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Cytokines and Cardiomyocyte Death

Kari J. Pulkki

Cytokines have been associated with the pathogenesis of acute coronary syndromes and chronic heart failure (CHF), which are both associated with cardiomyocyte loss. In CHF, increased serum concentrations of proinflammatory cytokines, including tumour necrosis factor α (TNF-α) and also soluble TNF receptor have been found. Both TNF and Fas-ligand have been able to induce programmed cell death (apoptosis) of cardiomyocytes in various experimental studies. In ischaemic conditions of the heart, increased serum levels of soluble Fas receptor have been found. The proinflammatory cytokines interleukin 1 (IL-1), IL-2 and interferon-γ can induce TNF production from target cells, including myocytes. TNF and some other cytokines are able to induce nitric oxide production, which depresses cardiac function and can induce apoptosis. However, anti-inflammatory cytokines such as IL-10, IL-4 and IL-13, secreted by T-helper type 2 lymphocytes and other cells, inhibit the production of proinflammatory cytokines. Preliminary studies suggest that cardiotrophin-1, produced by cardiomyocytes, is able to inhibit cytokine-induced cardiomyocyte apoptosis *in vitro*. As growth hormone is able to inhibit the production of proinflammatory cytokines in many cell types, it may also play an important role in the regulation of apoptosis induced by these cytokines. When the cytokine-induced pathways leading to altered gene expression of cardiomyocytes are understood, this knowledge may aid in the development of drugs that prevent progressive cardiomyocyte loss, in particular by inhibiting cytokine-induced apoptosis.

Key words: cytokines; heart diseases; heart failure; nitric oxide.

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Introduction

An understanding of the pathogenesis of myocardial diseases that lead to myocyte loss and deterioration of myocardial function is essential in order to target these processes with specific drugs. Diseases that may lead to myocyte death include viral myocarditis, rejection of transplanted heart, ischaemia-reperfusion injury, acute myocardial infarction and, as an end-stage, chronic heart failure (1, 2). As cardiomyocytes cannot proliferate in adults, it is essential to preserve the muscle mass of the heart (2). Therefore, therapeutic procedures directed to prevent additional myocyte loss should be initiated at an early stage. A potential process that may be stopped or reverted is programmed cell death, i.e. apoptosis (2, 3). Somewhat artificially, apoptosis may be contrasted with accidental or necrotic cell death (see below).

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A common feature of diseases leading to myocyte death is increased concentration of cytokines in the blood or the heart muscle itself (4, 6). This has been associated with depression of cardiac function and apoptosis, mainly through induction of nitric oxide production (7, 8). During the last five years it has

Abbreviations

CHF	Chronic heart failure
CRP	C-reactive protein
CT	Cardiotrophin
FGF	Fibroblast growth factor
GH	Growth hormone
IGF	Insulin-like growth factor
IFN	Interferon
IL	Interleukin
iNOS	Nitric oxide synthase
LIF	Leukaemia inhibitory factor
NO	Nitric oxide
TGF	Transforming growth factor
TH2 cells	T-helper type 2 cells
TNF	Tumour necrosis factor
TNFR	TNF receptor

become possible to understand the mechanisms of cell death induced by cytokines (9, 10). This paper reviews the current knowledge about cytokine-induced cardiomyocyte death.

Cytokines

Cytokines are targeted to myocardial cells as immune cells adhere to myocytes or as significant amounts of cytokines are delivered from other cells further away (11). According to current knowledge, cytotoxic lymphocytes rather than monocytes are found at sites of myocardial inflammation (12). Monocytes have been associated more with depression of cardiomyocyte function than necrotic death of these cells (12).

Cytokines are low-molecular-weight proteins that are produced by many cell types, particularly inflammatory cells. Approximately 100 molecules have been cloned to date (13). These cytokines bind to specific receptors and induce a specific gene expression in the target cells. As the molecules induce their effects through a secondary network of cytokines it is often hard to estimate the net effects of all cytokines on a specific function in this context. In terms of cardiac pathophysiology, the cytokines may be divided into three categories: proinflammatory, anti-inflammatory and cardioprotective. They may originate from inflammatory cells: polymorphonuclear cells, monocytes, lymphocytes, eosinophils or mast cells; and also from resident cells: fibroblasts, endothelial cells, other cells or even cardiomyocytes themselves (3–13).

The mediators leading to the death of the target cell (cardiomyocyte) may be secondary mediators or second messengers: nitric oxide (7, 8, 14), reactive oxygen species (15), cyclic nucleotides or intracellular ions (calcium overload and changes in other ions) (16).

TNF-α: The Prototype for Proinflammatory Cytokines

The function of a specific cytokine depends on its target cell. Cytokines that are able to induce the death of a cardiomyocyte are cytotoxic for other cell types, too. These cytokines are typically proinflammatory, e.g. interleukin-1 (IL-1), IL-2, interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α) (4–8, 11, 12). In addition to cell death in experimental sepsis of the rabbit, only IL-1 and TNF have also been shown to induce functional myocardial cell depression (17).

TNF-α, a pleiotropic cytokine, induces receptor-mediated death of its target cells (9, 10). TNF-α may induce a dual response as both apoptotic and necrotic types of cell death have been described (3). There are more than 10 members in the TNF-receptor (TNFR) family of cytokines, many of which mediate death signals. TNF-α is probably clinically the most important cytotoxic cytokine. Fas, also called APO-1, is a member of this family and shares a common cytoplasmic signalling motif, called the death domain, with TNFR. Fas signalling has been well-characterized: it needs two other molecules called FADD and FLICE for signalling and for forming complexes. FADD contains a cell death domain (D) in the C-terminus and uses this to interact with the death domain of Fas (9, 10). The N-terminus of FADD contains another novel motif, called the death effector domain (E), which is used for binding the third protein, FLICE. FLICE and FADD interact via their respective death-effector domains. Most interestingly, FLICE contains an interleukin-converting enzyme-like domain that may function as an initiator of the cystein protease cascade (10). Both FADD and FLICE play a critical role in TNF-induced apoptosis. However, another adapter molecule, TRADD, is needed to recruit FADD to the death domain of TNFR (9, 10, 18). This example illustrates the complexity and several check-points involved in cytokine-induced cell death.

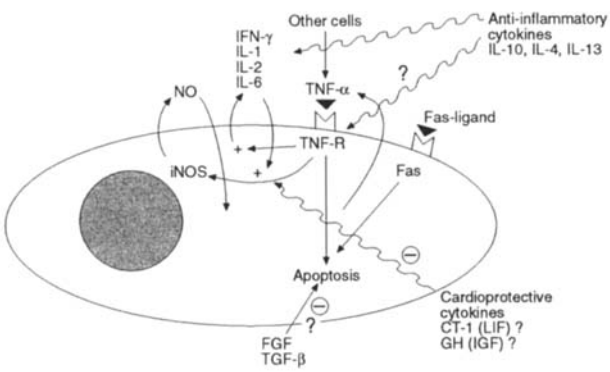

Figure 1. The cytokine network of cardiomyocytes. For definitions please refer to the list of abbreviations.

Other Proinflammatory Cytokines

The group of proinflammatory cytokines also includes IFN-γ, IL-1, IL-2, IL-8 and the chemokine family. Interest-

Table 1. The effects of cytokines on cardiac myocytes.

Cytokine	Function
TNF-α	Cytotoxic, induce NO
Fas-ligand	Cytotoxic
Other proinflammatory (IL-1, IL-2, IFN-γ)	May induce TNF and NO→cytotoxic
IL-4, IL-10, IL-13	May inhibit TNF
IL-6	Induce TNF, is induced by TNF
IL-8, other chemokines	Chemotactic and activate leucocytes*
CT-1	Inhibits myocyte apoptosis *in vitro*
LIF	Mimic effects of CT-1
TGF-α, β	Modulate functions of other cytokines*
Atriopeptide, adrenomedullin	Induce NO production of myocytes
GH→IGF-1	May inhibit apoptosis

*Functions that have not been shown in cardiomyocytes but in other experimental systems. For definitions please refer to the list of abbreviations.

ingly, IL-2 and IL-1 (α and β) are able to induce TNF-α through a network of actions (18). Furthermore, the apoptotic pathway may be induced by nitric oxide production in cardiomyocytes which in turn is induced by IL-1, IL-6, TNF-α and IFN-γ (7, 8, 14).

Anti-inflammatory and Cardioprotective Cytokines

A great deal of interest is directed towards anti-inflammatory cytokines, which inhibit the synthesis of proinflammatory cytokines, including TNF-α. Most members of this group are secreted by T-helper type 2 (TH2) cells: IL-4, IL-10 and IL-13. IL-4 secreted by TH2 cells induces IL-10 and IL-13 secretion from other cell types, including monocytes and TH1 cells (19). IL-10 seems to inhibit TNF-α production and decrease the area of myocyte death in experimental reperfusion injury (20).

Cardiac growth and survival factors induce differentiation-associated genes (21). Understanding of activation of these genes may aid definition of the genes that become activated in heart failure and other diseases associated with threatened cardiomyocyte survival. The role of transforming growth factors (TGF) in the pathogenesis of heart diseases is not clear, but they may modulate protective gene expression of cardiomyocytes. For example, TGF-β inhibits the production of proinflammatory cytokines in other cell types (22). Recently, growth hormone (GH) (23) and GH-induced insulin-like growth factor 1 (IGF-1) (24) has been shown to inhibit TNF-α production of monocytes and, correspondingly, TNF has been shown to inhibit growth hormone production and induction of IGF-1 (25). Therefore, experiments directed to study the mechanisms of the potentially beneficial effects of GH and IGF-1 on myocytes have become an area of main interest.

Cardiotrophin-1

A new member of the IL-6 receptor family of cytokines, cardiotrophin-1 (CT-1) was recently cloned (26). Other members of this family are IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin and ciliary neurotrophic factor. CT-1 binds to a heterodimer of receptors. The β-chain of this receptor, glycoprotein 130, is common to all cytokines in this family; the α-chain is specific for the particular cytokine. CT-1, however, binds to the α-chain of LIF. CT-1 acts as a nerve growth factor and also induces hypertrophy of cardiac myocytes (27, 28). Preliminary studies have suggested that CT-1 is able to inhibit cytokine-induced apoptosis *in vitro* (29). Therefore, further studies are needed to confirm its antiapoptotic role in diseases leading to cardiac myocyte loss.

The Cardiomyocyte as the Source and Target of Cytokines

Human myocardial cells are binucleated terminally differentiated mesodermal cells, which are not able to proliferate (1–3). They are also capable of secreting cytokines, including TNF-α (6). This is surprising as this proinflammatory cytokine might lead to increased cell death of the same myocytes ('suicide' through apoptosis). Another 'suicide' signal is the production of nitric oxide (NO) through induction of nitric oxide synthase (iNOS), by TNF, IL-1, IL-2 and IFN-γ. In addition, the recently discovered adrenomedullin produced by the heart (30) may induce NO production. Another well-established system that may jump-start apoptosis is local production of reactive oxygen species (15). Therefore it is likely that there are antiapoptotic mechanisms in the myocardial tissue with the role of protecting the heart from inappropriately initiated apoptotic cascade. As an example, observations of increased production of bcl-2 oncoprotein (31) may be considered as an attempt to control apoptosis-inducing signals and enhance survival (32). The fact that cell death induced by TNF (or Fas) may need other regulatory systems to fail in order to get the myocyte to enter apoptosis (33) also illustrates the multistep nature of this control.

Apoptosis and Necrosis

Two types of cell death have been described, which also concern the fate of cardiomyocytes. The former type is the random-type, nonphysiological, non-energy-dependent cell death called necrosis, which usually results in the death of many cells in tissues. The classic example of necrosis is the ischaemic necrosis in acute myocardial infarction. In contrast, the latter type of cell death, apoptosis, is an active process controlled by the nucleus, although it also leads to death of committed cells (34). Apoptosis has a central role in the development of tissues and in the regulation of the growth of both normal and malignant tissues (35). In recent years both the morphological and biochemical features of apoptosis have been well-characterized. A hallmark of apoptosis is fragmentation of nuclear DNA by specific endonucleases ('DNA ladder' in gel electrophoresis) and activation of specific proteases. This can also be found histochemically with DNA *in situ* end-labelling techniques. The current hypothesis is that cell death through apoptosis does not induce an inflammatory reaction, which is rather the result of necrotic cell deaths (36, 37). When the cardiomyocyte is induced by signals or faced by events, it has two choices, which are related to changes in its gene expression: hypertrophy or death (through apoptosis) (3).

Experimental hypoxia of the myocardium (38), ischaemia–reperfusion injury (39) and overstretching (40) are all able to induce apoptosis of cardiomyocytes. There is a layer of apoptotic cells around the necrotic area of acute myocardial infarction. The clinical importance of this phenomenon is under discussion (41–43) but it may have a role in the remodelling of the left ventricle (43). It has been established that increased percentages of apoptotic cardiomyocytes are found in patients with chronic heart failure (CHF) (44–46). However, the clinical importance of these findings in

patients with either ischaemic or dilated cardiomyopathy is not clear.

Circulating Cytokines and Heart Diseases

Myocarditis, rejection of the transplanted heart and acute myocardial infarction are associated with an inflammatory response and increased concentrations of proinflammatory cytokines both in the heart (6) and in the blood (4–6, 47, 48). In addition, many studies have demonstrated that patients with CHF have increased concentrations of cytokines, including TNF (4, 5), soluble TNFRs (6, 47) and soluble Fas receptors, but normal levels of Fas-ligand (48), in their blood. Because blood levels may not reflect the situation within the myocardial tissue, affirmative studies are needed to establish the clinical utility of serum level determinations.

Genetic Polymorphism of Cytokine Genes

It is well established that there is individual variation in the immune response between each person. This may be the result of individual variation in cytokine secretion, which may come from genetic polymorphisms in the regulation of cytokine secretion or function. Patients with increased serum concentrations of fibrinogen (49) and C-reactive protein (CRP) (50, 51) have increased risk for acute myocardial infarction. As fibrinogen and CRP are cytokine-induced, genetic polymorphisms of IL-6 or other proinflammatory cytokines may lie behind these associations. Polymorphic variation that may be of genetic origin has been described in the serum levels of TNF-α (52). The relation of such genetic polymorphism to the progression of CHF or acute myocardial infarction has not been studied.

Therapeutic Interventions and Future Directions

As this review has demonstrated, cytokines are relevant to the pathogenesis of many heart diseases. The most intriguing example is cardiomyocyte loss through apoptosis in CHF. Current knowledge suggest that inhibition of the synthesis, release or function of cytotoxic cytokines, e.g. TNF and Fas-ligand, might benefit the heart failure patient. This has become possible as there are plenty of data on the signalling of these cytokines. On the other hand, therapies to increase the synthesis, release or function of anti-inflammatory (e.g. IL-10) or cardioprotective (e.g. CT-1, IGF-1) cytokines may benefit the patient by leading to decreased function of proinflammatory cytokines. Another therapeutic avenue is the modulation of second messengers and mediators or the modulation of the triggering events leading to apoptosis. These areas will be of primary interest in experimental and clinical studies in the near future.

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