From Myocarditis to Cardiomyopathy: Mechanisms of Inflammation and Cell Death

Learning From the Past for the Future

Chuichi Kawai, MD

Abstract—A progression from viral myocarditis to dilated cardiomyopathy has long been hypothesized, but the actual extent of this progression has been uncertain. However, a causal link between viral myocarditis and dilated cardiomyopathy has become more evident than before with the tremendous developments in the molecular analyses of autopsy and endomyocardial biopsy specimens, new techniques of viral gene amplification, and modern immunology. The persistence of viral RNA in the myocardium beyond 90 days after inoculation, confirmed by the method of polymerase chain reaction, has given us new insights into the pathogenesis of dilated cardiomyopathy. Moreover, new knowledge of T-cell–mediated immune responses in murine viral myocarditis has contributed a great deal to the understanding of the mechanisms of ongoing disease processes. Apoptotic cell death may provide the third concept to explain the pathogenesis of dilated cardiomyopathy, in addition to persistent viral RNA in the heart tissue and an immune system–mediated mechanism. Beneficial effects of α1-adrenergic blocking agents, carteolol, verapamil, and ACE inhibitors have been shown clinically and experimentally in the treatment of viral myocarditis and dilated cardiomyopathy. Antiviral agents should be more extensively investigated for clinical use. The rather discouraging results obtained to date with immunosuppressive agents in the treatment of viral myocarditis indicated the importance of sparing neutralizing antibody production, which may be controlled by B cells, and raised the possibility of promising developments in immunomodulating therapy. (Circulation. 1999;99:1091-1100.)

Key Words: viruses ■ myocarditis ■ cardiomyopathy ■ immune system ■ apoptosis

Since Gore and Saphir demonstrated in 1947 that rheumatic and diphtheritic carditides each constituted only 10% of a series of 1402 cases of myocarditis, increasing attention has been paid to myocarditis occurring in various viral diseases. A number of agents induce myocarditis. Among them, viruses are believed to be the most common agents causing myocarditis in the developed countries. However, direct proof of the presence of a given virus in the heart is very difficult to obtain in clinical settings.

A 4-fold rise in neutralizing antibody titers to viruses in paired sera over a 2- to 4-week period has been generally believed to establish a viral pathogenesis in patients with myocarditis. The persistence of a higher incidence of neutralizing antibodies to coxsackievirus B (CVB) in patients with cardiomyopathy than in age-, sex-, race-, and living district–matched control subjects has prompted the theory of a viral cause underlying the pathogenesis of cardiomyopathy.2,3

The present review summarizes clinical and experimental studies on the viral origin of myocarditis and dilated cardiomyopathy and suggests directions for future investigations.

Host Factors That Influence Susceptibility to Viral Myocarditis

The virulence of murine viral infection is increased by malnutrition,4 exercise,5 sex and sex hormones,6–8 and age.4–10 More importantly, genetic factors, including immune states, which are heavily involved in the above-mentioned host factors, play a crucial role in susceptibility to infection.

Ample evidence suggests that the same strain of virus causes different lesions in the heart in different inbred strains of mice. Severe myocarditis was induced at high frequency in BALB/c, DBA/2, and C3H/He mice inoculated with EMC virus, but A/J and C57BL/6 mice treated similarly did not show any cardiac lesions.11,12 Susceptibility to viral infection may be regulated primarily by the major histocompatibility complexes, or H-2 complexes, in each strain of inbred mice.

Sequential Pathological Changes in Murine Viral Infection

After inoculation of mice with a virus, 2 principal pathogenic mechanisms, the direct viral and immunocyte-mediated pathogenic mechanisms, are implicated in the destruction of...
myocardial tissue. The outline of temporal changes in the murine myocytes inoculated with virus is depicted as a flow diagram in Figure 1.

Acute Phase of Viral Myocarditis (Days 0 to 3)
Mice injected intraperitoneally with a cardiotropic virus (e.g., encephalomyocarditis virus [EMCV] or CVB) exhibit a direct consequence of virus-induced cytoyticity, including focal necrotic myofibers in the absence of an inflammatory cell infiltrate within 3 days after infection. Cytokine mRNA, such as that of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, is already induced 3 days after inoculation, when few cell infiltrates are seen.

Subacute Phase of Viral Myocarditis (Days 4 to 14)
After viral invasion of the myocardium, the first wave of infiltrating cells in the heart consists mainly of natural killer (NK) cells. Various cytokines, including IL-1β, TNF-α, IFN-γ, and IL-2, are produced at this stage and persist as long as 80 days after the inoculation of EMCV. Circulating levels of plasma TNF-α, IL-1α, and IL-1β were also elevated in patients with acute myocarditis, dilated cardiomyopathy, and other cardiac patients with congestive heart failure. It is well known that most cytokines have multiple biological activities that overlap, and there is considerable redundancy in actions. A given cytokine can activate a variety of cell types. Such interactions are further amplified by the capacity of some cytokines to act as potent inducers of other cytokines. Thus, most cytokines exert both beneficial and deleterious effects on myocytes. Nitric oxide (NO) is also generated during this phase in response to cytokines that are induced by EMCV. Both beneficial and detrimental actions of NO have also been observed. NO is beneficial as a modulator of immunological self-defense mechanisms, and at the same time, it plays an important role in killing infectious agents. Conversely, NO is also associated with detrimental effects on the myocardial tissue in autoimmune myocarditis in rats.

NK Cells, Perforin, and IFN
NK cells, activated by IL-2, have protective effects against viral invasion by limiting virus replication. The hypothesis of the defensive role of NK cells during viral infection has been supported by the prolonged viral infection or increased viral titers and severe myocarditis in murine strains with decreased NK-cell responses and in NK-cell-deficient mice treated with antiserum against NK cells. In contrast, NK-like large granular lymphocytes and asialo GM1-positive cells both cause myocardial damage by releasing perforin molecules, which form circular pore lesions on the membrane surface of the cardiac myocytes in murine CVB3 myocarditis and in patients with acute myocarditis. However, there would seem to be no further contribution of NK cells to lesion pathology, because they interact only with virus-infected myofibers.

Viral myocarditis can be ameliorated in mice if IFN is administered before, simultaneously with, or within 24 hours of inoculation with EMCV or CVB3. NK cells and IFN frequently interact to control virus infection, but CVB-induced “native” IFN does not appear to be fully protective. As shown in the above-mentioned studies, “exogenous” IFN treatment was beneficial in ameliorating myocarditis only when IFN was administered before or during the very early stages of infection. Thus, it seems reasonable to assume that IFN evokes other antiviral processes. It is believed that the IFN-mediated induction of NO is important in controlling an enterovirus infection. “Knockout” mice rapidly succumbed to CVB3 myocarditis compared with normal infected mice carrying the IFN gene. As described previously, the IL-1β, TNF-α, and IFN-γ produced at this stage each induce inducible NO synthase (iNOS) in cardiac myocytes, but only the combination of IL-1β and IFN-γ causes contractile dysfunction in the presence of insulin in adult rat ventricular myocytes. IFN-γ, which by itself does not induce iNOS in cardiac microvascular endothelial cells, potentiates and accelerates iNOS induction by IL-1. Transforming growth factor-β (TGF-β) decreases iNOS activity, protein content, and mRNA in IL-1β- and IFN-γ-pretreated adult rat cardiac microvascular endothelial cells.

Viral Titers and Neutralizing Antibody
Viral titers in the myocardium were maximal on day 4 after EMCV inoculation in BALB/c mice. Almost no neutralizing antibodies to the virus were present until day 4, when the highest viral titer was detected. The neutralizing antibody titers were then elevated rapidly on days 8 and 10 and reached the highest level on day 14. The viral titers were still elevated on day 8 but rapidly decreased and disappeared after day 10.
EMCV; Lyt 1–positive cells (helper/inducer) were present in the myocardium of DBA/2 and BALB/c mice, Thy 1.2 (precursor) cells as the largest T-cell population, and peripheral blood and spleen B cells in both strains of mice.38 In other words, the B lymphocytes were 10% to 20% of the infiltrating lymphocytes in the myocardium of DBA/2 and BALB/c mice on days 7 to 14; thereafter, the levels of B cells decreased over 1 to 3 months. In other words, the B lymphocytes in the myocardium seem to show reciprocal changes of less intensity than those of the T lymphocytes; peripheral blood and spleen B cells in both strains of mice showed no significant changes throughout the experimental period.38

The inflammatory response continues at a lesser intensity at sites surrounding cardiac necrosis after a culturable virus has been eliminated. The cell-mediated immune mechanisms evoked by these infiltrating immune cells then play a pivotal role in the ongoing destruction of cardiac tissue. Cytotoxic T lymphocytes (CTLs; Lyt 2–positive cells) have been shown to be capable of lysing virus-infected cardiocytes in vitro.39,40 Foreign particles such as viruses are reduced to component peptides in the target cell cytoplasm, and the peptides are then placed on the surface of the target cell membrane in the groove of major histocompatibility complex (MHC) molecules for presentation to the T-cell receptors (TCRs), which consist mainly of a- and b-chain heterodimers (Figure 4), designated as Vα and Vβ, respectively. Through their TCRs, CTLs recognize virus-derived peptides presented by MHC class I antigen, which is strongly induced on cardiac myocytes in murine acute myocarditis caused by CVB3,41 and lead to further myocardial cell damage.42 A recent study demonstrated that TCR Vα and Vβ gene usage by infiltrating T cells in the murine heart on day 12 after virus inoculation is restricted, raising the possibility the myocardium to the same extent as they were in the peripheral blood and the spleen. Lyt 2–positive cells (suppressor/cytotoxic) were greatly increased in the myocardium of DBA/2 and BALB/c mice.43 These infiltrating immune cells also play a critical protective role in limiting viral replication in the heart and in eliminating infected myocardial cells. The B lymphocytes were 10% to 20% of the infiltrating lymphocytes in the myocardium of DBA/2 and BALB/c mice on days 7 to 14; thereafter, the levels of B cells increased, while those of Thy 1.2 (pan T) cells gradually decreased over 1 to 3 months. In other words, the B lymphocytes in the myocardium seem to show reciprocal changes of less intensity than those of the T lymphocytes; peripheral blood and spleen B cells in both strains of mice showed no significant changes throughout the experimental period.38

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of using anti-TCR antibodies or a vaccination with synthetic TCR V-region peptides to prevent T-cell–mediated myocardial damage after viral infection.43,44

Perforin particles expressed on virus-specific CTLs also cause myocardial injury. At the same time, virus-specific CTLs play an important role in the pathogenic immune mechanism in viral myocarditis and dilated cardiomyopathy. It is believed that cell-cell contact and adhesion are required in such immune responses. Intercellular adhesion molecule-1 (ICAM-1) expressed on the infected myocytes induced by IFN-γ and TNF-α plays a crucial role in the interaction with CTLs as the ligand of lymphocyte function–associated antigen-1 expressed on the lymphocytes. The enhanced expressions of HLA class I and ICAM-1 and the infiltration of perforin-expressing killer cells were demonstrated in the hearts of patients with acute myocarditis and dilated cardiomyopathy and in murine hearts with viral myocarditis.45 In addition, recent studies demonstrated that the adhesion molecules B7-1 (B7, CD80), B7-2 (B70, CD86), and CD40, expressed in some infected myocytes, bind to a counterreceptor, CD28 and CD40L (ligand, gp39), expressed on T lymphocytes, and play a role in the costimulation of T cells in a primary immune response through TCR.

Myocardial Injury in T-Cell–Depleted Mice

A marked reduction in myocardial damage was noted in T-cell–depleted mice inoculated with CVB3 pretreated with antithymocyte serum, in TXBM mice (thymectomized, irradiated, and reconstituted with bone marrow cells), and in mice treated with monoclonal antibody against total T cells.49,50 The reduction in myocardial damage in the mice was independent of myocardial CVB3 virus replications, because the viral titers were similar in the T-cell–depleted and intact mice.

Pathological changes in the myocardium were less prominent in T-cell–depleted nude mice (BALB/c-nu/nu) inoculated with EMCV.38 There was a mild mononuclear cell infiltration in the right and left ventricular myocardium; no cavity enlargement was visible (Figure 5). Myocardial damage in BALB/c-nu/+ mice inoculated with EMCV was markedly severe, demonstrating prominent mononuclear cell infiltration in the myocardium and cavity enlargement of both ventricles (Figure 6). Severe myocarditis was also found in BALB/c-nu/nu mice injected with spleen cells from BALB/c-nu/+ mice (Figure 7). The survival rate of the nude mice inoculated with EMCV on days 10 to 15 was significantly higher than that of nu/+ mice and nude mice injected with spleen cells from nu/+ mice. There were no significant differences in virus titrations of the heart and serum neutralizing antibody titers among the 3 varieties of mice. These results indicated that viral clearance from the myocardium is controlled by B lymphocytes, which are involved mainly in humoral immunity, and occurs independently of T-cell function in murine viral myocarditis. These studies and others support the view that the severity and development of myocarditis are mediated by T lymphocytes, which control cell-mediated immunity.

Figure 5. Hematoxylin-eosin stains of hearts obtained 16 days after inoculation with EMCV in BALB/c-nu/nu mice. There was neither cavity dilatation nor a decrease in wall thickness in right (RV) and left (LV) ventricles (top, magnification ×12). Myocardial necrosis and cellular infiltration were minimal (RV, lower left, magnification ×180; LV, lower right, magnification ×370). Modified with permission from Reference 38.

Chronic Phase of Viral Myocarditis (Days 15 to 90)

From day 15 after viral inoculation, after the culturable virus had been eliminated, myocardial damage persists insidiously.
In the DBA/2 mice surviving 90 days after acute EMCV myocarditis, both the heart weight and the heart weight/body weight ratio were significantly larger than those of control mice. The cavity dimensions of the left ventricle were enlarged. Myocardial fibrosis was prominent, particularly in the inner two thirds of the left ventricular free wall. There was no longer any inflammatory cell infiltration at this stage, thus resulting in cardiac lesions that resembled human dilated cardiomyopathy in mice 3 months after viral infection.\(^{14}\) IL-1 is said to be correlated with the increased heart weight/body weight ratio and the extent of fibrotic lesions in the chronic phase.\(^{15}\)

The pathogenic mechanisms involved in the transition from viral myocarditis to dilated cardiomyopathy have been a challenging puzzle. Because of the absence of culturable virus and viral capsid proteins after the initial phase of myocarditis, it has been suggested that a cell-mediated autoimmune mechanism(s) possibly triggered by virus infection may play a role in the pathogenesis of dilated cardiomyopathy.

**Persisting Viral RNA**

Recent evidence suggests that a viral mechanism contributes not only to the acute phase of myocarditis but also to the evolution of ongoing heart disease. With currently available molecular techniques, the role of persisting virus has been rediscovered in its interaction with the immune system to provide clinical and experimental evidence on the viral pathogenesis of myocarditis and dilated cardiomyopathy.\(^{52}\) In EMCV-infected mice, viral RNA was detected in the heart for 3 weeks and in the brain for 4 weeks by in situ hybridization.\(^{52}\) There is a hypothesis that in the chronic phase, the T lymphocytes infiltrate the myocardium in response to viral RNA in myocytes. This hypothesis is supported by the observation that 3 different strains of immunocompetent inbred mice in which viral RNA (detected by in situ hybridization) persists progressed to chronic heart disease. In contrast, DBA/1 J mice, which are capable of terminating the inflammatory processes by eliminating the virus from the heart, showed no evidence of viral RNA persisting in the myocardium.\(^{54}\) Because viral RNA diminishes during the chronic phase of the disease, its detection by in situ hybridization becomes progressively more difficult. Alternatively, polymerase chain reaction (PCR) provides a more sensitive method for the detection of viral RNA by a rapid amplification of specific DNA sequences, although a wide discrepancy exists in the reported results, probably because of the use of different procedures by different investigators.\(^{55,56}\) Using PCR in our laboratory, we were able to demonstrate that EMCV RNA persists in the absence of infectious virus beyond 90 days after inoculation,\(^{57}\) when cardiac lesions resembling human dilated cardiomyopathy in mice have been developed in the same animal model.\(^{11}\) Another group\(^{58}\) used PCR and showed that EMCV RNA signals were detectable in the myocardium up to day 42 after infection, at which point most of the inflammatory response had subsided in the murine model of viral myocarditis.

The levels of positive detection of enteroviruses by PCR are still low in myocardial biopsy specimens from patients with myocarditis and dilated cardiomyopathy.\(^{59,60}\) With the current technique, EMCV RNA was detected only in 2 of 7 mice 90 days after inoculation,\(^{57}\) even under excellent experimental conditions within inbred animals.

Some evidence suggests that the persisting viral RNA appears to be capable of replication.\(^{54}\) However, in the absence of detectable virus titers, it seems likely that the replication is done in a restricted or altered manner.\(^{54}\) Even such replication could produce new antigenic noninfectious or defective interfering viral particles, enough to cause ongoing myocardial injury.

Carrier state infections are an alternative mechanism to explain the virus-induced ongoing heart disease. These infections may occur in murine CVB heart disease, particularly under conditions in which the host defense mechanisms are depressed.\(^{4}\) Persistent viral infection has been observed in the spleen and lymph nodes,\(^{54}\) liver, and pancreas\(^{61}\) as extracardiac reservoirs of virus during the chronic phase of the disease.

However, a recent study\(^{62}\) demonstrated that enterovirus is not a primary cause of dilated cardiomyopathy. A significantly high frequency of the presence of anti–hepatitis C antibody associated with hepatitis C virus RNA in the sera was found in patients with dilated cardiomyopathy.\(^{63}\) Both positive- and negative-strand RNA of hepatitis C virus was present in myocardial and liver tissue samples at necropsy in 3 patients with chronic active myocarditis.\(^{64}\) Further studies of the increasing number of myocarditis patients with appropriate control subjects may establish whether these findings simply represent a coincidence and/or the uptake of viral material from the neighboring infected cells or plasma.
Animal models in which myocarditis is inducible by purified cardiac myosin have been established and serve as an exclusively virus-free system for investigations of the pathological mechanisms acting in autoimmune heart disease. Murine strains susceptible to chronic CVB-induced myocarditis developed myocarditis when cardiac myosin was injected. There are many other circulating heart-specific autoantibodies, including antisarcolemma antibodies. Some of them exhibit cross-reactivity to CVB3 capsid proteins. The immunological similarity between myosin and CVB3 capsid proteins could reflect the fact that they both share \( \approx 40\% \) identity in the amino acid sequence. Thus, some heart-specific autoantibodies that react with CVB3 and cardiac myosin demonstrate cell lysis capabilities and induce myocardial lesions when they are injected into mice. Although their origin and pathogenic role seem to depend on the experimental system used, further investigations of these cross-reacting autoantibodies through molecular mimicry may be important for understanding autoimmune mechanisms in viral heart disease and dilated cardiomyopathy. However, it has been reported that the myocardiogenic epitopes are located in the cardiac myosin rod in one study and in the head portion in another study. The identification of pathogenic epitopes on the cardiac myosin is important because of the future application of the peptide therapy.

Apoptosis

Thus far, the discussion in this review has been focused on virus- and immunocyte-mediated pathogenic mechanisms in the development of dilated cardiomyopathy from viral myocarditis. The cell death dealt with here, as James describes in his excellent review, “has almost universally been considered synonymous with necrosis, and necrosis was generally regarded as an abnormal or pathological condition.” In contrast to necrosis, apoptotic cell death demonstrates distinctive morphological characteristics that consist of the shrinkage instead of swelling of cells, early disintegration of the nucleolus with a typical form of cleavage into \( \geq 2 \) pieces of the entire nucleus forming apoptotic bodies, and rapid phagocytosis by local macrophages or even neighboring cells such as cardiac myocytes, with a total absence of inflammation. Very few reports had been published on the recognition of apoptosis in the human heart until James et al called attention to the significant participation of apoptosis in the postnatal morphogenesis of the human cardiac conduction system, arrhythmias including sudden unexpected death, cardiomyopathy, arrhythmogenic right ventricular dysplasia, Uhl’s anomaly, and complete heart block. Although electron microscopic examination of apoptotic bodies is the most reliable and direct diagnostic method for recognition of an apoptotic cell, indirect evidence of the presence of apoptosis has accumulated in necropsied ventricular myocytes of patients with myocardial infarction, by the expression of bcl-2 at the acute stage and the overexpression of Bax at the late stage; in human vascular pathology, including restenotic lesions and primary atherosclerotic lesions, by terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick-end labeling (TUNEL) staining; and in hypoxic cultured neonatal rat cardiomyocytes by DNA fragmentation, TUNEL staining, and the enhanced expression of Fas antigen messenger RNA. It is now known that in the absence of culturable viruses and with a characteristic avoidance of inflammatory changes, cardiac injuries persist and cardiac lesions resembling human dilated cardiomyopathy develop after murine viral myocarditis. A new and intriguing hypothesis is that apoptotic cell death is at least in part responsible for the disease process from acute viral myocarditis to dilated cardiomyopathy. Moreover, some reports indicate that several different viruses act as triggers of apoptosis. Aptoic cell death may provide the third mechanism, in addition to an immune-mediated mechanism initiated by viral infection and persistent viral RNA in the myocardium, to explain the development of dilated cardiomyopathy.

Future Therapeutic Implications

\( \alpha \)- and \( \beta \)-Adrenergic Blockers, Calcium Channel Blockers, ACE Inhibitors, and Amiodarone

The administration of prasozin or doxazosin, carteolol, verapamil, and captopril has been shown to be effective in the treatment of viral myocarditis and dilated cardiomyopathy, both clinically and experimentally. These results raise the possibility that microvascular spasm may underlie the evolution of viral myocarditis to dilated cardiomyopathy; these agents are effective in reducing myocardial injury at least in part by abolishing microvascular spasm.

In addition, some dihydropyridine calcium channel blockers, amiodipine in particular, prolonged survival and reduced myocardial damage without significant effect on viral replication in the murine heart; these beneficial effects of amiodipine may be due to altered inflammatory responses or immunomodulating effect by inhibiting NO production. Amlodipine improved survival of patients with heart failure due to nonischemic dilated cardiomyopathy.

A recent study also demonstrated that amiodarone, a well-known antiarrhythmic drug to prevent fatal arrhythmia in patients with heart failure, may contribute its beneficial effects through inhibition of TNF-\( \alpha \) and IL-6 production. Modulation of IL-1\( \beta \) production by amiodarone was biphasic.

Antiviral Agents

If viral myocarditis is a precursor of dilated cardiomyopathy, antiviral agents may be crucial in preventing the development of the disease.

Ribavirin (Virazole, 1-\( \beta \)-d-ribofuranosyl-1,2,4-triazole-3-carboxamide), a synthetic nucleoside analogue, has a broad antiviral activity against RNA and DNA viruses. The early administration of ribavirin after virus infection reduced EMCV replication in FL (human amnion) cells in vitro and inhibited virus replication in the heart, reduced myocardial damage, and decreased the mortality of treated mice.

Recombinant human leukocyte IFN-\( \alpha \) A/D, when administered before or simultaneously with the virus inoculation, inhibited virus replication and reduced the inflammatory response and myocardial damage in DBA/2 mice inoculated with EMCV and C3H/He mice inoculated with CVB3. The combined use of ribavirin and IFN-\( \alpha \) A/D showed a synergistic effect on the inhibition of myocardial virus...
replication and enhanced the survival of infected mice with the lower dose of each agent, which had no suppressive effect in a single use. Thus, this combination may be able to reduce the frequency of unfavorable effects of ribavirin and IFN by lowering the effective dose of both agents in future clinical use.

Immunosuppressive Agents

A wide variety of immunosuppressive agents have been used in both animal models and humans with no clear favorable effects.

Prednisolone given in the early stage aggravated the course of acute viral murine myocarditis with the increased viral titers because of its inhibition of the synthesis of neutralizing antibody. However, extrapolation of these results in mice to humans should be done with caution, because there are distinctive differences in susceptibility to steroids between mice, a steroid-sensitive species, and humans, a steroid-resistant species.

Cyclosporine, which preferentially inhibits helper T-cell functions, probably through the inhibition of IL-2 production, caused greater mortality when administered early in the illness and greater myocardial failure without an evident reduction of myocardial pathology when administered later during the early recovery phase in murine EMCV and CVB3 myocarditis models. Decreases in Thy 1.2 (pan T) and L3T4 (activated helper T) cells in the peripheral blood and thymus may account for the higher mortality in the cyclosporine-treated mice, in which the serum neutralizing antibody titers showed no reduction due to an incomplete depletion of the T- and B-cell zones in the spleen.

FK-506, a novel potent immunosuppressant at least 10-fold stronger than cyclosporine in vivo, induced an almost total depletion of T- and B-cell functions in mice inoculated with CVB3, resulting in lower titers of serum neutralizing antibody, higher virus titers in the heart, and a higher mortality rate, notwithstanding an apparent reduction of myocardial cellular infiltration compared with control subjects.

Cyclophosphamide suppressed mainly the B-cell region in lymphoid organs at a low dose (30 mg·kg\(^{-1}\)·d\(^{-1}\)); a high dose (300 mg·kg\(^{-1}\)·d\(^{-1}\)) caused total cellular depletion of the B-cell as well as T-cell regions. The treatment of CVB3 murine myocarditis with high-dose (100 mg·kg\(^{-1}\)·d\(^{-1}\)) cyclophosphamide in the early stage resulted in an increased mortality rate, probably due to the total depression of T- and B-cell functions or the subsequent decreased neutralizing antibody associated with an increase in virus titers, despite less severe myocardial cellular infiltration and necrosis. Treatment in the late stage had no effect.

The above results of immunosuppressant therapy indicated the importance of sparing the neutralizing antibody production in the host for the treatment of viral myocarditis. It has been reported that the serum neutralizing antibody production in experimental CVB3 virus infection in mice was not affected by the absence of T cells; it may thus be controlled only by B cells.

A recent multicenter clinical trial of immunosuppressive therapy for biopsy-proven myocarditis, consisting of prednisone with either cyclosporine or azathioprine, revealed no distinct benefit for patients with myocarditis.

Immunomodulating Therapy

It was reported that the administration of rat anti-mouse monoclonal antibodies against total T cells, Lyt 1 plus Lyt 2, during the viremic stage resulted in decreased mortality with less myocardial cellular infiltration and necrosis in mice with CVB3 myocarditis. The serum neutralizing antibody titers and virus replications showed no significant changes.

These results indicate that the inhibition of deleterious heightened T-cell activity without any effect on B cells by appropriately timed immunosuppressive treatment, if such an agent becomes available, would be helpful in ameliorating myocarditis. High-dose immunoglobulin treatment suppressed CVB3 murine myocarditis through the transfer of an antiviral antibody and by exerting an anti-inflammatory effect. High-dose intravenous γ-globulin has been effective in the treatment of patients with myocarditis and with myocarditis secondary to Kawasaki disease. In view of the absence of a general consensus on the effective treatment of myocarditis, high-dose immunoglobulin could be a candidate for the future treatment for myocarditis.

Levamisole, a promising immunopotentiating drug, increased the number of myocarditis lesions when it was administered to adolescent CD-1, ICR, and C57BL/6 mice at the time of or up to 4 days after an inoculation with CVB3. Exogenous administration of IL-1 or IL-2 restored myocarditis susceptibility in H310 AI virus–infected mice, which otherwise produce only minimal myocarditis; recombinant human TNF caused more severe myocardial changes in EMC viral myocarditis than in the control mice. Severe ventricular dysfunction has been reported in cancer patients treated with high-dose IL-2 immunotherapy.

Of note is a recent study demonstrating that the in vivo administration of anti-B7-1 monoclonal antibody alone or combined with anti-CD40L monoclonal antibody suppressed myocardial injuries, which in contrast were exacerbated by anti-B7-2 monoclonal antibody administration in murine viral myocarditis.

Future Studies

1. In the acute stage of viral myocarditis, the roles of NK cells and T-cell–mediated immune mechanisms should be clearly established. In particular, the contributions of cytokines such as tumor necrosis factors (TNF-α and -β), ILs, IFNs, NO produced by iNOS, perforins, adhesion molecules, and ligands such as ICAM-1, lymphocyte function–associated antigen-1, B7-1, B7-2, CD28, CD40, and gp39 as well as TCR (Vα and Vβ) should be clarified in relation to potential therapeutic implications. The applications of antiviral agents such as ribavirin and α-IFN, high-dose immunoglobulin, ACE inhibitors, and β-blockers are to be further investigated for clinical use.

2. In the chronic stages of viral myocarditis, it is crucial to determine the significance of persistent enterovirus RNA and other virus genomes such as hepatitis C virus RNA in the myocardium long after virus infection, in regard to carrier state infections, virus/immune interactions, and autoimmune
mechanisms with or without a triggering by a virus infection to develop diluted cardiomyopathy.

3. In the hypothesis that an underlying viral infection in acute myocarditis progresses to diluted cardiomyopathy in the chronic stage, a novel concept, apoptosis, has emerged. Although reliable diagnostic criteria for apoptosis remain to be established, this revolutionary concept may explain the ongoing cardiac damage in the absence of inflammatory responses.

Conclusions
This review summarizes the current status of clinical and experimental studies of the underlying viral pathogenesis of acute myocarditis that progresses to diluted cardiomyopathy. It should be borne in mind that the evolution of myocardial disease and the development of diluted cardiomyopathy clearly demarcated into stages in animal models cannot be extrapolated to humans. However, the concepts derived from the results of the animal experiments described here will no doubt contribute to the establishment of a new paradigm for the pathogenesis of viral myocarditis and diluted cardiomyopathy and thus to the elucidation of effective treatments for these diseases.

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