This Review is part of a thematic series on Chemokines and Cytokines, which includes the following articles:
Inflammatory Mediators and the Failing Heart: Past, Present and the Foreseeable Future

Inflammatory Cytokines and Postmyocardial Infarction Remodeling

Cytokines in Ventricular Function
Chemokines/Cytokines

Peter Liu, Guest Editor

Inflammatory Cytokines and Postmyocardial Infarction Remodeling

Min Nian, Paul Lee, Neelam Khaper, Peter Liu

Abstract—Inflammatory response and cytokine elaboration are particularly active after myocardial infarction and contribute to cardiac remodeling and eventual host outcome. The triggers of cytokine release in the acute postinfarction period include mechanical deformation, ischemic stimulus, reactive oxygen species (ROS), and cytokine self-amplification pathways. Acutely, the elaboration of tumor necrosis factor, IL-1 and IL-6, transforming growth factor families of cytokines, contribute to survival or deaths of myocytes, modulation of cardiac contractility, alterations of vascular endothelium, and recruitment of additional circulating cells of inflammation to the injured myocardium. This leads to further local oxidative stress and remodeling but also initiates the processes of wound healing. Chronically, sustained presence of cytokines leads to myocyte phenotype transition and activation of matrix metalloproteinases that modifies interstitial matrix, augmenting further the remodeling process. This in turn alters the local collagen composition and also the integrins that constitute the interface between myocytes and the matrix. These processes ultimately, when favorable, pave the way for angiogenesis and cellular regeneration. Thus, the insightful modulation of cytokines through current and future therapies could promote improved healing and cardiac remodeling postmyocardial infarction. (Circ Res. 2004;94:1543-1553.)

Key Words: remodeling ■ cytokines ■ inflammation ■ myocardial infarction ■ matrix ■ integrins

Inflammatory response and cytokine elaboration are integral components of the host response to tissue injury and plays a particularly active role after myocardial infarction. The degree of the inflammatory response in turn is an important determinant of the host’s outcome. Cytokines are released by the host myocardium to modulate tissue repair and adaptation after injury.

Cytokines such as tumor necrosis factor-alpha (TNF-α) or interleukin-6 (IL-6) are elaborated soon after myocardial ischemic injury and can acutely regulate myocyte survival or apoptosis and trigger additional cellular inflammatory response. Chronically, cytokines can mediate repair and remodeling through activating matrix metalloproteinases and collagen formation, and regulate integrins and angiogenesis and progenitor cell mobilization.

The consequences of inflammatory cytokine effects can be favorable, leading to healing and restoration of function, or unfavorable, leading to acute cardiac rupture or chronic dilatation, paving way for heart failure. The critical importance of the cardiac remodeling process postmyocardial infarction in the survival of the patient is well-documented from several postmyocardial infarction follow-up studies. Excessive ventricular dilatation readily leads to increased mortality.1

This review focuses on some of these recent developments and evolving concepts of cytokine activation postmyocardial infarction.
Triggers of Cytokine Production

Proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are not constitutively expressed in the normal heart. Up-regulation and production of these cytokines represent an intrinsic or an innate stress response against myocardial injury. In rodent models of myocardial infarction, within the first hours to 1 day, there are robust upregulations of intramyocardial cytokines including TNF-α, IL-1β, and IL-6 mRNA expression in the infarct area (up to 50-fold), as well as in the noninfarcted myocardium (up to 15-fold). This robust upregulation may return to baseline levels if the infarction is small. However, if the infarction is large, or if host inflammatory response is exuberant, there can be either sustained cytokine upregulation or a second wave of cytokine upregulation, corresponding to chronic remodeling phase. The second wave can also extend to involve the noninfarct remote zone, mediating important remodeling process in the entire myocardium. The elevation in cytokine expression precedes the consequent increase in local matrix metalloproteinase (MMP) activity (eg, MMP-2 and 9) in the infarct area, as well as the increase in natriuretic peptides (ANP and BNP) and collagen expression in the noninfarcted myocardium.

Mechanical Stress Triggers Cytokine Production

Cytokines such as TNF-α and IL-6 are rapidly released in the central ischemic zone during infarction but are usually maximal in the border zone. Ischemic stress represents a potent trigger for cytokine production, but direct myocardial mechanical stretch—maximal in the infarct and peri-infarct zone—is also a potent regulator (Figure 1). Kapadia et al have shown that direct hemodynamic stretch can trigger myocardial production of TNF-α de novo within 30 minutes. Mechanical stress associated with myocardial infarction leads to the prompt production of TNF-α and IL-6 in the myocardium. Mechanical stimulus acts through potential mechanosensors (integrins, cytoskeleton, and sarcolemmal proteins) and converts into 3 major intracellular cross-talking signal transduction pathways, mitogen-activated protein kinase (MAPK), JAK-signal transducer and activator of transcription (STAT), and calcineurin-dependent pathways. These pathways activate cognate downstream nuclear transcription factors, such as NF-κB and AP-1, which are required for the induction of most cytokine genes, including TNF-α and IL-6.

Intrinsic Response to External Stress, Including Ischemia

The robust upregulation of cytokines after ischemia also directly coincides with the transient induction of stress-
induced transcription factors such as C/EBP-β and STAT3 phosphorylation. This is also associated with local gp130 and IL-6 induction, again part of the host stress response signaling system, which can lead to phenotype transition, such as induction of hypertrophy. These signaling pathways that ultimately lead to cytokine induction are integrators of cellular stress signals and are upregulated in response to diverse stimuli such as hypoxia, free radical excess, osmotic dysregulation, and early membrane injury.

Other novel stress-induced inflammatory regulators, such as peroxisome proliferator-activated receptor (PPAR-γ), may also play a role in ischemic inflammation. PPARs have been implicated as regulators of cellular proliferation and host inflammatory response. In a murine postinfarction model, PPAR agonist pioglitazone was found to improve function and remodeling, and was associated with significant reductions in inflammatory cytokine levels in the myocardium. Rosiglitazone, another PPAR-γ agonist, can also reverse the upregulation of CD11b/CD18 and downregulation of cytokines in ischemia-reperfusion models.

Reactive Oxygen Species as Activators
Recent studies suggest that cytokines not only induce reactive oxygen species (ROS) production but also are themselves induced by ROS. In this regard, H₂O₂ can directly induce myocardial TNF production via the p38 MAPK pathway and, in turn, mediate myocardial dysfunction and apoptosis.

In a canine model of myocardial ischemia reperfusion, Kukielka et al have noted that cardiac IL-6 induction was minimal during 4 hours of ischemia but was markedly enhanced during 1 hour of ischemia followed by 3 hours of reperfusion, despite similar degrees of injury and blood flow reductions. The authors originally postulated that neutrophil infiltration was responsible for the difference. However, more recent information linking the induction of NF-κB and AP-1 to the intermediate and nonlethal levels of reactive oxygen radicals may be an exciting alternate hypothesis, because the latter are present in large quantities during reperfusion.

ROS during myocardial ischemia or reperfusion may originate from the mitochondria, xanthine oxidase, and the phagocytic nicotinamide adenine dinucleotide phosphate oxidase. ROS can participate as second messengers in host myocardial signaling events, including MAP kinases, small GTP-binding proteins, the Src family of tyrosine kinases, and cytokines cascades, activating stress-related nuclear transcription factors, leading to cellular hypertrophy and apoptosis.

Cytokine Amplification Pathways
Cytokines also have the unique ability to self-amplify through a positive feedback loop targeting the transcription factor NF-κB. For example, the upregulation of cytokines such as TNF-α in a localized area of the myocardium, eg, ischemic region, can easily induce TNF-α upregulation in neighboring normal myocardium, leading to amplified cytokine effects. Irwin et al from our group demonstrated that TNF-α mRNA was maximally detectable acutely in the infarct and peri-infarct zones during the first days postmyocardial infarction.

In contrast, by day 35 late postmyocardial infarction, the “contralateral normal zone” in the infarcted hearts showed the highest level of TNF-α expression.

To definitively demonstrate that cytokines such as TNF-α can upregulate remote regions in the heart through a “virtual” network, we have recently used a novel heterotopic cardiac transplant model, in which myocardial infarction was imposed on the heart in the abdomen, whereas the native recipient heart, totally undisturbed by surgery, is observed during subsequent days of follow-up. Most surprisingly, the otherwise infarcted and undisturbed recipient heart demonstrated a significant decrease in LV fractional shortening and increase in LV end-diastolic dimension after infarction in the abdominal heart. This was accompanied by increased TNF-α levels also in the recipient heart. This set of unusual observations was completely abolished by the intravenous administration of a TNF-α antagonist, Etanercept. This suggests that cytokines such as TNF-α can activate a remote amplification network that mediates potent remodeling effect not only in areas of acute injury but also in remote regions currently still surviving to recruit additional tissue for global repair and remodeling process.

Additional inflammatory cytokine amplification takes place through direct recruitment of inflammatory cells to the site of injury. Early after myocardial ischemia, adhesive cytokines such as monocyte chemoattractant protein, a potent mononuclear cell chemoattractant, is also induced in the myocardium. This promotes transmigration of macrophages from the blood and, in turn, provides an additional source of local cytokine production and amplification of the local inflammatory response.

Although mononuclear cells such as macrophages and neutrophils play a well-documented prominent role in early inflammation, other inflammatory cells also play important roles. Mast cell, a bone marrow-derived inflammatory cell with prepackaged inflammatory cytokines and growth factors, also accumulates in the ischemic-reperfused myocardium by 3 days postmyocardial infarction. Stem cell factor, secreted by a subset of macrophages in the reperfused area, may be an important factor for mast cell mobilization. In the early stage of injury, mast cell participates in the release of additional preformed TNF, which can further attract mononuclear cells to the injured tissue.

Cytokines and Acute Myocardial Remodeling Postinfarction
The stimulation of cytokine secretion in response to myocardial ischemia or infarction has profound effects on myocardium that provokes at least 4 changes directly in cardiac myocytes that contribute to the phenotype reprogramming or remodeling: progressive myocyte apoptosis, myocardial hypertrophy, defects in contractility, and inflammatory signal transduction. Chronically, cytokines have additional effects on extracellular matrix, integrins, and vascular and cardiac regeneration. Among the cytokines, TNF-α, IL-1β, and IL-6 are most commonly associated with the remodeling process postmyocardial infarction.
Paradoxic Effect of Cytokines on Myocyte Survival and Apoptosis

Cytokines such as TNF appear to have a significant pleiotropic effect on the host cells, with the potential for apoptosis versus cellular preservation and hypertrophy. The net balance between these 2 opposing processes defines the net cellular remodeling (Figure 1).

TNF ligand achieves all its different physiological and pathological effects by binding to 2 distinct surface receptors; TNF receptor 1 (TNF-R1) and TNF receptor 2 (TNF-R2).29,30 TNF-R1 and TNF-R2 share a conserved extracellular domain with both containing 4 tandemly repeated cysteine-rich motifs.29

TNF-R1 belongs to cell surface “death receptors” family, a growing subset of TNF-R superfamily that also includes Fas/CD95,31,32 death receptor 3 (DR3),33 DR4,34 DR5,35 and DR6.36 All death receptors contain a signature cytoplasmic death domain, a region of ~80 amino acids in length toward the carboxyl-terminus of the receptor.37 In response to TNF, TNF-R1 is trimerized, and death domain-containing molecule TNF receptor–associated death domain protein (TRADD) is recruited to the cytoplasmic regions of the receptors through homotypic death domain interactions.38 TNF receptor–associated death domain protein recruits the downstream signaling adaptor molecules Fas-associated death domain. Fas-associated death domain interacts with procaspase-8, thereby stimulating caspase-8 autoproteolytic activation and, in turn, initiate cell death by destroying cell’s own repair mechanisms. However, activation of the Bcl-2 family of proteins, such as Bcl-2, Bcl-x, may alter the release of cytochrome C and the net final balances of these signals will determine whether apoptosis will proceed to completion.

Beside the caspase-dependent apoptotic pathway, TNF has also been shown to induce cell death through the recruitment of a protein termed FAN (factor associated with neutral sphingomyelinase).39 FAN then interacts directly with membrane-bound neutral sphingomyelinase with resultant generation of ceramide.40 Ceramide triggers apoptosis via a mechanism postulated to involve ROS.41

Conversely, the cytoprotective effect of TNF-α may converge through the activation of the transcription factors NF-κB and stress-activated protein kinase/c-Jun N-terminal kinase (JNK), leading to the activation of cytoprotective gene expression. Tumor necrosis factor receptor–associated factors, such as TRAF2, interact with and activate downstream signaling molecules such as NF-κB–inducing kinase, a member of the serine/threonine MAPK kinase (MEK) kinase (MEKK) family.42,43 NF-κB–inducing kinase phosphorylates IKK (inhibitor of κB kinase) leads to NF-κB translocation to the nucleus and activation of genes involved in cellular inflammation, growth, and survival.44,45 Several other molecules, such as RIP, protein kinase B (PKB or AKT), apoptosis-stimulating kinase (ASK1), and germinal center kinase (GCK), also facilitate the TNF-induced stimulation of either NF-κB or stress-activated protein kinase/JNK activities to promote cell preservation and survival.46,47

The cardioprotective action of TNF-α is best illustrated in mice with combined genetic ablation of the TNF-R1 and TNF-R2 receptors. TNF-R1 and TNF-R2 double-receptor knockout mice undergoing left coronary ligation had significantly larger infarct size and increased myocyte apoptosis when compared with normal control mice. The hypertrophic effect of TNF-α was demonstrated in several independent studies. Physiologically relevant concentrations of TNF-α provoke a hypertrophic response by increasing the synthesis of structural and contractile protein in adult feline cardiac myocyte.26 Moreover, transgenic mice with cardiac-specific overexpressing TNF-α–developed cardiac hypertrophy.38

Similar finding have been observed for the so-called IL-6 family of cytokines that include IL-6, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, IL-11, and cardioprotein-1. The effects of this family of cytokines on multiple cell types, including myocytes, are achieved through gp130, a signal-transducing subunit shared by the receptors for the IL-6 family of cytokines. Dimerization of gp130 on IL-6–binding to its receptor leads to the activation of JAK and phosphorylation of gp130.19 These events lead to the activation of multiple signal transduction pathways, including the most prominent signal transducer and activator of transcription, Ras-MAPK, and phosphatidylinositol-3 kinase pathways that result in cytoprotective response in the heart. The significant role for IL-6 in tissue repair was demonstrated by experiments in which IL-6–/– knockout mice have significantly delayed cutaneous wound healing and worse outcome.50

Other cytokines are also potentially protective in the setting of myocardial infarction, including the bone marrow-derived cytokines such as granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO). In addition to their potential role of expanding and mobilizing bone marrow-derived progenitor cells, they may have direct myocardial-protective effects. G-CSF has been found to directly improve ventricular remodeling and function.51 EPO, important for erythrocyte survival and differentiation, has the ability to maintain vascular autoregulation and attenuating primary (apoptotic) and secondary (inflammatory) causes of cell death. In a rat model of infarction, EPO reduces cardiac myocyte loss by ~50%, sufficient to normalize hemodynamic function after reperfusion.52

Cytokines in Altering Cardiac Contractility

Cytokines are capable of decreasing left ventricle performance and myocyte contractility directly and indirectly. In the setting of injury, the reduction in contractility mediated by TNF and IL-6 may be an adaptive response to decrease myocardial energy demand. TNF-α and IL-6 can attenuate myocyte contractility directly through the immediate reduction of systolic cytosolic [Ca2+] via alterations in sarcoplasmic reticulum function, reversible by the removal of the cytokine exposure.53 However, TNF-α is also capable of decreasing myocardial contractility indirectly through nitric oxide-dependent attenuation of myofilament Ca2+ sensitivity.54

Alternatively, TNF provokes negative inotropic effects in myocytes partially through the neutral sphingomyelinase pathway. Early, within minutes after cardiac injury, TNF decreases systolic function by altering calcium-induced calcium release by the sarcoplasmic reticulum and by disrupting
the L-type calcium channel. In this phase, the binding of TNF to TNF-R1 leads to the release of sphingolipid metabolites (stress-induced second messenger) via sphingomyelin degradation. Oral et al also reported that sphingosine production correlated directly with calcium imbalance. Furthermore, blockade of sphingosine production negatively regulated TNF-α-induced contractile dysfunction. However, late (days) after MI, there is iNOS induction leading to increased production of nitric oxide, which further amplifies myofilament desensitization to calcium, resulting in sustained contractile abnormality. Furthermore, it has been shown that sustained expression of TNF can also lead to decreased SERCA2A expression, which is essential for the re-uptake of calcium in an energy-dependent manner after excitation–contraction of the cardiac myocyte.

Effects on Inflammatory Cell Infiltrate Through the Vasculature
Cytokines such as TNF and IL-1 and IL-6 are expressed at high levels in the vasculature in the ischemic myocardium. However, the upregulation of cytokines may alter the endothelial phenotype and set the stage toward an angiogenic program. Specifically, the presence of angiogenic factors, such as VEGF, HIF-α, and iNOS, both in animal models and human models after myocardial infarction, enhances the process of healing and angiogenesis.

In the setting of ischemia reperfusion, infiltration of neutrophils represents an important step in the local amplification of the initial inflammatory response triggered by the cytokines. Neutrophil migration depends on their interaction with the endothelial cells via L-selectin (originates from hematopoietic cells) and P-selectin (constitutively present in endothelial cells and platelets). The leukocyte β-integrins are, in turn, involved in adhesion to endothelial cells, particularly the β2 integrins (CD18), lymphocyte function antigen-1 (CD11a/CD18), macrophage antigen-1 (CD11b/CD18), or the very late antigen-4 (CD49d/CD29). Once inflammatory cell transmigration takes place, further cytokines are released to facilitate extravasation into the extravascular tissue. Recent data suggest that myosin light chain kinase-mediated myosin light chain phosphorylation is important in loosening the gap between endothelial cells and thereby increasing the vessel wall permeability for these cells.

Insight into the role of neutrophil infiltration in ischemic reperfusion has been gained mainly through loss of function studies. Ischemia reperfusion interventions in CD18−/− and ICAM-1−/− knockout murine models both resulted in decrease in infarct size and neutrophil infiltration, when compared with wild-type controls. This process has been similarly targeted in clinical trials involving the administration of monoclonal antibody against CD18. However, the result to date has been disappointing. A possible explanation is that leukocytes are secondary mediators of inflammation, and targeting the cause of inflammation is ultimately the more efficacious strategy.

Oxidative Stress Signaling
Cytokines and neutrophils collaborate to play an important role in ischemia reperfusion injury. They can cause tissue damages by at least 3 major mechanisms: (1) oxygen free radical release; (2) degranulation of elastase and proteases; and (3) release of arachidonic acid metabolites and platelet activating factors.

An intimate link between cytokines and oxidative stress has recently been established, in which transgenic mice overexpressing TNF-α increased the production of hydroxyl radical and impaired the antioxidant capacity of MnSOD. IL-6 can also induce oxidative stress and endothelial dysfunction in a model of atherosclerosis via angiotensin II receptor. TNF-α, IL-1β, and interferon-γ increased production of superoxide anion, which reacts with NO to form peroxynitrite and in turn desensitizes myofilament to calcium, leading to myocardial contractile failure. Suematsu reported recently that TNF-α directly induced mitochondrial ROS production within cardiac myocytes and caused mitochondrial DNA damage via a ceramide-dependent signaling.

Abnormal activation of the nonphagocytic NAD(P)H oxidase in response to neurohormones and cytokine activation (eg, angiotensin II, norepinephrine, TNF-α) is also a major source of ROS. ROS, with its potentiation role in physiological signaling, has also been shown to contribute to the development of cardiac myocyte hypertrophy. Furthermore, the subsequent chronic remodeling processes including fibrosis, collagen deposition, and matrix metalloproteinase activation are all dependent on ROS released during the phenotypic transformation of fibroblasts to myofibroblasts. The latter is particularly associated with pathological fibrosis, hallmarks of progression toward end-stage heart failure.

Initiation of Wound Healing
The acute remodeling process mediated by cytokines and inflammatory cells in the infarcted myocardium also initiate the wound repair process. This includes phagocytosis and resorption of the necrotic tissue, survival, and hypertrophy of the surviving myocytes, degradation and synthesis of matrix support such as collagens and integrins, proliferation of the myofibroblasts and angiogenesis/vasculogenesis, and, to a limited extent, progenitor cell proliferation.

Interestingly, during the acute phase of myocardial infarction, cytokines such as TNF-α and IL-1β appear to play a protective and coordinating role for wound healing. Anti–IL-1β treatment early postinfarction leads to poor wound healing and delayed collagen deposition. This is also concordant with the earlier observations that steroid treatment postmyocardial infarction leads to poor wound healing. Therefore, in balance, the acute and appropriate cytokine activation in the ischemically injured myocardium is fundamentally protective for the host, leading to improved wound healing and cell survival, at the expense of decreased contractility.

Chronic Remodeling Postinfarction
After the initial increase of gene expression of cytokine such as TNF-α, IL-1β, and IL-6 in the infarcted region, the cytokines normally begin to decrease toward baseline after 1 week. However, if the infarct size is large, or if there are other ongoing myocardial stress factors, the cytokine gene expression may remain significantly elevated or may recru-
ultimately leading to dilated cardiomyopathy, inflammatory models consistently demonstrate myocardial hypertrophy, collagenase), MMP-2 (gelatinase A), and MMP-9 (gelatinase dants, use of angiotensin-converting enzyme inhibitors, antiox-
dation can be attenuated significantly by the timely cycle of stress-cytokine-stress. The vicious cycle of cytokine amplification can be attenuated significantly by the timely use of angiotensin-converting enzyme inhibitors, antioxidants, β-blockers, or means to lower the mechanical load.

**Sustained Effects of Cytokines on Myocyte Morphology and Phenotype**

The influence of cytokines on myocardial cell phenotype initiated during the acute phase of remodeling can continue during the chronic phase if the cytokine elevations are sustained. The processes of myocyte hypertrophy, myocyte apoptosis, and triggering of additional inflammatory cell signaling are particularly prominent. The best biological validation of this effect is through a variety of transgenic mice superexpressing TNF-α in the myocardium. All these models consistently demonstrate myocardial hypertrophy, ultimately leading to dilated cardiomyopathy, inflammatory cell infiltrations, and increased interstitial fibrosis.

**Cytokines and MMP Activation**

The myocardium in the infarcted zone is ultimately replaced by scar tissue after gradual resorption of the necrotic tissue. Elevated cytokines also promote interstitial fibrosis and collagen deposition in the contralateral noninfarct zone. These remodeling processes involve synthesis and degradation of collagens as major components of the extracellular matrix, and, most important, mediators of the matrix remodeling are the MMPs. MMPs are present in the myocardial interstitium normally in the inactive form and can be readily activated within minutes of ischemia by free radicals, cytokines, and hypoxia, and they can be counter-regulated to a certain extent by tissue inhibitors of MMPs or TIMPs. Cytokines such as TNF-α and IL-1β can upregulate and activate MMPs that are initially responsible for collagen degradation and, subsequently, matrix deposition (Figure 1).

Studies in cultured neonatal and cardiac fibroblasts showed that IL-1β decreases the expression of procollagens α1(I), α2(I), and α1(III) mRNA and increases MMP-13 (type I collagenase), MMP-2 (gelatinase A), and MMP-9 (gelatinase B) protein levels. TNF can, in addition, increase the mRNA expression for pro-MMP-3. In fact, 2 activators of MMPs—extracellular MMP inducer and membrane-type MMP—have been shown to be present in myocytes and are upregulated in myocardial infarction and failure. In transgenic mice overexpressing TNF, which develops dilated cardiomyopathy with time, their myocardium demonstrates increased MMP activity by gelatin zymography.

Interestingly, the hearts expressing highest levels of MMP-2 and MMP-9 proteins also have the most amount of collagen deposition and impaired diastolic function. Anti-TNF-α treatment with adenoviral vector-expressing soluble TNF-α p55 receptor attenuated MMP-2 and MMP-9 expression, decreased collagen synthesis, and corrected left ventricle diastolic dysfunction.

It is possible that MMP inhibition presents an interesting novel strategy in attenuating cardiac remodeling postinfarction. Initial testing with MMP inhibitors with activity against a broad spectrum of MMP showed positive results in mice, but an unfortunate side effect is that it may also inhibit angiogenesis. A more selective MMP inhibitor reduced scar thinning, reduced left ventricle chamber dilatation, and increases angiogenesis in the infarcted area. Recently, we have tested inhibitors of the proinflammatory MMP, elastase, and found that elastase inhibition can significantly improve the ventricular function and remodeling in an ischemic model. This confirms our earlier testing of this strategy in blocking inflammation and ventricular function in a model of myocarditis.

**Cytokines and Collagen Production**

The cardiac matrix is an elaborate network of interconnected proteins, including collagen, elastin, and fibronectin, interacting with integrins and focal adhesion kinases at the cell/matrix junction. The collagen degradation and production postinfarction constitute the most dominant protein change in the myocardial matrix. Cytokines play an important role in regulating collagen deposition, including TNF, transforming growth factor (TGF-β), and osteopontin.

After myocardial infarction in a rat model, expression of TNF-α, IL-1β, IL-6, TGF-β1, and TGF-β3 as expected are increased maximally by 2-fold at ~1 week after myocardial infarction. These increases in cytokines correlate closely to the subsequent deposition of type I and type III collagens, and TGF-β3 appears to play a particularly active role in the regulation of late collagen deposition. TNF-α can also independently induce AT1 receptor upregulation, enhancing angiotensin II-mediated effects in favor of fibrosis.

Osteopontin (OPN), also called cytokine Eta-1, is also expressed in the myocardium and plays an important role in postmyocardial infarction remodeling by promoting collagen synthesis and accumulation. Osteopontin, a cell-secreted adhesive glycosphosphoprotein with embedded RGD motif, interacts with integrins αvβ3, αvβ5, αvβ1, and CD44 receptors. OPN is a multifunctional protein that can interact with fibronectin and collagen, suggesting a possible role in matrix organization or stability. OPN−/− (osteopontin-deficient) mice demonstrate disorganization of matrix and alterations in collagen fibrillogenesis. Increased expression of OPN postmyocardial infarction most likely coordinates remodeling by promoting collagen synthesis and accumulation through regulation of MMP activity via PKC-zeta.

**Cytokines and Integrins**

The interaction of matrix with the myocyte can take place through a number of nodes, the most important of which is
integrin. On the myocytes, integrin β1 is associated with anchoring of the myocyte in its matrix. β1 is found in 2 isoforms—a β1D form, which exists in the adult myocyte, is important for firm anchoring of myocyte in the matrix and leads to effective mechanical advantage during contraction.70 A β1A form exists in the fetal myocytes and promotes mobility and proliferation at the expense of efficient contractility. On exposure to cytokines such as TNF, the β1D isoform is transitioned to the fetal β1A isoform (Figure 2). This breaks the usual anchor of the myocyte to the matrix and promotes myocyte mobility. This transition is obliterated in the TNF-α/-β knockout mice, despite cardiac injury such as infarction.70 However, integrin β3 is found mainly in newly formed blood vessels and is also under cytokine control. This may provide 1 mechanism by which cytokines regulate the angiogenic pathways as well postinfarction, affecting the angiogenic potential.

**Cytokines and Angiogenesis and Cell Regeneration Postinfarction**

Part of the remodeling process postmyocardial infarction also involves regeneration of the lost components of the myocardium—blood vessels and cellular components such as the myocyte. The previous notion that after cardiac injury, the tissue will only be replaced by an inert scar is now overturned by current evidence support the fact that the heart can regenerate some of these components spontaneously and can be enhanced for an even more favorable remodeling.69

More recent studies suggest that cytokines elaborated during myocardial infarction can set the stage for subsequent angiogenesis. In a postinfarction model, expression of isoform VEGF120 was found at days 1 and 4 after myocardial infarction, whereas isoforms VEGF164 and VEGF188 along with expression of TNF-α and iNOS were noted for a much longer period of time.59 These findings suggest that angiogenic factors are elaborated at the same time as proinflammatory cytokines to mediate vascular repair. Macrophages may also participate in angiogenesis; a macrophage-derived peptide, PR39, inhibited the ubiquitin-proteasome–dependent degradation of hypoxia-inducible factor-1α protein, resulting in accelerated formation of vascular structures in vitro and increased myocardial vasculature in mice.90 Chemokines are chemotactic cytokines important in regulating angiogenesis. One class of the chemokine is the “CXCl” chemokines, which contain the 2 amino terminal cysteines are separated by a single nonconserved amino acid residue. CXCl chemokines that contain the so-called ELR motif, such as IL-8, are potent angiogenic factors, whereas CXC chemokines that lack the ELR motif, such as interferon-inducible protein 10 (IP-10), are potent angiostatic factors. In the first hours of reperfusion, TNF-α release from mast cells induces IP-10 synthesis in the microvascular endothelium. IP-10 expression inhibits angiogenic activity, until the injured area is debrided and a fibrin-based provisional matrix is formed. After the first 24 hours of reperfusion, TGF-β–mediated IP-10 downregulation shifts the balance toward angiogenesis.
Monocyte-derived macrophages, mast cells, and myofibroblasts secrete proteases and growth factors necessary for neovessel formation and optimal repair. In addition, TGF-β potently upregulates β-FGF and VEGF expression in endothelial cell and smooth muscle cells, thus enhancing angiogenic activity.

Furthermore, bone marrow cells from adult humans contain endothelial precursors with phenotypic and functional characteristics of embryonic hemangioblasts, and these can be used to directly induce new blood vessel formation in the infarct bed (vasculogenesis) and proliferation of preexisting vasculature (angiogenesis) after experimental myocardial infarction. The neangiogenesis resulted in decreased apoptosis of hypertrophied myocytes in the peri-infarct region, long-term salvage and survival of viable myocardium, reduction in collagen deposition, and sustained improvement in cardiac function.

Inflammatory mediators may induce recruitment of blood-derived primitive stem cells in the healing infarct, which may differentiate into endothelial cells and even lead to limited myocardial regeneration. The mechanisms by which cytokine cascade is activated in the infarcted myocardium have been recently elucidated. Several hematopoietic growth factors including IL-3, IL-6, granulocyte macrophage colony-stimulating factors, G-CSF, and stem cell factor have been reported to be positive regulators of granulopoiesis and act at different stages of myeloid cell development. G-CSF plays a critical role in regulation of proliferation, differentiation, and survival of myeloid progenitor cells. G-CSF also causes a marked increase in the release of hematopoietic stem cells into the peripheral blood circulation, a process termed mobilization. Recently, G-CSF has been reported to improve cardiac function and reduces mortality after acute MI. Although the mechanism by which G-CSF ameliorates cardiac dysfunction is not fully understood, there is the possibility that G-CSF may regenerate cardiac myocytes and blood vessels through mobilization of bone marrow stem cells. In the future, cytokine-mediated regeneration therapy may evolve to be a novel therapeutic strategy for myocardial infarction.

The hematopoietic growth factor EPO has been found to mediate repair and regeneration after brain and spinal cord injury, including the recruitment of stem cells into the region of damage. The EPO receptor interestingly is expressed in the fetal and adult heart as well. In models of myocardial ischemia and infarction, the administration of EPO has shown significant benefit by preventing myocyte apoptosis and attenuating deterioration in hemodynamic function. This benefit may also accrue from the mobilization of circulating stem cells, as observed in brain injury.

Clinical Implications: Cytokines as Diagnostic and Prognostic Markers and Potential Targets for Therapy

From the foregoing discussion, we can see that cytokines such as TNF are produced in significant quantities from the multiple sources postmyocardial infarction. They have effects on inflammatory cells, myocytes, and the matrix. These effects are pleiotropic and depend on the balance of other factors, the timing of release, and cell types involved. Therefore, the resulting phenotype can be very diverse.

More recently, the concept of inflammatory markers as a predictor of heart failure has gained more momentum. In the Framingham Heart Study, elderly patients who exhibited spontaneously elevated levels of inflammatory cytokines, including CRP, TNF, and particularly IL-6, have shown a much higher propensity for heart failure.

In a Finnish clinical study postmyocardial infarction, IL-6 levels increased by 44% and peaked at 24 hours. Peak IL-6 levels correlated positively with area under the curve of creatine kinase MB mass, peak troponin T level, and procollagan type III aminoterminal peptide measured at discharge. IL-6 is thus a potential marker of myocardium undergoing stress but may, in turn, regulate subsequent collagen formation and thus remodel the left ventricle after acute myocardial infarction.

However, the recent failure of TNF-α inhibitors in the treatment of CHF highlights the complexity of trying to inhibit this pleiotropic pathway in the heart, despite the success in the therapy of other inflammatory diseases. Therefore, for the cytokines to be a true therapeutic target, one would have to define the timing, the quantity, and what other cofactors need to be present to favorably influence the outcome. Until then, it is much better to treat the causative triggers of inflammation than to broadly block the effectors. However, the fact that many of the current modulators of benefit postinfarction already have important antiinflammatory activities, including angiotensin-converting enzyme inhibitors and β-blockers, suggests that ultimately they converge on the inflammatory mechanisms as part of the mechanism to exert their benefit in contractility, survival, and remodeling.

Acknowledgments

Supported in part by grants from the Heart and Stroke Foundation (HSF) of Ontario, the Canadian Institutes of Health Research (CIHR), CHFNET, and TACTICS Partnership Programs of the HSF and CIHR. Dr. Liu holds the Heart & Stroke/Polo Chair Professor of Medicine and Physiology at the University of Toronto. Dr. Nian is a CIHR TACTICS research fellow. Drs. Kshaper and Lee are recipients of the CIHR postdoctoral fellowship awards.

References


42. Nian et al Cytokines and Infarct Remodeling


Inflammatory Cytokines and Postmyocardial Infarction Remodeling
Min Nian, Paul Lee, Neelam Khaper and Peter Liu

Circ Res. 2004;94:1543-1553
doi: 10.1161/01.RES.0000130526.20854.fa
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/94/12/1543

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/