An overview of the inflammatory signalling mechanisms in the myocardium underlying the development of diabetic cardiomyopathy

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Abstract

Heart failure is a highly morbid and mortal clinical condition that represents the last stage of most cardiovascular disorders. Diabetes is strongly associated with an increased incidence of heart failure and directly promotes cardiac hypertrophy, fibrosis, and apoptosis. These changes, in turn, contribute to the development of ventricular dysfunction. The clinical condition associated with the spectrum of cardiac abnormalities induced by diabetes is termed diabetic cardiomyopathy. Myocardial inflammation has recently emerged as a pathophysiological process contributing to cardiac hypertrophy, fibrosis, and dysfunction in cardiac diseases. Myocardial inflammation is also implicated in the development of diabetic cardiomyopathy. Several molecular mechanisms link diabetes to myocardial inflammation. The NF-κB signalling pathway and the renin-angiotensin-aldosterone system are strongly activated in the diabetic heart, thereby promoting myocardial inflammation. Advanced glycation end-products and damage-associated molecular pattern molecules also represent strong triggers for inflammation. The mediators resulting from this inflammatory process modulate specific intracellular signalling mechanisms in cardiac cells that promote the development of diabetic cardiomyopathy. This review article will provide an overview of the signalling molecular mechanisms linking diabetic cardiomyopathy to myocardial inflammation.

Keywords

Inflammation • Diabetes • Cytokine • Cardiomyopathy • Heart failure

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1. Introduction

Despite continuous advances in treatment and prevention strategies, cardiovascular diseases still represent the main cause of death in Western countries.1 Among cardiovascular diseases, heart failure is a highly morbid and mortal condition, which represents the last stage of most cardiovascular disorders. Specific pharmacotherapy that can reverse heart failure is not yet available. For this reason, it is very important to understand the molecular mechanisms underlying the development of cardiac dysfunction and heart failure in order to find new therapeutic targets.

Diabetes is a major risk factor for cardiovascular diseases.2 Among these, previous large epidemiologic studies demonstrated that diabetic patients are at increased risk for developing heart failure.3 Diabetes may lead to of heart failure indirectly, by promoting the development of coronary artery disease, a major heart failure cause. However, it is now known that diabetes may cause heart failure also by eliciting a direct detrimental impact on the myocardium leading to the development of cardiac hypertrophy, diastolic, and systolic dysfunction.4 The clinical condition associated with the spectrum of cardiac abnormalities induced by diabetes is termed diabetic cardiomyopathy.4 Myocardial inflammation has recently emerged as a pathophysiologic process contributing to cardiac hypertrophy, fibrosis, and dysfunction in the context of heart disease.5–7 Interestingly, accumulating lines of evidence suggest that myocardial inflammation is also implicated in the development of diabetic cardiomyopathy.4–7
Several molecular mechanisms link diabetes to myocardial inflammation. It is known that in subjects with metabolic syndrome and type 2 diabetes, visceral adipocytes secrete cytokines and chemokines that lead to the development of a low-grade systemic inflammatory state. Circulating cytokines contribute to worsening insulin resistance and also directly elicit detrimental effects on the heart, leading to myocardial inflammation. High glucose levels and dyslipidemia directly induce the upregulation and secretion of cytokines, chemokines, and adhesion molecules in cardiac cells by modulating multiple signalling pathways that converge towards nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signalling. Activation of the renin-angiotensin-aldosterone system (RAAS) and accumulation of advanced glycation end-products (AGE) and damage-associated molecular pattern (DAMP) molecules also represent important mechanisms that mediate inflammation in the diabetic heart primarily by acting on the Toll-like receptors (TLRs). Following these initial molecular events, leucocytes infiltrate the myocardium and perpetuate the inflammatory process through secretion of cytokines and pro-fibrotic factors and increasing the production of reactive oxygen species (ROS). Mediators resulting from this inflammatory cascade in turn modulate specific intracellular signalling mechanisms in cardiac cells causing cardiomyocyte hypertrophy, mitochondrial dysfunction, endoplasmic reticulum (ER) stress and death, fibroblast proliferation, and collagen production. In addition, inflammatory factors may affect myocardial metabolic processes and interfere with cardiomyocyte contractile properties. These abnormalities together promote the development of diabetic cardiomyopathy. 

The impact of myocardial inflammation on diabetes-induced cardiac remodelling and development of heart failure is relevant to human disease. Patients with diabetes and metabolic syndrome display higher circulating levels of inflammatory mediators and these markers are more strictly correlated with the presence of cardiac abnormalities in these subjects, with respect to patients without metabolic syndrome. Importantly, previous studies demonstrated that the higher risk of heart failure conferred by metabolic syndrome is mediated by higher levels of inflammation.

This review article will provide an overview of the molecular mechanisms linking diabetic cardiomyopathy to myocardial inflammation.

2. Definition of diabetic cardiomyopathy

Diabetic patients are at increased risk for developing heart failure. In addition, diabetic patients with heart failure have a worse prognosis when compared to non-diabetic subjects. A major reason explaining the increased association of diabetes with heart failure relies on the fact that diabetes promotes the development of ischemic heart disease, which frequently leads to cardiac remodelling and dysfunction. Diabetes is also strongly associated with hypertension, another important cause of cardiac insufficiency. However, diabetes may also directly participate to the development of heart failure, independently of other concomitant conditions. Data from the Framingham Heart Study indicated that the pathophysiologic link between diabetes and heart failure is independent of the presence of coronary artery disease and hypertension, suggesting that diabetes may lead to cardiac insufficiency by directly affecting cardiac structure and function. These data were confirmed in several other large studies demonstrating that diabetes is associated with an increased incidence of heart failure independently of other concomitant conditions. Diabetes may cause heart failure by promoting the development of cardiac hypertrophy, diastolic, and systolic dysfunction. Mechanistically, diabetes induces oxidative stress, intracellular calcium abnormalities, metabolic alterations, mitochondrial dysfunction, and inflammation that directly contribute to the development on these structural abnormalities by modulating specific signalling pathways. The clinical condition associated with the spectrum of cardiac abnormalities induced by diabetes is termed diabetic cardiomyopathy. Diabetes-induced alterations may directly lead to the development of heart failure, particularly heart failure with preserved ejection fraction. On the other hand, there is no consensus yet regarding the possibility that diabetes alone, without concomitant coronary artery disease or hypertension, is sufficient to cause overt systolic heart failure. This is due to the lack of long-term clinical studies specifically designed to address this issue. However, previous work demonstrated that indexes of systolic function are subclinically reduced in diabetic patients without coronary artery disease. Diabetes is also associated with a reduction of myocardial flow reserve due to myocardial microvascular abnormalities, which may lead to subendocardial ischaemia and to systolic dysfunction in the long-term. In addition, diabetes-induced myocardial derangements make the heart more vulnerable to other concomitant conditions promoting systolic abnormalities, thereby favouring the development of systolic dysfunction and heart failure.

The causative relationship between diabetes and myocardial abnormalities has also been exhaustively studied in genetically-modified and diet-induced animal models of diabetes. It was shown that in addition to inducing cardiac hypertrophy, both type 1 and type 2 diabetes also markedly induce cardiac fibrosis and apoptosis, directly contributing to the development of ventricular dysfunction.

3. The role of myocardial inflammation in the development and progression of cardiac abnormalities in response to cardiac and metabolic stress

Several pathological insults are able to trigger myocardial inflammation, which initially represents an adaptive mechanism against stress, but becomes maladaptive if the stress persists. Various pathologic stressors directly induce the secretion of cytokines, chemokines (e.g. interleukin 8 and monocyte chemoattractant protein-1 [MCP-1]), and adhesion molecules (e.g. vascular cell adhesion molecule 1 and intercellular adhesion molecule-1 [ICAM-1]) in cardiomyocytes, fibroblasts, and endothelial cells that promote myocardial recruitment of monocytes and lymphocytes. Of note, a clear distinction should be made between this type of chronic myocardial inflammation and the myocardial inflammation associated with viral myocarditis. The first type of inflammation is subclinical and contributes to the development of cardiac abnormalities in the long-term. In contrast, the second typology of inflammation represents the main cause of cardiac dysfunction in viral myocarditis. It is massive, acute and may lead to systolic dysfunction and heart failure in a rapid manner.

Previous experimental studies demonstrated the causative role of interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α) and IL-6 in the development of cardiac hypertrophy and dysfunction. Exogenous administration of TNF-α has been shown to induce cardiac inflammation and dysfunction in vivo. Similar effects were observed in mice with cardiomyocyte-specific TNF-α overexpression. In contrast, genetic disruption of this cytokine exerted protective effects in a murine model of...
pressure overload, reducing hypertrophy, fibrosis, and cardiac dysfunction. IL-1β null mice display a milder hypertrophic phenotype in response to pressure overload. Abbate’s group demonstrated that exogenous administration of this cytokine induces cardiac dysfunction in mice, whereas blocking the cytokine with anakinra, an IL-1 receptor antagonist, improves peak oxygen consumption in patients with heart failure. Similarly, IL-6 infusion in rats was sufficient to induce cardiac hypertrophy, inflammation, fibrosis, and diastolic dysfunction, whereas IL-6 genetic deletion ameliorated angiotensin-II-induced (AT-II-induced) and norepinephrine-induced cardiac hypertrophy and fibrosis.

Leucocyte recruitment in the myocardium is also responsible for the development of cardiac abnormalities. Several studies showed that inhibition of monocyte recruitment in the heart may reduce chronic remodeling in response to stress. Hirsch’s group previously showed that inhibition of phosphoinositide 3-kinase gamma (PI3K-γ) activity in bone marrow cells reduces leucocyte infiltration in the myocardium in response to pressure overload, leading to reduced cardiac inflammation and fibrosis, and preservation of cardiac structure and function. Administration of MCP-1 neutralizing antibodies elicited the same effect on a pressure overload hypertensive model without affecting hypertrophy. MCP-1 gene deletion attenuates post-infarction remodelling. An antibody-mediated neutralization of ICAM-1 reduced infiltration of macrophages and suppressed cardiac fibrosis during pressure overload. However, it should be noted that a complete macrophage depletion with clodronate administration was found to be detrimental during chronic post-infarction remodelling, suggesting that physiologic macrophage levels in the heart are required for proper myocardial healing.

T-cells also play a role in the development of cardiac abnormalities in response to chronic stress. T-cell-depleted mice have reduced macrophage infiltration and myocardial fibrosis in response to mechanical stress.

Finally, mast cells are also recruited to the heart in response to cardiac injury and have a prominent role in the development of cardiac remodelling, hypertrophy, and fibrosis. Accumulating lines of evidence indicate that a chronic myocardial inflammatory status is associated with and contributes to diabetic cardiomyopathy. Such involvement of myocardial inflammation in the development of diabetic cardiomyopathy was proved to be significant in both type 1 and type 2 diabetes, although the majority of the data were collected in animal models of type 1 diabetes.

Previous work demonstrated that TNF-α antagonism with a monoclonal antibody reduces myocardial inflammation, leucocyte infiltration, and fibrosis in rats with streptozotocin-induced diabetic cardiomyopathy. Similarly, mice with genetic disruption of IL-6 showed reduced cardiac inflammation and fibrosis, and improved cardiac function in response to streptozotocin-induced type 1 diabetes, through the upregulation of microRNA 29 (miR-29) and downregulation of TGF-β. IL-6 gene deletion was also shown to reverse myocardial inflammation and cardiac metabolic abnormalities in response to high fat diet-induced diabetes. This beneficial effect was associated with reactivation of AMP-activated protein kinase (AMPK) and abrogation of suppressor of cytokine signalling 3 (SOCS3)-mediated inhibition of insulin receptor substrate (IRS)-1. In mice with type 1 or type 2 diabetes, pharmacological inhibition of stromal-cell-derived factor 1 (CXCR4) axis, which is known to promote inflammatory cell recruitment and inflammation, reduced cardiac fibrosis. Finally, recent evidence indicates that nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3)-dependent inflammasome contributes to the development of diabetic cardiomyopathy. Inflammasome inhibition through NLRP3 downregulation in vivo reduced type 2 diabetes-induced cardiac inflammation, pyroptosis, fibrosis, and improved ventricular function. Of note, NLRP3 inflammasome appears to be implicated also in the development of insulin resistance and diabetes. NLRP3 was found to be activated in adipose tissue and liver in response to obesity. Genetic or pharmacological inhibition of NLRP3 was seen to reduce obesity-induced insulin resistance and diabetes.

4. Signalling mechanisms underlying the development of cardiac inflammation in the diabetic heart

Several molecular mechanisms may be involved in the establishment of myocardial inflammation in the setting of diabetes. In general, these mechanisms converge towards the activation of the NF-κB pathway, which promotes the upregulation of cytokines, chemokines and adhesion molecules, and is highly active in the diabetic heart and vasculature contributing to cardiovascular damage. Cardiomyocyte-specific overexpression of IkB-α protein, which suppresses the canonical NF-κB signalling pathway, was found to prevent streptozotocin-induced diabetic cardiomyopathy through the inhibition of the renin-angiotensin system. Other work also showed that pharmacological inhibition of NF-κB mitigates cardiac oxidative stress induced by type 2 diabetes and reduces mitochondrial abnormalities. A systematic description of the mechanisms promoting myocardial inflammation in response to diabetes follows (Figure 1).

4.1 Direct effects of diabetes-related metabolic abnormalities

Diabetes-associated metabolic derangements can directly induce cytokine expression and release from cardiac cells. It was previously shown that hyperglycemia increases the expression of high-mobility group box 1 (HMGB1) in isolated cardiomyocytes, macrophages, and cardiac fibroblasts, thereby activating the MAPK and NF-κB pathways and inducing TNF-α and IL-6 secretion. Inhibition of HMGB1 decreased myocardial inflammation and fibrosis in the presence of type 1 diabetes in vivo and protected diabetic animals in response to post-infarction remodelling.

High glucose levels were also found to induce cytokine expression in cardiomyocytes through the activation of the Jun NH2-terminal kinase (JNK)/NF-κB pathway. Inhibition of this mechanism by a curcumin analog reduced diabetic cardiomyopathy induced by streptozotocin.

Diabetes also appears to increase the expression of cytokines, chemokines, and adhesion molecules in the heart through the activation of glycogen synthase kinase 3 beta (GSK-3B). Inhibition of this kinase was shown to reduce cardiac inflammation, nitrosative damage, and fibrosis in mice with type 2 diabetes.

Another intracellular mechanism underlying the pro-inflammatory effects of metabolic dysregulation is mediated by the enzyme O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT). OGT catalyzes a post-translational modification characterized by the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine or threonine residues of target proteins, and is amplified by the accumulation of glycolysis intermediates. Increased O-GlcNAc levels were found to be associated with diabetic cardiomyopathy and it was recently demonstrated that NF-κB p65 O-GlcNAcylation is increased by hyperglycemia and this...
modification decreases the binding of p65 subunit to IκB-α, thereby leading to NF-κB activation. In addition, hyperglycemia was previously found to modulate specific epigenetic changes, which would regulate NF-κB activity and thus cytokine expression in vascular cells and cardiomyocytes. Furthermore, dysregulation of sirtuin-1 (SIRT1) level/activity may also play a role in the development of myocardial inflammation. SIRT1 activity is reduced by high glucose levels and was shown to reduce cardiac inflammation through the deacetylation of the p65 subunit of NF-κB at Lys310, inhibiting NF-κB transcriptional activity.

An increase in circulating lipids may also contribute to cardiac inflammation in diabetes. Fatty acids activate TLR4 that strongly promotes inflammation through the NF-κB pathway. Mice with a TLR4 gene deletion demonstrate improved cardiac function and reduced cardiac intracellular lipid accumulation in response to streptozotocin-induced diabetes. Fatty acids induce the activation of NF-κB and the subsequent expression of TNF-α, IL-1, and IL-6, which can be reversed by peroxisome proliferator-activated receptor gamma beta/delta (PPAR-γ/β). Previous work showed that hyperglycemia and high circulating levels of lipids promote inflammation through the activation of protein kinase C (PKC), which activates MAPK pathway thereby inactivating IκB.

In addition, high glucose and lipid levels also induce ROS accumulation. This mechanism is partly mediated by PKC- and Rac1-dependent activation of NADPH oxidase. ROS promote NF-κB transcription and degradation of IκB, an inhibitor of NF-κB. NF-κB is also activated by oxidative stress through the activation of Erk1/2, which inhibits Nrf-2, a transcription factor controlling the expression of anti-oxidants and detoxifying enzymes. To that end, cardiac-specific overexpression of catalase reduced NF-κB signalling and reversed diabetes-related cardiac abnormalities.

Mitochondrial dysfunction is also another strong contributor to ROS generation and myocardial inflammation in diabetic cardiomyopathy. Mitochondrial dysfunction is a frequent alteration in the diabetic heart significantly participating to the development and progression of cardiac abnormalities and dysfunction. In fact, a mitochondrial defect leads to reduced ATP production, further impairing myocardial efficiency. It also determines an increased oxidative damage. Several mechanisms may cause mitochondrial abnormalities in diabetes. In both type 1 and type 2 diabetes, downregulation of the components of the electron transport chain was observed. In addition, mitochondrial protein nitration is increased in the diabetic heart indicating an increased oxidative damage altering both protein structure and function.
reduction by overexpression of scavengers was found to reduce mitochondrial dysfunction and cardiac abnormalities induced by diabetes. Overall, these mitochondrial derangements lead to mitochondrial uncoupling and further stimulate ROS production, which triggers NF-κB signalling and perpetuates myocardial inflammation.

Lastly, diabetes was also found to induce IL-1β and inflammation through the NF-κB-mediated activation of NLRP3 inflammasomes. Circulating saturated fatty acids have also been shown to induce NLRP3 activation. NLRP3 inflammasomes were markedly activated in the hearts of rats with type 2 diabetes through ROS-dependent activation of NF-κB and TXNIP, an activator of both NLRP3 and caspase-1.

Mice deficient for NLRP3 or for the other components of the inflammasome, ASC or caspase-1, showed decreased pro-inflammatory cytokines levels when exposed to a high-fat diet.

4.2 Role of the renin-angiotensin-aldosterone system

Myocardial inflammation in the diabetic heart is also mediated by the renin-angiotensin-aldosterone system (RAAS), which is strongly activated in diabetes and promotes cardiac remodelling. Administration of angiotensin-converting-enzyme (ACE)-inhibitors to different animal models affected by type 2 diabetes reduced fibrosis, collagen deposition, and TGF-β levels. Similar results were obtained in type 1 diabetes animal models.

Angiotensin-II and aldosterone promote the expression of adhesion molecules and chemokines which help recruit inflammatory cells to the myocardium, leading to inflammation. These effects are mediated by the ability of angiotensin-II and aldosterone to activate NF-κB. Angiotensin-II activates NF-κB either through the activation of NADPH oxidase-dependent ROS production or through the transactivation of TLR4.

Of note, endothelin-1, a vasoconstrictor and pro-fibrotic agent extensively produced by the endothelium in chronic inflammation, is also activated in the diabetic heart, exerting a synergistic effect with angiotensin-II in the induction of inflammation. In streptozotocin-induced diabetic mice, increased levels of endothelin-1 have been reported and contribute to inflammation, whereas endothelial-specific disruption of endothelin-1 can reduce cardiac fibrosis.

4.3 Role of AGEs

The cardiac accumulation of advanced glycosylated end-products (AGEs) in diabetes also represents an important inflammatory trigger. AGEs are the result of the reaction of glucose-derived dicarbonyls with the amino groups of arginine and lysine residues of proteins. Methylglyoxal, a highly reactive molecule, is one of the major non-enzymatic fragmentation products of triose-phosphate. It accounts for most of the inflammation-mediated mechanisms dependent on protein kinase A (PKA) activation and elevation of cAMP levels. In addition, adiponectin was found to increase intracellular cAMP levels. Dysregulation of adipokines may also be directly implicated in the promotion of myocardial inflammation.

Dysregulation of adipokines may also be directly implicated in the promotion of myocardial inflammation. It is known that the circulating adipokine profile is altered in diabetes and accumulating lines of evidence indicate that adipokines may interfere with the immune system. In particular, adiponectin is known to exert anti-inflammatory actions in the heart. These mechanisms are mediated by the binding of adiponectin to block the TNF-α-induced activation of NF-κB through a mechanism dependent on protein kinase A (PKA) activation and elevation of cAMP levels. In addition, adiponectin was found to increase intracellular NO levels, which exert anti-inflammatory actions through the activation of AMPK and PKB/AKT signalling. Both cardiac and circulating levels of adiponectin are reduced in diabetes, suggesting that this dysregulation may favour the establishment of cardiac inflammation, metabolic abnormalities, and susceptibility to stress.

4.4 Role of DAMPs

Another important mechanism contributing to inflammation in the diabetic heart is through recognition of ‘hidden-self’ damage-associated molecular patterns (DAMPs) signals, which are presented by cells that have undergone apoptosis, necrosis, or necroptosis, leading to sterile inflammation. DAMPs are recognized by TLR-2 and 4, which in turn trigger several inflammatory pathways, such as the NF-κB, activator protein 1 (AP-1), and interferon regulatory factor (IRF) pathways. In addition, a strong TLR activation can also activate the caspase-based inflammasome molecular complex.

4.5 Role of adipose tissue-derived cytokines

Type 2 diabetes and metabolic syndrome are characterized by a chronic inflammation of visceral adipose tissue. Nutrient overload leads to adipocyte hypertrophy, ER stress and subsequent activation of inflammatory signals and cytokine secretion. This event promotes leucocyte infiltration in the adipose tissue, further exacerbating the inflammatory process. Free fatty acids also actively participate in this process by activating leucocytes through stimulation of the TLRs/NF-κB pathway. Overall, this leads to a systemic low-grade inflammation with increased circulating cytokine levels. Circulating cytokines contribute to worsening insulin resistance and elicit direct detrimental effects on the heart resulting in myocardial inflammation.

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4.6 Role of SDF-1- and MCP-1-dependent inflammatory cell recruitment

Infiltration of the myocardium by leucocytes represents a key event in establishing and promoting the myocardial inflammatory process. Previous work indicated that SDF-1 and MCP-1 are required for vascular and myocardial infiltration by inflammatory cells. A pharmacological inhibition of the SDF-1/CXCR4 axis reduced cardiac fibrosis in response to diabetes. MCP-1 inhibition was found to reduce vascular inflammation and dysfunction in type 2 diabetes.

4.7 Role of NETosis

Interestingly, a new process involving lymphocytes, neutrophils, and monocytes may be critical for the acquisition of the pro-inflammatory phenotype of macrophages (M2 to M1 transition) and for the development of tissue inflammation in diabetes. This process is termed NETosis. ROS, inflammatory cytokines, glucose metabolites, and many other stimuli converging on NF-κB, transcriptionally up-regulate PAD-4,

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the initiating enzyme of the neutrophil extracellular trap (NET) molecular machinery. The process leads to nucleus digestion and the digestion products are released into the extracellular space, providing an extremely strong DAMP signalling, which further recruits and activates leucocytes.89

5. Inflammatory signalling mechanisms promoting cardiac abnormalities and dysfunction in the presence of diabetes

An elevated expression of mediators of inflammation in the diabetic heart directly promotes cardiac derangements through the modulation of multiple mechanisms. In fact, it is known that cytokines, chemokines, and ROS modulate specific intracellular signalling pathways in cardiac cells thereby promoting cardiomyocyte hypertrophy, death, and cardiac fibrosis. An itemized description of these signalling mechanisms follows (Figure 2).

5.1 Hypertrophy

Cytokines can directly induce cardiomyocyte hypertrophy. TNF-α induces protein synthesis and reduces protein degradation in feline cardiomyocytes through a mechanism dependent on a preserved interaction between cell integrins and the extracellular matrix.90 Condorelli et al. demonstrated that TNF-α induces cardiomyocyte hypertrophy by activating the AKT/NF-κB and JNK pathways.91 A recent study from Dawn’s group indicated that IL-6 also directly promotes cardiomyocyte growth through the activation of the Ca(2+)-calmodulin-dependent protein kinase II (CaMKII) and signal transducer and activator of transcription 3 (STAT3) pathway.92 In addition, IL-1β directly induces cardiomyocyte growth in a NO-independent manner93 and it was also shown to promote cardiac hypertrophy in vivo by inducing insulin-like growth factor-1 (IGF-1) release from cardiac fibroblasts through a paracrine mechanism involving the activation of STAT3.94 In line with this evidence, a genetic deletion of the TLR4 gene, which is involved in the initiation of intracellular cascades leading to cytokine production, attenuates pressure overload-induced hypertrophy.95

The effects of cytokines on cardiac hypertrophy are also mediated by the activation of the NF-κB and glycoprotein 130 (gp130) signalling pathways. NF-κB activation induces cardiomyocyte growth and the upregulation of fetal sarcomeric genes, whereas its inhibition reduces cardiac growth in vivo.95-97 Mechanistically, it was recently demonstrated that NF-κB cross-talk to nuclear factor of activated T-cells (NFAT) is critical in the promotion of cardiomyocyte growth.96 Gp130 overexpression was also shown to be sufficient to induce cardiomyocyte hypertrophy and to mediate the effects of IL-6, cardiotrophin-1 and leukemia inhibitory factor (LIF).98 The pro-hypertrophic effect of gp130 appears to be mediated by the activation of STAT3.

Figure 2. Inflammatory signals promoting cardiac abnormalities induced by diabetes. Schema representing the inflammatory signals through which diabetes-induced myocardial inflammation would contribute to the establishment of cardiac abnormalities. Cytokines in the diabetic heart may modulate multiple signalling mechanisms converging toward either NF-κB or STAT3 thereby leading to cardiomyocyte hypertrophy. Inflammatory mediators may also activate apoptotic pathways or affect contractility in cardiomyocytes through specific transduction cascades. In addition, they can induce fibrosis by acting directly on fibroblasts. Finally, inflammatory signalling mechanisms can also affect cardiomyocyte metabolism. The figure was made in part using tools provided by Servier Medical Arts. TAK1: transforming growth factor β-activated kinase 1. PKC: protein kinase C. ATF2: activating transcription factor 2. CAMKII: Ca2+/calmodulin-dependent protein kinase II. JNK: c-Jun N-terminal kinase. IGF1: insulin-like growth factor. iNOS: inducible nitric oxide synthase. WISP1: WNT1 inducible signalling pathway protein 1. Smad3: small mother against decapentaplegic homolog 3. SOCS3: suppressor of cytokine signalling 3. IRF1: interferon regulatory factor 1. PGC1α: peroxisome proliferator-activated receptor gamma coactivator 1-apha.
signalling, and by the GRB2-associated-binding protein 1-Src homology 2 domain-containing phosphatase 2 (Gab1-SHP2) interaction.

TGF-β also contributes to the development of cardiac hypertrophy induced by myocardial inflammation. It was previously shown that TGF-β induces cardiomyocyte hypertrophy through the TAK1-MKK3/6-p38MAPK signalling pathway. In addition, the hypertrophic effects of TGF-β on cardiomyocytes are also mediated by a PKC-dependent activating transcription factor 2 (ATF-2) activation.

Finally, chronic myocardial inflammation leads to increased ROS production in the heart, which in turn may contribute to cardiac hypertrophy.

5.2 Cell death
Increased myocardial inflammation also contributes to cardiac remodelling through the promotion of cardiomyocyte death. Mann’s group demonstrated that a sustained TNF signalling activation induces cardiomyocyte apoptosis and remodelling through the activation of both intrinsic and extrinsic cell death pathways, with consequent increases of cytotoxic levels of cytochrome c, caspase-3 and 8 activation, c-FLIP degradation and Bid cleavage. NF-κB activation also appeared to mediate the pro-apoptotic effects of TNF-α.

IL-1β-induced apoptosis in cardiomyocytes was shown to be mediated by inducible NO synthase (iNOS) activation and upregulation of Bcl-2 homologous antagonist/killer (Bak) and B-cell lymphoma-extralarge (Bcl-XL). IL-1β also induces apoptosis in cardiomyocytes through the induction of C/EBP homologous protein (CHOP) in response to diabetes. In addition, activation of the NLRP3 inflammasome induces apoptosis in cardiomyocytes through the activation of caspase-1. Finally, oxidative stress mediates in part the effects of inflammation on cell death.

5.3 Contractility
Previous work demonstrated that myocardial inflammation may directly affect cardiac contractility. TNF-α, IL-2, IL-6, and IL-1β exert a negative inotropic effect on the heart, inducing a rapid and reversible contractile dysfunction.

These effects were found to be mediated by the activation of iNOS and formation of peroxynitrites, which can interfere with the excitation-contraction coupling. In addition, it was demonstrated that both IL-6 and IL-1β are able to reduce the expression of SERCA2a in cardiomyocytes, suggesting that abnormalities in calcium handling may underlie the negative inotropic effects of these cytokines.

5.4 Fibrosis
During inflammation, cardiac cells and inflammatory cells secrete cytokines and pro-fibrotic factors that stimulate fibrosis. TNF-α directly stimulates cardiac fibroblast proliferation and collagen production through the activation of WISP1, whereas IL-6 exerts similar effects through the suppression of miR-29. On the other hand, IL-1β was found to stimulate matrix metalloproteinases in cardiac fibroblasts through the inhibition of endoglin signalling and activation of the bone morphogenic protein and activin membrane-bound inhibitor pathway. However, TGF-β is considered the major pro-fibrotic cytokine in the heart. It is mainly secreted by fibroblasts, macrophages, and T-cells, although cardiomyocytes are also able to secrete it particularly in response to angiotensin-II. TGF-β stimulates fibroblast activation into myofibroblasts, characterized by expression of alpha smooth muscle actin (α-SMA) and increased production of extracellular matrix proteins. It also promotes collagen production and inhibits the secretion of protease inhibitors, thereby reducing the degradation of the extracellular matrix. The effects on fibroblasts are mediated by Smad3 signalling. ROS may also mediate the pro-fibrotic effects of cytokines and growth factors on fibroblasts following myocardial inflammation.

5.5 Metabolism
Finally, chronic myocardial inflammation may contribute to cardiac dysfunction by inducing metabolic perturbations in the heart that can impair cardiac energetics, particularly in response to metabolic stress. Infusion of IL-6 was found to impair cardiac glucose metabolism through a SOCS3-dependent inhibition of IRS-1. In contrast, genetic disruption of IL-6 gene reduced inflammation and reversed glucose metabolism defects induced by high fat diet, which was paralleled by SOCS3 inhibition and IRS-1 reactivation.

In addition, cardiac expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial function and biogenesis, was found to be inhibited by a chronic inflammatory process, through a mechanism dependent on NF-κB activation. Exposure of AC16 cells to TNF-α reduced the levels of PGC-1α through the activation of NF-κB. This process may be explained by the evidence that p65 binds and sequesters PGC-1α, thereby leading to its inhibition and downregulation. PGC-1α inhibition in response to NF-κB activation was found to cause increased glucose utilization through the downregulation of pyruvate dehydrogenase lipomide kinase 4 (PDK4).

6. Clinical implications of cardiac inflammation in diabetic patients
The impact of myocardial inflammation on cardiac remodelling and heart failure development appears to be relevant to the human disease. Circulating cytokine levels are higher in patients with heart failure and are directly correlated with the severity of the disease and with a worse prognosis. Importantly, large epidemiological studies demonstrated that circulating cytokines and chemokines are independent predictors of the incidence of heart failure. The Framingham Heart Study demonstrated that patients without a prior acute myocardial infarction who display higher baseline levels of TNF-α, IL-6, and C-reactive proteins (CRP) have a significantly higher long-term risk to develop heart failure, independently of the occurrence of an acute myocardial infarction.

Interestingly, the impact of inflammation on cardiac abnormalities and heart failure appears to be higher in subjects with metabolic disorders. Patients with diabetes and metabolic syndrome display higher circulating levels of inflammation markers and cytokines. Circulating levels of CRP, TNF-α, and TGF-β were found to be directly correlated with higher left ventricular mass and with indexes of systolic and diastolic abnormalities in hypertensive subjects. Interestingly, this correlation resulted to be stronger in patients with metabolic syndrome. Subsequent studies demonstrated that higher levels of circulating CRP and IL-6 may explain the higher risk of heart failure conferred by obesity and metabolic syndrome, thereby suggesting that an increased inflammatory status is a mechanism through which metabolic disorders are associated with a higher development of cardiac abnormalities and heart failure in patients.

7. Perspectives
Several aspects regarding the role of myocardial inflammation in diabetic cardiomyopathy need to be clarified. First, is whether a myocardial
Inflammatory process induced by diabetes is exclusively maladaptive, or whether it may also be protective in allowing the heart to properly respond to metabolic stress. Data so far suggest only a detrimental effect. However, it was previously demonstrated that after acute myocardial injury, an acute inflammatory phase is important for the removal of damaged tissue and activation of the repair mechanisms that lead to scar formation.\textsuperscript{5–7} Suppression of this acute inflammatory phase by marked macrophage depletion was proven to be detrimental and to impair post-infarction remodelling.\textsuperscript{13,34} Carnevale et al.\textsuperscript{33,34} previously demonstrated that placental growth factor knockout mice rapidly develop cardiac dilation and dysfunction in response to pressure overload as a consequence of an impaired inflammatory response due to decreased activity of the TNF-\textit{\textalpha}-converting enzyme.\textsuperscript{120} In addition, TGF-\textit{\beta}-induced fibrosis is necessary for normal myocardial response to mechanical stress, as recently demonstrated by Kass’ group.\textsuperscript{121} Therefore, future studies should be focused on elucidating the various phases of myocardial inflammation induced by metabolic stress, and trying to dissect the mediators and cellular components that are important in each of these phases. In particular, the role of anti-inflammatory cytokines, such as IL-10, should be investigated.

Several pharmacological drugs might be beneficial for the treatment of diabetic cardiomyopathy by targeting inflammation. Obviously, RAAS inhibitors are among these drugs.\textsuperscript{10} In addition, pre-clinical studies suggested that metformin and fenofibrate may reduce myocardial inflammation and diabetic cardiomyopathy.\textsuperscript{126} However, previous clinical reports suggested that metformin should be avoided in subjects with severe heart failure because it may lead to lactic acidosis.\textsuperscript{124} The efficacy of other insulin sensitizer drugs in the reduction of inflammation and diabetic cardiomyopathy should be screened in future studies. Among these drugs, glitazones were found to have a negative impact on cardiac function and be associated with the development of heart failure.\textsuperscript{124} In contrast, statins also exert important anti-inflammatory effects and may be beneficial for the treatment of diabetes-induced myocardial inflammation.\textsuperscript{125} Inflammasome and NF-kB inhibitors may prevent diabetic cardiomyopathy by reducing myocardial inflammation. Anakinra, a recombinant IL-1 receptor antagonist, might also be beneficial for the treatment of diabetes-related cardiac abnormalities. Finally, it would be interesting to understand whether autophagy activators may reduce diabetes-induced cardiac abnormalities by decreasing myocardial inflammation.

Autophagy is an evolutionarily-conserved intracellular mechanism of protein and organelle degradation.\textsuperscript{126} Autophagy is required for protein and organelle turnover and for the cellular adaptation to stress.\textsuperscript{126} Autophagy is also required for the maintenance of cardiac function and cardiac adaptation to stress.\textsuperscript{122} Recent studies indicated that the autophagic process is impaired in the heart in the presence of metabolic disorders, such as obesity, diabetes and metabolic syndrome through a dysregulated activation of mammalian target of rapamycin complex 1 (mTORC1) signalling.\textsuperscript{128,129} Importantly, autophagy reactivation ameliorates diabetic cardiomyopathy and tolerance to stress, which is usually impaired by diabetes.\textsuperscript{128,129}

Future studies are warranted to investigate whether dysregulation of cardiac autophagy in the setting of diabetes contributes to more severe myocardial inflammation. Autophagy inhibition usually leads to ER stress, which is a strong inducer of autophagy.\textsuperscript{130} In addition, recent work from Otus’s laboratory demonstrated that myocardial inflammation in response to cardiac stress is triggered by mitochondrial DNA fragments which are not degraded by autophagy.\textsuperscript{131} In line with this work, it was recently demonstrated that NF-kB activation in response to inflammatory stimuli and mitochondrial stress promotes a mitophagy-dependent removal of damaged mitochondria, which is important for limiting the escape of mitochondrial DNA fragments and restraining the inflammatory process.\textsuperscript{132} Similarly, inflammasome activation was shown to stimulate autophagy, which in turn degrades inflammasome components and keeps inflammation within a physiological range.\textsuperscript{133} Therefore, it is reasonable to hypothesize that an impairment of autophagy would favour an exaggerated activation of inflammation in response to stress, which would exert maladaptive functions.\textsuperscript{134}

In conclusion, the role of inflammation in the development of diabetic cardiomyopathy appears to be significant. However, more effort is needed to understand the mechanisms promoting this pathologic process and how to modulate it in order to develop new therapeutic interventions aimed at the reduction of heart failure and mortality in diabetic patients.

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References


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