Myocarditis
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Abstract: Viral myocarditis remains a prominent infectious-inflammatory disease for patients throughout the lifespan. The condition presents several challenges including varied modes of clinical presentation, a range of timepoints when patients come to attention, a diversity of approaches to diagnosis, a spectrum of clinical courses, and unsettled perspectives on therapeutics in different patient settings and in the face of different viral pathogens. In this review, we examine current knowledge about viral heart disease and especially provide information on evolving understanding of mechanisms of disease and efforts by investigators to identify and evaluate potential therapeutic avenues for intervention. (Circ Res. 2016;118:496-514. DOI: 10.1161/CIRCRESAHA.115.306573.)

Key Words: cardiovascular infections ■ communicable diseases ■ heart diseases ■ myocarditis ■ virus diseases

Definitions
In 1749, inflammation of the heart and the difficulty in discerning such was described by a physician, Jean Baptiste Senac in Versailles, France, in his work entitled Traité des Maladies du Coeur (Treatise on Disease of the Heart). The term myocarditis was ultimately coined by Joseph Friedrich Sobernheim in 1837; however, the use of this term included other cardiomyopathies that were previously undocumented including ischemic heart disease and hypertensive heart disease. It was not until the 1980s that the World Health Organization and the International Society and Federation of Cardiology attempted to differentiate between myocarditis and other cardiomyopathies.1 In general, myocarditis is identified as an inflammatory disease of the heart muscle cells and is pathologically identified by conventional histology and immunohistochemical techniques as an infiltration of mononuclear cells to the myocardium. Myocarditis can be acute, subacute, or chronic and may either involve focal or diffuse areas of the myocardium. A recent update to the definition of myocarditis has been discussed by Caforio et al2 in defining myocarditis, using immunohistochemical data, as individuals who exhibit ≥14 lymphocytes/mm² including ≤4 monocytes/mm² with the presence of CD3-positive T lymphocytes ≥7 cells/mm². This definition uses immunohistochemical data that require endomyocardial biopsy (EMB) collection and thus is limited to a relatively smaller cohort of patients or postmortem autopsy samples. Moreover, although this definition of myocarditis has been widely accepted,2-4 it lacks information on the complexity of cellular infiltrates such as macrophage subtypes (classical/intermediate/nonclassical), effector (Th1/Th2/Th17), and regulatory (FoxP3+/CD4+) T-lymphocyte subtypes, and thus fails to differentiate a profibrotic response from a healing inflammatory response. Transcriptome-based analysis of biopsies may further our definition of myocarditis.5

Patients of suspected myocarditis are clinically evaluated to distinguish fulminant lymphocytic myocarditis from acute lymphocytic myocarditis. In the case of fulminant myocarditis, patients exhibit New York Heart Association class IV symptoms, such as flu-like symptoms with left ventricle (LV) systolic dysfunction and cardiogenic shock.6 Other characteristics include leukocytosis, eosinophilia (including rare cases of eosinophilic myocarditis), elevated erythrocyte sedimentation rate, and increased levels of cardiac troponin or the creatine kinase biomarker. Fulminant myocarditis may possess multiple foci of active myocarditis that typically can resolve within 6 months. Less frequently, giant cell myocarditis has been associated with fulminant acute myocarditis. In contrast, nonfulminant myocarditis may be acute or chronic myocarditis that often progresses in an insidious manner. Although both acute and chronic myocarditis can be inferred on echocardiography as heart failure (HF) with LV dysfunction, acute myocarditis may lead to complete resolution or stable dilated cardiomyopathy (DCM), whereas chronic active myocarditis is defined as an ongoing myocarditis with visible fibrosis and may include giant cells. The development of new molecular techniques such as miRNA profiling, nested polymerase chain reaction, and in situ hybridization has improved accuracy of diagnosis and prognostic value of EMB samples significantly, allowing for improved definitions of the various types of myocarditis, including less prevalent subtypes of myocarditis such as eosinophilic and giant cell myocarditis.7

In this review, we will highlight key research findings in the epidemiology, causes, presentations, mechanisms, and treatments of myocarditis. Furthermore, we will discuss the
use of traditional and emerging techniques to identifying suspected myocarditis patients. Consolidating our understanding of myocarditis may aid in new research that can spearhead challenging questions and overcome obstacles in the field, especially in better defining the wide range of causative agents and clinical presentations of myocarditis.

**Scope of Importance (Epidemiology, Cause, and Consequences)**

The clinical manifestations of myocarditis are heterogeneous, ranging from virtually asymptomatic states with vague signs and symptoms to severe myocardial destruction by virus and immune cells yielding cardiogenic shock and arrhythmias. Actual patient cases of myocarditis are estimated to be significantly underestimated. A recent study using International Classification of Diseases (ninth revision) codes estimated the global prevalence of myocarditis to be ≈22 of 100,000 patients annually. In addition, recent studies from the American Heart Association and American College of Cardiology rank myocarditis as the third leading cause of sudden cardiac death in competitive athletes. Approximately 1% to 5% of patients that test positive for acute viral infection(s) may exhibit a form of myocarditis. In a study investigating individuals suspected of myocarditis from >670,000 male military recruits with a mean age of 20 years, 98 cases had myocarditis symptomatically similar to myocardial ischemia, 1 case had sudden death, and 9 cases had DCM within the initial stages of clinical disease.

Furthermore, Japanese investigators revisited 20 years of autopsy records and reported an incidence of 0.11% and 0.007% for non-specific myocarditis and giant cell myocarditis, respectively. Another retrospective study of 17,162 autopsy post-mortem records demonstrated that myocarditis can be easily overlooked in an unsuspected number of cases if a standardized method of sampling is not followed, suggesting that myocarditis prevalence is often underestimated. In the European Study of Epidemiology and Treatment of Inflammatory Heart Disease, 3,055 patients with suspected acute or chronic myocarditis were screened, whereupon 72% had dyspnea, 32% had chest pains, and 18% had arrhythmias. The incidence rates for different demographics, ethnicities, sexes, and ages differ; however, young men have a relatively higher incidence rate. A study by Kyto et al evaluating 3,274 Finnish hospital treatments of myocarditis cases revealed that myocarditis is significantly more common in male than in female controls. Female patients with myocarditis often display a more severe disease presentation (worsened ventricular tachycardia, ventricular fibrillation, and resuscitation complicated myocarditis) at an older age. More specifically, patients with a clinical manifestation of lymphocytic myocarditis have a median age of 42 years, whereas those with giant cell myocarditis have a median age of 43 years. In contrast, neonates and children exhibit a more fulminant myocarditis and are typically more susceptible to virus-induced pathogenesis in myocarditis than adults. Most studies of acute myocarditis report a greater prevalence and severity in male patients, speculated to be caused by a protective effect of natural hormonal influences on immune responses in women when compared with men. A detailed overview of age and sex difference in myocarditis is provided by Fairweather et al.

Myocarditis can be caused by a broad range of infectious agents, including viruses, bacteria, Chlamydia, rickettsia, fungi, and protozoa, as well as noninfectious triggers, such as toxins and hypersensitive reactions. Among these triggers, viral infection has been documented to constitute the most prevalent cause of myocarditis, particularly in children. A wide spectrum of viral genomes in the endomyocardial specimens of patients with clinically suspected myocarditis or DCM has been identified by polymerase chain reaction and virus-specific in situ hybridization, which includes enterovirus, parvovirus B19 (PVB19), adenovirus, influenza A virus, human herpes virus (HHV), Epstein–Barr virus, cytomegalovirus, hepatitis C virus, and HIV (Table 1).

Between 1980 and 1990, virus-induced myocarditis was most associated with enterovirus and adenovirus infections. As molecular technologies continued to improve, viral-induced myocarditis epidemiology in certain geographical locations shifted predominantly toward PVB19 and HHV6 infections. However, the geographical distribution of cardiotropic viral infections that induce cardiomyopathy is still in debate. In a study performed by Andreoletti et al, active coxsackievirus type B (CVB) genomes and viral capsid proteins, particularly VP1, were observed in immunostained sections of myocardial tissue, and it was concluded that CVB had induced myocarditis. Furthermore, Yilmaz et al demonstrated that endomyocardial biopsies performed in Germany consistently, and almost exclusively, tested positive for PVB19 or HHV6, and that positive detection of enterovirus genomes was rare. These discrepancies are speculated to be either derived from technical issues involving unspecific cross-reactivity of antibodies with necrotic or apoptotic human cardiomyocytes or on the possibility that cardiotropic viruses exist along the French–German border. In fact, the waves of outbreaks in human viral diseases documented over the years by agencies like the Centers for Disease Control in the United States would suggest a variable series of outbreaks, from time to time, and from region to region. Coinfections by various cardiotropic viruses have also become an attractive hypothesis for the confusing epidemiological variability. In 2006, hepatitis C virus was detected in 4.4% of Japanese patients with myocardial manifestations by using antibodies specific for hepatitis C virus epitopes.
A virus–related myocarditis. These studies raise a striking concern over studies investigating the prevalence of synergistic viral infections in confirmed myocarditis patients would be of high value to understanding virus-induced myocarditis. Given that clinical manifestations elicited by such a wide spectrum of causative agents, ranging from asymptomatic to severe cardiac damage with conduction and other electrophysiological abnormalities, sudden death or HF; and a range of magnitude in immune responses, researchers must adopt an international, cooperative, and consistent approach to characterizing myocarditis. Ideally, this global approach must be comprehensive and standardized from a virological, immunologic, pathological, and clinical point of view; however, such comprehensive international study will be financially demanding and difficult to coordinate.

### Clinical Syndromes (Presentations)

#### Variability

No population-based epidemiological study has comprehensively documented the range of clinical presentations of acute and chronic myocarditis, likely because of 2 factors: the protein presentation of patients continues to challenge healthcare professionals in their differential diagnostic efforts, and the lack of reliable noninvasive tests reduces the yield conclusive diagnostics or accurate prognostic value. The gold standard for diagnosing suspected myocarditis remains the EMB; however, improved molecular and histological tests using tissue samples reveal great variability, partly because of a variety of factors including interobserver variability, tissue quality, stage of presentation, causes, and disease severity. Similarly, myocardial inflammation and injury may be diffuse or focal, involving any of the cardiac chamber walls to greater or lesser degrees concurrently, and at various stages of disease.

Differential diagnosis of myocarditis is a great concern because of the heterogeneous nature of the clinicopathologic scenarios. For example, Pieroni et al has recently demonstrated that patients with right ventricular myocarditis are indistinguishable from arhythmogenic right ventricular cardiomyopathy on the basis of clinical features, presence, and severity of structural and functional right ventricle abnormalities. Other study reported similar observations that myocarditis involving the right ventricle can mimic arhythmogenic right ventricular cardiomyopathy. Myocarditis may also present similarly to ischemic heart disease with chest pain, abnormalities on electrocardiograms, and elevated cardiac biomarkers. Furthermore, myocarditis may seem similar to other causes of HF, including cardiac amyloidosis and hypertrophic cardiomyopathy. In all, thorough consideration must be taken when firmly diagnosing suspected myocarditis. Although the real prevalence of myocarditis is likely underestimated, its presentation, features, and course remain a challenge to be resolved.

The majority of individuals with acute myocarditis who present with acute DCM have a surprisingly mild disease that may resolve within a few days. Frequently, these individuals are experiencing hypersensitivity myocarditis in the context of peripheral eosinophilia and fever, often with recent initiation of new medication(s). Yet, patients who present with giant cell myocarditis and eosinophilia myocarditis are at high risk of downstream difficulties. These patients may also present with thymoma, autoimmune disorders, ventricular tachyarrhythmia, or advanced heart block. Moreover, for patients who fail to respond positively to standard supportive care, cardiac sarcoidosis should be considered in patients with DCM, chronic HF, or ventricular arrhythmias.
Many cases of myocarditis likely go undetected because of asymptomatic presentations or nonspecific symptoms. For example, a recent study suggests that smallpox vaccination results in ≈1:200 patients having elevated troponin levels, whereas the prevalence of myocarditis has been estimated at ≈1:5500. Creatine kinase was previously a preferred biomarker for myocarditis; however, its sensitivity in late diagnosis is poor because studies show that creatine kinase decreases steadily back to basal levels by ≈36 hours. Elevated levels of soluble ST2, a truncated soluble receptor of interleukin-33, have also recently been demonstrated to be correlated with HF and DCM cases and have been gaining popularity for detecting HF. However, measuring levels of biomarkers in patients presenting with suspected myocarditis remains low in dependability but may still aid in confirming a diagnosis of myocarditis. Other nonspecific serological markers of inflammation including leukocyte count and C-reactive protein may be elevated in suspected myocarditis. However, non-elevated levels fail to rule out an inflammatory response in the myocardium, suggesting that serological tests for myocarditis remain a poorly developed method of diagnosis, with relatively low prognostic value.

**ECG**

One approach to distinguishing between patients presenting with acute myocardial infarction or myocarditis is by obtaining an ECG. Despite the low sensitivity of electrocardiograms, it is widely used as an initial screening tool. Electrocardiograms of confirmed myocarditis patients may exhibit nonspecific T waves and ST-segment changes including ST-segment elevation. Recently, a study by Ukena et al investigated the prognostic value of electrocardiography in patients suspected to have myocarditis and found that a prolonged QRS duration is a significant independent predictor for cardiac death or heart transplantation. In cases of acute myocarditis, electrocardiograms may show sinus tachycardia. In general, changes in electrocardiograms suggest signs of myocardial infarction with ST-segment elevation. Electrocardiograms provide a convenient tool for risk stratification and initial screening but provide weak diagnostic value.

**Echocardiography**

Echocardiography is a useful measurement tool in the diagnostic assessment of suspected myocarditis and to rule out other causes of HF. Echocardiography investigates cardiac chamber sizes, wall thicknesses, and systolic and diastolic functions, and thus does not provide direct evidence of myocarditis. However, patients with fulminant myocarditis often lack cardiac dilation and exhibit increased septal thickness, whereas patients with acute myocarditis may exhibit a spherical-shaped ventricle that remodels to a more elliptical shape over the period of a few months with normal wall thickness. In addition, patients with fulminant myocarditis exhibit substantial recovery in ventricular function by 6 months when compared with patients with acute myocarditis.

Speckle tracking has recently been a useful technique in diagnosing patients with acute myocarditis. A recent study by Hsiao et al demonstrated that LV strain and strain rate may be useful measurements for diagnosis of suspected myocarditis patients, even those with preserved LV ejection fraction (LVEF). In addition, speckle tracking is useful for predicting deterioration and overall event-free survival. In general, strain rate and global strain values were decreased in myocarditis patients when compared with healthy individuals.

**Cardiac Magnetic Resonance Imaging**

To date, cardiac magnetic resonance imaging (cMRI) is mostly used to detect significantly decreased EFs and abnormalities in wall motion. Contrast enhancement (CE) CMR is a more sensitive technique of cMRI and can detect areas of myocardial damage in patients with acute myocarditis. Studies evaluating the diagnostic value of cMRI demonstrate that late gadolinium enhancement imaging is a superior method in diagnosing myocarditis because of the improved difference in signal intensity between myocardial regions with and without gadolinium-diethylenetriaminepentaacetate. Moreover, cMRI sensitivity and specificity are as high as 100% and 90%, respectively, when compared with histology confirmed samples identified by CE-CMR. In patients with acute myocarditis, areas of CE are often located in the lateral wall originating from the epicardial quartile, even if the pattern of myocardial injury is influenced by viral infection. This technique also rules out ischemic causes because CE in ischemic infarction will include subendocardial layers of the myocardium. From our observations and understanding, eosinophilic myocarditis is the only form of myocarditis with striking involvement of the endocardium.

A study conducted by Mahrholdt et al demonstrated that late gadolinium enhancement imaging of confirmed virus-induced myocarditis exhibited either a rim-like pattern in the septal wall or a subepicardial distribution in the free LV lateral wall that recovered within months of detection in the case of PVB19, whereas patients coinfected with both PVB19 and HHV6 progressed toward chronic HF. CMRI can also provide indirect evidence of viral involvement because PVB19 patients exhibit CE most frequently in the lateral wall, whereas HHV6 exhibits CE in the midwall of the interventricular septum. The caveat for cMRI is that it lacks the ability to determine the magnitude of myocardial inflammation and depends heavily on the clinical context. Moreover, the high sensitivity of cMRI is only found in cases of acute myocarditis because diagnostic features of chronic myocarditis resolve after several weeks to months. However, because patients presenting with suspected chronic myocarditis often lack viremia, chronic myocarditis can be inferred when chronic HF or myocardial fibrosis can be excluded. CMRI is a reliable method of detecting myocardial fibrosis in DCM. A study by De Cobelli et al was able to use CE to identify areas of myocardial inflammation in 70% of biopsy-proven chronic myocarditis. To date, available data on the diagnostic accuracy of cMRI are limited although CE-CMR is becoming useful in obtaining accurate and reliable preliminary information in a noninvasive manner. Because of the ability of CE-CMR to locate areas of inflammation, it can serve as a guidance for EMB sampling when necessary, thus enhancing the diagnostic accuracy of biopsies for further testing using conventional histopathology and molecular...
polymerase chain reaction techniques for cellular infiltrates and viral presence.\textsuperscript{96}

**Endomyocardial Biopsy**

EMB positivity in acute myocarditis patients is defined as lymphocytic infiltration in association with myocyte necrosis/death (Dallas Criteria). Patients exhibiting borderline myocarditis are defined as those with lymphocytic infiltration in the absence of myocyte necrosis.\textsuperscript{60} EMB is currently the best method to differentiate between giant cell myocarditis and sarcoidosis. For example, giant cell myocarditis exhibits an abundance in eosinophils, the lack of sarcoidosis or granulomas, and never affects the pericardium. In addition, eosinophilic myocarditis is best identified by myocyte necrosis accompanied by the presence of intracavitary thrombi containing eosinophils observed in the lumen of intramyocardial coronary vessels. Also, an observational study investigating patients with progressive HF and EMB-confirmed lymphocytic myocardial infiltration showed that the positive detection of enterovirus genomes in ventricular myocardium correlated with worsened outcomes.\textsuperscript{61} A study by Frustaci et al\textsuperscript{60} treated 41 patients, presenting with progressive HF, with immunosuppressive therapy (prednisone and azathioprine) for 6 months. Of 41 patients, 21 exhibited improved LVEFs, whereas 20 failed to respond. Of these 20 patients who failed to respond to immunosuppressive therapy, 17 patients were detected positive for enteroviral genomes, whereas only 3 patients who responded to treatments tested positive for hepatitis C virus genomes. Consistent with this observation, Kühl et al\textsuperscript{27} demonstrated that viral persistence in the myocardium is associated with compromised LV function, whereas patients with viral clearance have improved LV function. In contrast, there are many examples of patients who tested positive for viral genomes including HHV6 and PVB19, yet had noted absence of infiltrating cellular immune responses in the myocardium and did not satisfy the Dallas criteria for myocarditis.\textsuperscript{62-65}

It is important to note that the use of EMB varies widely based on the physician and clinical presentation of the patients. For example, the European Society of Cardiology recommends the use of EMB as early as possible for histology,\textsuperscript{2} immunohistochemistry, and viral polymerase chain reaction analysis. Whereas the American College of Cardiology/American Heart Association recommends EMB based on 3 classes of comprehensive clinical presentations, risks, and measurements using noninvasive techniques.\textsuperscript{56} In brief, the use of EMB by the European Society of Cardiology and American College of Cardiology/American Heart Association both aim to clearly define myocarditis with the least amount of risk to the patient, but vary in the timing of EMB sampling. It is worth noting that the sensitivity of the EMB for detection of myocarditis is rather low.\textsuperscript{66a}

Immunohistochemistry on EMB samples has been gaining popularity in characterizing myocarditis. Caforio et al\textsuperscript{16} have demonstrated that immunohistochemistry yields increased sensitivity and shows that more than half of previous cases of borderline myocarditis diagnosis would be missed by hematoxylin-eosin alone. Moreover, Kindermann et al\textsuperscript{66} show that 69 of 181 (38%) EMB samples are positive for myocarditis using the Dallas criteria, whereas 91 of the 181 (50%) had positive staining for CD3, CD68 (macrophages), and human leukocyte antigen class II antibodies. Currently, endomyocardial biopsies provide relatively high diagnostic and prognostic value in suspected myocarditis patients with low complication rates of <1%.\textsuperscript{63,67}

**Micro-RNA Profiling**

Evolving molecular technologies have led to the characterization of miRNA profiles among acute, chronic, and fulminant myocarditis, including a relationship to the severity of myocardial damage. For example, miR-208b and miR-499 are upregulated upon myocardial damage and can be detected in plasma of myocarditis patients.\textsuperscript{68} Furthermore, although miR-208b is upregulated in myocarditis patients, simultaneous upregulation of both miR-208b and miR-499 is only observed in fulminant virus-induced myocarditis.\textsuperscript{68} Other miRNAs demonstrated to be altered in acute myocarditis patients include miR-155, miR-21, miR-146b, miR-511, and miR-212.\textsuperscript{69-71} Currently, no miRNAs have been found to be specific for patients with chronic myocarditis. The altered expression of specific miRNAs during the pathological progression of myocarditis makes miRNAs interesting future targets for treating and diagnosing suspected myocarditis patients with greater accuracy.

**Mechanisms**

A definitive cause–effect relationship between most cardiotropic viruses detected in the heart, including PVB19, the most commonly identified virus in EMB samples has not been established; however, the role of enterovirus infection, in particular CVB3, in the development of myocarditis and DCM has been studied thoroughly in both animal models and tissue culture cells.\textsuperscript{72} Here, we will discuss recent advances in understanding the molecular mechanisms of viral myocarditis and progression to DCM using CVB3 as a model system. At the cellular and tissue levels, the pathological progression of viral myocarditis consists of 3 stages: the acute stage triggered by viral entry and replication, the subacute stage characterized by inflammatory cell infiltration, and the chronic stage featuring cardiac remodeling.\textsuperscript{72} It is well known that the pathogenesis of viral myocarditis is caused by both direct injury mediated by viral infection and indirect damage secondary to the immune responses of the host.\textsuperscript{73} The pathogenic processes discussed here are recognized to be downstream of the encounter between host and virus that includes an enteric portal and involvement of the immune system structurally and functionally at early points in the deadly duel of virus and host.\textsuperscript{74,75}

**Virus-Induced Direct Damage**

There is considerable evidence that reflects an important role for the direct effect of viral infection on cardiomyocytes in the development of viral myocarditis. Early research has shown that CVB3 infection is sufficient to induce myocardial injury in severe combined immune deficient mice in which mature T and B lymphocytes are absent.\textsuperscript{76} It was also demonstrated that cardiac-specific expression of a replication-restricted CVB3 genome in mice is able to induce a DCM phenotype in the absence of an apparent host immune response.\textsuperscript{77} Other evidence of the contribution of viral direct damage on the
myocardium is provided by the findings that elimination of the coxsackievirus-adenovirus receptor (CAR) specifically in the adult mouse hearts completely blocks viral infection of myocardial cells and virus-induced inflammation in the myocardium.78 These CVB3-infected mice exhibit improved cardiac function, whereas other tissues (eg, pancreas and spleen) that also express CAR are CVB3 positive.78 This study suggests that viral infection of cardiomyocytes and consequent productive viral replication within the myocardium is required for the development of myocarditis. CAR, a transmembrane protein in the family of adhesion molecules, localized predominantly at the intercalated disc and the cell–cell junctions of the AV node in the adult heart is required for virus entry into different cell types.79,80 Studies from independent research groups using CAR-deficient mice have revealed an important role of the CAR in embryonic development and the maintenance of normal AV conduction and cardiac function.79-81 The observation of increased expression of CAR in the heart of patients with DCM and relatively high levels of CAR in young adult heart may partially explain the increased susceptibility of young hearts to myocarditis.82 In addition to CAR, viral entry or virulence of CVB3 is also determined by decay-accelerating factor (CD55) coreceptor in certain cell types.83 For instance, CVB3 binding with decay-accelerating factor on the apical surface of epithelial cells promotes the interaction between virus and CAR, which is localized to the tight junction; normally inaccessible to virus.84 The mechanisms underlying direct myocardial injury have been extensively studied. Enteroviruses encode 2 proteinases; 2A and 3C, which are crucial in the completion of the full viral lifecycle by processing viral polyprotein into individual structural and nonstructural proteins. These viral proteinases also contribute to the cellular and molecular phenotype of myocarditis by targeted cleavage of host proteins essential for the regulation of protein translation, transcription, cardiac contraction, signaling transduction, and host structural proteins.85,86 The list of protein substrates is growing rapidly (Table 2). Recently, a proteomic approach allowed for global screening of viral proteinase targets.12 Cardiac-specific expression of viral proteinase 2A is sufficient to induce a DCM phenotype.124 Notably, dystrophin and dysferlin deficiency confers increased susceptibility to CVB3 infection of the heart by enhancing viral propagation to adjacent cardiomyocytes and via disrupting membrane repair function.99,125 It has been suggested that these proteins may serve as genetic predisposing factors for viral myocarditis. Wong et al106 also revealed that serum response factor, a muscle-enriched transcriptional factor controlling the expression of many cardiac contractile and regulatory genes, is cleaved through the activity of viral proteinase 2A, partially contributing to virus-induced cardiac dysfunction. Virus can produce direct cytotoxicity in the heart through targeting host protein translational machinery,111-113 and inducing apoptosis by direct processing of caspases.126 Increasing evidence reveals that aberrant accumulation of protein aggregates plays an important role in the development of human heart diseases.127-129 Misfolded protein aggregates are frequently detected in common heart diseases and are thought to be highly toxic to cardiomyocytes.130,131 Abnormal accumulation of ubiquitin conjugates that occur in CVB3-infected cardiomyocytes has also been demonstrated, suggesting that protein homeostasis is disrupted in CVB3-infected hearts.136,132,133 In addition, cleavage of autophagic adaptor proteins sequestosome/p62 and neighbor of BRCA1 gene protein, as well as RNA-binding protein transactive response DNA-binding protein-43, has also been documented after CVB3 infection, contributing to the increased accumulation of protein aggregates.96,104,119 Together, the loss of cellular homeostasis, translation/transcription shut-off, contractile dysfunction, and apoptosis as a result of viral proteinase activity may contribute to direct damage of infected cells (Figure 1).

Analogous to many other viruses, CVB3 has evolved processes to subvert or manipulate the host cellular machinery to ensure productive viral infection. For example, virus uses the host signaling and miRNA system to enhance viral replication, contributing to the pathogenesis of myocarditis and the progression to end-stage DCM. Multiple cellular signaling pathways are known to be activated upon viral infection and play a critical role in regulating viral infectivity at various steps of viral lifecycle, including viral entry, replication, release, and evasion from host immune responses.78 Coyne and Bergelson84 demonstrated that viral entry into epithelial cells through tight junctions is mediated by Fyn and Abl kinases. Studies have also revealed that the activation of the tyrosine protein kinase p56lck, a member of the Src family kinases, the extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase, and protein kinase B signaling as a result of CVB3 infection of immune and myocardial cells is required for efficient viral replication and is associated with host susceptibility to viral myocarditis.134-138 In addition, virus-induced p38 mitogen-activated protein kinase and glycogen synthase kinase-3β activation induces increased cell death and consequent viral progeny release.139,140 Administration of a p38 inhibitor to CVB3-infected mice reduces viral replication, attenuates myocardial damage, and improves cardiac function, suggesting a potential value for CVB3-induced myocarditis.141 It is notable that these signaling pathways do not work independently; indeed, CVB3 can subvert the host cell signaling networks cooperatively to ensure optimal viral infection.142,143 A statistical model has been built to systematically analyze and predict the intracellular signaling responses to CVB3 infection.143

The interplay between CVB3 and host miRNAs also plays a pivotal role in viral pathogenesis (Figure 2). The miRNAs comprise a large, novel class of noncoding RNAs (20–24 nucleotides) that regulate gene expression by targeting messenger RNAs for degradation and translational repression.144,145 Ho et al146 conducted the first investigation on the role of miRNAs in CVB3 replication, where upregulation of miR-141 upon CVB3 infection is partially responsible for the shut-off of host protein translation via targeting the capped protein eukaryotic initiation factor 4E. Following this study, several other miRNAs were identified as induced after CVB3 infection. For instance, miR-203 is upregulated upon CVB3 infection through the activation of the protein kinase C/transcription factor AP-1 pathway, and in turn, enhances CVB3 replication by targeting zinc finger protein-148 to promote cell survival during early infectious stages.147 Similarly, miR-126, which is upregulated during
infection through a sprout-related EVH1 domain containing 1-extracellular signal-regulated kinase 1/2 positive feedback loop, facilitates viral propagation and contributes to viral cytopathogenicity by suppressing LRP6 and WRCH1 genes. In addition to negative regulation of gene expression profiles, miRNAs can also positively affect the translation of their targets. For example, it was shown that miR-10a* promotes CVB3 biosynthesis by targeting the 3-dimensional coding sequence of CVB3 genomic RNA. Moreover, certain miRNAs were found to contribute to the development of viral myocarditis without evidence of altering viral replication dynamics. One example is miR-21, whose change in expression levels during CVB3 infection is controversial because some studies indicated its upregulation, whereas others demonstrate downregulation. Nevertheless, it was found that a high level of miR-21 does not influence CVB3 replication but leads to disrupted myocyte interaction by suppressing and disorientating critical components of intercalated disks. Another example is provided by miR-1, which is induced in CVB3-infected mouse hearts. Upregulation of miR-1 leads to repression of its target gene GJA1 (connexin-43), suggesting a possible mechanism by which CVB3 impairs the function of cellular gap junctions and triggers arrhythmias. Although most miRNAs upregulated in CVB3 infection facilitate viral replication and pathogenesis, some can also act as host defensive components against viral infection. MiR-221 and miR-222, 2 miRNAs significantly elevated during CVB3-induced viral myocarditis, however, directly target several genes benefiting viral replication and inflammation, including genes encoding c-ets-1, interferon }

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CVB3 indicates coxsackievirus B3; EMCV, encephalomyocarditis virus; EV, enterovirus; HRV, human rhinovirus; and PV, poliovirus.
regulatory factor 2, bcl-2-interacting mediator of cell death, thymocyte selection-associated high mobility group box, bcl-2 modifying factor, and chemokine (C-X-C motif) ligand 12. Inhibition of miR-211/miR-222 augments viral load and strongly aggravates CVB3-induced cardiac injury, whereas a high level of these 2 miRNAs reduces viral replication.155 In brief, accumulated evidence indicates that CVB3-induced differential expression of miRNAs plays a crucial role in modulating host and viral gene expression, and in turn directly or indirectly promotes CVB3 pathogenesis.

**Immunopathogenesis of Viral Myocarditis**

Viral infection of the heart triggers the activation of the host antiviral immune response, which is characterized by the infiltration of natural killer cells and macrophages, followed by virus-specific T lymphocytes. The immune response functions as a double-edged sword: initial activation is beneficial to the host by limiting viral spread; however, a persistent and excessive immune response conveys harmful consequences contributing to the progression of myocarditis and DCM. The balance between the antiviral influences and the deleterious effects on cardiac function is an important determinant of the severity of myocarditis and the ultimate progression to DCM.

The innate immune response is essential for host defense early during an infection. It is mainly mediated through cytokines, including interleukins, tumor necrosis factor-α, and interferons (IFNs). The pattern recognition receptors, including the toll-like receptor (TLR), retinoic-acid inducible gene-I–like receptor, nucleotide-binding oligomerization domain-like receptor, and C-type lectin receptor, play a key role in the innate immune response by detecting specific pathogen-associated molecular patterns that are primarily present in invading microbes.136 These receptors can recognize the CVB3 genome and trigger an intracellular signaling cascade leading to the production of type I IFNs and proinflammatory cytokines.85 Type I IFNs, including IFN-α and IFN-β, serves as important antiviral mediators to inhibit CVB3 replication.157–159 It was reported that certain TLR3 polymorphisms are associated with increased susceptibility to enterovirus-myocarditis and DCM in patients.160 Animal studies using TLR3- and TLR4-deficient mice demonstrate a protective function for both TLR3 and TLR4 in CVB3-induced myocarditis and DCM.161–163 Moreover, animal studies suggest that sex differences in TLR signaling play an important role in differential susceptibility to CVB3-induced myocarditis in men and women.164–165

The antiviral significance of IFNs has also been observed at the level of their gene targets. For example, IFN-inducible protein 10 is upregulated in viral-infected myocardium, and overexpression of this gene specifically in cardiomycocytes decreases virus titers leading to improved cardiac function.166 Furthermore, ablation of IFN stimulated gene 15 results in decreased virus titers leading to improved cardiac function.166–168 It was also reported that the mRNA level of the suppressor of cytokine signaling proteins, which inhibit the innate immunity by suppressing the janus kinase/signal transducers and activators of transcription signaling, is upregulated in response to CVB3 infection to counteract the host defense mechanism.171,174

The initial beneficial role of the immune response can become detrimental to the myocardium if it is not properly regulated. For example, sustained production of the proinflammatory cytokines, such as tumor necrosis factor-α175–178 and interleukin-1,178,179 after viral infection can exacerbate cardiac damage, leading to impaired cardiac function. In addition, the balance between regulatory T (Treg) and interleukin-17–producing (Th17) cells has also been suggested to play an important role in the pathogenesis of viral myocarditis.180 Treg inhibits proinflammatory responses, resulting in reduced immunopathology, whereas Th17-mediated immunity induces enhancement of immunopathology.180

The adaptive immunity responses begin later in the acute and subacute phases of myocarditis. Lymphocyte infiltration can aid with the clearance of virus-infected cardiomyocytes, but it may also cause myocardial damage. The latter effects are highlighted by the early evidence that depletion of T lymphocytes decreases mortality rate and reduces cardiac inflammation and injury after CVB3 infection.181 It was subsequently shown that the absence of both CD4⁺ and CD8⁺ T-lymphocytes attenuates the pathological phenotype of myocarditis and significantly reduces mortality.182 Molecular mimicry between viral and host antigens has also been proposed to be a mechanism responsible for autoimmune-mediated destruction of the myocardium.183 After viral infection, exposure of cardiac antigens, such as cardiac myosin that cross-react with coxsackieviral antigens, to the immune system elicits an autoimmune response, leading to the damage of cardiomycocytes through production of autoantibodies and autoreactive immune cells.183 The miRNAs also play a role in the unfavorable inflammatory response to viral infection of the heart. It was reported that miR-155 is strongly induced on CVB3 infection and is colocalized with infiltrated inflammatory cells in virus-infected mouse heart and human myocarditis.151 Inhibition of miR-155 results in attenuated inflammatory infiltration, improved cardiac function, and reduced mortality in a mouse model of viral myocarditis.151

**Management and Therapeutics**

Currently, the effective treatments for advanced DCM are limited, mainly being heart transplantation and mechanical assistance devices. A sizeable gap in therapeutic management exists from the earliest stages of viral heart disease through the progressive inflammatory and healing stages of
myocarditis. Thus, identification and validation of more effective management strategies and therapeutics for myocarditis are critical for the prevention and interruption of heart muscle disease that manifests as HF and sudden death. A recent case study of PVB19-associated fulminant myocarditis reported successful recovery of a 27-year-old man treated with intravenous immunoglobulins (0.5 g/kg per day), aciclovir (2.1 g/d), and intravenous prednisolone (50 mg/d). Further antiviral studies of PVB19-associated myocarditis are lacking, whereas enterovirus-induced myocarditis has been extensively studied in this past decade. Below, we examine 3 major approaches that have been considered for the treatment of enterovirus-induced myocarditis: pathogen inhibition, immune modulation, and HF therapy.

Pathogen Inhibition

Early application of antiviral agents is a potential therapeutic avenue to halt the development of viral myocarditis. Several antiviral agents have shown favorable effects in clinical trials. IFNs, the most commonly used antiviral agent, have been assessed in several circumstances as a treatment for myocarditis. In a Phase II clinical study, IFN-β was administered to 22 patients presenting with persistent LV dysfunction coinciding with enterovirus or adenovirus infection. The outcome was positive demonstrating complete viral clearance indicated by untraceable viral genomes in all patients and improved LV function in 15 patients. In another instance, IFN-α2a was used in the treatment of 2 patients with confirmed myocarditis, as well as RNA-binding protein transactive response DNA-binding protein-43 (TDP-43) are cleaved by protease 2A and 3C, contributing to impaired autophagy and increased accumulation of protein aggregates (Illustration Credit: Ben Smith).
as astragaloside IV can significantly decrease CVB3 titers by upregulating IFN-γ as well as reducing mononuclear cell infiltration and cardiomyocyte injury in mice. Chen et al. showed that astragaloside IV significantly decreases the fibrosis of the heart muscle tissue and increases the murine survival rate caused by the inhibition of the TGFβ1-Smad signaling in DCM.

The clinical application of antiviral agents is limited by the nonspecific targeting, unclear mechanisms, and side effects. The development of nucleic acid–based antiviral agents has introduced more specific strategies against viral infection, which allows one to target the viral genome directly. Antisense oligodeoxynucleotides were found to inhibit gene expression by binding specific sequences of mRNA and through cleavage of target RNA strands in a DNA–RNA duplex by the cellular RNase H. Therefore, antisense oligodeoxynucleotides designed to target viral gene sequences can be used to inhibit viral gene expression and inhibit viral replication. An early study designed and tested 7 antisense oligodeoxynucleotides that target various regions of CVB3 genome RNA; all of which demonstrated strong antiviral activity in both cultured mammalian cells and a mouse myocarditis model. RNA interference is an another tool that can be used to diminish the infection of viruses. Previously, artificially designed small interfering RNAs targeting several regions of CVB3 transcripts have been shown to decrease cell death in cell culture models. In addition, host cellular genes involved in viral replication can also be used as potential therapeutic targets of small noncoding RNAs. For example, small interfering RNAs targeting components in critical pathways for viral pathogenesis, including the ubiquitin-proteasome pathway, the lysosome pathway, and the autophagy pathway, result in significant inhibition of CVB3 replication and myocarditis. However, such data are obtained from studies using cultured mammalian cells, and the therapeutic role of these small noncoding RNAs in myocarditis should be further verified in vivo. Fewer studies have substantiated the effects of small noncoding RNAs in the treatment of myocarditis. The limitation of using small noncoding RNAs is the lack of a specific mode of delivery to target viral-infected cells. An attempt to address this issue is the use of artificial miRNAs that specifically target CVB3 genomes. These studies indicate a potential trend in the targeted delivery of antiviral drugs although its data are limited.
Preventing viral entry is another strategy to confine viral spread and attenuate viral myocarditis. A previous study used the treatment of cell cultures with a modified CAR or decay-accelerating factor containing the human IgG1 fragment crystallization domain on the C-terminal region of the molecule, resulting in a dimeric antibody-like molecule. These recombinant CAR-fragment crystallization and decay-accelerating factor-fragment crystallization proteins competitively bind to soluble viral particles and aid in viral-specific activation of the innate and adaptive immunity. Simultaneous administration of soluble CAR-fragment crystallization and small interfering RNA targeting CVB3 genome exerts synergistic antiviral activity in the treatment of a persistently infected cardiac cell line in vitro. However, the utility of recombinant proteins in disease treatment needs more optimization to avoid unexpected side effects. Currently, there is an abundance of animal data on antiviral strategies in myocarditis and a lack of evidence in human trials.

**Immune Modulation**

The acute phase of myocarditis characterized by intense pathogen infection and myocyte necrosis and apoptosis lasts only for a few days. After the acute phase, the further development of myocarditis in the subacute phase is thought to be predominantly a result of exaggerated autoimmune responses. Thus, the application of immunosuppressive drugs seems plausible for the treatment of myocarditis. In a clinical trial, administration of immunosuppressant drug azathioprine

### Table 3. Studies of Antiviral and Supportive Treatments for Myocarditis

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CAR-Fc indicates coxsackievirus and adenovirus receptor fragment crystallization; CMV, cytomegalovirus; CVB3, coxsackievirus B3; DAF-Fc, decay-accelerating factor-fragment crystallization; ER, endoplasmic reticulum; HF, heart failure; HRV, human rhinovirus; IFN, interferon; IL, interleukin; IVIG, intravenous immune globulin; LV, left ventricle; LVEF, left ventricular ejection fraction; NSAID, nonsteroidal anti-inflammatory drug; siRNA, small interfering RNA; TIMP, tissue inhibitor of metalloproteinase; and TNF, tumor necrosis factor.
and prednisone improved the LVEF and New York Heart Association functional class of patients with myocarditis and symptoms for >6 months. Similar results were reported in another trial of patients with chronic nonviral myocarditis and symptomatic HF, in which both LVEF and New York Heart Association functional class were also improved after treatment with azathioprine and prednisone. Despite the favorable outcomes of immunosuppressant use for chronic myocarditis, most attempts to alleviate acute myocarditis using anti-inflammatory drugs or immunosuppressants have failed, with little positive or even adverse effects. Similarly, adiponectin mediates cardioprotection and favors positive outcome in patients with nonviral inflammatory cardiomyopathy. But in a recent study, administration of adiponectin in CVB3-induced acute myocarditis mouse models promoted the development of myocarditis by suppression of TLR-dependent innate immune responses.

Several other immune modulators have been tested in experimental animal models or cell cultures and exhibited inspiring therapeutic potentials. Nonsteroidal anti-inflammatory drugs are considered effective anti-inflammatory therapeutics and have been applied in the treatment of pericarditis. Nonsteroidal anti-inflammatory drugs have shown increased myocardial inflammation and mortality in murine models with acute viral myocarditis, however, should not be considered as an antiviral therapy. Intravenous immunoglobulin is another type of immunomodulatory agent that also shows antiviral effects in experimental models; however, data remain controversial.

Agents modulating specific genes can also be used to relieve exaggerated autoimmune responses in myocarditis. For instance, tissue inhibitor of metalloproteinase 1–specific small interfering RNA or polyclonal antisera ameliorates CVB3-induced myocarditis by enhancing the matrix metalloproteinase–dependent migration of inflammatory cells at sites of infection and thereby anatomically focusing the adaptive immune response.

In all, treatment with immunomodulatory drugs seems beneficial for nonviral myocarditis or the subacute stage of viral myocarditis, but for the early stage of acute infectious myocarditis, such treatment will bring little or possibly detrimental effects. Thus, it is rather crucial to detect pathogen-induced myocarditis with sensitive techniques and to treat at early stages to improve detrimental outcomes.

**HF Therapy**

Because antiviral therapy benefits little in the subacute stages of myocarditis and the effects of immunomodulatory drugs on infectious myocarditis are still controversial, therapy to relieve HF is still a major strategy used in the treatment of patients with myocarditis. HF drugs used for myocarditis include angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, β-blockers, aldosterone antagonists, and calcium-channel blockers. In different animal models with viral myocarditis or autoimmune myocarditis, angiotensin-converting enzyme inhibitors, captopril, and angiotensin II receptor blockers, losartan and olmesartan, significantly reduce inflammation, necrosis, and fibrosis of the myocardium and improve LV function. β-blockers generally improve LV function, relieve HF, and increase survival of patients with inflammatory cardiomyopathy, but different types of β-blockers show variable impact on myocarditis. Carvedilol shows a cardioprotective function in rats with autoimmune myocarditis by suppressing inflammatory cytokines and oxidants, whereas metoprolol exerts adverse effects on CVB3-induced myocarditis leading to increased inflammation and mortality in the murine model. Aldosterone antagonists were reported to reduce hospital admission for worsening HF and to increase survival of patients with acute or chronic HF. Calcium-channel blocker amlodipine shows a protective effect against myocardial injury in mice with congestive HF induced by viral myocarditis through blunting of the overproduction of nitric oxide. However, administration of calcium-channel blockers is not generally recommended in the management of acute HF.

Mechanical circulatory supports for heart function also aids in the recovery of myocarditis. Although such supports cannot cure myocarditis itself, the devices allow avoidance of fatality and increase the survival longevity of patients, especially in those with cardiogenic shock. A myocarditis and acute cardiomyopathy 2 (Intervention in Myocarditis and Acute Cardiomyopathy [IMAC] 2) study showed that myocarditis was the strongest predictor of bridge to recovery in recent onset nonischemic cardiomyopathy patients requiring the support of LV assist devices. Furthermore, female sex was associated with even greater likelihood of bridge to recovery in myocarditis patients on LV assist device support. As for fulminant myocarditis, a ventricular assist device is associated with complete recovery of myocardial function. Moreover, external pulsatile mechanical ventricular assistance also improves survival in patients with acute myocarditis. If myocarditis leads to end-stage HF, cardiac transplantation is the ultimate measure that should be considered.

Although different strategies for the therapy of myocarditis have been introduced, most are still yet to be verified in the research laboratory and are far from clinical application. As well, most clinical assessments performed on myocarditis treatment are still related to symptoms and depend much on the clinical presentation. Development of new monitoring and therapeutic techniques, such as molecular diagnoses, nucleic acid–based antiviral drug development, and targeted drug delivery, will greatly enhance the therapy for myocarditis in the future. An exhaustive list of current treatments for viral and nonviral-associated myocarditis is included in Table 3.

**Summary and Prospect**

Myocarditis remains an important clinical condition from perinatal to adult timeframes. Significant challenges remain in regards to firm diagnoses, clear management and treatment approaches, and ultimate consequences of acute disease. Viruses play an important role in causing myocarditis, yet their precise contributions are masked by varied clinical presentations and progression, and the widely varied type and quality of molecular tools and samples used to establish an association of the disease phenotype with certain cardiotropic viral agents.
This scrambled situation in humans stands in contrast to the huge body of sterling work that has been conducted in in vitro and in vivo models wherein viruses and their mechanistic impact on host cells and immune systems has been documented elegantly. Similarly, many promising avenues of therapeutic intervention, pursued in model systems, have yet to be studied in humans given the relative infrequency of a viral heart disease diagnosis, the highly variable timepoint at which patients present after the initiation of their illness, and the near impossibility, to date, in establishing the actual onset of viral myocarditis in people.

A global approach to human studies is long overdue. A global cohort, with standardized clinical, imaging and immunovirolological techniques, highly SOP-driven sample accrual, and a reset on our collective views of pathogenesis and disease course would open a new avenue for effective reduction and prevention, detection, and intervention.

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