FOCUS SEMINAR: PERICARDIAL AND MYOCARDIAL DISEASE

STATE-OF-THE-ART REVIEW

The Quest for New Approaches in Myocarditis and Inflammatory Cardiomyopathy

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ABSTRACT

Myocarditis is a diverse group of heart-specific immune processes classified by clinical and histopathological manifestations. Up to 40% of dilated cardiomyopathy is associated with inflammation or viral infection. Recent experimental studies revealed complex regulatory roles for several microribonucleic acids and T-cell and macrophage subtypes. Although the prevalence of myocarditis remained stable between 1990 and 2013 at about 22 per 100,000 people, overall mortality from cardiomyopathy and myocarditis has decreased since 2005. The diagnostic and prognostic value of cardiac magnetic resonance has increased with new, higher-sensitivity sequences. Positron emission tomography has emerged as a useful tool for diagnosis of cardiac sarcoidosis. The sensitivity of endomyocardial biopsy may be increased, especially in suspected sarcoidosis, by the use of electrogram guidance to target regions of abnormal signal. Investigational treatments on the basis of mechanistic advances are entering clinical trials. Revised management recommendations regarding athletic participation after acute myocarditis have heightened the importance of early diagnosis. (J Am Coll Cardiol 2016;68:2348–64) © 2016 by the American College of Cardiology Foundation.

Myocarditis refers to multiple heart-specific inflammatory conditions with a spectrum of clinical and histopathological manifestations [1]. In the late 19th century, the term *myocarditis* included myocardial infarction and chronic ischemic heart disease. The term has been refined, and presently applies to acute or chronic inflammatory responses of the heart to environmental or endogenous triggers, most commonly viruses, and less frequently bacteria, fungi, and parasites. Important noninfectious causes include giant cell myocarditis (GCM), drug-induced hypersensitivity, and cardiac manifestations of systemic autoimmunity, such as sarcoidosis or systemic lupus erythematosus [2,3]. This paper seeks to frame recent clinical advances in the management of myocarditis within the latest understanding of pathogenesis from experimental myocarditis models.

The incidence of myocarditis, as ascertained by International Classification of Diseases, 9th Revision diagnoses, was 22 per 100,000 people or approximately 1.5 million cases in the 2013 world population [4]. The burden of myocarditis as a percentage of prevalent heart failure varies by age and region from approximately 0.5% to 4.0% [5]. In 2015, there were approximately 354,000 deaths from myocarditis and cardiomyopathy, with a death rate of 4.8 per 100,000 people (**Figure 1**). Myocarditis is responsible for sudden cardiovascular death in approximately 2% of infant, 5% of childhood, and 5% to 12% of young athlete sudden death [6]. The overall rate of myocarditis was 3% (6 of 200) in autopsies of patients experiencing sudden death in Japan [7].

Specific forms of myocarditis occur less frequently. Between October 1998 and December 2014,

histologically verified cardiac sarcoidosis (CS) was diagnosed in 233 patients in Helsinki, Finland [8]. The annual detection rate of CS was 0.6 per 100,000 adults (>18 years of age) in the last full 2-year study period between 2013 and 2014. The prevalence of CS in 2012 was 2.2 per 100,000 people. Over the study period, the detection rate of CS increased more than 50-fold. From case series, CS affects 2.3% to 11% of patients with sarcoidosis and 2.5% of unexplained cardiomyopathy in a referral population (diagnosed by heart biopsy, 31 of 1,235). Asymptomatic sarcoid heart involvement is more common. In autopsy studies, myocardial involvement has been seen in 20% to 27% of consecutive sarcoidosis patients [9]. Also, other granulomatous systemic diseases may involve the heart in a subclinical way. In antinuclear cytoplasmic antibody-associated vasculitis, cardiac involvement is found in up to 65% of patients in remission, the majority caused by nonischemic injury of the heart [10].

GCM is a rare, rapidly progressive, and clinically important form of myocarditis [11]. The estimated annual detection rate for GCM in Finland between 2013 and 2014 was 0.13 per 100,000 people [12,13]. Over a 23-year period between 1991 and 2015, 55 cases of GCM were diagnosed. The detection rate increased 10-fold over this period. The cause for the increased rate of detection of CS and GCM in Finland is uncertain, and may be related to increased awareness of the diseases, increased referral to an advanced heart failure center, or an increased use of biventricular biopsies over the study timeframe. In autopsy registries, histological evidence of GCM was found in up to 6.6 per 100,000

people or 3% to 5.6% of all myocarditis autopsies [14,15]. In endomyocardial biopsy (EMB) studies of dilated cardiomyopathy (DCM) at a tertiary referral center, GCM was found in 0.2% to 1.2%, and 2% of cardiac transplant patients had GCM [16].
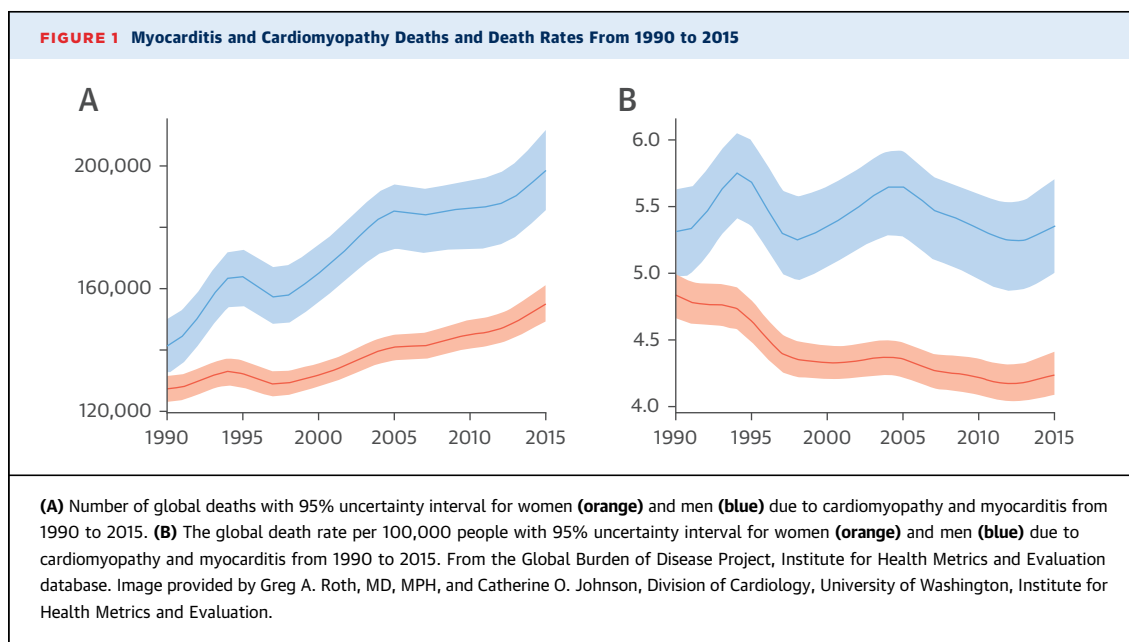
ETIOLOGY

The most commonly identified pathogens in acute myocarditis are viruses [17]. The immune response triggered by injury and autoantigen-specific mechanisms leads to organ dysfunction, pathological remodeling, and heart failure. From the 1950s to the 1990s, enteroviruses were the most frequently identified pathogen, particularly coxsackie virus in North America and Western Europe [18,19]. In the past 2 decades, polymerase chain reaction and in situ hybridization have identified a range of cardiotropic viruses, including H1N1 strains of influenza [20], adenovirus [21,22], hepatitis C [23,24], cytomegalovirus, echovirus, parvovirus B-19, and Epstein-Barr virus [25] in heart biopsy samples. In subjects with more chronic symptoms and "inflammatory cardiomyopathy," parvovirus B19 [26] and human herpes virus 6 genomes [27] predominate.

In select populations, specific nonviral infections and autoimmune syndromes remain important causes of myocarditis. Autoimmunity following untreated streptococcal infection results in rheumatic carditis [28]. Bacteria, such as diphtheria and *Borrelia burgdorferi* (Lyme disease), or parasites, such as

FIGURE 1 Myocarditis and Cardiomyopathy Deaths and Death Rates From 1990 to 2015

(A) Number of global deaths with 95% uncertainty interval for women **(orange)** and men **(blue)** due to cardiomyopathy and myocarditis from 1990 to 2015. **(B)** The global death rate per 100,000 people with 95% uncertainty interval for women **(orange)** and men **(blue)** due to cardiomyopathy and myocarditis from 1990 to 2015. From the Global Burden of Disease Project, Institute for Health Metrics and Evaluation database. Image provided by Greg A. Roth, MD, MPH, and Catherine O. Johnson, Division of Cardiology, University of Washington, Institute for Health Metrics and Evaluation.

Trypanosoma cruzi (Chagas disease), are important in specific regions (29,30). Hypersensitivity myocarditis may result from drug- or vaccine-related heart-specific autoimmunity. Methyldopa, hydrochlorothiazide, furosemide, ampicillin, tetracycline, azithromycin, aminophylline, phenytoin, benzodiazepines, tricyclic antidepressants, and tumor necrosis factor antagonists all have a low rate of myocarditis (31). Although most cases of hypersensitivity myocarditis develop early in the course of drug use, up to 15% of clozapine-induced myocarditis develops later, up to 2 years after initiation of drug therapy (32,33). Vaccination against smallpox infection is associated with myopericarditis in up to 6 per 10,000 vaccines (34). Other noninfectious causes of myocarditis include cardiac inflammation in the context of radiation or systemic autoimmune diseases, including antinuclear antibody-related vasculitis, systemic sclerosis, lupus erythematosus, and celiac disease (35). A total of 20% of GCM patients have a history of other autoimmune disorders. In GCM, the risk of transplantation and death has decreased since the introduction of prompt calcineurin inhibitor-based immunosuppression (12). Biopsy-documented GCM recurs up to 8 years after successful treatment in 12%, in both the native heart and in the allograft following transplantation (36).

PATHOGENESIS

Most of the current mechanistic understanding of human myocarditis derives from experiments in susceptible rodent strains. Inflammatory cellular infiltrates develop either after cardiomyocyte infection with a cardiovirulent virus strain (37,38), parasites, or bacteria, or after delivery of cardiac antigens with a strong adjuvant or carried within dendritic cells in noninfectious models (39-41). Models that do not rely on infection only provide insights to the latter phases of myocarditis.

The transition from acute viral infection through active inflammation to DCM can be conceptualized as a multiphase model (**Central Illustration**). The opportunities for therapeutic intervention vary by stage of disease. Depending on the inciting infective agent, the mechanisms of early tissue injury as well as the resulting patterns of inflammatory mediators and cytokines differ. In virus-mediated myocarditis, for example, viral entry into cardiomyocytes leads to the production of type 1 interferons within hours and to myocyte death through apoptosis and autophagy (42). It requires specific cell surface receptors, such as the coxsackie-adenovirus receptor and decay-accelerating factor (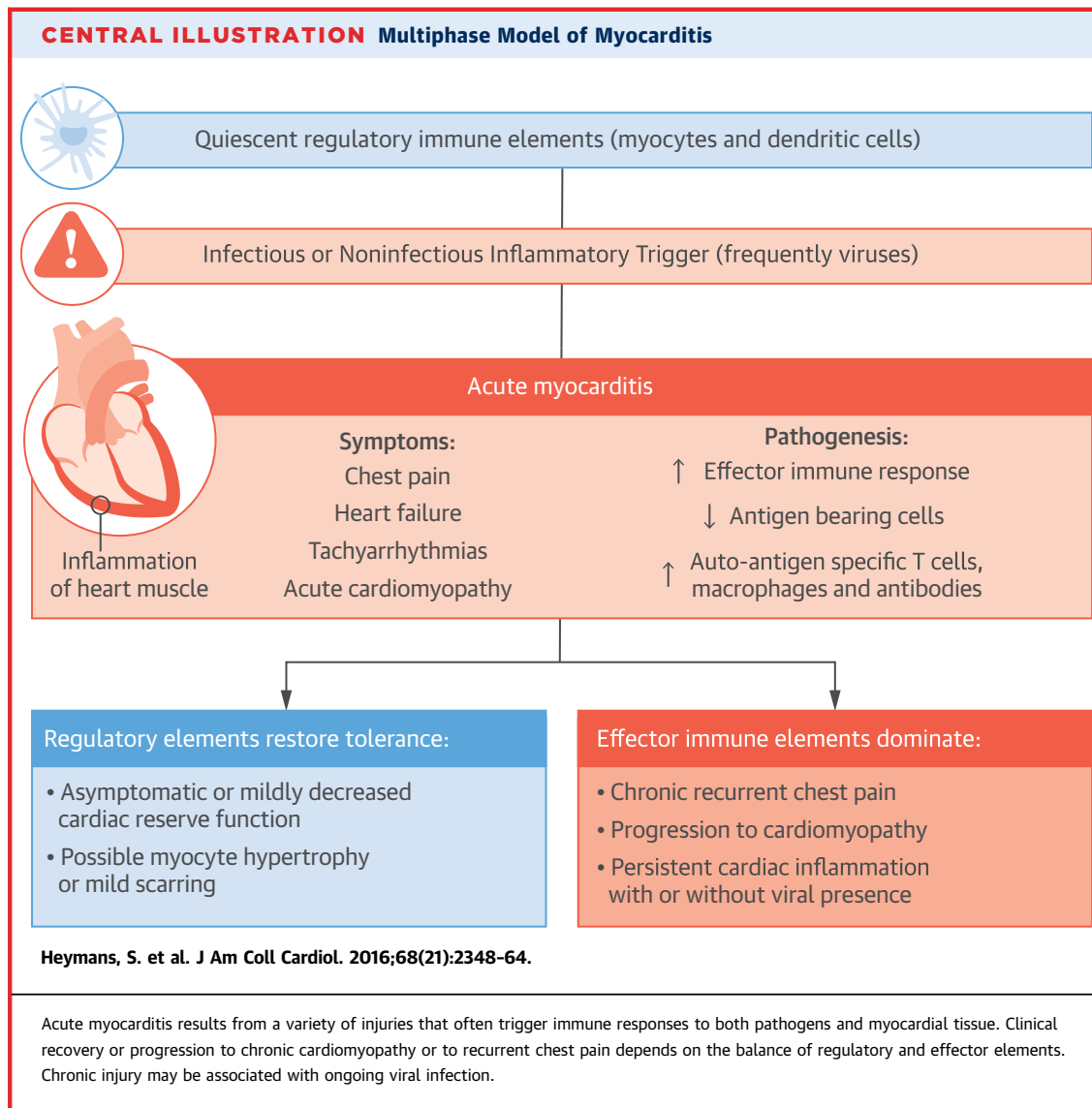CD55) for some coxsackie B virus strains (43,44). The activity of inflammatory signaling pathways in cardiac myocytes contributes to coxsackie B virulence via effects on viral replication (45,46). The result of the initial damage is presentation of normally sequestered cardiac protein fragments in the context of tissue damage. The pathogenesis of myocarditis varies by pathogen. For example, in Chagas myocarditis, tissue damage results not only from the associated invasion of inflammatory cells, but also directly, through parasites releasing bioactive compounds and promoting oxidative stress (47). Cardiac parasympathetic neurons are also damaged by the parasite, leading to unopposed sympathetic activation (48).

A second phase that evolves over hours to days involves activation of an innate immune response, consisting of nitric oxide, altered regulatory T-cell function, natural killer (NK) cells, release of mediators such as type I interferon in viral myocarditis (49-51), or granule components from eosinophils and polymorphonuclear cells in bacterial and parasitic myocarditis. The exodus of antigen-bearing cells to regional lymph nodes within the appropriate cytokine milieu triggers a third phase, characterized by cellular cardiac infiltrates with pathogen- and autoantigen-specific T cells, macrophages, and antibodies (52-54). Efficient clearance of the infective agent usually results in recovery of normal cardiac function. In genetically susceptible experimental animals and humans, a breakdown of T-cell tolerance to cardiac self-antigens may occur (37,55,56). This leads to a process of chronic autoantigen-driven inflammation, which can progress to DCM and end-stage heart failure (57-60). The significance of cardiac autoimmunity in disease progression depends on genetic susceptibility of the host, genetic variations of the inciting microbes, molecular mimicry between cardiac structures and microbial proteins, and environmental factors. Phases of autoimmune inflammation progression cannot be triggered only through infective agents, but also through noninfective microbial particles, heat shock proteins, or immunogenic fragments released after tissue injury, drug exposure, or in the context of systemic autoimmune diseases. In the transition from acute inflammation to chronic fibrosis, inflammation can persist in the absence of symptoms (61,62).

CELLULAR AND EXTRACELLULAR COMPARTMENTS OF CARDIAC INFLAMMATION

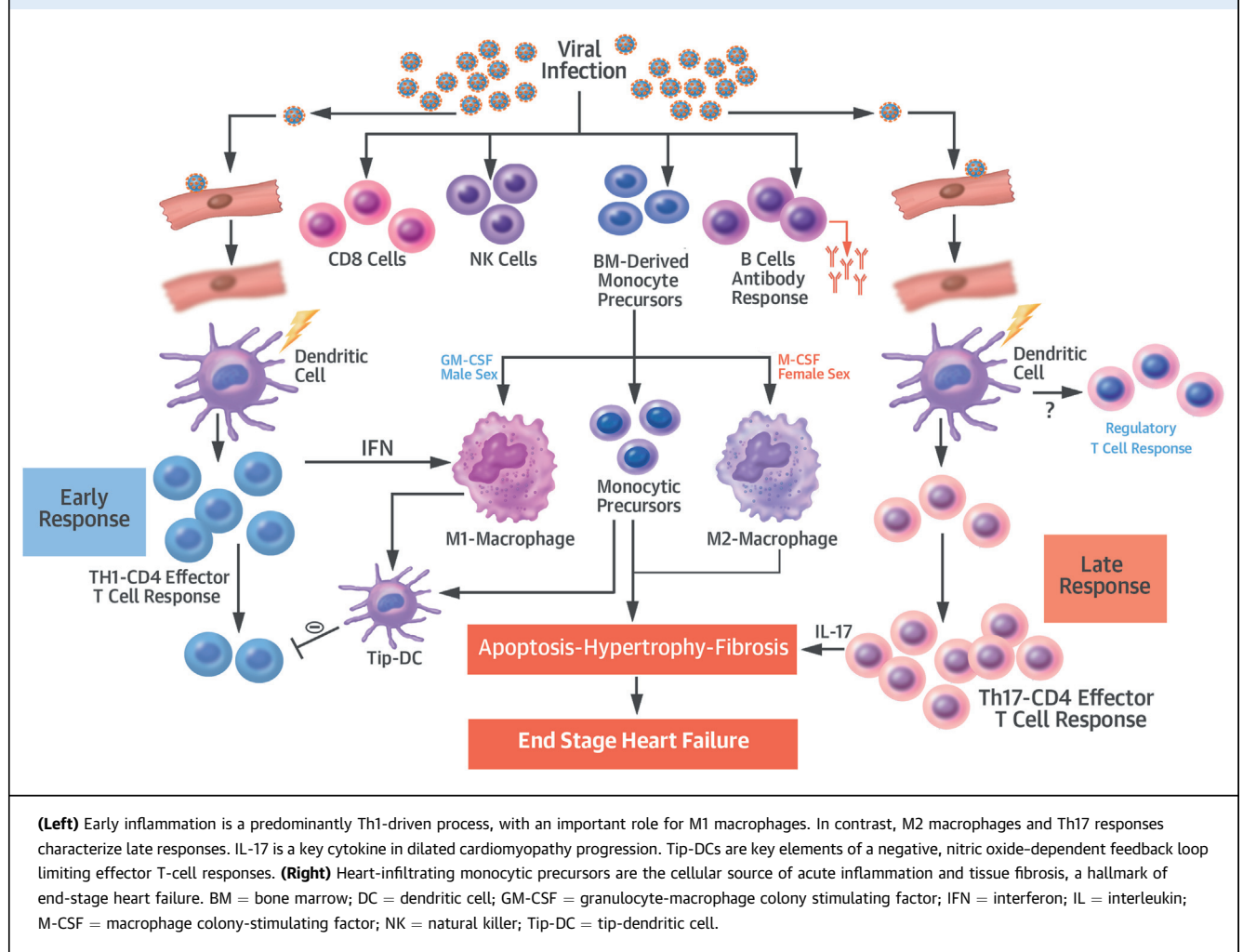Similar to other organs, different cellular compartments are involved in myocardial inflammation and remodeling: 1) the bone marrow-derived

CENTRAL ILLUSTRATION Multiphase Model of Myocarditis

Quiescent regulatory immune elements (myocytes and dendritic cells)

Infectious or Noninfectious Inflammatory Trigger (frequently viruses)

Acute myocarditis

Inflammation
of heart muscle

Symptoms:
Chest pain
Heart failure
Tachyarrhythmias
Acute cardiomyopathy

Pathogenesis:
↑ Effector immune response
↓ Antigen bearing cells
↑ Auto-antigen specific T cells,
macrophages and antibodies

Regulatory elements restore tolerance:

• Asymptomatic or mildly decreased
cardiac reserve function
• Possible myocyte hypertrophy
or mild scarring

Effector immune elements dominate:

• Chronic recurrent chest pain
• Progression to cardiomyopathy
• Persistent cardiac inflammation
with or without viral presence

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Acute myocarditis results from a variety of injuries that often trigger immune responses to both pathogens and myocardial tissue. Clinical recovery or progression to chronic cardiomyopathy or to recurrent chest pain depends on the balance of regulatory and effector elements. Chronic injury may be associated with ongoing viral infection.

compartment, including effector cells of adaptive immunity, such as T- and B-cells, and a myeloid-derived population of astonishing plasticity, including subsets of macrophages, dendritic cells, granulocytes, eosinophils, mast cells, and an immature precursor population with the capacity to differentiate not only into inflammatory cells, but also into myofibroblasts; 2) the endothelial compartment, which serves as a critical barrier regulating the access of circulating bone-marrow-derived cells to the heart; 3) the interstitial compartment, consisting of fibroblasts, myofibroblasts, and other stromal cells, embedded in an interstitial matrix that contributes to and interacts with the specific local milieu to shape the inflammatory phenotype; and, finally, 4) the cardiomyocyte compartment, which is not only a target of early infection, but also shows specific adaptive responses to inflammatory stimuli, such as hypertrophy and altered calcium signaling (63,64). Changes in these cellular compartments following an inciting event of heart tissue damage, incurred by infections, toxins, ischemia, or immunological imbalance, define the pathological anatomic phenotype of the inflamed heart. Changes in the cellular compartments, including activation, deactivation, or transdifferentiation of specific cell subsets, are regulated by cell-cell interactions and by autocrine and paracrine mediators, including chemokines, cytokines, hormones, and degradation or danger products, acting to activate or inhibit gene expression through more or less specific receptors and their intracellular downstream pathways (65).

FIGURE 2   Important Cellular Compartments Involved in Early Myocarditis and Progression to End-Stage Heart Failure

(Left) Early inflammation is a predominantly Th1-driven process, with an important role for M1 macrophages. In contrast, M2 macrophages and Th17 responses characterize late responses. IL-17 is a key cytokine in dilated cardiomyopathy progression. Tip-DCs are key elements of a negative, nitric oxide–dependent feedback loop limiting effector T-cell responses. **(Right)** Heart-infiltrating monocytic precursors are the cellular source of acute inflammation and tissue fibrosis, a hallmark of end-stage heart failure. BM = bone marrow; DC = dendritic cell; GM-CSF = granulocyte-macrophage colony stimulating factor; IFN = interferon; IL = interleukin; M-CSF = macrophage colony-stimulating factor; NK = natural killer; Tip-DC = tip-dendritic cell.

Other cellular regulatory mechanisms, such as gene silencing, contribute to the post-transcriptional regulation of myocarditis (62). The extracellular matrix, an intricate proteinaceous network that fills the extracellular spaces and provides structural support and tissue organization, is the fifth element that modulates cardiac inflammation, remodeling, and tissue fibrosis (**Figure 2**) (66,67).

INNATE MECHANISMS IN MYOCARDITIS AND INFLAMMATORY CARDIOMYOPATHY

Cells of the innate immune system mainly include macrophages, granulocytes, dendritic cells, histiocytes, mast cells, and eosinophils. These cells present a broad array of intracellular and surface pattern-recognition receptors that recognize conserved microbial structures that are not shared by host molecules (pathogen-associated molecular patterns) (68).

In myeloid cells, these pathogen-associated molecular patterns induce the inflammasome, a multiprotein oligomer including caspase 1, PYCARD, and nucleotide-binding oligomerization domain (NOD)-like receptors. Pathogen-associated molecular patterns also form inflammasomes by binding to intracellular pattern recognition receptors, such as NOD-like receptor subsets, and to caspase-1. The inflammasome activates the caspase-1 cascade, leading to production of proinflammatory cytokines. In contrast, Toll-like receptors (TLRs) represent a family of pattern recognition receptor molecules that are usually expressed on the cell surface (**Figure 2**).

TLRs play a key role in the early activation of the innate immune response against viral and other infections. TLRs are predominantly, but not exclusively, expressed in macrophages and dendritic cells. Eleven TLRs have been identified in humans. Thus far, specific roles in inflammatory heart disease and myocarditis

have been attributed to TLR2, TLR3, TLR4, TLR7, and TLR9, and their downstream adaptors, MyD88 and TRIF (45,69-73). Accordingly, TLR3-deficient mice show markedly increased mortality after infection with enteroviruses (74). Interestingly, a common TLR3 polymorphism has been found in patients with enteroviral myocarditis/cardiomyopathy (75). Expression of the TLR3 variant results in significantly reduced TLR3-mediated signaling after exposure to enterovirus-specific pathogen-associated molecular patterns, and promotes viral replication. In non-ischemic cardiomyopathy, cardiac expression of pro-tease activated receptor 2 (PAR2), which interacts with TLR3, correlates positively with inflammation and negatively with interferon (IFN)-β expression and cardiac function (76).

Activation of TLRs and the inflammasome results in release of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-18, or high-mobility group box 1 protein in myocarditis and DCM (77,78). TLR2 and TLR4 activation mediates TNF release, and TLR2, together with TLR4, promotes IL-1β secretion. Moreover, self-proteins, such as cardiac myosin, can directly activate TLR2 and TLR4 (79). TLR4 is up-regulated in macrophages and mast cells during the early innate immune response following acute cox-sackie virus B3 infection. In patients with myocarditis, TLR4 messenger ribonucleic acid (mRNA) expression is up-regulated and directly correlates with viral ribo-nucleic acid (RNA) levels. However, TLR4-deficient mice show reduced cardiac inflammation and IL-1β levels in the heart during acute coxsackie virus B3 myocarditis. The IL-1β axis is directly involved in car-diac fibrosis, a hallmark of pathological remodeling and heart failure progression (61). In contrast, high-mobility group box 1 protein promotes cardiac inflammation in a mouse model of troponin I–induced experimental autoimmune myocarditis by a complex interaction involving the receptor for advanced glycation end products and TLRs (77).

ROLE OF MACROPHAGE SUBSETS IN MYOCARDITIS DEVELOPMENT

Mononuclear phagocytic cells, termed *macrophages*, play an important role as scavengers, microbicidal effectors, and regulatory cells in cardiac inflamma-tion. Cells of the monocyte and macrophage lineages represent the majority of infiltrating inflammatory cells in human and experimental myocarditis. The spleen is an important reservoir of monocytic pre-cursors in inflammatory conditions. Cardiac injury upon myocarditis results in early recruitment of Ly6C^{hi} inflammatory macrophages (80,81). Blockade

of chemokines associated with the recruitment of Ly6C^{hi} inflammatory monocytes, such as the CCR2 ligands CCL2/MCP1 or CCL3/MIP1α, ameliorates autoimmune myocarditis (82,83).

CD4^{+} T (type 1 T helper [Th1]) cells strongly influ-ence the functional differentiation of monocytes to-ward proinflammatory or classical M1 macrophages (84). The Th1 IFN-γ potentiates the microbicidal ac-tivity of macrophages and, as such, promotes antigen presentation (85).

Alternatively-activated Ly6C^{low} M2 macrophages predominate during the process of myocardial heal-ing (86,87). These M2 macrophages blunt the in-flammatory response and promote cardiac fibrotic healing (68,86). Here, Th2-associated cytokines, mainly IL-4 and -13, activate the anti-inflammatory M2 macrophage phenotype (87). These M2 cells are also hallmarks of the transition from acute myocar-ditis to chronic pathological remodeling, where the macrophages are replaced by profibrotic myofibro-blasts (88,89). Overall, macrophage phenotypes in vivo are variable, due to their high plasticity and capacity to adopt varying degrees of specialization, including microbicidal activity, antigen presentation, immunoregulation, and fibrosis, depending on the local cytokine and chemokine milieu.

ROLE OF T-CELL SUBSETS IN MYOCARDITIS

T cells play a central role in the development of myocarditis, despite the fact that they account for a minority of heart infiltrating cells in mouse models of myocarditis, as well as biopsy-proven human myocarditis (90). CD4^{+}, as well as γδ T cells, CD8^{+} T-cell, and NK cell responses are critical to overcome early acute viral infections in rodents and humans (53,91,92). Importantly, T-cell responses, in partic-ular CD4^{+} cells, can turn initial myocarditis into an autoimmune response, even after clearance of the infectious agent (56), in part due to thymic intoler-ance to alpha-myosin heavy chain (α-MyHC) (93). Activated, self-antigen-expressing dendritic cells promote expansion of autoreactive CD4^{+} T-cells and heart-specific inflammation (39,94). Transgenic mice overexpressing α-MyHC–specific T-cell receptors (TCR) in nearly all CD4^{+} T cells develop spontaneous autoimmune myocarditis, even in the presence of minimal antigen-presenting cell activation (95).

CD4^{+} effector T cells can be divided into 3 major subsets, according to the cytokines they produce: Th1 CD4^{+} cells mainly produce IFN-γ; Th2 CD4^{+} T cells mainly produce IL-4, -5, and -13; and Th17 CD4^{+} T cells mainly produce IL-17. Those subsets play distinct roles in myocarditis and its progression to

DCM (45,96-98). IFN-γ-producing CD4$^+$ T cells pre-dominate in autoimmune myocarditis in mice and human patients, the latter in α-MyHC–responding CD4$^+$ T cells. Also, IFN-γ–overexpressing mice develop spontaneous cardiac inflammation (95). These findings all point toward a crucial role of IFN-γ–producing T1 CD4$^+$ cells in myocarditis.

Besides promoting inflammation, IFN-γ has a par-adoxical role in preventing exaggerated inflamma-tion, as mice undergoing viral, parasitic, and autoimmune myocarditis in its absence present with increased cardiac inflammation (99-101). Mechanisti-cally, IFN-γ is a key mediator of a negative feedback loop limiting activated T-cell expansion. IFN-γ pro-motes nitric oxide production by TipDCs, a distinct, monocyte-derived CD68$^+$CD11b^{hi}CD11c$^+$ dendritic cell subset (102). Nitric oxide directly induces apoptosis of activated Th1 and Th17 effector helper cell subsets.

Th17 cells have been recognized as an important effector T-cell population in myocarditis. Il-17 depletion results in decreased severity of autoim-mune myocarditis in mice (54,103). However, its complete absence in IL-17A knockout mice does not affect myocarditis (104), but is associated with a sig-nificant reduction in cardiac fibrosis and progression to DCM (104). Also, mice lacking IL-23 or signal transducer and activator of transcription 3, critical for Th17 differentiation, have reduced severity of myocarditis (97,100). Taken together, Th17 cells and associated cytokines appear as major drivers mainly in chronic myocarditis. Importantly, the cardiac myosin-Th17 response promotes heart failure in hu-man myocarditis (105). In contrast, Th1 cells play a dual role: as mediators in early autoimmune T effector cell expansion, and as regulators through a negative feedback mechanism that protects from overwhelming cardiac inflammation.

Finally, heart-specific, T-helper, cell-dependent immunoglobulin G autoantibodies are present in hu-man and rodent myocarditis and in DCM. Their presence predicts the outcome of DCM patients (106-109). Anti-beta-1 adrenoceptor antibodies are detected in up to 38% of DCM patients (110) and predict the development of cardiac dysfunction in relatives of DCM patients (107). To better understand the role of autoantibodies in myocarditis, compari-sons of patient outcomes with and without myocar-ditis will be needed.

SEX DIFFERENCES IN MYOCARDITIS AND INFLAMMATORY CARDIOMYOPATHY
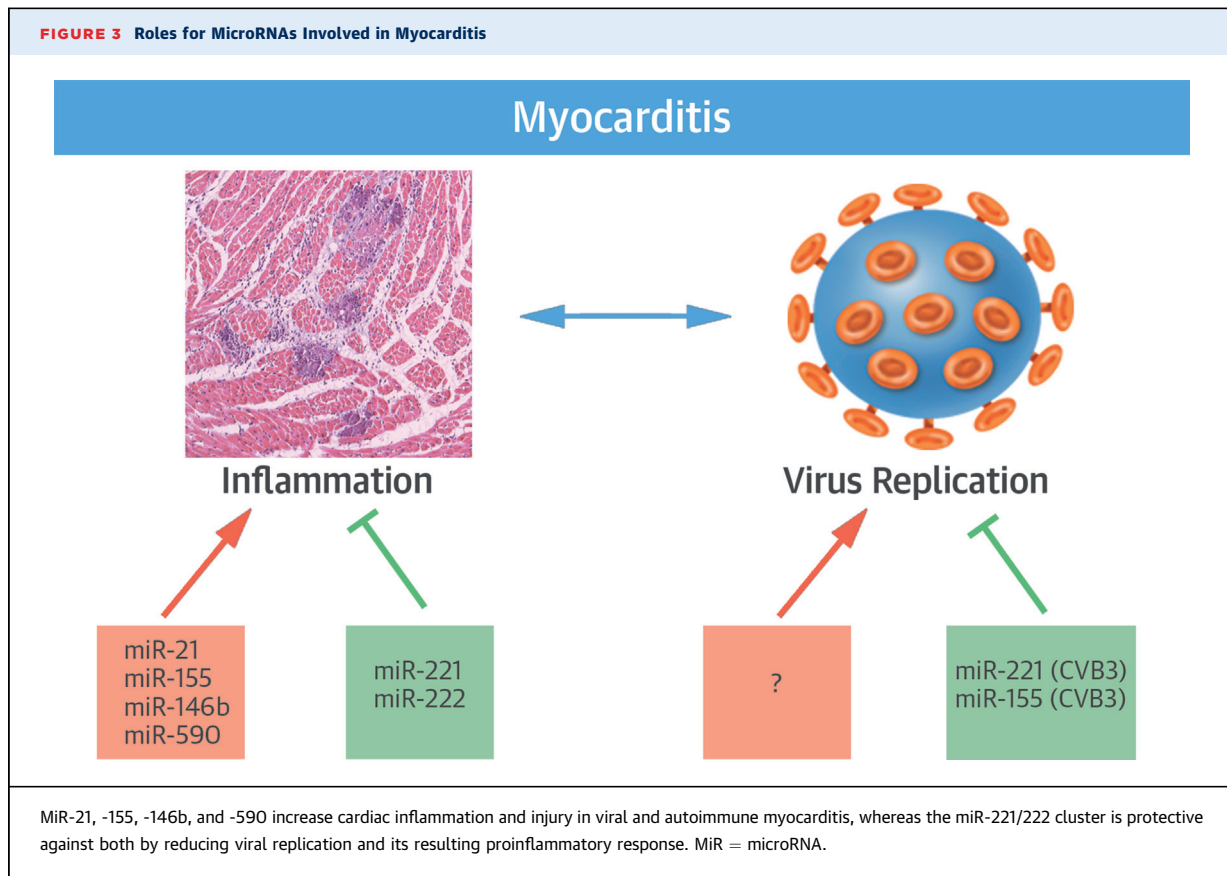
Men are diagnosed with myocarditis more often and have a worse prognosis than women, perhaps due to a more pronounced fibrotic response (111). A key question is whether this difference can lead to sex-specific therapies for acute and chronic myocarditis. In murine models, male subjects have increased coxsackie B3-induced myocarditis compared with females, due to differences in innate immune re-sponses to coxsackie B3, rather than increased viral replication (112,113). Male mice have increased γδ T cells; increased TLR4$^+$ CD11b$^+$ inflammation, including macrophages, neutrophils, mast cells, and DCs; and an increased Th1 response compared with females (114). In contrast, protective Th2 responses, increased B cells, more inhibitory Tim-3$^+$ CD4$^+$ T cells, and more T regulatory cells dominate the pic-ture in female animals (114,115). Consistent with these findings, intracardiac macrophages from coxsackie B3-infected male mice preferentially expressed inducible nitric oxide synthase, IL-12, TNFα, and CD16/32, markers associated with M1 activation (116,117). In contrast, heart-infiltrating macrophages in female animals showed a M2 activation pattern, including arginase 1, IL-10, and the macrophage mannose receptor expression. Specific targets in the inflammasome are under investigation.

POST-TRANSLATIONAL REGULATION OF INFLAMMATION IN MYOCARDITIS

Epigenetic factors influence the expression of polygenetic susceptibility. For example, microribo-nucleic acids (miRNAs) have emerged as epige-netic regulators of the cardiac immune response (Figure 3). miRNAs are noncoding, ~21-nucleotide-long, endogenous, small RNA molecules that post-transcriptionally regulate gene expression by imperfectly binding to the 3′ untranslated region, the 5′ untranslated region, or the coding region within a gene. A single miRNA can modulate the expression of transcripts for different active proteins, which are frequently clustered within a single biological process or pathway, such as inflammation or fibrosis (118,119). They may also result in new therapies, as miRNAs can easily be inhibited by their antisense complements (anti-miRs) injected systemically or locally. For example, the first phase II clinical trial with anti-miRs against miRNA-122 successfully reduced the RNA load of hepatitis C virus in patients with chronic hepatitis C virus infection (120).

Recent translational human and experimental miRNA expression studies support causal links be-tween miRNA dysregulation and human myocarditis, and suggest novel miRNA therapeutic targets. miRNA-155, -146b, and -21 are up-regulated in hearts during acute human and murine coxsackie B3

FIGURE 3 Roles for MicroRNAs Involved in Myocarditis

MiR-21, -155, -146b, and -590 increase cardiac inflammation and injury in viral and autoimmune myocarditis, whereas the miR-221/222 cluster is protective against both by reducing viral replication and its resulting proinflammatory response. MiR = microRNA.

myocarditis (121). In human right ventricular (RV) myocarditis samples, a total of 107 miRNAs are differentially expressed between viral myocarditis and control hearts. Of these miRNAs, 21 are significantly up-regulated and 37 are down-regulated by more than 1.5-fold. Inhibition of miRNA-155 (121-123), miRNA-21, and miRNA-146b (124) by systemically delivered anti-miRs attenuates cardiac inflammation and myocardial damage in CVB3 or autoimmune myocarditis in mice. Cardiac overexpression of miR-590-3p reduced cardiac injury and dysfunction: it inhibits cardiac nuclear factor-κB p50 expression, suppresses nuclear factor-κB activity, and blocks IL-6/TNF-α expression. Furthermore, in a murine model of *Trypanosoma cruzi*–induced myocarditis, expression of 113 of 641 miRNAs was significantly altered. miR-146b, miR-21, miR-142-3p, miR-142-5p, miR-145-5p, and miR-149-5p correlated with the severity of the disease (125), and are potential therapeutic targets for immunomodulation.

The miRNAs may also modulate the virulence of cardiotropic viruses. Following cardiac infection, the enterovirus CVB3 enters the cardiomyocytes via the coxsackie and adenovirus receptor, replicates, and

activates immune pathways. The miRNA-221/222 cluster in cardiomyocytes regulates both virulence and inflammatory pathways in the heart (126). Systemic inhibition of miR-221/-222 in mice increases cardiac viral load, prolongs the viremic state, and aggravates cardiac inflammation and injury. miR-221/-222 works through targeting proteins that orchestrate viral replication and inflammation, including ETS1/2, interferon regulatory factor 2, Bcl2-like 11, TOX, Bcl2 modifying factor, and CXCL12. Similarly, miRNA-155 inhibits PU.1 and suppressor of cytokine signaling 1 in the heart and, as such, de-represses the production of proinflammatory cytokines, enhancing T-cell and monocyte activation (126,127). Together, these results support the concept that a single miRNA or cluster may orchestrate immune activation and modulate myocarditis.

The miRNAs are under investigation as tools for myocarditis diagnosis and prognosis. In blood, miRNAs reflecting cardiomyocyte injury (including miRNA-208 and -499) are increased in acute myocarditis (128). These cardiomyocyte injury markers are not specific for myocarditis, but are also increased in acute ischemic or hypertensive

cardiac events (128,129). Interestingly, inflammatory miRNAs, including miRNA-155, -146b, and -21, did not increase in blood in acute myocarditis (128), possibly reflecting the lack of cellular release of those miRNAs upon inflammation. In a recent study comparing cardiac miRNA profiles in myocarditis patients with CVB3 persistence to CVB3 clearance, 8 miRNAs (miR-135b, -155, -190, -422a, -489, -590, -601, and -1290) were strongly induced only in the hearts of patients with late viral persistence and progressive cardiac dysfunction (130). Profiling of miRNA groups together with their messenger RNA/protein targets in cardiac biopsies is an area of active investigation.

CLINICAL PRESENTATIONS AND PROGRESS IN CARDIAC IMAGING

Acute myocarditis most commonly presents with chest pain or dyspnea. A viral syndrome, such as an upper respiratory tract or gastrointestinal illness, often precedes the clinical syndrome by several days to weeks. Chest pain syndromes may be broadly divided into those resembling pericarditis, with an associated rise in troponin, and those resembling an acute myocardial infarction. Angina may be due to coronary vasospasm or microvascular dysfunction. Myopericarditis recurs in about 11% of patients (131). Palpitations and syncope may result from ventricular arrhythmias. Men have, on average, a more severe course with less complete recovery than women (112).

When myocarditis is suspected, we recommend use of the diagnostic algorithm illustrated in Figure 4. EMB should be performed if the patient requires inotropic or mechanical circulatory support, develops Mobitz type 2 second-degree or higher heart block, has sustained or symptomatic ventricular tachycardia, or fails to respond to guideline-based medical management within 1 to 2 weeks. Disorders that have a greater likelihood of high-grade heart block or ventricular tachycardia include GCM, CS, and necrotizing eosinophilic myocarditis. Other clinical scenarios where EMB may be useful include suspected eosinophilic myocarditis and myocarditis associated with systemic inflammatory disorders. If EMB is infeasible or is not clearly indicated, noninvasive diagnostic criteria may be used to diagnose probable acute myocarditis. A diagnosis of probable myocarditis leads to a recommendation to avoid athletics for at least 3 to 6 months (6) and counseling on the likelihood of left ventricular (LV) and clinical recovery, discussed later (132). At medical centers with specialized expertise in myocarditis care, a tailored evaluation involving specialized studies on biopsy
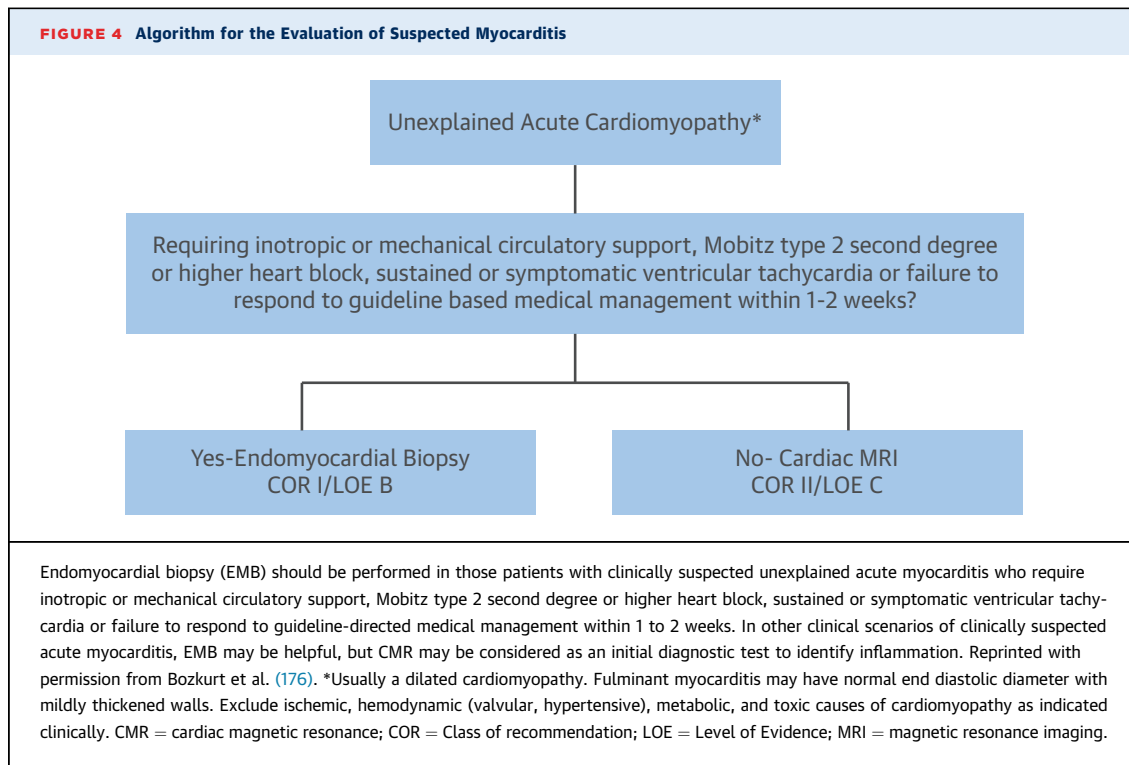
tissue or advanced imaging techniques may be appropriate.

In myocarditis presenting as acute or fulminant DCM with heart failure, cardiac troponins are often detectable in blood and can be supportive of the diagnosis (3). With subacute or chronic myocarditis presentations, biomarkers of myocardial injury are usually normal (17,133). We recommend that cardiac troponin levels be obtained in patients with clinically suspected acute myocarditis. Although most electro-cardiographic and echocardiographic changes are nonspecific, speckle-tracking-derived strain may identify early myocarditis (134-136). An echocardiogram should be performed in all patients with clinically suspected myocarditis to exclude hemo-dynamic, pericardial, and congenital causes of cardiomyopathy.

Newer sensitive and specific cardiac magnetic resonance (CMR) sequences have improved diagnostic and prognostic value. T_2-weighted CMR sequences detect edema or water, and T_1-weighted sequences detect inflammation or fibrosis. Standard tissue characterization by CMR relies on qualitative visual estimates of signal intensity in native T_2-weighted and post-contrast T_1-weighted sequences. In contrast, native mapping sequences using T_1- or T_2-weighted techniques allow for quantitative, reproducible, pixel-level relaxation times that are compared with standard values derived from normal subjects. In the largest $T_1 + T_2$ mapping trial to date, an elevated native T_1 yielded the best diagnostic performance of all CMR parameters in patients with acute symptoms. In patients with <2 weeks of symptoms, native T_1 yielded the best area under the receiver-operating characteristic curve (0.82) followed by T_2 (0.81). The standard Lake Louise Criteria did not perform as well (0.56). Native T_1 had little value; however, in the chronic (>2 weeks) patient group, these parameters could not differentiate patients with inflammation from those without (137).

The most typical late gadolinium enhancement (LGE) pattern in CS consists of multiple, patchy midmyocardial lesions in a noncoronary (i.e., sparing the endocardium) distribution with septal and RV involvement. Diagnostic findings may fade over weeks to months (138).

Although LGE imaging can help in distinguishing nonischemic patterns of myocyte damage and fibrosis from ischemic injury, T_2-weighted and early gadolinium enhancement imaging detect other inflammatory features of edema, capillary leakage, and hyperemia. Myocardial edema in a water-sensitive T_2-weighted sequence is a typical feature of active inflammation. Hyperemia is a regular feature of

FIGURE 4 Algorithm for the Evaluation of Suspected Myocarditis

Endomyocardial biopsy (EMB) should be performed in those patients with clinically suspected unexplained acute myocarditis who require inotropic or mechanical circulatory support, Mobitz type 2 second degree or higher heart block, sustained or symptomatic ventricular tachycardia or failure to respond to guideline-directed medical management within 1 to 2 weeks. In other clinical scenarios of clinically suspected acute myocarditis, EMB may be helpful, but CMR may be considered as an initial diagnostic test to identify inflammation. Reprinted with permission from Bozkurt et al. (176). *Usually a dilated cardiomyopathy. Fulminant myocarditis may have normal end diastolic diameter with mildly thickened walls. Exclude ischemic, hemodynamic (valvular, hypertensive), metabolic, and toxic causes of cardiomyopathy as indicated clinically. CMR = cardiac magnetic resonance; COR = Class of recommendation; LOE = Level of Evidence; MRI = magnetic resonance imaging.

inflammation that may be imaged noninvasively with early, regional post-gadolinium enhancement (139).

In acute DCM, CMR features suggestive of myocarditis predict a greater likelihood of recovery, defined as a left ventricular ejection fraction (LVEF) >55% after 24 months (138). In a second study of 37 patients with acute myocarditis, patients with greater regional or global T_2-weighted "edema-sensitive" sequences also had a significant improvement in LVEF at 12-month follow-up (140). In contrast, delayed gadolinium enhancement has been associated with a higher (3.7%/year) risk of a composite of cardiovascular adverse events. The extent of delayed gadolinium enhancement also predicted a composite endpoint of cardiac death, heart failure hospitalization, ventricular tachycardia, and sudden death (141).

The extent of myocardial LGE is also inversely related to survival free of transplantation and life-threatening ventricular arrhythmias in CS. In 59 patients with CS (38 women, mean age 46 ± 10 years), the extent of myocardial LGE, measured as a percentage of LV mass; the LV and RV volumes and ejection fractions; and the thickness of the basal interventricular septum were determined and analyzed for prognostic significance. In univariate analysis, myocardial LGE extent predicted event-free survival, as did scar-like thinning (<4 mm) of the

basal interventricular septum and the ejection fraction of the RV (p < 0.05 for all). In multivariate analysis, LGE extent remained the only independent CMR predictor of the composite outcome (hazard ratio: 2.22 per tertile; 95% confidence interval: 1.07 to 4.59). The extent of LGE >22% (third tertile) had positive and negative predictive values for the composite endpoints of 75% and 76%, respectively (142).

^{8}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) is also useful for detecting active myocardial inflammation, in particular for suspected CS (143,144). Combining FDG-PET imaging with myocardial perfusion detects active inflammation and scarring. Early in CS, ^{18}F-FDG-PET may show a focal FDG increase in the absence of a perfusion defect. In a more advanced stage, FDG accumulation may correlate with a perfusion defect. After granulomas have "burned out" or inflammation has been therapeutically suppressed, a perfusion defect may persist without active inflammation. ^{18}F-FDG-PET imaging may also detect extracardiac inflammation and identify lymph nodes that are amenable to biopsy (145,146).

In addition to diagnostic use, ^{18}F-FDG-PET can inform prognosis. Mismatch of FDG and perfusion measurements predicts adverse cardiac events, and RV involvement is associated with high risk for

arrhythmias (147). Patients with either a PET mismatch or RV involvement had a 3-fold increase in the rate of adverse events. Newer imaging data from CMR and PET should be integrated with established prognostic risk variables, such as QRS width >120 ms, LVEF, and New York Heart Association functional class (147,148).

THE ROLE OF EMB

Although EMB is essential for the diagnosis of specific histological disorders, the broad use of EMB as a gold standard has been limited because of cost, availability of experienced centers, and sensitivity (3,149). The sensitivity of EMB for GCM is approximately 80% to 93% (with repeated LV and RV samples), but is much lower, estimated to be around 20% to 30%, for more common lymphocytic and sarcoid myocarditis due to sampling limitations and focal distribution of sarcoid granuloma and clusters of inflammatory cells (12,150). Immunohistochemistry to visualize specific inflammatory cell types and viral genome analysis have improved the sensitivity to 40% for DCM. An ESC position statement recommends "immunohistochemical criteria," such as the presence of an inflammatory infiltrate consisting of ≥14 leukocytes/mm^2, including up to 4 monocytes/mm^2, with the presence of CD3$^+$ T-lymphocytes ≥7 cells/mm^2 (3). Immunohistochemical criteria seem to be particularly useful in subacute or chronic myocarditis, also called inflammatory cardiomyopathies, where the rate of normalization of LV function is only about 20%. LV biopsy increased the yield in disorders that affect the LV, with a stroke risk of about 1:300 to 1:500 (151). Intracardiac electrograms can increase the diagnostic yield of suspected CS from between 20% and 32% (152,153) to between 60% and 80% (144,154).

Cardiac biopsies may be used to identify and quantify viral genomes. Because serological tests for antiviral antibodies correlate poorly with viral genomes detected on heart biopsies, serology cannot be used to substitute for a biopsy-based diagnosis of viral infection (155). Because viral genomes may be detected in normal hearts, and in ischemic and valvular heart disease (156-159), assays for replicative RNA intermediates (mRNA for deoxyribonucleic acid [DNA] viruses) may be required to define active infection. For example, CpG dinucleotide methylation regulates parvovirus 19 (PVB19) viral genome expression (160). A minimum of 4 samples is needed to achieve an acceptable sensitivity for molecular diagnosis of erythrovirus PVB19 (161,162).

Significant cardiac virus presence (possibly related to the development of cardiac dysfunction) has only been described for PVB19 (>250 copies/μg DNA) and for human herpesvirus 6 (>500 copies/μg DNA). Yet, the simple presence of virus DNA/RNA does not reflect active replication of a virus. Looking for mRNA intermediates to score active virus replication is still not part of routine practice. Only a few studies focused on determining the replicative status of cardiotropic viruses, mainly for the detection of PVB19 mRNA intermediates (162). Active PVB19 replication was only seen in cardiac samples with increased inflammation, either in acute myocarditis or chronic inflammatory cardiomyopathy, and was absent in DCM hearts without inflammation. As antiviral therapies have the highest benefit in patients with active virus replication, its detection would be of great value, but methods are not yet established, standardized, or implemented in clinical practice. In the setting of myocarditis, the presence of multiple viral genomes has been associated with progression to a DCM. Clinical studies of the use of molecular assays for active viral replication to enhance the clinical benefit of EMB are underway. Other features on EMB may affect diagnosis and prognosis. GCM may be distinguished from CS by transcriptome analysis (163). The severity of necrosis and fibrosis are associated with an increased risk of death and transplant in GCM (164).

HOW TO TREAT MYOCARDITIS?

For all patients with myocarditis presenting as heart failure with reduced systolic function, we recommend following the current guidelines and scientific statements for heart failure management. The current guidelines emphasize gradual titration of neurohormonal-blocking medications, including inhibitors of angiotensin-converting enzyme, angiotensin-receptor blocking agents, and beta-blockers, to doses used in clinical trials (165,166). Tests for specific causes of myocarditis, such as human immunodeficiency virus, Lyme, or Chagas disease are indicated in patients who have heightened pre-test probability. Serological tests for specific systemic rheumatological conditions, such as lupus erythematosus, may be useful in select groups. The 2015 American Heart Association/American College of Cardiology Foundation scientific statement on sports participation after myocarditis recommends 3 to 6 months abstinence from competitive sports. Before clearance, freedom from exercise-induced arrhythmias and normalization of heart function should be assessed with a symptom-limited exercise test, Holter monitor,

and echocardiogram (6). The treatment of patients with myocarditis depends on the clinical scenario and: 1) the presence or absence of inflammation; 2) the presence or absence of viral infection; and 3) one of the specific histological entities, including GCM, listed in the following text.

VIRUS-NEGATIVE INFLAMMATION-POSITIVE PATIENTS.

In virus-negative patients with chronic idiopathic DCM and increased cardiac inflammation despite guideline-directed medical treatment, immunosuppression with azathioprine and prednisone for up to 12 months can be an option to improve cardiac function (167,168). To confirm the positive outcomes in the first randomized single-center studies using immunosuppression for virus-negative and inflammation-positive DCM (167,168), a multicenter trial using azathioprine and prednisone for 6 months is underway (NCT01877746). Enrollment criteria include more than 6 months of symptoms and a DCM at the time of screening.

VIRUS-POSITIVE PATIENTS.

Viral genome analysis of EMBs suggests that up to 70% of chronic DCM patients may carry 1 or more cardiotropic viruses (10). A study of IFN-β in virus persistence-related cardiomyopathy, revealed that IFN-β effectively eliminates enteroviral RNA, with a beneficial effect on New York Heart Association functional class ($p = 0.013$ at follow-up week 12), improvement in quality of life (Minnesota Heart Failure score; $p = 0.032$ at follow-up week 24), and patient global assessment (follow-up weeks 12 to 24; $p = 0.039$) (169). However, one-half of patients spontaneously eliminated their enterovirus without specific treatment, suggesting that in addition to the presence of viral genomes, evidence of active virus replication should be a criterion for antiviral therapy. Retrospective case series indicate that patients with chronic cardiomyopathy, a higher virus genome copy number, and evidence of virus replication may benefit from intravenous immunoglobulin (IVIG) (170,171). Although IVIG was ineffective for the treatment of acute DCM in general (172), a single-center randomized trial using IVIG for chronic PVB19-related cardiomyopathy to reduce PVB19 viral load is ongoing (NCT00892112). Chronic viral infection in the heart can cause chest pain or heart failure in the absence of cellular inflammation. The clinical term for a cardiac syndrome associated with being virus genome-positive and without inflammation on biopsy is *viral heart disease* (17).

Corticosteroid therapy is the mainstay of immunosuppressive therapy in CS, even though evidence is on the basis of retrospective observations. Published clinical series suggest that corticosteroid therapy may halt the progression of LV dysfunction, but there are no data on the effect of immunosuppression on survival (173,174). If treatment is initiated early, development of systolic heart failure seems rare. Observational, uncontrolled data suggest an improvement in LV function with immunosuppression in CS patients with severely impaired LVEF (<35%) (8). Atrioventricular conduction block appears to recover in only 10% to 30% of patients after initiation of steroid therapy.

The treatment of GCM includes immunosuppression. Since the first report of the Multicenter GCM Study Group, treatment has relied on the combination of cyclosporine and prednisone, sometimes with azathioprine (175). Retrospective observations from the Multicenter GCM Registry and a prospective study with repeat biopsies suggest that cyclosporine-based combined immunosuppression attenuates myocardial inflammation and improves clinical outcomes (11). Abrupt cessation of immunosuppression within the first 2 years of treatment has been associated with fatal disease relapse (36).

High-grade heart block in acute lymphocytic myocarditis may be temporary and may require only short-term, transvenous pacing until the acute inflammation subsides. In the setting of likely reversible LV dysfunction associated with acute myocarditis, a wearable external defibrillator vest may be used pending reassessment of LV function. In contrast, high-grade heart block often is permanent, and is associated with frequent ventricular arrhythmias in CS. The Heart Rhythm Society expert consensus statement gave a Class IIa recommendation for an implantable cardioverter-defibrillator in patients with suspected CS, regardless of LV function or reversibility of heart block (145).

CONCLUSIONS

In summary, myocarditis represents many diseases with distinct immunophenotypes. Most advances in our understanding of inflammatory heart disease pathogenesis have not yet translated into therapeutic approaches or even better diagnostic tests. Multicenter trials examining pathway- and pathogen-specific treatments are underway presently, using immunohistochemical and molecular analysis as part of the enrollment criteria. Peripheral T-cell, DC, monocyte, and NK-cell activation patterns may differ in myocarditis compared with noninflammatory DCM, or between different viruses and purely autoimmune processes. Multiple groups are searching for

peripheral blood immune markers in proteins, and in coding or noncoding RNAs for diagnosis and to guide emerging therapies, such as RNA interference. The path toward personalized treatment in acute myocarditis requires deep clinical and immunological phenotyping. More specific therapeutic approaches proposed or under clinical investigation include individualized antiviral strategies, miRNA-based gene-silencing strategies, cytokine targeting, and pharmacological modulation to alter tissue monocytic precursors of cardiac fibrosis.

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REFERENCES

1. Cooper LT Jr. Myocarditis. N Engl J Med 2009; 360:1526–38.

2. Dennert R, Crijns HJ, Heymans S. Acute viral myocarditis. Eur Heart J 2008;29:2073–82.

3. Caforio ALP, Pankuweit S, Arbustini E, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J 2013;34:2636–48.

4. Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;386:743–800.

5. Cooper LT Jr., Keren A, Sliwa K, et al. The global burden of myocarditis: part 1: a systematic literature review for the Global Burden of Diseases, Injuries, and Risk Factors 2010 study. Glob Heart 2014;9:121–9.

6. Maron BJ, Udelson JE, Bonow RO, et al. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 3: hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy and other cardiomyopathies, and myocarditis: a scientific statement from the American Heart Association and American College of Cardiology. J Am Coll Cardiol 2015;66:2362–71.

7. Matoba R, Shikata I, Iwai K, et al. An epidemiologic and histopathological study of sudden cardiac death in Osaka Medical Examiner's Office. Jpn Circ J 1989;53:1581–8.

8. Kandolin R, Lehtonen J, Airaksinen J, et al. Cardiac sarcoidosis: epidemiology, characteristics, and outcome over 25 years in a nationwide study. Circulation 2015;131:624–32.

9. Roberts WC, McAllister HA Jr., Ferrans VJ. Sarcoidosis of the heart. A clinicopathologic study of 35 necropsy patients (group I) and review of 78 previously described necropsy patients (group II). Am J Med 1977;63:86–108.

10. Hazebroek MR, Moors S, Dennert R, et al. Prognostic relevance of gene-environment interactions in patients with dilated cardiomyopathy: applying the MOGE(S) classification. J Am Coll Cardiol 2015;66:1313–23.

11. Cooper LT Jr., Hare JM, Tazelaar HD, et al., for the Giant Cell Myocarditis Treatment Trial Investigators. Usefulness of immunosuppression for giant cell myocarditis. Am J Cardiol 2008;102: 1535–9.

12. Kandolin R, Lehtonen J, Salmenkivi K, et al. Diagnosis, treatment, and outcome of giant-cell myocarditis in the era of combined immunosuppression. Circ Heart Failure 2013;6:15–22.

13. Ekström K, Lehtonen J, Kandolin R, et al. Long-term outcome and its predictors in giant cell myocarditis. Eur J Heart Failure 2016 Jul 13 [E-pub ahead of print].

14. Whitehead R. Isolated myocarditis. Brit Heart J 1965;27:220–30.

15. Kytö V, Saukko P, Lignitz E, et al. Diagnosis and presentation of fatal myocarditis. Hum Pathol 2005;36:1003–7.

16. Davidoff R, Palacios I, Southern J, et al. Giant cell versus lymphocytic myocarditis. A comparison of their clinical features and long-term outcomes. Circulation 1991;83:953–61.

17. Schultheiss HP, Kühl U, Cooper LT. The management of myocarditis. Eur Heart J 2011;32:2616–25.

18. Andréoletti L, Lévêque N, Boulagnon C, et al. Viral causes of human myocarditis. Arch Cardiovasc Dis 2009;102:559–68.

19. Pauschinger M, Phan MD, Doerner A, et al. Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. Circulation 1999; 99:889–95.

20. Baruteau AE, Boimond N, Ramful D. Myocarditis associated with 2009 influenza A (H1N1) virus in children. Cardiol Young 2010;20:351–2.

21. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol 2003;42:466–72.

22. Pauschinger M, Bowles NE, Fuentes-Garcia FJ, et al. Detection of adenoviral genome in the myocardium of adult patients with idiopathic left ventricular dysfunction. Circulation 1999;99: 1348–54.

23. Matsumori A, Yutani C, Ikeda Y, et al. Hepatitis C virus from the hearts of patients with myocarditis and cardiomyopathy. Lab Invest 2000;80: 1137–42.

24. Omura T, Yoshiyama M, Hayashi T, et al. Core protein of hepatitis C virus induces cardiomyopathy. Circ Res 2005;96:148–50.

25. Chimenti C, Russo A, Pieroni M, et al. Intramyocyte detection of Epstein-Barr virus genome by laser capture microdissection in patients with inflammatory cardiomyopathy. Circulation 2004; 110:3534–9.

26. Breinholt JP, Moulik M, Dreyer WJ, et al. Viral epidemiologic shift in inflammatory heart disease: the increasing involvement of parvovirus B19 in the myocardium of pediatric cardiac transplant patients. J Heart Lung Transplant 2010;29: 739–46.

27. Kühl U, Pauschinger M, Noutsias M, et al. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with "idiopathic" left ventricular dysfunction. Circulation 2005;111:887–93.

28. Ellis NM, Kurahara DK, Vohra H, et al. Priming the immune system for heart disease: a perspective on group A streptococci. J Infect Dis 2010; 202:1059–67.

29. Karatolios K, Maisch B, Pankuweit S. Suspected inflammatory cardiomyopathy. Prevalence of Borrelia burgdorferi in endomyocardial biopsies with positive serological evidence. Herz 2015;40 Suppl 1:91–5.

30. Marin-Neto JA, Cunha-Neto E, Maciel BC, et al. Pathogenesis of chronic Chagas heart disease. Circulation 2007;115:1109–23.

31. Burke A, Saenger J, Mullick F, et al. Hypersensitivity myocarditis. Arch Pathol Lab Med 1991; 115:764–9.

32. Haas SJ, Hill R, Krum H, et al. Clozapine-associated myocarditis: a review of 116 cases of suspected myocarditis associated with the use of clozapine in Australia during 1993-2003. Drug Safety 2007;30:47–57.

33. Cook SC, Ferguson BA, Cotes RO, et al. Clozapine-induced myocarditis: prevention and considerations in rechallenge. Psychosomatics 2015;56:685–90.

34. Engler RJ, Nelson MR, Collins LC Jr., et al. A prospective study of the incidence of myocarditis/pericarditis and new onset cardiac symptoms following smallpox and influenza vaccination. PLoS One 2015;10:e0118283.

35. Zawadowski G, Klarich K, Moder K, et al. A contemporary case series of lupus myocarditis. Lupus 2012;21:1378–84.

36. Maleszewski JJ, Orellana VM, Hodge DO, et al. Long-term risk of recurrence, morbidity and mortality in giant cell myocarditis. Am J Cardiol 2015; 115:1733–8.

37. Ciháková D, Sharma RB, Fairweather D, et al. Animal models for autoimmune myocarditis and

autoimmune thyroiditis. Methods Mol Med 2004;
102:175–93.

38. Chow LH, Beisel KW, McManus BM. Entero-viral infection of mice with severe combined immunodeficiency. Evidence for direct viral pathogenesis of myocardial injury. Lab Invest 1992;66:24–31.

39. Eriksson U, Ricci R, Hunziker L, et al. Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. Nat Med 2003;9:1484–90.

40. Kaya Z, Katus HA, Rose NR. Cardiac troponins and autoimmunity: their role in the pathogenesis of myocarditis and of heart failure. Clin Immunol 2010;134:80–8.

41. Watanabe K, Arumugam S, Sreedhar R, et al. Small interfering RNA therapy against carbohy-drate sulfotransferase 15 inhibits cardiac remod-eling in rats with dilated cardiomyopathy. Cell Signal 2015;27:1517–24.

42. Kandolf R, Canu A, Hofschneider PH. Cox-sackie B3 virus can replicate in cultured human foetal heart cells and is inhibited by interferon. J Mol Cell Cardiol 1985;17:167–81.

43. Bergelson JM, Cunningham JA, Droguett G, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 1997; 275:1320–3.

44. Martino TA, Petric M, Brown M, et al. Car-diovirulent coxsackieviruses and the decay-accelerating factor (CD55) receptor. Virology 1998;244:302–14.

45. Abston ED, Coronado MJ, Bucek A, et al. Th2 regulation of viral myocarditis in mice: different roles for TLR3 versus TRIF in progression to chronic disease. Clin Dev Immunol 2012;2012: 129486.

46. Fairweather D, Frisancho-Kiss S, Rose N. Vi-ruses as adjuvants for autoimmunity: evidence from Coxsackievirus-induced myocarditis. Rev Med Virol 2005;15:17–27.

47. Bonney K, Engman D. Autoimmune patho-genesis of Chagas heart disease: looking back, looking ahead. Am J Pathol 2015;185:1537–47.

48. Mocelin A, Issa V, Bacal F, et al. The influence of aetiology on inflammatory and neurohumoral activation in patients with severe heart failure: a prospective study comparing Chagas' heart dis-ease and idiopathic dilated cardiomyopathy. Eur J Heart Failure 2005;7:869–73.

49. Yuan J, Liu Z, Lim T, et al. CXCL10 inhibits viral replication through recruitment of natural killer cells in coxsackievirus B3-induced myocarditis. Circ Res 2009;104:628–38.

50. Jenke A, Holzhauser L, Löbel M, et al. Adipo-nectin promotes coxsackievirus B3 myocarditis by suppression of acute anti-viral immune responses. Basic Res Cardiol 2014;109:408.

51. Deonarain R, Cerullo D, Fuse K, et al. Protec-tive role for interferon-β in coxsackievirus B3 infection. Circulation 2004;110:3540–3.

52. Huber SA, Sartini D, Exley M. Vγ4+T cells promote autoimmune CD8+ cytolytic T-lymphocyte activation in coxsackievirus B3-induced myocarditis in mice: role for CD4+ Th1 cells. J Virol 2002;76:10785–90.

53. Noutsias M, Rohde M, Göldner K, et al. Expression of functional T-cell markers and T-cell receptor Vbeta repertoire in endomyocardial bi-opsies from patients presenting with acute myocarditis and dilated cardiomyopathy. Eur J Heart Failure 2011;13:611–8.

54. Valaperti A, Marty RR, Kania G, et al. CD11b+ monocytes abrogate Th17 CD4+ T cell-mediated experimental autoimmune myocarditis. J Immunol 2008;180:2686–95.

55. Caforio AL, Marcolongo R, Jahns R, et al. Immune-mediated and autoimmune myocarditis: clinical presentation, diagnosis and management. Heart Fail Rev 2013;18:715–32.

56. Fairweather D, Kaya Z, Shellam GR, et al. From infection to autoimmunity. J Autoimmun 2001;16: 175–86.

57. Mahrholdt H, Wagner A, Deluigi C, et al. Pre-sentation, patterns of myocardial damage, and clinical course of viral myocarditis. Circulation 2006;114:1581–90.

58. Caforio AL, Mahon NJ, McKenna WJ. Cardiac autoantibodies to myosin and other heart-specific autoantigens in myocarditis and dilated cardio-myopathy. Autoimmunity 2001;34:199–204.

59. Cunha-Neto E, Bilate AM, Hyland KV, et al. Induction of cardiac autoimmunity in Chagas heart disease: a case for molecular mimicry. Autoim-munity 2006;39:41–54.

60. Lauer B, Schannwell M, Kühl U, et al. Anti-myosin autoantibodies are associated with dete-rioration of systolic and diastolic left ventricular function in patients with chronic myocarditis. J Am Coll Cardiol 2000;35:11–8.

61. Blyszczuk P, Kania G, Dieterle T, et al. Myeloid differentiation factor-88/interleukin-1 signaling controls cardiac fibrosis and heart failure pro-gression in inflammatory dilated cardiomyopathy. Circ Res 2009;105:912–20.

62. Kania G, Blyszczuk P, Eriksson U. Mechanisms of cardiac fibrosis in inflammatory heart disease. Trends Cardiovasc Med 2009;19:247–52.

63. Frieler RA, Mortensen RM. Immune cell and other noncardiomyocyte regulation of cardiac hy-pertrophy and remodeling. Circulation 2015;131: 1019–30.

64. Delgado C, Ruiz-Hurtado G, Gómez-Hurtado N, et al. NOD1, a new player in cardiac function and calcium handling. Cardiovasc Res 2015;106:375–86.

65. Gratchev A, Kzhyshkowska J, Kothe K, et al. Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology 2006;211:473–86.

66. Cheung C, Luo H, Yanagawa B, et al. Matrix metalloproteinases and tissue inhibitors of met-alloproteinases in coxsackievirus-induced myocarditis. Cardiovasc Pathol 2006;15:63–74.

67. Rienks M, Papageorgiou AP. Novel regulators of cardiac inflammation: matricellular proteins expand their repertoire. J Mol Cell Cardiol 2016; 91:172–8.

68. Epelman S, Liu PP, Mann DL. Role of innate and adaptive immune mechanisms in cardiac injury and repair. Nat Rev Immunol 2015;15:117–29.

69. Jenke A, Wilk S, Poller W, et al. Adiponectin protects against Toll-like receptor 4-mediated cardiac inflammation and injury. Cardiovasc Res 2013;99:422–31.

70. Frisancho-Kiss S, Davis SE, Nyland JF, et al. Cutting edge: cross-regulation by TLR4 and T cell Ig mucin-3 determines sex differences in inflam-matory heart disease. J Immunol 2007;178: 6710–4.

71. Fairweather D, Yusung S, Frisancho S, et al. IL-12 receptor β1 and Toll-like receptor 4 increase IL-1β- and IL-18-associated myocarditis and cox-sackievirus replication. J Immunol 2003;170: 4731–7.

72. Riad A, Meyer zu Schwabedissen H, Weitmann K, et al. Variants of Toll-like receptor 4 predict cardiac recovery in patients with dilated cardiomyopathy. J Biol Chem 2012;287:27236–43.

73. Pagni PP, Traub S, Demaria O, et al. Contri-bution of TLR7 and TLR9 signaling to the sus-ceptibility of MyD88-deficient mice to myocarditis. Autoimmunity 2010;43:275–87.

74. Abston ED, Coronado MJ, Bucek A, et al. TLR3 deficiency induces chronic inflammatory cardio-myopathy in resistant mice following coxsack-ievirus B3 infection: role for IL-4. Am J Physiol Regul Integr Comp Physiol 2013;304:R267–77.

75. Gorbea C, Makar KA, Pauschinger M, et al. A role for Toll-like receptor 3 variants in host susceptibility to enteroviral myocarditis and dilated cardiomyopathy. J Biol Chem 2010;285: 23208–23.

76. Weithauser A, Bobbert P, Antoniak S, et al. Protease-activated receptor-2 regulates the innate immune response to viral infection in a coxsackievirus B3-induced myocarditis. J Am Coll Cardiol 2013;62:1737–45.

77. Su Z, Zhang P, Yu Y, et al. HMGB1 facilitated macrophage reprogramming towards a proin-flammatory M1-like phenotype in experimental autoimmune myocarditis development. Sci Rep 2016;6:21884.

78. Eriksson U, Kurrer MO, Sonderegger I, et al. Activation of dendritic cells through the inter-leukin 1 receptor 1 is critical for the induction of autoimmune myocarditis. J Exp Med 2003;197: 323–31.

79. Zhang P, Cox CJ, Alvarez KM, et al. Cutting edge: cardiac myosin activates innate immune re-sponses through TLRs. J Immunol 2009;183: 27–31.

80. Barin JG, Baldeviano GC, Talor MV, et al. Macrophages participate in IL-17-mediated inflammation. Eur J Immunol 2012;42:726–36.

81. Leuschner F, Courties G, Dutta P, et al. Silencing of CCR2 in myocarditis. Eur Heart J 2015; 36:1478–88.

82. Cooper LT Jr., Fairweather D. Nano-scale treatment for a macro-scale disease: nanoparticle-delivered siRNA silences CCR2 and treats myocarditis. Eur Heart J 2015;36:1434–6.

83. Göser S, Ottl R, Brodner A, et al. Critical role for monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α in induction of experimental autoimmune myocarditis and

effective anti-monocyte chemoattractant protein-1 gene therapy. Circulation 2005;112:3400-7.

84. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010;11: 889-96.

85. Martinez FO, Sica A, Mantovani A, et al. Macrophage activation and polarization. Front Biosci 2008;13:453-61.

86. Barin JG, Rose NR, Cihákova D. Macrophage diversity in cardiac inflammation: a review. Immunobiology 2012;217:468-75.

87. Cihakova D, Barin JG, Afanasyeva M, et al. Interleukin-13 protects against experimental autoimmune myocarditis by regulating macrophage differentiation. Am J Pathol 2008;172: 1195-208.

88. Kania G, Blyszczuk P, Stein S, et al. Heart-infiltrating prominin-1+/CD133+ progenitor cells represent the cellular source of transforming growth factor β-mediated cardiac fibrosis in experimental autoimmune myocarditis. Circ Res 2009;105:462-70.

89. Blyszczuk P, Müller-Edenborn B, Valenta T, et al. Transforming growth factor-β-dependent Wnt secretion controls myofibroblast formation and myocardial fibrosis progression in experimental autoimmune myocarditis. Eur Heart J 2016 Apr 20 [E-pub ahead of print].

90. Afanasyeva M, Georgakopoulos D, Belardi DF, et al. Quantitative analysis of myocardial inflammation by flow cytometry in murine autoimmune myocarditis: correlation with cardiac function. Am J Pathol 2004;164:807-15.

91. Cihakova D, Rose NR. Pathogenesis of myocarditis and dilated cardiomyopathy. Adv Immunol 2008;99:95-114.

92. Huber SA. Viral myocarditis and dilated cardiomyopathy: etiology and pathogenesis. Curr Pharm Des 2016;22:408-26.

93. Lv H, Havari E, Pinto S, et al. Impaired thymic tolerance to α-myosin directs autoimmunity to the heart in mice and humans. J Clin Invest 2011;121: 1561-73.

94. Marty RR, Dirnhofer S, Mauermann N, et al. MyD88 signaling controls autoimmune myocarditis induction. Circulation 2006;113:258-65.

95. Nindl V, Maier R, Ratering D, et al. Cooperation of Th1 and Th17 cells determines transition from autoimmune myocarditis to dilated cardiomyopathy. Eur J Immunol 2012;42:2311-21.

96. Chen P, Baldeviano GC, Ligons DL, et al. Susceptibility to autoimmune myocarditis is associated with intrinsic differences in CD4+ T cells. Clin Exp Immunol 2012;169:79-88.

97. Eriksson U, Kurrer MO, Sebald W, et al. Dual role of the IL-12/IFN-γ axis in the development of autoimmune myocarditis: induction by IL-12 and protection by IFN-γ. J Immunol 2001;167:5464-9.

98. Barin JG, Baldeviano GC, Talor MV, et al. Fatal eosinophilic myocarditis develops in the absence of IFN-γ and IL-17A. J Immunol 2013;191: 4038-47.

99. Eriksson U, Kurrer MO, Bingisser R, et al. Lethal autoimmune myocarditis in interferon-γ receptor-deficient mice: enhanced disease severity by impaired inducible nitric oxide synthase induction. Circulation 2001;103:18-21.

100. Afanasyeva M, Wang Y, Kaya Z, et al. Interleukin-12 receptor/STAT4 signaling is required for the development of autoimmune myocarditis in mice by an interferon-γ-independent pathway. Circulation 2001;104:3145-51.

101. Silverio JC, Pereira IR, Cipitelli Mda C, et al. CD8+ T-cells expressing interferon gamma or perforin play antagonistic roles in heart injury in experimental Trypanosoma cruzi-elicited cardiomyopathy. PLoS Pathog 2012;8:e1002645.

102. Kania G, Siegert S, Behnke S, et al. Innate signaling promotes formation of regulatory nitric oxide-producing dendritic cells limiting T-cell expansion in experimental autoimmune myocarditis. Circulation 2013;127:2285-94.

103. Rangachari M, Mauermann N, Marty RR, et al. T-bet negatively regulates autoimmune myocarditis by suppressing local production of interleukin 17. J Exp Med 2006;203:200-19.

104. Baldeviano GC, Barin JG, Talor MV, et al. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. Circ Res 2010;106:1646-55.

105. Myers JM, Cooper LT, Kem DC, et al. Cardiac myosin-Th17 responses promote heart failure in human myocarditis. JCI Insight 2016;1:e85851.

106. Ameling S, Herda LR, Hammer E, et al. Myocardial gene expression profiles and cardiodepressant autoantibodies predict response of patients with dilated cardiomyopathy to immunoadsorption therapy. Eur Heart J 2013;34: 666-75.

107. Staudt A, Böhm M, Knebel F, et al. Potential role of autoantibodies belonging to the immunoglobulin G-3 subclass in cardiac dysfunction among patients with dilated cardiomyopathy. Circulation 2002;106:2448-53.

108. Caforio AL, Angelini A, Blank M, et al. Passive transfer of affinity-purified anti-heart autoantibodies (AHA) from sera of patients with myocarditis induces experimental myocarditis in mice. Int J Cardiol 2015;179:166-77.

109. Okazaki T, Tanaka Y, Nishio R, et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. Nat Med 2003;9:1477-83.

110. Limas CJ, Goldenberg IF, Limas C. Autoantibodies against beta-adrenoceptors in human idiopathic dilated cardiomyopathy. Circ Res 1989; 64:97-103.

111. Cocker MS, Abdel-Aty H, Strohm O, et al. Age and gender effects on the extent of myocardial involvement in acute myocarditis: a cardiovascular magnetic resonance study. Heart 2009;95: 1925-30.

112. Fairweather D, Cooper LT Jr., Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. Curr Problems Cardiol 2013;38: 7-46.

113. Frisancho-Kiss S, Nyland JF, Davis SE, et al. Sex differences in coxsackievirus B3-induced myocarditis: IL-12Rβ1 signaling and IFN-γ increase inflammation in males independent from STAT4. Brain Res 2006;1126:139-47.

114. Frisancho-Kiss S, Davis S, Nyland J, et al. Cutting edge: cross-regulation by TLR4 and T cell Ig mucin-3 determines sex differences in inflammatory heart disease. J Immunol 2007;178: 6710-4.

115. Frisancho-Kiss S, Nyland JF, Davis SE, et al. Cutting edge: T cell Ig mucin-3 reduces inflammatory heart disease by increasing CTLA-4 during innate immunity. J Immunol 2006;176:6411-5.

116. Li K, Xu W, Guo Q, et al. Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3 defines susceptibility to viral myocarditis. Circ Res 2009;105: 353-64.

117. Liu L, Yue Y, Xiong S. NK-derived IFN-γ/IL-4 triggers the sexually disparate polarization of macrophages in CVB3-induced myocarditis. J Mol Cell Cardiol 2014;76:15-25.

118. van Rooij E, Marshall WS, Olson EN. Toward microRNA-based therapeutics for heart disease: the sense in antisense. Circ Res 2008;103:919-28.

119. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature 2011; 469:336-42.

120. Janssen HL, Reesink HW, Lawitz EJ, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med 2013;368:1685-94.

121. Corsten MF, Papageorgiou A, Verhesen W, et al. MicroRNA profiling identifies microRNA-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. Circ Res 2012;111:415-25.

122. Yan L, Hu F, Yan X, et al. Inhibition of microRNA-155 ameliorates experimental autoimmune myocarditis by modulating Th17/Treg immune response. J Mol Med (Berl) 2016;94: 1063-79.

123. Zhang Y, Zhang M, Li X, et al. Silencing microRNA-155 attenuates cardiac injury and dysfunction in viral myocarditis via promotion of M2 phenotype polarization of macrophages. Sci Rep 2016;6:22613.

124. Liu YL, Wu W, Xue Y, et al. MicroRNA-21 and -146b are involved in the pathogenesis of murine viral myocarditis by regulating TH-17 differentiation. Arch Virol 2013;158:1953-63.

125. Navarro IC, Ferreira FM, Nakaya HI, et al. MicroRNA Transcriptome profiling in heart of Trypanosoma cruzi-infected mice: parasitological and cardiological outcomes. PLoS Negl Trop Dis 2015;9:e0003828.

126. Corsten M, Heggermont W, Papageorgiou AP, et al. The microRNA-221/-222 cluster balances the antiviral and inflammatory response in viral myocarditis. Eur Heart J 2015;36:2909-19.

127. Heymans S, Corsten MF, Verhesen W, et al. Macrophage microRNA-155 promotes cardiac hypertrophy and failure. Circulation 2013;128: 1420-32.

128. Corsten MF, Dennert R, Jochems S, et al. Circulating MicroRNA-208b and MicroRNA-499 reflect myocardial damage in cardiovascular disease. Circ Cardiovasc Genet 2010;3:499-506.

129. Devaux Y, Vausort M, Goretti E, et al. Use of circulating microRNAs to diagnose acute myocardial infarction. Clin Chem 2012;58:559–67.

130. Kuehl U, Lassner D, Gast M, et al. Differential cardiac microRNA expression predicts the clinical course in human enterovirus cardiomyopathy. Circ Heart Fail 2015;8:605–18.

131. Imazio M, Brucato A, Barbieri A, et al. Good prognosis for pericarditis with and without myocardial involvement: results from a multicenter, prospective cohort study. Circulation 2013; 28:142–9.

132. Foerster SR, Canter CE, Cinar A, et al. Ventricular remodeling and survival are more favorable for myocarditis than for idiopathic dilated cardiomyopathy in childhood: an outcomes study from the Pediatric Cardiomyopathy Registry. Circ Heart Failure 2010;3:689–97.

133. Gilotra NA, Minkove N, Bennett MK, et al. Lack of relationship between serum cardiac troponin I level and giant cell myocarditis diagnosis and outcomes. J Card Fail 2016;22:583–5.

134. Escher F, Kasner M, Kühl U, et al. New echocardiographic findings correlate with intramyocardial inflammation in endomyocardial biopsies of patients with acute myocarditis and inflammatory cardiomyopathy. Mediators Inflamm 2013;2013:875420.

135. Uppu SC, Shah A, Weigand J, et al. Two-dimensional speckle-tracking-derived segmental peak systolic longitudinal strain identifies regional myocardial involvement in patients with myocarditis and normal global left ventricular systolic function. Pediatr Cardiol 2015;36:950–9.

136. Ukena C, Mahfoud F, Kindermann I, et al. Prognostic electrocardiographic parameters in patients with suspected myocarditis. Eur J Heart Failure 2011;13:398–405.

137. Lurz P, Luecke C, Eitel I, et al. Comprehensive cardiac magnetic resonance imaging in patients with suspected myocarditis: the MyoRacer-Trial. J Am Coll Cardiol 2016;67:1800–11.

138. Raimondi F, Iserin F, Raisky O, et al. Myocardial inflammation on cardiovascular magnetic resonance predicts left ventricular function recovery in children with recent dilated cardiomyopathy. Eur Heart J Cardiovasc Imaging 2015; 16:756–62.

139. Friedrich MG, Sechtem U, Schulz-Menger J, et al., for the International Consensus Group on Cardiovascular Magnetic Resonance in Myocarditis. Cardiovascular magnetic resonance in myocarditis: a JACC White Paper. J Am Coll Cardiol 2009;53:1475–87.

140. Vermes E, Childs H, Faris P, et al. Predictive value of CMR criteria for LV functional improvement in patients with acute myocarditis. Eur Heart J Cardiovasc Imaging 2014;15:1140–4.

141. Schumm J, Greulich S, Wagner A, et al. Cardiovascular magnetic resonance risk stratification in patients with clinically suspected myocarditis. J Cardiovas Magn Reson 2014;16:14.

142. Ekström K, Lehtonen J, Hänninen H, et al. Magnetic resonance imaging as a predictor of survival free of life-threatening arrhythmias and

transplantation in cardiac sarcoidosis. J Am Heart Assoc 2016;5:e003040.

143. Youssef G, Leung E, Mylonas I, et al. The use of 18F-FDG PET in the diagnosis of cardiac sarcoidosis: a systematic review and metaanalysis including the Ontario experience. J Nucl Med 2012;53:241–8.

144. Tung R, Bauer B, Schelbert H, et al. Incidence of abnormal positron emission tomography in patients with unexplained cardiomyopathy and ventricular arrhythmias: the potential role of occult inflammation in arrhythmogenesis. Heart Rhythm 2015;12:2488–98.

145. Birnie DH, Sauer WH, Bogun F, et al. HRS expert consensus statement on the diagnosis and management of arrhythmias associated with cardiac sarcoidosis. Heart Rhythm 2014;11:1305–23.

146. Kadkhodayan A, Chareonthaitawee P, Raman SV, Cooper LT. Imaging of inflammation in unexplained cardiomyopathy. J Am Coll Cardiol Img 2016;9:603–17.

147. Blankstein R, Osborne M, Naya M, et al. Cardiac positron emission tomography enhances prognostic assessments of patients with suspected cardiac sarcoidosis. J Am Coll Cardiol 2014;63: 329–36.

148. Aggarwal NR, Snipelisky D, Young PM, et al. Advances in imaging for diagnosis and management of cardiac sarcoidosis. Eur Heart J Cardiovasc Imaging 2015;16:949–58.

149. Sagar S, Liu PP, Cooper LT Jr. Myocarditis. Lancet 2012;379:738–47.

150. Shields RC, Tazelaar HD, Berry GJ, et al. The role of right ventricular endomyocardial biopsy for idiopathic giant cell myocarditis. J Card Fail 2002; 8:74–8.

151. Cooper LT Jr. Role of left ventricular biopsy in the management of heart disease. Circulation 2013;128:1492–4.

152. Kandolin R, Lehtonen J, Kupari M. Cardiac sarcoidosis and giant cell myocarditis as causes of atrioventricular block in young and middle-aged adults. Circ Arrhythm Electrophysiol 2011;4:303–9.

153. Sperry BW, Oldan J, Hachamovitch R, et al. Insights into biopsy-proven cardiac sarcoidosis in patients with heart failure. J Heart Lung Transplant 2015;35:392–3.

154. Liang JJ, Hebl VB, DeSimone CV, et al. Electrogram guidance: a method to increase the precision and diagnostic yield of endomyocardial biopsy for suspected cardiac sarcoidosis and myocarditis. J Am Coll Cardiol HF 2014;2:466–73.

155. Mahfoud F, Gärtner B, Kindermann M, et al. Virus serology in patients with suspected myocarditis: utility or futility? Eur Heart J 2011;32: 897–903.

156. Donoso Mantke O, Nitsche A, Meyer R, et al. Analysing myocardial tissue from explanted hearts of heart transplant recipients and multi-organ donors for the presence of parvovirus B19 DNA. J Clin Virol 2004;31:32–9.

157. Kuethe F, Lindner J, Matschke K, et al. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. Clin Infect Dis 2009;49:1660–6.

158. Moimas S, Zacchigna S, Merlo M, et al. Idiopathic dilated cardiomyopathy and persistent viral infection: lack of association in a controlled study using a quantitative assay. Heart Lung Circ 2012; 21:787–93.

159. Lotze U, Egerer R, Glück B, et al. Low level myocardial parvovirus B19 persistence is a frequent finding in patients with heart disease but unrelated to ongoing myocardial injury. J Med Virol 2010;82:1449–57.

160. Bonvicini F, Manaresi E, Di Furio F, et al. Parvovirus B19 DNA CpG dinucleotide methylation and epigenetic regulation of viral expression. PLoS One 2012;7:e33316.

161. Escher S, Kuhl U, Lassner D, et al. Analysis of endomyocardial biopsies in cardiomyopathies: diagnostic value of left versus right ventricular biopsy (abstr). J Am Coll Cardiol 2015;65 Suppl 10: A930.

162. Bock CT, Klingel K, Kandolf R. Human parvovirus B19-associated myocarditis. N Engl J Med 2010;362:1248–9.

163. Lassner D, Kühl U, Siegismund C, et al. Improved diagnosis of idiopathic giant cell myocarditis and cardiac sarcoidosis by myocardial gene expression profiling. Eur Heart J 2014;35:2186–95.

164. Kandolin R. Cardiac Sarcoidosis and Giant Cell Myocarditis in Finland [dissertation]. Helsinki, Finland: Universitatis Helsinkiensis, 2015.

165. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013;62:e147–239.

166. Pinto YM, Elliott PM, Arbustini E, et al. Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases. Eur Heart J 2016;37: 1850–8.

167. Wojnicz R, Nowalany-Kozielska E, Wojciechowska C, et al. Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. Circulation 2001;104: 39–45.

168. Frustaci A, Russo MA, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study. Eur Heart J 2009;30:1995–2002.

169. Schultheiss H, Piper C, Sowade O, et al. Betaferon in chronic viral cardiomyopathy (BICC) trial: effects of interferon-β treatment in patients with chronic viral cardiomyopathy. Clin Res Cardiol 2016;105:763–73.

170. Dennert R, Velthuis S, Schalla S, et al. Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high PVB19 viral load. Antivir Ther 2010;15:193–201.

171. Maisch B, Hufnagel G, Kölsch S, et al. Treatment of inflammatory dilated cardiomyopathy and

(peri)myocarditis with immunosuppression and i.v. immunoglobulins. Herz 2004;29:624–36.

172. McNamara DM, Holubkov R, Starling RC, et al. Controlled trial of intravenous immune globulin in recent-onset dilated cardiomyopathy. Circulation 2001;103:2254–9.

173. Blauwet LA, Cooper LT. Idiopathic giant cell myocarditis and cardiac sarcoidosis. Heart Fail Rev 2012;18:733–46.

174. Kandolin R, Lehtonen J, Kupari M. Cardiac sarcoidosis. J Intern Med 2016;280:129–31.

175. Cooper LT, Berry GJ, Shabetai R, for the Multicenter Giant Cell Myocarditis Study Group Investigators. Giant cell myocarditis: natural history and treatment. N Engl J Med 1997;336:1860–6.

176. Bozkurt B, Colvin M, Cooper LT, et al., on behalf of the American Heart Association Committee on Heart Failure and Transplantation of the Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Epidemiology and Prevention; and Council on Quality of Care and Outcomes Research. Current diagnostic and treatment strategies for specific dilated cardiomyopathies: a scientific statement from the American Heart Association. Circulation 2016 Nov 3 [E-pub ahead of print].

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