is directly involved in the early mechanical dysfunction and remodeling that follow MI and play a role in the development of CHF.

The aim of this study was therefore to investigate the pathophysiological relevance of myocardial production of TNF-α early after MI and its possible implication in the subsequent establishment of CHF.

With this objective in mind, we have evaluated the functional and morphological cardiac modifications in the 9 days after left coronary artery ligation in rats, together with the profile of TNF-α production. We also have investigated the effects of early TNF-α inactivation (3 days after surgery) by a single injection of monomeric recombinant human soluble TNF-α receptor type II (sTNF-RII) on early (1 wk after surgery) cardiac dysfunction and remodeling and late (10 wk after surgery) CHF.

METHODS

Experimental MI and Heart Failure

Adult male Wistar rats (280–320 g body wt; IFFA CREDO, Lyon, France) were maintained on a standard diet and cared for according to the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The rats were subjected to in vivo left coronary artery ligation as originally described by Selye et al. (30). After the rats were anesthetized intramuscularly with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg), they were intubated and ventilated via a tracheal cannula by a constant-volume ventilator (Apexlex; 70/min, 1 ml/100 g). A left thoracotomy was performed at the fourth intercostal space, and the heart was briefly exteriorized by digital pressure on the chest wall. The left coronary artery was then ligated 1–2 mm from its origin with a 5-0 silk suture (Autosuture). The heart was quickly returned to the chest cavity; then the chest cavity was compressed to remove any air before being hermetically sealed. This ligation induces a reproducible, large, lateral wall infarction. To further ensure uniformity of the infarct, only hearts with infarcts >40% of midwall circumference, which were identified postmortem and measured by morphometry, were included in the final analysis. The overall mortality of this model was 25% for the infarction group; ~80% of this mortality occurred during surgery or during the first 5 min after coronary artery ligation mainly because of pulmonary edema or ventricular fibrillation, and the remaining 20% occurred within the first 24 h after the procedure. The same procedure was followed for sham-operated control animals, but the coronary ligation was not tied. No mortality was observed in these sham groups.

Experimental Design

After surgery, the rats were housed in polyethylene cages (3 or 4 rats per cage), were fed standard laboratory food, had free access to tap water, and were studied after 5 days, 7 days, 9 days, or 10 wk of permanent coronary artery ligation. Rats of the protocol were randomly assigned (6–15 per group) to the experimental groups described below.

Chronological study. In this study, functional and morphological cardiac modifications were evaluated 5–9 days after MI and the profile of TNF-α production was assessed. Rats with MI were randomly assigned to one of three groups according to the duration of left coronary artery ligation: 5 days after MI (MI-5d), 7 days after MI (MI-7d), and 9 days after MI (MI-9d). The same protocol was followed for sham-operated (sham) rats, but the ligation was not tied. Other animals subjected to the same duration of coronary artery ligation (5, 7, or 9 days) were used for determination of myocardial TNF-α localization by immunohistochemistry.

Pharmacological study. In this study, we investigated the effects of early TNF-α inhibition on early (7 days) and late (10 wk) cardiac alterations after MI in rats. At 3 days after MI, a single intravenous injection of sTNF-RII (40 μg/kg; Clinisciences, Montrouge, France) or vehicle solution was administered. Coronary ligation was maintained for 7 days or 10 wk. MI and sham rats were treated with sTNF-RII or vehicle alone. Rats were randomly distributed into eight groups: sham-or MI-vehicle-7d, sham-or MI-sTNF-RII-7d, sham-or MI-vehicle-10w, and sham-or MI-sTNF-RII-10w.

In Vivo Measurement of Cardiac Function

Hemodynamic studies were performed 5 days, 7 days, 9 days, and 10 wk after coronary artery ligation or sham operation. Animals were anesthetized with pentobarbital sodium (60 mg/kg, 1 ml/kg ip). After tracheotomy, the rats were immediately ventilated and heparin was injected via a saphenous vein (400 IU/kg). Body temperature was kept constant at 37°C with a heating blanket controlled by a thermostat and connected to a rectal thermocouple (Homeothermic Blanket System, Harvard Apparatus, Edenbridge, UK). A PE-10 catheter was inserted into the right femoral vein to deliver a continuous infusion (3.6 mg·min⁻¹·kg⁻¹) of pentobarbital sodium, and the left femoral vein was also catheterized to allow volume overload with thermostated Ringer solution (Aguetant). Mean arterial blood pressure (MAP) was measured via a PE-50 arterial catheter (left carotid) connected to a pressure transducer (Statham P23XL, Hugo Sacks Electronic,hungtry, Germany), and a catheter (SRP-407 Mikro Tip, Millar Instruments, Houston, TX) was inserted into the LV via the right carotid artery to monitor LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), heart rate (HR), and maximal and minimal first derivatives of developed pressure (dP/dtmax and dP/dtmin) and to calculate LV developed pressure (LVDP). The rat was then positioned in the lateral decubitus position, and the abdomen was opened. The abdominal aorta was isolated just below the level of the diaphragm, a standard electromagnetic flow probe (1.5 or 2 mm ID; model MDL-1401, SolarM) was placed around the vessel, and mean abdominal aortic blood flow (AaO) was monitored and considered equivalent to cardiac blood flow. Stroke volume (SV) was estimated from AaO and HR.

After baseline measurements had been carried out over a 10-min period to reach a steady state, thermostated Ringer solution was infused into the left femoral vein at a rate of 17 ml·kg⁻¹·min⁻¹ for 60 s. This volume overload induces an immediate rise in cardiac output followed by a plateau and reveals underlying cardiac dysfunction.

All hemodynamic variables were recorded for 15 min after volume overload, until they had returned to baseline levels.

Serum levels of TNF-α. At the end of the hemodynamic study, the chest was opened, and a blood sample was collected directly in the coronary common vein of the myocardium. This sample was stored at 4°C overnight and then centrifuged at 15,000 g for 10 min (4°C). The supernatant corresponding to the serum was isolated and stored at −80°C until analysis.

Serum TNF-α was determined using an ELISA kit (Quantikine, R & D Systems, Abingdon, UK). Briefly, a standard curve was generated for each set of samples assayed by standards of various concentrations. Serum TNF-α levels were within the range of linearity in each assay. Assays were performed in duplicate. Development of a colored reaction caused by the conversion of chromogenic substrate (tetramethylbenzidine) that is directly proportional to the amount of the analyte assayed was followed for 5–20 min with analysis via an ELISA plate reader (Titertek Multiskan Plus, MK II, Labsystems) at 450 nm. Results were expressed in picograms per milliliter.

Ex Vivo Determination of LV Pressure-Volume Curves

Passive pressure-volume characteristics of the LV were defined as previously described by Fletcher et al. (8). A saturated potassium chloride solution was introduced directly into the vena cava until the heart stopped in diastole. The heart was then carefully excised and rinsed, and a cannula was inserted 5 mm into the LV through the ascending aorta. The right and left atrioventricular junctions, as well as the pulmonary artery and vena cava, were ligated. The LV was compressed manually to expel blood and create a negative pressure, which was taken as zero volume. Physiological saline was infused at 0.68 ml/min via the cannula while intraventricular pressure was continuously monitored at 0–30 mmHg. At least two reproducible pressure-volume curves were obtained within 10 min after cardiac arrest, well before the onset of rigor mortis. Ventricular volumes at 0, 2.5, 5, 10, 15, 20, and 30 mmHg were determined from the pressure-volume curves. The operating LV end-diastolic volume was derived from the LV pressure-volume curve and defined as the volume on the pressure-volume curve corresponding to a filling pressure equal to in vivo end-diastolic pressure.

Assessment of Infarct Size and Cardiac Geometry 5, 7, and 9 Days or 10 Weeks After Coronary Ligation

After the pressure-volume data had been recorded, the heart was slowly frozen in liquid nitrogen and then cut transversely at −20°C with a cryostat (microtome; model RM2165, Leica). Six transverse 5-μm sections were obtained at different levels of the ventricle at...
1.5-mm increments from the apex of the heart to the base of the ventricles.

**Estimation of cardiac necrosis.** Each section was stained using nitro blue tetrazolium (0.04% in 0.05 mol/l sodium succinate buffer, pH 7.6) as described by Nachlas and Schnitka (22). The colorant acts as a substrate for mitochondrial succinate dehydrogenase. In the presence of NADH and succinate, it is reduced by the enzyme into a deep-blue insoluble deposit of formazan. On each section, necrotic and nonnecrotic tissues were distinguished by the absence or presence of staining, respectively, and infarct size was estimated by planimetry from the six sections as previously described by Pfeffer et al. (25).

Briefly, the lengths of necrotic tissue and noninfarcted muscle for the endocardial and epicardial surfaces of all histological sections were determined by planimetry of the projected histological slides. Lengths of the scars on the endocardial and epicardial surfaces of all histological sections were numerically summed separately, as were the endocardial and epicardial circumferences. The ratio of the sums of the scar lengths and surface circumference defined the infarct size for each of the myocardial surfaces. Final infarct size was expressed as a percentage (i.e., average of infarct sizes on endocardial and epicardial surfaces × 100).

**Assessment of cardiac geometry.** Cardiac architecture was studied on histological sections 4, 5.5, and 7 mm from the apex, as described by Sulprice et al. (33) with the use of picture analysis software (Biomax Visiolab® 2000). For each section, the cross-sectional area of LV cavity dilation (DI). The thickness of the infarcted wall, septum, and right ventricular wall were measured on cross sections. The thining index (TI) was defined as the ratio of the thickness of the infarcted wall to the thickness of the septum. The infarct expansion index (EI) was estimated from the ratio of DI to TI. Only the data from 5.5 mm from the apex are presented in **RESULTS**.

**Localization of TNF-α by Immunohistochemistry.**

At 5, 7, or 9 days after surgery, the animals were anesthetized with pentobarbital sodium (60 mg/kg, 1 ml/kg ip), and the hearts were excised, rinsed with saline buffer, and then frozen in liquid nitrogen and stored at −80°C until further analysis. Frozen 5-μm-thick sections obtained with a cryostat (model CM3000, Leica) at median levels of the heart were incubated in acetone for 10 min at 4°C. After they were washed with PBS in the presence of 0.1% saponin, slides were incubated in a blocking solution (PBS containing 0.1% saponin and 10% rabbit serum) for 20 min. The specific goat polyclonal primary antibody (R & D Systems) or negative control goat antibody (Rockland) was added to the sections at 2 μg/ml, and the sections were incubated for 1 h at room temperature. The slides were washed with PBS and 0.1% saponin and incubated for 1 h at room temperature with rabbit anti-goat secondary antibody (Cy3, red fluorescence; Sigma). The slides were counterstained with Hoechst 33258 (0.1 mg/ml) and observed by fluorescence microscopy using a DMLB microscope (Leica) equipped with a video camera (model DXC-950P, Sony). For each section, three images were acquired: border infarct zone, interventricular septum, and right ventricular wall.

**Statistical Analysis**

Not normally distributed data (serum TNF-α levels) are expressed as median values. Normally distributed values are expressed as means ± SE. One-way analysis of variance was performed to determine significant differences between the various groups for hemodynamics, infarct size, geometric variables, and passive pressure-volume curves. The significance of the difference between the mean of the groups was tested with Fisher’s post hoc protected least significant difference test.

**RESULTS**

**Hemodynamic Measurements**

**Chronological study.** Under basal conditions, LVDP was significantly increased in MI-7d compared with sham: 7.1 ± 2.1 vs. 1.5 ± 1.3 mmHg (P < 0.05; Table 1). MAP, LVSP, and LVDP were reduced in MI-7d compared with sham: MAP = 110 ± 4 vs. 125 ± 5 mmHg (P < 0.05), LVSP = 122 ± 5 vs. 150 ± 9 mmHg (P < 0.05), and LVDP = 115 ± 5 vs. 148 ± 8 mmHg (P < 0.05). dP/dt(max) and dP/dt(min) were significantly altered, particularly in MI-7d: dP/dt(max) = 4.7 ± 0.8 × 10^6 vs. 7.4 ± 0.4 × 10^6 mmHg/s (P < 0.01) in MI-7d vs. sham and dP/dt(min) = −3.5 ± 0.4 × 10^6 vs. −6.0 ± 0.5 × 10^6 mmHg/s (P < 0.01) in MI-7d vs. sham. No significant differences were found between the groups for HR, AAO, and SV.

**Volume overload.** LVDP was dramatically increased after volume overload in MI-7d compared with sham: 27.6 ± 1.7 vs. 13.8 ± 3.1 mmHg (P < 0.01). MAP, LVSP, LVDP, and dP/dt(max) and dP/dt(min) were significantly reduced in all MI groups compared with sham-operated groups and more markedly in MI-7d. Volume overload induced a major increase in AAO in sham-operated animals. This variable was particularly

**Table 1. Hemodynamic variables at baseline and after 1-min infusion of Ringer solution: chronological study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>MI-5d</th>
<th>MI-7d</th>
<th>MI-9d</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Overload</td>
<td>Baseline</td>
<td>Overload</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>433 ± 8</td>
<td>436 ± 6</td>
<td>444 ± 13</td>
<td>442 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>125 ± 5</td>
<td>174 ± 6</td>
<td>110 ± 7</td>
<td>141 ± 10</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>150 ± 9</td>
<td>205 ± 8</td>
<td>128 ± 7</td>
<td>167 ± 15*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>1.5 ± 1.3</td>
<td>13.8 ± 3.1</td>
<td>4.8 ± 1.9</td>
<td>20.9 ± 2.6</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>148 ± 8</td>
<td>191 ± 6</td>
<td>123 ± 8</td>
<td>146 ± 16</td>
</tr>
<tr>
<td>dP/dt(max), × 10^6 mmHg/s</td>
<td>7.4 ± 0.4</td>
<td>10.9 ± 0.5</td>
<td>6.6 ± 0.5</td>
<td>8.6 ± 0.8*</td>
</tr>
<tr>
<td>dP/dt(min), × 10^6 mmHg/s</td>
<td>−6.0 ± 0.5</td>
<td>−8.4 ± 0.5</td>
<td>−5.2 ± 0.5</td>
<td>−5.8 ± 0.3</td>
</tr>
<tr>
<td>AAO, μl/min</td>
<td>29.5 ± 1.8</td>
<td>67.5 ± 3.0</td>
<td>32.6 ± 4.5</td>
<td>60.6 ± 5.4</td>
</tr>
<tr>
<td>SV, μl/beat</td>
<td>73 ± 4</td>
<td>164 ± 7</td>
<td>75 ± 11</td>
<td>138 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7–15 per group. Ringer solution was infused at 17 ml/kg into ligated and sham-operated rats 5, 7, and 9 days after myocardial infarction (MI-5d, MI-7d, and MI-9d). HR, heart rate; MAP, mean arterial pressure; LVSP, LVEDP, and LVDP, left ventricular systolic, end-diastolic, and developed pressure, respectively; dP/dt(max) and dP/dt(min), 1st derivatives of developed pressure; AAO, aortic arterial flow; SV, stroke volume. *P < 0.05; †P < 0.01 vs. sham. ‡P < 0.05; §P < 0.01 vs. MI-5d.
compared with sham-operated animals, and HR and MAP were significantly lower in MI-sTNF-RII-7d than in MI-vehicle-7d: 5.8 ± 0.9 mmHg vs. 164 ± 7 mmHg/L (P < 0.01). AAo was also significantly reduced in MI-sTNF-RII-7d compared with sham: 106 ± 7 vs. 164 ± 7 µl/beat (P < 0.01). AAo and SV were also less reduced (20%) in MI-sTNF-RII-7d than in MI-vehicle-7d, although not significantly (P = 0.21 and P = 0.11, respectively). MAP, LVSP, LVDP, and dP/dt were significantly decreased in both MI groups compared with sham-operated animals (P < 0.01), and HR was reduced by 10% in MI-sTNF-RII-7d compared with sham-sTNF-RII-7d (P < 0.05).

Effect of sTNF-RII on cardiac function 7 days after MI. Basal conditions. Under basal conditions, LVEDP was 60% lower in MI-sTNF-RII-7d than in MI-vehicle-7d: 5.8 ± 0.9 vs. 14.5 ± 2.4 mmHg (P < 0.01; Table 2). No significant difference in LVEDP was observed between the sTNF-RII-treated MI and sham-operated groups. AAo was significantly altered in both ligated groups treated with sTNF-RII or with vehicle alone 7 days after coronary artery ligation compared with the corresponding sham-operated groups (P < 0.05 and P < 0.01, respectively). A 29% decrease in SV was also observed in MI-sTNF-RII-7d compared with sham-sTNF-RII-7d (P < 0.05), but no significant difference was measured between MI-sTNF-RII-7d and sham-sTNF-RII-7d. Finally, LVSP, LVDP, and dP/dt were reduced in both ligated groups compared with sham-operated animals, and HR and MAP were also reduced in MI-sTNF-RII-7d compared with sham-sTNF-RII-7d.

Volume overload. After volume overload, LVEDP was significantly improved in MI-sTNF-RII-7d compared with MI-vehicle-7d: 25.2 ± 1.9 vs. 39.7 ± 1.7 mmHg (P < 0.01). AAo and SV were less reduced (20%) in MI-sTNF-RII-7d than in MI-vehicle-7d, although not significantly (P = 0.21 and P = 0.11, respectively). MAP, LVSP, LVDP, and dP/dt were significantly decreased in both MI groups compared with sham-operated animals (P < 0.01). LVSP, LVDP, and dP/dt were significantly decreased in both MI and sham-sTNF-RII-10w groups compared with MI-vehicle-10w and sham-sTNF-RII-10w. AAO was significantly improved by sTNF-RII in MI rats compared with animals treated with vehicle alone: 31.7 ± 4.4 vs. 20.1 ± 2.1 ml/min (P < 0.05). Again, no significant difference in this variable was observed between MI-sTNF-RII-10w and sham-sTNF-RII-10w: 31.5 ± 3.2 ml/min. SV was not significantly different between MI and sham-operated groups; nevertheless, this variable was 27% lower in MI-vehicle-10w than in MI-sTNF-RII-10w: 63 ± 8 vs. 86 ± 13 µl/min (P = 0.1, not significant). dP/dt was significantly altered in MI-sTNF-RII-10w compared with sham-sTNF-RII-10w, and dP/dt was reduced in both MI groups compared with sham-operated animals. Finally, HR, MAP, LVSP, and LVDP were not significantly different between the groups.

Table 2. Effect of sTNF-RII on early cardiac dysfunction 7 days after MI: pharmacological study

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>sTNF-RII</td>
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<tr>
<td></td>
<td>Baseline</td>
<td>Overload</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>407 ± 11</td>
<td>396 ± 10</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>113 ± 7</td>
<td>151 ± 11</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>132 ± 10</td>
<td>189 ± 11</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>0.2 ± 0.8</td>
<td>12.4 ± 2.8</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>135 ± 10</td>
<td>177 ± 10</td>
</tr>
<tr>
<td>dP/dt, mmHg</td>
<td>0.0 ± 0.0</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td>AAo, ml/min</td>
<td>39.7 ± 3.2</td>
<td>63.2 ± 4.5</td>
</tr>
<tr>
<td>SV, µl/beat</td>
<td>74 ± 6</td>
<td>162 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–7 sham rats per group and 9–11 MI rats per group. *P < 0.05; †P < 0.01 vs. sham. ‡P < 0.01 vs. MI-vehicle.
VOLUME OVERLOAD. After volume overload, LVEDP was significantly improved by sTNF-RII in rats subjected to left coronary artery ligation compared with MI-vehicle-10w: 22.5 ± 4.0 vs. 39.8 ± 4.0 mmHg (P < 0.01). AaO was markedly altered in MI-vehicle-10w compared with sham-vehicle-10w, but this variable was preserved in MI-sTNF-RII-10w after MI compared with sham-sTNF-RII-10w. The same phenomenon was observed for SV. Finally, HR, MAP, LVSP, LVDP, and dP/dmax and dP/dmin were significantly decreased in all MI groups compared with sham-operated animals.

LV Volume

Chronological study. LV pressure-volume curves measured in potassium-arrested hearts are shown in Fig. 1A. A significant rightward shift of the MI-7d and MI-9d curves was found.
compared with the sham-operated group, indicating an increase in LV volume. MI-7d and MI-9d curves were also significantly shifted toward larger volumes at transmural pressures of 10–30 mmHg compared with MI-5d (P < 0.05). Moreover, no difference was observed between MI-5d and sham curves. Operating LV end-diastolic volume was also significantly increased 7 days after coronary occlusion compared with 5 and 9 days after MI and with sham-operated animals: 0.34 ± 0.06 ml (MI-7d) vs. 0.19 ± 0.03 ml (MI-5d) and 0.18 ± 0.07 ml (MI-9d, P < 0.05) and 0.09 ± 0.02 ml (sham, P < 0.001; Fig. 2A).

**Pharmacological study.** Passive pressure-volume curves of the LV measured 7 days after surgery in sham-operated and MI rats treated with sTNF-RII or vehicle are shown on Fig. 1B. No difference was observed between the two sham-operated groups treated with sTNF-RII or vehicle. A significant rightward shift of the MI-vehicle-7d pressure-volume curve was found compared with the sham-operated group (P < 0.001), whereas no significant difference was observed between MI-sTNF-RII-7d and the sham-operated group. Therefore, sTNF-RII treatment significantly shifted pressure-volume curves to smaller LV volumes at any given pressure in MI-sTNF-RII-7d compared with MI-vehicle-7d (P < 0.01). Moreover, operating LV end-diastolic volume was also significantly less affected in MI-sTNF-RII-7d than in MI-vehicle-7d: 0.21 ± 0.04 vs. 0.44 ± 0.03 ml (P < 0.001; Fig. 2B).

No difference was observed between the two MI groups treated with sTNF-RII or vehicle 10 wk after ligation (Fig. 1C). Pressure-volume curves were significantly shifted toward larger volumes from 0 to 30 mmHg compared with sham curves (P < 0.001). Operating LV volume was also significantly increased in both MI groups compared with sham (P < 0.001; Fig. 2C).

**Cardiac Geometry Measurements**

**Chronological study.** Infarct size and morphometric variables are presented in Table 4. Infarct size was similar among rats that underwent 5, 7, and 9 days of coronary artery ligation. Only morphometric variables measured 5.5 mm from the apex are presented. Permanent ligation 5–9 days after surgery led to a progressive significant decrease in the thickness of the LV free wall: 0.72 ± 0.11, 0.61 ± 0.06, and 0.48 ± 0.05 mm in MI-5d, MI-7d, and MI-9d, respectively, vs. 1.50 ± 0.11 mm in sham (P < 0.01). Septal and right ventricular wall thicknesses were identical in all groups. DI was similar in sham and MI-5d.

![Graph](http://ajpheart.physiology.org/)

**Fig. 2.** Operating LV end-diastolic volume. A: chronological study 5, 7, and 9 days after MI and in sham-operated rats. B: pharmacological approach 7 days after MI and in sham-operated rats receiving sTNF-RII or placebo (vehicle). C: pharmacological approach 10 wk after MI and in sham-operated rats receiving sTNF-RII or placebo. Values are means ± SE. Differences in mean values among groups were determined by post hoc comparison with Fisher’s protected least significant difference method.

<table>
<thead>
<tr>
<th>Infarct size, % of total LV</th>
<th>Sham (n = 18)</th>
<th>MI-5d (n = 11)</th>
<th>MI-7d (n = 11)</th>
<th>MI-9d (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST, mm</td>
<td>1.96±0.08</td>
<td>2.20±0.13</td>
<td>1.91±0.13</td>
<td>1.88±0.21</td>
</tr>
<tr>
<td>RVT, mm</td>
<td>0.97±0.05</td>
<td>1.05±0.09</td>
<td>1.14±0.09</td>
<td>0.77±0.08</td>
</tr>
<tr>
<td>LVT, mm</td>
<td>1.50±0.11</td>
<td>0.72±0.11†</td>
<td>0.61±0.06*</td>
<td>0.48±0.05*</td>
</tr>
<tr>
<td>DI</td>
<td>0.26±0.03</td>
<td>0.30±0.04</td>
<td>0.38±0.04*</td>
<td>0.47±0.04†</td>
</tr>
<tr>
<td>EI</td>
<td>0.76±0.05*</td>
<td>0.34±0.05*</td>
<td>0.32±0.02*</td>
<td>0.27±0.02*</td>
</tr>
<tr>
<td>El</td>
<td>0.33±0.04</td>
<td>1.12±0.19*</td>
<td>1.27±0.21*</td>
<td>1.84±0.20†</td>
</tr>
</tbody>
</table>

Values are means ± SE. ST, mean thickness of septum; RVT, mean thickness of right ventricular wall; LVT, minimal thickness of infarcted LV wall; DI, dilation index; EI, expansion index; Tl, thickening index. *P < 0.01 vs. sham. †P < 0.05 vs. MI-5d.
A staining was more intense than at 5 days (Fig. 4).

**B** indicates the presence of TNF-α/H9251 in the border zone, and compared with sham: 0.38/H11006 significantly increased in MI-7d and MI-9d compared with sham (P < 0.01). This index was significantly reduced in MI-5d, MI-7d, and MI-9d compared with sham (P < 0.01).

**C** staining was detected in the septal wall 7 days after MI, and moderate staining was detected only 9 days after MI. Finally, TNF-α was undetectable in hearts from sham-operated animals (Fig. 4D).

**DISCUSSION**

We have shown a progressive contractile dysfunction associated with pronounced LV dilation that peaks 7 days after MI in rats. Moreover, our results suggest a strong correlation between these deleterious effects and the increase in cardiac blood TNF-α, probably as a consequence of overproduction of this cytokine in the border zone of the infarct.

In addition, a single intravenous infusion of sTNF-RII, which binds to TNF-α and functionally inactivates this cytokine, led to a significant improvement in LV function and remodeling 7 days after ligation in rats. Finally, this therapeutic approach also showed beneficial effects in rats with CHF 10 wk after MI.

Thus our study demonstrates that TNF-α is expressed in the heart and directly contributes to myocardial dysfunction and ventricular dilation 7 days after MI in rats. Moreover, this early effects in rats with CHF 10 wk after MI.

Thus our study demonstrates that TNF-α is expressed in the heart and directly contributes to myocardial dysfunction and ventricular dilation 7 days after MI in rats. Moreover, this early effect of sTNF-RII on morphometric variables 7 days or 10 wk after MI: pharmacological study

<table>
<thead>
<tr>
<th></th>
<th>Sham 7 days</th>
<th>MI 7 days</th>
<th>Sham 10 wk</th>
<th>MI 10 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size, % total LV</td>
<td>58 ± 4</td>
<td>55 ± 3</td>
<td>41 ± 1</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>ST, mm</td>
<td>1.71 ± 0.17</td>
<td>1.55 ± 0.15</td>
<td>1.90 ± 0.09</td>
<td>1.92 ± 0.11</td>
</tr>
<tr>
<td>LV, mm</td>
<td>1.56 ± 0.06</td>
<td>1.35 ± 0.10</td>
<td>0.38 ± 0.04</td>
<td>0.65 ± 0.07</td>
</tr>
<tr>
<td>DI</td>
<td>0.31 ± 0.04</td>
<td>0.33 ± 0.03</td>
<td>0.55 ± 0.02</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td>TI</td>
<td>0.96 ± 0.09</td>
<td>0.90 ± 0.09</td>
<td>0.27 ± 0.02</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>EI</td>
<td>0.32 ± 0.05</td>
<td>0.38 ± 0.05</td>
<td>2.24 ± 0.23</td>
<td>1.42 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05; †P < 0.01 vs. sham. ‡P < 0.05; §P < 0.01 vs. MI-vehicle.

This index was significantly increased in MI-7d and MI-9d compared with sham: 0.38 ± 0.04 and 0.47 ± 0.04 in MI-7d and MI-9d vs. 0.26 ± 0.03 in sham (P < 0.01). TI was significantly reduced in MI-5d, MI-7d, and MI-9d compared with sham (P < 0.01). EI, which reflects the thinning of the infarcted wall and the DI of the cavities, significantly increased from 5 to 9 days after MI compared with sham operation: 1.12 ± 0.19, 1.27 ± 0.21, and 1.84 ± 0.20 in MI-5d, MI-7d, and MI-9d vs. 0.33 ± 0.04 in sham (P < 0.01, MI vs. sham; P < 0.05, MI-9d vs. MI-5d).

**Effects of sTNF-RII 7 days and 10 wk after coronary artery ligation.** Infarct size and morphometric variables are reported in Table 5. In all ligated groups, infarct size was similar 7 days and 10 wk after MI. All morphometric parameters measured in sham-operated groups treated with sTNF-RII or vehicle were identical (cross section at 5.5 mm from the apex) 7 days as well as 10 wk after surgery. Minimal thickness of the LV free wall was significantly decreased in MI groups compared with sham operation (P < 0.01); nonetheless, this parameter was significantly preserved in MI-sTNF-RII-7d vs. MI-vehicle-7d: 0.65 ± 0.07 vs. 0.38 ± 0.04 mm (P < 0.01). TI was similar in both MI groups 7 days and 10 wk after ligation. Finally, EI was significantly improved in MI-sTNF-RII-7d vs. MI-vehicle-7d (1.42 ± 0.21 vs. 2.24 ± 0.23, P < 0.01) but was similar in both groups after 10 wk of permanent ligation.

**Serum Levels of TNF-α**

TNF-α serum level (Fig. 3) measured by ELISA in the coronary common vein was markedly increased in MI-7d compared with sham, in which the TNF-α level was below the detection threshold of the kit (i.e., 5 pg/ml): 34 pg/ml vs. not detectable (<5 pg/ml) in sham-operated animals. In rats subjected to 5 and 9 days of permanent coronary artery ligation, TNF-α was detectable in few serum samples but at lower levels than in rats subjected to 7 days of ligation.

**In Situ Localization of TNF-α by Immunohistochemistry**

Immunostaining of TNF-α at 5, 7, and 9 days after MI indicates the presence of TNF-α in the border zone, and particularly 7 days after ligation (Fig. 4B), when the immunostaining was more intense than at 5 days (Fig. 4A) and 9 days (Fig. 4C) after MI. Low TNF-α staining was detected in the septal wall 7 days after MI, and moderate staining was detected 9 days after MI. In the right ventricular wall, weak immunostaining of TNF-α was present only 9 days after MI. Finally,
cardiac contractile dysfunction was shown to contribute, at least in part, to the development of CHF.

Infarct Model Leading to CHF

The rat model of large MI leading to severe LV dysfunction and subsequent CHF is commonly used to study the mechanisms that are involved in the pathogenesis of this disease. This model evolves more or less rapidly toward heart failure, depending on the size of infarct (28). During the early phase after MI, a variety of compensatory mechanisms are activated to maintain cardiac function, including the adrenergic nervous system and the renin-angiotensin-aldosterone system, the activation of which has been well established in MI (31) and CHF (34). Elevated LV filling pressure, which favors ventricular dilation, can be considered an initial adaptive response (Frank-Starling mechanism) by restoring SV, despite a persistently depressed ejection fraction. Nevertheless, by virtue of Laplace’s law, this dilation increases diastolic and systolic wall stress and, thereby, stimulates further enlargement. Furthermore, this increase in LV cavity volume is associated with a poor prognosis and constitutes a major predictor of postinfarct mortality (26).

In parallel to these phenomena, a major inflammatory process occurs in the infarcted zone to eliminate necrotic tissue and initiate scar formation. During this period of extensive deposition of collagen and fibroblast proliferation, tensile strength increases and the infarcted region can thin and elongate. This process, described by Hutchins and Bulkey (9) and termed “infarct expansion,” is defined as an acute dilation and thinning of the area of infarction. In rats, the major factors that contribute to ventricular dilation and infarct expansion have been extensively described (26).

In our study, mean infarct sizes measured in rats subjected to 5 days, 7 days, 9 days, or 10 wk of coronary artery ligation were sufficient to lead to significant LV cardiac remodeling and correspond to an infarct size >40% of LV according to the method described by Fletcher et al. (8). Infarct size was determined histochemically on the basis of the failure of necrotic tissue to exhibit a positive tetrazolium staining. Although this staining does not allow evaluation of fibrotic tissue, it provides a good indication of scar formation. In our experiments, infarct size was similar among all MI groups.

Chronological Study

The first demonstration of our study is that left coronary artery ligation in rats induces a progressive cardiac contractile dysfunction that peaks 7 days after MI. These abnormalities of contractile function were detectable under baseline conditions and were exacerbated after hemodynamic overload at this time point. Inasmuch as infarct size and systemic arterial pressures were not significantly different between MI groups, the deterioration of contractile function was probably the result of decreased contractile capacity of noninfarcted tissue. In parallel to these alterations, we have shown a peak of coronary venous TNF-α at 7 days after MI, whereas this cytokine was undetectable in most serum samples from rats subjected to 5 or 9 days of permanent ligation.

Moreover, as shown by immunohistochemistry, this increase in serum TNF-α might be the result of the overexpression of this cytokine in the border infarct wall. Indeed, many authors...
have demonstrated that the myocardium itself can be a major source of TNF-α in pathological cardiovascular situations. Fibroblasts can synthesize TNF-α in response to angiotensin II after MI in rats (11, 37), and cardiomyocytes themselves produce TNF-α after mechanical stretch (13). Moreover, Irwin et al. (10) showed that TNF-α mRNA and protein are persistently expressed by myocytes in the noninfarcted region of the rat myocardium from 1 day to 5 wk after infarction.

Our data also confirm the absence of septal and right ventricular wall hypertrophy early after MI in accordance with Rubin et al. (29), who demonstrated that hypertrophy of the noninfarcted zone begins ~15 days after infarction. The histomorphometric study also indicated major LV dilation from 7 days after MI, with a tendency to increase 9 days after MI. These findings are supported by pressure-volume curves obtained ex vivo. Moreover, EI also progressively increased from 5 to 9 days after permanent ligation, indicating that this period after MI is probably crucial in the process of cardiac remodeling. LV dilation and infarct expansion might be explained by contractile dysfunction, because we observed a chronological correlation between increased LVEDP and the unfavorable evolution of morphometric indexes.

**TNF-α and Early Cardiac Dysfunction After MI**

Many studies have postulated that TNF-α might directly contribute to myocardial remodeling and dysfunction after MI, progressively leading to CHF. For example, Ono et al. (24) showed that an increase in LV wall TNF-α gene expression correlated with progressive ventricular dilation and with LV end-diastolic diameter after MI in rats. Other experimental studies have shown that pathophysiologically relevant peripheral and/or elevated intramyocardial levels of TNF-α are sufficient to mimic many aspects of the CHF phenotype, including LV dilation, LV dysfunction, cardiac hypertrophy and fibrosis, and activation of fetal gene programs (2, 4, 16).

Experimental and clinical studies previously demonstrated that specific anti-TNF-α is effective in reducing some of the deleterious cardiovascular effects of TNF-α in vitro and in vivo (2, 3, 12). Thus, to verify our first hypothesis of the direct involvement of TNF-α in early cardiac alterations after MI, we have studied the effects of sTNF-RII to establish whether TNF-α is a marker of or an actor in the geometric modifications and cardiac dysfunction observed 7 days after MI.

Our data demonstrate that sTNF-RII treatment improves LVEDP and operating LV end-diastolic volume 7 days after MI. This treatment also reduces geometric cardiac rearrangements initiated in the early phase after infarction, as shown by the improvement of DI and EI. sTNF-RII was administered intravenously 3 days after coronary artery ligation to avoid interference with the early postinfarction inflammatory process. Thus, in our model, the beneficial effects observed with sTNF-RII cannot be the consequence of reduced bioactivity of TNF-α in the infarcted zone, because there is no residual perfusion in this necrotic zone. Moreover, infarct size was comparable in sTNF-RII-treated and untreated MI rats. The dose of 40 μg/kg sTNF-RII in a single intravenous injection was used in the present study following the experimental procedure described by Xianzhong et al. (35), who demonstrated that cardiodepression in endotoxemic rats was totally abolished after specific anti-TNF-α injection at 40 or 80 μg/kg. Moreover, a single administration of this compound was shown to be sufficient to maintain pharmacological activity for ≥15 days (3).

Although mean dP/dmax and dP/dmin were not significantly different between MI rats receiving sTNF-RII and those receiving vehicle alone, the preservation of LVEDP and SV in sTNF-RII-treated MI rats and the lesser dilation of LV suggest a better contractility of the nonischemic regions of the myocardium. This improved contractile reserve was, moreover, highlighted under volume-overload conditions.

The limitation of ventricular dilation and the reduction of infarct expansion produced by sTNF-RII could be the result of an improvement of cardiac function or of an action on mechanical wall resistance. Indeed, besides reducing the deleterious consequences of ventricular filling pressures on dilation, sTNF-RII might modify the structural cohesion of the extra-cellular matrix of the border infarct zone. This hypothesis is supported by the literature showing that TNF-α, when infused continuously in rats at levels that occur in CHF, can reduce the fibrillar collagen weave in the LV myocardial wall (2) and increase the activity of metalloproteinases (19, 32). Therefore, in our study, interaction between metalloproteinases and TNF-α might occur, but without inducing any modification of mechanical wall resistance, because rigidity constants calculated from pressure-volume curves were not different in the MI groups (data not presented).

With regard to these data, we suggest that the beneficial effects of sTNF-RII on cardiac remodeling 7 days after infarction could be mainly due to a better intrinsic contractility of the myocardium, which limits the LVEDP increase.

**Effect of Early Inhibition of TNF-α After MI on Development of CHF**

On the basis of our first findings showing that TNF-α can induce a profound cardiac dysfunction and remodeling 7 days after MI, we have verified whether this early detrimental period after infarction plays a deleterious role in the subsequent development of CHF.

Thus the same protocol was performed 10 wk after MI, because it is generally considered that rats subjected to left coronary artery ligation exhibit CHF symptoms from 8 wk after large and transmural MI.

The present study demonstrates that the specific inhibitor of TNF-α, sTNF-RII, administered as a single injection 3 days after left coronary artery ligation in rats, improves LVEDP, AAo, and SV 10 wk after MI, when CHF has developed.

Because all geometric data were similar in both MI groups of rats, we can suggest that the cardioprotective action of sTNF-RII might be the consequence of a better preservation of the intrinsic functional capacity of myocytes of the noninfarcted regions of the heart, rather than an improvement of long-term cardiac remodeling. Further studies are required to verify this point. Finally, because rats were subjected to a single intravenous injection of sTNF-RII 3 days after MI and because this inhibitor can remain pharmacologically active for 2 wk, it is conceivable that the treatment has only delayed the progression of CHF and that the cardiac function of treated rats would have progressively deteriorated 10 wk after MI. This last hypothesis would be consistent with the lack of beneficial effect on long-term remodeling. Moreover, the aim of this
long-term study was to investigate the role that early (7 days after MI) events could play in the evolution toward CHF. For this reason, the anti-TNF-α intervention was limited to this period of time. The dual effects of TNF-α depending on TNF-α levels might also explain the lack of effect of our treatment on remodeling and pressure-volume curves. Indeed, as suggested by Mann (20), high TNF-α levels might be deleterious, whereas low physiological levels might be beneficial, in post-MI remodeling. Thus, in our model, TNF-α antagonism might attenuate the deleterious and beneficial effects of TNF-α. Nonetheless, our results clearly indicate that the 7-day period after MI is critical in the development of CHF.

Although no study has investigated the pathological role of TNF-α in the early phase after MI, diverse preclinical (2) and clinical trials (3) have attempted to demonstrate improved cardiac function in moderate-to-advanced CHF after TNF-α inhibition. Preliminary studies on a small number of patients have shown an improvement in LV ejection performance and quality of life after treatment with etanercept (a soluble TNF-α receptor type II fusion protein) for up to 3 mo (3). Nevertheless, the two multicenter clinical trials initiated in patients with CHF on the basis of these preliminary reports were stopped prematurely because of discouraging results (1). The two hypotheses formulated by Mann (20) to explain this lack of efficacy of the treatment were as follows. 1) Cytokine binding proteins such as etanercept might act as agonists for the cytokine that they bind (14). 2) Antagonism of TNF-α may result in the loss of one or more of the cytoprotective effects of TNF-α, as previously suggested (17, 23). However, the results of these clinical studies might also be explained if, during advanced CHF, TNF-α is only a marker of the pathology.

In contrast to the above-mentioned studies, the present work was designed to investigate the potential ability of an anti-TNF-α treatment to prevent the evolution of the ischemic myocardium toward CHF (rather than to improve the function of the already failing heart). Moreover, in the present study, the treatment was given to rats as a single injection, which might have avoided toxic effects of this compound resulting from stabilization of TNF-α at high concentrations in the peripheral circulation.

In conclusion, our data highlight the pathological role of TNF-α early after MI in rats. Profound cardiac function depression and cardiac geometric alterations occurred simultaneously with a peak of TNF-α 7 days after coronary occlusion. Moreover, this critical period contributed to the subsequent development of CHF. Thus, although this anti-TNF-α therapeutic strategy has been shown to be ineffective in the treatment of CHF, it could be beneficial in the prevention of this pathology if acute treatment is initiated early after MI.

GRANTS

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