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Could interferon-gamma be a therapeutic target for treating heart failure?

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Abstract The cytokine interferon-gamma (IFN-γ) is the only known member of the type II family of interferons, and as such, binds to its own distinct receptor. It is important in host defense against infection, as well as adaptive immune responses. While a wide array of cytokines are known to be involved in adverse remodeling of the heart and the progression to heart failure, the role of IFN-γ is unclear. Recent evidence from clinical studies, animal models of myocarditis and hypertension, as well as isolated cell studies, provide conflicting data as to whether IFN-γ is pathological or protective in the heart. Thus, it is important to highlight these discrepant findings so that areas of future investigation can be identified to more clearly determine the precise role of IFN-γ in the heart. Accordingly, this review will (1) discuss the source of IFN-γ in the diseased heart; (2) summarize the data from animal studies; (3) discuss the effects of IFN-γ on isolated cardiac fibroblasts and cardiomyocytes; (4) identify signaling mechanisms that may be invoked by IFN-γ in the heart; and (5) present the clinical evidence supporting a role for IFN-γ in heart failure.

Keywords Interferon-gamma · Heart failure · Hypertrophy · Fibrosis · Inflammation

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Introduction

Since Levine et al. [1] identified a positive correlation between tumor necrosis factor-alpha (TNF-α) and chronic heart failure, many other cytokines have been identified as contributing to the causative processes of this disease. These include, but are not limited to the following: regulated upon activation, normal T cell expressed, and secreted (RANTES); interleukin (IL)-6; cardiotropin; IL-8; IL-1; and macrophage inflammatory protein (MIP)-1α [2, 3]. These cytokines have effects on virtually all aspects of adverse myocardial remodeling and function. Targeting cytokines, predominantly the inhibition of TNF-α, has emerged as a powerful tool to treat autoimmune diseases, and this approach is currently being used with relative success in rheumatoid arthritis and inflammatory bowel disease for example. However, despite the clear role for TNF-α in the pathogenesis of heart failure, targeting this cytokine has not become part of standard clinical practice due to the lack of success of several clinical trials aimed at inhibiting TNF-α [4, 5].

The lack of success targeting TNF-α means that certain cytokine-based approaches that are efficacious in other autoimmune diseases may not be applicable to heart failure. Thus, there is a need to identify other cytokines that may serve as novel targets in this context. There is emerging evidence that interferon-gamma (IFN-γ) may represent a possible target either through inhibition, or conversely, as a treatment itself. The literature regarding IFN-γ is conflicted with some studies indicating that it is important in driving adverse myocardial remodeling leading to heart failure, and others suggesting that it has a protective function. Consequently, it is important to highlight these discrepant findings so that areas of future investigation can be identified to more clearly determine

the precise role of IFN-γ in the heart. Accordingly, this review will (1) discuss the source of IFN-γ in the diseased heart; (2) summarize the data from animal studies; (3) discuss the effects of IFN-γ on isolated cardiac fibroblasts and cardiomyocytes; (4) identify signaling mechanisms that may be invoked by IFN-γ in the heart; and (5) present the clinical evidence supporting a role for IFN-γ in heart failure. This review does not attempt to place IFN-γ among other cytokines or attempt to discuss its interactions with other cytokines because, while this undoubtedly occurs, as yet we do not understand the basic actions IFN-γ in the heart, let alone its complex relationships with other cytokines.

Inflammatory cells as the source of IFN-γ in the diseased heart

Inflammatory cells play a critical role in adverse remodeling of the heart in response to chronic and acute stress and injury and therefore ultimately the onset of heart failure. For example, young spontaneously hypertensive rats (SHR; 8 week old) develop small foci of inflammatory cells, consisting of $CD4^+$, $CD8^+$, $OX6^+$ (B cell) lymphocytes, as well as $ED1^+$ macrophages, in perivascular and interstitial regions of the heart [6]. As these SHR age (12 months of age), fibroblasts expressing collagen I are consistently associated with inflammatory cells. In addition, all of the aforementioned inflammatory cell types are increased in areas of fibrosis. In humans, myocardial biopsies from patients with dilated cardiomyopathy show myocardial damage, severe interstitial fibrosis, and the influx of lymphocytes and macrophages [7]. Peak monocyte counts also correlate positively with left ventricular diastolic volume and negatively with ejection fraction in patients with acute myocardial infarction [8]. As cells in the damaged heart die by necrosis, they release their contents, which in turn stimulate this inflammatory response, including the release of cytokines and chemokines [9]. For example, IL-1 cytokines regulate the synthesis of other cytokines as well as leukocyte infiltration [10], while inhibition of monocyte chemoattractant protein-1 (MCP-1) with a neutralizing antibody successfully reduces macrophage infiltration and fibrosis of the remote region of the heart in rats' post-myocardial infarction [11]. Furthermore, anti-MCP-1 was found to prevent macrophage accumulation and attenuate perivascular and interstitial fibrosis in hypertension [12].

These infiltrating inflammatory cells represent the primary, if not the only, source of IFN-γ in the heart. This is summarized in Table 1. Peripheral blood mononuclear cells (PBMCs) isolated from dogs infected with the trypanosoma cruzi parasite, produced excess amounts of IFN-γ when cultured [13]. Similarly, Cheng et al. [14] isolated PBMCs from congestive heart failure patients and incubated those cells with increasing concentrations of atorvastatin (0, 0.4, 1.0, and 4.0 μmol/L) for 72 h. Atorvastatin suppressed IFN-γ production by the PBMCs in a concentration-dependent manner. While neither study attempted to identify the specific inflammatory cell type(s) producing IFN-γ, we have detected IFN-γ in the media of cultured bone marrow-derived mast cells (unpublished data). Further, we have reported in the SHR that elevated myocardial levels of IFN-γ are returned to normal when these animals are treated with the mast cell stabilizing compound nedocromil, suggesting cardiac mast cells as a potential source [15]. Although, in that same study, we also found that mast cells were responsible for the recruitment of other inflammatory cells into the hypertensive heart, suggesting that mast cell stabilization may also have the effect of preventing recruitment of other inflammatory cells that produce IFN-γ. In fact, using flow cytometry, Han et al. [16] recently identified that $CD4^+$ and $CD8^+$ T cells were a major source of IFN-γ in the hypertensive mouse heart. They also determined that macrophages contributed to the IFN-γ pool. An interesting observation was that when isolated and cultured alone, both T cells and macrophages produced almost no IFN-γ. However, when co-cultured, IFN-γ levels dramatically increased (~20-fold increase). Co-culture of IFN-$\gamma^{-/-}$ T cells with wild-type macrophages resulted in significantly reduced IFN-γ production. The reciprocal experiment produced similar findings. Thus, T cell macrophage interactions appear to be important in determining IFN-γ levels.

In an elegant series of experiments, Han et al. [16] further found that deletion of IFN-γ in mice infused with angiotensin II resulted in a decreased infiltration of Mac-2^+ macrophages. In keeping with that finding, the chemokine MCP-1 was found predominantly in macrophages in the hearts from angiotensin II-infused mice, with the number of Mac-1^+ cells being reduced by deletion of IFN-γ [16]. This was reflected in the tissue levels of MCP-1, which were elevated in the hearts of angiotensin II-infused mice and dramatically reduced by deletion of IFN-γ. Culture experiments also found that co-culture of

Table 1 Cellular sources of IFN-γ in cardiac pathology

Cell type	Source	Disease	Reference
PBMC	Dog	Myocarditis	[13]
PBMC	Human	Congestive heart failure	[14]
Mast cell	Rat	Hypertension	[15]
$CD4^+$ T cell	Mouse	Hypertension	[16]
$CD8^+$ T cell	Mouse	Hypertension	[16]
Macrophage	Mouse	Hypertension	[16]

PBMC peripheral blood mononucleocytes

wild-type T cells with wild-type macrophages increased macrophage migration, while co-culture of IFN-$\gamma^{-/-}$ T cells with wild-type macrophages did not.

Interestingly, IFN-γ may also play a role in selectively regulating pro-inflammatory cytokine production in the heart since TNF-α mRNA levels were elevated in wild-type mice infused with angiotensin II, but were attenuated in IFN-$\gamma^{-/-}$ mice [16]. This was not the case for MIP-1α, where IFN-γ deletion had no effect. Together, this series of experiments by Han et al. [16] makes it is clear that IFN-γ is an important regulator of the complex interplay of inflammatory cells, chemokines and cytokines, all of which ultimately contribute to adverse myocardial remodeling. Given these collective findings, IFN-γ may be an important mediator in any cardiac pathology that involves an inflammatory component such as myocardial infarction, hypertension, or myocarditis.

Animal studies

Studies of the whole heart

Only a handful of studies have looked at the role of IFN-γ in adverse myocardial remodeling in whole animals. These are summarized in Table 2. In dogs infected with various strains of the trypanosoma cruzi parasite, serum levels of IFN-γ and TNF-α increased dramatically, with the greatest increase being in IFN-γ (∼six to sevenfold) [13]. The authors also noted that those dogs with increased IFN-γ displayed myocardial remodeling including hypertrophy and fibrosis. Likewise, in the SHR model of hypertension, we have found that myocardial IFN-γ levels are significantly elevated in 20-week-old animals, an age where hypertrophy and fibrosis were present [15]. In mice, induction of a Th1 T cell phenotype resulted in a 12-fold increase in IFN-γ levels in the heart and was associated with concentric hypertrophy, increased total collagen, extensive collagen cross-linking, and a stiffer left ventricle [17]. Overexpression of IFN-γ in the liver of transgenic mice increased circulating levels of the cytokine and caused myocardial inflammation, interstitial fibrosis, and a trend toward increased apoptosis [18]. Interestingly, there was a significant up-regulation at the gene level of TNF-α, IL-12, MCP-1, and MIP1α. This alone appears to clearly indicate that IFN-γ is capable of regulating inflammatory cell recruitment as well as cytokine and chemokine production in the heart. Ultimately, these mice developed a thin walled, dilated cardiac phenotype with impaired systolic function. In support of those findings, Han et al. [16] found that IFN-γ mRNA and protein levels were increased in the hearts of wild-type mice infused with angiotensin II for 7 days and that infusion of angiotensin II into IFN-$\gamma^{-/-}$ mice resulted in a comparable level of cardiac hypertrophy as the wild type, but a significant reduction in cardiac fibrosis. This was also associated with suppressed T cell and macrophage infiltration into the hearts of the IFN-$\gamma^{-/-}$ mice, following angiotensin II infusion. Not surprisingly then, deletion of IFN-γ also had profound effects on cytokine and chemokine production. Myocardial TNF-α mRNA levels, but not MIP-1α mRNA, were reduced in the knockout mice following angiotensin II infusion. MCP-1 was also reduced. While this study examined the acute effects of IFN-γ gene deletion on adverse remodeling, the investigators did not investigate the role of IFN-γ in the subsequent development of heart failure.

In complete contrast to those findings, Garcia et al. [19] reported that 4 weeks of aldosterone infusion in mice caused a greater degree of hypertrophy in IFN-$\gamma^{-/-}$ mice than in the wild type, following removal of one kidney and high salt intake (1 % in drinking water). This was characterized by an increased thickening of the ventricle wall and septum with no changes in chamber size detected. Administration of aldosterone also led to increased left ventricular end diastolic pressure, and impairment of diastolic function, which was again significantly worse in the IFN-$\gamma^{-/-}$ mice compared to the wild-type mice. In this model, fibrosis was unaffected by deletion of IFN-γ in that it remained elevated, similar to the wild type. It is unclear as to why there are such dramatic differences in the remodeling response between angiotensin II and aldosterone infusion/high salt models. Both studies used mice, although Han et al. used IFN-$\gamma^{-/-}$ mice on a B6.129 background for their angiotensin II infusions, while Garcia et al. conducted their aldosterone infusions/high salt on the BALB/c background. It has been shown that hearts from mice with a Th1 T cell background (C57B/6) remodel differently in response to pathologic stimuli than mice with a Th2 T cell background (BALBc). For example, C57B/6 mice develop concentric hypertrophy and fibrosis in response to angiotensin II, whereas BALBc mice develop a dilated phenotype with dramatically reduced ejection fraction [20]. Also, administration of L-NAME increased cardiac fibrillar collagen and percentage of fibrillar collagen cross-linking in BALB/c mice, but not in C57B/6 mice [21]. Alternatively, it is possible that the disparate findings related to IFN-γ could represent differences in the disease etiology in that angiotensin II induces hypertension due to vasoconstriction, whereas the aldosterone model is salt induced.

Of further interest, infusions of IFN-γ (0.08 mg/kg, SQ, twice daily) attenuated but did not prevent myocardial hypertrophy in the rat aortic banding model of pressure overload (14 days) [22]. This was independent of blood pressure. These results in part support Garcia et al.'s findings. Collectively, the studies presented in this section

Table 2 Summary of studies investigating IFN-γ and myocardial remodeling

Species	Model	Outcome	Reference
Studies showing association			
Canine	Trypanasoma cruzi parasite	↑ IFN-γ levels associated with ↑ hypertrophy and fibrosis	[13]
Rat	Hypertension	↑ IFN-γ levels associated with ↑ hypertrophy and fibrosis	[15]
Mouse	Induction of Th1 T cell phenotype	↑ IFN-γ levels associated with concentric hypertrophy, fibrosis, and increased myocardial stiffness	[17]
Studies showing detrimental effects			
Mouse	Liver overexpression of IFN-γ	Myocardial inflammation, fibrosis, ventricular wall thinning and dilatation, reduced systolic function	[18]
IFN-$\gamma^{-/-}$ mouse	Hypertension-angiotensin II infusion	Deletion of IFN-γ reduced inflammation, cytokine production, and fibrosis	[16]
		No effect on hypertrophy	
Rat	Isolated atria preparation	IFN-γ inhibits contraction	[23]
Mouse	Embryonic cardiomyocytes	IFN-γ caused release of MIP-1α, RANTES, and CXCL1	[24]
Mouse	Fetal cardiomyocytes	IFN-γ caused ↑ ANF	[25]
Rat	Adult cardiomyocytes	IFN-γ caused ↑ α- and β-myosin heavy chain transcription	[26]
Rat	Cardiac fibroblasts from post-transplantation hearts	IFN-γ caused ↑ hyaluronan production and proliferation in confluent cultures	[28]
		No effect on non-confluent cultures	
Rat	Cardiac fibroblasts	IFN-γ caused ↑ phosphorylation of Stat1 and Stat3	[29]
Studies showing beneficial effects			
IFN-$\gamma^{-/-}$ mouse	Hypertension-aldosterone infusion, 1 kidney removed, 1 % salt in drinking H_2O	Deletion of IFN-γ caused greater hypertrophy (increased wall thickening), and worse diastolic dysfunction	[19]
		No effect on fibrosis	
Rat	Pressure overload-aortic banding	IFN-γ infusion (0.16 mg/kg/d) attenuated hypertrophy	[22]
Rat	Adult cardiomyocytes	IFN-γ prevented increased width induced by prostaglandin F2α	[22]
Rat	Neonatal cardiomyocytes	IFN-γ decreased cell area	[27]
		IFN-γ decreased α- and β-myosin heavy chain	

clearly underscore the insufficiency of our understanding of the role of IFN-γ in the heart and highlight a need for more studies using rats and mice of differing backgrounds, as well as models of disease.

Cardiomyocytes

There is also evidence that IFN-γ has direct effects on cardiomyocytes. These are summarized in Table 2. Studies using an isolated rat atria preparation have shown that concentrations of IFN-γ ranging between 2 and 10 U/mL have an inhibitory effect on contraction [23]. This appears to be mediated by cholinergic pathways because atropine was able to completely abolish this effect. IFN-γ was also able to increase intracellular levels of cGMP and abolish activation of adenylate cyclase by the β-adrenergic receptor agonist, isoproterenol. In addition to effects on contractility, IFN-γ also appears to influence release of specific products by cardiomyocytes. Murine embryonic cardiomyocytes responded to IFN-γ (100 U/mL, 48 h) by producing MIP-1α (CCL3), RANTES (CCL5), and CXCL1 [24]. Further, stimulation of murine fetal cardiomyocytes with IFN-γ resulted in a 15-fold increase in atrial natriuretic factor (ANF) expression [25]. It is important to keep in mind that these studies were conducted in embryonic and neonatal cardiomyocytes, and how these results translate to the adult cardiomyocyte is unknown. However, Patten et al. [26] did investigate the effect of IFN-γ on adult rat cardiomyocytes cultured for 24 h. They found that IFN-γ (2 ng/mL) induced increased transcription of both β-myosin heavy chain (MHC) and α-MHC to a level equal to that achieved with IL-1β stimulation. In contrast with this, Jin et al. [22] found that IFN-γ (500 U/mL) prevented prostaglandin F2α-induced increases in width in cardiomyocytes isolated from rat hearts. Similarly, treatment of neonatal rat cardiomyocytes with IFN-γ (200 U/mL) resulted in a decrease in area (~26 %) of these cells [27]. This was concurrent with equivalent decreases in α- and β-MHC isoforms. Thus, similar to the whole animal experiments, the effects of IFN-γ on cardiomyocytes are conflicting and may differ depending on the source of the cardiomyocytes and concentration of IFN-γ.

Cardiac fibroblasts

Cardiac fibroblasts are the cells responsible for maintenance of the extracellular matrix (ECM) in the heart, and their activation is essential to the formation of scars and fibrosis in the damaged or stressed myocardium. The effects of IFN-γ on these cells are summarized in Table 2. In the study mentioned in the section "Studies of the whole heart", Han et al. [16] found that α-SMA^{+} cells were decreased in number in the hearts of IFN-γ$^{-/-}$ mice infused with angiotensin II. While this could be indicative of decreased proliferation of smooth muscle cells in the heart, it may also suggest a reduction in fibroblasts converting to the more active and contractile myofibroblast phenotype for which α-SMA is a marker. In one of the few studies of the direct effects of IFN-γ on cardiac fibroblasts, Hellkvist et al. [28] found that IFN-γ increased hyaluronan production, as well as induced proliferation of confluent cultures of isolated cardiac fibroblasts from rat hearts undergoing post-transplantation rejection. Interestingly, they also found that IFN-γ did not have the same effects on non-confluent cardiac fibroblasts. IFN-γ also transiently increases phosphorylation of Stat1 and Stat3 in cardiac fibroblasts, causing translocation of these transcription factors to the nucleus [29]. Peak activation occurred at 15 min for both. Unfortunately, neither of these studies looked at a comprehensive array of phenotypic or functional outcomes. Thus, there is still a need to know exactly what effects IFN-γ has on cardiac fibroblasts ability to synthesize collagen and other ECM products, whether IFN-γ stimulates the release of other pro-fibrotic mediators, and the intracellular pathways involved in these events. This may help resolve some of the conflicting findings related to the ECM, described in whole animals in the section "Studies of the whole heart".

Possible IFN-γ-induced signaling and heart failure

IFN-γ receptor (IFN-γR)1 is the ligand-binding receptor that couples with IFN-γR2. Once the ligand binds to IFN-γR1, the intracellular domains of the two receptor chains open out to allow association of downstream signaling components [30].

Jak/Stat

The classical IFN-γ signaling pathway is via Janus tyrosine kinase (Jak) 1 and signal transducer and activator of transcription (Stat) 1 [31]. Activation of the receptor by IFN-γ induces autophosphorylation of Jak2, which then phosphorylates Jak1. Jak1, in turn, phosphorylates the IFN-γ receptor complex [30]. This allows for subsequent phosphorylation of Stat1 at the C-terminus at Y701. Stat1 then dissociates from the IFN-γ receptor and enters the nucleus to bind to the promoter regions of IFN-γ-inducible genes [30]. Jaks are non-receptor tyrosine kinases, and although Jak1 and Jak2 are prominent in cardiomyocytes [32], most work in the heart has focused only on Jak2. Whether Jak2 is pathological or protective is unclear, which is interesting given the requirement of Jak2 for Jak1 activation. Stat1 is known to be activated in cardiomyocytes by numerous stimuli including angiotenisin II, cardiotrophin-1, stretch, pressure overload, myocardial infarction, and ischemia reperfusion and has been linked with cardiomyocyte apoptosis [33, 34]. Beyond this, very little is known about the role of Jak1/Stat1 in the heart.

MAP kinases

Recently, MAP kinase pathways have also been described as regulating IFN-γ-induced genes [31, 35]. Specifically, up-regulation of iNOS and interferon-activated gene 205 by IFN-γ required extracellular signal-regulated kinase (ERK1/2), leading to c-Jun and AP-1 activation in mouse embryonic fibroblasts [35]. c-Jun N-terminal kinase (JNK) and p38 signaling were not required. Consistent with this, we previously reported that ERK1/2 activity was up-regulated in fibroblasts isolated from SHR hearts, while p38 and JNK1/2 activity was not [36]. In terms of cardiac hypertrophy, the role of ERK1/2 is not clear [37]. ERK1/2 is activated in cardiomyocytes in response to a broad range of stimuli, including cytokines, and constitutive activation of ERK1/2 induces myocardial hypertrophy consisting of a thicker septum and left ventricular posterior wall. However, this model has been criticized as being non-physiological due to the fact that under normal circumstances ERK1/2 activation is cyclical [37]. Deletion of the gene for dual-specificity phosphatase 6 (DUSP6), the phosphatase that inactivates ERK1/2, leads to increased ERK1/2 phosphorylation and an increase in heart size. However, this appeared to be due to hyperplasia since cardiomyocyte cross-sectional area decreased in these mice [37].

mda-9

The IFN-γ-inducible gene, mda-9 (syntenin), is up-regulated in hearts from dilated cardiomyopathy patients [25]. mda-9 is a member of the family of scaffolding PDZ domain-containing proteins [38], which makes it important in localizing signaling proteins to specific areas within the cell. mda-9 is most recognized in cancer metastasis [39]; however, mda-9 also regulates transcription factor activation and cell adhesion [40], likely making it important in both cardiac fibroblasts and cardiomyocytes where cell to

ECM attachments are critical to mediating cell phenotype and function. mda-9 binding to cSrc results in the activation of NF-κB [41], which leads to the transcription of genes regulating cell adhesion and migration as well as cytokines.

PKC

This family of serine/threonine kinases can have effects on contractile performance as well as ventricular remodeling in the heart [42]. IFN-γ has the potential to regulate PKC function. There is evidence that IFN-γ signals through PKC isozymes in different cell types through mechanisms involving other signaling complexes. Incubation of both murine bone marrow-derived mast cells and the human mast cell line (HMC-1) with IFN-γ (100 U) increased phosphorylation of PKC-α and -β isoforms in addition to MAP kinases, Jak1/2, and Stat1 [43]. Inhibition of PKC prevented phosphorylation of p38 kinase and Stat1, as well as inhibiting NF-κB and AP-1 activity. An important event in IFN-γ-dependent gene transcription is phosphorylation of Stat1 on Ser727, and there is evidence that PKC isozymes modulate the cellular responses to IFN-γ by regulating Stat1 phosphorylation. In human promyelocytic leukemia cells, PKCδ appears to be responsible for Stat1 phosphorylation downstream of PI3 kinase based on pharmacological inhibition studies [44]. In rat mesangial cells, pharmacological inhibition studies identified PKCε as being a critical isoform in mediating Stat1 phosphorylation and IFN-γ-dependent gene expression [45, 46]. In monocytes, IFN-γ stimulation induces nuclear translocation of PKC-α and PKC-βI (but not PKC-βII), which phosphorylate the transcription factor PU.1 to enhance gp91 (phox) gene expression [47]. However, the direct mechanism by which IFN-γ stimulation induces PKC isozyme activation and nuclear translocation may involve a multi-step process requiring multiple signaling molecules. In IFN-γ-stimulated bone marrow-derived macrophages, PI3K and p38 MAPK are required for PKC-α translocation (independent of JAK2 activity), but phosphorylation of PKC-α, which is synonymous with activation, is independent of PI3K and p38 MAPK. Phosphorylation of Stat1 at Ser727 and IFN-γ-induced gene expression required both PKC-α and PI3K, suggesting the existence two distinct steps (activation and targeting) in the regulation of PKC-α-mediated gene expression in IFN-γ-stimulated bone marrow-derived macrophages [48]. Adding another level of complexity are studies showing mechanisms involved in the activation of PKC isozymes in response to IFN-γ, may be occurring through integrin-mediated interactions. The regulation of PKCε phosphorylation in mouse embryonic fibroblasts by cell–matrix interactions is correlated with the changing responsiveness to IFN-γ [49]. These data illustrate that PKC-mediated effects of IFN-γ are based on cell type with multiple mechanisms associated with the activation and targeting of the different isozymes. Indeed, PKCs play a central role in the development and progression of multiple forms of heart failure and have been extensively reviewed elsewhere [50]. Given the complexity of these signaling networks, attention has turned to the convergence of these pathways at the level of Stat1-mediated gene expression and its role in propagating the effects of IFN-γ in ischemic, inflammatory, and atherosclerosis induced in heart failure [51–54].

Clinical evidence for IFN-γ

Even though our knowledge regarding the role of IFN-γ in pathogenesis of heart failure is limited and contradictory,

Table 3 Clinical associations between IFN-γ and cardiac disease

Disease	Source	Outcome	Reference
Chagas'	Myocardial sample at autopsy	Inflammatory cell number increased in heart failure patients	[55]
		8 of 11 heart failure patients had IFN-γ^+ cells	
Chagas'	Left ventricle free wall	Many IFN-γ-related genes up-regulated	[25]
Dilated cardiomyopathy (non-ischemic)	Left ventricle free wall	2 IFN-γ-related genes up-regulated (mda-9 and polyubiquitin UbC)	[25]
Congestive heart failure (CHF)	Serum	IFN-γ levels increased compared to control (92.69 vs 66.41 pg/mL)	[14]
Peripartum cardiomyopathy	Serum	IFN-γ decreased in patients with improved function	[56]
		Lower levels of IFN-γ associated with less severe disease	

there are clinical data suggesting an association. This data is summarized in Table 3. Rocha Rodrigues et al. [55] studied at autopsy, myocardial samples from 21 patients with chronic Chagas' Disease. The samples were divided into patients with chronic heart failure and patients with no clinical or pathological signs of heart failure. The authors found an increased mononuclear inflammatory cell infiltrate in subjects with heart failure and a positive correlation between IFN-γ-containing cells and heart failure ($P = 0.032$). The number of IFN-γ-containing cells was compared to the number of IL-4-containing cells as an indicator of T cell phenotype. The number of IFN-γ-containing cells was higher than the number of IL-4-containing cells ($P = 0.02$), indicating a Th1 T cell response. Another study comparing mRNA taken from the free wall of the left ventricle of end stage heart failure patients with Chagas' disease cardiomyopathy and dilated cardiomyopathy (non-ischemic) found that while numerous IFN-γ-induced genes were up-regulated in Chagas' disease, two distinct IFN-γ-induced genes were up-regulated in dilated cardiomyopathy [25]. The two genes were polyubiquitin UbC and mda-9. A study of 72 individuals with congestive heart failure secondary to non-ischemic disease (48 with dilated cardiomyopathy and 24 with hypertensive heart disease) [14], none of whom were receiving statin therapy, found increased serum levels of IFN-γ compared to controls (92.69 vs. 66.41 pg/mL). Administration of atorvastatin at 10 mg/day for 2 weeks reduced serum IFN-γ levels, providing yet another possible mechanism by which statins are effective. An interesting study assessed IFN-γ levels in patients with peripartum cardiomyopathy [56]. Peripartum cardiomyopathy is the onset of heart failure in previously healthy women between 1-month antepartum and 5 months post-delivery. Forster et al. found that most peripartum cardiomyopathy patients that showed improved cardiac function over the 6-month follow-up period had decreasing serum levels of IFN-γ.

IFN-γ as an appropriate target for therapy

An important factor when considering targeting IFN-γ as a potential therapy is the possible side effects, since IFN-γ is important in immune system function. While IFN-$\gamma^{-/-}$ and IFNGR1$^{-/-}$ mice show normal development including their immune system, they do show deficiencies in natural resistance to bacterial, parasitic, and viral infections [30]. This appears relevant to the human population where patients with mutations of the human IFNGR1 or IFNGR2 chains that render these receptors inactive and have similar deficiencies in their immune responses to these infectious agents. Despite this downside of interfering with the IFN-γ system, of relevance to heart failure is that possession of the +874A allele in the IFN-γ gene has been associated with longevity in women [57]. This allele, which is a single-nucleotide polymorphism from T to A, coincides with a putative NF-κB binding site, possibly impacting NF-κB-regulated transcription of IFN-γ [57]. Thus, a dampened inflammatory response caused by an IFN polymorphism may not have major impacts on an individual's ability to fight infection, but may prevent an over activation of the immune system leading to diseases such as cardiovascular disease [30]. Interestingly, this allele was also associated with an increased risk of severe acute respiratory syndrome [58]. However, the +874A allele was associated with tuberculosis infection in patients [59], indicating that it may have an effect on immune response.

Perspective: IFN-α and IFN-β

IFN-α and IFN-β are type I interferons, unlike IFN-γ which is the only known type II interferon. As such, IFN-α and IFN-β bind to receptors distinct from IFN-γ. In contrast to IFN-γ, the effects of IFN-α and IFN-β in the heart are clear. Lutton et al. [60] demonstrated that the number of myocardial lesions could be reduced in mice infected with coxsackievirus B3 by IFN-β. Further, IFN-β immunotherapy reduced $CD8^+$ T cells in chronic myocarditis induced by cytomegalovirus in mice [61]. This is important because these are the principal cell type detected in this autoimmune disease. In the reciprocal experiment, deletion of IFN-β in mice infected with coxsackievirus B3 resulted in increased infection as well as cardiomyocyte breakdown [62]. In humans, 6-month treatment of myocarditis patients with IFN-β1a completely eliminated enterovirus and adenovirus [63]. Further, these patients had decreased inflammation, reduced heart size, improved ventricular function, and reduced symptoms of heart failure. Of note, myocarditis derived from parvovirus B19 and human herpes virus 6 do not respond as well to IFN-β in terms of virus clearance, although patients do improve clinically [64].

Similarly, administration of IFN-α to mice infected with coxsackievirus B3 resulted in reduced virus levels and less myocardial necrosis and inflammatory infiltration [65]. Daliento et al. [66] reported two case studies in which administration of IFN-α improved heart failure in patients suffering enterovirus-induced myocarditis. The first was a 65-year-old male who had worsened clinically from New York Heart Association (NYHA) class II to IV. The individual tested positive for enterovirus from myocardial biopsy. Following 12 months of IFN-α therapy (in addition to the heart failure medication that the patient was already on), left ventricular end diastolic volume had decreased, ejection fraction had improved (from 28 to 40 %), clinical

status had improved (NYHA IV to II), and right ventricular biopsy showed complete resolution of inflammation. In the second example, a 37-year-old male patient with no prior history of cardiovascular disease presented with congestive heart failure (NYHA III). Three months of heart failure medication (ACE inhibitors, digoxin, diuretics, and carvedilol) provided no improvement. PCR assessment was positive for enterovirus. Additional treatment with recombinant IFN-α for 6 months did not improve cardiac dimension or function; however, clinical status did improve (NYHA Class II). Biopsy revealed no signs of active lymphocytic myocarditis and PCR demonstrated no myocardial enterovirus genome.

The evidence is clear that IFN-α and -β are protective against myocarditis. Yamamoto et al. [67], however, compared the effectiveness of IFN-γ and IFN-α/β treatment in encephalomyocarditis virus-induced myocarditis in mice. Interestingly, while IFN-α/β improved survival, IFN-γ prevented any deaths from occurring and thus was far more effective than IFN-α/β. Only IFN-γ was effective in reducing cardiac lesions in the infected mice. Similarly, a mouse model overexpressing IFN-γ in pancreatic β cells was protected from developing myocarditis in response to coxsackievirus B3 [68]. Thus, in the situation where a pathogen is involved, IFN-γ may in fact have a protective function. However, as detailed, the role of IFN-γ in adverse myocardial remodeling and heart failure of non-pathogen etiology is far from clear.

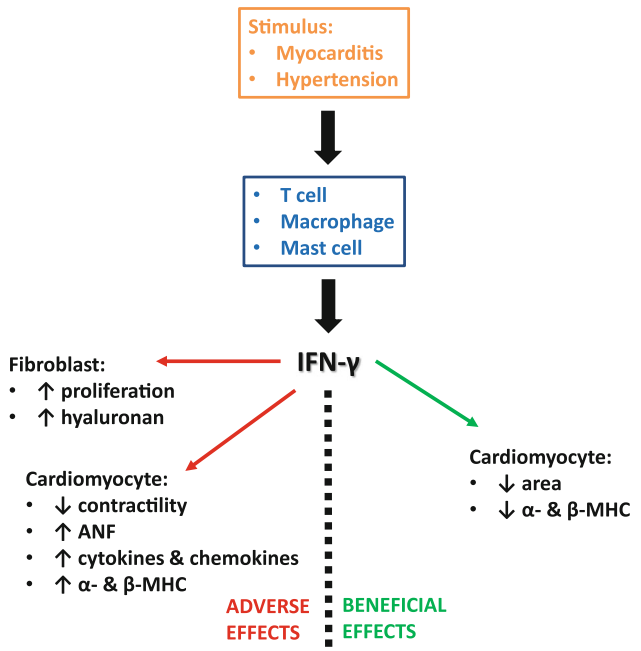

Fig. 1 Schematic summarizing the beneficial and adverse effects attributed to IFN-γ in the heart. Adverse stimuli, such as myocarditis or hypertension, initiate the release of IFN-γ from inflammatory cells recruited into the heart. Multiple studies have concluded that IFN-γ initiates adverse effects on cardiac fibroblasts, leading to fibrosis, and cardiomyocytes, leading to hypertrophy. Other studies have alternatively found that IFN-γ exerts protective effects limiting cardiac hypertrophy. *ANF* atrial natriuretic factor, *MHC* myosin heavy chain

Summary

Figure 1 is a schematic summarizing the major research findings to date, highlighting a number of contradictory observations. IFN-γ has the potential to regulate a wide range of functions in the heart due to its transcriptional control over a large number of genes related to proliferation, migration, and cytokine production. Evidence from a limited number of animal and human studies is contradictory in that it is unclear if IFN-γ is detrimental or protective in the development of heart failure. Certainly, studies in IFN-γ overexpressing mice strongly suggest a role for this cytokine in inflammatory cell recruitment, cytokine and chemokine production, and development of heart failure. However, mouse background strain may be important, given the differences in underlying T cell phenotype in C57B/6 and BALB/c mice. Thus, we need further studies to determine whether this is indeed a contributing factor that determines whether IFN-γ has adverse or protective effects on the heart. At the cellular level, the overall effect of IFN-γ on cardiac fibroblast phenotype and function, the mechanisms behind these effects, and the ultimate role of IFN-γ in heart failure is also not clear. At present, the effects of IFN-γ on cardiomyocyte function are unclear due to discrepant findings possibly due to the types of cardiomyocytes studied and/or concentrations of IFN-γ used. Cytokine therapy is not a part of the standard treatment regime for heart failure. There is a need to identify novel cytokine candidates, and thus, we would propose that a more thorough examination of IFN-γ and its effects on the heart are warranted for consideration as a future target for heart failure treatment.

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Conflict of interest Drs. Scott Levick and Paul Goldspink have no conflicts of interest or financial ties to disclose.

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