MDA5
The Almighty for Myocardium

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Myocarditis is an inflammation of the heart muscle, which frequently follows microbial infections. Picornaviruses and adenoviruses are the most commonly identified viral etiologic agents.1 Cardiac injury results from both virus infection/replication and host inflammatory responses, but in either case, elimination of the virus from the heart can have a beneficial effect on disease outcome as is evident from clinical trials treating biopsy-proven, virus-positive patients with interferon β (IFNβ).2 The success of type I IFN therapy in myocarditis may partly depend on the initiating etiologic agent, as viral clearance and cardiac improvement was greater in patients with enteroviruses than with parvovirus B19 or human herpesvirus 6.2 This finding is not surprising as it is well known that virus families vary dramatically in their sensitivity to type I IFN, in part because many viruses have developed immune evasion mechanisms targeting this innate host defense mechanism.1 Enteroviruses of the Picornavirus family are small, nonenveloped, positive-sensed, single-stranded RNA viruses, which are classically among the most sensitive to type I IFN, with encephalomyocarditis virus (EMCV) being one of the most IFN sensitive of the enteroviruses.4

Type I IFN are induced through virus-sensing pattern recognition receptors (PRR) of the innate immunity system, which recognize shared pathogen-associated molecular patterns between many infectious agents.5 PRRs include Toll-like, retinoic acid-inducible gene I (RIG-I)-like, nucleotide-binding oligomerization domain receptors (NOD)-like, C-type lectin receptors; a diverse subcellular compartmentalization of the pattern recognition receptors allows optimal recognition of their relevant pathogen-associated molecular patterns. Toll-like receptors (TLR) are primarily implicated in reacting to extracellular bacterial pathogen-derived molecules (TLR5 [flagellin], TLR4 [lipopolysaccharide], TLR2/1, or TLR2/6 [lipoprotein]) and are located in the plasma membrane; in contrast, RIG-I-like (single-stranded/double-stranded RNA) and NOD-like (muramyl tripeptide/muramyl dipeptide) receptors are located in the cytosol; finally, TLR3/TLR9 (double-stranded RNA) and TLR7/TLR8 (single-stranded RNA) are located in endosomes where microbial RNA and DNA would most likely be found.5 The innate antimicrobial immunity is not solely dependent on individual pattern recognition receptors, but rather multiple receptors coordinate activation in a particular infection to amplify the host defense. For example, during rhinovirus infection, TLR3 initially recognizes the pathogen, which leads to upregulation of RIG-I expression, and thus even more effective viral control.6

The two known RIG-I-like receptors (RIG-I and melanoma differentiation-associated protein 5 [MDA5]) recognize and bind viral genomic RNA. Although RIG-I plays a prime role in host defense, picornaviruses are recognized predominantly by MDA5. This receptor specificity is partially determined by characteristics of the viral genome, since MDA5 recognizes long double-stranded RNA (>1–2 kb in length).7 MDA5 consists of a conserved ATP-dependent helicase domain, which is connected at the N-terminus to 2 caspase activation and recruitment domains, and at the C-terminus to an RNA binding and regulatory domain. On binding to viral double-stranded RNA, MDA5 is recruited through its caspase activation and recruitment domains to the mitochondrial antiviral-signaling protein on the outer membrane of the mitochondria.8 This activates IFN response factors-3 and -7 and nuclear factor-κB, leading to transcription of type I IFN genes.8 Comparative studies of MDA5 recognition within the picornavirus family show that incoming genomic plus-strand RNA is not recognized by MDA5, but minus-strand RNA synthesis and association of the replicative double-stranded RNA initiates a strong IFNα/β response.9 All this suggests that MDA5 requires the virus to undergo active replication to stimulate type I IFN secretion. The latest crystal structure of MDA5 in complex with RNA10 indicates, in conjunction with previous biochemical analysis, that MDA5 forms long filaments, which enhance downstream signaling via mitochondrial antiviral-signaling protein.9–11

Although induction of type I IFNs is the most beneficial result of pattern recognition receptor activation, autophagy is also activated by these pathways and can protect cells from pathogens and regulate immune responses.12 Autophagy is a cellular process which maintains homeostasis by degradation of long-lived cellular proteins and organelles under normal conditions, but can provide the cell with nutrients during periods of starvation. Signaling through TLRs, NOD-like, and RIG-I-like receptors induce the formation of autophagosomes which deliver pathogens to lysosomes and lead to intracellular elimination of the microbes. Autophagosome delivery of viral nucleotides to endosomes also facilitates stimulation of TLRs 3, 7, 8, and 9. These steps seem to be essential to host defense as dendritic cells lacking autophagy-related proteins (Agt5)
show impaired production of IFNα.12 Interestingly, autophagy was also proposed to be hijacked by picornaviruses, to induce autophagosome-like membrane structures for genomic RNA replication.13,14 While stimulation through TLRs is a positive regulator of autophagy, stimulation of RIG-I-like receptors is inhibitory. Interaction of Agt5-Agt12 autophagy-related proteins with the caspase activation and recruitment domains of the RIG-I-like molecules suppresses secretion of type I IFN. The true relevance of RIG-I-like molecules and autophagy may reside in the role of autophagy in antigen presentation and the developing immune response as discussed below.

In this issue of Circulation: Heart Failure, Philip et al15 describe the production of a transgenic mouse in which MDA5 is constitutively overexpressed in cardiac myocytes, using the α-myosin heavy chain promoter (αMHC-MDA5 mice). One of the surprising observations was that overexpression of MDA5 leads to increased expression of tumor necrosis factor α, IFNβ, and IFNγ in the heart, even in the absence of infection and spontaneous inflammatory cell (primarily macrophage) infiltration of the myocardium.15 However, despite the presence of these cytokines and infiltrating lymphoid cells, no evidence of cardiac injury or dysfunction was noted. Transgenic mice overexpressing MDA5 in the cardiac myocytes showed significant protection from EMCV-induced contractile dysfunction and myocarditis. These animals also had significantly decreased cardiac virus titers early (day 3) but not later (day 5) after infection.11 Cardiac myocyte apoptosis was also decreased in the transgenic animals. This work originated from the observation that cardiac-specific overexpression of the TLR3/4 adaptor molecule TIR-domain–containing adapter-inducing interferon-β (TRIF), not only protected β

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Of high interest is the fact that apoptosis and cleavage of caspase-3 after EMCV infection were attenuated in MHC-MDA5 mice.15 Earlier studies have shown that overexpression of MDA5 increases the rate of apoptosis18 and proteasome activation leads to MDA5 cleavage,19 which was also shown to happen on infection with picornaviruses.20 It was proposed that the MDA5 cleavage and consequent induction of apoptosis during picornavirus infection could be a protective mechanism by limiting viral replication and spread of the virus. However, apoptosis in the context of cardiac myocytes leads to acute heart failure and in severe cases, death.12,17 Therefore, it is possible that cardiac-specific overexpression of MDA5 might upregulate antiprototic proteins, for example, FLICE-like inhibitory protein, caspase-8 protein regulator (FLIP). Overexpression of cellular FLIP long form was shown to be protective against coxsackievirus B3 (CVB3) myocarditis23 and cellular FLIP together with caspase-8 has a regulatory role in the RIG-I pathway.24 It would be of interest to analyze the gene expression profile of cardiac cells overexpressing the MDA5 gene.

Most experimental studies of picornavirus-induced myocarditis implicate immune/autoimmune responses to cardiac antigens as major factors in cardiac injury. Similarly, in clinical myocarditis/dilated cardiomyopathy, the presence of autoantibodies increased human leukocyte antigen (HLA) expression in the heart. Furthermore, certain levels of HLA haplotype association in patients, and a positive response of at least some of the patients to immunosuppressive therapy, argue for an immunopathogenic aspect to the disease process.1,17 The evidence for autoimmunity is even stronger in mouse models of picornavirus-induced myocarditis.1 The indication for autoimmunity derives not only from the demonstration of T cells reactive to heart antigens in infected mice, but by the ability to transfer myocarditis adoptively to uninfected animals with T cells from myocarditic mice. Picornaviruses are lysogenic and therefore should directly kill infected cells; however, if the total number of myocytes which are infected in the heart is small, then the amount of injury due to direct virus lysis ought to be limited, especially if the virus is rapidly cleared from the heart. As shown in Figure 6A of the article by Philip et al.,16 cardiac virus titers are rapidly decreasing by day 5 after infection even in littermate control (LM) mice. Thus one could ask whether the dramatic protection observed in the αMHC-MDA5 mice might reflect the known ability of RIG-I-like receptors to suppress autophagy processing of intracellular proteins for presentation by major histocompatibility complex class II (MHC II) antigens and abortion of CD4+ cell-dependent autoimmunity.25 Under these circumstances, MDA5 overexpression could have a 2-prong effect in viral myocarditis by both suppressing virus replication and suppressing autoantigen processing and presentation to the adaptive immune system. This would be a new concept in the field of viral myocarditis.

Disclosures

None.

References


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