Chapter 4

IFN-γ versus IL-17: A Battle During Cardiac Autoimmunity Evolution

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Abstract

Cardiovascular diseases are the leading global cause of death. Cardiomyopathies are the most prevalent forms of heart failure diseases currently. They may have genetic or environmental etiology, and the development of an autoimmune process is essential for the progression of the disease. During an autoimmune response, there is the breakdown of self-tolerance and generation of a T-lymphocytes-mediated cellular autoimmune response and B-lymphocytes-mediated humoral autoimmune response. Lymphocytes perpetuate the autoimmune response throughout the release of cytokines, expansion of autoreactive clones, and attenuation of regulatory mechanisms. Increasing evidences indicate that interferon (IFN)-γ and interleukin (IL)-17 participate during autoimmune disorders development. The use of autoimmune cardiomyopathy models revealed antagonistic functions for both cytokines during the evolution of autoimmune cardiomyopathy: while enhanced IFN-γ levels are associated to a lower disease severity, the levels of IL-17 are inversely correlated to a favorable prognosis. More precisely, recent findings indicate that the IFN-γ/IL-17 ratio in combination with other cytokine levels dictates heart's autoimmunity development and dilatation. In this chapter, we discuss the role played by the autoimmune response in the development of cardiomyopathy. We also discuss some immune mechanisms focused on IFN-γ and IL-17's ability to induce and perpetuate cardiac autoimmunity.

Keywords: autoimmunity, cardiomyopathy, IL-17, IFN-γ and immunological response

1. Introduction

Cardiovascular diseases are responsible for over 17 million deaths per year worldwide, representing the leading cause of deaths globally (WHO 2015—http://www.who.int). Among the main disorders that directly affect the heart and/or circulatory system, there are the coronary heart diseases, cerebrovascular accident, hypertension, peripheral arterial diseases, congenital heart diseases, and heart failure [1]. Currently, cardiomyopathies are the most prevalent form of heart failure [2].

About 30% of cardiomyopathies have genetic origins, most of them are autosomal dominant, but there are also cases of X-linked-recessive inheritance and even mitochondrial DNA mutations. In 2015, more than 110 nuclear and 24 mitochondrial genes were correlated with cardiomyopathies [3–5]. Cardiomyopathy patients showed enhanced expression of the mutated genes *TTN* (titin, 27%), *LLMNA* (laminin A/C, 6%), *MYBPC* (myosin-binding protein C, 3%), *TNNT2* (cardiac troponin C, 3%), *MYH6* (myosin heavy chain 6, 3%), and *SCN5A* (sodium channel voltage-dependent-α5, 3%) [6–8]. In addition to the classic cases of sarcomeric protein mutations, an association between single nucleotide polymorphisms (SNPs) and predisposition to cardiomyopathies in some specific populations has also been reported. These SNPs were mainly observed in genes related to the immune response, such as CTLA-4 (cytotoxic T-lymphocyte antigen-4), IL-6 (interleukin-6), TNF-α (tumor necrosis factor alpha), and HLA (human leukocyte antigen) [9, 10].

Cardiomyopathy can also be induced by excessive alcohol consumption, poisoning by heavy metals or medications (e.g., doxorubicin), metabolic abnormalities, and microbial infections (**Figure 1**) [11]. Among the non-infectious etiologies, alcohol consumption is probably the main cause of cardiomyopathy in the Occidental world [10, 12, 13]. Viral infections are the most common form of microbial-mediated cardiomyopathy in Europe and North America [14]. Analysis of patient biopsies revealed that coxsackie B3 virus infection is the leading cause of cardiomyopathy, followed by parvovirus B19, enterovirus, adenovirus, human herpes virus 6, and HIV infection [15, 16].

In Latin America, Chagas′ cardiomyopathy is one of the most common forms of morbidity and mortality in *Trypanosoma cruzi*-infected people, now estimated in the order of 5.7 million people. Approximately one-third of these patients will develop a dilated chronic form of heart failure associated to a worst clinical prognosis [16, 17]

During the development of cardiomyopathy, these genetic or environmental conditions are considered initiators that cause local damage. The disease progression will activate immune response mechanisms, which may lead to the clearance of the infectious agent and/or the defective cardiomyocytes. However, this initial trigger can also activate an immune status that modulates disease progression to a chronic state of cardiomyopathy **(Figure 1)**. The literature suggests that the development of autoimmune processes is the key element in the progression of cardiomyopathy regardless of its etiological origin [11, 18]. Nonetheless, the specific role of the complex immune system response in the induction of cardiac autoimmunity and perpetuation of cardiomyopathy is poorly understood. Recent findings that shed some light in the immune mechanisms of cardiomyopathy induction will be described below.

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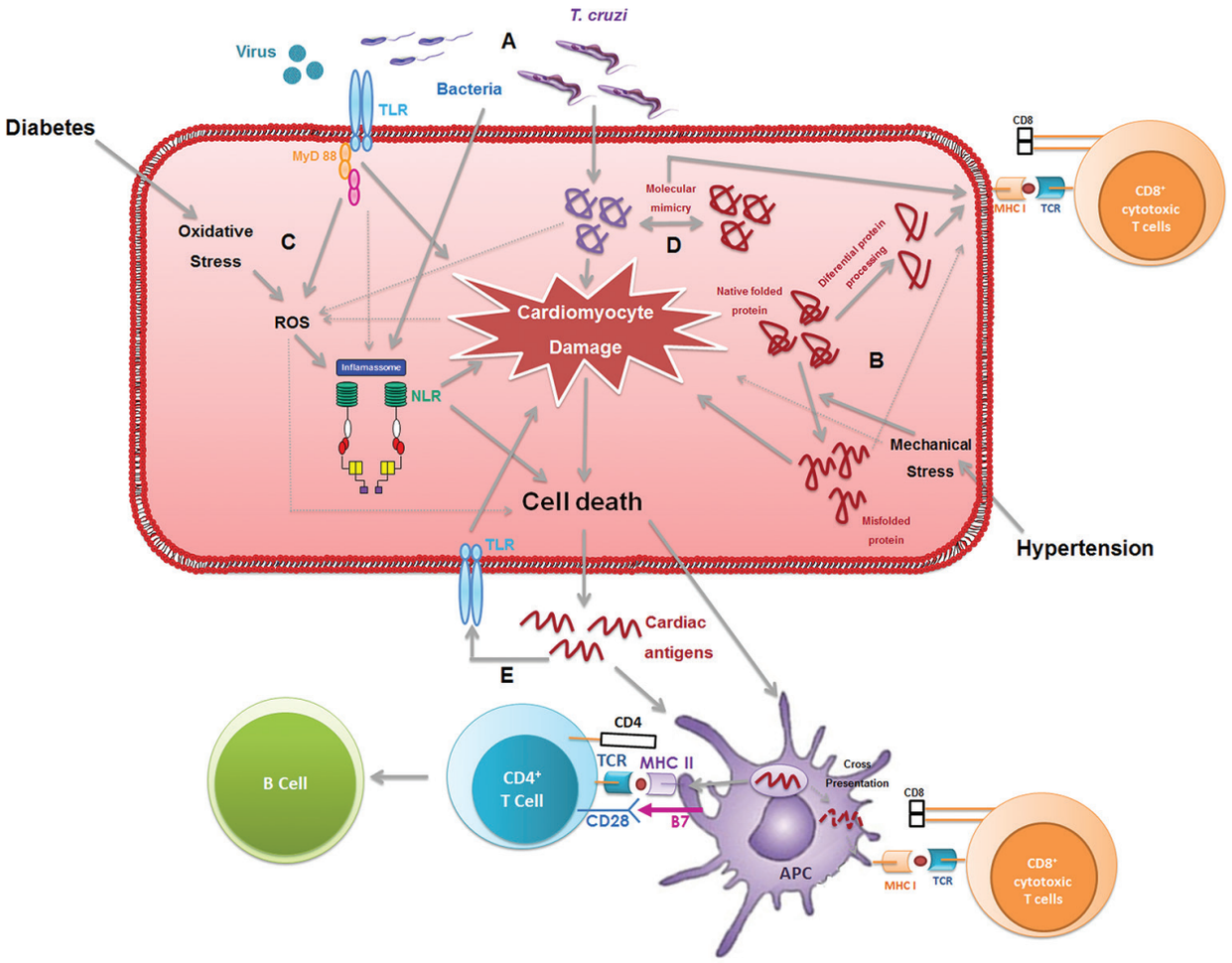

Figure 1. Proposed mechanisms in the development of cardiac autoimmunity. Infection caused by virus, bacteria, protozoa, and other stimuli (A) are recognized by receptors of the innate response, such as TLR and NLRs, leading to cardiomyocytes damage and death. During this process, the assembly of a multiprotein complex known as inflammasome may occur, responsible for secretion of cytokines and the amplification of an autoimmunity cascade. Also, diseases as diabetes and hypertension may activate oxidative and mechanical stress responses, respectively (B and C). These can lead to a redox imbalance and a change in protein processing, amplifying the heart damage. Proteins from infectious agents may exhibit similarity with host proteins, a process known as molecular mimicry (D). All these processes jointly or separately will provoke the exposure of cardiac antigens via MHC I by cardiomyocytes or via MHC II by antigen-presenting cells (APCs) after cardiac antigens or apoptotic cardiomyocytes phagocytosis. These processes will stimulate B-cells, $CD4^+$ and $CD8^+$ T-cells to mount humoral and cellular autoimmune responses. Besides, cardiac antigens can stimulate additional damages via TLR activation (E), as positive feedback. Gray continuous arrows represent mechanisms described in this review while gray dashed arrows not.

2. Development of cardiac autoimmunity

Cardiomyocyte cellular damage may be induced by infection with pathogens, endogenous stress from mechanical or oxidative traumas, or from mutated proteins (**Figure 1A–C**) [19–22]. These insults promote activation of the innate immune response through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs). PRRs are expressed in immune cells, such as macrophages, dendritic cells and lymphocytes, cardiac fibroblasts, and cardiomyocytes (**Figure 1**) [23–26]. Activation of

heart receptors such as TLR2, TLR4, and TLR5 induces the expression of IL-6, MIP-2 (macrophage inflammatory protein 2), KC (CXCL 1 – homolog of human IL-8), and ICAM-1 (intercellular adhesion molecule-1), and is associated with a decrease in cardiomyocyte contractility [26, 27]. The absence of host immunity receptors or associated pathway components inhibits the development of cardiomyopathies, as observed using an autoimmune animal model in mice depleted for TLR-$7^{-/-}$, TLR-$9^{-/-}$, and $MyD88^{-/-}$ [28].

The listed factors may cause a chronic condition and lead to the activation of cell death mechanisms by apoptosis or necrosis [29–31]. Apoptotic cardiomyocytes are processed by antigen-presenting cells, such as dendritic cells and macrophages, resulting in immune system activation, myocarditis, and production of autoantibodies against heart protein, as anti-myosin antibodies [32, 33]. The death of cardiomyocytes releases autoantigens which are captured by dendritic cells and leads to a "self-aggressive" state through the activation of CD40 and TLR receptors. This dendritic cell-mediated activation mechanism is an important regulator of $CD4^+$ T-cell function [32, 34]. The endocytosed cardiac antigens will be processed and coupled to a molecule of major histocompatibility complex class II (MHC II) and presented via T-cell receptor (TCR) to a $CD4^+$ T-cell **(Figure 1)**. In addition to this initial interaction, the correct activation of the $CD4^+$ T-cell requires a second positive signal that is stimulated by costimulatory molecules such as CD28 and B7 (CD80 and CD86). However, inhibitory signals, such as mediated by CTLA4 molecules, are capable of competing with CD28 for binding to B7, reducing T-cell expansion and production of cytokines [35]. T-cells that do not express the CTLA4-B7-inhibitory signal exhibit an unregulated proliferation of lymphocytes in the heart, which can lead to a severe damage of myocardium, and the development of cardiomyopathies [36, 37]. Cardiomyopathy patients present higher levels of CTLA4 SNP (+49A>G; Thr17Ala), which lead to a loss of function of CTLA4, than healthy subjects (14.7% vs. 7.4%, $p = 0.005$) [38]. Therefore, the correct activation of $CD4^+$ T-cells in cardiac autoimmunity is mediated by (1) antigen-presenting cells that phagocytosed death cardiomyocytes or cardiac autoantigens and (2) a second co-stimulatory signal mediated by B7 **(Figure 1)**. In addition to antigen-presenting cells, other cell types of non-hematopoietic lineage can also present antigens to $CD4^+$ T-cells via MHC II under inflammatory stimuli [39]. It has been shown in patients and in rodent models that the MHC II expression by non-hematopoietic cells, in particular endothelial cells, contributes to the development of cardiomyopathy [40, 41]. Mice developed lower cardiac commitment when they did not express MHC II in endothelial cells [41]. Finally, properly activated $CD4^+$ T-cells are able to activate B-lymphocytes to produce and secrete antibodies against cardiac antigens. The role of B-cells during the development of heart disease has been extensively studied [42]. The absence of programmed cell death protein-1 (PD-1), a key factor in the differentiation of B cells, can lead to the development of a severe form of dilated cardiomyopathy, with high levels of IgG that specifically binds to cardiomyocytes and induce apoptosis [43, 44]. Depletion of these B-cells recovers the heart failure phenotype in mice [42]. The production and release of autoantibodies is dependent of B-cell differentiation to plasma cells. During this process, there is a decrease in CD19 expression and maintenance of high levels of CD138 and transcription factor Blimp-1 [45]. The expression of these two factors is dependent of high levels of IL-17 and activation of autophagy. After this differentiation, it is possible to observe high titers of anti-myosin antibodies in BALB/c mice

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immunized with α-myosin heavy chain peptide (α-Myhc), particularly IgG2a and IgG2b subtypes, and the development of some cardiac impairment characteristics, such as the increase in the ratio of heart and body weight [46]. These antibodies can recognize and bind to specific cardiac antigens and deposited into the myocardium [47]. A wide range of evidence suggests that these autoantibodies directly affect cardiac function and physiology [48].

In addition to the response mediated by $CD4^+$ T- and B-cells, $CD8^+$ T-cytotoxic lymphocytes may be activated through the recognition of conjugated epitopes on MHC I. Damage induced by pathogen infection or cell stress mechanisms can alter intracellular protein processing resulting in misfolding, which will expose it to MHC I molecules. This will expose self-epitopes to autoreactive $CD8^+$ T-cells (**Figure 1A**, **B** and **D**) [13]. Also, molecular mimicry may occur between pathogens and heart proteins (**Figure 1B** and **D**) [49]. This latter mechanism is well described in Chagas' patients where antibodies against the B13 *T. cruzi* protein can also recognize cardiac α-Myhc (**Figure 1D**) [50]. This step via MHC I will promote the release of cytotoxic agents, such as perforin and granzyme B by $CD8^+$ T-cells that could promote cardiomyocytes apoptosis and can amplify the release of cardiac antigens [51]. Throughout the modulation of immune system, lymphocytes will release cytokines that will expand autoreactive clones propagating the autoimmune response [11]. The autoimmune process formed by humoral and cellular responses can amplify the cardiac damage through the secretion of autoantibodies, cytokines, and other immune factors, despite initial stimuli.

Analysis of cardiomyopathy patients revealed the presence of autoantibodies against self-myocardial protein in up to 80% of the patients [52], indicating that autoimmunity is a central element for cardiomyopathy development. Evidence indicates that these autoantibodies affect the heart rather than other organs. Serum or IgG purified from these patients can induce negative inotropic effects on the heart of chicken embryos [53] and decrease heart contraction, the calcium transport [54, 55], and the diastolic relaxation in mice [48, 56]. The transference of IgG purified from patients serum with cardiac dysfunction to healthy mice induced significant necrosis in cardiomyocytes and mediated inflammatory effects with the aid of immune cells [57]. The characterization of these antibodies started in the 1980s and continues until today. Some of these antigens are listed in **Table 1** [58–69]. Most of produced autoantibodies directly recognize one specific cardiac antigen. But it has been demonstrated in rats that anti-myosin antibodies are capable to recognize the β1-adrenergic receptor and promotes their activation [70].

As briefly discussed above, the mechanisms that trigger the development of autoimmune cardiomyopathy are orchestrated by humoral and cellular responses. Immune cells such as granulocytes, monocytes, T-cells, B-cells, and mast cells infiltrate into the heart and promote the secretion of the cytokines, IL-17, -6, -1, -10, -12, IFN-γ, TGF-β, TNF-α, and chemokines that will generate an amplification loop, recruiting new inflammatory cells to the heart [71–77]. However, the precise elucidation of this complex immune response mechanism remains unclear due to the difficulty to determine the precise order that immune cells infiltrate the heart [78–80]. Once activated, this cellular response will undergo both beneficial and harmful effects as the disease progresses.

Data obtained in experimental autoimmune cardiomyopathy models using immunization with α-Myhc showed a predominance of $CD4^+$ T-cell response [41, 77, 78, 81]. The transfer

Class of cardiac protein	Protein	First citation
G protein–coupled receptors	β1 adrenergic receptor	Limas et al., [58]
	2 muscarinic receptor	Fu et al., [59]
Mitochondrial	M7 antigen	Klein et al. [60]
	Adenine nucleotide translocase (ANT)	Schultheiss et al. [61]
Structural	α-myosin heavy chain (MyHC)	Neu et al. [62]
	Troponin I	Okazaki et al. [63]
	Laminin	Wolff et al. [64]
	Myosin-binding protein-C	Müller et al. [65]
	Dystrophin	Müller et al. [65]
Others	Na-K ATPase	Baba et al. [66]
	Hsp-60	Latif et al. [67]
	Proteasome 20 S	Voigt et al. [68]
	Calreticulin	Sánchez et al. [69]
	RNA-binding protein 20	Müller et al. [65]

Table 1. Cardiac antigens characterized in autoimmune cardiomyopathies.

of $CD4^+$ T-cells from mouse spleen that produced anti-myosin antibodies and developed cardiomyopathy mimics the disease in severe combined immunodeficiency (SCID) mice. On the contrary, $CD8^+$ T-cell transference did not induce changes [32, 82]. Moreover, the depletion of $CD4^+$ T-cells or treatment with anti-CD4 antibody prevents acute myocarditis with a decrease in the antibodies production and heart size [18, 83], confirming a prominent $CD4^+$ T-cell role in this autoimmune cardiomyopathy model.

$CD4^+$ T-lymphocytes can be biased to different profiles: Th1, Th2, Th17, Th9, Th22, follicular T (Tfh cells), and induced regulatory T (Treg cells) cells, each of which has a specialized function and is adapted to suppress a specific class of injuries or counteract the excessive activation of the immune system [84]. For autoimmune cardiomyopathy, the involvement of Th1, Th2, and Th17 cells has been characterized. The most relevant studies in this area are focused on Th1 and Th17 responses and their respective cytokines [32, 71–73, 76, 85–88].

3. IL-17 *versus* IFN-γ

Early studies using immunohistochemical assays identified the presence of cells producing mainly TNF-α and IL-1 in heart [89, 90]. In the early 2000 era, novel cytokines were identified

in cardiomyopathic animal as IL-2 and IL-1-β [91]. In 2006, the first evidence of the IL-17 participation in autoimmune cardiomyopathy [76] was described.

In the last decade, Th17 cells have been extensively characterized in various autoimmune diseases [92, 93], including autoimmune cardiomyopathy [3, 80, 94]. Th17 $CD4^+$ T-cells have been named after the discovery of their classical cytokine, IL-17A, but they also produce other effector cytokines including IL-17F, IL-22, and granulocyte macrophage-colony-stimulating factor (GM-CSF) [95]. Additionally, IL-17 may also be secreted by other cell types as Th17 $CD8^+$ T-cells (Tc17), γδ T-cells (mainly in the skin and intestine), mucosal-associated invariant T-cells (MAIT), among other resident T-cells in different tissues [96, 97]. The polarization of $CD4^+$ T-cells to Th17 profile initially requires the presence of TGF-β. This cytokine induces the expression of ROR-γt (RAR-related orphan receptor-γt) and FoxP3 (Forkhead box P3) transcriptional factors [98]. The co-expression of these two factors allows the physical connection between FoxP3 and ROR-γt inhibiting their differentiation to Th17. In the presence of IL-6, STAT3 is activated and interrupts the inhibition induced by FoxP3, resulting in the expression of IL-23 receptor and initiating the differentiation to Th17. Nevertheless, in the absence of IL-6, the inhibition of ROR-γt induced by FoxP3 will favor the development and expansion of Treg cells [99]. Beyond IL-6, IL-1-β is also capable of inhibiting FoxP3, generating an amplification loop [100, 101]. This cytokine polarization is very well characterized to $CD4^+$ T-cells, but also appear to be responsible for differentiation of $CD8^+$ T-cells to Tc17 profile [102, 103]. Recently, it was demonstrated that the differentiation of $CD8^+$ T for IL-17 producing $CD8^+$ T-cells is also dependent on the inhibition of Blimp-1 and T-bet [104]. Today, it is well accepted that the release of cytokines required for this process of differentiation, such as IL-6, IL-12, TNF-α, and IL-23 by antigen-presenting cells, such as dendritic cells and $CD14^+$ monocyte, is induced by the recognition of cardiac autoantigens by TLR and by the presence of GM-CSF [105, 106]. As described, IL-6 and IL-23 will induce the differentiation of T helper (Th) cells to Th17 profile, where the release of IL-17 and more GM-CSF occurs, forming a positive feedback [88, 94].

Several works published in the last 10 years, using animal models and patients with cardiomyopathy, tried to establish IL-17 as a cytokine inducer of autoimmune cardiomyopathy. IL-17 was described as an important factor responsible for cardiac remodeling, fibrosis, and many other effects in the heart [72, 78, 86, 88]. An increase in IL-17 and IFN-γ transcriptional levels in mice that develop experimental autoimmune myocarditis (EAM) induced by subcutaneous inoculation with α-Myhc was observed. In this case, the copy number of IL-17 mRNA was about 20–30 times higher than those to IFN-γ [107].

It has been shown that the presence of IL-17 increases the expression of MMP-1 (matrix metalloproteinase-1), promoting the migration of cardiac fibroblasts *in vitro* and cardiac remodeling *in vivo* [108]. Furthermore, it was shown that Th17 cells and IL-17 were involved in survival, proliferation, and differentiation of B-cells [109]. In this direction, sera of patients with dilated cardiomyopathy showed an increase in IL-17 levels and in the frequency of Th17 cells when compared to health donors [109]. IL-17 neutralization or depletion slowed the development of autoimmune response and reduced the generation of cardiac autoantibodies in EAM myosin model [76, 110]. Also, mice treated with anti-IL-6, which were not capable of promoting the polarization of $CD4^+$ T-cells to Th17 profile, do not develop autoimmune cardiomyopathy [111].

But these facts seem to be true only in the acute phase of the disease. After the establishment of a chronic condition, patients with cardiomyopathy presented lower levels of IL-17 and Th17 cells subtype [112]. So, high IL-17 levels are essential in acute phase of cardiomyopathy and for the progression to the final stage of the disease. But when the cardiomyopathy reaches this final stage, where heart dilatation is found, IL-17 seems not necessary [86, 87]. And even more, the reduction of IL-17 levels after the establishment of cardiac damage did not appear to be beneficial. In fact, mice infected with *T. cruzi* presenting typical functional cardiomyopathy changes, when treated with anti-IL17, developed an acute exacerbation of inflammation and cardiac dysfunction [113]. The absence of IL-17 receptor on infected mice also leads to the development of a fatal cardiomyopathy [114]. Additionally, it was demonstrated that individuals infected with *T. cruzi* who developed severe cardiac dysfunction had lower levels of IL-17 when compared with infected patients presenting moderate symptoms [115]. Recently, a study analyzed IL-17 levels in blood sample of 41 patients with dilated cardiomyopathy, without differentiating the etiology of the disease. They observed an increase in IL-17 levels up to 6 months after the diagnosis, but after 1 year of monitoring, IL-17 levels reduced close to those found in healthy patients, even in the presence of high levels of IL-6 and TGF-β [94]. This association between reduction in IL-17 levels and worse prognosis has also been found in patients who suffered acute myocardial infarction [116]. Finally, our research group recently showed that this relationship between IL-17 late decrease and worse symptoms occurred in heart disease of autoimmune origin. Mice that produce anti-M_2AChR antibodies induced by gene immunization showed dilated cardiomyopathy and an increase in IL-17 production in the heart at 20-week postimmunization; however, with the progression of the disease to a final dilated stage, about 40 weeks after immunization, the IL-17 levels become comparable to the levels produced by the respective control animals [117]. The literature did not present explanations about the mechanisms involved in decreasing IL-17 levels. But IL-17 reduction, after the achievement of the disease, seems to be more harmful than beneficial to the development of cardiomyopathy. Thus, it is crucial to emphasize that the use of anti-IL-17 therapies for heart disease and other autoimmune diseases needs to be employed in a precise time to avoid harmful effects in the patients.

The immune response via Th1 cells and their cytokine marker IFN-γ is also largely related to autoimmune cardiomyopathy. During the innate immune response, IFN-γ is produced by natural killer cells and natural killer T-cells [118], as well as macrophages and dendritic cells [119]. In adaptive immunity, IFN-γ is mainly produced by $CD8^+$ T-cells and Th1 $CD4^+$ T-cells [120, 121]. After TCR stimulation, $CD8^+$ T-cells produce higher levels of IFN-γ than $CD4^+$ T-cells [122]. This is possible due to the interaction between TCR-MHC interaction that occurs between the $CD8^+$ T-cells and MHC I-expressing antigen-presenting cells is sufficient to induce the secretion of IFN-γ and differentiation to a cytotoxic profile, whereas $CD4^+$ T-cells need TCR recognition and a series of other stimuli [120]. IFN-γ also contributes to the switch process of IgG subclass in B-cells to a more pathogenic profile (IgG2a and IgG3 subclasses), activation of the complement system, inflammation, and tissue damage [123, 124]. But the IFN-γ expression is not static and confined to these classic subtype cells described above. Th17 cells can also produce IFN-γ concomitantly with IL-17 and even can become an exclusive IFN-γ producer [125, 126].

IFN-γ is an indicator of pathogenicity for autoimmune diseases [127], but its role in cardiomyopathies is still controversial [128] and it appears to be more protective than inducer of disease [76, 85, 129–131]. For instance, mice treated with anti-IFN-γ antibodies or genetic deleted for T-bet, IFN-γ, or IFN-γR presented an exacerbated inflammatory infiltrates and increased heart size and its cavities [71–73, 76, 85, 130]. One of the possible protective mechanisms mediated by IFN-γ involves the inhibition of autoreactive T-cell proliferation through the induction of nitric oxide synthase 2 enzyme (NOS2) and nitric oxide (NO) release [77, 132]. Although today this mechanism seems simple, its elucidation was very contradictory and troubled for some years. Initially, IL12Rβ1, one of the IL-12 receptor subunits, knockout mice were used for the study of participation of the IFN-γ in autoimmune cardiomyopathy. The inhibition of this classically Th1-polarizing pathway decreased the development of autoimmune cardiomyopathy in knockout mice, indicating the pathogenicity of Th1 cells [85]. However, as already mentioned, the IFN-$\gamma^{-/-}$ and IFN-$\gamma R^{-/-}$ mice showed an exaggerated and lethal disease [72, 73], an apparent contradictory result. This impasse was resolved when it was shown that β1 subunit of the receptor for IL-12 was shared with the IL-23 receptor, inducer of Th17 response [110]. Despite all this characterization of IFN-γ-protective role, it is unclear which mechanisms are activated during this process. It is known that high levels of IFN-γ induce the production of NO by NOS2 with consequently inhibition of $CD4^+$ T-cells autoreactive proliferation [77]. HL-1 cell line and primary cardiomyocytes treated with IFN-γ showed an activation of absent in melanoma 2 (AIM-2), an intracellular receptor of the PRRs family, which reduces IL-6, IP-10 (inducible protein 10, CXCL10), and TNF-α transcription in cardiomyocytes and limits inflammation in cardiomyocytes, but not in cardiac fibroblasts [133]. Also, high IFN-γ levels secreted by γδ cells could kill pathogenic $F4/80^+$ macrophages in heart and control cardiac damage [134]. There could be some explanation for how IFN-γ protects mice from the development of autoimmune cardiomyopathy.

Meanwhile, several other studies demonstrated the ability of high IFN-γ levels in inducing myocardial inflammation, interstitial fibrosis, apoptosis, wall thinning, systolic dysfunction, dilatation, and cardiomyopathy [128]. And more recently, it has been shown that IFN-γ has the capacity to induce cardiac damage in autoimmune cardiomyopathy model. High IFN-γ levels were associated with cardiorespiratory commitment, electrical abnormalities, and cardiac dilatation. This situation was more prominent in the absence of purinergic receptor P2X7 [117]. These evidences show that it is not possible to withdraw the IFN-γ participation as a cardiomyopathy inducer.

4. Immune cells' function on autoimmune cardiomyopathy

The entire description and discussion made so far focused mainly on the presence and polarization of $CD4^+$ T-cells; however, as already pointed out, the presence and participation of other immune cells can be decisive in disease severity. In genetically susceptible mouse model, preferably BALB/c, immunization with the α-Myhc in the presence of a strong adjuvant, like complete Freund's adjuvant (CFA), the disease is mediated almost exclusively by $CD4^+$ T-cells. However, it has been recently identified in EAM-induced A/J mice that the

α-Myhc$_{338\text{-}348}$ epitope was the immune dominant for CD8$^+$ response [135]. In this case, they showed antibodies production, cardiomyopathy development, and the presence of inflammatory infiltrate composed of 35% of CD8$^+$ T-cells [135]. As previously mentioned, this infiltration of CD8$^+$ T-cells has a high cytotoxic role as a source of IFN-γ, perforin, and granzyme that will induce irreversible damage to cardiomyocytes [51].

Despite the great importance of T-cells, it is believed that the major cells infiltrating the hearts during the development of cardiomyopathy are monocytes, especially CD11b$^+$ [77]. These cells can differentiate into different profiles ranging from dendritic cells, macrophages, and fibroblasts depending on the immune environment (including cytokines, chemokines, and growth factors) present in the heart [77, 132, 136]. The more severe cardiomyopathy is found when there is the presence of eosinophils in the heart infiltration [131]. It is believed that NK cells control the exacerbated proliferation of eosinophils in the heart through direct induction of apoptosis [137]. The recruitment of eosinophils to the heart can also be controlled by cardiac fibroblasts and F4/80$^+$ macrophages through the release of CCL11 and CCL24 (eotaxin-1 and eotaxin-2), respectively [138].

In the healthy heart, it is possible to find a population of resident macrophages, but the number of these cells can be expanded after the infiltration of new macrophages under some stimulus such as initiators of the autoimmune cardiomyopathy, cited at the beginning of this review (Figure 1). Macrophage infiltration is a well-known step, but it is little studied in cardiomyopathy [139]. These cells can differentiate into various profiles depending on the cytokine present in the medium. Some of these profiles are classically activated, pro-inflammatory M1 macrophages and alternatively activated, anti-inflammatory M2-polarized macrophages, tumor-associated macrophages (TAM), "immature" monocyte-like (GR1/Ly6C$^+$) or "mature" neutrophil-like (GR1/Ly6G$^+$), and suppressor cells derived from myeloid precursor (MDSCs) [79]. The functional properties and secretory profile of macrophages likely promote myocardial health or disease. In some cases, their influence on acute inflammation and chronic fibrosis is well described, and in others, their cardioprotective function seems to be almost indisputable, being proposed as a good source of treatment [140–142]. In models of cardiac autoimmunity, there is a predominance of M2 macrophages, around 70%, which promoted the resolution of the disease in heart tissue after damage. And with the presence of M1 macrophages, there is the expansion of Th17 cells and cardiac dilation [79, 143–145]. Therefore, further studies about the importance and function of macrophages in cardiomyopathies, in particular autoimmune ones, are needed.

5. Conclusion

The findings described in this chapter demonstrate the existence of a precise balance of the immune response, where a complex network of factors creates the conditions for the progression of autoimmune cardiomyopathy and dictates its severity. The combination of present and future knowledge on this line of study can ultimately guide to a possible effective and non-general treatment. However, two factors must be taken into consideration: (1) the correct

association between anti-humoral and anti-cytokine therapies and (2) the period where the treatment must be applied.

Therefore, the participation of IFN-γ and IL-17 in the autoimmunity development recalls us a dance instead of an arms race, where a fine temporal and quantitative control of these cytokines can determine the cardiomyopathy evolution.

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