Editorial

Two Sides to Every Proinflammatory Coin

New Insights Into the Role of Dendritic Cells in the Regulation of T-Cell Driven Autoimmune Myocarditis

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Myocarditis is a major cause of heart failure in young adults that is typically precipitated by cardiac infection with organisms such as Coxsackie B virus or the parasite Trypanosoma Cruzi.[1] Myocarditis has a variety of clinical presentations but is often characterized by severe ventricular dysfunction and risk of fatal arrhythmia. Tissue injury during myocarditis is caused by direct infection of cardiomyoctes and immune-mediated responses to microbial antigens; in addition, autoimmune T cell and antibody responses to myocardial antigens can develop and persist even after the inciting infection has been cleared. The autoimmune component of myocarditis indicates a failure of self-tolerance mechanisms and may be driven by molecular mimicry between microbial and myocardial self-antigens. Although there has been an emphasis on the role of autoantibodies in autoimmune myocarditis, such as those targeting the β1 adrenergic receptor or the α myosin heavy chain (αMHC), this may reflect the relative ease of their experimental detection as compared with assays of T cell activation by specific self-antigens. Nonetheless, the relevance of T cells is supported by the fact that lymphocytic infiltrates, including $CD4^+$ helper T cells, can be demonstrated in endocardial biopsy or autopsy sections taken from patients with myocarditis, and by the fact that many of the cardiac autoantibodies in human myocarditis have undergone IgG class switching, which reflects T helper–dependent B cell responses.

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Rodent models have been instructive in further defining how T cells may contribute to the pathophysiology of myocarditis. Murine viral myocarditis, induced by Coxsackie B infection of susceptible mouse strains, leads to dilated cardiomyopathy and elevated levels of serum antibodies specific for cardiac proteins, including αMHC. In addition, Neu et al[2] developed the experimental autoimmune myocarditis (EAM) model, in which immunization with murine αMHC in strong adjuvant results in a self-limiting acute myocarditis followed by chronic dilated cardiomyopathy. EAM was shown to be dependent on αMHC specific $CD4^+$ T cells,[3] and the relevant peptide epitope and presenting MHC alleles recognized by these T cells were subsequently determined.[4] The presence of αMHC-specific $CD4^+$ T cells in normal mice, which can be activated by highly immunogenic delivery of self-antigen, reflects a lack of expression of the antigen in thymic antigen-presenting cells, leading to a failure of central (thymic) tolerance. Importantly, the same lack of thymic expression of αMHC is found in humans, and circulating αMHC-specific $CD4^+$ T cells are found in normal individuals without myocarditis,[5] suggesting that peripheral mechanisms of tolerance are required to prevent these T cells from targeting the heart. Lastly, there are likely genetic factors (eg, HLA type) that also predispose to the failure of peripheral T cell tolerance to αMHC, as evidenced by the fact that only some mouse strains are susceptible to EAM (eg, BALB/c but not C57BL/6), and that non-obese diabetic mice genetically engineered to express DQ8, a human class II MHC allele associated with high risk for type 1 diabetes mellitus, spontaneously develop autoimmune myocarditis mediated by αMHC-specific $CD4^+$ T cells.[6]

The Role of TLR Signaling in T Cell Responses, Autoimmunity, and Myocarditis

Dendritic cell (DC) activation and antigen presentation to naïve T cells are well-established roles of the innate immune system in promoting T cell responses. DCs recognize and respond to a variety of danger signals that reflect the presence of infection or tissue damage. These include pathogen-associated molecular patterns, which are present on microbes but not host cells, and damage-associated molecule patterns, which are self-molecules expressed by infected or otherwise damaged cells. These pathogen-associated and damage-associated molecular patterns bind to pattern recognition receptors (eg, Toll-like receptors [TLRs]) on DCs and promote an increase in antigen processing, lymph node homing, and the expression of B7 family costimulators and cytokines, which facilitate naïve T cell activation and differentiation. In fact, the necessity of DC TLR activation for the initiation of strong T cell responses is 1 reason why immune responses are not more frequently mounted against healthy tissue, given that central tolerance to many self-antigens, such as α-MHC, is often incomplete. Autoimmunity may develop, therefore, as a result of strong TLR activation of self-antigen presenting DCs that are present at the site of infection or tissue injury. Indeed, this is the basis for the induction of EAM in mice, whereby peripheral tolerance is broken in susceptible mouse strains by immunization with peptide fragments of α-MHC in

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complete Freund's adjuvant, which provides the appropriate innate signaling necessary to initiate the T cell response.

Evidence for and Against a Pathological Role of Interferon-γ in Autoimmune Myocarditis

After antigen presentation by TLR-activated DCs, naïve T cells can differentiate into one of several functional phenotypes, so called helper T cell subsets, depending largely on cytokine cues delivered at the time of activation. The 2 major proinflammatory subsets are Th1 and Th17 cells, which are characterized by their production of the cytokines interferon-γ (IFNγ) and interleukin-17 (IL-17), respectively. Each of these subsets is thought to play a unique role in host defense against infection, with the Th1/IFNγ axis providing immunity against viruses and intracellular organisms, and the Th17/IL-17 axis combating extracellular fungi and bacteria. In light of its proinflammatory role, therefore, it is reasonable to hypothesize that IFNγ would act to enhance EAM severity, and indeed some evidence is consistent with this prediction. For example, IFNγ-producing $CD4^+$ T cells are present in cardiac infiltrates during EAM in mice, and αMHC responding $CD4^+$ T cells found in the blood of myocarditis/dilated cardiomyopathy patients produce IFNγ at high levels.[5,7] In addition, TCR transgenic mice in which most T cells are specific for αMHC develop spontaneous myocarditis and dilated cardiomyopathy, which is prevented if IFNγ is blocked or knocked out genetically[8]; similarly, IFNγ-overexpressing mice also go on to develop spontaneous chronic myocarditis.[9]

On the other hand, there is also strong evidence that IFNγ can play a negative regulatory role during myocarditis. In murine models of viral, parasitic, and autoimmune myocarditis, enhanced disease severity is observed in mice with genetic deficiency of IFNγ.[10–12] Likewise, in a model of myocarditis mediated by $CD8^+$ T cells, IFNγ deficiency also led to more severe disease.[13] Furthermore, many studies have observed an increase in IL-17–driven inflammation in the absence of IFNγ, suggesting that Th17 cells may be important for EAM in this setting. In support of this notion, studies using genetic deficiency of molecules critical for Th17 differentiation, such as IL-23 and STAT3, have demonstrated reduced EAM severity.[14,15] Interestingly, in another study of EAM, IL-17A deficiency had no effect on acute disease relative to either WT (WT versus $Il17a^{-/-}$) or IFNγ knockout mice ($Ifng^{-/-}$ versus $Ifng^{-/-}Il17a^{-/-}$), but did produce a clear reduction in cardiac fibrosis and progression to dilated cardiomyopathy in the chronic phase.[7] This study raised the interesting possibility that the Th1/IFNγ and Th17/IL-17 axes could play distinct roles in controlling the onset and progression of myocarditis.

Dendritic Cells Activate Then Inhibit Myocarditic T Cells Through IFNγ-, TLR-, and NO-Dependent Pathways

Despite the severity of the disease phenotype observed in EAM as a result of IFNγ deficiency, the mechanism by which IFNγ might restrict the onset of disease is not currently known. Nitric oxide (NO) has been postulated as a potential mediator because of its established role in T cell suppression and its clear downregulation in the absence of IFNγ. For example, inducible nitric oxide synthase (iNOS) is upregulated in hearts of mice with EAM, and its expression is dependent on IFNγ signaling.[12] In addition, treatment of WT mice with the iNOS inhibitor L-NAME also caused an increase in disease severity, suggesting a role for iNOS induction in mediating the inhibitory effect of IFNγ on EAM.[12] Interestingly, this finding is consistent with previous research on experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis, in which IFNγ induction of iNOS/NO was postulated as a central mechanism of disease regulation.[16] Although not addressed specifically in either of these studies, the plausibility of NO suppression of T cell activation is supported by previous work that suggests a mechanism involving disrupted STAT5 signaling.[17]

In this issue of *Circulation*, Kania et al[18] present data showing that IFNγ, TLR, and NO signaling cooperate to limit disease severity in EAM. This finding is particularly interesting in light of the necessity for dendritic cell TLR activation during the initiation of cardiac specific T cell responses in EAM. The authors demonstrate a mechanism for this negative regulation that involves NO production by so-called TipDCs, a monocyte-derived DC subset named for their robust production of tumor necrosis factor-α and iNOS that can be identified by flow cytometry as $CD68^+CD11b^{hi}CD11c^+$. The authors show that TipDCs are preferentially induced in EAM after immunization with α-MHC and complete Freund's adjuvant, and are subsequently found in draining lymph nodes and foci of myocardial inflammation. Along with gp-38+ fibroblasts in the heart, these cardiac infiltrating TipDCs were shown to be the predominant source of NO present during EAM. The authors further observed that TipDC generation and NO production was dependent on IFNγ signaling, in conjunction with the TLR2- and NFκB-dependent activation of monocytes by heat-killed *Mycobacterium tuberculosis* (a component of complete Freund's adjuvant). Through the use of bone marrow chimeras, the authors go on to show that NO derived from either hematopoietic (eg, TipDCs) or nonhematopoietic compartments (eg, stromal fibroblasts) was sufficient to limit T cell expansion and EAM severity, whereas only a complete lack of NO resulted in uncontrolled disease. Finally, the authors also observed that IFNγ and TipDC associated tumor necrosis factor-α exert a paracrine effect on stromal fibroblasts to increase NO expression, further limiting T cell expansion and EAM severity. Thus, although TLR activation of DCs is essential for the initiation of EAM, the study authors nicely show how this process also leads to the development of a DC subset (TipDCs) that ultimately serves a counter-regulatory role in limiting disease severity. These findings suggest a model (Figure) whereby IFNγ produced by autoreactive Th1 cells contributes both to inflammatory tissue damage in myocarditis and to enhanced TipDC formation, which in turn suppresses the extent of T cell–mediated tissue damage through NO-dependent mechanisms.

Summary and Unresolved Questions

The results presented by Kania et al[18] add to our general understanding that proinflammatory molecules in the immune system can also have important counter-regulatory effects. Interestingly, an anti-inflammatory effect of DC TLR

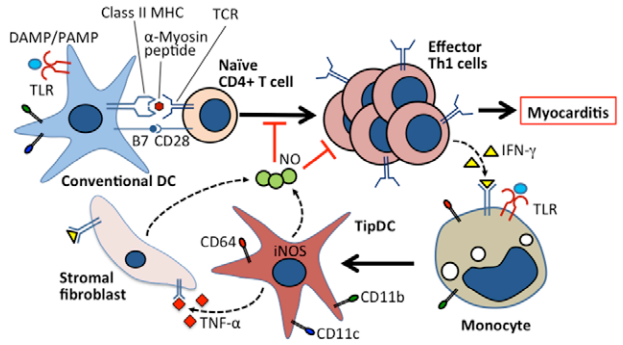

Figure. Schematic representation of the model proposed by Kania et al[18] for nitric oxide (NO)–mediated negative regulation of autoreactive T cells by tumor necrosis factor-α (TNF-α) and inducible NO synthase producing dendritic cells (TipDCs) during autoimmune myocarditis. First, conventional dendritic cells (DCs) present antigen (α-Myosin peptide) to naïve CD4+ T cells with a specific T cell receptor. In the presence of toll-like receptor (TLR) activation by pathogen-associated molecular patters (PAMPs) or damage-associated molecular patterns (DAMPs), DCs will provide the appropriate costimulatory (eg, B7) and cytokine signals (eg, interleukin [IL]-12) to promote the differentiation of naïve CD4+ cells into effector T cell subsets, such as T helper type 1 cells (Th1). Th1 cells then produce high levels of interferon gamma (IFN-γ), which in turn acts to promote the differentiation of monocytes into CD64+CD11b^{hi}CD11c+ TipDCs in conjunction with TLR2 activation. TipDCs then produce NO directly, and through indirect mechanisms involving TNF-α activation of stromal fibroblasts. NO then acts to prevent effector T cell activation and clonal expansion, thus limiting the extent of T-cell–mediated myocardial damage.

activation was also demonstrated recently in a mouse model of atherosclerosis, and was found to result from the induction of regulatory T cells (Treg).[19] The possibility that Treg responses are enhanced by TLR/NO dependent mechanisms in EAM was not explored in the current study but would represent an interesting future direction of research. In addition, the role of other T cell inhibitory pathways in EAM, which are known to be upregulated by IFNγ, were not explored in the current study. For example, IFNγ strongly induces expression of programmed death ligand 1 (PD-L1), a well-known coinhibitory molecule that can be expressed both by antigen-presenting and stromal cells. Through interaction with its coreceptor PD-1, PD-L1 limits TCR-mediated activation of T cells and has been shown to reduce disease severity in CD8+ T cell myocarditis and in EAM.[13,20] Although it is difficult to know to what extent these mechanisms contribute to the control of myocarditis relative to TipDC-mediated NO production, it is likely that multiple and sometimes overlapping mechanisms may work in concert to protect against autoimmunity. Of interest in this regard is a previous study showing that disruption of PD-L1/PD-1 signaling in macrophages resulted in increased T cell IFNγ production but decreased T cell proliferation.[21] This seemingly contradictory observation was reconciled by the finding that increased IFNγ expression also led to a robust induction of macrophage iNOS and NO production, which in turn inhibited T cell proliferation. In any case, the study by Kania et al[18] highlights the fact that therapeutic strategies for immunologic diseases require a careful consideration and manipulation of the balance between the activating and regulatory effects of specific immune pathways. In particular, research into the human relevance of TipDC biology is lacking and would represent a critical first step in translating the current findings for eventual clinical application.

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Disclosures

None.

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