I
fections by viruses, such as adenoviruses, enteroviruses, or parvoviruses, can affect the heart, resulting in myocardial inflammation.1 Viral myocarditis encompasses a wide spectrum of clinical presentations, ranging from oligosymptomatic infections to the rapid development of terminal heart failure.

Clinical diagnosis of viral myocarditis remains a challenge. Although modern imaging modalities, such as late gadolinium enhancement MRI, can be used to estimate the likelihood of viral heart disease in suspected cases, the definite diagnosis still requires histopathological examination of endomyocardial biopsies.2 Viral heart disease seems to account for a relevant proportion of otherwise unexplained dilated cardiomyopathies. In large series of patients with this diagnosis who underwent endomyocardial biopsy, myocarditis was found in ≈10% of cases.3,4 Because of the unavailability of specific noninvasive tests, the exact prevalence of viral myocarditis is unknown, and it is likely that many cases remain undiagnosed.5

One of the most prevalent and most intensively studied myocarditis-causing viruses is coxsackievirus B3, a member of the enterovirus genus of unenveloped single-stranded positive-sense RNA viruses. The entry of coxsackieviruses into cardiomyocytes depends on surface expression of the coxsackievirus and adenovirus receptor, a transmembrane protein with 2 extracellular immunoglobulin domains that is highly expressed on the cell membrane of cardiomyocytes.6

Upon replication in the host myocardium, a protein called enteroviral protease 2A is synthesized, which was demonstrated to cleave the sarcolemmal protein dystrophin specifically, disrupting the cytoskeletal integrity of the cardiomyocytes.7

Much of our knowledge of the pathomechanisms contributing to the onset and progression of human viral myocarditis is based on studies of experimental cellular and murine models. Such models comprise immortalized, proliferative cell lines, such as HEK293T and HeLa cells, or genetically susceptible mice that are infected with coxsackievirus B3.8–10

In this issue of Circulation Research, Sharma et al11 report on a different approach at modeling viral myocarditis. They obtained either skin fibroblasts or mononuclear blood cells from healthy individuals and, by viral transduction with a specific cocktail of reprogramming factors, reprogrammed them to induced pluripotent stem cells (iPSCs). These iPSCs, which can be propagated in culture indefinitely, were subjected to cardiac differentiation to obtain human spontaneously beating cardiomyocytes (Figure [A]). The coxsackievirus and adenovirus receptor was present on the plasma membrane of iPSC-derived cardiomyocytes, albeit mRNA expression level was ≈30-fold lower than in primary human myocardial tissue.

The iPSC-derived cardiomyocytes were then infected with a coxsackievirus B3 strain that was modified to induce expression of luciferase to monitor viral gene expression by luciferase luminescence indirectly. Within 6 hours of infection, the cardiomyocytes started to beat irregularly and displayed disrupted intracellular calcium transients. Moreover, they ceased to beat after ≈12 hours and detached from the culture dishes after 24 hours. When the number of virus particles applied to the cells was reduced, these cytopathic effects developed during a longer time frame. However, even low virus numbers eventually resulted in the death of all iPSC-derived cardiomyocytes.

In addition, the authors used this system to investigate the effect of several drugs on virus replication as measured by luciferase luminescence. Interferon beta 1 (IFNβ1), ribavirin, fluoroxetine, and pyrrolidine dithiocarbamate all reduced virus replication in iPSC-derived cardiomyocytes. The small-molecule compounds ribavirin and pyrrolidine dithiocarbamate, in contrast to IFNβ1, were also effective if administered concurrently with or even after infection with the virus. IFNβ1 was more effective if given 12 hours in advance, presumably because its mechanism of action involves the activation of transcription of downstream target genes. To elucidate the role of IFNβ1-mediated transcriptional regulation in its antiviral activity further, gene expression analysis was performed on coxsackievirus B3–infected iPSC-derived cardiomyocytes either pretreated with IFNβ1 or untreated. Among the 139 genes found to be differentially expressed, 22 genes were previously implicated in viral elimination pathways, suggesting that this approach might be suitable to identify additional genes with a role in the cellular defense against cardiac coxsackievirus infection.
The study by Sharma et al extends the field of iPSC-based cardiovascular disease models to infectious diseases. To date, genetically caused channelopathies and cardiomyopathies have been the focus of this emerging technology. Although animal models are an important fundament of cardiovascular research, it is often desirable to investigate disease mechanisms or drug effects in human cells to exclude that species differences result in wrong conclusions. Primary human myocardial tissue can be obtained only by invasive procedures and cannot be propagated or kept in culture for a long time period. Cardiomyocytes derived from iPSC are one possible solution to these obstacles because they represent a theoretically unlimited source of human cardiomyocytes. In their study, Sharma et al have shown that, in the case of coxsackievirus B3-mediated myocarditis, this system can be used not only to model the disease in vitro but also as a platform to evaluate drugs that interfere with the disease, and—by gene expression analysis—to investigate the mechanism of drug action further (Figure B).

In principle, iPSC technology would offer the possibility to investigate other important aspects of viral myocarditis. It is well known that the susceptibility to viral myocarditis is genetically determined. The progression of viral myocarditis to dilated cardiomyopathy may be also influenced by genetic susceptibility factors. By deriving iPSCs not only from healthy individuals but also from patients affected by viral myocarditis (assuming their susceptibility to the disease) and from patients who have developed dilated cardiomyopathy after viral myocarditis, it might be possible to elucidate the molecular mechanisms of this susceptibility further. Knowledge of the signal transduction pathways that confer resistance to virus-mediated myocardial damage might inform the development of specific drugs to prevent the potentially devastating consequences of this disease.

Nevertheless, it should be noted that the study by Sharma et al models only 1 aspect of viral myocarditis—the cell-autonomous interaction between the cardiomyocytes and the virus particles. Earlier studies in experimental models have suggested 3 still controversial mechanisms by which viral myocarditis causes damage to the heart: (1) direct virus-induced cardiomyocyte injury; (2) destruction of the myocardium by infiltrating immune cells targeting virus-infected cardiomyocytes; (3) autoimmune-mediated destruction of cardiac cells by circulating autoantibodies and autoreactive immune cells. In its present state, the iPSC-derived experimental myocarditis model only allows investigation of the first aspect. It may be an advantage to be able to study the virus–cardiomyocyte interaction in an isolated fashion. However, to gain a complete picture of the pathogenesis of viral myocarditis, human disease models that also capture the other above-mentioned aspects of the disease will be necessary in the future.

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