

## Myocarditis: past, present and future

Akira Matsumori

President, Asian Pacific Society of Cardiology

Myocarditis is caused by various etiologic agents, and it was believed that the most common cause was enteroviruses especially coxsackieviruses. Recent collaborative studies conducted by Dr. Tohru Izumi and coworkers have shown that influenza H1N1 virus infection is associated with various heart diseases, including severe heart failure, pericarditis, and arrhythmias.

Hepatitis C virus (HCV) is the cause of many different forms of heart disease worldwide, yet few cardiologists are aware of HCV as an etiology of myocarditis. The burden of HCV myocarditis is global, with a higher prevalence in Asia, Africa, and South America. HCV myocarditis is a chronic, persistent, and devastating disease.

"Global Alert and Response Network for HCV-Derived Heart Diseases" showed a high prevalence of electrocardiographic abnormalities, including QT prolongation, abnormal Q waves, and various arrhythmias. Prevalence of HCV infection was higher in patients with ischemic heart disease, hypertension, and diabetes suggesting that HCV infection is a risk factor of these diseases. Immunohistochemical analysis showed that HCV core and nonstructural antigens were found mostly in infiltrating cells in the heart, liver, kidney, and bone marrow; and these cells were mostly macrophages, and suggested that macrophages are the major target of HCV. Chronic persistent inflammation might cause myocardial fibrosis and myocyte hypertrophy, and lead to hypertrophic and dilated cardiomyopathies and ARVC (arrhythmogenic right ventricular cardiomyopathy).

Viral infection could lead to increased synthesis of free immunoglobulin light chains (FLCs). Serum levels of FLCs were increased in myocarditis, and exogenously given FLCs inhibited viral replication and improved myocarditis. FLCs may be promising for the diagnosis and treatment of viral myocarditis. Furthermore, anti-allergic drugs, and a new anti-inflammatory agent, Fingolimod (FTY720) were effective in an animal model of viral myocarditis.

We suggest that a strategy of drug development specifically addressing inflammation in myocarditis may provide increased benefit considering target organ damage.

### Introduction

The myocardium is involved in a wide range of viral infections.<sup>1</sup> The clinical presentation of viral myocarditis is variable. When myocardial necrosis occurs diffusely, congestive heart failure develops and later, dilated cardiomyopathy. If myocardial lesions are localized, a ventricular aneurysm may form. When complicated with arrhythmias, myocarditis presents as arrhythmogenic right ventricular cardiomyopathy.<sup>2</sup> When myocardial necrosis is localized to the subendocardium, restrictive cardiomyopathy may develop. While it has not been established that hypertrophic cardiomyopathy may be a complication of viral myocarditis, asymmetrical septal hypertrophy has sometimes been observed in patients with myocarditis.

The myocardium may be the target of several types of viral infections. Recently, the importance of influenza virus in fulminant myocarditis has been noted,<sup>3</sup> and hepatitis C virus (HCV) has been associated with hypertrophic cardiomyopathy, dilated cardiomyopathy, and myocarditis.<sup>4-6</sup>

### Hepatitis C virus-derived heart diseases

HCV has infected an estimated 170 million individuals worldwide and, in the next few years, the number of annual deaths from HCV-related liver disease and cancer in the United States may exceed the number of deaths caused by the human immunodeficiency virus.<sup>7</sup> A screening test developed in 1990 has nearly eliminated the spread of HCV through blood transfusions in industrial

countries, and the sharing of contaminated needles is now by far the most common mode of infection. As a result, the US Centers for Disease Control and Prevention estimates that new infections have decreased from approximately 230,000/year in the 1980s to fewer than 36,000 in the United States in 1996. However, because most individuals infected in earlier decades are alive, it is estimated that 1.8% of the US population harbors the virus.<sup>8</sup> As these patients become older, HCV-related liver disease, now accounting for 8,000-10,000 deaths/year in the United States, and the single most common indication for liver transplants, is likely to increase.<sup>7</sup>

HCV is the cause of many different forms of heart disease worldwide, and yet few cardiologists are aware of it as an etiology of heart disease, or its treatment. HCV infection is seen globally, and is often undetected and, therefore, untreated. The burden of HCV-derived heart disease is global, with a higher prevalence in Asia, Africa, and low-and middle-income countries. HCV-derived heart diseases are chronic, persistent, and devastating diseases.

The global prevalence of HCV carriers is estimated to average 3%, ranging from 0.1 to  $\geq 10\%$  among different countries. In Asia, the virus is highly prevalent in Mongolia, Vietnam, Myanmar, and China. In Africa, a high prevalence is present in the central countries and in Egypt; and in South America, a high prevalence is observed in Brazil. The highest prevalence ( $\geq 10\%$ ) has been recorded in Mongolia, Egypt, Tanzania, Guinea, and Cameroon. Therefore, HCV infection is an important and treatable health concern in developing countries in Asia, Africa, and South America.<sup>9</sup>

The present study showed that in more than 10% of Japanese patients, cardiomyopathies were associated with HCV infection. More recently, we found that up to 15% of patients with heart failure with myocarditis in the USA have HCV infection. In contrast, 79% of patients with hepatocellular cancer and 37% of hepatitis C patients in China have heart disease, as detected by measuring a proven and sensitive biomarker of heart disease, NT-ProBNP.<sup>10</sup> In Pakistan, 17% of hepatitis C patients have heart disease as measured by this biomarker.<sup>11</sup> Based on these data, 3% of 6.6 billion (198 million) persons worldwide are infected with HCV, and 17%-37% (34-73 million) persons are suffering from HCV heart diseases. These figures may be comparable to the number of patients with hepatitis C.

HCV infection causes only hepatitis in some patients, only heart diseases in some patients, and both hepatitis and heart diseases in other patients. In addition, HCV infection is associated with enhanced atherosclerosis, and

more than 20% of the people with HCV infection have diabetes mellitus, which is an important risk factor of cardiovascular disease.<sup>12</sup>

### **Assessment of cardiac involvement of hepatitis C virus; tissue Doppler imaging and NT-proBNP study in Egypt**

Hepatitis C disease burden is substantially increasing in Egyptian communities, and it is estimated that the prevalence of HCV in Egyptian communities has reached 22% of the total population. We evaluated left ventricular diastolic functions of HCV patients using tissue Doppler imaging and NT-proBNP.<sup>13</sup>

The HCV group showed significance in the QTc interval, significant increase in A wave, deceleration time; highly significant decrease in tissue doppler  $E_a$ , highly significant decrease in  $A_a$ , highly significant increased  $E/E_a$  ratio, significant decrease in the  $E_a/A_a$  ratio, and a significant increase in  $SR_u$ . NT-proBNP levels showed a highly significant increase with mean value  $222 \text{ pg/mg} \pm 21.2$  in the control group. No statistical differences in  $SR_e/SR$  or  $E/SR_a$  or  $E/SR_e$  ratio were observed; however, they had significant correlation with NT-proBNP level and tissue doppler parameters. These data provide the direct evidence that HCV infection causes diastolic dysfunction without any other predisposing factor. Tissue Doppler was more sensitive to diagnose diastolic dysfunction than conventional Doppler.<sup>13</sup>

### **Leukocytes are the major target of hepatitis C virus infection**

HCV has been associated with several extrahepatic manifestations, among which the best characterized are mixed cryoglobulinemia associated with a risk of developing B-cell non-Hodgkin's lymphoma, and glomerulonephritis. Porphyria cutanea tarda is commonly associated with HCV infection, and other disorders have been loosely associated with the virus, including secondary Sjögren's syndrome, Moorthen's corneal ulcer, hyper- and hypothyroidism, and myositis. However, the pathogenesis of these extrahepatic complications is not well understood.

In the present study, an antibody against the HCV-core antigen stained peripheral blood mononuclear cells (PBMC), and the majority positive staining, was seen in CD68-positive macrophages. HCV-core antibody stained mostly mononuclear cells in the liver, heart, kidney, and bone marrow, but not hepatocytes, myocytes, or globular cells. Positive staining was found in PBMC and

mononuclear cells of various tissues by antibody against NS4 protein, which also supports that HCV replicates in mononuclear cells. Therefore, mononuclear cells may be a primary target of HCV infection.<sup>14</sup> The notion that HCV is a lymphotropic virus is supported by findings of replicating HCV found in B-cells, T cells, and PBMC from patients with chronic hepatitis C. Recently, occult HCV infection, defined as the presence of low levels of HCV genomes in serum, PBMC, and/or liver in the absence of clinically evident liver disease, was identified in patients years after apparent complete resolution of hepatitis C. More recently, immune cells have been shown to be reservoirs of HCV in symptomatic and occult infections. The present study suggests that CD68 monocytes/macrophages are the major target of HCV, and that CD3-positive T cells or CD20-positive B-cells are not major targets. Pharmacologic preparations targeting leukocytes infection might be a reasonable approach. Clinical applications to test this new concept include evaluating effects of anti-viral agents *ex vivo* using the patient's mononuclear cells before treatment. Or, the effects of new therapeutic agents can be assessed *in vitro* by cultured mononuclear cells. Also, apheresis of mononuclear cells can be a possible treatment of HCV infection.

### **Autoantibodies against cardiac troponin I in patients presenting with myocarditis**

Anticardiac autoantibodies have been observed in patients with myocarditis and dilated cardiomyopathy. Several immunofluorescent patterns have been described, including fibrillary, sarcolemmal, cytoplasmic and others. The presence of antifibrillary antibodies suggested myocarditis. An antibody to cardiac myosin has often been observed in patients with dilated cardiomyopathy, and alpha and beta myosin heavy chain isoforms both react with that autoantibody.<sup>15</sup> Antibodies to the mitochondrial ADP/ATP translocator or  $\beta_1$ -adrenoceptor, often present in patients with dilated cardiomyopathy, enhance the  $Ca^{2+}$  current.<sup>16</sup> We have shown that dihydropyridine-insensitive  $Ca^{2+}$ -permeable cation channels, but not voltage-gated  $Ca^{2+}$  channels, are activated by anticardiac antibodies associated with myocarditis in ventricular myocytes. It is likely that the excess  $Ca^{2+}$  entry, caused by the activation of this channel by disease-associated antibodies, is involved in the pathogenesis of myocarditis and dilated cardiomyopathy.<sup>17</sup>

We have recently discovered that mice lacking the T-cell receptor costimulatory molecule PD-1 spontaneously develop autoimmune myocarditis,<sup>18</sup> along with the

production of high titers of antibodies to cardiac troponin I (cTnI). The passive transfer of monoclonal antibodies to cTnI induced myocardial dysfunction in mice.<sup>19</sup> Our experimental observations support the hypothesis of an interaction between cTnI-specific antibodies and cTnI on the surface of cardiomyocytes, enhancing the  $Ca^{2+}$  current and, eventually, causing cardiac dysfunction and dilation. However, we cannot exclude the possibility that antibodies to cTnI penetrate the plasma membrane and recognize cTnI in sarcomeres. The present study provided the first direct evidence of an implication of autoimmunity, autoantibodies in particular, in the development of dilated cardiomyopathy in an animal model.

In our recent study, we developed a new method to detect autoantibodies against cTnI, and we measured these autoantibodies in patients with myocarditis.<sup>20</sup> Myocarditis, defined by the Dallas criteria as "the presence of an inflammatory infiltrate in the myocardium with necrosis and/or degeneration of adjacent myocytes" remains an etiologic dilemma and a therapeutic challenge. Different microorganisms can cause the same pathologic manifestations, although they mandate different therapies. Several microorganisms have been identified as possible pathogens, including enteroviruses, adenoviruses, and HCV. We detected higher anti-cTnI antibody titers in patients with myocarditis whose biopsy satisfied the Dallas criteria than in those whose biopsy was negative. Furthermore, among those whose biopsy satisfied the Dallas criteria, those in whom an anti-HCV antibody was detected, had higher titers than those who were not infected with the HCV. The present study showed that anti-cTnI antibody is often present in patients with active myocarditis, suggesting that their presence correlates with ongoing inflammation.

In our previous studies, we found that HCV infection is often associated with myocarditis, and that myocardial injury is particularly severe in patients infected with the HCV. In this study, the anti-cTnI antibody titers were higher in patients with, than in those without, HCV infection. One might hypothesize that infection with the HCV increases the production of anti-cTnI antibody and further increases the severity of myocardial injury. Therefore, the detection of anti-cTnI antibody might be helpful in the diagnosis, as well as the evaluation and follow-up, of patients presenting with active myocarditis.

### **Role of mast cells in myocarditis**

Role of mast cells have been implicated in the pathogenesis of cardiovascular and atherosclerotic

disorders. In particular, we have observed that mast cells cause apoptosis of cardiac myocytes and proliferation of nonmyocytes *in vitro*.<sup>21</sup> In our model of viral myocarditis, the number of mast cells was increased. We observed that mast cell deficiency had beneficial effects in the disorder. The gene expression of mast cell chymase and tryptase was unregulated in the acute phase of viral myocarditis and rose further in the subacute phase of heart failure.<sup>22</sup> This activation coincided with the development of myocardial necrosis and correlated with the upregulation of MMP-9 and type-I procollagen, suggesting that mast cell chymase and tryptase participate in the acute inflammatory reaction as well as the remodeling process associated with acute viral myocarditis.

Cetirizine administration improved the survival of mice, congestion of the lungs, and myocardial necrosis, suppressed the expression of pro-inflammatory cytokines, and decreased expression of MMP-2. Thus, these may be the mechanisms by which cetirizine decreases inflammation and fibrosis. These results suggest that histamine released from mast cells may play a pivotal role in the pathogenesis of viral myocarditis. However, antihistaminic agents, such as cetirizine, not only act via mediation of H1 receptors, but may also attenuate various steps in the inflammatory process.<sup>23</sup> Although the exact molecular mechanisms of the beneficial effect of cetirizine remains to be clarified, cetirizine is a promising agent for the treatment of viral myocarditis and merits further study.

### **Free immunoglobulin light chains as a biomarker and therapist agent for viral myocarditis**

Immunoglobulin is composed of two identical heavy chains and two identical light chains and provides defense against all extracellular and some intracellular pathogens. In mammals, immunoglobulin light chain genes generally exist in two distinct isotypes called  $\kappa$  and  $\lambda$ . The genes for the two light chain isotypes are encoded at separate and unlinked loci, and the organization of the  $\kappa$  and  $\lambda$  chain locus differs significantly. Free immunoglobulin light chains (FLC) are produced by plasma cells and can be found in various body fluids. Interestingly, viral infection has been shown to increase the occurrence of immunoglobulin free light chains in various body fluids.

We have recently shown that FLC production is greatly enhanced during infection with the encephalomyocarditis (EMC) virus in mice. Furthermore, FLC are shown to have protective effects in viral myocarditis, most likely through direct antiviral activity and anti-inflammatory effect by the increased expression

of IL-10.

Immunoglobulin free light chains exert a range of biological activities including enzymatic activity (proteolysis of diverse substrates), specific binding activity for substrates and antigens and binding to different cells, although its immunologic function remains to be clarified. We found that circulating  $\kappa$ FLC monomers and dimers are slightly increased in mice with EMC viral myocarditis when myocardial necrosis becomes apparent, but are significantly increased when heart failure developed. Infection with EMC virus was also associated with an increased expression of FLC in mouse heart tissue at the stage of heart failure.<sup>24</sup> Recently, we found that circulating FLC was elevated in patients with myocarditis and heart failure (Matsumori A, et al. unpublished observation).

Application of the FLC antagonist F991 during the first period of viral infection worsened pathology of viral myocarditis suggesting a protective role of FLC. The latter was further substantiated by the protective effect of supplementation of FLC at the start of the viral infection leading to reduced necrosis and greatly enhanced survival. Our data indicate that FLC may directly inhibit viral replication as shown in an *in vitro* plaque assay. Indeed, in FLC-treated animals viral load of the heart tissue was significantly reduced.

### **A new anti-inflammatory immunomodulatory agent, fingolimod (FTY720/Gilenya)**

The discovery of fingolimod, an orally active immunomodulatory drug, has opened up new approaches to the treatment of multiple sclerosis, the most common inflammatory disorder of the central nervous system. Elucidation of the effects of fingolimod mediated by the modulation of sphingosine 1-phosphate (S<sub>1</sub>P) receptors has indicated its effects to the central nervous system and direct effects on neural cells, particularly astrocytes.

The structure of fingolimod (FTY720/Gilenya) was first described in 1995-1996, following a chemical derivatization programme based on the fungal metabolite myriocin. Early studies in animals showed that fingolimod synergized with calcineurin inhibitors, which inhibit the proliferation of T cells and prolong organ graft survival. In a clinically relevant model of bone marrow transplantation, fingolimod prevented the development of graft-versus-host disease. Together, these data indicated that fingolimod might act differently to classical immunosuppressants (such as cyclosporine, tacrolimus, corticosteroids, methotrexate, mitoxantrone, and lymphocyte-depleting antibodies), an intriguing

observation that prompted extensive follow-up.<sup>25</sup>

We showed that the immunosuppressor FTY720 had notable therapeutic effects in our murine model of viral myocarditis, by a prolongation of survival and attenuation of histologic abnormalities. These effects were exerted by cardiac immunosuppression, without the induction of excessive virus replication, despite the use of relatively high doses of FTY. Virus concentrations in the heart were significantly increased by cyclosporine A, compared with control, but FTY 720 did not enhance viral replication. Therefore, Fingolimod is a promising agent for the treatment of myocarditis.<sup>26</sup>

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