Significance of changes in TNF-α and IL-10 levels in the progression of heart failure subsequent to myocardial infarction

K. Kaur, A. K. Sharma, and P. K. Singal
Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Center, and Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada
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Kaur, K., A. K. Sharma, and P. K. Singal. Significance of changes in TNF-α and IL-10 levels in the progression of heart failure subsequent to myocardial infarction. Am J Physiol Heart Circ Physiol 291: H106–H113, 2006. First published February 3, 2006; doi:10.1152/ajpheart.01327.2005.—We tested whether a decrease in the ratio of interleukin-10 (IL-10) to tumor necrosis factor-α (TNF-α) correlates with the decrease in cardiac function in heart failure. It has been suggested that TNF-α plays a role in the progression of heart failure, and the effect of TNF-α in many tissues is modulated by IL-10. Any relation of these two cytokines to heart failure has never been examined. Cardiac function was assessed by echocardiographic and hemodynamic techniques in coronary artery-ligated rats at 1, 4, 8, and 16 wk after myocardial infarction (MI). Membrane-bound and soluble fractions of TNF-α and IL-10 proteins, the ratio of TNF-α to IL-10, and TNF-α and IL-10 mRNA levels were analyzed. Losartan was used to modify cardiac function in rats 4 wk after MI to further validate the relation between the IL-10-to-TNF-α ratio and cardiac function. Cardiac function deteriorated with time in all coronary artery-ligated groups, with severe failure at 16 wk after MI. Membrane-bound and soluble TNF-α protein fractions were increased 1 and 4 wk after MI, whereas TNF-α mRNA was increased 4 and 8 wk after MI. Membrane-bound IL-10 protein and mRNA levels were decreased 4, 8, and 16 wk after MI. The decrease in the IL-10-to-TNF-α protein ratio in all coronary artery-ligated groups correlated with the depressed cardiac function. Losartan improved cardiac function, membrane-bound and soluble TNF-α and IL-10 protein levels, the ratio of IL-10 to TNF-α, and IL-10 mRNA. This study suggests that a decrease in IL-10 and IL-10-to-TNF-α ratio correlates with depressed cardiac function.

Exogenous administration of IL-10 protects against TNF-α-mediated oxidative stress-induced acute lung injury (33), which was augmented by IL-10 antibody (37). IL-10 has been reported to mitigate the other negative effects of TNF-α (5). Furthermore, IL-10 is known to shift the protease-antiprotease balance, favoring matrix preservation and, thus, promoting the healing of injured myocardium (25). These studies strongly suggest that the two cytokines may interact in a complex manner, such that the final physiological or pathophysiological effect is the sum total of a TNF-α-IL-10 interaction. Therefore, using a coronary ligation model of heart failure in rats, we examined the ratio of IL-10 to TNF-α in relation to cardiac function.

METHODS

Animals. All animal study protocols were approved by the University of Manitoba Animal Care Committee following the guidelines established by the Canadian Council on Animal Care. Male Sprague-Dawley rats (150 ± 10 g body wt) were maintained on standard rat chow and water ad libitum. Myocardial infarction (MI) was produced by occlusion of the left anterior descending coronary artery according to a method described elsewhere (23). Sham control animals were similarly handled, except the suture around the coronary artery was not tied. Body weight, general behavior, and mortality of the animals were monitored on a regular basis. Cardiac function was assessed at 1, 4, 8, and 16 wk after MI or sham operation. In the losartan-treated group, the drug (2 mg/ml) was added to the drinking water, starting on the day of surgery and continuing for 4 wk (31). Daily average water consumption was ~30 ml in losartan-treated sham-operated and MI animals.

Function assessment. Transthoracic echocardiographic images of the hearts of all groups were obtained using a 12-MHz ultrasound probe (model s12, Agilent Technologies) and an echograph (Sonos 5500, Agilent Technologies). For M-mode recordings, the parasternal short-axis view was used to image the heart in two dimensions at the level of the papillary muscles. Left ventricular fractional shortening (FS) and ejection fraction (EF) were recorded.

For hemodynamic measurements, the rats were anesthetized and left ventricular end-diastolic pressure (LVEDP), left ventricular peak systolic pressure (LVSP), and the maximum rate of isovolumic pressure development and decay (+dP/dt and −dP/dt) were recorded as described elsewhere (23). After these assessments, the rats were killed and the hearts were removed and processed (see below).

Myocardial TNF-α and IL-10 protein and mRNA levels. Hearts were washed with PBS. Viable ventricular tissue was flash frozen in liquid nitrogen. Frozen tissue (0.5–1.0 g) was homogenized, and membrane-bound and soluble fractions of TNF-α and IL-10 proteins were collected (39) and analyzed by ELISA using a commercially available kit (R & D Systems, Minneapolis, MN).

Despite intense research efforts, the death rate due to heart disease remains at an unacceptable level. A better understanding of the molecular mechanisms of myocyte death and/or heart dysfunction is necessary to develop specific therapeutic strategies to slow or prevent the progression of heart failure. The proinflammatory cytokine TNF-α is overexpressed in humans and animals with left ventricular dysfunction, cardiomyopathy, and heart failure (40). The anti-inflammatory cytokine IL-10 is reported to downregulate the expression of several proinflammatory cytokines, including TNF-α (32). Although proinflammatory cytokines are generally thought to be produced by cells of the immune system, recent observations have confirmed TNF-α production by the myocardial cells (22). TNF-α is expressed in two forms: a 26-kDa membrane-bound fraction and a 17-kDa soluble fraction (30). IL-10 is also expressed as membrane-bound and soluble fractions (7).

Address for reprint requests and other correspondence: P. K. Singal, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Center, 351 Tache Ave., Rm. 3022, Winnipeg, MB, Canada R2H 2A6 (e-mail: psingal@sbrc.ca).

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RNA was isolated from the frozen myocardial tissue using TriReagent (Molecular Research Center) according to the manufacturer’s instructions. RT-PCR was performed on total RNA (4 μg), which was subjected to first-strand synthesis of cDNA using a commercially available kit (Ambion, Austin, TX). The reaction mixture was stored at −20°C until the PCR step.

The cDNA (5 μl) was subjected to PCR. The PCR mixture contained 10 μl of 10× PCR buffer, 2 μl of 25 mM MgCl2, 2 μl of 10 mM dNTP mix, 2.5 U of thermostable DNA polymerase, and each primer at 100 pM as follows: β-actin (bp 315), 5′-TGGAGAAGAGCTATGAGCTGC-3′ (sense) and 5′-CTCACAGAGATCTTTGCG-3′ (antisense); IL-10 (bp 469), 5′-GCTCAGACTGTATGGTG-3′ (sense) and 5′-TTTGTGGCTTGAGACAC-3′ (antisense); and TNF-α (bp 318), 5′-CACCCTCCTCCAGCTGGAGAAGG-3′ (sense) and 5′-GGCCACTCTCAGCTGCTATGTT-3′ (antisense). Each sample was subjected to denaturation at 94°C for 30 s, 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 30 s, 35 cycles of denaturation at 94°C for 60 s, and extension at 72°C for 120 s, with final extension at 72°C for 10 min. The PCR mixture was analyzed on a 2% agarose gel by electrophoresis.

Protein and statistical analysis. Total protein concentration was determined by the Bradford method (6). Values are means ± SE. For statistical analysis of the data, group means were compared by one-way ANOVA, and Bonferroni’s test was used to identify differences between groups. P < 0.05 was considered significant.

RESULTS

Mortality in the coronary artery-ligated animals, during or immediately after the surgery, was ~20%. Another 13% of the animals died within 24 h after the surgery. There was no significant difference in body weight gain between coronary artery-ligated animals and their respective sham-operated controls. At 16 wk, animals subjected to MI appeared lethargic, with clear signs of heart failure, as indicated by dyspnea and cyanosis of the peripheral extremities.

Cardiac function assessment. Echocardiographic studies revealed differences between sham-operated controls and their respective MI groups as early as 4 wk after coronary ligation (Fig. 1, see Fig. 6). At 1 wk after surgery, FS was 39.5 ± 2.2% and EF was 70.7 ± 4.6% in the sham control group, and these values did not change. In the animals subjected to MI, FS and EF decreased significantly at all time points starting at 4 wk (Tables 1 and 2; Fig. 1, see Fig. 6). LVSP, +dP/dt, and −dP/dt were significantly decreased at 4, 8, and 16 wk after MI. LVEDP was significantly increased in all animals subjected to MI (Tables 1 and 2).

Myocardial TNF-α and IL-10 protein and mRNA levels. The control baseline value for membrane-bound TNF-α was 8.17 ± 1.46 pg/mg protein. Membrane-bound TNF-α was significantly (P < 0.008) increased by 88% at 1 wk after MI and by 198% at 4 wk after MI (P < 0.003; Fig. 2A). At 8 and 16 wk after MI, these TNF-α levels were still higher by 59 and 29%, respectively, but the differences were not statistically significant (Fig. 2A). In the sham control group, soluble TNF-α was 4.11 ± 0.50 pg/mg protein. This value was significantly increased by 49% in the 1 wk post-MI group (P < 0.042) and...
by 197% in the 4-wk post-MI group ($P < 0.001$; Fig. 2B). Although there was 74% increase in soluble TNF-α at 8 wk after MI, this difference was not significant ($P > 0.06$). At 16 wk after MI, there was no change in soluble TNF-α compared with the sham control (Fig. 2B).

Membrane-bound IL-10 in the sham control group was $28.74 \pm 6.51$ pg/mg protein. Although membrane-bound IL-10 was decreased by $\sim 50\%$ in the 1-wk post-MI group, this decrease was not significant ($P = 0.07$; Fig. 3A). Membrane-bound IL-10 at 4, 8, and 16 wk after MI was significantly decreased by 73, 77, and 79% of sham control values, respectively. The baseline value for soluble IL-10 protein in the sham control group was $4.40 \pm 0.74$ pg/mg protein. There was no significant difference in soluble IL-10 protein levels between different post-MI groups and baseline (Fig. 3B).

The ratio of membrane-bound IL-10 to membrane-bound TNF-α protein in the sham control group was $3.45 \pm 0.21$. This value was significantly decreased at all times after MI.

### Table 2. Effects of losartan on cardiac function in 4-wk sham and 4-wk post-MI rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4 wk Losartan</th>
<th>4 wk Post-MI</th>
<th>4 wk Sham</th>
<th>4 wk Post-MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF, %</td>
<td>73.15 ± 3.31</td>
<td>49.56 ± 6.39*</td>
<td>76.32 ± 4.20</td>
<td>62.36 ± 5.23</td>
</tr>
<tr>
<td>FS, %</td>
<td>39.54 ± 2.15</td>
<td>26.98 ± 3.87*</td>
<td>42.36 ± 3.26</td>
<td>39.56 ± 3.36</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>117.94 ± 4.24</td>
<td>87.21 ± 9.05*</td>
<td>108.65 ± 5.69</td>
<td>96.56 ± 8.95</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>3.33 ± 0.49</td>
<td>14.38 ± 1.22†</td>
<td>3.65 ± 1.2</td>
<td>7.56 ± 0.98‡§</td>
</tr>
<tr>
<td>$+\Delta p/\Delta t$, mm Hg/s</td>
<td>9.49 ± 422.87</td>
<td>6.80 ± 844.99*</td>
<td>8.95 ± 562</td>
<td>7.89 ± 759</td>
</tr>
<tr>
<td>$-\Delta p/\Delta t$, mm Hg/s</td>
<td>$-10.30 \pm 602.81$</td>
<td>$-7.35 \pm 774.32$*</td>
<td>$-9.89 \pm 759$</td>
<td>$-8.26 \pm 689$</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4–6 rats. Significantly different from sham: *$P < 0.05$; †$P < 0.005$. ‡Significantly different from 4 wk post-MI ($P < 0.05$). §Significantly different from sham-losartan.

Fig. 2. Myocardial membrane-bound (A) and soluble (B) TNF-α protein expression in rat hearts at 1, 4, 8, and 16 wk after myocardial infarction by ELISA. 4W PMI, 4 wk after myocardial infarction. Values are means ± SE ($n = 4$). Significantly different from Sham: *$P < 0.05$; **$P < 0.005$.

Fig. 3. Myocardial membrane-bound (A) and soluble (B) IL-10 protein expression in rat hearts at 1, 4, 8, and 16 wk after myocardial infarction by ELISA. Values are means ± SE ($n = 4$). *Significantly different from Sham ($P < 0.05$).
The 4-wk post-MI group showed a maximum decrease to 0.34 ± 0.08 (Fig. 4A). This ratio of soluble IL-10 to soluble TNF-α was significantly decreased to 19.33% only at 4 wk after MI (Fig. 4B).

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TNF-α mRNA was significantly increased by 89% at 4 wk after MI (P < 0.01) and by 131% at 8 wk after MI (P < 0.001) compared with sham control. Changes in TNF-α mRNA in the 1- and 16-wk post-MI groups were not significantly different from the sham control group (Fig. 5A). IL-10 mRNA was significantly reduced at all times after MI compared with sham control (Fig. 5B), with the maximum decrease in the 16-wk post-MI group.

Effects of losartan on myocardial function and cytokines. Losartan treatment had no apparent effect on general appearance and weight gain in the sham control and MI groups. There was no significant difference in body weight between sham and MI groups. Losartan treatment improved FS and EF in the 4-wk post-MI group, such that there was no significant difference between the drug-treated sham and post-MI groups (Fig. 6, Table 2). In the untreated 4-wk post-MI group, LVEDP was increased by 331%. In the losartan-treated group, this increase was only 107%. LVPSP, +dP/dt, and −dP/dt were close to normal in the losartan-treated 4-wk post-MI group (Table 2).

Membrane-bound and soluble TNF-α levels were significantly decreased in the losartan-treated 4-wk post-MI group compared with the untreated 4-wk post-MI group (P < 0.049; Fig. 7). However, these values were significantly higher in the losartan-treated post-MI group than in the losartan-treated sham group. There was no effect of losartan on membrane-bound and soluble TNF-α protein in the sham animals.

Levels of membrane-bound and soluble IL-10 in the losartan-treated 4-wk post-MI group were significantly improved compared with the untreated 4-wk post-MI group (Fig. 8). Membrane-bound IL-10 levels in losartan-treated post-MI and sham groups were not different (Fig. 8A). Soluble IL-10 was increased by losartan treatment in the sham and 4-wk post-MI groups. However, this increase was significant only in the post-MI group (Fig. 8B).

Losartan treatment significantly improved the ratio of membrane-bound and soluble fractions of IL-10 to TNF-α protein (Fig. 9). The ratio of membrane-bound IL-10 to membrane-bound TNF-α protein was significantly less in the losartan-treated 4-wk post-MI group than in the losartan-treated sham group (Fig. 9A).

There was no effect of losartan treatment on TNF-α mRNA in the sham control group. The difference in TNF-α mRNA...
Fig. 6. Echocardiographic images of rat heart at 4 wk after myocardial infarction and sham operation with and without losartan (Los) treatment.

Fig. 7. Effects of losartan on myocardial membrane-bound (A) and soluble (B) TNF-α protein expression at 4 wk after myocardial infarction in rat hearts by ELISA. Values are means ± SE (n = 4). #Significantly different from Sham + Los (P < 0.05). **Significantly different from sham (P < 0.005). #Significantly different from 4W PMI (P < 0.05).

Fig. 8. Effects of losartan on myocardial membrane-bound (A) and soluble (B) IL-10 protein expression at 4 wk after myocardial infarction in rat hearts by ELISA. Values are means ± SE (n = 4). #Significantly different from Sham + Los (P < 0.05). **Significantly different from sham (P < 0.005). #Significantly different from 4W PMI (P < 0.05).
levels in the treated and untreated 4-wk post-MI groups was not significant (Fig. 10A).

There was no significant difference in the levels of IL-10 mRNA between treated and untreated sham groups. IL-10 mRNA was significantly improved toward control levels in the losartan-treated 4-wk post-MI group (Fig. 10B). However, this value was still less in the losartan-treated 4-wk post-MI group than in the losartan-treated sham-operated animals.

**DISCUSSION**

Cytokines have been suggested to play an important role in the pathogenesis of cardiovascular diseases. Membrane-bound TNF-α has been suggested to have a more localized effect than soluble TNF-α and has also been implicated in several other disease conditions, such as acute hepatitis, rheumatoid arthritis, and neurological disorders (2, 19, 24). Several clinical studies have examined circulating levels of TNF-α and IL-10 in cardiac patients (20, 38). In animal studies, changes in TNF-α subsequent to coronary ligation have been examined for only shorter time intervals (10, 17, 36), whereas changes in myocardial levels of IL-10 subsequent to MI have not been reported. The present study describes changes in TNF-α and IL-10 at different times after MI in functionally assessed hearts. The study shows that the depressed cardiac function, subsequent to coronary ligation, correlates with a decrease in IL-10 and the ratio of IL-10 to TNF-α.

The echocardiographic, as well as hemodynamic, data showed depressed function at early time points, and the condition progressed to clear signs of severe congestive heart failure in the 16-wk post-MI animals. Heart failure in this model of coronary ligation has also been reported by many others (11, 15, 23).

The baseline values for membrane-bound and soluble TNF-α are in the range reported for a normal heart (10, 16, 36). A significant increase in membrane-bound and soluble TNF-α in the 1- and 4-wk post-MI groups is in agreement with the data from other laboratories reporting a rise in this cytokine during early stages of heart failure (10, 17, 18, 22). Overexpression of TNF-α in heart failure has been reported (40). However, our data show that at later stages of heart failure, i.e., 8 and 16 wk after MI, TNF-α levels return close to baseline. It is known that
anti-TNF-α therapy is beneficial only if instituted in the early stages of heart failure (4). Etanercept, a soluble TNF-α receptor (28), and infliximab, a TNF-α monoclonal antibody (8), were ineffective in patients with frank heart failure. It is likely that the early rise in membrane-bound TNF-α in this study has some role in the depressed cardiac function at these early stages, where anti-TNF-α therapy is seen to be beneficial (4). Lack of any change in TNF-α at later stages of heart failure in our study may explain the failure of anti-TNF-α therapy in the late stages of heart failure (8, 28).

The early rise in TNF-α protein may have been due to an increase in TNF-α mRNA at these stages. Interestingly, TNF-α mRNA was significantly higher, even at 8-wk after MI, when TNF-α protein declined toward control levels. A delayed increase in myocardial TNF-α mRNA during MI has also been reported by others (34). In losartan-treated animals, TNF-α protein showed a significant decrease, whereas there was no drop in TNF-α mRNA. In this regard, IL-10 downregulates the synthesis of TNF-α (12, 14, 26, 43), and an early drop in IL-10 may have supported the increase in TNF-α at these time points. Furthermore, any increase in IL-10 induced by losartan may have interfered with the synthesis of TNF-α. Because angiotensin is known to have proinflammatory effects, losartan may also have an anti-inflammatory influence by blocking receptors for angiotensin (9). At any rate, there appears to be a disconnect between transcription and translation of TNF-α at later stages of heart failure, and/or there is more degradation of this protein with the severity of the disease.

The levels of membrane-bound IL-10 were reduced at 1 wk after MI, and this reduction was significant at 4, 8, and 16 wk after MI, which correlated with the depressed cardiac function. A direct correlation between myocardial IL-10 mRNA and heart failure with time has been reported (41). This decrease in IL-10 protein could be due to a drop in IL-10 mRNA in these stages of heart failure. Interestingly, there was no change in soluble IL-10 at any time point. Most of the clinical studies in cardiac patients show no change in the plasma level of IL-10 (21), which most likely represents soluble IL-10. IL-10 production by mononuclear cells and monocytes in cardiac patients was also not changed (3, 42). Consistency in circulating levels of this cytokine may be due to the lack of change in the soluble fraction of IL-10 in the heart as well as a lack of change in the production of soluble IL-10 by blood cells. Thus our study also suggests that the change in IL-10 during progression of heart failure mainly occurs in the membrane fraction, highlighting the importance of localized effects of this protein.

The IL-10-to-TNF-α ratio in the membrane-bound fraction was significantly decreased to ~0.5 at all times after MI. Patients with advanced congestive heart failure (New York Heart Association classes III and IV) also show a low (i.e., 0.312) IL-10-to-TNF-α ratio (38). Improvement in cardiac function after dexamethasone, growth hormone, or steroid treatment has been associated with an increase in IL-10 and/or a decrease in TNF-α, thus shifting the cytokines in the anti-inflammatory direction (1, 13, 20). Growth hormone treatment in idiopathic dilated cardiomyopathy improved the cytokine IL-10-to-TNF-α ratio from 1.9 to 3.5 (1). To further test the validity of the IL-10-to-TNF-α correlation with the post-MI cardiac function, we used losartan to improve cardiac function and examined its relation to the IL-10-to-TNF-α ratio. These data provide the first experimental evidence that improved function is associated with improved IL-10-to-TNF-α ratio. An imbalance between IL-10 and TNF-α has been suggested to play a role in atherosclerotic lesions in stable and unstable angina (27, 29, 42).

The change in the balance between anti-inflammatory (IL-10) and proinflammatory (TNF-α) cytokines in this study suggests the importance of the ratio of IL-10 to TNF-α, rather than changes in TNF-α levels alone. Although no change was observed in TNF-α protein in the later stages of heart failure, the IL-10-to-TNF-α ratio was greatly reduced. This correlated with depressed cardiac function, suggesting that even though there was no increase in the inflammatory cytokine TNF-α, a decrease in the anti-inflammatory cytokine IL-10 can also cause the disease to progress by affecting other inflammatory proteins that are transcriptionally controlled by NF-kB (14). It has been suggested that IL-10 may exert anti-inflammatory properties by inhibiting NF-kB as follows: 1) blocking NF-kB translocation by inhibitory kB kinase activity, thus preventing degradation of inhibitory kB (26, 42), and 2) inhibiting DNA binding of NF-kB, which is present in the nucleus, without increasing the nuclear levels of inhibitory kB (35). It is also important to note that for describing the role of complex molecules, such as cytokines, in heart failure, membrane-bound vs. soluble fractions should also be recorded.

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REFERENCES
Cytokines and Heart Failure


