Virally induced myocarditis (VM) is an inflammatory disease of the myocardium initiated by common viral infections, such as the enterovirus coxsackie group B, parovirus B19, and human herpes virus 6, and sustained by postviral immune-mediated responses. While acute myocarditis is typically self-limited in most individuals, the development of severe heart muscle injury may lead to dilated cardiomyopathy, heart failure, or even death in others. The mechanisms underlying why some individuals are more susceptible to cardiotropic viruses than others are not well established. Furthermore, therapies for VM are generally directed at supportive care of the myocardium through hematogenous or lymphangitic spread. During the first few days of viral exposure, the virus gains entry into cells, including cardiomyocytes, using viral specific receptors and replication proceeds which itself can cause direct myocardial tissue injury (Figure, A, left panel). However, viral infectivity also strongly activates the innate immune system by inducing proinflammatory Toll-like receptors (TLRs), the inflammasome, and the release of cytokines such as TNF-α or IL-1β (Figure, A, middle panel). Indeed, cardiac TLR4 mRNA expression and signaling is higher in human subjects with VM than control subjects and has been associated with the presence of active viral proliferation and reduced left ventricular systolic function. Activation of TLR signaling induces transcriptional activity of nuclear factor κB (NF-κB), which amplifies the initial release of cytokines by inducing NF-κB–responsive genes including endothelial adhesion molecules (eg, VCAM-1, E-selectin), chemokines (eg, MCP-1), and other inflammatory mediators (eg, IL-6, tissue factor). Activated endothelial cells (ECs) begin to recruit a range of immune cell types including neutrophils, natural killer cells, and antigen-presenting cells such as macrophages.

The majority of information about the pathogenesis of myocarditis has been derived from experimental models in rodents. Using mouse models of enterviral myocarditis, the course of VM has been characterized into 3 phases: (1) viral entry; (2) innate and adaptive immune activation; and (3) chronic/resolution (Figure, A). First, the virus may proliferate in a variety of tissues in the susceptible host and eventually reach the myocardium through hematogenous or lymphangitic spread. During the first few days of viral exposure, the virus gains entry into cells, including cardiomyocytes, using viral specific receptors and replication proceeds which itself can cause direct myocardial tissue injury (Figure, A, left panel). However, viral infectivity also strongly activates the innate immune system by inducing proinflammatory Toll-like receptors (TLRs), the inflammasome, and the release of cytokines such as TNF-α or IL-1β (Figure, A, middle panel). Indeed, cardiac TLR4 mRNA expression and signaling is higher in human subjects with VM than control subjects and has been associated with the presence of active viral proliferation and reduced left ventricular systolic function. Activation of TLR signaling induces transcriptional activity of nuclear factor κB (NF-κB), which amplifies the initial release of cytokines by inducing NF-κB–responsive genes including endothelial adhesion molecules (eg, VCAM-1, E-selectin), chemokines (eg, MCP-1), and other inflammatory mediators (eg, IL-6, tissue factor). Activated endothelial cells (ECs) begin to recruit a range of immune cell types including neutrophils, natural killer cells, and antigen-presenting cells such as macrophages.

The final phase constitutes a resolution phase in which the virus is cleared and the inflammatory response is signaled to turn off by anti-inflammatory factors (eg, IL-10) from regulatory T cells (Tregs) or alternatively activated macrophages (Figure, A, right panel). Injured cardiac tissues subsequently remodel in response to cytokine-induced matrix metalloproteinases (MMPs) that degrade interstitial collagen and elastin and replacement fibrosis ensues over weeks in response to TGF-β1 signaling. Failure to resolve the inflammatory response and/or persistence of the viral genome within the myocardium may lead to dilated cardiomyopathy and predispose to congestive heart failure. Given the need of the immune response to maintain equipoise between limiting viral proliferation while avoiding unintended myocardial injury, it remains unclear if therapies directed at suppressing aspects of the innate or immune response would be beneficial or injurious in the pathogenesis of myocarditis.

MicroRNAs (miRNAs) belong to a family of evolutionarily conserved, small noncoding RNAs that bind to the 3′-untranslated regions of target mRNAs and repress gene expression by either promoting mRNA degradation or suppressing translation. Emerging studies suggest that miRNAs are important regulators in inflammatory and immune responses. In this issue of Circulation Research, Corsten et al found that the miRNA mir-155 is increased in cardiac samples from mice or human subjects with VM. Using elegant profiling methodologies in myocarditis susceptible (C3H strain) or resistant (C57Bl/6N strain) mice treated with Coxsvackie B3 viruses, Corsten et al identified differentially expressed miRNAs that became most robust during the inflammatory phase (day 4) compared with the viral replication phase (day 4). These miR-
miR-155 was identified from studies of animal models of myocarditis. In particular, miR-155 inhibition results in a decrease in immune cell infiltration and myocardial injury, as well as an improvement in cardiac function. This is consistent with previous studies showing that miR-155 expression is dysregulated in myocarditis and that its inhibition can reduce inflammation and improve outcomes.

Consistent with the beneficial effect of miR-155 inhibition revealed in this study, miR-155 deficiency protects mice from experimental autoimmune encephalomyelitis and collagen-induced arthritis, whereas miR-155 overexpression leads to a myeloproliferative disorder. To date, miR-155 has been involved in several aspects of immune cell development and function in macrophages, B cells, dendritic cells, natural killer cells, and T cells. For example, miR-155 promotes differentiation of hematopoietic stem cells toward the myeloid lineage and regulates B-cell differentiation and antibody production.

In the present study, it is unknown if miR-155 inhibition may reduce the generation of autoreactive antibodies and protect the myocardium from damage. MiR-155 also regulates T-cell development and function. Overexpression of miR-155 in activated CD4+ T cells promotes Th1 differentiation, whereas miR-155 deficiency leads to polarization toward Th2 differentiation. Deletion of miR-155 reduced Treg cell number and proliferative potential by increasing the expression of suppressor of cytokine signaling 1 (SOCS1) without apparently changing the sensitivity of Treg cells to induced apoptosis and without impairing Treg suppressor function. Finally, miR-155 also regulates proinflammatory functions in dendritic cells, natural killer cells, and Th17 cells. Coletti et al found that miR-155 inhibition significantly attenuated the accumulation of CD11b-positive macrophages and Ly6-positive neutrophils, whereas CD3-positive T cells exhibited a nonsignificant reduced trend. To better characterize the effects of miR-155 inhibition on immune cell subtypes, the authors performed flow cytometry on the cardiac immune cell fraction at day 7 of VM, which revealed that LNA-anti-miR-155 treatment markedly reduced the activation of CD4+ and CD8+ T lymphocytes. Surprisingly, the percentages of anti-inflammatory Foxp3+CD25+ Treg cells and proinflammatory Ly6C<sup>hi</sup> monocytes did not differ between treatment groups. Taken together, these data suggest that the attenuation of the immune response after miR-155 suppression is primarily due to less activation of proinflammatory immune subsets and not due to an increase in anti-inflammatory Treg cells.

Several questions and issues arise from the study by Corsten et al. In particular, the cellular location and mechanism by which miR-155 inhibition exerts its protective effects remain unclear. For example, because systemic delivery of miRNA mimics and inhibitors can target the vascular endothelium, reduced EC activation and leukocyte transendothelial migration may be another potential mechanism underlying the effects of miR-155 on myocardial leukocyte influx. Although the authors point out the relative expression of miR-155 in mouse-derived macrophages and T cells in vitro is several-fold higher than miR-155 expression in SVECs (a simian virus-transformed mouse EC line), closer examination of relative miR-155 expression from primary myocardial ECs compared with peripheral blood mononuclear cells after systemic delivery of LNA-anti-miR-155 and effects on EC-leukocyte functional interactions may provide further insights in this regard. Furthermore, does miR-155 inhibition in other nonimmune cell types, such as the cardiomyocyte or cardiac fibroblast, confer a favorable microenvironment for early left ventricular remodeling? In support, miR-155 re-
duces human cardiomyocyte progenitor cell migration in vitro by directly targeting MMP-16 and subsequently MMP-2 and MMP-9 activities, raising the provocative possibility that miR-155 inhibition may concomitantly reduce extracellular matrix degradation and promote cardiac progenitor cell migration in response to myocardial injury. Bone marrow transplant studies could shed light on the relative contribution of the bone marrow and non–bone marrow cellular constituents for mediating the protective effects of miR-155 inhibition in VM. Recent studies examining the role of miR-155 in cell apoptosis support both proapoptotic or antiapoptotic effects, in part, depending on the cell type and stimulus. The study by Corsten et al showed no difference in myocardial apoptosis in response to miR-155 inhibition; however, a role for miR-155-mediated apoptosis specifically in macrophages or other immune subsets may still be operative. Previous studies demonstrate that miR-155 expression is induced in response to a broad range of inflammatory stimuli including TLR ligands. Proinflammatory signaling pathways activate transcription factors NF-κB and AP-1, which bind the miR-155 promoter to induce its expression. How miR-155 is generally induced in macrophages and T lymphocytes during acute VM and whether, specifically, NF-κB and AP-1 are involved in the upregulation of miR-155 will require future investigation. In addition, miR-155 has been shown to target multiple proteins involved in inflammation. Further studies will be needed to determine the relative functional significance of these miR-155 targets and if one or more are necessary for conferring the protective effect of miR-155 inhibition in VM. Finally, the study by Corsten et al also revealed a panel of miRNAs expressed only after viral infection in myocarditis-resistant C57Bl/6N mice. Future studies interrogating the function and targets of these miRNAs may provide important mechanistic insights for host susceptibility to VM.

Although in metazoans the innate and adaptive immune response evolved as a beneficial response to pathogens, accumulating studies now highlight that, if uncontrolled, a diverse range of injurious effects on host tissue ensues. The ideal anti-inflammatory therapies will prevent tissue damage without affecting antipathogenic clearance. Therefore, the study by Corsten et al suggests that pharmacological inhibition of miRNA-155 may provide a novel therapeutic approach for the treatment of VM without affecting antiviral immunity. However, some caution should be noted. Because patients often present after several days to weeks of progressive symptoms such as dyspnea or chest pain, an important question is whether the timing of administration of LNA-antimiR-155 in later phases of myocarditis will also achieve a similar level of efficacy for ameliorating myocardial injury. Furthermore, it remains unclear if miR-155 inhibition would be similarly protective against other viral subtypes. Finally, viral genomes that harbor miRNA binding sites may provide additional therapeutic opportunities for VM. Studies in larger animals will be required to test these possibilities. Nonetheless, this study demonstrates proof-of-principle an important framework that miR-155 is markedly increased with acute VM and orchestrates adverse cardiac immune cell activation and deleterious cardiac remodeling. It is clear that we can consider miRNA therapeutics a reality, with miR-122 inhibitors inching toward the clinic after the recent completion of a Phase II clinical trial for patients infected with chronic hepatitis C virus. Targeted therapeutics for acute VM using miR-155 inhibition or other miRNA therapies may provide novel approaches to this potentially devastating disease.

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None.

References


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