



Do cytokines and endothelial function have an impact on myocardial activity?

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Background: cytokines mediate cell-to-cell interactions via specific surface receptors regulating activities such as growth and cell death. The cardiac endothelium separates circulating blood from the myocardium; dysfunction of vascular endothelium upregulates enzymatic processes ascribed to modulation of nitric oxide (NO). **Aims:** to assess the role of cytokine-mediated immunologic responses in heart failure (HF). **Results:** patients with HF have high levels of cytokines that correlate with HF severity. Cytokines can affect endothelial function, reducing the vasodilator response, this action being exerted through NO, oxidative stress, and apoptosis. **Conclusions:** cytokines may contribute to the progression of HF through endothelial dysfunction, left ventricular dysfunction, and remodeling. New treatments capable of modulating cytokines should be investigated for the management of HF.

Keywords: cytokine, endothelial function, heart failure, nitric oxide synthase, oxidative stress, apoptosis

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This article looks at the hypothesis that the immunologic responses mediated by cytokines play an important pathogenetic role in the development of heart failure (HF). This “cytokine hypothesis” does not imply that cytokines cause HF per se, but rather that they contribute to the progression of the disease through endothelial dysfunction, left ventricular (LV) dysfunction, and remodeling.

Growing evidence of the complexity of the endothelium has led a large number of cardiovascular diseases to be reinterpreted with regard to their pathophysiology. In particular, endothelial dysfunction is now considered as a very important initial etiopathogenetic event in HF, both at the cardiac and vascular levels (Figure 1, next page).

CYTOKINES

Cytokines are small soluble protein molecules with molecular weights between 6000 and 60 000. They mediate cell-to-cell interactions via specific cell surface receptors, and regulate the activation, differentiation, growth, death, or acquisition of effector functions of immune cells. They are not produced by specialized glands, but are discontinuously produced by individual cells and different tissues in response to specific stimuli. Cytokines do not have general systemic effects, but

exert their effects mainly in either a paracrine (toward adjacent cells) or autocrine (toward the producing cell itself) way. It was originally thought that each cytokine exerted a specific effect on its specific target cell. However, it is today well established that most cytokines exhibit a wide range of biological effects on various tissues and cells. The manifestations of the actions of the cytokines are attributable to the functional pleiotropism due to the similarities among their receptors. The classification of cytokines is based on the structural relationships among the molecules. Some of them retain their historical name,

SELECTED ABBREVIATIONS AND ACRONYMS

| | |
|---------------|--|
| eNOS | constitutive (endothelial) nitric oxide synthase |
| HF | heart failure |
| HUVECs | human umbilical vein endothelial cells |
| IFN- γ | interferon gamma |
| IL | interleukin |
| iNOS | inducible nitric oxide synthase |
| LV | left ventricle/ left ventricular |
| NO | nitric oxide |
| TNF- α | tumor necrosis factor α |

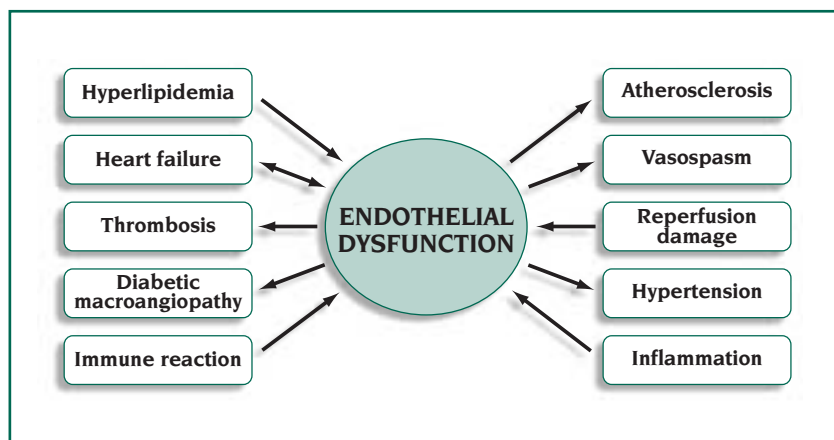


Figure 1. Causes and consequences of endothelial dysfunction.

eg, tumor necrosis factor α (TNF- α), while others have been termed interleukins (IL) and have been assigned numbers in sequence (IL-1 to IL-18). To date, more than 100 genetically unrelated cytokines have been identified (Table I).

ENDOTHELIAL FUNCTION

At the cardiac level, the endocardial endothelium plays an important role, as it covers the complex inner surface of the cardiac cavities, separating the circulating blood from the myocardium itself. It modulates the mechanical performance of the adjacent myocardium through its electrophysiological and transendothelial transport properties and through the release of a number of mediators, such as nitric oxide (NO), prostacyclin, and endothelin. These mediators may also play a role as growth factors, promoting or inhibiting the proliferation of endocardial interstitial cells and myocardial cells. In addition, the endocardium plays a role in coagulation and thrombotic processes, and expresses adhesion molecules and other antigens during inflammatory processes. Impairment of any of these functions may result in endocardium dysfunction. At the vascular level, activation, hence dysfunction, of the vascular

endothelium results in upregulation of adhesion molecules and enzymatic processes, which are mainly ascribed to the modulation of the NO pathway (ie, activation of inducible NO synthase [iNOS]).

CYTOKINES AND ENDOTHELIAL FUNCTION IN HEART FAILURE

Even though several symptoms of HF can be explained or mimicked by cytokines, only few among these (TNF- α , IL-1, and IL-6) have generated interest among cardiologists with respect to their potential role in the pathogenesis, clinical course, and treatment of congestive HF.

HF is considered as a systemic syndrome rather than a disease of the heart alone. In fact, the clinical features of HF are the resultant of structural and functional changes occurring both at the cellular and molecular levels in different systems such as heart, skeletal muscle, kidney, and endothelial cells.

Proinflammatory cytokines modulate cardiovascular function through a variety of mechanisms, resulting in progressive LV dysfunction and remodeling, pulmonary edema, fetal gene expression, and cardiomyopathy. Thus, the production of cy-

tokines—similar to neurohormone production—represents one of the possible biochemical mechanisms responsible for the development of symptoms in patients with HF.

A number of clinical studies have demonstrated that patients with HF express excessive levels of cytokines in plasma.¹ The systemic increase in cytokines produces a series of pathologic reactions. Thus, continuous infusion of TNF- α in rat was shown to induce a time-dependent depression in LV function and structure.² Injection of recombinant TNF- α increased mortality in the murine model of myocarditis.³ It has been reported that serum levels of TNF- α may correlate with the severity of HF, but the net biologic effect of TNF- α seems to be determined primarily by the body compartment in which it is produced.⁴⁻⁶ The source of cytokines in patients with HF is controversial; clinical studies suggest that the heart may be the source of intracardiac TNF- α , as evidenced in excised hearts, right atrial specimens from patients during cardiac surgery, and, recently, in failing human cardiac myocytes.

Moreover, during HF, cytokines affect endothelial function, contributing to a marked reduction in the vascular dilator response. This action is exerted through several pathways, which involve NO, oxidative stress, and apoptosis.

NO-dependent pathway

There is clear evidence that, both in experimental animals with HF of differing etiology⁷ and in patients with HF,^{8,9} the vasodilator response to acetylcholine is reduced, compared with control groups. Several reasons may explain this reduced NO production, the main one being reduced activity of constitutive (endothelial) nitric oxide synthase



(eNOS), which produces NO from arginine. This hypothesis is supported by evidence of reduced synthesis and expression of this enzyme in experimental models of HF.⁷ Such NO synthase downregulation seems to be the resultant of chronic cytokine activation, as suggested by experimental data showing that TNF- α , IL-6, interferon gamma (IFN- γ), and other cytokines¹⁰ can inhibit the transcription of this enzyme in vitro.

Furthermore, several studies from different groups of researchers have shown that, in the advanced stages of HF, an increase in the circulating levels of these cytokines occurs. Fi-

nally, cytokines have been shown to be the most powerful indicator of short-term negative prognosis in patients with HF.^{11,12}

In vivo expression of the iNOS messenger RNA and protein has been evidenced in most cardiovascular tissues, including vascular smooth muscle, endothelial cells, and cardiac myocytes.¹³⁻¹⁶ Overproduction of NO could, at least in theory, account for many of the clinical features described in HF, since NO is a potent vasodilator and is involved in blood flow regulation; it can also depress myocardial function and impair cellular respi-

ration. However, in many in vivo settings—especially in the heart—a major proportion of iNOS expression and activity is present in infiltrating inflammatory cells (*Figure 2, next page*). Therefore, in the case of cardiac iNOS expression in vivo, it is quite difficult to distinguish the actual cellular contribution of iNOS and determine the corresponding functional consequences.

Oxidative stress

IL-1, IL-6, and particularly TNF- α may induce endothelial and LV dysfunction and remodeling either directly or via oxidative stress, ie,

| | | |
|-----------------------------|--|--|
| TNF- α | <i>Tumor necrosis factor α</i> | Contains highly conserved carboxy terminal domains; can induce receptor trimerization influencing signaling pathways |
| IL-1 | <i>Interleukin-1</i> | Synthesized as glycosylated proforms lacking signal peptides |
| IL-6 family | <i>Interleukin-6</i> | Uses receptors homologous to or containing the gp 130 subunit as the common signaling component |
| IL-4 IL-13 | <i>Interleukins 4 and 13</i> | Bind to shared heteromultimeric receptor complexes |
| IGF | <i>Insulin-like growth factor</i> | Shares sequence homology with the insulin family of proteins |
| HGF MSP | <i>Hepatocyte growth factor</i> <i>Macrophage-stimulating protein</i> | Contain a 4-kringle domain and a pseudoserine protease domain that lacks enzymatic activity |
| FGF | <i>Fibroblast growth factor</i> | Heparin-binding polypeptide |
| NGF BDNF | <i>Nerve growth factor</i> <i>Brain-derived neurotrophic factor</i> | Induce signal transduction through ligand-induced dimerization and activation of trk receptors |
| Tpo Epo | <i>Thrombopoietin</i> <i>Erythropoietin</i> | Share sequence homology |
| PDGF VEGF PlGF | <i>Platelet-derived growth factor</i> <i>Vascular endothelial growth factor</i> <i>Placenta growth factor</i> | Dimeric angiogenic factors containing an 8-cysteine motif |
| EGF | <i>Epidermal growth factor</i> | Contains at least one extracellular EGF structural unit (conserved 6-cysteine motif that forms 3 disulfide bonds) |
| SCF Flt-3L, M-CSF | <i>Stem cell factor</i> ; <i>Fms-like tyrosine kinase 3 ligand</i> <i>Macrophage colony-stimulating factor</i> | Contain a 4-helix bundle structure in the extracellular domain and 4 conserved cysteines; receptors are tyrosine kinases |
| MK PTN | <i>Midkine</i> <i>Pleiotrophin</i> | Products of retinoic acid-responsive genes; developmentally regulated molecules |
| TGF- β superfamily | <i>Transforming growth factor-β</i> | Contain a highly conserved 7-cysteine domain that forms a characteristic cysteine knot |

Table 1. Cytokine family key.

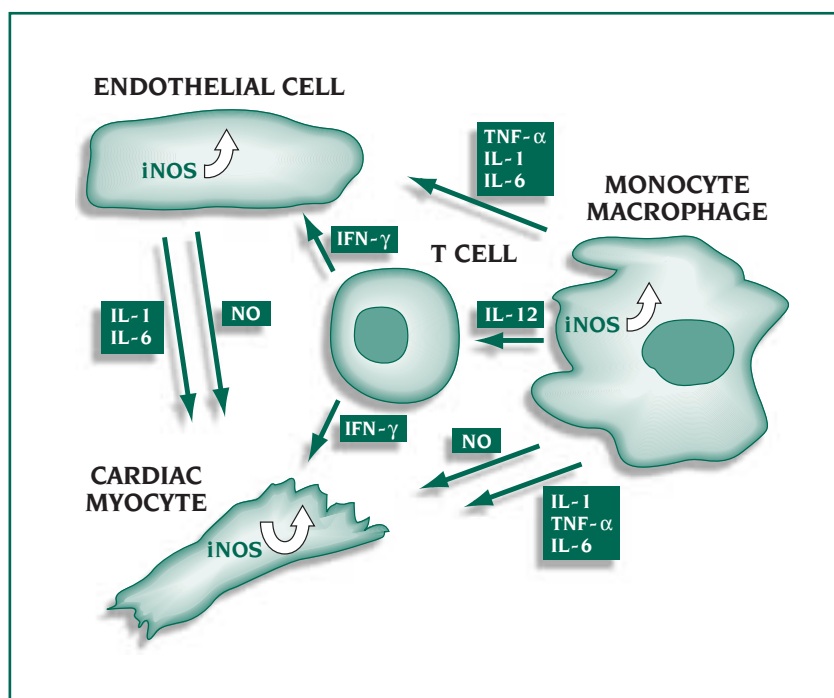


Figure 2. Relation between nitric oxide production and cytokine network in myocardium.

Abbreviations: IL-1, -6, -12, interleukin-1, -6, -12; iNOS, inducible nitric oxide synthase; IFN- γ , interferon gamma; NO, nitric oxide; TNF- α , tumor necrosis factor α .

through the toxic effect of reactive oxygen species. Oxidative stress occurs in patients with HF as a consequence of increased production of reactive oxygen free radicals and/or alteration of cellular mechanisms of antioxidant protection.¹⁷ Oxidative stress activates a family of transcription factors involved in cardiac and vascular remodeling. Oxygen free radicals are also involved in apoptosis, which is characterized by a continuous loss of myocardial and endothelial cells. This phenomenon may result in a progressive decrease in myocardial and endothelial function over time in patients with HF, and is a hallmark of the syndrome.

The myocardium is exposed to oxidative stress both during myocardial ischemia, where the antioxidant reserve of the heart is consumed and oxygen free radical production

increases, and particularly during reperfusion, where the heart is re-exposed to molecular oxygen.¹⁸ It has also been suggested that a mismatch between oxygen free radical production and antioxidant defense mechanisms may play a role in the transition from hypertrophy to the decompensated state of the heart. Interestingly, compensatory hypertrophy is accompanied by an increased myocardial antioxidant reserve, a redox state, and a greater resistance to oxidative stress.¹⁹ Conversely, in the decompensated state, there is a relative deficit in the myocardium's antioxidant capacity, accompanied by a decrease in its redox state.²⁰

Apoptosis

In contrast to necrosis, apoptosis is a genetically regulated death process characterized by cell shrinkage,

DNA fragmentation, cytoplasmic blebbing, and cellular disassembling into small apoptotic bodies that are then digested by neighboring cells and macrophages.²¹ Apoptosis has been demonstrated in pathologic conditions affecting the endothelium and the adult human heart. In vitro studies have shown that the incubation of human umbilical vein endothelial cells (HUVECs) with serum from patients with severe HF induces a downregulation of constitutive eNOS protein expression and an increase in apoptosis, and that these effects are partially counteracted by the addition of an anti-TNF- α antibody.²² Apoptosis has also been demonstrated in the heart of patients with severe end-stage HF.^{23,24} However, in spite of the evidence of apoptosis in the failing human heart, its clinical significance remains controversial.

Recent findings suggest that apoptosis, which is associated with HF and thus has a negative prognosis, may be counteracted by the activity of "good" cytokines, such as cardiotrophin 1. The latter acts on different cellular pathways than the other cytokines (gp 130 receptor), resulting in hypertrophy, thereby favoring cell survival instead of apoptosis.

CONCLUSIONS

The interest in the role of stress-induced cytokines in myocyte function in HF is increasing. Researchers are currently investigating new treatments designed to modulate the actions of the cytokines, by either neutralizing the proteins secreted or inhibiting their synthesis.²⁵ Such treatments, should they prove effective, hold the prospect of becoming the future gold standard in the management of HF.



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