invited review

Tumor necrosis factor in the heart

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Meldrum, Daniel R. Tumor necrosis factor in the heart. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R577–R595, 1998.—The heart is a tumor necrosis factor (TNF)-producing organ. Both myocardial macrophages and cardiac myocytes themselves synthesize TNF. Accumulating evidence indicates that myocardial TNF is an autocrine contributor to myocardial dysfunction and cardiomyocyte death in ischemia-reperfusion injury, sepsis, chronic heart failure, viral myocarditis, and cardiac allograft rejection. Indeed, locally (vs. systemically) produced TNF contributes to postischemic myocardial dysfunction via direct depression of contractility and induction of myocyte apoptosis. Lipopolysaccharide or ischemia-reperfusion activates myocardial P38 mitogen-activated protein (MAP) kinase and nuclear factor kappa B, which lead to TNF production. TNF depresses myocardial function by nitric oxide (NO)-dependent and NO-independent (sphingosine dependent) mechanisms. TNF activation of TNF receptor 1 or Fas may induce cardiac myocyte apoptosis. MAP kinases and TNF transcription factors are feasible targets for anti-TNF (i.e., cardioprotective) strategies. Endogenous anti-inflammatory ligands, which trigger the gp130 signaling cascade, heat shock proteins, and TNF-binding proteins, also control TNF production and activity. Thus modulation of TNF in cardiovascular disease represents a realistic goal for clinical medicine.

ACCUMULATING EVIDENCE indicates that cytokines are important mediators of cardiovascular disease (27, 104, 105, 154, 161, 216, 223, 224, 272–274, 303, 304). A working understanding of inflammatory cytokines and their relationship to myocardial disease is of growing importance to basic and clinical cardiovascular scientists, immunologists, and clinicians. In this regard, tumor necrosis factor (TNF) is a proinflammatory cytokine that has been implicated in the pathogenesis of cardiovascular diseases, including acute myocardial infarction, chronic heart failure, atherosclerosis, viral myocarditis, cardiac allograft rejection, and sepsis-associated cardiac dysfunction (27, 154, 161, 216, 223, 224, 272–274, 303, 304). Although initially described solely as a lipopolysaccharide (LPS)-induced macrophage product, evidence now indicates that cardiac myocytes themselves produce substantial amounts of TNF in response to ischemia (Fig. 1) as well as LPS (104, 137). Indeed, ischemia-provoked myocardial TNF production may prove more clinically significant than sepsis-induced myocardial TNF production by an order of magnitude.

Ischemia and LPS are two of several clinically relevant stimulants that induce TNF production in the heart. The intracellular signal pathways that provoke TNF production are being elucidated with increasing clarity (265). The discovery of the mitogen-activated protein kinases (MAPKs) and TNF transcription factors offer feasible targets for anti-TNF strategies. Furthermore, activation of endogenous anti-inflammatory strategies such as ligands for the gp130 subunit, induction of heat shock proteins (HSPs), and infusion of TNF-binding proteins now hold therapeutic promise.

Control of TNF’s destructive role in cardiovascular disease represents a realistic goal for clinical medicine. The purposes of this review are to 1) examine evidence implicating the myocardium as an important source of TNF, 2) dissect the mechanisms of TNF-induced cardiac dysfunction, 3) outline the mechanisms of TNF-induced apoptosis, 4) delineate clinically accessible signaling steps that lead to TNF production, and 5) identify therapeutic strategies for preventing TNF-mediated cardiovascular disease.

TNF

TNF is a proinflammatory cytokine. Proinflammatory cytokines act to increase their own production and the synthesis of small inflammatory mediators such as platelet-activating factor (PAF), eicosanoids, and oxidative radicals. Proinflammatory cytokines also recruit and stimulate cellular components of the immune system. TNF is an endogenous pyrogen that stimulates
the production of other endogenous pyrogens, such as interleukin 1β (IL-1) (77). Both forms of TNF, TNF-α and TNF-β, share similar inflammatory activities. TNF-β, first described as “lymphotoxin” (241), is a larger molecule, less potent, not as abundant, and produced mainly by T cells, whereas macrophages are the predominant source of TNF-α. For the purposes of this review, TNF refers to TNF-α, which is the form associated with septic shock, ischemia-reperfusion injury, and traumatic end organ damage.

Myocardial TNF production. Most cell types do not produce TNF, but the ubiquitous macrophage allows for TNF production in nearly every organ (10, 265). Not surprisingly, the heart contains resident macrophages (137, 200) and is a rich source of several inflammatory cytokines, including TNF (27, 112, 122, 137, 144, 151, 172, 216, 272–274, 285, 291). Levine and associates (161) correlated circulating levels of TNF with the severity of chronic heart failure in patients and postulated that TNF may contribute to the pathogenesis of heart failure. An increase in circulating TNF, soluble TNF receptor, and IL-1 receptor antagonist each follows myocardial infarction (154). In fact, the myocardium produces as much TNF per gram tissue (in response to endotoxin) as either the liver or the spleen, both of which possess large macrophage populations.
and are major sources of TNF (137). Unexpectedly, cardiac myocytes themselves produce TNF. Kapadia and co-workers (137) demonstrated that production of endotoxin-induced myocardial TNF is nearly evenly distributed between cardiomyocyte and resident cardiac macrophage cell types. Thus local myocardial TNF production is a potential source of TNF affecting myocardial function.

Clinically relevant stimulants of myocardial TNF production. Although infection and endotoxemia are potent stimulants of myocardial TNF production, studies have also documented increases in myocardial TNF and other cytokines following experimental ischemia-reperfusion (112, 122, 258), burn trauma (126), clinical myocardial infarction (27, 154, 216), cardiopulmonary bypass (51, 119, 291), and chronic heart failure (161, 135, 228, 229, 295). Before the discovery of TNF, several investigators suspected that sepsis-induced myocardial depression was mediated by a circulating myocardial depressant factor(s) (65, 158, 159). Parillo and colleagues (231) demonstrated that the sera from septic patients with myocardial depression consistently depressed in vitro myocyte performance, whereas sera from septic patients without a compromised ejection fraction did not. The first experimental evidence suggesting that TNF mediates endotoxin-induced myocardial depression was provided by Tracey and associates (275). They observed that TNF administration resulted in hypotension, metabolic acidosis, hemococoncentration, diffuse pulmonary infiltrates, hyperglycemia, hyperkalemia, pulmonary and gastrointestinal petechial hemorrhages, acute tubular necrosis, and death (275). Although myocardial function was not examined, the hypotension and shock suggested myocardial depression. These investigators further substantiated the link between sepsis and TNF by utilizing anti-TNF monoclonal antibodies to neutralize the circulating TNF and thereby prevent its adverse effects (276). Gulick and co-workers (111) demonstrated that TNF (or IL-1) inhibited cardiac myocyte adrenergic responsiveness in vitro. Similarly, TNF (or IL-1)-induced depression of myocardial function in an ex vivo, crystalloid-superfused papillary muscle preparation was observed by Finkel and colleagues (92).

Mechanisms of TNF-induced contractile dysfunction. Because calcium homeostasis is of paramount importance to the normal myocardial contraction-relaxation cycle, several investigators have examined the effects of TNF on myocardial calcium handling. Indeed, coordinated and precise regulation of the oscillating intracellular calcium mediates systolic contraction, diastolic relaxation, enzymatic activity, and mitochondrial function (187, 191). TNF-induced disruption of calcium handling may lead to dysfunctional excitation-contraction coupling and, thereby, systolic and/or diastolic dysfunction. Assessment of myocardial calcium handling can be accomplished in one of four ways: 1) the cardiac contractile state can be assessed as developed force or pressure, 2) sarcolemmal calcium handling is reflected in the action potential, 3) sarcoplasmic reticulum calcium handling is demonstrated by the calcium transient, and 4) the myofilament-regulatory complex is exhibited by the association between the calcium transient and the force of contraction. The calcium transient represents the transition from the resting state to contraction, which occurs when a small amount of calcium enters the cytosol via voltage-gated L-type calcium channels, which in turn results in a much greater release of calcium from sarcoplasmic reticulum ryanodine receptor calcium release channels. These two calcium channels have microarchitectural communication, and calcium entry through one influences the other. Yokoyama and colleagues (304) determined that, soon after TNF administration, the amplitude of the calcium transient was decreased during systole. TNF appears to depress systolic function by disrupting calcium-induced calcium release by the sarcoplasmic reticulum. Indeed, TNF disrupts L-type channel-induced calcium influx and thereby depresses calcium transients (150). Corroborating these findings, Oral et al. (223) demonstrated that TNF’s early effects on the calcium transient and systolic function were mediated by sphingosine (Fig. 2). NO does, however, appear to mediate TNF-induced desensitization of myofilaments to intracellular calcium (107). These findings (110, 141, 223) indicate that TNF-induced, sphingosine-mediated disruption of calcium-induced calcium release occurs early and that NO mediates TNF-induced desensitization of myofilaments to increased intracellular calcium (Fig. 2). Although the association between massive calcium influx and myocellular ischemic injury has been established, the source of the elevated intracellular calcium remains controversial and may have important therapeutic significance. The most likely scenarios involve either ineffective sarcolemmal calcium extrusion and/or inadequate sarcoplasmic reticulum calcium sequestration (191). Either seems plausible because both exhibit energy-dependent kinetics, i.e., posts ischemia, the ATP-hungry sarcolemmal calcium-adenosine-triphosphatase (ATPase) and/or sarcoplasmic reticulum calcium-ATPase would be unable to bring intracellular calcium back to the basal levels required for muscle relaxation (191). This, in turn, would decrease muscle shortening during a contraction, leading to both systolic and diastolic dysfunction.

In addition to calcium dyshomeostasis, the mechanisms by which TNF causes myocardial dysfunction include direct cytotoxicity, oxidant stress, disruption of...
Excitation-contraction coupling, and myocyte apoptosis, as well as the induction of other cardiac depressants such as IL-1 (72, 75), IL-2 (92, 106, 257), and IL-6 (92, 233). Indeed, IL-1 synergistically enhances TNF-induced myocardial depression (151) and cytotoxicity (153). Finkel and associates (92) demonstrated that NO synthase (NOS) inhibition prevented the myocardial depressive effects of either TNF or IL-1, concluding that the negative inotropic effects were mediated by NO. LPS, TNF, and IL-1 each induce NOS and augment guanosine 3′,5′-cyclic monophosphate, which mediates NO’s effects in other cell types (55, 98, 100, 167, 218, 255, 261). Nitric oxide has also been implicated in the pathogenesis of myocardial infarction (6), hypoxia-induced cardiomyocyte damage (144), and autoimmune myocarditis (131). Wang and Zweier (293) observed increased NO and peroxynitrite release from the isolated rat heart after 30 min of global ischemia. Pretreatment with a NOS inhibitor resulted in a fourfold increase in the postischemic functional recovery. Nitric oxide also appears to mediate the β-adrenoceptor unresponsiveness (281) that occurs during sepsis (21).

The biphasic (immediate and delayed) nature of TNF-induced myocardial depression suggests that TNF induces negative inotropic effects by at least two different mechanisms (208, 223). As depicted in Fig. 2, the early phase of TNF-induced functional depression occurs within minutes, whereas the delayed phase appears to require hours of TNF exposure (223). TNF may not induce high levels of NO rapidly enough to account for the early phase of myocardial depression (223). In this regard, sphingolipid metabolites are stress-induced second messengers that participate in intracellular signal transduction after TNF binding to the TNF type 1 receptor (TNFR1) (118). Two important characteristics of sphingolipid metabolites led to the hypothesis (223) that sphingosine mediates TNF-induced myocardial contractile dysfunction: 1) it is rapidly produced by cardiac myocytes (via sphingomyelin degeneration) after TNF’s triggering of TNFR1 (148, 300) and 2) sphingosine decreases calcium transients by blocking the ryanodine receptor, which mediates calcium-induced calcium release from the sarcoplasmic reticulum (71, 242). These investigators (223) reported that myocardial sphingosine production occurred within minutes of TNF administration and temporally correlated with myocardial dysfunction and calcium dyshomeostasis in cardiac myocytes. Blockade of sphingosine production abolished TNF-induced contractile dysfunction, and sphingosine administration replicated TNF-induced contractile depression in a dose-dependent fashion. Thus it appears likely that sphingosine mediates the early depression (NO independent) and that NO mediates the late dysfunction induced by TNF (Fig. 2).

Although several investigators have implicated NO in TNF-induced myocardial dysfunction (32, 33, 107, 139, 250), others have been unable to attribute all of TNF’s depressive effects to NO (140, 146, 198, 304). In fact, it has been reported that NO can protect the myocardium during ischemia-reperfusion injury (210, 247), possibly by decreasing leukocyte-mediated endothelial cell injury (28, 31, 68, 218) or decreasing myocardial oxygen consumption (256). This discrepancy may be due to differences in the quantities of NO produced during injury. The relative contribution of NO production by the calcium-dependent, constitutive form of NOS (cNOS) is at least two orders of magnitude less than the calcium-independent, cytokine-inducible form of NOS (iNOS). The low levels of NO produced by cNOS may serve a protective role, whereas the high levels produced by iNOS may be injurious (167, 218). Thus the role of NO as a mediator of this process remains controversial; however, it is likely that TNF-induced myocardial depression occurs via both NO-dependent and NO-independent mechanisms (141).

TNF-induced myocardial apoptosis. Programmed cell death (apoptosis) is a process by which cells undergo inducible nonnecrotic cellular suicide (263, 268, 287). In contrast with necrotic cell death, programmed cell death is dependent on de novo synthesis of proteins that initiate a cellular suicide program in response to specific stimuli (227, 263, 268, 287). For most cells of hematopoietic lineage, apoptosis is a constitutive process but can also be induced by noxious stimuli (11). Although originally described as a process by which the immune system “quietly” deleted autoreactive cells, it...
Cardiac myocyte apoptosis (versus necrosis) is characterized by cell death with the maintenance of cell membrane integrity (149), therefore, apoptotic cardiac myocytes do not release creatine kinase and do retain their ability to exclude dyes such as Trypan blue (149). This feature likely results in the underestimation of myocyte death during myocardial infarction. Indeed, it has been proposed that the degree of myocardial apoptosis, rather than necrosis, is a more accurate reflection of myocardial infarct size (136). Apoptotic cardiac myocytes also retain their ability to contract in response to calcium ionophores (149). After the high influx of calcium associated with necrosis (191), necrotic myocytes are maximally contracted and are unable to further contract in response to a calcium ionophore; however, apoptotic myocytes maintain myofilament responsiveness when calcium entry is induced (149).

TNF induces apoptosis in many cell types, including the myocardium (11, 219, 220, 245). Krown and colleagues (149) demonstrated that TNF induced cardiac myocyte apoptosis by a sphingosine-dependent mechanism. TNF induces sphingosine formation (220, 223), which also mediates apoptosis in other cell types (220). TNF-induced NO production also may play a role (246).

TNF induces cardiac myocyte apoptosis via TNFR1. The “death domain” of the cytosolic component of TNFR1 has been linked to apoptosis in many cell types and likely mediates TNF-induced cardiac myocyte apoptosis (283). TNF binding to either TNFR1 or Fas activates a pathway favoring apoptosis, whereas the type 2 TNF receptor (TNFR2) activates a pathway leading to nuclear factor kappa B (NFκB) induction (Fig. 3). TNFR1 and Fas are linked to cytoplasmic proteins that are referred to as the death domains TRADD (128) and FADD (56), respectively the TNF receptor-associated death domain and the Fas-associated death domain. Interaction between the death domain proteins is accomplished by receptor-interacting protein (RIP) (64). RIP, but not TRADD or FADD, contains a kinase domain that communicates the signal. TNF binding to TNFR1 or Fas may result in conformational changes in TRADD and FADD that allow RIP binding (30). RIP initiates the intranuclear communication that activates endonucleases to destroy the cell’s nuclear DNA (Fig. 3). The TNFR2 is linked to the TNF receptor-associated factors (TRAFs) (206). Although their biological function remains unknown, most TRAFs contain two protein motifs called “zinc” and “ring” fingers that likely convey proliferative signals by activating transcription factors such as NFκB (17). These pathways are not absolute, however, and cross-activation occurs (17, 227).

TNF PRODUCTION

Understanding of the intracellular mechanisms that lead to TNF production may allow targeted disruption of TNF-mediated pathological conditions.

LPS-induced macrophage intracellular signaling leading to TNF production. In most cell types, the TNF gene is silenced (24) but can be accessed in cell types that express the signaling mechanisms and transcriptional apparatus required for TNF production. Because macrophages are the main TNF source, mechanisms of TNF production have been studied almost exclusively in these cells. Although there are undoubtedly different cell types and different stimuli, the following signaling mechanisms describe what is presently known concerning LPS-stimulated macrophage TNF production (265).

The mechanisms leading to LPS-stimulated macrophage TNF production appear multiple. Intracellular pathways that participate in the LPS-activated signaling cascade and contribute to the production and release of macrophage-derived proinflammatory media-
LPS interaction with CD14 leads to rapid intracellular tyrosine phosphorylation of Ras [by phosphotyrosine kinase (PTK)], a process that initiates the protein kinase cascade, leading to TNF production. Ras activates Raf-1/mitogen-activated protein kinase kinase (MAPK) family of protein kinases, extracellular signal-related kinase, stress-activated protein kinase, and Jun nuclear kinase. The P38 MAPK appears to be an important MAPK in the cascade leading to TNF gene induction. NF-kB is activated by inhibitory kappa B (IxB) phosphorylation (which disengages its inhibitory subunit, IxB) and translocates to the nucleus to activate TNF promoters. At least 2 TNF promoter sites must be occupied by NF-kB for TNF gene transcription to occur. Once translocated to pro-TNF in the cytosol, myristoylation permits membrane insertion, where pro-TNF remains until it is cleaved to its mature form by TNF-α-converting enzyme (TACE). Ischemia-reperfusion, oxidant stress, and hydrogen peroxide directly activate P38 MAPK and NF-kB to induce TNF production. LBP, LPS binding protein.

Ischemia-reperfusion-induced TNF production. Although considerable information exists concerning the mechanisms by which LPS induces TNF production, little is known about the mechanisms of ischemia-reperfusion-induced myocardial TNF production. Reperfusion of ischemic myocardium imposes an oxidant burden in which the reduction product of molecular oxygen reacts with reduced glutathione, causing increased formation of reactive oxygen species, which trigger TNF and other proinflammatory mediator synthesis. Ischemia-reperfusion injury involves multiple signaling events that initiate the proinflammatory response (278). Of these, LPS-binding protein (LBP) is the best characterized. LBP facilitates LPS binding to the macrophage CD14 (Figs. 4 and 5). LBP facilitates the interaction of LPS with its receptor, CD14, which is required for LPS-CD14 interaction. Under normal circumstances, LPS-LBP interaction with CD14 is an obligate trigger of the intracellular signals that transmit LPS-induced TNF production. Indeed, Lee and colleagues (157) demonstrated that anti-CD14 monoclonal antibody abolished LPS-induced macrophage activation and TNF release. Although CD14 is macrophage-specific, soluble (shed) CD14 enhances LPS-induced activation of both endothelial cells and epithelial cells (236). Endogenous, shed CD14 acts at distant sites to enhance LPS-induced activation of other cell types, and, seemingly paradoxically, high pharmacological doses of exogenous soluble CD14 decreases LPS interaction with the intact macrophage CD14 receptor. Therefore, using saturating concentrations of soluble CD14, Haziot and associates (120) prevented mortality in LPS-treated mice.

As shown in Fig. 4, LPS-LBP-CD14 interaction provokes rapid activation of protein tyrosine kinase (PTK) causing tyrosine phosphorylation of several intracellular protein kinases (116, 244, 252, 296–298). PTK activates a pathway involving Ras/Raf-1/mitogen-activated protein kinase kinase (MEK)/MAPKs/NF-kB (46, 70, 162, 163). Several studies have demonstrated that LPS activates PTK and that PTK inhibition abolishes downstream activation of MAPKs, TNF and IL-1 production, and macrophage-mediated tumoricidal activity (46, 127, 205, 237, 282). Ras is an early target of activated PTK and is able to interact directly with Raf-1 (127, 205, 282). Raf-1 is an important intermediate to MAPK activation (46, 127, 205, 282). Studies by Reimann and colleagues (237) demonstrated that LPS resulted in PTK-dependent rapid phosphorylation and activation of Raf-1. These findings were supported by Geppert and associates (102), who observed that transfection of the dominant-negative repressors of Ras and Raf-1 into macrophages resulted in a suppression of LPS-induced activation of the TNF promoter. However, transfection with a constitutively active form of Raf-1 did not reproduce LPS-induced macrophage activation, suggesting that Raf-1 activation is necessary but not sufficient to induce macrophage TNF production (102).

Raf-1/MEK appears to activate members of the MAPK family of protein kinases; of these the P38 MAPK appears to be a pivotal MAPK in the cascade leading to TNF gene induction (115, 117, 155, 156, 253). Han and colleagues (116) isolated a 38-kDa macrophage protein kinase that was phosphorylated following LPS treatment. Subsequent cloning and sequence analysis (115, 117) revealed that the novel MAPK homologue was closely related to the osmolar-sensitive Hog-1 gene in yeast. During the same period, Lee and co-workers (155) were searching for the target proteins of a novel class of anti-inflammatory drugs (pyridinyl imidazoles) capable of abolishing LPS-induced proinflammatory monokine production. Photoaffinity labeling of drug analogs identified proteins that proved to be the mammalian equivalent of the Hog-1 gene product. The 38-kDa protein was identical to the MAPK cloned by Han and co-workers (115, 117). Pyridinyl imidazoles, which abolish proinflammatory monokine production, are highly selective inhibitors of P38 MAPK and its downstream products, but not of Ras or Raf-1, activation (156). Thus LPS interaction with CD14 leads to rapid intracellular tyrosine phosphorylation of Ras, a process that initiates the protein kinase cascade leading to NF-kB activation and TNF production.

Ischemia-reperfusion-induced TNF production. Although considerable information exists concerning the mechanisms by which LPS induces TNF production, little is known about the mechanisms of ischemia-reperfusion-induced myocardial TNF production (112). Reperfusion of ischemic myocardium imposes an oxidant burden in which the reduction product of molecular oxygen reacts with reduced glutathione, causing increased formation of reactive oxygen species, which trigger TNF and other proinflammatory mediator synthesis.
lar oxygen, hydrogen peroxide, contributes to myocardial injury (41). Hydrogen peroxide-induced activation of P38 MAP kinase may contribute to ischemia-reperfusion-induced TNF production (113, 129). Oxidant stress also activates NFκB, which may also play a role in the sequence of ischemia-reperfusion-induced TNF production (Fig. 4).

In most cells, NFκB exists in a latent state, unable to induce TNF production (169). In this state, NFκB is bound to its inhibitory proteins, collectively called inhibitory κB (IκB), which mask its nuclear localization site; however, after activation by ischemia-reperfusion (or LPS), phosphorylation of IκB results in disruption of the NFκB-IκB complex and degradation of IκB (165, 265, 277). Once liberated from IκB, NFκB translocates from the cytoplasm to the nucleus, where it docks to DNA at one of four NFκB binding (TNF promoter) sites (251). Site-directed mutation studies by Shakov et al. (251) demonstrated that at least two of these TNF promoter regions must be bound by NFκB for TNF transcription to occur. Deletion of all four NFκB binding sites abolished macrophage TNF production (251).

IκB may be phosphorylated by MAPKs (169); however, it is noteworthy that Raf-1, a relatively upstream component of this pathway, is also capable of activating NFκB (127, 165). Redundant or “skip” activation sequences may be designed to ensure NFκB activation following LPS challenge. Thus NFκB is activated by either IκB phosphorylation or directly by oxidant stress, after which it translocates to the nucleus to activate TNF gene transcription.

Posttranslational processing of TNF. Once TNF gene transcription occurs, TNF mRNA is translated into the 26-kDa TNF precursor (pro-TNF) in the cytoplasm (80, 177, 203). Myristoylation in the cytoplasm facilitates membrane insertion/association, where it is cleaved by...
TNF-α-converting enzyme (TACE). The mature 17-kDa TNF is then released into the extracellular space. This process is similar to the posttranslational processing of pro-IL-1β, which is converted to its mature form by IL-1β-converting enzyme (ICE) (53). Metalloproteinase inhibitors suppress TACE processing of pro-TNF and decrease LPS-stimulated TNF release (80, 177, 203). More importantly, McGeehan et al. (177) and Mohler et al. (203) independently observed that these inhibitors decrease LPS-induced lethality in mice. Metalloproteinase inhibitors are nonspecific and inhibit the enzymatic activity of various collagenses. Interestingly, ICE inhibitors do not prevent the release of the IL-1β precursor into the extracellular space, whereas TACE inhibitors do prevent the release of pro-TNF (80). This suggests that, in contrast to IL-1β, only the mature form of TNF is released. Thus TACE may be a rate-limiting step in TNF release.

**Therapeutic Strategies**

TNF contributes to myocardial contractile dysfunction and cardiomyocyte death, whether produced as a result of ischemia or sepsis. Future perspectives on blocking transcription and/or biological activity are discussed, as each suggests potentially therapeutic options (1-4, 22-26, 48, 49, 72-80, 85-88, 104, 105, 152, 163, 173-175, 182, 186, 193, 194, 196).

Inhibition of TNF transcription. Selective interference with TNF transcription may be an effective strategy of inhibiting TNF production (47). Although most anticytokine strategies have focused on preventing postrelease activity, agents that prevent TNF synthesis may provide additional benefit when used alone or in combination with postrelease neutralizing agents. Theoretically, disruption of the signaling cascade at any level would inhibit TNF production at the pretranscriptional level. This, however, may not provide selective inhibition of TNF production. Because the signaling cascade “fans out,” proximal signaling enzymes also initiate other vital pathways, as well as those for TNF production. For these reasons, more distal inhibition, such as P38 MAPK or NFκB inhibition, have theoretical appeal.

The P38 MAPK inhibitors selectively prevent macrophage proinflammatory monokine production (66, 155, 156, 253). P38 MAPK is activated by endotoxin (155, 156), cytokine (66, 156), hyperosmotic (253), hydrogen peroxide (113, 129), and ischemia-reperfusion stress (29). Bogoyevitch and colleagues (29) demonstrated that P38 MAPK is present in heart and that even brief ischemia-reperfusion of isolated rat heart activates P38 MAPK. These investigators also demonstrated that the degree of P38 MAPK activation correlates with the severity of injury. Pyridinyl-imidazole compounds, known as cytokine-suppressive anti-inflammatory drugs, complex with and inhibit P38 MAPK with high potency and specificity (66, 155, 156). Indeed, the half-maximal effective concentration values for P38 MAPK are in the nanomolar range (66, 155, 156, 271). Tong and associates (271) determined the crystal structure of the P38-pyridinyl-imidazole complex and reported that the high specificity of these compounds is due to its unique binding to the ATP pocket in P38 MAPK with a nearly perfect fit.

NFκB is a TNF transcription factor (16, 207) that is present in the heart and activated by myocardial oxidant stress (Fig. 4) (193, 196). Pyrroolidine dithiocarbamates (PDTC) are effective NFκB antagonists (47, 55, 138, 166, 201, 207, 248, 290); however, they lack specificity and little is known about their bioactivity or side effects. NFκB is activated by oxidant stress (160, 201, 207, 248); therefore, powerful antioxidants (e.g., PDTC) prevent NFκB activation. Indeed, vitamin E and glutathione also prevent NFκB activation via their antioxidant properties (138, 166, 201, 207, 248, 290). Binding NFκB with HSP70 should prevent nuclear translocation of NFκB and thereby also accomplish pretranscriptional inhibition of TNF production. Indeed, Feinstein and associates (91) demonstrated that, in astroglial cells, HSP70 binds NFκB in the cytosol and prevents its translocation to the nucleus. HSPs also inhibit TNF production at the posttranscriptional level (see HSPs) (238).

Inhibition of TNF activity. TNF and IL-1 synergistically depress myocardial function (75, 151, 208, 221). Therefore, blocking the activity of either TNF or IL-1 should have a synergistic beneficial effect. Phase I, II, and III clinical studies have examined the safety and efficacy of anti-TNF monoclonal antibodies, soluble TNF receptors, and IL-1 receptor antagonists in the treatment of patients with sepsis (1, 4, 26, 79, 89, 90, 95, 103, 221, 299). Although the overall results of these studies were somewhat disappointing, encouraging information has been obtained in specific subgroups of the patient population at risk. Anticytokine manipulation appears to be safe. Maximal TNF and IL-1 activity occurs within a few hours of insult, most often preceding the administration of anticytokine agents by hours or days in clinical studies (79). Pretreatment of animals with various anti-TNF binding protein strategies prevents endotoxin-induced myocardial dysfunction and lethality (26, 89, 105, 208, 286). Therefore, the most efficacious use of these strategies would be in those clinical settings allowing pretreatment. Because TNF has been implicated as a potential mediator of myocardial ischemia and reperfusion injury, preischemic administration of TNF binding proteins may reduce myocardial damage. Cardiac bypass, heart transplantation, and coronary angioplasty are three clinical scenarios that permit a scheduled myocardial ischemia-reperfusion/inflammatory event. Indeed, these events are more common clinically than sepsis.

TNF undergoes posttranslational myristoylation promoting membrane insertion and interaction within TACE (80, 101). Metalloproteinase inhibitors suppress TACE processing of TNF and decrease LPS-stimulated TNF release (80, 177, 203). These inhibitors decrease LPS-induced lethality in mice and may represent a therapeutic strategy to reduce TNF activity (177, 203). Protease inhibitors such as aprotinin may exert similar beneficial effects (124). Using exhaled NO and lung epithelial iNOS expression as markers of inflamma-
tion, Hill and colleagues (124) demonstrated that aprotinin decreased cardiopulmonary bypass-induced inflammation in humans. Indeed, aprotinin may exert its anti-inflammatory effects by decreasing TNF processing by TACE and thereby reduce TNF-induced iNOS expression and NO production.

HSPs. Many animal studies have demonstrated that prior thermal stress reduces myocardial infarct size following prolonged ischemia. Hutter et al. (130) postulated the induction of a class of “housekeeping” (heat shock) proteins that could repair or prevent stress-induced cellular damage. TNF, adrenergic, oxidant, endotoxin, or heat stress can induce the production of HSPs. The tolerance that evolves to the lethal metabolic and pyrogenic effects of endotoxin (12, 288) is temporally correlated with the appearance of the HSPs (43, 78, 96, 114, 307) and antioxidants (36, 38, 39, 41). Indeed, TNF-induced production of HSPs may be a mechanism by which TNF production is regulated (211, 212). LPS or TNF-induced production of HSPs (35, 37) can also provoke cross-tolerance (protective preconditioning) against other forms of injury (188, 197). We noted that LPS (39, 188, 197), TNF, or IL-1 (42) 24-h pretreatment conferred protection against subsequent myocardial ischemia-reperfusion injury. Induction of HSPs by heat, LPS, cytokine, or adrenergic stress results in similar postischemic protection (186).

Indeed, Hutter and colleagues (130) demonstrated a direct correlation in the degree of HSP induction (after various degrees of thermal stress) and the volume of myocardial infarct reduction following ischemia and reperfusion. We found that endotoxin (200) or adrenergic stress (199, 239) induced HSP70, which was associated with protection against endotoxemic or ischemic myocardial depression. Furthermore, this protection is cycloheximide inhabitable (188), suggesting that the mechanism of protection requires de novo protein synthesis. A differential effect of HSPs on cardiomyocyte survival following heat stress or ischemia and reperfusion was reported by Cumming and colleagues (67). They demonstrated that transfection of isolated cardiomyocytes with HSP70 increased cell survival following either lethal ischemia or lethal heat stress, that HSP90 protected against heat stress but not ischemia, and that HSP60 offered no protection against either stress. Plumier et al. (235) have convincingly linked HSP70 with infarct size reduction by overexpressing human HSP70 in transgenic mice with a dramatic improvement in posts ischemic myocardial recovery. Suzuki and colleagues (264) utilized an in vivo HSP70 gene delivery system to decrease myocardial ischemia-reperfusion injury. Liposomes containing the human HSP70 gene were used to facilitate in vivo transfer of HSP70 to rat heart. Furthermore, increased cardiomyocyte susceptibility to hypoxia and reoxygenation was observed when Nakano and associates (212) used antisense to HSP72 (inducible form of HSP70) to block endogenous HSP72 production. Thus HSPs, regardless of how they are induced, appear to decrease subsequent injury in part by downregulating subsequent TNF production (238).

Histological examination of protected hearts has localized the increased HSP70 to myocardial macrophages (200). It is possible that stimuli that induce HSP protect against injury by decreasing macrophage/TNF-mediated tissue injury. Indeed, HSP induction is temporally coincident with the onset of macrophage endotoxin-tolerance (96, 114). Feinstein and associates (91) demonstrated that HSP70 may reduce inflammation by decreasing NFκB activation. They demonstrated that HSP70 blocks iNOS expression by binding its transcription factor, NFκB, and preventing its translocation to the nucleus. Although not examined, HSP70 binding to NFκB may also decrease TNF and IL-1 production by the same mechanism (Fig. 5). In addition to its pretranscriptional effects, Ribeiro and colleagues (238) showed that HSP72 prevents posttranslational release of TNF. They demonstrated physical interaction between cytosolic TNF and HSP72, which was associated with decreased TNF release (Fig. 5). Thus heat shock or endotoxin induces HSPs, which appear to protect against subsequent insults by binding cytosolic NFκB and TNF, thereby limiting destructive inflammation. Liposomal delivery or gene transfer (40, 240) of HSPs into cells may allow the induction of HSP-mediated protection without the deleterious physical consequences of heat shock.

gp130 Subunit-linked agonists. The gp130 receptor subunit was first identified as a component of the IL-6 signal transduction pathway (5, 142). Six different cytokines that share gp130 as a receptor subunit downregulate TNF production (Fig. 5) (5, 7, 19, 279, 294). These cytokines are IL-6, IL-11, cardiotrophin-1 (CT-1), leukemia inhibitory factor, ciliary neurotrophic factor, and oncostatin M. Of particular interest are the recent findings of Benigni and colleagues (18) who demonstrated that CT-1 decreases TNF production by the heart. The mechanism by which gp130 agonists decrease TNF production is unknown, but may involve MAPK interaction at a pretranscriptional level (125). In addition to the transduction of anti-inflammatory signals, recent observations suggest that this receptor subunit has important implications in cardiovascular development and disease. Targeted disruption of gp130 (gp130 knockout mice) results in hypoplastic development of the ventricular myocardium and is incompatible with life (305), whereas continuous activation of gp130 leads to ventricular hypertrophy (232). Furthermore, Hirota and associates (125) have recently proposed that CT-1 is released by cardiac myocytes during ischemia to enhance cellular survival by an unknown mechanism. Thus gp130-linked agonists decrease TNF production and may be of therapeutic benefit in decreasing TNF-mediated myocardial injury.

Preconditioning: anti-TNF actions of adenosine, noradrenaline, HSPs, and antioxidants. The heart has intrinsic defense mechanisms against ischemic or endotoxemic injury, which can be elicited by brief periods of ischemia. We (15, 34, 37, 39, 42, 58, 62, 63, 185, 190, 195, 200, 202) and others (147, 164, 176, 209, 234, 259, 260, 269, 270, 289, 306) have termed this protective phenomenon ischemic cardiac preconditioning. Al-
though these findings are paradoxical, recent clinical evidence suggests that the myocardium adapts to the stresses of repeated sublethal ischemia. Indeed, Kloner and Shook (147), Otani et al. (225), and Andreotti et al. (9) have independently observed a protective role for angina, which precedes myocardial infarction by 24–48 h. We have recently confirmed these observations in an ex vivo model of human myocardial ischemia-reperfusion injury (57, 61, 63). These findings suggest that the stress of angina induces an endogenous adaptive mechanism that protects the myocardium. Endogenous myocardial protection against ischemia-reperfusion injury can be induced by early and delayed mechanisms, which have been respectively referred to as first and second window preconditioning (39, 209). The temporal discrepancy between early and delayed preconditioning suggests that different adaptation mechanisms exist (209, 269, 302). Early preconditioning occurs within minutes, is transient, and may be independent of de novo protein synthesis (269). The second window of protection requires hours for complete induction, is more sustained (up to 7 days), and may require de novo protein synthesis (176, 199, 200). Mechanistic examination of early and delayed preconditioning has permitted pharmacological induction of similar, if not identical, endogenous protection. In this regard, components of early preconditioning can be mimicked by preischemic stimulation of either adenosine A1 (121, 301), α1adrenergic (15, 202), or bradykinin B2 (34, 108) receptor stimulation. This receptor-inducible protection is mediated via the activation of KATP channels (110) and protein kinase C isoforms (145, 202); however, ultimate effectors remain unknown. Delayed myocardial adaptation, or second window preconditioning, has been induced by transient ischemia (302), endotoxemia (39, 176), inflammatory mediators (42), hyperthermia (81), rapid ventricular pacing (266), or α1-adrenoceptor stimulation (199) ≥24 h after stimulation.

Stress hormones, adenosine and norepinephrine (15, 34, 58–62, 178, 185, 195, 199, 202), are released during transient ischemia and can induce the early phase of preconditioning via intracellular kinases. The induced hydrolysis of phosphatidyli-4,5-bisphosphate by phospholipase C produces inositol trisphosphate and diacylglycerol, the intracellular targets of which have been identified as PKC and calcium-storage organelles (13, 14, 99, 178, 180, 181, 183–185, 188–190, 192, 197, 199, 200, 217, 292). Interrogation of preconditioning's mechanisms has focused on adenosine or norepinephrine signaling within the cardiac myocyte. However, it is possible that these ischemic stress hormones partly contribute to protection by their anti-inflammatory effects (45, 69, 175, 179, 230, 249, 254, 284). Adenosine's anti-inflammatory effects include 1) decreased TNF production in LPS-challenged mice (230), 2) decreased LPS-stimulated iNOS expression (69), 3) inhibition of neutrophil adhesion and injury to cardiac myocytes (45), and 4) decreased intestinal neutrophil accumulation after ischemia-reperfusion of the small intestine (249). Indeed, it has recently been reported that adenosine decreases cardiac TNF levels and bioactivity after ischemia and reperfusion of the isolated rat heart (182). Similarly, adrenergic agents decrease TNF production during human endotoxemia (284), as well as decrease macrophage superoxide and NO release (254). Thus 1) preconditioning induces the release of adenosine and norepinephrine, 2) adenosine and norepinephrine exhibit anti-inflammatory properties, 3) adenosine reduces cardiac TNF following ischemia and reperfusion, and 4) adenosine and norepinephrine pretreatment each protects myocardium against ischemia and reperfusion injury.

The mechanisms of endotoxin-induced delayed myocardial protection (39) and adenosine- or phenylephrine-induced acute myocardial protection (59, 63, 82, 97, 110, 143, 152) have been studied extensively. Endotoxin-induced delayed myocardial protection against the deleterious consequences of ischemia and reperfusion is only one of several noxious stimuli reported to induce protection >24 h after the original insult (39, 176). Indeed, transient ischemia (302), hyperthermia (170), rapid ventricular pacing (266), and norepinephrine each induce delayed myocardial protection against ischemia and reperfusion (199). Additionally, mediators of endotoxin-induced systemic effects, IL-1 and TNF, have been reported to independently induce similar, delayed cardioadaptive effects. The detoxified endotoxin derivative, monophosphoryl lipid-A, also instigates delayed cardioprotection (215). Induction of antioxidant enzymes and HSPs have been implicated in the mechanisms of delayed preconditioning. In this regard, it is known that ischemic stress induces myocardial adipation to ischemia and reperfusion (20, 39, 176). It has been hypothesized that the oxidant stress associated with ischemic preconditioning or endotoxin induces oxidant enzymes that scavenge free radicals and thereby decrease oxidant-induced myocardial damage during subsequent ischemia and reperfusion injury. Induction of antioxidant enzymes may act to limit oxidant-induced activation of NFκB or P38 MAPK. Delayed preconditioning may also act to decrease myocardial TNF production via HSPs (see HSPs).

Potential risks of cytokine inhibition: lessons learned from sepsis trials. Anti-TNF therapy should be interpreted with several important caveats. TNF likely plays an important role in the execution of a normal immune response. Effectiveness of antigen presenta-tion is enhanced when the T cell receives a costimulatory signal such as TNF or IL-1 (48, 49, 54). Many of the animal models that have demonstrated beneficial effects of anti-TNF therapy have chosen to use endotoxin, not live bacteria, as the insult. It is likely that in those situations anti-TNF therapy may limit the clearance of live bacteria and thereby increase mortality. The anti-TNF clinical trial that employed anti-TNF therapy in the form of soluble p75 TNFR was associated with increased mortality (94). Studies have demonstrated that TNF may be required to fight intracellular pathogens (Listeria) and fungi (Candida), and to survive abdominal sepsis. It appears that TNF signal transduction through the p55 receptor is necessary to clear
Candida infection (262). Likewise, TNF appears to be necessary for survival from sepsis in a murine peritonitis model (83, 123). TNF knockout mice are also unable to clear bacteremia after Listeria monocytogenes infection (8). TNF-deficient mice develop normally but are highly susceptible to Candida albicans infection, have impaired granuloma development, and do not form germinal centers after antigen challenge (171). However, these mice are also resistant to endotoxin challenge (171). Thus anti-TNF therapy may be most useful in those situations that constitute a pure inflammatory insult (e.g., ischemia and reperfusion injury). In this regard, the doses in the phase II p75 TNFR study (94, 214, 243) may have been excessive. The p75 TNFR may act as a TNF carrier and prolong the circulating half-life of TNF in vivo (204). The molecular difference between the p55 and the p75 TNFR may well prove important in that p55 TNFR may allow TNF to exit the circulation while p75 TNFR may prolong TNF circulation, stimulating fixed tissue receptors. This hypothesis is supported by a study in which mice were administered Escherichia coli and then either the p55 or the p75 TNFR. In mice treated with p75 TNFR, there appeared to be a “TNF carrier state” induced by p75 that was absent with p55 TNFR treatment (89). Thus TNF has been implicated in the pathogenesis of myocardial ischemia and reperfusion injury; therefore, employment of anti-TNF strategies during and after inflammatory insults may prove beneficial. However, we must be cognizant of the lessons learned from sepsis trials, which may suggest that we should avoid anti-TNF therapy in those patients with concurrent infection.

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