Regulating Viral Myocarditis: Allografted Regulatory T Cells Decrease Immune Infiltration and Viral Load

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Coxsackievirus was first discovered as a filterable agent associated with a paralytic syndrome, so named for its identification in Coxsackie, New York (coxsackievirus type A). Coxsackievirus type B (CVB) was isolated the following year from patients with aseptic meningitis, and by the mid-1950s, an association with acute myocarditis in humans was becoming clear. Many other viruses have since been shown to cause myocarditis and its long-term sequelae, arrhythmias, dilated cardiomyopathy, and heart failure. By example, adenovirus, herpesviruses, and influenza can cause myocarditis in humans and models of heart failure. Because immune cell infiltration is an easily seen feature of myocarditis, it is perhaps not surprising that the inflammatory response would be posited as a major cause of tissue injury during viral myocarditis. However, the virus itself has lytic and destructive potential, and a range of data indicate both viral and immune contributions to cardiomyocyte and interstitial damage during myocarditis. The question is often posed: Is damage primarily virus-mediated or more so the product of an overzealous immune response and autoimmunity? Perhaps the greatest error in the discussion has been approaching these 2 facets of the disease process in a mutually exclusive manner. Shi et al now show in this issue of Circulation that virus replication and the immune response are exquisitely intertwined in myocarditis pathogenesis.

Over the last 3 decades, it has become apparent that viral load is a primary determinant of viral myocarditis severity. The virus causing extensive, early, direct injury to the myocardium through its replication. Results cited here all indicate significant injury to the myocardium before cardiac immune infiltration, supporting the view that considerable damage to the heart is directly mediated by the virus alone. An argument has been made previously for the importance of chronic virus replication in myocarditis and as a basis of dilated cardiomyopathy and chronic heart failure. On the other hand, enhanced immunoproteasome expression in cardiomyocytes during CVB3-induced myocarditis may increase the expression and presentation of autoantigens, leading to more severe myocarditis through autoimmune avenues. However, not all T-cell infiltration is harmful, and Li et al recently reported that allografted M2 (anti-inflammatory) macrophages led to improvement of virus-induced myocarditis associated with enhanced levels of regulatory T cells (T-reg). Huber et al have also reported decreased viral load and immune infiltration after adoptive transfer of a CD4+CD25+ regulatory-like T-cell population into a mouse model of CVB3 infection.

In the face of the uncertainties about the pathogenesis of viral myocarditis, the work of Shi et al is most welcome. These investigators show that cardiac immunity and viral replication are mechanistically linked, and that viral replication and the degree of immune infiltration are interrelated. They set out to determine whether the immunosuppressive effect of allografted T-regs would cause an increased viral load due to a decreased immune response. T-regs are CD4+ cells that express the α subunit of the interleukin-2 receptor CD25 and are negative for CD127 (Figure). The hallmark protein expressed in these cells is the transcription factor FoxP3, and they circulate as a functionally distinct T-cell subpopulation, preventing autoimmune and other aberrant responses to innocuous environmental antigens. The T-reg population prevents the proliferation of self-reactive effector T-cell populations and were aptly named suppressor T cells when first discovered for their ability to secrete interleukin-10 and moderate T-helper 1 immunity. Thus it would be reasonable to postulate that adoptive transfer of T-regs into a mouse model of viral myocarditis would suppress the immune response and allow virus replication to proceed unchecked.

The authors infected mice with CVB3 after adoptive transfer of allogeneic T-regs from virus-naive mice. Included in the study were PBS injection controls, but also naïve T cells. Decreased immune infiltration and enhanced Akt activation, as compared with the naïve CD4 T-cell and PBS-grafted controls, were notable in the T-reg--adopted mice. Perhaps the most surprising of the results was the decreased viral load, not only in the heart, but also in the pancreas and spleen, associated with lower expression of the coxsackie-adenovirus receptor (CAR) (Figure). Of course, the authors adoptively transferred not only T-regs, but also CD25+ CD4 T cells (naïve), into a separate group of mice. In fact, the naïve CD4 T-cell--treated control group fared worse than either the PBS-treated or T-reg--treated groups, related to less Akt activation and higher virus CAR expression. As a result, the naïve T-cell population brought on a more severe myocarditis phenotype due to higher viral loads. Taken altogether,
Shi et al describe the protective role that T-regs play in immune infiltration of the heart during viral myocarditis, suggesting that there is a balance to be struck between clearance of infection and immune-associated damage to the myocardium. In fact, the authors observed decreased viral loads consonant with a decrease in immune infiltration due to reduced tumor necrosis factor α release and lower CAR expression; such findings suggest a more intimate link between inflammation and virus replication than previously posited. Thus moderating the immune response may be critical for prevention of chronic virus replication. This issue should receive attention given the fact that diluted cardiomyopathy is thought to result from repeated rounds of injury and repair that ultimately weaken the heart muscle.

Although the dominant factor in virus suppression during T-reg treatment was most likely downregulation of CAR expression, we propose that the alteration in signaling evoked by T-regs also plays a significant role in modulating virus replication. The basis of our argument is that CAR expression was suppressed 3-fold in vivo and 5-fold in a dividing cardiomyocyte cell line in vitro, but the suppression of viral load in the T-reg group was almost 2-logarithms reduced as compared with the control group. Although the correlation between cell receptor expression and virus infection may not be linear, the decreased levels of CAR may not explain the entire antiviral and protective effect of T-regs in the myocardium during virus infection. It is entirely possible that the signaling environment altered by the adoptive transfer of T-regs may have also contributed to a less favorable environment for virus replication. The authors reported the altered, prosurvival activation of Akt in the T-reg–treated groups, which suggests that the activation of other signaling proteins may have been altered by T-reg transfer. We have reported that Akt activation is required for successful CVB3 replication, a prosurvival protein required by the virus to optimize the longevity of the infected cell to promote optimal progeny virus production. On the other hand, the Akt activation reported by Shi et al may merely be a reflection of a virus-protective environment created by allografted T-regs in the myocardium. It is quite clear that an unbraked immuno-logically active environment supports enhanced cellular signaling driven by viruses for successful replication. For example, we have previously reported that the powerful immune-stimulating protein p38 is required for effective virus replication in a similar CVB3 myocarditis mouse model. The activation of p38 mitogen-activated protein (MAP) kinase was not investigated by Shi et al, but we would predict less net activation of p38 in the presence of allogeneic T-regs and thus an environment that is less conducive to virus replication. Although p38 MAP kinase activation is required for suppressor (T-reg) T-cell activity and function, we propose that adoptive transfer of T-regs may have decreased the immune infiltrate in the myocardium with less activation of p38 MAP kinase.

Figure. Regulatory T cells alleviate virally induced myocarditis by reducing viral load via transforming growth factor (TGF) β and reduced CAR expression. (A) Adoptive transfer of regulatory T cells (T-regs; CD4+, CD25+, FoxP3+, CD127−) caused enhanced expression of (B), TGF-β, and consequently reduced CAR receptor expression in cardiomyocytes, resulting in (C), lower viral loads and immune infiltration. (D) Greater activation levels of Akt may be an indicator of the improved cell survival after T-reg delivery and subsequent virus infection. (E) Activation of p38 MAP kinase was not investigated by Shi et al; however, we could predict lower net levels of p38 MAP kinase activation due to T-reg transfer, resulting in a less favorable environment for virus replication. IL-10 indicates interleukin-10; Mac3, macrophage specific marker.

Our laboratory has reported that inhibition of p38 MAP kinase is an effective antiviral strategy in vitro and in vivo. Thus the intimate link of this molecule to the immune response is perhaps not surprising; one might expect that Akt activation, p38 inhibition, and T-reg immune control lead to improved outcome and result in lower viral load and immune infiltration. With regard to new treatment strategies, this does not necessarily mean that we should be injecting allogeneic T-regs into patients with myocarditis, but methods should be pursued that mediate the immune response to swing the pendulum in favor of viral clearance and repair and away from immune infiltration of the myocardium that favors higher viral loads. The work of Shi and several other recent studies have demonstrated the evolved ability of the virus to bias immune activation and associated signaling queues for the benefit of virus replication. Given the results presented by Shi et al, one might initially conclude that immune suppression would be a sound therapeutic strategy. However, the Myocarditis Treatment Trial, wherein patients were treated with immunosuppressive agents, showed that such administration had no significant benefit for outcome of human myocarditis. As such, pan-immune suppression is not the way forward, and better targeted methods of immune modulation are needed. Shi et al demonstrate that transforming growth factor β secreted by T-regs may have been responsible for the decreased CAR expression and enhanced Akt activation.
(Figure). This suggests that we need not inhibit the immune response entirely, but rather target, modulate, and encourage the arm of the immune system that promotes virus clearance. The way forward may not be adoptive transfer of T-regs, but administration of an as yet undiscovered drug that can promote T-reg differentiation and function. These findings may even translate to approaches for lessening immune rejection of allografts through induction of immune senescence after transplantation; cotransplantation of T-regs might induce tolerance of grafted tissue.

In conclusion, the novel studies of Shi and colleagues help us to reconcile the complex relationships between the degree of immune infiltration and viral load. With more support for a model wherein virus replication is driven by the signaling queues provided by the immune response, we may move toward treatments that modulate the immune response in favor of resolution of infection and earlier repair of the damaged tissue.

Disclosures

None.

References


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