Cytokine-Induced Modulation of Cardiac Function

Sumanth D. Prabhu

Abstract—Cytokines act in an autocrine and/or paracrine fashion to induce a diverse variety of biological responses. Several cardiac diseases are associated with cytokine activation, and such activation significantly influences several physiologic parameters, including cardiac mechanical function. This review summarizes the current concepts regarding the modulation of myocardial function by cytokines and provides rationale for the sometimes-conflicting results in the literature regarding underlying mechanisms and patterns of dysfunction. Although traditionally considered cardiodepressant mediators, contractile responses are complex and bimodal, with an early response (within minutes) of variable direction, stimulatory or depressant, depending on the ambient physiologic milieu and relative contributions of the underlying signaling pathways that are activated. These pathways include sphingomyelinase-, nitric oxide (NO)-, and phospholipase A2-dependent signaling with resultant combined effects on contraction and the Ca\(^{2+}\) transient. This is subsequently followed by a profoundly cardiodepressant late response lasting hours to days, depending on the production of secondary mediators and the combined influence of NO generated from inducible NO synthase, reactive oxygen species, and alterations in \(\beta\)-adrenergic receptor signaling. The interrelationships between these pathways and the time-dependence of their activation are important considerations in the evaluation of cytokine-dependent dysfunction during both acute cardiac injury and chronic cardiac pathologies. (Circ Res. 2004;95:1140-1153.)

Key Words: cytokines ■ myocardial contraction ■ nitric oxide ■ sphingosine ■ free radicals

Cytokines are polypeptide or glycoprotein factors that under normal conditions act in an autocrine and/or paracrine fashion via specific membrane-bound receptors to signal a variety of physiological responses. Although traditionally classified as pro- or antiinflammatory proteins, cytokines impart diverse and pleiotropic biologic effects, and in response to environmental stress, these proteins are elaborated by a variety of cells, including all resident cell types in the myocardium. A broad range of cardiac disease has been associated with cytokine activation. These include heart failure,\(^1,2\) cardiac reperfusion injury,\(^3\) myocarditis,\(^4\) cardiac allograft rejection,\(^5\) and sepsis-associated cardiac dysfunction.\(^6\)–\(^8\)

Cytokines can impact myocardial function via effects on both myocyte contractility and the extracellular matrix. This review focuses on the former, ie, the manifestations and mechanisms of cytokine effects on intrinsic contractility and mechanical function. Although modulation of the interstitial matrix is also an important contributor to cytokine-mediated myocardial dysfunction (see Bradham et al\(^9\) and Li et al\(^10\) for review), proper consideration of these events is beyond the scope of this review. The ensuing discussion will primarily examine the proinflammatory cytokines tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), interleukin (IL)-1\(\beta\), and IL-6 and the immunomodulatory cytokine IL-2, insofar as these mediators have received the greatest attention in the literature. Although
these cytokines are typically considered to impart negative inotropic effects, the nature and pattern of the inotropic response is complex, consisting of an immediate response within minutes that can be either stimulatory or depressant, depending on the experimental conditions and physiologic milieu, and a delayed response lasting hours to days that is uniformly cardiodepressant and dependent on the production of secondary mediators. The cellular mechanisms underlying these effects are multiple. Nitric oxide (NO) derived from constitutive NO synthase (cNOS), sphingolipid mediators, arachidonic acid (AA), and alterations in intracellular Ca2+ handling have all been shown to contribute to the immediate contractile effects, whereas the delayed response results primarily from the combined influence of NO generated from inducible NOS (iNOS), the production of reactive oxygen species (ROS), and alterations in β-adrenergic receptor (β-AR) signaling.

**Hemodynamic and Mechanical Effects of Proinflammatory Cytokines**

**In Vivo Studies**

The notion that proinflammatory cytokines induce left ventricular (LV) dysfunction was first gleaned from animal and human studies of sepsis. These established that sepsis-associated cardiac dysfunction was caused by circulating myocardial depressant substances, later identified, in large part, to be inflammatory cytokines (including TNF-α, IL-1β, IL-6, IL-2, and interferon-γ [IFN-γ]).11-13 In intact animals, intravenous administration of either TNF-α11-13 or IL-1β12 recapitulated the hemodynamic changes of endotoxemia and endotoxic shock, whereas the administration of either IL-1β receptor antagonist14 or anti–TNF-α antibodies15,16 ameliorated the cardiovascular abnormalities and mortality. In addition to these insights from studies of sepsis, it was noted that immunomodulatory therapy for cancer using TNF-α and IL-2 was limited by dose-dependent cardiovascular depression and negative inotropy.16,17 These observations were extended by studies in rats demonstrating that sustained infusion of TNF-α caused time-dependent contractile dysfunction and LV dilatation that was partially reversed by stopping the infusion or on concomitant treatment with a TNF-α antagonist,18 and studies in dogs showing that intracoronary injection of IL-1β coated microspheres induced sustained LV dysfunction.19 This concept was subsequently confirmed by the observation that cardiac-specific TNF-α overexpression in mice induced LV dilatation, reduced ejection fraction, depressed catecholamine responsiveness, and premature mortality.20

Several studies have specifically evaluated the in vivo effects of cytokines on cardiac function, often using TNF-α as a prototypic proinflammatory cytokine (Table 1). Early studies in conscious dogs indicated that a single infusion of recombinant human (rh) TNF-α resulted in the delayed appearance (2 hours to 2 days postinfusion) of LV systolic dysfunction, as assessed by the load-sensitive index LV ejection fraction, that could persist for up to 10 days, depending on the dose administered.13,21 The decline in left ventricular ejection fraction was not normalized by volume resuscitation, suggesting intrinsic cardiac dysfunction rather than a preload-dependent response. Subsequent investigations using LV pressure-volume (or pressure-dimension) indexes confirmed the delayed onset of LV dysfunction following TNF-α infusion (from 2 to 24 hours after initiation of the infusion, depending on the contractile parameter examined), as assessed by the relatively load-independent parameters end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), or peak dP/dt normalized for end-diastolic volume (EDV)22-25 (Figure 1). Reduced LV performance has also been associated with several diastolic abnormalities including slowing of relaxation, LV dilatation without changes in

**TABLE 1. Mechanical Effects of Cytokines on LV Function In Vivo**

<table>
<thead>
<tr>
<th>Effects/Reference</th>
<th>Cytokine</th>
<th>Dose</th>
<th>Route</th>
<th>Species</th>
<th>Inotropic Effects</th>
<th>Parameter(s) Examined</th>
<th>Mechanism Suggested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early effects (0 to 3 hours)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>rhTNF-α</td>
<td>100</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Positive</td>
<td>dP/dtmax (normalized)</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>23</td>
<td>rhTNF-α</td>
<td>60</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>No change</td>
<td>dP/dtmax (normalized)</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>24</td>
<td>rhTNF-α</td>
<td>40</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Positive</td>
<td>Ees, dP/dtmax (normalized)</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>28</td>
<td>rhTNF-α</td>
<td>50–200</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Positive</td>
<td>PRSW</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>Delayed effects (hours to days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>rhTNF-α</td>
<td>60</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>LV contractility</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>18</td>
<td>rhTNF-α</td>
<td>2.5</td>
<td>μg/kg/min</td>
<td>15 days osmotic infusion Rat</td>
<td>Negative</td>
<td>LV and myocyte shortening</td>
<td>Sphingolipid mediators</td>
</tr>
<tr>
<td>21</td>
<td>rhTNF-α</td>
<td>30–120</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>LV contractility</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>22</td>
<td>rhTNF-α</td>
<td>100</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>dP/dtmax, PRSW</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>23</td>
<td>rhTNF-α</td>
<td>60</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>dP/dtmax, Ees</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>24</td>
<td>rhTNF-α</td>
<td>40</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>Ees, PRSW, dP/dtmax</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>25</td>
<td>rhTNF-α</td>
<td>40</td>
<td>μg/kg</td>
<td>Intravenously over 1 hour Dog</td>
<td>Negative</td>
<td>Ees, dP/dtmax</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>19</td>
<td>rhIL-1β</td>
<td>1</td>
<td>μg/kg</td>
<td>Intracoronary Dog</td>
<td>Negative</td>
<td>LV contractility</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>26</td>
<td>rhIL-1β</td>
<td>1</td>
<td>μg/kg</td>
<td>Intracoronary Dog</td>
<td>Negative</td>
<td>LV contractility</td>
<td>Reflex independent</td>
</tr>
<tr>
<td>27</td>
<td>rhIL-1β</td>
<td>1</td>
<td>μg/kg</td>
<td>Intracoronary Dog</td>
<td>Negative</td>
<td>LV contractility</td>
<td>Reflex independent</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; Ees, maximal elastance; dP/dtmax, slope of the dP/dtmax-end diastolic volume relation; ONOO-, peroxynitrite.
end-diastolic pressure (myocardial creep), and a leftward shift of the end-diastolic pressure–strain relation, indicating increased diastolic stiffness. Prolonged depression of LV performance in vivo has also been observed in a canine model using intracoronary administration of IL-1β coated microspheres. Generally, cytokine-mediated contractile dysfunction is reversible over an extended time period of several days following exposure.

In contrast, the few studies that have examined the immediate contractile effects of cytokines in vivo have generally reported a distinct early stimulatory effect of TNF-α on inotropy and lusitropy (Table 1). (One other study reported no detectable early effect.) Specifically, within the first 1 to 3 hours after TNF-α administration, there is an augmentation of several contractility indexes including Ees, the slope of the dP/dt max–EDV relation, and dP/dt max normalized for EDV, together with a leftward shift of the PRSW relation and a reduction in the time constant of relaxation, τ (Figure 1). Importantly, the increase in contractility and lusitropy was not related to variations in heart rate or to cardiovascular reflex responses, as they persisted in the presence of β-AR and vagal blockade, and occurred before the maximal surge in circulating catecholamines.

Thus, taken together, the aforementioned in vivo studies indicate that proinflammatory cytokines induce a biphasic effect on intrinsic cardiac function comprised of an early positive inotropic and lusitropic effect of relatively short duration (demonstrated primarily for TNF-α), followed by a delayed and prolonged phase of profound systolic and diastolic dysfunction (shown for both TNF-α and IL-1β). The rapid onset of the early stimulatory response (as early as 5 to 15 minutes in one study) suggests a direct myocardial effect via mechanisms not requiring gene expression, whereas the delayed onset and prolonged duration of the late cardiodepressant response suggest an indirect effect requiring the induction of de novo gene expression and protein synthesis and the activation of secondary mediators. Generally, proinflammatory cytokines can be considered to impart negative inotropic and cardiodepressant effects in pathophysiological states with sustained, chronic augmentation of cytokine expression.

**In Vitro and Ex Vivo Studies**

Analogous to investigations in vivo, a biphasic contractile response has also been observed in single myocyte, muscle strip, and isolated heart preparations, with early effects generally within 30 minutes and delayed effects thereafter and up to 48 to 72 hours (Tables 2 and 3). These studies have uniformly reported delayed cytokine-mediated depression of either basal or stimulated myocardial function, but have reported conflicting results regarding the immediate effects, with the preponderance of in vitro and ex vivo studies also indicating early cytokine-induced cardiodepression.

Pressure-volume studies of the isolated, blood-perfused canine heart have shown that TNF-α acutely impacts LV mechanoenergetics by increasing the oxygen cost of contractility, an effect ascribed to alterations in energy utilization for excitation-contraction (E-C) coupling. Coronary vasoconstriction also contributes indirectly to TNF-α-induced early or late contractile dysfunction in isolated, crystalloid-perfused rat hearts. In contrast, fewer studies support either an early stimulatory (or dual) effect or no detectable immediate contractile effect of specific proinflammatory cytokines.

The basis for the sometimes-discrepant results in the aforementioned studies regarding the immediate inotropic effects of cytokines is not fully clear but may be related to several factors. First, although single myocyte, papillary

---

**Figure 1.** TNF-α induces a bimodal inotropic response in vivo. TNF-α (40 μg/kg over 1 hour) was administered to conscious dogs and LV function was monitored over 24 hours. The left graph plots dP/dt max at a common EDV as an index of LV contractility. The right graph plots V100, or the end-systolic (ES) volume (V) at an ES pressure (P) of 100 mm Hg derived from the ESPV relation, as an index of LV performance. This was subsequently followed by a delayed and profound reduction in preload-adjusted dP/dt max and a shift of the ESPVR to the right, indicative of negative inotropy and LV dilatation. Reprinted from Murray DR, Freeman GL. Tumor necrosis factor-α induces a biphasic effect on myocardial contractility in conscious dogs. Circ. Res. 1996;78:154–160, by permission of the American Heart Association © 1996.
muscle, and isolated heart models can be manipulated with greater ease and higher specificity, they are inherently less physiological than the intact state, which is subject not only to direct myocardial effects but also to complex and integrated vascular, neural, hematologic/immunologic, and endocrino-logic changes effected by the cytokine(s) of interest. Thus, that the cardiac responses seen in the intact animal do not precisely parallel those in vitro is perhaps not surprising. Second, in physiological contexts, cytokines primarily act locally to induce effects that are determined not only by the

<table>
<thead>
<tr>
<th>Effect/Mechanism Suggested</th>
<th>Cytokine</th>
<th>Effective Concentration Range*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate cardiodepressant effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General (all studies)</td>
<td>TNF-α</td>
<td>0.005–2000 ng/mL</td>
<td>3,7,55–58,63–72,75,78</td>
</tr>
<tr>
<td>IL-1β (and IL-1α)</td>
<td></td>
<td>0.4–1000 ng/mL</td>
<td>7,33,58,59,64,70,76,79,80</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.1–3 ng/mL</td>
<td>35,60,68,75</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.5–1000 U/mL</td>
<td>58,61,62,68,77</td>
<td></td>
</tr>
<tr>
<td>IL-1β/TNF-α/IFN-γ ng/mL: IL-1β, 5; TNF-α, 20; IFN-γ, 9</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β transient depression</td>
<td>TNF-α</td>
<td>10–900 ng/mL</td>
<td>3,73–75,78</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5 ng/mL</td>
<td>79.80 (both lα)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1 ng/mL</td>
<td>35,75</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>2.5–1000 U/mL</td>
<td>62,77</td>
<td></td>
</tr>
<tr>
<td>cNOS-derived NO</td>
<td>TNF-α</td>
<td>0.125–2000 ng/mL</td>
<td>64,66,68–70,72</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.05–100 ng/mL</td>
<td>33,64,70</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.15–3.2 ng/mL</td>
<td>35,68,75</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>1–1000 U/mL</td>
<td>61,68</td>
<td></td>
</tr>
<tr>
<td>Sphingolipid mediators</td>
<td>TNF-α</td>
<td>0.125–50 ng/mL</td>
<td>30,63–66,75,84</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.05–5 ng/mL</td>
<td>64,80</td>
<td></td>
</tr>
<tr>
<td>Altered mechanoenergetics</td>
<td>TNF-α</td>
<td>90 μg/min for 20 minutes</td>
<td>66 (increased unloaded MVO₂)</td>
</tr>
<tr>
<td>Coronary vasoconstriction</td>
<td>TNF-α</td>
<td>20 ng/mL</td>
<td>65,81</td>
</tr>
<tr>
<td>Impaired β-AR response</td>
<td>TNF-α</td>
<td>25–50 ng/mL</td>
<td>71,75</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.4–4 ng/mL</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1 ng/mL</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>200 U/mL</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Immediate cardiostimulatory (or dual) effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General (all studies)</td>
<td>TNF-α</td>
<td>5–50 ng/mL</td>
<td>30,69,84</td>
</tr>
<tr>
<td>IL-1β and TNF-α ng/mL: IL-1β, 5; TNF-α, 20</td>
<td>2 U/mL</td>
<td>82,83 (both atrial muscle)</td>
<td>31</td>
</tr>
<tr>
<td>IL-2</td>
<td>2 U/mL</td>
<td>82,83 (both atrial muscle)</td>
<td></td>
</tr>
<tr>
<td>PLA2 and arachidonic acid (PKC also suggested)</td>
<td>TNF-α</td>
<td>10–50 ng/mL</td>
<td>30</td>
</tr>
<tr>
<td>IL-2</td>
<td>2 U/mL</td>
<td>82,83 (both atrial muscle)</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>IL-1β and TNF-α</td>
<td>ng/mL: IL-1β, 5; TNF-α, 20</td>
<td>31</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>TNF-α</td>
<td>10 ng/mL</td>
<td>69</td>
</tr>
<tr>
<td>GSH- and redox-related</td>
<td>TNF-α</td>
<td>25 ng/mL</td>
<td>84</td>
</tr>
<tr>
<td>No detectable immediate contractile effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General (all studies)</td>
<td>TNF-α</td>
<td>100 ng/mL</td>
<td>32</td>
</tr>
<tr>
<td>IL-1β</td>
<td>25 ng/mL</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.5–3000 ng/mL</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.1–10 000 U/mL</td>
<td>7,85,86 (atrial muscle)</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>4–1000 ng/mL</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>IL-1β/TNF-α/IFN-γ ng/mL: IL-1β, 5; TNF-α, 20; IFN-γ, 9</td>
<td>38,39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Assuming 50 pg/U for TNF-α and 1 pg/U for IL-6. ONOO⁻ indicates peroxynitrite; MVO₂, myocardial oxygen consumption.
target cell type but also by the prevailing cellular and biological milieu.87 Furthermore, cytokines are generally not elaborated in isolation but rather as part of a complex network involving multiple other cytokines and biologically active molecules. (For example, concomitantly elevated levels of several inflammatory mediators characterize pathologic states such as heart failure, myocarditis, and sepsis.1,2,4,7,8) As different cytokines can have additive, synergistic, or antagonistic effects,4,7,12,29,47,88 this will complicate the responses seen in vivo or in blood-perfused ex vivo models, such that the end-effects may not be determined solely by the index cytokine. Third, the ambient cellular redox and/or metabolic state should be considered. As a case in point, one suggested mechanism of cytokine-mediated immediate cardiodepressant effect (when reported) generally occurs very early (within 5 to 15 minutes) after exposure and at lower cytokine concentrations,30,31,69,84 whereas negative inotropic effects become pronounced with longer periods of exposure and/or higher concentrations. Finally, Stamm et al78 have reported significant species-specificity with regard to the inotropic effects of recombinant cytokines; this factor may also account for some of the variability among studies that have used cross-species sources of cytokines.

### Mechanisms of Cytokine-Induced Modulation of Contractile Function

#### Immediate Effects

**E-C Coupling**

In general, cytokine-mediated alterations of the Ca$^{2+}$ transient parallel the inotropic response reported by the particular study. In adult mammalian cardiomyocytes, low concentrations of TNF-α (200 to 500 U/mL) acutely decrease action potential duration, the peak Ca$^{2+}$ transient amplitude, and the rate of Ca$^{2+}$ decline (suggesting alterations of sarcoplasmic reticular [SR] function), but without influencing the resting Ca$^{2+}$ level or the inward Ca$^{2+}$ current (ICa).74,75 Similar effects of TNF-α on the Ca$^{2+}$ transient were reported in the isolated heart model.5,78 Studies using very high concentrations of TNF-α (≥10 000 U/mL) have reported variable effects. One study reported depression of peak Ca$^{2+}$ and inhibition of ICa,73 whereas another reported no change in Ca$^{2+}$ levels despite

---

### TABLE 3. Delayed Cardiodepressant Mechanical Effects (>30 Minutes) of Cytokines: In Vitro and Ex Vivo Studies

<table>
<thead>
<tr>
<th>Effect/Mechanism Suggested</th>
<th>Cytokine</th>
<th>Effective Concentration Range*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression of basal function</td>
<td>Ca$^{2+}$ transient depression</td>
<td>TNF-α</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-1β</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-6</td>
<td>1 ng/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-1β and TNF-α</td>
<td>ng/mL: IL-1β, 5; TNF-α, 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ/LPS</td>
<td>IFN-γ, 100 U/mL; LPS, 100 μg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-1β/TNF-α/IFN-γ</td>
<td>ng/mL: IL-1β, 5; TNF-α, 20; IFN-γ, 9</td>
</tr>
<tr>
<td></td>
<td>Sphingolipid mediators</td>
<td>TNF-α</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td></td>
<td>Not specified</td>
<td>IL-1β</td>
<td>0.3–5 ng/mL</td>
</tr>
</tbody>
</table>

*Assuming 50 pg/U for TNF-α and 1 pg/U for IL-6. ONOO$^-$ indicates peroxynitrite.
reduced contraction, suggesting reduced myofilament Ca$^{2+}$ sensitivity.\textsuperscript{72} The depressant effects of TNF-\textalpha on E-C coupling appear to be selectively mediated by TNF receptor 1.\textsuperscript{73,94} In contrast, the few studies \textsuperscript{30,84} reporting an early cardiostimulatory effect of cytokines have shown TNF-\textalpha-mediated augmentation of the Ca$^{2+}$ transient and hastening of Ca$^{2+}$ decline, suggesting increased, rather than reduced, SR Ca$^{2+}$ ATPase activity. IL-6\textsuperscript{35,75} and IL-2\textsuperscript{62,77} have also been shown to acutely reduce peak intracellular Ca$^{2+}$ levels and Ca$^{2+}$ transient amplitude without changing $I_{Ca}$. IL-2 specifically prolonged intracellular Ca$^{2+}$ decline, blunted the positive Ca$^{2+}$ frequency response and frequency dependence of caffeine-induced SR Ca$^{2+}$ release (indicating reduced SR Ca$^{2+}$ content and availability), and enhanced the Na$^{-}$Ca$^{2+}$ exchange current.\textsuperscript{77} In contrast, Liu and Schreuer\textsuperscript{79,80} have shown that IL-1\beta reduces the L-type Ca$^{2+}$ current in adult rat ventricular myocytes via a pertussis toxin–insensitive G protein pathway, although the overall effects on the Ca$^{2+}$ transient and SR function were not described. Thus, there is strong evidence that proinflammatory cytokines rapidly alter E-C coupling and Ca$^{2+}$ homeostasis, albeit with demonstrable cytokine specificity (Figure 2). TNF-\textalpha, IL-2, and IL-6 primarily modulate SR Ca$^{2+}$ flux and Na$^{-}$Ca$^{2+}$ exchange, with minimal effects on the L-type Ca$^{2+}$ channel (at least at low concentrations), whereas IL-1\beta has a more pronounced effect on $I_{Ca}$.

cNOS and NO

NO imparts cardiac effects via two general signaling modalities: (1) the activation of soluble guanylate cyclase and the formation of cGMP, which in turn activates protein kinase G (PKG) and PKG-dependent phosphorylation events; and (2) the direct oxidation of thiol residues on critical regulatory proteins (S-nitrosylation).\textsuperscript{92,93,95} These events induce complex myocardial functional responses that include bimodal,
concentration-dependent effects on contractility and the Ca\(^{2+}\) transient, desensitization of the myofilaments to Ca\(^{2+}\), and suppression of mitochondrial respiration. A large number of studies, performed in a variety of experimental models, have examined whether NO, derived from cNOS, contributes to early cytokine-induced contractile dysfunction. These investigations have yielded variable (and sometimes conflicting) results. In a study of isolated, superfused hamster papillary muscles, Finkel et al\(^6\) demonstrated that relatively high concentrations of rhTNF-\(\alpha\), IL-2, or IL-6 (but not IL-1\(\beta\)) profoundly depressed contractile function within 5 minutes, and that these effects were fully reversible on washout. The nonspecific NOS inhibitor \(N^G\)-monomethyl-L-arginine blocked these effects, a response that was overcome by the addition of excess L-arginine. The rapid time course and reversibility of these effects implicated the activation of myocardial cNOS. Since this initial report, several additional studies have supported a role for cNOS-derived NO in the early negative inotropic effects of the following: (1) IL-2 in isolated, blood-perfused rabbit hearts\(^6\); (2) IL-6 in avian\(^5\) and mammalian\(^7\) myocytes; and (3) TNF-\(\alpha\) and/or IL-1\(\beta\) (alone or in combination) in adult\(^2\) and neonatal\(^7\) mammalian myocytes, guinea pig\(^6\) and rat\(^6\) muscle strips, isolated crystalloid-perfused rat hearts,\(^3\) and human atrial trabeculae.\(^6\)

In addition to acute contractile dysfunction, cNOS-derived NO has been linked to a variety of functional abnormalities including IL-6–mediated depression of the Ca\(^{2+}\) transient,\(^3\)\(^5\)\(^7\)\(^5\) TNF-\(\alpha\)–mediated reductions in myofilament Ca\(^{2+}\) sensitivity,\(^7\) and the deterioration of mechanical efficiency induced by a combination of IL-1\(\beta\), TNF-\(\alpha\), and IFN-\(\gamma\) (Figure 2).\(^3\)\(^4\) Several authors have further shown that these NO-mediated responses were cGMP dependent, requiring activation of soluble guanylate cyclase.\(^3\)\(^5\)\(^7\) Importantly, Kumar et al\(^7\) demonstrated that although TNF-\(\alpha\) and IL-1\(\beta\) increased the myocyte generation of NO gas, there was no difference in Ca\(^{2+}\)–dependent (or independent) NOS activity measured in vitro. This suggested that cytokines impact cNOS activity by regulating cofactor and substrate conditions rather than via increases in abundance or fixed post-translational enzyme modifications. The above-mentioned studies have evaluated a wide range of cytokine concentrations (picomolar to nanomolar) and have uniformly used pharmacological NOS blockers and/or NO scavengers to probe the role of NO. To date, no studies have been performed in mice with genetic ablation of the cNOS isoforms, although this design would provide the most conclusive delineation of the role of cNOS in early cytokine-mediated contractile dysfunction.

Although these studies persuasively argue that cNOS-derived NO has an important role in the pathogenesis of acute cytokine-mediated contractile dysfunction, they stand in direct contrast to several reports that dispute this notion.\(^5\)\(^2\)\(^4\)\(^0\)\(^7\)\(^5\) In isolated adult feline LVs and cardiomyocytes, Yokoyama et al\(^7\) demonstrated that TNF-\(\alpha\)–dependent immediate reductions in contractility and Ca\(^{2+}\) transients were not accompanied by increased NO oxidation products or cGMP production. Furthermore, the contractile dysfunction was not prevented by two different pharmacological NOS inhibitors. Other studies examining TNF-\(\alpha\) and/or IL-1\(\beta\) in isolated rat hearts\(^4\)\(^5\)\(^6\) and guinea pig myocytes\(^3\) have similarly failed to show an acute increase in NO oxidation products or cGMP and have also reported no effect of NOS inhibition on early cytokine-mediated cardiodepression.\(^6\)

The basis for the discrepancies between these studies is not entirely clear but may be related to species differences and differences in experimental models and conditions. Importantly, the magnitude of cNOS activation may be cytokine specific. In this regard, Sugushita et al\(^7\) have demonstrated that in guinea pig myocytes, whereas both IL-6 and TNF-\(\alpha\) reduced cell shortening and peak systolic Ca\(^{2+}\), NOS blockade prevented IL-6-mediated effects but had no effect on the response to TNF-\(\alpha\). These and other investigators\(^3\)\(^5\)\(^6\)\(^5\) have put forth a primary role for sphingosine as a mediator of the immediate negative inotropic effects of TNF-\(\alpha\).

**Sphingomyelinase-Dependent Signaling**

In several cell types, cytokines such as TNF-\(\alpha\) and IL-1 activate acid and neutral sphingomyelinases that hydrolyze the membrane lipid sphingomyelin to form ceramide.\(^6\)\(^3\)\(^8\)\(^9\)\(^0\)\(^6\) Ceramide in turn can be deacylated by acid or neutral ceramidase to yield sphingosine, which on subsequent phosphorylation by sphingosine kinase yields sphingosine 1–phosphate. In mammalian cardiomyocytes, exogenous sphingosine depresses contraction, reduces action potential duration, inhibits the inward L-type Ca\(^{2+}\) current, and blocks Ca\(^{2+}\) release from the SR ryanodine receptor,\(^7\)\(^9\)\(^7\)\(^8\) thus recapituating several cytokine-mediated alterations in E-C coupling. Membrane-permeable ceramide analogs have also been shown to inhibit the amplitude and inactivation rate of \(I_{Ca}\).\(^8\)

Several studies have demonstrated that sphingolipid-based signaling contributes to the negative inotropic effects of TNF-\(\alpha\) (Figure 2). In adult feline myocytes, Oral et al\(^6\) showed that TNF-\(\alpha\) rapidly activated (within 15 minutes) both neutral and acidic sphingomyelinase in a concentration-dependent manner, resulting in increased ceramide and free sphingosine levels. This effect correlated with TNF-\(\alpha\)–induced contractile depression, and was selectively mediated by TNF receptor 1, which parallels the receptor specificity for the induction of negative inotropy.\(^3\)\(^4\) Furthermore, the contractile effects were abolished by ceramidase inhibition and overcome by the addition of sphingosine. The importance of sphingolipid-dependent signaling in the genesis of TNF-\(\alpha\)–mediated myocardial dysfunction has been further confirmed in studies of adult guinea pig myocytes,\(^7\) isolated rat\(^6\)\(^7\)\(^8\)\(^1\) and rabbit\(^3\) hearts, and rat right ventricular trabeculae.\(^6\)

Studies evaluating the role of sphingolipids in the mechanical effects of other proinflammatory cytokines are few. In a study of human atrial trabeculae, inhibition of either sphingosine generation or NO release prevented the early (and delayed) negative inotropic effects of TNF-\(\alpha\) and IL-1\(\beta\), either alone or in combination.\(^8\) Ceramide has also been proposed as the cellular mediator of IL-1\(\beta\)–induced suppression of the L-type Ca\(^{2+}\) current.\(^8\)

Although these studies have aptly demonstrated that sphingosine is an intracellular mediator of the negative inotropic effects of TNF-\(\alpha\), several important points must be considered. First, neutral sphingomyelinase activity is inhibited by
GSH, and TNF-α-dependent activation of neutral sphingomyelinase requires the upstream event of GSH depletion also induced by TNF-α. Thus, the intracellular redox state is likely to be an important determinant of the magnitude of negative inotropy. Indeed, in a recent study performed in adult rat cardiomyocytes, Cailleret et al demonstrated that antecedent in vivo administration of the GSH precursor N-acetylcysteine increased cardiac GSH content, prevented TNF-α-induced free radical generation and neutral sphingomyelinase activation, and uncovered a sustained positive inotropic response (over 30 minutes) to TNF-α, consisting of increased Ca²⁺ transient amplitude, increased contraction and accelerated relaxation, and increased phospholamban phosphorylation, changes that were directionally opposite from those observed in control rat myocytes. Second, neutral sphingomyelinase activity is enhanced by exogenous free fatty acids, including AA, as well as by the endogenous activation of phospholipase A2 (PLA2), thus implicating a link between these two signaling pathways in cytokine-mediated contractile changes (vide infra). Lastly, it is intriguing to note that in endothelial cells, sphingosine 1-phosphate signals through the endothelial differentiation gene (EDG)-1 receptor to activate Akt and phosphoinositide 3-kinase, resulting in cNOS phosphorylation and NO release. As cardiomyocytes also express the EDG-1 receptor, this raises the interesting possibility that sphingomyelinase and cNOS are activated in a parallel fashion and that both sphingosine and NO mediate the ensuing contractile response, although this hypothesis has not been tested directly.

**PLA2 and AA**

On binding to their cognate cell-surface receptors, TNF-α and IL-1β rapidly stimulate PLA2 within 5 to 10 minutes and subsequently increase AA release. In vitro and isolated cell studies indicate that cytokine-mediated PLA2 activation precedes sphingomyelin hydrolysis and that AA in turn activates neutral sphingomyelinase. In isolated myocytes, AA has a dual effect on contraction and Ca²⁺ transients that is both concentration and time dependent, with stimulatory responses evident at low micromolar (<10 μmol/L) concentrations and shorter duration of exposure (5 to 30 minutes), and depressant responses evident at higher concentrations and longer exposure times. A recent study in adult rat cardiomyocytes suggested that AA is responsible for the dual contractile effect of TNF-α, as PLA2 inhibition abrogated both the positive and negative inotropic responses attributable to TNF-α, an effect that could be overcome with exogenous AA. There was no demonstrable effect with either lipoxigenase or cyclooxygenase inhibitors, the latter confirming a previous study of isolated feline myocytes, implicating AA rather than a downstream metabolite. Further, ceramidase inhibition abolished the negative inotropic effects and unmasked a sustained stimulatory effect on contraction, even at higher TNF-α concentrations, a finding echoed by another study using GSH repletion to inhibit neutral sphingomyelinase. These few studies suggest that cytokine-mediated PLA2 activation is upstream of sphingomyelinase activation and that the AA generated initially induces a direct cardiotimulatory effect that is subsequently followed by AA-mediated sphingomyelinase activation, the formation of sphingolipid messengers, and negative inotropy (Figure 2). Earlier studies in isolated rat atria also suggested that PLA2-derived AA was required for the development of an early IL-2-induced positive inotropic response. Further studies are thereby warranted to delineate the role of PLA2 and AA in cytokine-induced contractile effects.

**Other Mechanisms**

One well-known facet of delayed cytokine-induced contractile dysfunction is impaired β-AR responsiveness (vide infra). However, some studies have suggested that this also occurs acutely after exposure to cytokines (Figure 2). In isolated mammalian myocytes, within minutes of exposure to cytokines such as TNF-α, IL-1β, and IL-6, there is an inhibition of cGMP-dependent contractility, cAMP generation, and Iₐ₃ augmentation. The mechanism for this defect in β-AR signal transduction is not clear, although one study suggested a mechanism unrelated to alterations in β-AR binding, adenylyl cyclase (AC) activity, or cAMP. Finally, in another isolated myocyte study, IL-2 was shown to reduce cell contraction and Ca²⁺ transients via the cardiac opioid receptor, a mechanism dependent on pertussis toxin-sensitive G protein and phospholipase C. Further studies are needed to determine the mechanistic role of these signal transduction pathways.

**Delayed Effects**

**Altered β-AR Signaling**

A hallmark of the delayed contractile dysfunction induced by cytokines is impaired β-AR sensitivity, mediated both by NO-dependent mechanisms as well as by an apparently NO- and cGMP-independent functional uncoupling of the β-AR to AC (Figure 3). Elegant studies by Gulick et al and Chung et al in neonatal rat myocytes demonstrated that prolonged exposure (>24 to 48 hours) to IL-1β and TNF-α in the supernatants from activated immune cells reduced contractility augmentation and cAMP accumulation in response to β-AR stimulation with isoproterenol, but had no adverse effect on Ca²⁺-mediated contractility or forskolin-stimulated cAMP accumulation. This occurred without changes in β-AR density or binding affinity, was unaffected by phosphodiesterase (PDE) inhibition, and was entirely reversible by 72 hours after supernatant removal. Subsequent studies also demonstrated uncoupling of β-AR stimulation to both cAMP accumulation and Iₐ₃ augmentation after prolonged (>1 hour) exposure to the cytokines IL-1β and TNF-α, despite intact responses of both to forskolin stimulation. Although several groups have reported that the cytokine-mediated loss of β-AR responsiveness is dependent on iNOS-derived NO and cGMP activation of PDE II and PDE (vide infra), the preserved forskolin-stimulated AC activity and lack of effect of PDE inhibition in these studies suggested mechanisms independent of cGMP.

Chung et al further showed that Gᵢ inhibition restored cytokine-mediated alterations in the β-AR response toward normal, supporting delayed uncoupling of agonist occupied β-ARs to cAMP production at the level of G-protein interaction with AC. The precise mechanism of this uncoupling...
has not been fully defined. Some, but not all, studies have shown that prolonged exposure to cytokines such as TNF-α upregulates membrane G proteins in cardiomyocytes. Alternatively, structural and modeling studies have shown that via S-nitrosylation of a surface accessible cysteine residue, NO can directly modulate the function of the small G-protein p21 and trigger guanine nucleotide exchange.

Theoretically, NO may analogously modulate β-AR–coupled G proteins, although whether such a mechanism exists to link NO generation and β-AR uncoupling after prolonged cytokine exposure is unknown. Furthermore, in neonatal rat cardiomyocytes, it has been demonstrated that prolonged exposure to low nanomolar concentrations of TNF-α is insufficient to induce iNOS and increase NO content and yet still results in loss of β-AR responsiveness, supporting, at least in part, a NO-independent mechanism for reduced β-AR sensitivity.

**iNOS-Derived NO and Loss of β-AR Responsiveness**

Several studies have implicated NO, derived from iNOS, as a mediator of delayed cytokine-induced contractile dysfunction, either under basal conditions and/or on β-AR or rate-related inotropic stimulation. In a series of experiments, Balligand, Ungureanu-Longrois, and coworkers demonstrated that on prolonged (24-hour) exposure to either culture supernatants conditioned by endotoxin-activated rat macrophages or to combinations of specific cytokines, adult rat ventricular myocytes lose β-AR responsiveness but maintain contraction under basal conditions. Such exposure was coincident with cardiomyocyte induction of iNOS, increased cGMP and nitrite content in the medium, and directly measured NO release. Furthermore, β-AR hyporesponsiveness was completely reversibly by concomitant treatment with NOS inhibitors and could be reproduced on coculture of myocytes with cytokine-treated, iNOS-expressing cardiac endothelial cells, strongly implicating iNOS-derived NO in the pathogenesis of delayed contractile dysfunction. Other groups also demonstrated that adult cardiomyocytes exposed to a combination of TNF-α, IL-1β, and IFN-γ for 24 hours had iNOS induction, increased NO synthesis, and increased cell death. These effects were prevented either by the addition of NO inhibitors or cotreatment with transforming growth factor-β (TGF-β), which has been shown to downregulate iNOS. TGF-β has also been shown to antagonize IL-1β–induced suppression of beating rate in neonatal rat myocytes, suggesting that TGF-β, via its effects on iNOS-derived NO, can be an endogenous modulator of myocyte functional responses to proinflammatory cytokines.

Importantly, no depression of basal (unstimulated) contractility was evident in these isolated myocyte studies, although this feature is a central component of the cytokine response in the intact animal and in isolated hearts. Additionally, the functional results depended not just on iNOS induction but also on the specific combinations of cytokines used, findings consistent with the synergistic cardiodepressant effects of the proinflammatory cytokines IL-1β, TNF-α, and IFN-γ noted in several studies, whereby these cytokines in combination impart significantly greater negative inotropy and at substantially lower concentrations than if given alone.

NO-mediated depression of the β-adrenergic response is primarily dependent on cGMP mechanisms, including stimulation of PDE II with attendant augmentation of cAMP degradation and activation of PKG with downregulation of L-type Ca²⁺ currents stimulated by cAMP-dependent PKA. The signal transduction pathways that link cytokines and iNOS induction are cytokine- and cell-type specific. In myocardium, upregulation of iNOS activity is evident by 2 hours of exposure to a combination of IL-1β and TNF-α or to IL-6 alone. IFN-γ acts synergistically with IL-1β and TNF-α to potentiate the induction of iNOS, whereas TGF-β antagonizes this re-
spontaneous.\textsuperscript{29,45,46,111,116} Furthermore, as compared with IL-1\(\beta\), TNF-\(\alpha\) is a less potent inducer of \(\text{iNOS}\).\textsuperscript{32,42,47} In adult rat ventricular myocytes, IL-6–induced activation of \(\text{iNOS}\) proceeds via the JAK2/STAT3 pathway,\textsuperscript{48} whereas of extracellular signal-regulated kinases 1 and 2 and PKC activation are necessary for \(\text{iNOS}\) induction by IL-1\(\beta\) and IFN-\(\gamma\).\textsuperscript{114}

Recent insights from transgenic mice with cardiac-specific overexpression of TNF-\(\alpha\) further underscore the importance of \(\text{iNOS}\)-derived NO in the pathogenesis of long-term cytokine-mediated contractile dysfunction.\textsuperscript{109,110} In these animals, the short-term administration of a selective \(\text{iNOS}\) inhibitor (ON-1714) significantly improved \(\beta\)-AR responsiveness but did not alter the depressed contractility at baseline. Similar effects were seen on crossing these mice with \(\text{iNOS}\) null mice: \(\beta\)-AR stimulated inotropy improved in TNF-\(\alpha\)-transgenic mice with \(\text{iNOS}\) disruption, but the cardiac dysfunction, hypertrophy, and inflammation evident at baseline did not. Similarly, we have shown that after the establishment of TNF-\(\alpha\)-mediated cardiac dysfunction in conscious dogs, systemic NO inhibition does not improve baseline contractile function but rather exacerbates it because of increased afterload.\textsuperscript{25} The lack of a beneficial effect of \(\text{NOS}\) blockade on basal cytokine-induced myocardial dysfunction has also been reported in a study of myocytes isolated from rats receiving a 15-day continuous infusion of TNF-\(\alpha\).\textsuperscript{18} Taken together, these results support the idea that \(\text{iNOS}\)-derived NO contributes importantly to reduced \(\beta\)-AR responsiveness in chronic cytokine-mediated cardiac dysfunction and that these effects are acutely reversible on \(\text{NOS}\) blockade, but that other mechanisms (either \(\text{iNOS}\)-independent or \(\text{iNOS}\)-mediated effects that are less readily reversible) are of greater importance in baseline cardiac dysfunction.

**Depression of Basal Contractility: Role of Nitrosative and Oxidative Stress**

Although delineated in some studies of isolated cardiomyocytes,\textsuperscript{29,32,35,37,116} delayed cytokine-mediated depression of basal, unstimulated contractility has been more uniformly described at higher experimental organizational levels, including the intact LV (isolated hearts or whole animals).\textsuperscript{13,18,19,21–27,31,34,38–40} Multicellular preparations such as papillary muscles and atrial trabeculae,\textsuperscript{33,36,64} and in transgenic mice with cardiac-specific overexpression of TNF-\(\alpha\).\textsuperscript{109,110} Most studies have implicated cytokine-mediated induction of \(\text{iNOS}\) gene expression, de novo \(\text{iNOS}\) protein synthesis, and increased NO generation in the depression of basal mechanical function (Figure 3).\textsuperscript{19,27,31–39,64,116}

In this context, modulation of basal function by NO may be more dependent on \(\text{S-nitrosylation}\) of thiol residues of important regulatory proteins.\textsuperscript{92,93,112,117} Consistent with this notion, studies in isolated myocytes have demonstrated that the late (24 to 48 hours of exposure) contractile dysfunction induced by IL-6 is linked to NO-dependent depression of the Ca\(^{2+}\) transient\textsuperscript{15} and that within a similar time frame, IL-1\(\beta\) causes a NO-dependent (but cGMP-independent) inhibition of oxidant-sensitive mitochondrial enzymes and the inward Ca\(^{2+}\) current, resulting in inhibition of mitochondrial respiration, energy depletion, and depressed contraction.\textsuperscript{37} As \(\text{S-nitrosylation}\) is a redox-driven event,\textsuperscript{118} ambient levels of ROS significantly modulate the ensuing covalent protein-thiol Modifications and the ultimate balance between nitrosative and oxidative stress.\textsuperscript{81} If cytokine stimulation induces high-output \(\text{iNOS}\), the high levels of NO generated would be expected to result in secondary oxidative modifications in the presence of superoxide.\textsuperscript{91,118} Indeed, several studies have established that cytokine-mediated contractile dysfunction results not only from increased \(\text{iNOS}\)-derived NO but also from increased ROS generation and peroxynitrite formation (Figure 3).\textsuperscript{19,27,34,38,39} In the isolated working rat heart model, Ferdinandy et al.\textsuperscript{18} demonstrated that the combination of IL-1\(\beta\), IFN-\(\gamma\), and TNF-\(\alpha\) induced in a marked decline in contractility over 20 minutes that was preceded by an augmentation of the activity of the superoxide sources xanthine oxidoreductase and NAD(P)H oxidase, together with enhanced activity of \(\text{iNOS}\). This was accompanied by increased myocardial content of NO, superoxide, and the peroxynitrite markers nitrotyrosine and dityrosine. Furthermore, cardiac dysfunction and nitrotyrosine levels were inhibited by concomitant administration (individually) of a \(\text{NOS}\) inhibitor, superoxide scavenger, and peroxynitrite decomposition catalyst.

Studies using an in vivo canine model of IL-1\(\beta\)-mediated chronic cardiac dysfunction have similarly demonstrated the importance of superoxide and peroxynitrite.\textsuperscript{19,27} Coronary injection of IL-1\(\beta\) coated microspheres (but not control microspheres) resulted in increased myocardial superoxide, nitrotyrosine, and persistent LV dysfunction at 7 days, effects that were prevented by concomitant treatment with dexamethasone, \(\text{iNOS}\) inhibition with aminoguanidine, or pharmacological inhibition of superoxide production. These authors also suggested that inflammatory cells infiltrating in response to cytokines may contribute to superoxide generation as inhibition of the adhesion molecule P-selectin ameliorated the LV dysfunction.\textsuperscript{26} These findings are consistent with the demonstration that cell-to-cell contact between inflammatory cells (macrophages) and myocytes enhances the delayed contractile dysfunction induced by the TNF-\(\alpha\) and IL-1\(\beta\) because of the production of ROS and NO (Figure 3).\textsuperscript{119} As oxidative modifications attributable to peroxynitrite would tend to be irreversible,\textsuperscript{118} post hoc inhibition of NO generation would not be expected to reverse the mechanical dysfunction. Indeed, whereas \(\text{NOS}\) inhibitors given concurrently with cytokines improves the delayed contractile dysfunction that is manifested in vivo,\textsuperscript{19} NO inhibition after the establishment of cytokine-mediated dysfunction does not acutely reverse the loss of contractile function.\textsuperscript{25}

**Other Mechanisms**

Two studies have also suggested that sustained activation of sphingomyelinase pathways also contributes to chronic TNF-\(\alpha\)-induced contractile dysfunction. Ceramidase inhibition, but not NO inhibition, partially restored the depressed cell contraction evident in cardiomyocytes isolated from rats administered a sustained infusion of TNF-\(\alpha\).\textsuperscript{18} Similarly, ceramidase inhibition improved TNF-\(\alpha\)-dependent delayed cardiac dysfunction in an isolated rat heart model.\textsuperscript{40} Additionally, it has been shown that sphingomyelinase-dependent signaling stimulates the expression of \(\text{iNOS}\) in cytokine- or...
lipopolysaccharide (LPS)-stimulated astrocytes. Thus, the possibility exists that sphingolipid mediators may contribute to cytokine-induced expression of iNOS, although whether this occurs in myocytes has not been examined.

Conclusion

Proinflammatory cytokines impact cardiac mechanical function in a temporally dependent, biphasic manner. The early phase is characterized by the rapid activation of cellular signaling mechanisms that are likely interrelated, involving sphingolipid, phospholipid, and cNOS-derived NO-dependent pathways. The response evoked may be either cardiostimulatory or cardiodepressant, depending on the prevailing cellular and biological milieu, specifically with regard to the redox and metabolic state, the magnitude of extracardiac adaptive and reflex responses, and the synergistic and/or counter-regulatory effects of several cytokines acting in concert. All of these variables will thus influence cytokine-mediated cardiac responses triggered by acute stress. The early response is subsequently followed by a more prolonged, delayed phase that uniformly demonstrates depression of both basal and stimulated contractility. This response is dependent on a variety of factors including β-AR uncoupling, de novo induction of iNOS, and oxidative and nitrosative stress, together with persistent activation of sphingomyelinase-dependent pathways. These responses are likely to be of greater prominence in pathological states that are characterized by sustained and pronounced elevations of an extensive portfolio of proinflammatory cytokines and have broader implications in myocardial dysfunction and remodeling in rats. Pathophysiologically relevant concentrations of tumor necrosis factor-α promote progressive left ventricular dysfunction and remodeling in rats. Circulation. 1998;97:1382–1391.

Acknowledgments

This work was supported by a Veterans Affairs Merit Award, NIH P01 ES011860-01A1, and the Commonwealth of Kentucky Research Challenge Trust Fund.

References


Cytokine-Induced Modulation of Cardiac Function
Sumanth D. Prabhu

Circ Res. 2004;95:1140-1153
doi: 10.1161/01.RES.0000150734.79804.92
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/95/12/1140

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/