Chapter 2

Viral Myocarditis from Animal Models to Human Diseases

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Abstract

Almost all viruses cause myocarditis. The influenza virus and enteroviruses such as coxsackievirus, echovirus, and poliovirus cause myocyte necrosis and acute myocarditis. In severely injured cases, cardiac fibrosis replaces myocyte loss while cardiac dysfunction persists, and the remaining myocytes become hypertrophic to compensate for the decreased cardiac function during chronic stages. Hepatitis C virus (HCV) infects monocytes and macrophages and causes chronic persistent inflammation, and this inflammatory reaction may cause myocyte necrosis, fibrosis, and myocyte hypertrophy. Recently, SARV-CoV2 has been reported to cause myocarditis.

Animal models of viral myocarditis are useful to study the natural history, genetics, pathogenesis, and clinical manifestations of viral myocarditis in humans, and to develop new methods for the prevention and treatment of the disease. Using animal models, it has been shown that mast cells, angiotensin II, nuclear factor κB (NF-κB), and cytokines may play important roles in the pathogenesis of viral myocarditis, and that mast cell-stabilizing agents, the inhibitors of NF-κB and renin–angiotensin–aldosterone system; fingolimod; interleukin (IL)-10 and IL-12; some calcium channel blockers; carvedilol; pycnogenol; and

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immunoglobulin free light chains (FLCs) prevented viral myocarditis, and these agents could be promising for the treatment of viral myocarditis in humans.

Cellular infiltration is not prominent in certain types of viral myocarditis including COVID-19 and HCV myocarditis, and the Dallas criteria typically relied on to diagnose myocarditis may not be appropriate for the diagnosis of these types. The presence of viral genomes in the heart does not necessarily indicate the cause of the disease, and the development of the disease relies on inflammatory and immune responses. In fact, anti-inflammatory agents, even those that do not have anti-viral effects, improve viral myocarditis. Therefore, a combined therapy of antiviral and anti-inflammatory agents could be an effective treatment for viral myocarditis in humans. FLCs that reflect the activation of B cells and plasma cells may have potential in the diagnosis of viral myocarditis.

Introduction

Almost all viruses cause myocarditis, but coxsackievirus and influenza virus are thought to be the most common causes of acute myocarditis. On the contrary, hepatitis C virus (HCV) usually causes chronic myocarditis and cardiomyopathy. Coronaviruses commonly cause diseases in animals, and while there were a few reports of myocarditis and cardiomyopathies caused by this viral genus in humans prior to the 2020 pandemic, myocarditis has been seen frequently in patients diagnosed with COVD-19.

Enteroviruses, such as coxsackievirus, echovirus, and poliovirus, as well as the influenza virus, may cause myocyte necrosis directly, and acute myocarditis follows myocyte injury. In cases with severe myocardial injury, cardiac fibrosis replaces injured myocytes, which leads to persistent cardiac dysfunction, and the remaining myocytes develop compensatory hypertrophy (Matsumori, 2003a). The persistence of the viral genomes of the enteroviruses (e.g., coxsackieviruses), parvovirus B19, human herpes virus 6, adenoviruses, Epstein–Barr virus, human cytomegalovirus, and human immunodeficiency virus (HIV) have been detected in endomyocardial biopsy specimens in human myocarditis and cardiomyopathies (Pollack et al., 2015, Schultheiss et al., 2019, and Tschope et al., 2021). However, the presence of only viral genomes

does not necessarily show the cause of the diseases; inflammatory and/or immune responses are required to develop myocarditis.

Clinically, viral myocarditis may present in a variety of ways, ranging from a lack of clinical manifestations to acute heart failure, arrhythmias, cardiogenic shock, and sudden death, and is associated with substantial morbidity and mortality (Cooper, 2009, Pollack et al, 2015). In addition to the broad spectrum of clinical features associated with acute viral myocarditis, possible complications and late sequelae may give cause for concern. A subacute or even chronic myocarditis may lead to progressive myocardial failure such as dilated cardiomyopathy and death (Matsumori, 1993). However, the relative infrequency of cases of acute viral myocarditis, together with the difficulty obtaining appropriate clinical materials, makes it difficult to study the pathogenesis of myocarditis in humans. Therefore, experimental models have been used to study various aspects of the pathogenesis of viral myocarditis.

Animal Models of Viral Myocarditis

Small Animal Models of Viral Myocarditis

Animal models provide an opportunity to study the natural history and the pathogenesis of viral myocarditis, as well as the possible interventions and preventions (Matsumori, 2012). An animal model was developed using encephalomyocarditis virus（EMCV), which causes congestive heart failure in the acute and chronic stages of the infection in mice (Matsumori and Kawai, 1982a). Dilatation and hypertrophy, as seen in dilated cardiomyopathy, has been shown to develop during the chronic stage of the infection in an animal model (Matsumori and Kawai 1982b). Right ventricular aneurysms, as seen in human arrhythmogenic right ventricular cardiomyopathy, were also observed in this model (Matsumori et al., 1983). In addition, mural thrombi were observed in the atria in a murine model, suggesting that acute viral myocarditis carries a risk of thromboembolism (Tomioka et al., 1986a) (Figure 1). Coxsackievirus myocarditis has also been studied in mice and hamsters (Matsumori and Kawai, 1980, Hoshino et al., 1982). Both coxsackievirus and EMCV cause acute myocarditis, but successive infections of coxsackievirus B3 (CVB3) and EMCV in mice showed that they developed similar lesions to those seen in humans with chronic myocarditis (Okada et al., 1992).
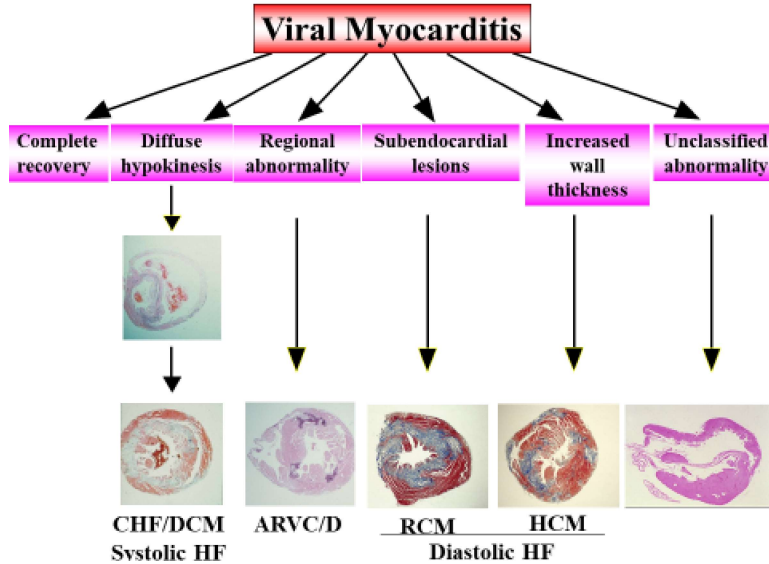
Figure 1. Viral myocarditis and its sequelae. Animal models of viral myocarditis show that similar lesions develop in viral myocarditis as seen in dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), restrictive cardiomyopathy (RCM), and hypertrophic cardiomyopathy (HCM). CHF: congestive heart failure; HF: heart failure. (Reproduced with permission, Matsumori, 2009; copyright Elsevier).

Genetic Background and Viral Myocarditis

Like other experimental autoimmune diseases, myocarditis is dependent on the genetics of the host. The major histocompatibility (MHC) locus of the mouse, H-2, is an important determinant of the severity of disease induced by CVB3 infection or cardiac myosin, but multiple non-MHC immunoregulatory genes are critical in determining susceptibility (Rose, 2016). We studied EMCV myocarditis in inbred strains of A/J, BALB/c, C3H/He, C57BL/6, and DBA/2 mice with different H-2 complexes. Myocardial lesions were frequently seen in BALB/c mice (48.7%), C3H/He mice (61.8%), and DBA/2 mice (66.1%), but no pathologic findings were noted in A/J or C57BL/6 mice. In C3H/He and DBA/2 mice, dilatation and hypertrophy of the heart along with myocardial lesions persisted up to the eighth month after virus inoculation. This study suggests that MHC genes are critical in the development of viral myocarditis (Matsumori et al., 1982c).

Arrhythmias in Viral Myocarditis

Hoshino et al., (1982) studied the serial electrocardiograms of acute viral myocarditis in Syrian golden hamsters inoculated with coxsackievirus B1. Various electrocardiographic abnormalities, similar to those reported in human viral myocarditis, were observed. The reciprocal ST displacement and/or T-wave flattening were detected in 80% of the animals. Atrioventricular (AV) block, left bundle branch block (LBBB), or extrasystoles were recorded. A histopathological study showed myocarditis in the endocardial third of the myocardium, particularly in the interventricular septum. These lesions were more widespread in the left ventricle than in the right.

In 47 mice infected with EMCV myocarditis, serial electro-cardiograms showed atrial and ventricular premature beats and complete AV block in 12.8%, 17.0%, and 53.2%, respectively, of the mice. Mononuclear cell infiltrations into the His bundle were noted in the conduction system of the mice with complete AV block. The QRS voltage decreased after day 6 and reached a minimum on day 13, when myocardial necrosis and the congestion of the lungs and the liver were most prominent (Kishimoto et al., 1984).

Cytokines and Mast Cells in Viral Myocarditis

The expression of the immunoregulatory cytokines interferon (IFN)- γ and interleukin (IL)-2 and the proinflammatory cytokines IL-1β and tumor necrosis factor (TNF)- α increased in the heart tissue during the acute stage of EMCV myocarditis. The expression of these cytokine genes decreased thereafter but persisted for three months after virus inoculation. Gene expression of IL-1β was high, as compared to other cytokines during the chronic stage, and was correlated with the ratio of heart weight–body weight and the extent of fibrotic lesions. Immunohistochemical analysis revealed that some of the mononuclear cells, endothelial cells, and interstitial macrophages were positive for either IL-1β or TNF-α, and the fibroblasts were positive for IL-1β in the heart tissue. Persistent expression of these cytokines may have important implications in the pathogenesis of dilated cardiomyopathy (Shioi et al., 1996).

Earlier, we mentioned that the gene expression of the mast cell chymase was upregulated during the acute stage of EMCV myocarditis, and it was even higher during the subacute stage of heart failure. This activation correlated with the development of myocardial necrosis and fibrosis and with the upregulation of matrix metalloproteinase (MMP)-9 and type I procollagen, suggesting that chymase may participate in the acute inflammatory reaction

and the remodeling process associated with acute viral myocarditis (Higuchi et al., 2008). Mast cell-deficient mice had less pronounced myocarditis than normal mice; in contrast, the myocardial lesions were more severe and the expression of chymase and MMP-9 was increased in mast cell-reconstituted mice. In the heart tissue of the viral myocarditis model, activated mast cells release many proinflammatory cytokines such as IL-6, TNF-α and MMPs as well as fibrogenic mediators including chymase and tryptase. Furthermore, these fibrogenic factors have been shown to increase the fibroblasts at the site of infection and may produce stem cell factor (SCF) (Fireman et al., 1999). SCF can mature and differentiate more mast cell precursors in the heart. Thus, mast cells play a critical role in the pathogenesis of viral myocarditis (Figure 2). The functions of mast cells can be controlled using anti-allergic or anti-chemical mediator drugs. In fact, a histamine H1-receptor antagonist improved EMCV myocarditis. Our study, therefore, offers hope that the control of mast cells, for example, the interaction between SCF and c-kit or the control of mast cell proteases, may be effective in the management of viral myocarditis and the subsequent dilated cardiomyopathy or heart failure (Higuchi et al., 2008).
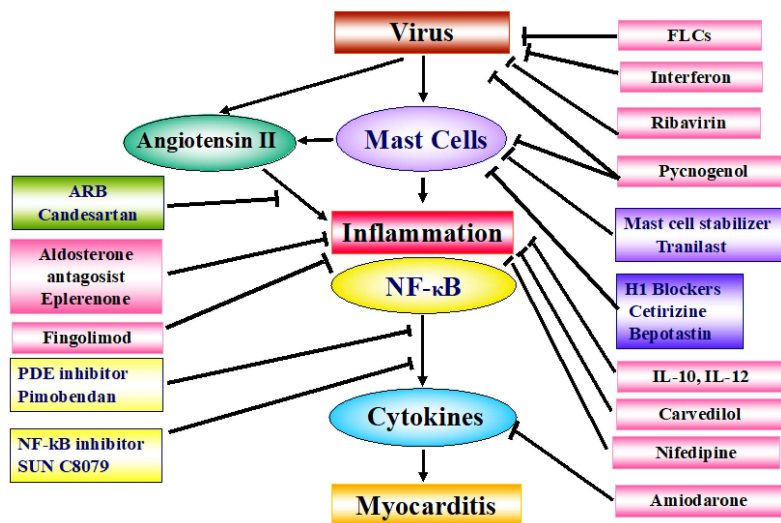

Figure 2. Anti-inflammatory therapy for viral myocarditis. Antiviral drugs; mast cell-stabilizing agents; the inhibitors of nuclear factor (NF)-κB; the renin–angiotensin–aldosterone system; interleukin (IL)-10 and IL-12; fingolimod; carvedilol; nifedipine; amiodarone; pycnogenol and immunoglobulin free light chains (FLCs) prevented viral myocarditis in experimental murine models of viral myocarditis, and show potential in the treatment of viral myocarditis

Therapeutic Trials of Viral Myocarditis in an Animal Model

Antiviral Agents

Passive immunization with anti-EMCV hyperimmune rabbit serum given shortly after inoculation with EMCV protected mice from viral myocarditis (Matsumori et al., 1987). A virus-specific formalin-inactivated vaccine also prevented the development of myocarditis. Neutralizing antibodies were detected after two doses of vaccine. After subsequent challenge with the virus, all vaccinated mice survived without developing myocarditis, whereas all unvaccinated animals developed fatal disease (Matsumori et al., 1987).

Natural human IFN preparations do not show significant activity in mouse cells. However, a recombinant human leukocyte IFN subtype-α A/D was shown to have relatively high antiviral activity in murine cells and also *in vivo*. Human leukocyte IFN-α A/D given one day before or simultaneously with EMCV inoculation inhibited multiplication of virus in the heart and protected mice from developing myocarditis. Prevention was dependent on dosage and on the time of treatment (Matsumori et al., 1987). Using an animal model of coxsackievirus myocarditis, human leukocyte IFN-α A/D was also effective for the protection of coxsackievirus B3 myocarditis (Matsumori et al., 1988).

Ribavirin is a synthetic nucleoside analogue, and has a broad antiviral activity against RNA and DNA viruses. When administered subcutaneously immediately after EMCV inoculation, ribavirin-treated mice survived longer, and cellular infiltration and myocardial necrosis were less severe than controls. Ribavirin was also effective at a high dose
beginning 1 day or 3 days after viral inoculation (Matsumori et al., 1985). Ribavirin effectively inhibited myocardial viral replication, and reduced the inflammatory response and myocardial damage in an experimental model of coxsackievirus B3 myocarditis in mice (Matsumori, 1993).

Combined therapy of ribavirin and IFN-α A/D achieved its effects at lower concentrations than either preparation was used alone *in vitro* and *in vivo* (Matsumori, 1993). It is possible that such a combination would reduce the frequency of undesirable side effects for either drug used at a high concentration. The use of combinations of antivirals deserves further careful study for other serious viral infections.

Immunosuppressive Agents

Tomioka et al. (1986b) studied the effects of prednisolone on EMCV myocarditis in a murine model; however, the survival rate of mice treated with prednisolone was significantly lower than that of the controls. The viral

concentration in the heart was higher, the myocardial necrosis was more severe, and the neutralizing antibodies were lower in the prednisolone-treated group. Therefore, corticosteroids may aggravate the course of acute viral myocarditis. Cyclosporine is a fungal metabolite with immunosuppressive properties. The mortality of mice infected with EMCV myocarditis and treated with cyclosporine was higher, and they had more severe inflammation (Monrad et al., 1986). Thus, immunsuppressive therapy for acute viral myocarditis was detrimental. FTY720 (fingolimod) is a known sphingosine-1-phosphate (S1P) receptor agonist that exerts strong anti-inflammatory effects and was approved as the first oral drug for the treatment of multiple sclerosis by the US Food and Drug Administration (FDA) in 2010. FTY720 is mainly associated with unique functional antagonistic and agonistic mechanisms. The functional antagonistic mechanism is mediated by the transient down-regulation and degradation of S1P receptors on lymphocytes, which prevents lymphocytes from entering the blood stream from the lymph node. This subsequently results in the development of lymphopenia and reduces lymphocytic inflammation. (Wang et al., 2020). We previously showed that FTY720 had a notable therapeutic potential in the treatment of EMCV myocarditis due to the increased rates of survival and the attenuation of histologic abnormalities. These effects were exerted without instigating excessive virus replication (Miyamoto et al., 2001).

Nuclear Factor Kappa B (NF-κB) Inhibitors

Angiotensin II and viral infection have been shown to activate NF-κB, and to increase the expression of TNF-α and IL-1β in the hearts of normal mice, but not in the AT$_1$ receptor of knockout mice (Yamamoto et al., 2003). Therefore, the authors surmised that AT$_1$ receptor signaling may be required in the development of virus-induced myocardial injury through the pro-inflammatory angiotensin II and NF-κB/cytokine pathway. Furthermore, aldosterone has also been shown to activate NF-κB and increase the expression of cytokines and adhesion molecules (Sun et al., 2002). Therefore, the renin–angiotensin–aldosterone system could play an important role in inflammation (Figure 2). Inhibitors of angiotensin-converting enzyme (Suzuki et al., 1993) and the AT-1 receptor (Tanaka et al., 1994), as well as an aldosterone antagonist, eplerenone, improved EMCV myocarditis (Xiao et al., 2009). These beneficial effects of eplerenone were associated with a decreased number of mast cells and the decreased expression of chymase, procollagen type I, and MMP-9. These results suggested that eplerenone has an anti-

inflammatory effect by inhibiting mast cell-derived proteinases and improving myocardial remodeling by suppressing fibrosis (Xiao et al., 2009).

Phosphodiesterase inhibitors, which were originally developed for heart failure therapy, inhibit the production of cytokines. Among these, pimobendan, which has been shown to improve the quality of life and decrease events in patients with heart failure, improved survival, attenuated inflammatory lesions, and decreased the expression of IL-1β, IL-6, TNF-α, and nitric oxide in mice with EMCV myocarditis (Iwasaki et al., 1999). Pimobendan blocked the activation of NF-κB; however, other phosphodiesterase III inhibitors did not have the same effect (Matsumori et al., 2000a). Furthermore, we found that another NF-κB inhibitor, SUN C8079, suppressed cytokine production and protected against viral myocarditis (Matsumori et al., 2004a). The anti-arrhythmic agent amiodarone, which has been shown to improve the long-term prognosis of heart failure, was shown to inhibit the production of TNF-α and IL-6 (Matsumori et al., 1997, Ito et al., 2002). Therefore, the therapeutic effects of drugs used to treat heart failure may be related to modulating inflammatory responses.

Cytokines

In a study by Yamada et al., (1994), viral myocardial concentrations were higher in TNF-α-treated mice, and myocardial necrosis and cellular infiltration were more prominent in the TNF-α group, as compared to the control group. The anti-TNF-α monoclonal antibody improved survival and myocardial lesions. TNF-α may play an important role during the very early stages of the immune response, and the anti-TNF-α monoclonal antibody may prevent the early pathway of acute viral myocarditis (Yamada et al., 1994).

In a later study by Shioi et al. (1997), the gene expression of IL-12 was enhanced in the hearts of mice infected with EMCV myocarditis, and treatment with neutralizing anti-IL-12 resulted in increased mortality. The administration of IL-12 reduced mortality, myocardial lesions, and viral concentration in the hearts of mice infected with EMCV. Thus, endogenous and exogenous IL-12 play protective roles in murine viral myocarditis.

We have also studied the effect of IL-10, an anti-inflammatory cytokine, in the treatment of EMCV myocarditis, and while we found that IL-10 decreased inflammation, it did not affect viral replication (Nishio et al., 1999). Cytokine gene therapy with viral IL-10 and IL-1 receptor antagonist using electroporation has been shown to be effective in the same model (Nakano et al., 2001, Adachi et al., 2002).

Calcium Channel Blockers

Calcium channel blockers (CCBs) have been shown to suppress the activation of various immune reactions such as macrophages, mast cells, and T cells. Our previous study showed that nifedipine inhibited the activation of NF-κB (Matsumori et al., 2000b, Liu and Matsumori, 2011). Different modulations of cytokine production were observed in various CCBs, and the suppressive effect was most prominent in nifedipine (Matsumori et al., 2010c). Myocardial necrosis was decreased, and the mast cell density was lower in the nifedipine group. The expressions of MMPs, mast cell proteases, TNF-α, IL- 6, SCF, and procollagen I were lower in the nifedipine group (Liu et al., 2009). These results suggested that nifedipine inhibited the activation of various participants in the inflammatory and immune reactions in EMCV myocarditis and may be applicable for the treatment of viral myocarditis.

Another CCB, amlodipine, inhibited nitrite formation in macrophages infected with EMCV and decreased myocardial lesions significantly in a murine model. Immunohistochemistry revealed that the number of cells stained with an antibody against an inducible nitric oxide synthase (iNOS) decreased significantly in the amlodipine-treated group, as compared to the control group. Amlodipine appeared to have a protective effect against myocardial injury in EMCV myocarditis. The therapeutic effect of amlodipine may result from the inhibition of nitric oxide overproduction (Wang et al., 1997).

Beta-Adrenergic Blockers

The third-generation, nonselective beta-blocker carvedilol was the first among several beta-blockers to reduce mortality in heart failure in clinical trials. We compared the effects of carvedilol, the selective beta (1)-blocker metoprolol, and the nonselective beta-blocker propranolol in EMCV myocarditis. Plasma catecholamine levels were increased by the EMCV infection. Carvedilol improved the survival of the animals, attenuated the myocardial lesions, increased myocardial IL-12 and IFN-γ, and reduced myocardial virus replication. Propranolol decreased myocardial lesions, but to a lesser extent, and increased the IL-12 and IFN-γ levels. Metoprolol had no effect in this study. Carvedilol may be effective in patients with viral myocarditis by boosting IL-12 and IFN-γ production (Nishio et al., 2003).

Pycnogenol

Pycnogenol (PYC), a natural plant extract derived by a standardized extraction process from the bark of the French maritime pine (*Pinus pinaster, subsp.*

atlantica), is a highly concentrated source of flavanols as well as phenolic and cinnamic acids (Rodewald, 2015). In a previous study, we investigated the anti-inflammatory properties of PYC in a mouse model of EMCV myocarditis. The results showed that PYC inhibited the replication of EMCV both *in vitro* and *in vivo* in animals and improved inflammation and preserved myocardial tissue by decreasing necrosis (Matsumori et al., 2007). Administration of PYC significantly inhibited the gene expression of pro-inflammatory cytokines while also inhibiting the expressions of mast cell-related tryptase and SCF. More investigation is needed to determine mechanisms behind the antiviral effect of PYC; however, our results confirmed its prominent anti-inflammatory effect. In related research, our group reported that inhibiting the activation of NF-κB and iNOS provided significant beneficial effects in EMCV myocarditis. As PYC displayed a similar inhibitory activity against NF-κB and iNOS, this may be a part of the mechanisms by which the extract improved EMCV myocarditis (Figure 2). Effects of PYC on hepatitis B virus (HBV) and hepatitis C virus (HCV) will be discussed in the next section.

Immunoglobulin Free Light Chains (FLCs)

Immunoglobulin is composed of two identical heavy chains and two identical light chains and provides a defense against all extracellular and some intracellular pathogens. In mammals, immunoglobulin light chain genes generally exist in two distinct isotypes, κ and λ, and are produced by B cells and plasma cells. Viral infection has been shown to increase FLCs in various body fluids, and FLCs production was greatly enhanced in mice infected with EMCV. Furthermore, FLCs have been shown to have protective effects in viral myocarditis, most likely through direct antiviral activity and an anti-inflammatory effect caused by the increased expression of IL-10 (Matsumori et al., 2010a).

FLCs influence various biological activities such as enzymatic activity, specific binding activity for substrates and antigens, and binding to different cells, but their immunological function is not yet fully understood. In a previous study, we found that circulating κ FLC monomers and dimers were increased in mice with EMCV myocarditis, particularly when myocardial necrosis became apparent, and was further increased during the heart failure stage (Matsumori et al., 2010a). The application of the FLC antagonist F991 during the first stage of the viral infection caused the mice infected with EMCV to deteriorate quickly, suggesting that FLCs play a protective role. In contrast, FLCs given at the start of the viral infection were shown to reduce necrosis and greatly enhance survival and inhibit viral replication directly (as

shown in an *in vitro* assay). In fact, the viral concentration in the hearts of FLC-treated mice was significantly reduced. Recently, we found that circulating FLC λ was increased in patients with myocarditis and heart failure (Matsumori et al., 2020a), so the roles of FLCs as biomarkers for viral myocarditis will be discussed in the next section.

In summary, mast cells, angiotensin II, NF-κB, and cytokines may play important roles in the pathogenesis of viral myocarditis. Anti-viral drugs; mast cell-stabilizing agents; the inhibitors of NF-κB; the renin–angiotensin–aldosterone system; IL-10 and IL-12; fingolimod; carvedilol; nifedipine; amiodarone; pycnogenol and FLCs prevented viral myocarditis in experimental murine models of viral myocarditis, and show potential in the treatment of viral myocarditis (Figure 2).

Large Animal Model of Viral Myocarditis

Gwathmey et al. (1992) studied 26 young pigs infected with EMCV. All the infected animals appeared ill, manifesting decreased appetite, lethargy, and fever. Spontaneous death occurred either 1– 4 days or 20 –21 days after infection. Electrocardiographic abnormalities were seen in most of the animals and included ST-T wave changes, conduction disturbances, and ventricular ectopic rhythms. Echocardiography showed that the majority of animals had left ventricular dilation and decreased systolic function that improved with time, but only in some animals. Hemodynamic testing revealed elevated biventricular filling pressures in some animals. At autopsy, the heart weight–body weight ratio was significantly elevated in the infected animals (Gwathmey et al., 1992). Histologically, there was a proliferation of fibrous connective tissue, calcification-associated necrosis, and mononuclear infiltration in pigs 30 days post-infection. Interestingly, the presence of giant cells in the left ventricle was noted, along with cardiac dysfunction, which is similar to the giant cell myocarditis that can be found in humans (Njenga et al, 2003). The hearts of the infected animals showed active myocarditis associated with fibrosis and focal calcification during the later stages. In general, the cardiovascular manifestations were analogous with those seen in acute and subacute myocarditis in humans. It was concluded that EMCV infections in pigs could function as a large animal model of viral myocarditis and may be suitable for assessing alterations in the structure and function of the cardiovascular system and the effects of interventions (Gwathmey et al., 1992).

HCV Myocarditis

HCV infection has been associated frequently with patients diagnosed with myocarditis, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and hypertrophic cardiomyopathy (Matsumori et al., 2000c, Matsumori, 2005, Matsumori, 2009). Various arrhythmias, conduction disturbances, and QT prolongation were also associated with HCV infection (Haykal et al., 2021) (Figure 3). In a previous study, we found that CD68-positive monocytes/macrophages are a primary target of HCV infection (Matsumori et al., 2010b). HCV-core antibodies stained mostly mononuclear cells in different body organs such as the liver, the heart, the kidney, and the bone marrow, but not hepatocytes, myocytes, or globular cells. Antibodies against NS4 protein, nonstructural antigen of HCV stained the peripheral blood mononuclear cells and the mononuclear cells of various tissues, which also confirmed that HCV replicates in mononuclear cells (Matsumori et al., 2010b).
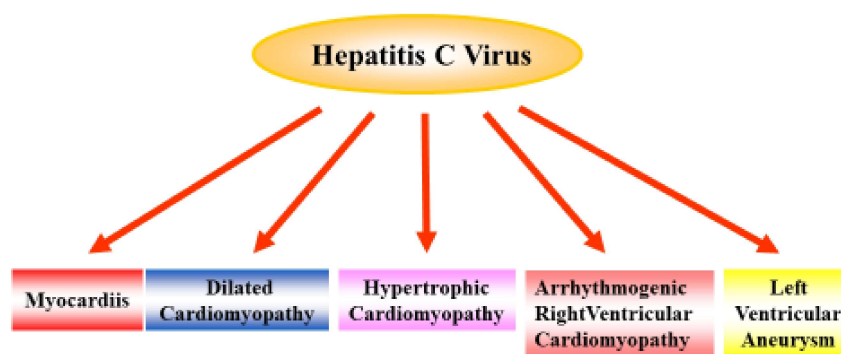

Figure 3. Hepatitis C virus causes various heart diseases.
(Reproduced with permission, Matsumori, 2005;
copyright Lippincott Williams & Wilkins).

The major human histocompatibility complex (MHC) is located on the short arm of chromosome 6 and codes for several cell surface proteins involved in immune function, such as complement system components, TNF-α, and the human leukocyte antigen (HLA) complex. In HCV hepatitis, DQB1*0301 and DRB1*1101 were associated with viral clearance. DQB1*0401 and DRB1*0405 were more prevalent in patients with chronic liver disease. We also found that DPB1*0401 and DPB1*0901 were significantly associated with an increased risk of HCV-associated

hypertrophic cardiomyopathy (Matsumori et al., 2003b, Shichi et al., 2008) (Figure 4). The susceptibility to HCV-associated dilated cardiomyopathy was mapped to a non-HLA gene locus spanning from NFKBIL1 to MICA gene loci within the MHC class III–class I boundary region. Our results showed that HCV-associated dilated cardiomyopathy had a stronger association to non-HLA genes over HLA genes. This marked difference in the MHC-related disease susceptibility for HCV-associated cardio-myopathies suggested that the development of HCV-associated dilated cardiomyopathy and hypertrophic cardiomyopathy was controlled by different pathogenic mechanisms (Shichi et al., 2005).
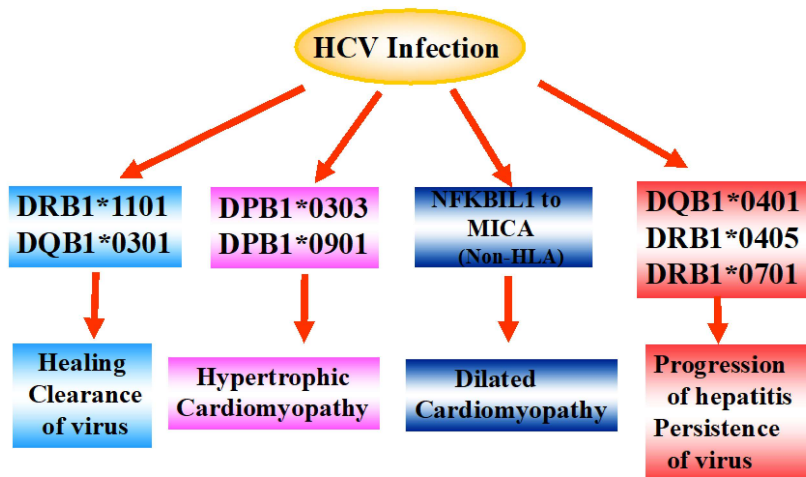

Figure 4. Major histocompatibility complex genes and HCV infection.
(Modified from Matsumori, 2005).

Haykal et al., (2021) found that patients with HCV infections had impaired left atrial functions as well as systolic and diastolic functions of the left ventricle (LV), and this finding could explain the atrial arrhythmias frequently observed in HCV patients.

Saleh et al. (2011) used tissue Doppler measurements to show that patients with HCV infections had atrial and ventricular functional abnormalities, and a significant correlation was detected between N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and tissue Doppler parameters. Haykal et al. (2021) studied anti-inflammatory therapies with cetirizine, which had been effective in animal models of viral myocarditis, in human patients

with HCV infections into heart failure and showed that myocardial function was substantially improved in patients who took cetirizine. There was a significant decrease in global LV average strain and a significant increase in LV ejection fraction.

Anti-Viral Effects of PYC on HCV

PYC treatment showed antiviral effects against the HCV and showed synergistic effects with IFN-α or ribavirin *in vitro* (Ezzikouri et al., 2015). PYC worked additively with the antiviral preparation, telaprevir, to lower levels of HCV RNA in wild-type HCV replicon cells while showing no cytotoxic effects. Further, PYC was shown to inhibit viral replication in telaprevir-resistant replicon cells as well. Investigations into the efficacy of PYC extract fractions showed that PYC had significantly higher antiviral activity, as compared to its individual components, procyanidin and taxifolin. *In vivo* investigations into HCV-infected chimeric mice revealed that PYC again inhibited HCV replication. Further, the extract showed a synergistic antiviral effect with IFN-α. Lastly, the addition of PYC to HCV replicon cell lines resulted in a dose-dependent reduction in reactive oxygen species (Ezzikouri et al., 2015).

Anti-Viral Effects of PYC on HBV in Humans

We performed a double-blind, placebo-controlled study involving patients with hepatitis B virus. A total of 200 mg of PYC was given daily over 12 weeks to the experimental group while the control group was given a placebo. In the PYC group, the circulating HBV was reduced, and hepatic function was improved (Matsumori, 2021b).

PYC as an Anti-Viral and Inti-Inflammatory Agent

Since PYC has broad spectrum anti-viral effects against several viruses, *in vitro* and *in vivo*, as well as anti-inflammatory effects against many inflammatory diseases, PYC is a promising agent for the prevention and treatment of viral myocarditis in humans.

Animal Model of HCV Cardiomyopathy

Transgenic mice for the HCV-core gene were developed. After the age of 12 months, mice showed left ventricular dilatation and systolic and diastolic dysfunction as seen in dilated cardiomyopathy, which was assessed by

Doppler echocardiography. Histologically, hypertrophy of cardiomyocytes, cardiac fibrosis, disarray and scarcity of myofibrils, vacuolization and deformity of nuclei, myofibrillar lysis, streaming of Z-bands, and an increased number of bizarre-shaped mitochondria were found (Omura et al., 2005).

Biomarkers of Viral Myocarditis

Natural killer cells, macrophages, and, eventually, T lymphocytes are recruited to the site of infection, causing myocardial injury. Autoimmune reactions activate virus-specific T cells that target host myocardium. High levels of cytokines, particularly TNF-α, IL-1α, IL-1β, IL-2, and IFN-γ, are produced during this phase. These cytokines, together with antibodies to viral and cardiac proteins, can further potentiate cardiac damage and compromise systolic function through the derangement of the contractile apparatus and/or interstitial cells and matrix proteins (Pollack et al., 2015).

Circulating cardiac biomarkers such as troponin I and troponin T, are increased in myocarditis and are helpful for the diagnosis, but have low sensitivity. In the U.S. Multicenter Myocarditis Treatment Trial, the sensitivity of elevated troponin I levels in patients with biopsy-proven myocarditis was 34%, specificity was 89%, with a positive predictive value of 82% (Smith, et.al., 1997). Troponin I and troponin T are more frequently elevated than creatinine kinase-MB, with increased levels of troponin I at presentation and, therefore, portending a worse prognosis. However, in patients who were treated in hospital with acute or fulminant myocarditis, creatine kinase-MB concentrations of greater than 29.5 ng/mL predicted in-hospital mortality with a sensitivity of 83% and a specificity of 73%. In acute or fulminant myocarditis, higher IL-10 and soluble Fas concentrations have been associated with an increased risk of death; however, tests for these markers are not commonly used in clinical practice (Sagar et al., 2012). In acute myocarditis, the presence of anti-heart antibodies could predict the risk of death or need for transplantation (Caforio et al., 2008). These findings have led to the routine measurement of cardiac biomarkers in patients with suspected myocarditis. Other serum markers of inflammation, such as white blood cell count, erythrocyte sedimentation rate, and C-reactive protein levels, can be elevated in acute myocarditis, but they are neither sensitive nor specific in terms of determining the presence or absence of active myocardial inflammation (Smith, et al., 1997, Mahfoud et al., 2011).

In 1995, we developed a new method to detect autoantibodies against cardiac troponin I (cTnI), and we measured these autoantibodies in patients enrolled in the U.S. Multicenter Myocarditis Treatment Trial (Mason et al., 1995). Myocarditis, as 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 etiological 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 biopsies satisfied the Dallas criteria than in those whose biopsies were negative. Furthermore, among those whose biopsies satisfied the Dallas criteria, those with anti-HCV antibodies had higher titers than those who were not infected with HCV. Our study showed that anti-cTnI antibodies are often present in patients with active myocarditis, suggesting that their presence is correlated with ongoing inflammation. Therefore, the detection of anti-cTnI antibodies may be helpful in the diagnosis, as well as the evolution and follow-up, of patients presenting with active myocarditis with viral infection (Matsumori et al., 2011).

Increased inflammatory cells and expressions of pro-inflammatory cytokines in heart disease has attracted attention since the elevation of circulating TNF-α and cardiac cachexia were reported. We demonstrated that blood levels of IL-α, IL-β, and TNF-α are frequently elevated in myocarditis, and that the level of TNF-α is frequently elevated in dilated or hypertrophic cardiomyopathy (Matsumori et al., 1994). These findings suggested that these cytokines play an important role in the pathogenesis of myocarditis and cardiomyopathies. In addition, the elevation of blood levels of soluble TNF-α receptors, IL-2 receptors, IL-1 receptor antagonists, and IL-18 in patients with cardiomyopathy and heart failure has been reported. In patients with heart failure, the blood levels of chemokines or macrophage chemotactic factors (e.g., monocyte chemo-attractant protein-1 (MCP-1), macrophage inflammatory protein-1α, and RANTES) were reported to be elevated; blood levels of MCP-1 and macrophage inflammatory protein-1α, were negatively correlated with a LV ejection fraction. Furthermore, the number of macrophages have been shown to increase in animal models of hypertension (Reviewed by Matsumori, 2004b).

Elevated serum levels of the soluble suppression of tumorigenicity-2 (sST2) have been correlated with the severity of heart failure in patients with myocarditis, dilated cardiomyopathy, and other cardiovascular conditions (Coronado, 2019). The cytokine sST2 is induced via the biomechanical strain in cardiac fibroblasts, cardiomyocytes, and vascular endothelial cells. In several recent studies, sST2 has been found to predict the risk of developing heart failure in patients with dilated cardiomyopathy. When combined with NT-proBNP, serum sST2 levels provide additive value in predicting sudden death in patients with heart failure along with decreased LV systolic function (reviewed by Scultheiss et al., 2019).

The New FLC Biomarker of Viral Myocarditis

FLCs are synthesized de novo and secreted into circulation by B cells and plasma cells. As FLCs emerge as an excess byproduct of antibody synthesis by B cells and plasma cells, elevated FLCs have been proposed as a biomarker of B cell activity in many inflammatory and autoimmune conditions (Hampson and Turner, 2014). Polyclonal FLCs are reported to be a predictor of mortality in the general population by measuring the sum of κ and λ concentrations (Dispenzieri et al., 2012). Increased κ levels occurred in rheumatic diseases, and the κ/λ ratio was higher than in healthy blood donors (Gulli et al., 2020). FLCs in inflammatory and autoimmune diseases were correlated with disease activity, suggesting their role as potential therapeutic targets in such conditions.

Earlier we mentioned that we observed that FLCs were increased in a murine model of heart failure due to EMCV myocarditis (Matsumori et al., 2010a). Recently, we conducted additional research with patients in heart failure, and we observed that circulating FLC λ were increased while the κ/λ ratio was decreased in sera from patients with heart failure resulting from myocarditis, as compared to a group of healthy controls. These findings demonstrated that the FLC λ and κ/λ ratio together showed good diagnostic potential for the identification of myocarditis (Figure 5). In addition, the FLC κ/λ ratio could also be used as an independent prognostic factor for overall patient survival (Figure 6) (Matsumori et al., 2020a).
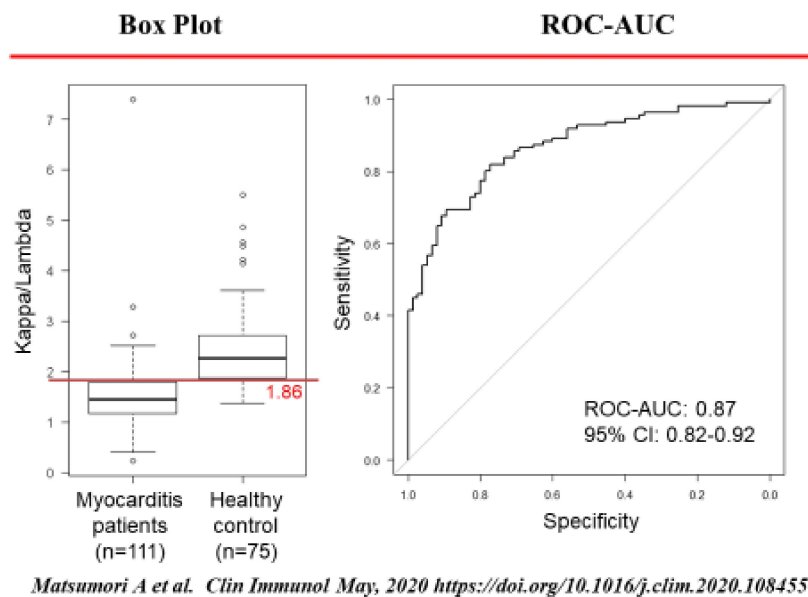
Figure 5. Ability of immunoglobulin free light chains (FLC) κ/λ ratio to distinguish between patients with myocarditis into heart failure and healthy controls. (Reproduced with permission, Matsumori, 2020a; copyright Elsevier). a: Boxplot for FLC κ/λ ratio in myocarditis patients with heart failure and healthy controls. The optimal cutoff with minimum p-value is 1.86. b: ROC-AUC (area under the receiver operating characteristic curve) indicated ability of FLC κ/λ ratio to distinguish between myocarditis patients with heart failure and healthy controls.

High concentrations of FLC κ have been observed in HCV-positive patients, and an alteration in the κ/λ ratio has been positively correlated with increasing severity of HCV-related lymphoproliferative disorder (Terrier et al., 2009). Furthermore, it has been suggested that the κ/λ ratio may be useful in the evaluation of therapeutic efficacy (Basile et al., 2015). As we discussed in a previous section, HCV infection has often been associated with myocarditis. In our study on FLCs using sera from the U.S. Multicenter Myocarditis Treatment Trial, myocardial injury was more severe in patients with HCV infection than in non-infected patients. The level of FLC κ was lower, FLC λ was higher, and the κ/λ ratio decreased in patients with myocarditis, both with and without biopsy-confirmation according to the Dallas criteria, as compared to normal volunteers.

These changes were more prominent in patients with HCV infection, as compared to those without infection. HCV infection may enhance the production of FLC λ while decreasing FLC κ (Matsumori, 2008, Figure 7).

Although the mechanisms of these changes require clarification, the detection of FLCs might be helpful for the diagnosis of myocarditis with heart failure and also be useful in differentiating patients with HCV infection from those without infection (Matsumori, 2008). In heart failure patients, LV end-diastolic and end-systolic diameters, pulmonary arterial pressure, and NT-proBNP correlated positively with FLC λ and negatively with the κ/λ ratio. Left ventricular ejection fraction was also negatively correlated with the κ/λ ratio (Matsumori, 2008).
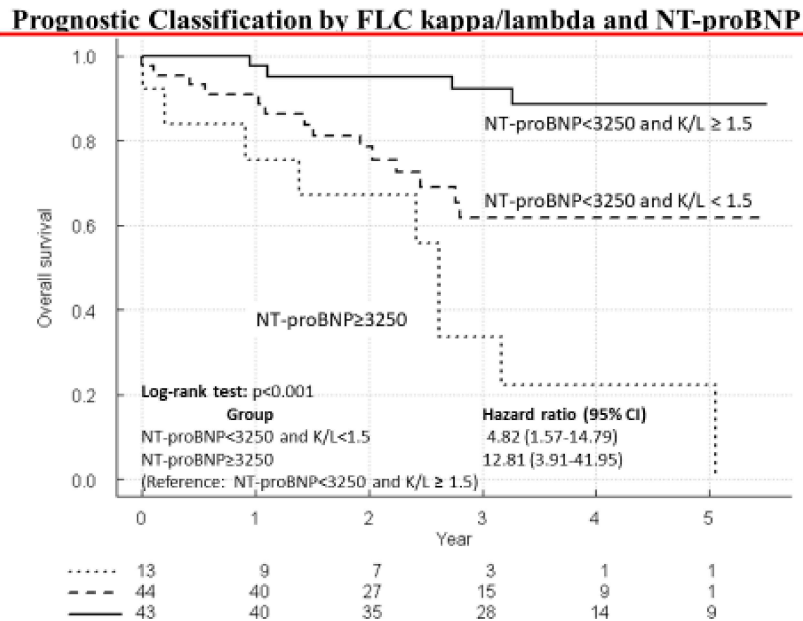

Figure 6. Prognostic classification by FLC κ/λ and NT-proBNP (pg/mL). Kaplan-Meier curve for overall survival was stratified by three groups with NT-proBNP ≥ 3250, NT-proBNP < 3250, and κ/λ ≥ 1.5; and NT-proBNP < 3250 and κ/λ < 1.5. (Reproduced with permission, Matsumori, 2020a; copyright Elsevier).
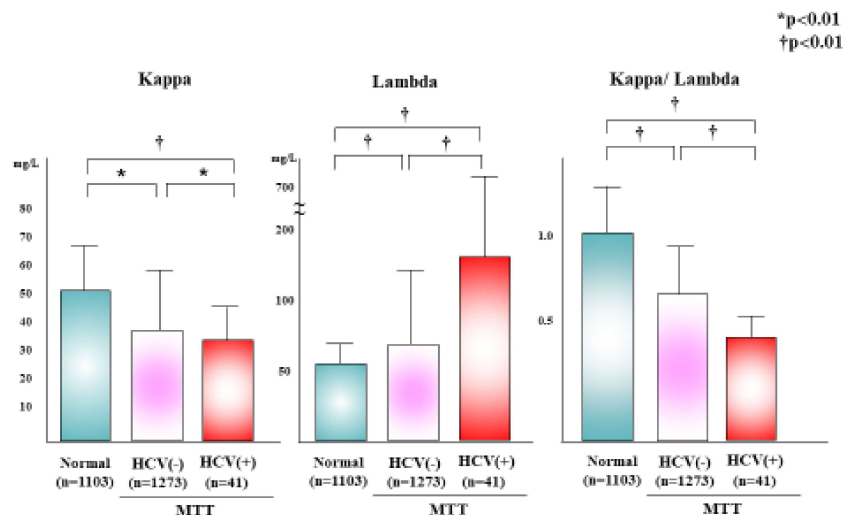
Figure 7. Comparisons of mean FLCs concentrations and κ/λ ratios in 1103 normal volunteers (NV) versus in 1273 patients with heart failure without antibody to hepatitis C virus (HCV) versus 41 patients with heart failure with antibody to HCV.

FLCs and COVID-19 Myocarditis

The recent review of 316 cases of postmortem examination of COVID-19 patients demonstrated that cardiac abnormalities either on gross pathology or histology were identified in almost all cases. Most autopsies demonstrated chronic cardiac pathologies such as hypertrophy (27%), fibrosis (23%), amyloidosis (4%), cardiac dilatation (20%), acute ischemia (8%), intracardiac thrombi (2.5%), pericardial effusion (2.5%), and myocarditis (1.5%). SARS-CoV-2 was detected within the myocardium of 47% of studied hearts by Roshdy et al. (2021). However, the Dallas criteria was satisfied in only five of these cases. In an additional 35 cases, minimal lymphocytic or mononuclear infiltration was reported, and they did not satisfy the Dallas criteria for myocarditis. Lymphocytic infiltration was scarce but could be detected in the pericardium, myocardium, epicardium, or endothelium. Therefore, cellular infiltration may be rare in COVID-19 myocarditis, and, therefore, the Dallas criteria may not be accurate in the diagnosis of COVID-19 myocarditis as in the case of HCV myocarditis (Hykal et al., 2021).

An increase in blood troponin levels in COVID-19 is an indicator of myocardial damage. Several studies have documented a strong association

between COVID-19 progression and elevated blood troponin. Reports from China found that elevated circulating cardiac troponin was present in 7%–28% of COVID-19 patients, suggesting the existence of myocardial injury or myocarditis (Chung et al., 2021, Matsumori and Mason, 2021). In hospitalized patients with COVID-19, mortality in the elevated-blood-troponin group was 51.2%–59.6%, a range markedly higher than in the 4.5%–8.9% in the normal-blood-troponin group (Komiyama et al., 2019).

We have studied how frequently myocardial injury or myocarditis occurs in COVID-19 patients (Saleh et al., 2020). Troponin T was positive in 63% of patients, NT-proBNP was elevated in 68% of patients, and elevated creatine kinase was noted in 43% of patients at admission (modified from Saleh et al, 2020, Table1). NT-proBNP showed a significant correlation with the length of hospital management and the severity of pulmonary CT findings. In addition, the existence of enhanced inflammatory biomarkers such as C-reactive protein and ferritin suggested that myocardial injury may be caused by inflammatory myocardial processes. D-dimer was also elevated frequently, suggesting that coagulation abnormality occurs frequently in COVID-19 patients (Saleh et al., 2020). Thus, COVID-19 has been frequently associated with myocardial injury, suggesting that SARS-CoV-2 causes myocarditis.

We also measured FLCs and IL-6 in COVID-19 patients. FLC κ and λ was elevated in 73% and 80%, respectively, and the frequency of the elevated levels was higher than those of troponin T, NT-proBNP, creatine kinase, and IL-6. IL-6 has been frequently measured in COVID-19 patients, but elevated levels of IL-6 were less frequent, as compared to other parameters (Saleh and Matsumori, unpublished observation, Table 1).

Table 1. Myocardial involvement in COVID-19.
Biomarkers in 40 patients who admitted to the hospitals

	Patients with Elevated Levels		Normal range
	n (%)	mean	
Troponin (pg/ml)	25 (63%)	28	<14
NT-proBNP (pg/ml)	27 (68%)	1848	<486
Creatinine kinase (U/l)	17 (43%)	243	<190
FLC kappa (mg/l)	29 (73%)	38.5	8.3–27.0
FLC lambda (mg/l)	32 (80%)	41.1	6.7–22.4
D-dimer (mg/l)	33 (83%)	2.3	<0.44
IL-6 (pg/ml)	6 (15%)	7.1	<8

FLCs and Atrial Fibrillation

Atrial fibrillation is most common arrhythmia and is a key factor in strokes. It is important to develop a method to detect early or asymptomatic atrial fibrillation. Abnormal atrial histology compatible with a diagnosis of myocarditis was uniformly found in patients with lone atrial fibrillation. Patients with atrial fibrillation exhibited a higher concentration of cytokines, higher NF-κB activity, and more severe lymphomonocyte infiltration than those with sinus rhythm. These observations imply local immunologic inflammatory responses in the atria in atrial fibrillation (Matsumori et al., 2020b). The concentrations of circulating FLC κ and λ in patients with lone atrial fibrillation were significantly different from the healthy volunteer group. The area under the curve of the receiver-operating characteristic curve analysis showed that FLC κ and λ were helpful in differentiating atrial fibrillation from healthy volunteers and that the cutoff value of FLC κ or λ may be beneficial to distinguish the two groups (Matsumori et al., 2020b). The mechanism by which FLCs may cause atrial fibrillation is not yet fully understood, but the inflammation associated with FLCs directly may induce atrial fibrillation, or FLCs may cause a change in membrane fluidity, which in turn could alter ion channel function (Matsumori et al., 2020b).

Conclusion

Animal models of viral myocarditis are useful to study the natural history, genetics, pathogenesis, and clinical manifestations of viral myocarditis in humans, and to develop new methods for the prevention and treatment of the disease. By using animal models, it was shown that mast cells, angiotensin II, NF-κB, and cytokines may play important roles in the pathogenesis of viral myocarditis, and that mast cell stabilizing agents, the inhibitors of NF-κB and the renin–angiotensin–aldosterone system; fingolimod; IL-10 and IL12; some CCBs; carvedilol; PYC; and FLCs prevented viral myocarditis, and these agents could be promising for the treatment of viral myocarditis in humans.

Cellular infiltration is not prominent in certain types of viral myocarditis including HCV and COVID-19 myocarditis, and the use of the Dallas criteria for the diagnosis of viral myocarditis may not be appropriate for the diagnosis of these diseases. The presence of viral genomes in the heart does not necessarily indicate the cause of the disease, and inflammatory and immune responses are needed to develop the disease. Interestingly, anti-inflammatory agents that do not have anti-viral effects improve viral myocarditis. Therefore,

combined therapy employing anti-viral and anti-inflammatory agents may have potential as a treatment for viral myocarditis in humans. FLCs, which reflect the activation of B cells and plasma cells, are promising for the diagnosis of viral myocarditis.

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Research and Professional Experience: Akira Matsumori received his MD degree from Kyoto University School of Medicine and a PhD degree from the Graduate School Kyoto University, Japan. Dr. Matsumori is President of a nonprofit organization the Asian Pacific Society of Cardiology, Kyoto, Japan, He is also the founder and current president of the International Society of Cardiomyopathies, Myocarditis and Heart Failure as well as serves as a Visiting Director at the Clinical Research Center of the Kyoto Medical Center. He has served as Secretary of the World Heart Federation, President of the Asian Pacific Society of Cardiology, and has also been an Instructor and Visiting Professor at the Harvard Medical School. Dr. Matsumori's research interest includes pathogenesis of heart disease, biomarkers and therapy of heart failure, cardiomyopathies and myocarditis, and the roles of cytokines, immunity and inflammation in cardiovascular diseases.

Dr. Matusmori is known as a leader in the study of cardiomyopathies and myocarditis. He is known for his landmark work on viral heart diseases and the pathogenesis and therapy of cardiomyopathies, heart failure and myocarditis. He is known for the identification of new biomarkers of cardiovascular diseases.

Dr. Matsumori published 234 peer reviewed articles, 60 reviews and 29 book chapters in English in clinical as well as top tier scientific journals. He has also served on 14 editorials boards including the highly prestigious journals. Dr. Matsumori has given 241 presentations at national and international forums.

Professional Appointments:

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