Viral Myocarditis

Is Infection of the Heart Required?*

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Coxsackieviral myocarditis continues to be a disease that is difficult to diagnose and treat in spite of decades of research in both patients and mice. The prominence of the inflammatory response combined with the challenge in definitively identifying viral infection within the myocardium in a given patient has stimulated a question regarding the role of virus infection of the cardiac myocyte versus activation of a humoral and cellular autoimmune process that is independent of infection of the cardiac myocyte. Is it possible that primary infection of other organs or nonmyocytes within the heart (1) is sufficient to activate an autoimmune phenomenon directed against myocardial cells and myocardial proteins without direct infection of the cardiac myocyte?

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There are a number of lines of evidence that suggest the importance of an autoimmune phenomenon (2,3) in both patients with myocarditis and mice infected with the Coxsackievirus (2,4–7). In contrast, there are considerable data demonstrating that enteroviruses can be detected within the myocardium of patients with acute onset of myocarditis and in mice infected with Coxsackievirus. Virus infection has also been associated with a direct myocyte damage (8–10). Altogether, the evidence suggests that both the direct effects of myocardial infection and the activation of the immune process either in an autoimmune or antiviral manner are likely to have significant roles in the development of myocarditis. However, it has not been clearly demonstrated whether infection of the myocardium is required for activation of the viral myocarditis phenotype.

Elucidation of the mechanisms by which virus infection can affect the host organism has been greatly facilitated by identification of the Coxsackievirus-adenovirus receptor (CAR). In addition to its role as a virus receptor, CAR is a transmembrane immunoglobulin domain-containing adhesion molecule that belongs to the family of adhesion molecules such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM). CAR has been shown to be expressed at relatively high levels on myocardial cells, but it is also expressed in other organs such as the liver, pancreas, and brain (11–13). In the adult myocardium, CAR is primarily localized in the intercalated disc and the cell–cell junctions of the atrioventricular (AV) node. Recently, CAR expression has been shown to be required for normal embryonic development, and when disrupted only in the heart, electrical conduction between the atria and ventricles is interrupted, leading to complete heart block and loss of connexin-45 in the AV node (14,15).

In cultured cells, infection by most Coxsackieviruses requires expression of CAR. However, less is known about whether CAR is required for infection of cells in the intact animal or in the intact heart. The importance of a potential alternative mechanism of viral infection has been demonstrated with adenovirus where infection of cultured cells has been shown to be mediated by the expression of CAR (11,13). However, efficient hepatocyte infection can occur through a CAR-independent manner in vivo that is dependent on Factor X of the coagulation cascade (16).

In this issue of the Journal, Shi et al. (17) report a series of experiments that demonstrate important points in relation to the pathogenesis of Coxsackieviral myocarditis. The first is that CAR is required for infection of myocardial cells in vivo, and the second is that infection of myocardial cells is required to induce myocarditis in the Coxsackievirus-infected mouse. To demonstrate these points, the authors generated a mouse in which the first exon of CAR was flanked by lox-p sequences that allow recombinant and deletion of intervening deoxyribonucleic acid in the presence of the bacteriophage-derived CRE recombinase. Because the authors had previously demonstrated that in their model cardiac-specific deletion of CAR during development led to embryonic lethality (15), they used a somewhat more sophisticated strategy that allowed for deletion of CAR in the adult heart. This was accomplished by expressing a modified CRE under the direction of the cardiac-specific, α-myosin heavy chain promoter. The modified CRE contains a mutated estrogen receptor on each side of the CRE recombinase. This mutated CRE is maintained in the cytoplasm until it binds to tamoxifen, at which point it translocates from the cytoplasm to the nucleus, stimulating recombination of the genome at the lox-p sites. In the current case this deletes the first exon of CAR, preventing expression of CAR. Because the modified CRE is expressed only in the cardiac myocytes, the deletion occurs only in the myocardial cells. This strategy leads to reduction of CAR expression in the myocardium by 90% at 3 months after administration of tamoxifen. At this point the mice were infected with Coxsackievirus B3 (CVB3), and their hearts were analyzed at days 10 and 28 after infection.

Key results from these experiments included the findings that the mice that lacked CAR in the cardiac myocytes had...
no significant viral infection or cellular inflammation in the heart. In addition, ventricular function was not significantly affected by viral infection of the mouse. This demonstrated that CAR expression is required for viral infection of the cardiac myocytes in vivo and that the myocarditis does not occur in the absence of infection of the cardiac myocyte. Secondary findings include a marked decrease in the messenger ribonucleic acid levels for the cytokines interleukin-6, interleukin-10, and tumor necrosis factor-α in infected CAR knockout mice compared with infected wild-type mice. However, tumor necrosis factor-α messenger ribonucleic acid levels were still somewhat elevated in the infected CAR knockout mice compared with uninfected mice. Another finding was that the level of CVB3 infection of different tissues did not correlate with the level of CAR expression in the wild-type mouse but that all tissues that were infected expressed CAR. There are a number of mechanisms by which viral infection might be inhibited in certain tissues. For example, replication of CVB3 in the liver is markedly increased in the absence of type I interferon signaling (18), thus demonstrating the importance of the innate immune function within the cell in determining a tissue’s susceptibility to viral infection.

Although the conclusions from the current article are quite clear in that viral infection of the heart was completely inhibited in the absence of CAR, there are some caveats that should be kept in mind. To be certain that the differences in infectivity and myocardial inflammation are only secondary to knockout of CAR rather than difference in mouse strain background, it is generally most suitable to perform the analysis in an inbred strain of mice. The experiments performed in the study by Shi et al. (17) used outbred mice that were derived from crosses of 129SVJ and C57black6. However, to control for infectivity between the groups, the authors analyzed the level of infection in noncardiac tissues and found that infection of the pancreas and spleen was not different between the mice that lacked CAR in the heart compared with infected wild-type mice. It is also notable, that the immune response within a mouse after CVB3 infection is at least partially dependent on the strain of mice analyzed (19,20). This outcross might not be representative of all mouse models of CVB3 infection. Finally, one cannot exclude the possibility that there is activation of some form of autoimmune process that might occur at later stages after infection.

In summary, Shi et al. (17) have demonstrated that CAR is required for Coxsackievirus infection of the cardiac myocyte and that in the absence of infection of the heart, there is no significant evidence of myocarditis after CVB3 infection in spite of evidence of infection of other tissues. These findings demonstrate the importance of the interaction of the virus with the cardiac myocyte in the initiation of viral myocarditis. Strategies that can interfere with these interactions will likely affect the severity of viral myocarditis after Coxsackieviral infection.

**REFERENCES**


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