Clinical Cardiology: New Frontiers

Advances in the Understanding of Myocarditis

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The Many “Faces” of Myocarditis

Myocarditis is a poorly understood disease because it progresses through stages with distinctly different mechanisms and manifestations. The objective of this article is to better define myocarditis for both clinicians and clinical scientists by setting it in the framework of 3 phases of disease. In phase 1, the viral stage, we review recent discoveries about the way viruses gain access to target tissue and how they trigger immune responses. In the second, autoimmune phase of disease, we examine the roles of autoreactive T cells, cytokines, and cross-reacting antibodies and reconsider the relevance of recent therapy trials. In the third phase of the disease, dilated cardiomyopathy, we consider the remodeling processes. We then offer current recommendations for diagnosis and therapy and conclude with a look to the future.

Myocarditis is a continuum of 3 distinct disease processes, one evolving into the other with transitional periods of indistinctness. For each of the 3 processes, pathogenesis, diagnosis, and treatment differ considerably. Without precise knowledge of the point to which an individual patient’s myocarditis has evolved in this continuum, the clinician can only use diagnostic tools and therapeutic interventions haphazardly.

It is likely that the majority of cases of myocarditis, except in countries in which Chagas’ disease or diphtheria is common, result from viral infection, which may progress to an autoimmune phase after resolution or reduction of the initial infection, and then finally to progressive dilatation after resolution or reduction of the autoimmune injury (Figure 1). A viral cause can only be proved by direct molecular or indirect methods. Appropriate treatment at the viral stage is eradication of virus and amelioration of viral injury. The autoimmune phase can be diagnosed by endomyocardial biopsy, supplemented by serological markers of immune activation. Immune suppression is probably the most appropriate therapy in this stage, unless significant viral replication persists. The third phase of the disease, dilated cardiomyopathy, is largely a result of viral and autoimmune injury but may progress after cessation of injury, much as idiopathic dilated cardiomyopathy does. This stage of disease is usually recognized by imaging and other diagnostic procedures that exclude other causes of dilatation. Treatment centers on reversal of the continued remodeling process by promotion of myocyte survival, attenuation of continued neurohormone and cytokine activation, and reduction of hemodynamic stress.

Confusion in diagnosis and errors in therapy are particularly likely during the transitional periods among the 3 phases. This confusion is further compounded by the potential for reinfection and autoimmune reactivation in any given individual patient, resulting in simultaneous coprogression of different phases of the disease.

Phase I: Viral Infection

Traditional and Novel Viral Etiologic Agents

The enteroviral species, such as Coxsackie virus B3 and B4, are the dominant viruses detected either serologically or by direct molecular techniques such as the polymerase chain reaction (PCR) or in situ hybridization in patients with myocarditis. More recently, in part because of better molecular detection techniques and in part owing to changing epidemiology, additional viral agents have gained prominence. These include adenoviruses, which are being detected frequently in the younger population, and hepatitis C virus. Human immunodeficiency viral infection is frequently associated with myocardial decompensation. It appears that myocarditis is present in most of these cases. It is not yet clear whether this form of myocarditis fits into the triphasic pathogenetic scheme proposed herein.

New Understanding of the Enteroviral Receptor: CAR and DAF

The preponderance of coxsackieviruses and adenoviruses as etiologic agents in clinical myocarditis is now better understood with the recent identification of a common mammalian viral receptor for both viruses (Figure 2). The coxsackie–adenoviral receptor (CAR) allows internalization of the coxsackieviral genome after attachment and is a critical step for viral infection. Similarly, CAR protein is a facilitatory receptor for adenoviruses 2 and 5 fiber protein. CAR belongs to the immunoglobulin superfamily, which most likely has an adhesion molecule function yet to be identified precisely. CAR apparently can act as the multifunctional internalizing receptor for all known members of the coxsackie B family of viruses and many other members of the enteroviral family.

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1076
In mammalian cells, CAR coreceptors determine efficiency in host cell targeting by coxsackie and adenoviruses. Coxsackievirus B (CVB) utilizes the complement deflecting protein decay accelerating factor (DAF, CD55) as its coreceptor, whereas adenovirus uses integrin \( \alpha_v\beta_3 \) and \( \alpha_v\beta_5 \) as its coreceptors. DAF serves an important function as a coreceptor by significantly increasing the binding efficiency of coxsackievirus onto the DAF-CAR receptor complex to facilitate its internalization by CAR.

Triggering of the Immune Response
The close link between viral infection and the attendant immune response is a common theme in many virally induced diseases, but it is particularly pertinent to enteroviral myocarditis. In fact, the secondary immune response to viral infection likely plays a greater role in disease pathogenesis than the primary infection. Throughout the entire process of coxsackieviral myocardial infection, there is a direct interplay with the immune system. After gaining a portal of entry into the host, through the gut in the case of enteroviruses and through the respiratory tract for both the enteroviruses and adenoviruses, the virus is harbored in the immune cells of lymphoid organs, temporarily escapes immune clearance, and is secondarily transported to other target sites such as the heart and pancreas, in a manner analogous to the Trojan horse paradigm.

Subsequent activation of the immune system may be accomplished by direct activation of the coreceptor-associated signaling pathways, such as the tyrosine kinase p56\( ^{14,15} \) associated with DAF (Figures 3 and 4). Or, the immune system may be activated by viral antigens presented at the cell surface through major histocompatibility complex–restricted pathways. Thus, viruses such as coxsackievirus take advantage of our immune system for their own survival. Once the immune system is activated, a second phase of the disease, the autoimmune process, ensues.

Phase 2: Autoimmunity
Phase 1 of the disease is triggered by the entry and proliferation in the myocardium of the causative virus. Phase 1 concludes with activation of the host immune system, which attenuates viral proliferation but may also enhance viral entry. Ideally, the immune system should downregulate to a resting state once viral proliferation is controlled. However, if host immune activation continues unabated despite elimination of the virus, autoimmune disease may result, initiating phase 2 of the disease. T cells may then target the host’s own tissue through molecular mimicry. Cytokine activation and cross-reacting antibodies may further accelerate the process.

Autoreactive T Cells
T cells are triggered in the setting of viral infection of the myocardium through classic cell-mediated immunity. Viral peptide fragments are processed in the Golgi apparatus of the host cell and presented to the cell surface in a major histocompatibility complex–restricted manner. This immune activation is teleologically protective, as the T cells seek out...
virus-infected cells and destroy them by either cytokine production or perforin-mediated cell cytolysis. However, continuous, exuberant activation of the T cells is ultimately detrimental to the host, because both cytokine-mediated and direct T-cell–mediated myocyte damage reduce the number of contractile units. The cumulative effect causes impairment of contractile function, which leads to long-term remodeling and phase 3 of the disease, dilated cardiomyopathy. Persistent T-cell activation is induced by antigens intrinsic to the myocardium that cross-react with viral peptides (molecular mimicry). The virus may also trigger a Th2 response, activating more CD8 killer cells in the process. This explains in part why CD4/CD8 or p56lck knockout animals have a much improved survival after exposure to Coxsackievirus infection.14,15,17

Cytokine Activation
A major mediator of immune activation and its maintenance are cytokines. Matsumori et al18 showed that patients with myocarditis have marked activation of cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6. The pattern of activation may indeed determine the type of T-cell reaction and the subsequent degree of autoimmune perpetuation.19 Similar patterns of cytokine activation are seen in murine models of myocarditis, with many of these cytokines directly detectable in heart tissue.20 Cytokines contribute in a major way to disease phenotype. For example, mice deficient in the TNF p55 receptor (TNF-R1−/−) have milder autoimmune myocarditis.21 Indeed, the frequently observed full recovery of some patients with severe left ventricular dysfunction is probably the result of short-term exposure to cytokines. A recent natural history study in which patients with fulminant myocarditis were compared with those with nonfulminant acute myocarditis showed that at 5.6 years of follow-up, 93% of the former group were alive compared with only 45% of those with milder myocarditis.22 This suggests that aggressive hemodynamic support is important in this potentially reversible, presumably cytokine-mediated disease.

Cross-Reacting Antibodies
Activation of CD4 cells also leads to B-cell clonal expansion and antibody production. Antibodies may not be the critical initiating factor that leads to the progression of disease. In T-cell knockout animals, the disease is dramatically modified despite presence of antibody.14,15 But, antibodies are an important modifier of the disease phenotype. In patients with histologically proven myocarditis or familial dilated cardiomyopathy, autoreactive antibodies to components of the myocardium are often present, including intracellular targets such as the ADP/ATP translocator and other mitochondrial proteins.23 These can reproduce the disease when transferred to immunodeficient mice.24

Immunosuppression Therapy Trials
Several clinical trials based on the concept of autoimmunity have been conducted in humans. In 1993, Parrillo et al25 randomized patients with dilated cardiomyopathy and evidence of immune activation to either steroids or placebo. They reported a temporary improvement in ventricular function, but there was no sustained benefit. This was followed by the National Institutes of Health–supported US Myocarditis Treatment Trial reported by Mason et al.26 One hundred eleven patients met eligibility criteria, including the Dallas histopathological standard for diagnosis of myocarditis (simultaneous presence of myocyte necrosis and mononuclear infiltrate on myocardial biopsy). The patients were randomized to conventional therapy or an immunosuppressive regimen of steroids combined with either azathioprine or cyclosporine. The primary objective of the study was to determine whether immunosuppression affects the severity of left ventricular dysfunction. There was a similar degree of recovery of ventricular function in both arms of the study, and there was no difference in mortality, which was 20% overall at 1 year and 56% at 4.3 years of follow-up.
The Myocarditis Treatment Trial investigators monitored several components of immune activation,\textsuperscript{36} Their observations are consistent with the foregoing explanation of the basic pathophysiology of the disease and with the triphasic nature of myocarditis. They found that at the time of presentation, patients with a more aggressive early immune response to infection (with, for example, more circulating IgG) had better left ventricular function, smaller left ventricular size, lower pulmonary capillary wedge pressure, longer exercise time, less need for heart failure drug therapy, and a shorter duration of illness (see Table 3 of Mason et al\textsuperscript{26} for the parameter estimates for each variable; probability values for each association were \textless{}0.05). On the other hand, patients with greater late immune activation potentially mediating an autoimmune response, such as higher levels of circulating CD2\textsuperscript{+} T cells, had significantly poorer survival. Those patients passed into phase 3 of the disease process and succumbed to dilated cardiomyopathic failure.

Phase 3: Dilated Cardiomyopathy
Some remodeling mechanisms leading to dilated cardiomyopathy may be specific to myocarditis. Badorff and Knowlton\textsuperscript{27} have shown that a coxsackieviral protease can directly modify the sarcoglycan complex in myocytes. This may provide one of the potential mechanisms explaining the significant ventricular dilation that may be seen soon after viral infection. They have also shown that mice expressing the Cox sackievirus genome, devoid of the capacity to replicate, develop cardiac dilatation, which could be explained by many mechanisms, including that of the viral protease mentioned above. That persistent myocyte viral gene expression, after infectivity has passed, might be a cause of progressive dilated cardiomyopathy is an intriguing possibility that conjures up an array of diagnostic and therapeutic opportunities.

Cytokines may also contribute to the development of dilated cardiomyopathy. During the autoimmune phase, they activate the matrix metalloproteinases, such as gelatinase, collagenases, and elastases.\textsuperscript{28} The dilated cardiomyopathy seen in experimental models can be significantly attenuated by treatment to interfere with matrix degradation, such as elastase inhibitors.\textsuperscript{29}

Viruses may also directly cause myocyte apoptosis. Ongoing viral persistence is associated with much worse outcome (early death or requirement for transplantation).\textsuperscript{30} Cytokines may activate death-domain or ceramide-mediated signaling pathways as part of the remodeling process.\textsuperscript{31} In later stages of immune activation, cytokines play a leading role in adverse remodeling and progressive heart failure. This is demonstrated well by the study of Nakamura et al.\textsuperscript{32} They introduced a viral infection that initially only produced mild cardiac pathology. However, on second introduction of the virus at a much later date, the heart became rapidly dilated. Cardiomyopathy developed despite the absence of viral proliferation but was correlated with elevated levels of cytokines such as TNF.

Current Recommendations for Diagnosis and Treatment
The following recommendations draw on current knowledge but are unproved. We propose that appropriate therapy of myocarditis requires knowledge of the phase of the disease at the time therapy is contemplated. Phase specificity of therapy is probably important because each therapy has the potential to worsen rather than improve myocarditis if administered at the wrong time.

Phase 1: Viral Replication
In phase 1 of myocarditis, the presence of viral replication, under most circumstances, is difficult to prove quickly enough to allow application of appropriate antiviral therapies because of the rarity of obtaining a positive culture, the impracticality of obtaining serial viral titers, the unavailability of an established, rapid, noninvasive screening tool for the immediate detection of viral protein or genetic material, and the need to know the specific infectious agent for optimal selection of antiviral therapy. This phase may transpire unnoticed, without symptomatic myocardial failure. When cardiac involvement is apparent during phase 1, in most patients the diagnosis of myocarditis is based on the constellation of signs and symptoms caused by viral infection, including fever, lymphocytosis, and symptoms of upper respiratory or gastrointestinal infection. The patient may also have chest pain and atrial or ventricular arrhythmias. ECG findings in the acute phase of clinical myocarditis can include widened QRS complex, left bundle-branch block, ST-segment and T-wave changes, and heart block. Echocardiography may reveal decreased systolic ventricular function and wall-motion abnormalities. Recent investigation of texture analysis suggests that specific diagnostic information may be available through such image-processing techniques.\textsuperscript{33}

Endomyocardial biopsy is generally not necessary in this phase, but a virological diagnosis with myocardial biopsy is possible through recombinant DNA techniques. Two methods have been used: PCR and in situ hybridization. PCR techniques have used highly conserved regions of the Enterovirus genome as targets and achieved positive results in \textasciitilde{}20% of patients with clinically suspected myocarditis or dilated cardiomyopathy.\textsuperscript{1,4,34–38} The frequency is much higher in phase 1 of the disease.\textsuperscript{2} In situ hybridization detects viral genome in \textasciitilde{}35% of such patients.\textsuperscript{3,5} Detection of viral genome in normal controls is low to absent, which attests to the specificity of current methods.

At the present time, treatment during this phase includes avoidance of potentially harmful immunosuppression, nonspecific antiviral measures, and direct antiviral therapy in the few cases in which an organism has been identified or in the context of a known viral epidemic. The reduction of viral entry, attachment, and proliferation diminishes the severity of myocarditis in experimental models. Potentially effective antiviral agents include nucleoside analogues such as ribavirin,\textsuperscript{39} boosters of the intrinsic immune defenses such as immune globulin and interferon,\textsuperscript{40–43} and agents that can block viral entry at the receptor site through CAR.\textsuperscript{44} We look forward to the results of the European ESETCID trial (European Study of Epidemiology and Treatment of Cardiac Inflammatory Disease),\textsuperscript{45} which addresses the roles of immune globulin and interferon and is discussed later.
Phase 2: Immune Activation

Phase 2 of the disease, a result of immunologic activation, can be definitively diagnosed by endomyocardial biopsy. The diagnosis is most secure when it is made only a few days to weeks after resolution of a symptomatic viral infection and many foci of lymphocytic infiltration are found on histology. The diagnosis is less secure when the viral prodrome was remote, or no such prodrome occurred, and only 1 or a few small inflammatory foci are present. This was the case in a portion of the subjects in the US Myocarditis Treatment Trial and may have contributed to the lack of efficacy of immunosuppressive therapy in that trial. Many of those patients may have already entered phase 3.

Endomyocardial biopsy samples can be analyzed with histological, immunologic, and molecular techniques. Data from large case series involving >4000 patients suggest that the frequency of positive biopsy in patients with myocarditis or dilated cardiomyopathy is low (≈10%). Series that include only acute, early presentations of myocarditis demonstrate an inflammatory infiltrate more frequently. Positivity on biopsy increases with increasing number of samples.

Markers of immunologic activation, including intercellular adhesion molecule-1 (ICAM-1), soluble FAS ligand, and markers of T-cell activation, are higher in patients with myocarditis than in controls. Cardiac-specific autoantibodies, such as anti-alpha-myosin, are also more common in patients with clinical myocarditis than in controls. None of these is, however, sensitive or specific enough to qualify as a noninvasive diagnostic tool. Viral serology is often positive in this phase, but high background and noncardiac specificity detract from its significance.

Molecular diagnosis with myocardial biopsy establishes both the origin and the molecular epidemiology of the disease. These techniques remain investigational but very promising.

Noninvasive imaging techniques such as gallium- and indium-labeled anti-myosin antibody scanning have been shown to have either inadequate specificity or sensitivity. The latter technique’s higher sensitivity may obviate the need for endomyocardial biopsy in selected patients with negative scans.

A variety of immunomodulatory therapies have been proposed for the autoimmune phase of disease, including immunosuppression, manipulation of cytokines, and anti-T-cell–receptor vaccines. Steroids, azathioprine, cyclosporine, and OKT-3 have been used as immunosuppressive agents in humans with myocarditis. Immunoglobulin, which may have immunomodulatory effects independent of its potential direct antiviral effects, has also been used. No anticytokine regimens have been studied in humans with myocarditis, although several have been proposed. At present, only the well-studied immunosuppressive drugs should be used in this phase of the disease, and only when the phase is well defined.

It is important to recognize that patients with fulminating myocarditis who develop severe hemodynamic compromise suddenly may have a much better prognosis than those with mild acute or chronic forms of myocarditis. These patients should be supported aggressively, with left ventricular assist devices if necessary, because there is a high likelihood of recovery. Peripartum cardiomyopathy due to myocarditis also may develop relatively suddenly and may cause severe heart failure. It is likely an autoimmune phenomenon, probably unrelated to viral infection. When its development is rapid and aggressive, it too has a good prognosis. Efficacy of immunosuppression is not proven in this group.

Phase 3: Dilated Cardiomyopathy

In the absence of chronic, ongoing viral infection or recurrent autoimmune activity, patients in phase 3 should be managed like those with idiopathic dilated cardiomyopathy and congestive heart failure. There is no evidence that patients who develop chronic dilated cardiomyopathy as a result of myocarditis should be treated differently from others with dilated cardiomyopathy. The assumption is that further ventricular remodeling and clinical deterioration may be prevented with ACE inhibitors, beta-blockers, spironolactone, and possibly amiodarone. An implantable defibrillator should be considered for those with documented life-threatening ventricular arrhythmias. Monitoring for recrudescence of viral infection or autoimmunity should be undertaken. This is best done with frequent history, examination, and echocardiography.

Future Directions

Novel diagnostic strategies that more fully reflect the underlying pathophysiological process are needed. The advent of methods to characterize viral, cardiac, and immunologic interactions may allow more accurate diagnosis, more effective prognostication, and more appropriate therapy. Molecular diagnosis with cardiac tissue may increase the utility of endomyocardial biopsy in the short term, but new noninvasive imaging techniques with phase specificity are expected to reduce the need for biopsy. As more data are collected, an effort to establish new clinical diagnostic criteria will be needed.

Improved understanding of pathophysiology will lead to new therapeutic insights. For example, T-cell tyrosine kinase p56 may evolve as a target for future drug development. With the identification of the coxsackie-adenovirus receptor/coreceptor complex for this disease, more precise therapeutic targeting of the viral entry site may prove fruitful.

Although attention must be directed toward currently recognized cardiotropic viruses, we must remain alert to the possibility that other viruses are common cardiac pathogens. Hepatitis C infection is so common and pervasive throughout the world that it could prove to be an important cause of myocarditis and dilated cardiomyopathy. Because congestive heart failure is a common cause of death in the AIDS population, attempts to elucidate the mechanism(s) of myocarditis in this condition are needed and will undoubtedly expand our understanding of common forms of myocarditis. The ability to modify the remodeling process with anticytokine or antimetallomatrix protein therapy may be particularly effective in preventing myocarditis-induced cardiomyopathy.

In future clinical studies, the status of viral persistence in the myocardium should be clarified, and immunosuppression with a targeted approach, as in the ESETCID trial, should be tested. Another interesting approach is immunoglobulin adsorption. Functional improvement has been observed in patients with idiopathic dilated cardiomyopathy after removal
of IgG. This method might be especially effective during phase 2 of myocarditis, but this hypothesis remains to be tested.

Effective vaccines could prevent many of the adverse consequences of viral infections, including myocarditis. They could be included in childhood vaccination programs, because the disease appears to have a predilection for children and young adults. However, a more cost-effective strategy would be to identify those individuals who are particularly at risk. Other more specific vaccination or tolerance-induction approaches might be included in childhood vaccination programs, because the disease appears to have a predilection for children and young adults. However, a more cost-effective strategy would be to identify those individuals who are particularly at risk. Other more specific vaccination or tolerance-induction strategies are being evaluated. Vaccination against receptors of specific T-cells implicated in autoimmune myocarditis has therapeutic potential because it is now possible to rapidly identify pathogenic T cells. Specific tolerance to myosin antigens, which induce autoimmune myocarditis, has been achieved in mouse models by intranasal administration of cardiac myosin. This is a promising potential therapy, because myosin may be a common autoantigen in human myocarditis. Its release into the circulation after myocardial injury due to any of a variety of insults is theorized to initiate an autoimmune process that could be a final common pathway for many forms of myocarditis.

Dramatically differing results among the many murine models of myocarditis used to study autoimmune aspects of the disease reduce our ability to apply those results to human pathophysiology and therapy. These differences relate in large part to the differing genetic backgrounds of the animals. Knowlton and Badorff highlighted this problem nicely in their recent editorial. There must be important modifier genes that affect phenotype and virus-immune interaction. This is an important area for future work, because similar genetic variations probably occur in humans. Recent availability of cDNA microarrays, proteomic technology, and the human genome map will help shed light on unique virus-host interactions.

In the meantime, care must be taken in extending observations in animals to humans.

Understanding the triphasic nature of myocarditis helps both the scientist and the clinician study and treat the disease. In fact, that understanding of the disease is surely too simple and in the future must incorporate recognition of the chronological overlap of disease phases, ie, the fact that each phase may recur when 1 of the other 2 phases is already well established. The model becomes even more complex with the knowledge that the original invading virus may often persist well into phase 3, and that by doing so, it can cause cardiac failure not only by ongoing replication, with the attendant direct immune-mediated injury, but also by noninfectious transcriptional expression (Figure 5). The diagnostic complexity created by the multifaceted pathophysiology of continued viral presence might best be deciphered by cDNA microarray analysis of message from endomyocardial biopsy tissue.

We are entering an exciting era in the understanding of viral myocarditis and cardiac inflammation. An acceleration of relevant scientific discoveries will greatly change our management of this disease process in the near future.

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