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Interferons and interferon-related pathways in heart disease

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Interferons (IFNs) and IFN-related pathways play key roles in the defence against microbial infection. However, these processes may also be activated during the pathogenesis of non-infectious diseases, where they may contribute to organ injury, or function in a compensatory manner. In this review, we explore the roles of IFNs and IFN-related pathways in heart disease. We consider the cardiac effects of type I IFNs and IFN-stimulated genes (ISGs); the emerging role of the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway; the seemingly paradoxical effects of the type II IFN, IFN- γ ; and the varied actions of the interferon regulatory factor (IRF) family of transcription factors. Recombinant IFNs and small molecule inhibitors of mediators of IFN receptor signaling are already employed in the clinic for the treatment of some autoimmune diseases, infections, and cancers. There has also been renewed interest in IFNs and IFN-related pathways because of their involvement in SARS-CoV-2 infection, and because of the relatively recent emergence of cGAS-STING as a pattern recognition receptor-activated pathway. Whether these advances will ultimately result in improvements in the care of those experiencing heart disease remains to be determined.

KEYWORDS

interferon, interferon-stimulated gene, heart failure, myocardial infarction, cGAS-STING, interferon regulatory factor, type I interferon (IFN), interferon gamma (IFN- γ)

Introduction

It is over sixty five years since a substance that interferes with viral replication in host cells, termed “interferon”, was first reported by Isaacs and Lindemann (1). Since that first description, our knowledge of interferons (IFNs), their upstream regulators, downstream effects, and related regulatory factors continues to expand well beyond the field of virology. IFNs and IFN-related pathways are emerging as critical determinants of the pathogenesis of heart disease, or indeed on occasion protection against it. In this review article, we discuss the emerging state-of-the-art of IFNs and IFN-related pathways in the heart focusing on type I IFNs, the type I IFN response, and interferon-stimulated genes (ISGs); the related upstream cyclic GMP-AMP synthase (cGAS)- stimulator of interferon genes (STING) signaling pathway; the seemingly paradoxical actions of the type II IFN, IFN- γ ; and the interferon regulatory factor (IRF) family of transcription factors.

The IFN family, IFN function and IFN induction

IFNs belong to the Class II cytokine family, a group of α -helical cytokines with modest sequence homology but structural similarity (2). The IFN family itself is made up of three classes, distinguished from one another according to the type of receptor that they bind to: type I IFNs, type II IFN (of which there is only one), and type III IFNs (Figure 1). The type

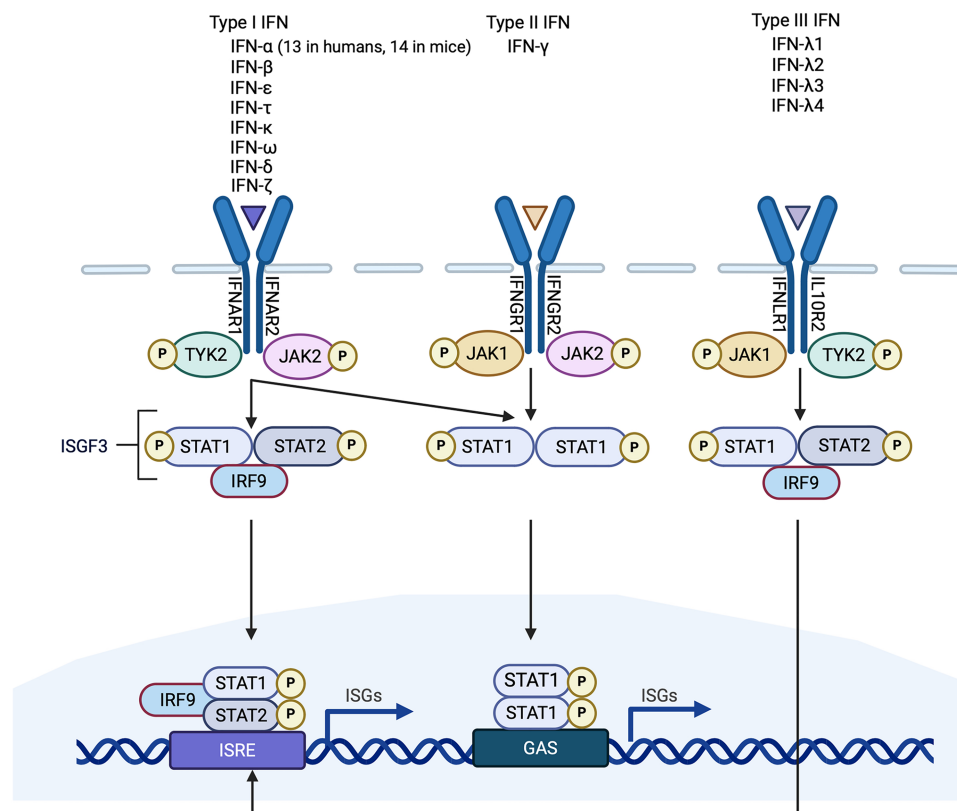


FIGURE 1

Simplified schematic of interferon (IFN) signaling pathways. Type I IFNs are the largest of the three classes of IFN. They signal through IFNAR1/2 which activates JAK/STAT signaling. STAT1/2 phosphorylation causes the release of STATs from IFNAR and the formation of a complex containing phosphorylated STAT1/2 and IRF9, called interferon-stimulated gene factor 3 (ISGF3). ISGF3 binds to a promoter sequence called interferon-stimulated response element (ISRE) and induces interferon stimulated genes (ISGs). There is only one Type II IFN (IFN- γ). It binds to IFNGR1/2 to activate JAK/STAT signaling and the formation of phosphorylated STAT1 homodimers which bind to IFN- γ -activated sites (GAS) elements in gene promoters. Type I IFNs can also induce STAT1 homodimerization. Type III IFNs signal through IFNLR1/IL10R2 and, like type I IFNs, induce the formation of an ISGF3 complex. Non-canonical signaling pathways are not shown.

I IFNs consist of five α -helices (3), and bind to a ubiquitously expressed heterodimeric receptor that is made of two chains called IFNAR1 and IFNAR2. Within the type I IFN class, and the most extensively studied of the IFNs, are IFN- α and IFN- β . Thirteen genes encode for human IFN- α (in mice there are 14), whereas IFN- β is encoded by a single gene (3, 4). Other type I IFNs are less well characterized. These include IFN- ϵ , IFN- τ , IFN- κ , IFN- ω , IFN- δ , and IFN- ξ (3). There is only one member of the type II IFN class, IFN- γ . Whereas type I IFNs are monomers, IFN- γ is an intercalated dimer (3). IFN- γ is structurally unrelated to the type I IFNs and it binds to a different receptor, which is made up of IFNGR1 and IFNGR2 subunits (5). The type III IFNs [made up of IFN- λ 1, IFN- λ 2 and IFN- λ 3, also called interleukin-28A (IL-28A), IL-28B and IL-29 respectively, and IFN- λ 4] are structurally related to type I IFNs and also to IL-10 (5). They bind to a heterodimeric receptor made up of IL10R2 and IFNLR1 subunits (5), and they are the least characterized IFN class.

Almost all cells can be induced to express type I IFNs, although the main sources of type I IFNs are innate immune cells (6). IFN- γ

expression, in contrast, is more restricted, being primarily expressed by T cells [CD8+ cytotoxic T cells and T helper type 1 (Th1) cells] and natural killer (NK) cells (5). However, most cells express IFNGR and therefore most cells respond to IFN- γ (7). IFN- γ can induce the expression of genes that prime the type I IFN response, and type I IFNs can potentiate IFN- γ signaling (7, 8). Type III IFNs, like type I IFNs, can also be expressed by most cells, although they mainly act at epithelial surfaces (5, 9, 10).

The primary function of the IFNs is in the host defence against microbial infection. IFN gene expression is induced by the binding of pattern recognition receptors (PRRs) to pathogen-associated molecular patterns (PAMPs), which are molecules unique to microbes such as viruses or bacteria [e.g., nucleic acids, bacterial endotoxin (lipopolysaccharide, LPS), certain glycoproteins, bacterial peptides, and fungal glucans] (Figure 2). PRRs, however, can also be activated by endogenous molecules that are released by damaged or dying host cells. These endogenous molecules are called damage-associated molecular patterns (DAMPs). Outside of the setting of viral myocarditis or other microbial infections, induction of the IFN response in heart disease is mediated by

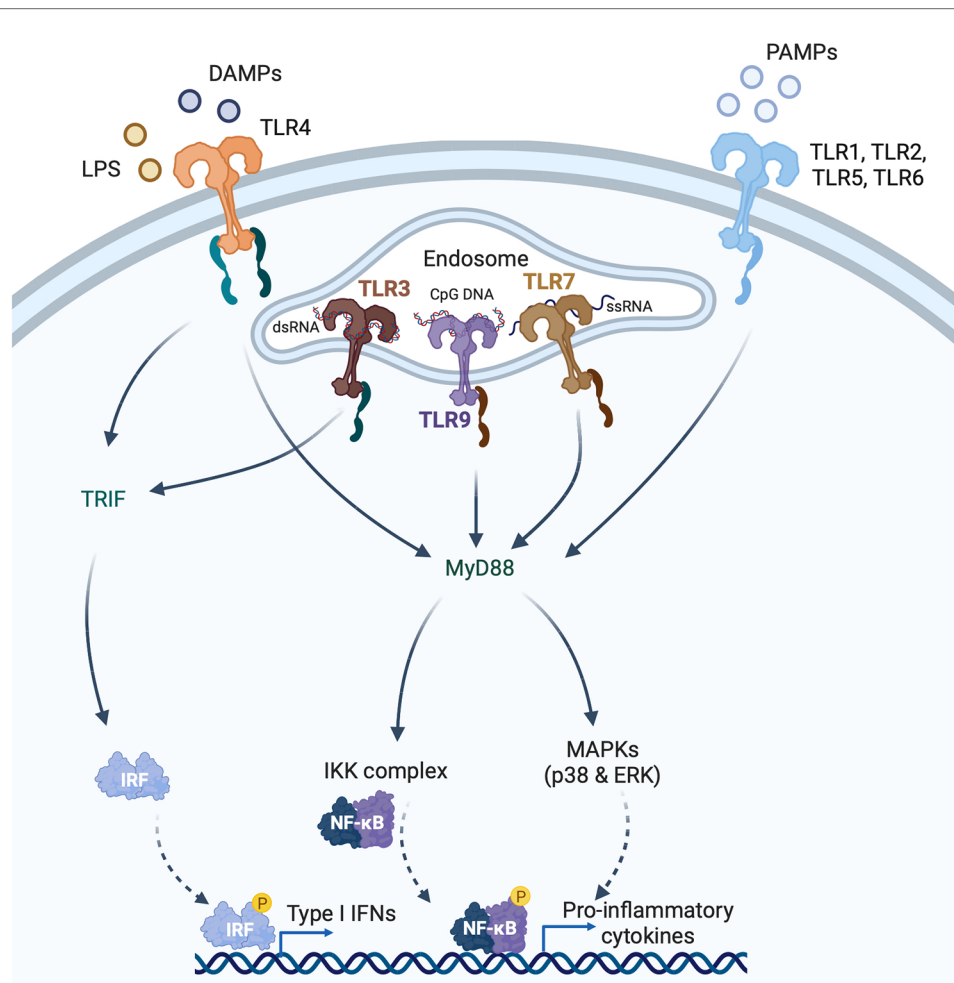


FIGURE 2

Induction of type I interferons (IFNs) by pattern recognition receptors (PRRs). PRRs recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are either intracellular or extracellular. Toll like receptors (TLRs) are membrane bound PRRs and major inducers of the IFN response. TLR4 is expressed on the cell surface and recognizes bacterial lipopolysaccharide (LPS) and endogenous DAMPs. TLR1, 2, 5 and 6 recognize PAMPs. TLR3 is localized to endosomes, and recognizes viral double-stranded RNA (dsRNA), whereas TLR7 recognizes single-stranded RNA, and TLR9 recognizes CpG DNA. TLRs induce signaling via either the myeloid differentiation primary response 88 (MyD88)-dependent or TIR domain-containing adaptor inducing IFN β (TRIF)-dependent pathways. TLR3 and TLR4 signal through the TRIF-dependent pathway, and TLR4 can also signal through the MyD88-dependent pathway. MyD88-signaling involves mitogen-activated protein kinase (MAPK) and nuclear factor κ -light chain enhancer of activated B cells (NF κ B), whereas TRIF-signaling is mediated through IRF3 phosphorylation.

DAMPs. PRRs can sense PAMPs (or DAMPs) that are either outside the cell (through membrane bound PRRs) or inside the cell (through cytoplasmic PRRs). Membrane bound PRRs include Toll like receptors (TLRs) and C-type lectin receptors (CLRs). TLRs are major inducers of the IFN response. TLRs can be present on the cell membrane (mediating extracellular signaling) or on the membrane of endosomes (mediating intracellular signaling) (Figure 2). For instance, TLR3 is localized to endosomes, and recognizes viral double-stranded RNA (dsRNA), small interfering RNAs (siRNAs) and host RNAs derived from damaged cells (11). TLR4, on the other hand, is expressed on the cell surface and recognizes LPS derived from invading bacteria, and DAMPs produced by dying cells (5) (Figure 2). TLR7 recognizes single-stranded RNA (ssRNA). Its encoding gene is located on the X chromosome and frequently avoids X chromosome inactivation, which may be responsible for sex

differences in type I IFN responses (12). TLR9 is an intracellular TLR expressed on endosomes and the endoplasmic reticulum that senses DNA, especially unmenthylated CpG DNA, which is more common in viruses and bacteria. After ligand-binding by TLRs, signal transduction is mediated by either the myeloid differentiation primary response 88 (MyD88)-dependent or TIR domain-containing adaptor inducing IFN- β (TRIF)-dependent pathways (11) (Figure 2). TLR3 and TLR4 signal through the TRIF-dependent pathway, and TLR4 can also signal through the MyD88-dependent pathway (11). Signaling by TLR7 and TLR9 is also MyD88 dependent. The MyD88-dependent pathway involves activation of mitogen-activated protein kinase (MAPK) and nuclear factor κ -light chain enhancer of activated B cells (NF κ B), whereas TRIF-dependent signaling is mediated through phosphorylation of IRF3. Both pathways ultimately result in the induction of proinflammatory genes, including IFNs (11).

Cytosolic PRRs recognize nucleic acids, such as viral nucleic acids, but also nucleic acids arising from damaged host cells or damaged mitochondria. For instance, RIG-I and melanoma differentiation-associated gene 5 (MDA5) sense viral dsRNA in the cytosol (13, 14), relaying signals via the adaptor protein mitochondrial antiviral sensing protein (MAVS) (15). Double-stranded DNA (dsDNA) aberrantly present in the cytosol can arise from invading microbes or from the host, including through leakage of mitochondrial DNA (mtDNA) from damaged mitochondria. Cytosolic dsDNA is sensed by cGAS, which relays signals via the adaptor protein, STING (16, 17). There has been substantial recent interest in the role that cGAS-STING pathway activation plays in organ injury, and the literature describing the contributions of cGAS-STING to heart disease is reviewed in the relevant section later in this review.

IFN signaling, IRFs and ISGs

All IFNs signal through the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (Figure 1). Briefly, the intracellular domains of IFNAR1 and IFNAR2 are associated with two JAKs called non-receptor tyrosine-protein kinase 2 (TYK2) and JAK1 respectively. Ligand binding of IFNAR results in phosphorylation of the JAKs, which in turn phosphorylate tyrosine residues in the intracellular domains of the receptor subunits, as well as the STATs, STAT1 and STAT2 (18). STAT1/2 phosphorylation causes the release of the STATs from the IFNAR receptor and the formation of a trimeric complex that is comprised of STAT1, STAT2 and IRF9. This trimeric complex is called interferon-stimulated gene (ISG) factor 3, or ISGF3. ISGF3 translocates to the nucleus and acts as a transcriptional activator by binding to an interferon-stimulated response element (ISRE) in the promoter region of ISGs (5). Other type I IFN signaling pathways include the formation of STAT homodimers and heterodimers which can initiate gene transcription by binding to IFN- γ -activated site (GAS) elements in gene promoters (19). It has also been reported that IFNAR signaling can be mediated by the MAPK/c-Jun amino-terminal kinase (JNK) signaling pathway (8). Type II IFN signaling is also primarily JAK/STAT-mediated. IFN- γ binding of IFNGR1 and IFNGR2 induces association and phosphorylation of the receptor subunits with JAK1 and JAK2 respectively (5) (Figure 1). This results in STAT1 homodimerization and the binding of the STAT1 homodimers to GAS elements in gene promoter regions (5). Type III IFN signaling is similar to type I IFN signaling, involving activation of TYK2 and JAK1, recruitment of STAT1 and STAT2 and the formation of an ISGF3 complex which mediates gene transcription (20, 21) (Figure 1).

IFN responses are regulated by a group of 9 (in humans and mice) transcription factors, called IRFs. IRF proteins all contain an amino-terminal DNA binding domain that recognizes a consensus DNA sequence element called the interferon-stimulated response element (ISRE), present in the genes encoding IFNs and ISGs (5). IRFs have differing roles in regulation of IFN responses. For instance, IRF3 mediates

downstream signaling relayed by the adaptor proteins TRIF, MAVS, and STING to induce the production of type I IFNs (22). In contrast, IRF2 attenuates IRF3-mediated transcriptional activation (23). The effects of IRFs, however, are not necessarily limited to their roles in the immune response. The actions of several of the individual IRFs in the heart have been described, and these actions are summarized in the relevant section later in this review.

IFNs mediate their effects by stimulating the induction of several hundred ISGs (24), which can have unique and overlapping functions. Teleologically, the end effects of IFNs and IFN genes can be considered as ways in which an infected cell can limit the damage caused to itself and to neighbouring cells by an invading microbe. For instance the ISG, protein kinase R (PKR) is a stress induced kinase that restricts protein synthesis via phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) (25). One of the most strongly induced ISGs is the ubiquitin-like (Ubl) protein ISG15 (ISG15; also called interferon-induced 15 kDa protein). ISG15 limits the cellular damage caused by viral myocarditis (26); whereas our group recently reported induction and a pathogenetic role of ISG15 in the adverse ventricular remodeling that occurs in response to pressure overload (27). These findings illustrate the divergent effects of IFN-related pathways in the presence or absence of microbial infection, and they are elaborated upon later. In addition to its role in modulating cellular and viral protein synthesis, the IFN response also regulates the host response to viral infection by stimulating the upregulation of major histocompatibility complex class I and II antigens (28, 29), promoting programmed cell death (30–32), regulating cellular differentiation (33), suppressing angiogenesis (34), and activating other immune cells (35, 36).

The type I IFN response and the heart

Induction of a type I IFN response has been reported to occur in both ischemic (37) and non-ischemic (27) cardiomyopathy, as well as in viral myocarditis (38). However, the contributions of the type I IFN response (being ostensibly protective or detrimental) depend on the underlying cause of the IFN response. In the context of viral infection, induction of type I IFNs has a largely protective effect (38), whereas in the absence of infection and in the setting of ischemia (37), or pressure overload (27), the type I IFN response may have deleterious consequences. Evidence of the direct cardiac effects of type I IFNs themselves can be sought through the study of genetic conditions and through clinical experience with therapeutic use of recombinant IFNs.

Cardiac involvement in monogenic and autoimmune interferonopathies

The monogenic type I interferonopathy Aicardi-Goutières syndrome (39) may present with an inflammatory cardiomyopathy (40); and type I IFNs have also been implicated in the pathogenesis of autoimmune congenital heart block (41). That being said, cardiac disease is not always a feature of

interferonopathy. Cardiac involvement is not, for instance, a common occurrence in STING-associated vasculopathy with onset in infancy (SAVI). Likewise, whereas systemic lupus erythematosus (SLE) is associated with both an IFN gene signature (42) and an increased risk of cardiovascular disease (CVD) (43), there are insufficient data to establish a causal association between the two (44).

Adverse cardiac effects of recombinant IFNs

Recombinant human IFN β 1b (e.g., Betaseron) is approved for the management of multiple sclerosis. The product monograph states that there is no evidence of a direct cardiotoxic potential of Betaseron, although cases of cardiomyopathy have been reported (45). Recombinant IFN α 2b (e.g., INTRON A) has regulatory approval for the management of chronic hepatitis C, chronic active hepatitis B, chronic myelogenous leukemia, multiple myeloma, non-Hodgkin's lymphoma, malignant myeloma, AIDS-related Kaposi's sarcoma, hairy cell leukemia, basal cell carcinoma, and condylomata acuminata (46). The product monograph for INTRON A states that adverse reactions associated with the cardiovascular system are mostly correlated with pre-existing CVD and prior cardiotoxic therapy, although transient reversible cardiomyopathy has been reported rarely in patients without prior evidence of cardiac disease (46). The interpretation from this experience of the use of recombinant IFNs for other indications is that the likelihood of deleterious cardiac effects of type I IFNs in otherwise normal hearts is relatively low. However, their effects in the presence of concurrent cardiac illness may be more notable. This is perhaps best exemplified by a report describing the role of IRF3 and type I IFNs in the response to myocardial infarction (MI) (37).

IRF3 and the type I IFN response to MI

In a landmark study published in 2017, King and co-workers used single cell RNA sequencing (scRNA-seq) to profile leukocytes isolated from the hearts of mice after MI (37). In doing so, they observed that a subtype of cardiac macrophages was characterized by IRF3/type I IFN activation, and that disruption of either IRF3 or IFNAR signaling resulted in improved survival following MI, decreased inflammation and improved cardiac function (37). The authors concluded that the high level of cell death that occurs in MI results in disruption of the compartmentalization of DNA in the cell nucleus and mitochondria and interferes with the housekeeping actions of self-DNases. This leads to the release of damage signals, especially dsDNA from dying cells (37). dsDNA, in turn, is sensed by cGAS in infiltrating phagocytes leading to activation of an IRF3-dependent type I IFN response (37). Secreted IFNs then diffuse in the local microenvironment, binding to IFNARs on neighbouring cells and amplifying the type I IFN response through induction of ISGs (37). In our opinion, this postulated mechanism most clearly exemplifies how type I IFNs may be induced during myocardial injury and how the type I IFN response may, in turn, contribute to adverse cardiac outcomes.

ISG15 in viral myocarditis and pressure overload

Whereas the example above illustrates the potentially deleterious effects of the type I IFN response in MI, type I IFNs are protective against viral myocarditis. For instance, mice deficient in IFNAR are susceptible to coxsackievirus B3 (CVB3) infection (47), and mice deficient in IFN- β experience exacerbated CVB3-induced myocarditis (48). The example of ISG15 illustrates how a downstream effector ISG of the type I IFN response also may play a context-dependent role in the protection from or development of cardiac injury. ISG15 is a Ubl protein and, in this role, once it is induced ISG15 can post-translationally modify lysine residues on actively translated proteins, including viral proteins (49) (Figure 3). The post-translational modification of proteins by conjugation with ISG15 is termed, ISGylation. In addition to its intracellular effects, ISG15 also exists in an intracellular free form and it can also be secreted. In its secreted form, ISG15 binds to its receptor, lymphocyte function-associated antigen 1 (LFA-1) expressed especially by T cells and NK cells, where it stimulates IFN- γ production (50, 51) (Figure 3). Absence of ISG15 has been reported to exacerbate CVB3 myocarditis (26), and this effect was also mimicked in mice lacking ubiquitin-activating enzyme E1-like (UBE1), the E1-activating enzyme necessary for protein ISGylation (26). The authors of that article attributed the protective actions of ISG15 to the ISGylation of CVB3 2A protease, limiting CVB3-induced cleavage of host eukaryotic initiation factor 4 γ (eIF4G) in cardiomyocytes, which ordinarily promotes viral infection by restricting host cell protein translation (26). A recent study, further illuminated the role of ISG15 in viral myocarditis, concluding that induction of ISG15 in myocarditis functions to counter cardiac atrophy and dysfunction by increasing the heart's metabolic capacity through downregulation of cardiac glycolysis and enhancing the respiratory activity of mitochondria (52).

Viral infection, however, is not the only cause of cardiomyocyte ISG15 induction. For instance, Maier and coworkers reported that cardiomyocytes with constitutively active I κ B kinase/NF κ B signaling exhibited a type I IFN response that is characterized by activation of the ISG15 pathway (53). In the absence of viral infection, the conjugation of ISG15 to actively translated host proteins can affect several cellular processes including cytoskeletal dynamics, DNA damage responses, cytokine release, and immune modulation (54). In recent work by our group, we set out to determine the mechanism(s) by which proinflammatory CCR2-expressing macrophages contribute to pressure overload-induced ventricular remodeling (27). We found that exposure of cardiomyocytes to the secreted products of CCR2+ cardiac macrophages isolated from mouse hearts, induced a profound type I IFN response, characterized by ISG15 induction. Cardiac ISG15 induction was also observed in mouse hearts after transverse aortic constriction (TAC) or following infusion with angiotensin II, the left ventricles (LVs) of uninephrectomized rats treated with deoxycorticosterone acetate (DOCA) salt, and the right ventricles of rats after pulmonary artery banding (27). We observed that, in pressure overload, ISG15 induction results in the ISGylation of newly translated cardiomyocyte proteins, including the myofibrillar protein, filamin-C (27), and that absence of ISG15 attenuated adverse ventricular remodeling after TAC (27). Interestingly, in that

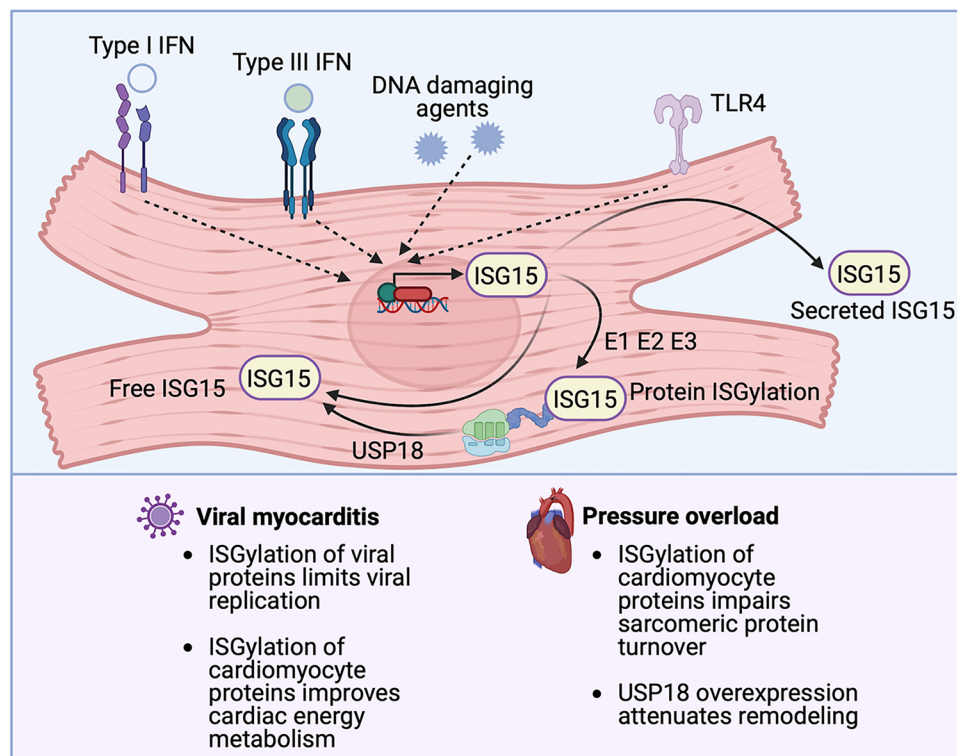


FIGURE 3

The emerging role of ISG15 in heart disease. ISG15 is one of the most strongly inducible interferon (IFN) stimulated genes, the expression of which can be triggered by IFNs themselves, DNA damaging agents or TLR signaling. ISG15 exists in 3 forms, an intracellular free form, a secreted form and a protein-bound form. ISG15 binds to newly synthesized viral or host proteins through an energy consuming process, termed ISGylation, that requires E1-activating, E2-conjugating, and E3-ligating enzymes. Removal of ISG15 from proteins (de-ISGylation) is mediated by the protease USP18. ISG15 induction protects against viral myocarditis, likely through the ISGylation of both viral and cardiomyocyte proteins. Conversely, in the absence of microbial infection, ISG15 induction can have detrimental effects, as has been observed in pressure overload, where the ISGylation of newly synthesized cardiomyocyte proteins impairs sarcomeric protein turnover.

study, we also found cardiac ISG15 levels to be markedly reduced in *Ifnar1* deficient mice even in the absence of pressure overload (27). This observation illustrates that constitutively expressed IFNs can have important physiological roles even in the absence of induction and even though they are present at very low levels (55).

Protein ISGylation is mediated by an energy-consuming process involving E1-activating enzymes, E2-conjugating enzymes, and E3-ligating enzymes and it is reversed by an ISG15-specific protease called ubiquitin-specific protease 18 (USP18), which itself is an ISG (56–58) (Figure 3). Whereas we reported a pathogenetic role for protein ISGylation in pathological ventricular hypertrophy, Ying and coworkers described a protective effect of USP18 overexpression, which would be expected to reverse protein ISGylation (59). In brief, the authors observed that cardiomyocyte-specific overexpression of USP18 attenuated myocardial hypertrophy, fibrosis, ventricular dilatation, and ejection fraction decline induced by aortic banding, whereas USP18 knockout exacerbated remodeling (59). The authors attributed the cardioprotective effects of USP18 to inhibition of transforming growth factor β -activated kinase 1 (TAK1)/MAPK/JNK signaling (59). They had focused on this pathway because USP18 had previously been reported to deubiquitinate TAK1 (60), whereas the polyubiquitination of TAK1 is important for its autoactivation and

downstream activation of p38 MAPK/JNK signaling (61). Other studies, though, have reported that USP18 is specific for ISG15 and that USP18 does not remove ubiquitin from substrate proteins (62, 63). Accordingly, it is feasible that the phenotypes observed in the USP18 overexpressing/knockout mice were mediated through altered protein ISGylation, although this possibility was not explored in the original report (59).

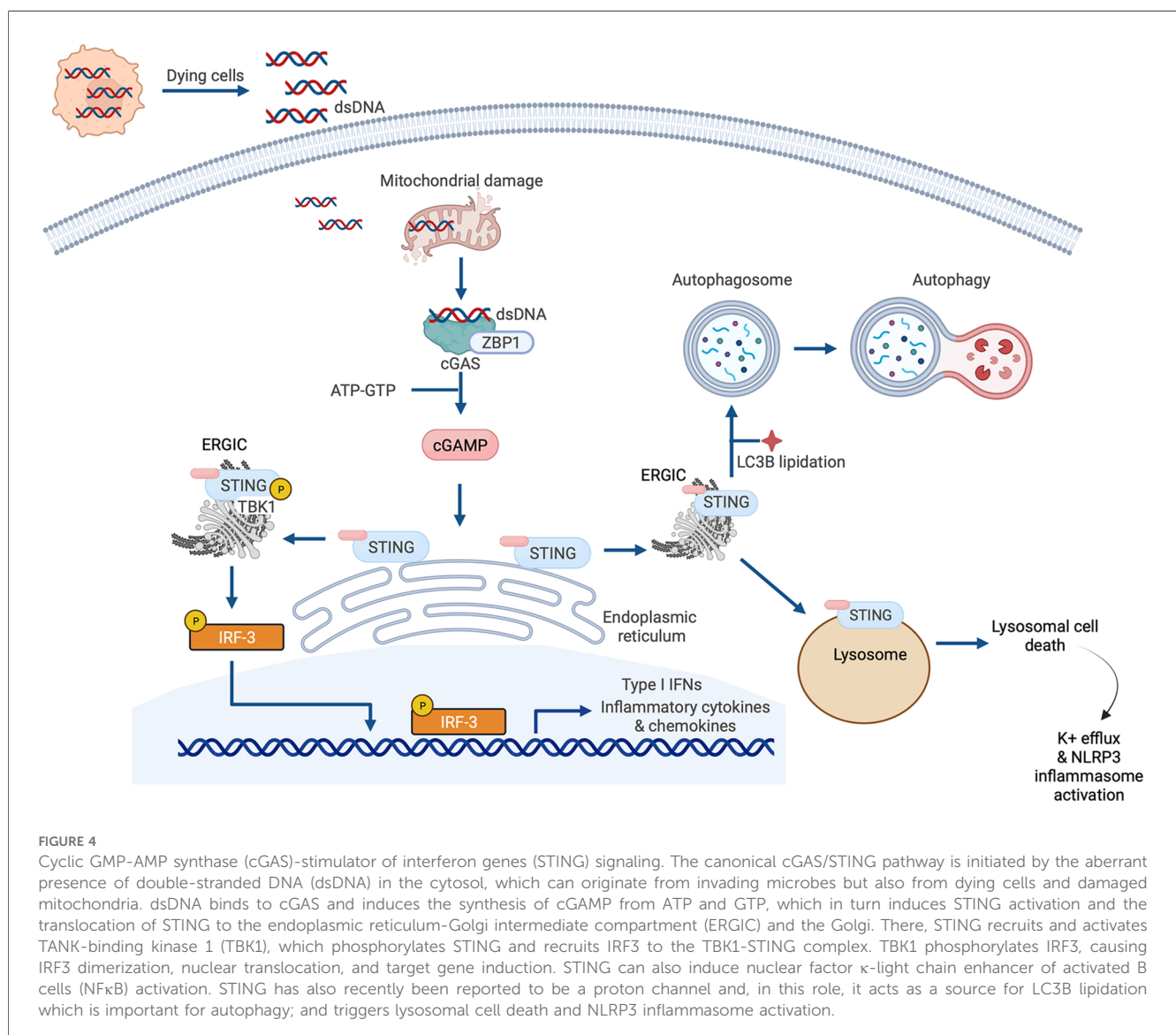
Lastly, whereas the role of ISG15 in the defence against viral infection has been known about for decades (64), its biological function in this capacity has gained increasing attention of late because of the involvement of ISG15 in COVID-19, with potentially intracellular and extracellular proviral and antiviral effects (65). The contribution of an insufficient or augmented IFN response to COVID-19 severity and its potential cardiac complications is discussed later in this review.

The emerging role of cGAS-STING pathway activation in the pathogenesis of heart disease

The earlier summarized study by King et al. that reported the importance of IRF3 and the type I IFN response in MI described

a central role for cGAS-STING signaling in myocardial injury (37). This is one of several recent studies espousing the significance of cGAS-STING pathway activation in cardiac disease that have emerged since the original description of the pathway in 2013 (66, 67). Briefly, the cGAS-STING signaling pathway exists to mediate the immune response to displaced dsDNA which can originate from invading microorganisms, dead cells, extracellular vesicles, or leakage of DNA from damaged mitochondria (Figure 4). dsDNA binds to cGAS and activates the synthesis of the second messenger 2'3' cyclic GMP-AMP (cGAMP) from ATP and GTP. cGAMP, in turn, binds to the active pocket site of the dimeric adaptor protein STING, causing STING activation and downstream signaling (68). Once it is activated, STING translocates from its residing place in the endoplasmic reticulum (ER) to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and the Golgi where it recruits and activates TANK-binding kinase 1 (TBK1), which in turn phosphorylates STING [on serine residue 366 (Ser366) in humans, Ser365 in mice], and recruits IRF3 to the TBK1-STING

complex (22, 69) (Figure 4). TBK1 phosphorylates IRF3, causing IRF3 dimerization, nuclear translocation and induction of its target genes (69), including type I IFNs, ISGs and inflammatory cytokines (70) (Figure 4). In addition to this canonical mechanism of gene induction by cGAS-STING, STING can also induce NFκB activation (68). The role of TBK1 in NFκB activation is unclear. It has been suggested that TBK1 is dispensable for STING activation of NFκB, and that this process requires TAK1 and IκB kinase (IKK) complexes in myeloid cells (71). However, other authors have reported that TBK1 recruitment is necessary for STING-mediated NFκB activation (72). Aside from (and independent of) its role in mediating the induction of IFNs and cytokines, STING also plays an important role in autophagy induction (73), and inflammasome activation (74). For instance, when STING binds cGAMP and translocates to the ERGIC, the STING-containing ERGIC acts as a source for non-canonical LC3B lipidation which is important for the biogenesis of autophagosomes (73) (Figure 4). STING-induced autophagosome formation is dependent on a direct interaction



between STING and WD repeat domain, phosphoinositide interacting 2 (WIPI2) (75). Separately, activated STING traffics to the lysosome where it triggers membrane permeabilization and lysosomal cell death, and potassium efflux which promotes NLRP3 inflammasome activation (74) (Figure 4). Interestingly, human STING has also recently been reported to act as a proton channel, and its effects in both L3B lipidation and inflammasome activation have been attributed to this property (76).

Experimental studies implicating cGAS-STING pathway activation in the pathogenesis of heart disease are summarized in Table 1. Some of the findings from these studies are elaborated upon below.

cGAS-STING in MI

In 2018 Cao and coworkers reported that MI caused by ligation of the left anterior descending (LAD) artery induced upregulation of the cGAS-STING pathway, which sustains the inflammatory “M1-like” macrophage phenotype (77). Furthermore, inactivation of the pathway through knockout of cGAS prompted a more “M2-like” macrophage phenotype that was accompanied by improved wound healing, enhanced angiogenesis, diminished remodeling, and improved survival (77). Similarly, treatment of mice with the STING antagonist H-151 has been reported to improve outcomes after experimental MI (82, 83).

cGAS-STING in non-ischemic cardiomyopathy

Zhang and coworkers reported STING upregulation in heart tissue of humans with dilated cardiomyopathy or HCM, and in

the hearts of mice with pressure overload induced by aortic banding (90). In that study, knockout of STING attenuated pathological hypertrophy and ejection fraction decline induced by aortic banding (90). Similarly, Hu et al. also observed activation of the cGAS-STING pathway in mice with ventricular remodeling caused by TAC, with a preservation of LV function (and improved survival) when cGAS was knocked down using adeno-associated virus 9 (AAV9) gene transfer of short hairpin RNA (shRNA) (78).

cGAS-STING in diabetes and in sepsis

Yan et al. reported that oxidative damage-induced mtDNA leak was accompanied by cGAS-STING pathway activation in the hearts of diabetic high fat diet-fed mice, and that AAV9-mediated knockdown of STING with shRNA preserved cardiac function (84). In a model of sepsis-induced cardiac injury, Li et al. observed that global STING knockout attenuated LV systolic dysfunction and improved survival in mice injected with LPS (91).

Noncanonical actions of cGAS-STING in the heart

The above examples, and those additional studies summarized in Table 1, illustrate how a substantial body of literature has arisen in recent years indicating that cGAS-STING pathway activation takes place in both ischemic and non-ischemic cardiomyopathy and that blockade of cGAS-STING signaling improves cardiac outcomes in experimental models. More recently still, new fundamental insights into cGAS-STING biology have emerged through the study of the actions of the pathway in the heart. For

TABLE 1 Experimental studies implicating the cGAS-STING pathway in heart disease.

Years	Disease or experimental context	Summary of principal findings	Citation
2017	Myocardial infarction	Mice with functionally deficient STING mimic IRF3 knockout mice in their gene expression patterns after MI	(37)
2018	Myocardial infarction	cGAS knockout improved survival in mice after MI, decreased pathological remodeling, enhanced angiogenesis, and preserved contractile function	(77)
2020	Pressure overload	AAV9-mediated knockdown of cGAS with shRNA improved survival, preserved LV contractility, and attenuated pathological remodeling in mice after TAC	(78)
2020	High fat diet	High fat diet upregulated cGAS-STING and augmented cardiac remodeling in Akt2-AMPK double knockout mice, and cGAS-STING inhibition attenuated cardiomyocyte contractile dysfunction	(79)
2020	Alzheimer's disease	Knockdown of cGAS or STING negated the beneficial effects of melatonin on neonatal cardiomyocyte mitophagy and apoptosis in response to APP/PS1 mutation	(80)
2020	Cigarette smoke	cGAS-STING inhibition rescued smoke-induced contractile dysfunction in wildtype and <i>Becn1</i> haploinsufficient (<i>Becn1</i> ^{+/-}) neonatal cardiomyocytes, except cGAS inhibition in <i>Becn1</i> ^{+/-} cardiomyocytes	(81)
2022	Reperused myocardial infarction	Small molecule STING inhibition decreased infarct expansion, attenuated cardiac function decline, and reduced myocardial hypertrophy	(82)
2022	Myocardial infarction	Small molecule STING inhibition preserved cardiac function and attenuated fibrosis in mice with MI	(83)
2022	Diabetic cardiomyopathy	AAV9-mediated knockdown of STING with shRNA preserved cardiac function and hypertrophy and alleviated cardiac pyroptosis in streptozotocin-diabetic high fat diet-fed mice	(84)
2023	Doxorubicin-induced cardiotoxicity	AAV9-mediated knockdown of STING with shRNA improved survival and cardiac function and reduced inflammation in mice with doxorubicin cardiotoxicity	(85)
2023	Cardiac transplantation	Graft survival was prolonged in donor hearts from cGAS knockout mice	(86)
2023	Doxorubicin-induced cardiotoxicity	Global deficiency of cGAS, STING or IRF3 each ameliorated doxorubicin-induced cardiotoxicity in mice; and endothelial-specific STING deficiency attenuated cardiotoxicity and endothelial dysfunction	(87)
2023	Doxorubicin-induced cardiotoxicity	ZBP1 stabilizes Z-form mtDNA and cooperates with cGAS to induce type I IFN signaling and cardiotoxicity induced by doxorubicin	(88)
2023	Cholesterol metabolism	Carnitine acetyltransferase depletion induced mtDNA stress and a cardiomyocyte innate immune response mediated by cGAS-STING	(89)

cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; MI, myocardial infarction; IRF, interferon regulatory factor; AAV9, adeno-associated virus 9; shRNA, short hairpin RNA; LV, left ventricle; TAC, transverse aortic constriction; AMPK, AMP-activated protein kinase; APP, amyloid precursor protein; PS1, presenilin 1; ZBP1, Z-DNA binding protein 1; mtDNA, mitochondrial DNA.

example, one recent study reported that cGAS interacts with the innate immune sensor protein Z-DNA binding protein 1 (ZBP1) to promote type I IFN responses and cardiotoxicity (88). Briefly, because mtDNA is circular and lacks free ends it cannot rotate to relieve torsional stress. As a result, mitochondrial genome instability promotes the accumulation of a form of DNA called Z-DNA which differs from classical Watson-Crick B-DNA in its conformation including (but not limited to) that Z-DNA is a left-handed double helix, and B-DNA is a right-handed double helix. Mitochondrial Z-DNA is stabilized by ZBP1 which nucleates a complex that contains cGAS, as well as the mediators of cell death and inflammation, receptor-interacting protein 1 (RIPK1) and RIPK3 (88). This, in turn, augments STAT1 phosphorylation, and induces type I IFN signaling that is dependent on ZBP1, STING and IFNAR (88). Illustrating the importance of the interaction between ZBP1 and cGAS in mtDNA-sensing and downstream signaling, mice lacking ZBP1, STING or IFNAR1 were protected from doxorubicin-induced cardiotoxicity (88). Interestingly though, ZBP1 is not required for sensing of cytosolic B-form mitochondrial DNA (88). Separately, in another recent study, Mao et al. implicated cGAS-STING activation as an important mediator of the mechanisms by which

altered cholesterol metabolism may affect innate immune responses in the heart (89). In that study, the investigators reported that depletion of carnitine acetyltransferase (CRAT) from cardiomyocytes promoted cholesterol catabolism, and accumulation of bile acid and the intermediate 7α -hydroxyl-3-oxo-4-cholestenoic acid (89). This, in turn, induced mitochondrial stress and cGAS-STING-dependent type I IFN responses, which contributed to myocardial inflammation and heart failure (89).

IFN- γ

IFN- γ in the heart arises from infiltrating inflammatory cells, primarily CD4+ and CD8+ T cells and NK cells (92, 93), with some contribution from macrophages (92, 94) (Figure 5). Interaction between T cells and macrophages is important in driving IFN- γ production by each cell-type, and IFN- γ plays a central role in mediating crosstalk between innate and adaptive immune cell populations (92, 95). Cardiac IFN- γ levels are increased in a range of different diseases including myocarditis (96), Chagas disease cardiomyopathy (97–99), MI (100),

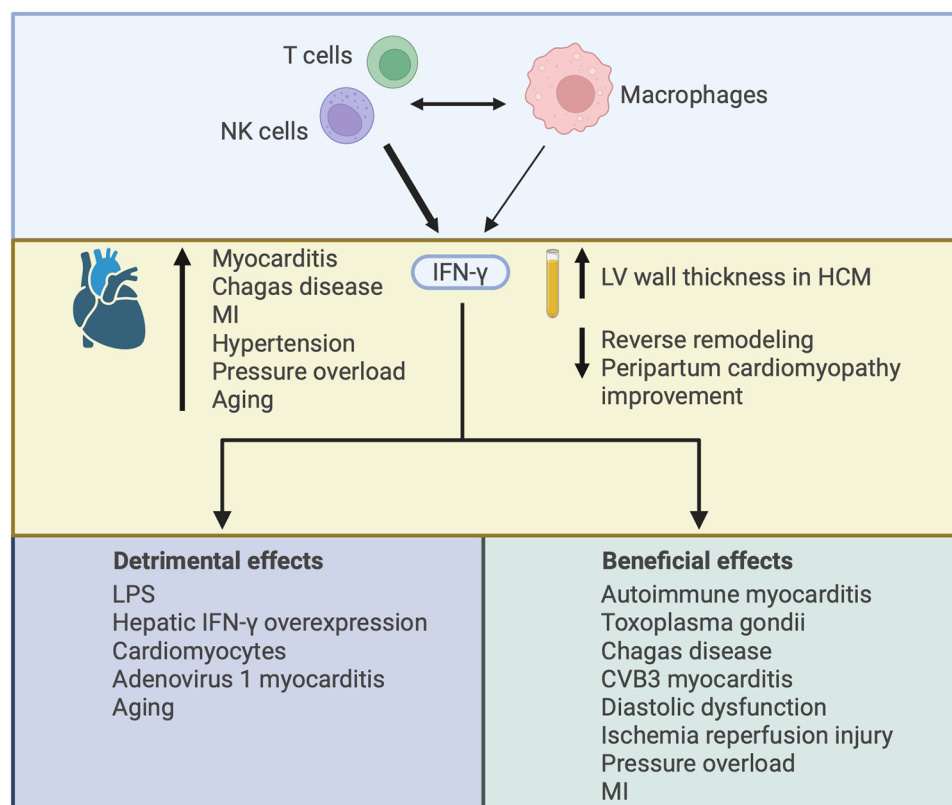


FIGURE 5

Illustrating the varied effects of interferon- γ (IFN- γ) in the heart. IFN- γ is mainly produced by CD4+ and CD8+ T cells and NK cells, with some contribution from macrophages. Interaction between adaptive and innate immune cells can drive IFN- γ production by each cell-type. IFN- γ expression in the heart has been described in several disease settings and increased plasma levels of IFN- γ have been associated with worsened cardiac outcomes. The increase in IFN- γ may contribute to cardiac injury or it may be compensatory, with both detrimental and beneficial effects of IFN- γ described according to the nature of IFN- γ augmentation or deficiency and the context in which it has been studied. HCM, hypertrophic cardiomyopathy; MI, myocardial infarction; LPS, lipopolysaccharide.

hypertensive heart disease (101), pressure overload (94), and aging (102) (Figure 5). In comparison to the type I IFNs, a much larger body of literature exists attesting to the actions of IFN- γ in the heart, with overall conflicting reports describing both beneficial and detrimental context-dependent effects of IFN- γ . These studies are summarized in Table 2 and Figure 5, and some of the key observations are discussed below.

Studies reporting deleterious actions of IFN- γ in the heart

Transgenic mice that overexpress IFN- γ in their livers, and thus with high circulating serum levels of IFN- γ , have been reported to develop a chronic active myocarditis and cardiomyopathy characterized by accumulation of CD4+ and CD8+ T cells, macrophages and dendritic cells (105). This was accompanied by upregulation in the expression of proinflammatory cytokines including TNF α , IL-12, CCL2 and CCL3 (105), illustrating the role of IFN- γ in inflammatory gene induction. Furthermore, in patients with LV assist device (LVAD) implantation, low serum IFN- γ (and TNF α) predicted cardiac improvement (116). This is interesting because, in experimental studies, co-exposure of cardiomyocytes to IFN- γ and TNF α has also been reported to induce mitochondrial dysfunction and nitro-oxidative stress (99).

These findings suggesting deleterious actions of IFN- γ in the heart are supported by several other studies in experimental animals (92), isolated atria (117), cultured cardiomyocytes (99), and cultured cardiac fibroblasts (118), reviewed in (95). Given the production of IFN- γ by infiltrating adaptive immune cells, and the role of IFN- γ in orchestrating the interaction between innate and adaptive immune cells, this seems teleologically appropriate. However, several other studies have described a protective role for IFN- γ in the heart.

Studies reporting protective actions of IFN- γ in the heart

IFN- γ knockout mice have been reported to develop worsened hypertrophy, cardiac fibrosis and cardiac dysfunction after TAC (94). This has been attributed to an essential role of IFN- γ in Stat5-dependent activation of phosphoinositide 3-kinase/Akt signaling during compensatory hypertrophy (94). Cardiac inflammation has also been reported to be increased in IFN- γ knockout mice injected with cardiac myosin and accompanied by a constrictive pericarditis (111). Furthermore, cardiac hypertrophy and diastolic dysfunction were exacerbated in IFN- γ knockout mice with aldosterone, uninephrectomy and salt water feeding (114), whereas recombinant IFN- γ attenuated cardiac

TABLE 2 Experimental studies reporting the detrimental or beneficial effects of IFN- γ in the heart.

Years	Disease or experimental context	Reported actions of IFN- γ	Citation
Detrimental effects of IFN-γ			
1998	Rat papillary muscle	Cardiodepressant effect of IFN- γ in the presence of LPS	(103)
2001	Isolated retrograde-perfused rat hearts	Augmented depression of inotropic and lusitropic effects of LPS and IFN- γ in aged hearts	(104)
2007	Hepatic IFN- γ overexpression	Chronic active myocarditis and cardiomyopathy in IFN- γ overexpressing mice	(105)
2012	Hepatic IFN- γ overexpression	TNF α knockout attenuated myocarditis and cardiomyopathy in IFN- γ overexpressing mice	(106)
2012	Neonatal rat ventricular myocytes	Cardiac myocyte atrophy induced by recombinant IFN- γ due to degradation of myosin heavy chain protein	(107)
2015	Mouse adenovirus 1 (MAV-1)	Attenuated myocarditis with neutralizing antibody mediated IFN- γ depletion	(96)
2021	AC16 cardiomyocytes	Co-incubation with IFN- γ and TNF α induced cardiomyocyte mitochondrial dysfunction and nitro-oxidative stress	(99)
2023	Aging	IFN- γ response signature in aging mouse hearts mimicked by recombinant IFN- γ treatment of induced pluripotent stem cell derived cardiomyocytes, accompanied by reductions in oxidative phosphorylation and glycolysis	(102)
Beneficial effects of IFN-γ			
2001	Autoimmune myocarditis	Exacerbated cardiac α -myosin heavy chain induced autoimmune myocarditis in IFN- γ receptor knockout mice	(108)
2001	Toxoplasma gondii	Augmented accumulation of Toxoplasma gondii in the hearts of IFN- γ knockout mice	(109)
2001	Chagas disease	Augmented cardiac Trypanosoma cruzi parasitism in IFN- γ knockout mice	(110)
2004	Autoimmune myocarditis	Constrictive pericarditis and augmented myocarditis in IFN- γ knockout mice injected with cardiac myosin	(111)
2004	Viral myocarditis	Worsened CVB3-induced myocarditis in IFN- γ knockout mice	(112)
2005	PGF(2 α)-treated rat cardiac myocytes and abdominal aortic constriction	Recombinant IFN- γ attenuated myocardial hypertrophy	(113)
2012	Aldosterone infusion, uninephrectomy and 1% saline water	Augmented LV hypertrophy and worsened diastolic dysfunction in IFN- γ knockout mice	(114)
2013	Porcine cardiopulmonary bypass-associated myocardial ischemia-reperfusion injury	Preconditioning with IFN- γ improved recovery of ventricular function after ischemia reperfusion injury	(115)
2018	Pressure overload	Worsened hypertrophy, cardiac fibrosis and dysfunction in IFN- γ knockout mice after TAC	(94)
2019	Left anterior descending artery ligation	IFN- γ orchestrated the sequential cellular immune response after MI and IFN- γ knockout impaired cardiac function and survival after MI	(100)

Table does not include studies reporting solely an association of IFN- γ with cardiac or atherosclerotic disease, or the role of IFN- γ in the immune response to transplantation.

IFN- γ , interferon- γ ; LPS, lipopolysaccharide; TNF α , tumor necrosis factor α ; MAV-1, mouse adenovirus 1; CVB3, coxsackievirus B3; PGF(2 α), prostaglandin F2 α ; LV, left ventricle, TAC, transverse aortic constriction; MI, myocardial infarction.

hypertrophy in rats with abdominal aorta banding (113). Other reports also describing beneficial actions of IFN- γ in the heart are also summarized in Table 2.

In sum, cardiac IFN- γ is increased in several different disease states. Its role in these diseases may contribute to their pathogenesis, or it may be compensatory, with experimental studies reporting both deleterious and protective actions of IFN- γ . These actions appear to be dependent on the models studied, timing of intervention, mechanism of upregulation or inhibition, and the experimental endpoints employed.

Interferon regulatory factors

The IRFs are a family of 9 transcription factors that play an important role in the immune response, also regulating other cellular processes. IRF1 regulates gene expression by binding to ISREs in their promoter regions (119) and, although it is best studied as a transcriptional activator (119), IRF1 can also function as a transcriptional repressor (120). IRF2 competitively inhibits IRF1-mediated gene transcription (121). IRF3, IRF5 and IRF7 are important for the production of type I IFNs in response to PRR-mediated signaling (122). IRF9 regulates IFN-induced gene expression, and IRF4, IRF5 and IRF8 control myeloid cell development and responses (122). IRF6 is important for early development (123). Most of the IRFs have been studied individually for their role in the heart, and these actions are summarized below and in Table 3.

Several of these studies were published by the same team in 2013–2014, and followed a similar pattern of investigation involving: exploration of change in IRF protein levels in diseased

hearts; cardiac-specific IRF overexpression and global IRF knockout; and elucidation of a cellular mechanism underlying the beneficial or detrimental cardiac effects of IRF overexpression and knockout (124, 128, 129, 132, 134, 135).

IRF1

IRF1 expression has been reported to be reduced in the hearts of humans with dilated cardiomyopathy or hypertrophic cardiomyopathy, whereas in mice with pressure overload caused by banding of the thoracic aorta there was an early upregulation of IRF1 after 3–7 days, followed by a reduction in IRF1 protein in the heart by weeks 4–6 (124). Cardiac-specific IRF1 overexpression exacerbated pressure overload-induced hypertrophy, ventricular dilatation and dysfunction, whereas cardiac hypertrophy was attenuated in IRF1 knockout mice and rats (124). These prohypertrophic effects of IRF1 were attributed to induction of inducible nitric oxide synthase (iNOS) caused by binding of IRF1 to the promoter region of the iNOS gene (124). IRF1 has also been implicated in the pathogenesis of cardiac dysfunction that can occur because of chronic kidney disease (CKD), termed cardiorenal syndrome type 4 (125). In that study, the authors reported that high phosphate levels in CKD impair myocardial energy metabolism by downregulating the transcription of the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma activator 1-alpha (PGC1 α) (125). Mechanistically, high phosphate was observed to epigenetically regulate the expression of IRF1 by inducing acetylation of histone protein H3 on lysine residue 9 (H3K9) (125). IRF1 has a repressor domain and, in some circumstances, can bind to the IRF response element of target genes to repress gene expression (136, 137). In the case of

TABLE 3 Experimental studies exploring the actions of interferon regulatory factors (IRFs) in cardiac disease.

IRF	Years	Disease or experimental context	Reported actions of IRFs	Citation
IRF1	2014	Pressure overload	Cardiac IRF1 overexpression exacerbated hypertrophy, ventricular dilatation and dysfunction, whereas IRF1 knockout attenuated cardiac hypertrophy	(124)
IRF1	2020	Cardiorenal syndrome type 4	IRF1 mediated cardiac PGC1 α downregulation and consequent dysfunction of myocardial energy metabolism	(125)
IRF2	2021	Myocardial infarction	IRF2 contributed to cardiac dysfunction in MI by inducing gasdermin D-mediated pyroptosis	(126)
IRF3	2011	Angiotensin II induced cardiac fibrosis	IRF3 knockout attenuated cardiac fibrosis	(127)
IRF3	2013	Aortic banding	IRF3 knockout exacerbated cardiac hypertrophy, whereas cardiac IRF3 overexpression attenuated it	(128)
IRF3	2017	Myocardial infarction	MI induced activation of an IRF3-type I IFN axis in cardiac macrophages which was caused by release of DNA from damaged cells, and which impaired cardiac function	(37)
IRF4	2013	Aortic banding	Cardiac specific overexpression of IRF4 exacerbated hypertrophy, fibrosis, and dysfunction and IRF4 knockout attenuated cardiac hypertrophy	(129)
IRF5	2014	Myocardial infarction	Nanoparticle-delivered siRNA against IRF5 supported infarct healing and attenuated heart failure after MI	(130)
IRF5	2019	Viral myocarditis	IRF5 interference attenuated viral myocarditis	(131)
IRF7	2014	Aortic banding	Cardiac specific overexpression of IRF7 attenuated hypertrophy, fibrosis, and dysfunction and IRF7 knockout augmented cardiac hypertrophy and fibrosis	(132)
IRF7	2022	Cardiac autoinflammation	IRF7 knockout attenuated cardiac autoinflammation induced by ADAR1 inactivation	(133)
IRF8	2014	Aortic banding	Cardiac specific overexpression of IRF8 attenuated hypertrophy and fibrosis, and IRF8 knockout augmented cardiac hypertrophy and fibrosis	(134)
IRF9	2013	Aortic banding	Cardiac-specific overexpression of IRF9 attenuated cardiac hypertrophy whereas hypertrophy, fibrosis and cardiac dysfunction were augmented in IRF9 knockout mice	(135)

Table does not include studies reporting solely an association of IRFs with cardiac or atherosclerotic disease, or the role of IRFs in the immune response to transplantation. No experimental studies were identified that examined the role of IRF6 in cardiac disease.

IRF, interferon regulatory factor; PGC1 α , peroxisome proliferator-activated receptor gamma activator 1-alpha; MI, myocardial infarction; siRNA, short interfering RNA; ADAR1, adenosine deaminase acting on RNA-1.

cardiorenal syndrome type 4, IRF1, induced by high phosphate, was observed to bind directly to the promoter region of the PGC1 α encoding gene inhibiting PGC1 α transcription (125).

IRF2

IRF2 levels have been reported to be increased after experimental MI, whereas lentiviral mediated silencing of IRF2 with shRNA attenuated cardiac dysfunction after MI, an effect attributed to the role of IRF2 in gasdermin D-mediated pyroptosis (126).

IRF3

Several studies have explored the effects of IRF3 in the heart. In 2011, Tsushima and coworkers reported that IRF3 knockout attenuated cardiac fibrosis and ventricular chamber shrinkage in mice infused with angiotensin II, whereas cardiac hypertrophy was unaffected (127). In that study, the authors attributed IRF3 activation by angiotensin II in cardiac fibroblasts to be mediated by ERK signaling rather than canonical TBK1/IKK signaling (127). Lu and coworkers performed aortic banding in IRF3 knockout mice and in mice with cardiac-specific IRF3 overexpression, observing that IRF3 knockout exacerbated cardiac hypertrophy and IRF3 overexpression attenuated it, and concluding that IRF3 is a negative regulator of pathological cardiac hypertrophy (128). They attributed this effect to an interaction between IRF3 and ERK2, which inhibited ERK1/2 signaling (128). The authors also observed that IRF3 levels are increased early after aortic banding and return to basal levels by day 28, whereas IRF3 is also upregulated in human failing hearts (128). Considering the more recent findings of King et al, using scRNA-seq (37), it seems likely that the early increases in cardiac IRF3 levels in mice after aortic banding and in human heart failure may represent accumulation of cardiac macrophages, which is known to occur early after aortic banding (138).

IRF4

Jiang et al. reported that IRF4 is downregulated in human dilated cardiomyopathy, in mouse hearts 4 and 8 weeks after aortic banding, and in cardiomyocytes exposed to angiotensin II or phenylephrine (129). They found that cardiac specific overexpression of IRF4 exacerbated pressure overload-induced hypertrophy, fibrosis, and dysfunction, whereas IRF4 knockout attenuated cardiac hypertrophy (129). The authors attributed this effect to a role for IRF4 in inducing the transcription of cAMP response element-binding protein (CREB) in cardiomyocytes, which is known to promote cardiac hypertrophy (139, 140).

IRF5

IRF5 plays a key role in macrophage polarization favoring an “M1-like” phenotype (141, 142). In 2014, Courties et al. used nanoparticle-delivered siRNA to silence IRF5 in infarct macrophages and observed that IRF5 knockdown attenuated the development of heart failure after MI in ApoE knockout mice (130). Whereas most IFNs and IFN-related pathways have shown to have protective roles in viral myocarditis, IRF5 may play a role in the pathogenesis of cardiac injury in this setting.

Specifically, Nie et al. observed upregulation of the TLR9-IRF5 pathway in the hearts of humans and mice with CVB3 myocarditis, and they observed that an AAAG-rich oligodeoxynucleotide that interferes with IRF5 alleviated myocarditis in CVB3-infected mice (131).

IRF7

Jiang et al. also studied the actions of IRF7, observing that IRF7 negatively regulates cardiac hypertrophy (132). Briefly, the authors reported that either angiotensin II or phenylephrine decreased IRF7 protein levels in neonatal rat cardiomyocytes, and IRF7 protein levels were also observed to be reduced in the hearts of mice 2 and 4 weeks after aortic banding (132). Cardiac specific IRF7 overexpression attenuated pressure overload-induced hypertrophy, fibrosis, and dysfunction, whereas IRF7 knockout augmented cardiac hypertrophy and fibrosis (132). These effects were attributed by the authors to binding of IRF7 to inhibitor of κ B kinase- β (IKK β) and consequent inactivation of NF κ B (132). Elsewhere, IRF7 expression levels have been reported to be markedly increased in the hearts of mice with CVB3 myocarditis (143). More recently, inactivation of adenosine deaminase acting on RNA-1 (ADAR1) (which acts as an RNA sensing inhibitor) was found to induce a late-onset autoimmune myocarditis, dilated cardiomyopathy, and heart failure (133). This phenotype was attenuated by IRF7 knockout, indicating that IRF7 is the principal mediator of cardiac autoinflammation induced by ADAR1 absence (133).

IRF8

When studying the cardiac effects of IRF8, Jiang and coworkers observed a reduction in cardiac IRF8 protein levels in the hearts of humans with dilated cardiomyopathy or hypertrophic cardiomyopathy, mice with pressure overload caused by aortic banding, and in neonatal rat cardiomyocytes incubated with angiotensin II or phenylephrine (134). Mice with cardiac-specific overexpression of IRF8 were resistant to hypertrophy and fibrosis induced by pressure overload, whereas either global- or cardiomyocyte-specific IRF8 knockout aggravated adverse remodeling (134). The investigators attributed this effect to an interaction between IRF8 and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) which inhibits the nuclear translocation of NFATc1 (134). The authors speculated that the interaction between IRF8 and NFATc1 may prevent dephosphorylation of NFATc1 by calcineurin which ordinarily promotes nuclear translocation and facilitates pathological hypertrophy (134, 144).

IRF9

Lastly, Jiang et al. also studied the effects of IRF9 in the heart (135). In that study IRF9 protein levels were observed to be increased in the hearts of mice 2 and 4 weeks after aortic banding and in neonatal rat cardiomyocytes exposed to angiotensin II or isoproterenol (135). Cardiac-specific overexpression of IRF9 attenuated cardiac hypertrophy whereas hypertrophy, fibrosis and cardiac dysfunction were augmented in IRF9 knockout mice (135). These effects were attributed by the authors to an action of IRF9 in competing with p300 for binding to the transcription activation

domain of myocardin (135), a transcriptional coactivator and inducer of cardiac hypertrophy (145).

The IFN response and the heart in COVID-19

The COVID-19 pandemic has shone the spotlight on the role that IFNs play in the defence against viral infection, and potentially also the contribution of IFNs to adverse outcomes. COVID-19 can result in several different cardiovascular complications including heart failure, arrhythmia, acute coronary syndrome, MI, myocarditis, and acute myocardial injury (146). Indeed, myocardial injury may be the most common extrapulmonary complication of COVID-19, affecting over 70% of those with severe disease (146). Most studies and commentaries discussing the role of IFNs in COVID-19 do not distinguish the cardiac effects specifically from systemic effects associated with severe disease or critical illness. Furthermore, whereas cardiac complications are common in severe COVID-19, the relative contributions of direct viral infection and the immune response to viral infection (including the IFN response) have not been disentangled. It is similarly challenging to disentangle the relative contributions of either individual IFNs or the IFN response from hyperinflammation in general (147, 148). Nevertheless, both a delayed persistent type I IFN response and diminished capacity to produce type I IFNs have been linked to COVID-19 severity (147). Thus, in COVID-19, the optimal IFN response is one that is finely balanced and dependent on host factors, stage and severity of disease and site of infection, amongst other factors (147, 148). For instance, a genome-wide association study has linked genes encoding members of IFN signaling pathways to critical illness in COVID-19 (149). However, Mendelian randomization revealed that life-threatening COVID-19 was associated with low expression of *IFNAR2*, but high expression of *TYK2* (149). Therapeutically, recombinant IFN β 1a did not reduce mortality in hospitalized patients with COVID-19 (150). In contrast, the anti-inflammatory therapy baricitinib, which has activity against JAK1/2 and moderate activity against TYK2, reduced mortality amongst hospitalized patients with COVID-19 by about 20%, and it has received U.S. Food and Drug Administration (FDA) approval for this indication (151). As discussed below, however, although JAK inhibitors may block IFN signaling, their effects are not limited to this pathway. In sum, the IFN response likely has a complex, bidirectional role in COVID-19, including the cardiac complications of COVID-19, both functioning in the host defence against viral infection, and contributing to the deleterious consequences of hyperinflammation in severe disease.

IFNs and atherosclerosis

Both type I IFNs and IFN- γ have also been implicated in the pathogenesis of atherosclerosis. In brief, type I IFNs may affect plaque formation through several different processes, including through the formation of foam cells and macrophage

extracellular traps, endothelial dysfunction and through influencing the actions of dendritic cells and T cells (152). IFN- γ affects cholesterol accumulation in macrophages and macrophage activation, induces foam cell formation and apoptosis, affects Th1-mediated immune responses, and promotes oxidative stress, endothelial activation, smooth muscle cell proliferation and plaque development (153, 154). Like its paradoxical actions in the heart, however, IFN- γ has been reported to have both pro- and anti-atherogenic effects (154). In this review, we have focused on the actions of IFNs in the heart and in heart disease. It should be noted that a comparably sized body of experimental evidence exists outlining the roles of both type I IFNs and IFN- γ in atherosclerosis. For an in-depth exposition, the reader is referred to reviews specifically on this topic (152–154).

IFNs as prognostic markers

Whereas IFNs and interferon-related pathways play several different roles in heart disease, evidence supporting circulating IFNs as biomarkers of cardiac disease is scant. Those data that do exist support a greater role for the measurement of plasma levels of IFN- γ than for type I IFNs. For instance, in a study of patients with HCM, increased plasma levels of IFN- γ were associated with LV wall thickness (155) (Figure 5). Furthermore, lower circulating levels of IFN- γ have been associated with greater likelihood of reverse remodeling following LVAD implantation (116), and of less severe peripartum cardiomyopathy (156) (Figure 5). For type I IFNs, plasma proteomic analysis revealed that higher circulating levels of IFNA5 were associated with a higher relative wall thickness in women, but not in men (157). It may be that determination of a type I IFN signature, as has been done in SLE for instance (42, 158), may offer greater prognostic value than measurement of individual IFNs. However, even amongst patients with SLE who often exhibit a strong type I IFN response, a type I IFN signature has not been robustly associated with CVD (44). In sum, extensive experimental evidence and correlative clinical studies support roles of IFNs and IFN-related pathways in the pathogenesis of heart disease (or protection against it). However, there is an absence of evidence that would suggest routine measurement of IFNs (or their downstream effectors) will offer utility as biomarkers of cardiac disease, certainly above already established measures, and even amongst at-risk groups.

Therapeutically targeting IFNs to improve outcomes in heart disease

To date, several different therapeutic approaches that augment or inhibit IFN pathways have received regulatory authority approval, although not for the treatment of heart disease. Most notably, these approaches include recombinant IFNs and small molecule JAK inhibitors. Elsewhere, there is fervent medicinal chemistry activity in the development of agents that interfere (or augment) cGAS-STING signaling, and newer strategies to modulate the actions of cytokines are under development.

Recombinant IFNs

As discussed earlier, recombinant IFN β 1b and recombinant IFN α 2b are approved for other indications, including multiple sclerosis, chronic hepatitis, and hematological malignancy. Whereas clinical studies have suggested improvements in some outcomes for patients treated with recombinant IFN in viral myocarditis (159–162), recombinant IFN therapy is not part of usual viral myocarditis management, with somewhat disappointing trial results attributed possibly to a relatively poor response to IFN of parvovirus B1 and HHV6 myocarditis (163). Similarly, as already discussed, recombinant interferon IFN β 1a did not affect mortality in hospitalized patients with COVID-19 (150).

JAK inhibitors

Whereas the goal of recombinant IFN therapy is to augment signaling through IFN pathways, JAK inhibitors block IFN signaling. These therapies have received regulatory approval principally for the treatment of inflammatory arthropathies, and they have shown promise in other disease settings associated with inflammation or an augmented IFN response. However, by virtue of blocking JAK/STAT signaling, the effects of these agents extend to antagonizing the actions of several proinflammatory cytokines whose receptors signal through this pathway and they are not limited to antagonizing IFNs or IFN-related pathways. Tofacitinib is a JAK1/3 inhibitor, with less efficacy vs. JAK2 and TYK2, and it is approved for the treatment of rheumatoid arthritis, psoriatic arthritis and ulcerative colitis (164). Baricitinib is an inhibitor of JAK1/2, with moderate activity vs. TYK2, with an indication for the treatment of rheumatoid arthritis (165). As already discussed, baricitinib has also received FDA approval for use in the treatment of COVID-19. Upadacitinib is selective for JAK1 and is approved for the treatment of rheumatoid arthritis, psoriatic arthropathy, axial spondyloarthritis, atopic dermatitis, ulcerative dermatitis and Crohn's disease (166). In a small clinical trial, baricitinib was shown to improve disease severity amongst patients with monogenic IFN-mediated autoinflammatory diseases [including chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE), SAVI, and other interferonopathies] (167). Baricitinib also reduced albuminuria and improved inflammatory markers in patients with diabetic kidney disease (168). However, the product monograph for baricitinib carries warnings as to a potential increased risk of infection, malignancy, major adverse cardiovascular events, and thrombosis (165). Accordingly, whereas JAK inhibitors offer a repurposing opportunity, their adverse effect profiles may preclude their use in the treatment of heart disease.

Strategies that antagonize cGAS-STING signaling

The development of inhibitors of cGAS or STING (70), or of TBK1 (169), is an area of active investigation from both the academic and the industrial sectors. Over 20 compounds have already been reported to have cGAS- or STING-inhibitory effects (70), and activators of the cGAS-STING pathway are being developed as cancer therapeutics (170, 171). cGAS inhibitors are being developed that exert their effects by either blocking the

catalytic site of cGAS (e.g., RU.521, Compound S3, G150, PF-06928125) or by interfering with cGAS binding to DNA (e.g., suramin, oligonucleotides, antimalarials) (70). Antagonists of STING have been developed that exert their effects by targeting the cGAMP binding site of STING (e.g., Astin C, tetrahydroisoquinolones) or by targeting STING palmitoylation which is necessary for its activation [e.g., indole ureas (especially H-151), nitrofurans, acrylamides, and nitro fatty acids] (70). Given the wealth of preclinical data attesting to the importance of cGAS-STING in the pathogenesis of cardiac disease, it will be interesting to see if any of these medicinal chemistry breakthroughs reach the clinical trial arena for this indication.

Future strategies to modulate signaling by IFNs and IFN-related pathways

Looking ahead, several different strategies are being trialed to either augment or antagonize cytokine action. The reader is directed to an excellent recent review on this topic (172). Briefly, various approaches are being developed to augment cytokine activity including the use of fusion proteins, PEGylation, polymeric matrices, microparticles, immune complexes and immunocytokines, orthogonal cytokines, mutagenesis, neokines, and surrogate agonists (172). Strategies to antagonize cytokine signaling that are under development include antibodies to cytokines or their receptors, JAK inhibitors, STAT inhibitors, proteolysis-targeting chimeras (PROTACs) and cytokine receptor targeting chimeras (or KineTACs) (172).

Summary and future directions

In this review, we have considered the actions of type I IFNs and ISGs, cGAS-STING pathway activation, IFN- γ , and IRFs in the heart. From the body of experimental evidence, it is clear that each of these biological processes plays important roles in either protection against heart disease or in the pathogenesis of diseases of the heart. How might it be possible to exploit these discoveries for the benefit of patients?

It is unlikely that measurement of plasma IFNs, certainly single IFNs, will offer prognostic value in the management of heart disease. Alternatively though, within the biomedical research space, recent years have witnessed enormous advances in single cell technologies. These single cell technologies, and in particular scRNA-seq, are especially helpful in defining immune cell subpopulations (173), and identification of immune cell subpopulations that are enriched for IFN-related genes has been important to several recent studies elucidating the molecular pathological basis of cardiac diseases (37, 102, 174–180). Fundamental studies exploiting single cell technologies and defining immune cell subpopulations based on IFN-related gene enrichment are likely to continue to advance our understanding of the role of inflammation in heart disease in years ahead.

Therapeutically, various strategies may be employed to dampen signaling by IFNs or IFN-related pathway signaling. However, immunosuppressive therapies may be associated with an increased risk of malignancy and infection. For prevalent diseases

such as heart disease, where therapies that improve outcomes already exist, the challenge will be the development of a therapeutic strategy that has an acceptable side effect profile. In this respect, the therapeutic targeting of a downstream pathway, such as cGAS-STING signaling, may offer theoretical advantages in that it leaves other immune defense pathways intact (70). Alternatively, a niche for therapies that antagonize IFN-related pathways may be found in the acute setting. Elsewhere, there has been renewed interest in the complex pathobiology of ISGs, and especially ISG15, since the emergence of SARS-CoV-2, and the recognition that ISG15 plays a key role in the defence against viral infection and in the immune response to viral infection (65). This renewed interest could pave the way for therapeutic advances for chronic diseases, such as heart disease, in the future. The biology of ISG15 is complex, the protein exerting different effects in its intracellular free or conjugated forms, and as a secreted protein, with species-specific differences between mice and humans (181). Similarly, it appears that IFN- γ can exert both beneficial and detrimental effects on the heart. However, just because the biology may be complicated, does not mean that it is not important.

In summary, IFNs and IFN-related pathways play important roles in the inflammation that commonly accompanies diseases of the heart. These roles are complex, being dependent on the nature of the underlying cardiac insult, the pathway itself, stage of disease, and host factors. As technological breakthroughs continue to advance the study of fundamental biology, a more nuanced understanding of the actions of IFNs and IFN-related pathway is sure to follow. Whether these advances will ultimately lead to improved outcomes for those affected by heart disease awaits to be seen.

Author contributions

DT: Writing – original draft, Writing – review & editing. SB: Writing – original draft, Writing – review & editing. AA: Writing – original draft, Writing – review & editing.

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