Toll-like receptor 3 is an essential component of the innate stress response in virus-induced cardiac injury

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Toll-like receptor 3 is an essential component of the innate stress response in virus-induced cardiac injury. Am J Physiol Heart Circ Physiol 292: H251–H258, 2007. First published August 25, 2006; doi:10.1152/ajpheart.00398.2006.—Enterovirus-induced myocarditis is an important cause of heart failure in children and young adults (8, 14). The pathogenesis of viral heart disease is not completely understood, and specific therapies are not available. Recently, some investigators have suggested that myocarditis should be viewed as a continuum that is composed of three separate phases, namely, acute viral infection (phase I), autoimmunity (phase II), and dilated cardiomyopathy (phase III) (16). The host genetic factors responsible for the changes observed in these three phases of myocarditis have yet to be completely defined. However, Woodruff and Woodruff (31) were the first to demonstrate a role for T lymphocytes in the pathogenesis of viral myocarditis. Subsequently, Opavsky et al. (21) described the specific contributions of T-cell subsets (CD4 and CD8) and the T-cell receptor β chain to the pathogenesis of viral myocarditis. These same investigators have since shown that the T-cell receptor-associated tyrosine kinase p56

Toll-like receptor 3 (TLR3) is an important component of the innate antiviral response. We investigated the involvement of TLR3, which recognizes viral double-stranded RNA, on encephalomyocarditis virus (EMCV) infection. To examine the contribution of TLR3 in protection from EMCV infection, we infected mice deficient in TLR3 with 50 plaque-forming units of EMCV. TLR3-deficient (TLR3−/−) mice were more susceptible to EMCV infection and had a significantly higher viral load in the heart compared with TLR3+/+ mice. Histopathological examination showed that the inflammatory changes of the myocardium were less marked in TLR3−/− than in TLR3+/+ mice. TLR3−/− mice had impaired proinflammatory cytokine and chemokine expression in the heart following EMCV infection. However, the expression of interferon-β was not impaired in EMCV-infected TLR3−/− mice. EMCV infection leads to a TLR3-dependent innate stress response, which is involved in mediating protection against virus-induced myocardial injury.

Toll-like receptors: myocardial inflammation; innate immunity

ACUTE VIRAL MYOCARDITIS can lead to heart failure, arrhythmias, and sudden death, especially among infants and young adults (8, 14). The pathogenesis of viral heart disease is not completely understood, and specific therapies are not available. Recently, some investigators have suggested that myocarditis should be viewed as a continuum that is composed of three separate phases, namely, acute viral infection (phase I), autoimmunity (phase II), and dilated cardiomyopathy (phase III) (16). The host genetic factors responsible for the changes observed in these three phases of myocarditis have yet to be completely defined. However, Woodruff and Woodruff (31) were the first to demonstrate a role for T lymphocytes in the pathogenesis of viral myocarditis. Subsequently, Opavsky et al. (21) described the specific contributions of T-cell subsets (CD4 and CD8) and the T-cell receptor β chain to the pathogenesis of viral myocarditis. These same investigators have since shown that the T-cell receptor-associated tyrosine kinase p56

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MATERIAL AND METHODS

Mice. TLR3-deficient (TLR3<sup>−/−</sup>) mice were produced using gene targeting as described previously (2). Briefly, to generate TLR3<sup>−/−</sup> mice, we isolated the genomic DNA of the Tlr3 gene from a 129SV mouse genomic library. A targeting vector was constructed from a liver sample homogenized in 800 µl of RPMI 1640, and homologous recombinants were identified using Southern blot analysis. Homozygous TLR3<sup>−/−</sup> and TLR3<sup>+/−</sup> mice were generated by intercrossing heterozygous (TLR3<sup>+/−</sup>) mice.

Viral inoculation. A myocarditic variant of EMCV was generously provided by Dr. Sally Huber (University of Vermont). The virus stock was stored at −80°C in Hanks’ balanced salt solution with 0.1% BSA until use. Mice were inoculated intraperitoneally with 50 plaque-forming units (pfu). Four- to six-week-old male and female mice were used for these studies and were housed in an isolated room. The day of virus inoculation was defined as day 0. Mice were observed twice daily for the duration of the experiments. Mice that became moribund were considered to have reached the end point of the experiment and were killed. All experiments were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine and were performed in compliance with the National Institutes of Health regulations for animal handling and usage.

Viral titer. The titer of infectious EMCV was determined in individual heart and liver homogenates of TLR3<sup>+/+</sup> and TLR3<sup>−/−</sup> infected mice by plaque assay, using HeLa cells. Briefly, heart and liver samples were homogenized in 800 µl of RPMI 1640, and supernatants were stored at −80°C until used in the plaque assay. Dilutions of tissue supernatants were incubated on 80% confluent HeLa cell monolayers for 2 h at 37°C and 5% CO<sub>2</sub> to allow virus attachment and then incubated for 2 days to allow plaque formation. All viral titers are expressed as the mean ± SEM pfu per milligram of myocardial or liver tissue.

EMCV reverse transcriptase-polymerase chain reaction. RT-PCR was performed using a commercially available kit (Roche Diagnostics, Indianapolis, IN). The following oligonucleotide primer pairs were synthesized: EMCV sense, 5′-GGACTCTATGGGTGGAAGG-3′; antisense, 5′-GGACGGCATGAGCCTGAT-3′; and β-actin sense, 5′-GGACTCTATGGGTGGAAGG-3′; and antisense, 5′-GGACGGCATGAGCCTGAT-3′. PCR products were analyzed using agarose gel electrophoresis with ethidium bromide staining. The optimum number of cycles was determined experimentally for each gene product, as well as to verify uniform amplification.

Histopathological study. Hearts for TLR3<sup>+/+</sup> and TLR3<sup>−/−</sup> mice were fixed in 10% buffered formalin and embedded in paraffin. Transverse sections of the ventricles were stained with hematoxylin and eosin and observed using microscopy at ×20 magnification. The extent of cellular infiltration was graded blindly by an experienced individual who had no knowledge of the study design. The inflammatory score was determined as described by Kanda et al. (11) and was as follows: 0, no lesions; 1+, lesions involving <25%; 2+, lesions involving 25–50%; 3+, lesions involving 50–75%; and 4+, lesions involving 75–100%.

Immunohistochemistry. For cryosections (6 µm), mouse hearts from EMCV-infected (3 days and 5 days) wild-type (WT) and TLR3<sup>−/−</sup> mice were equilibrated in 20% sucrose solution and mounted in OCT. Sections were fixed with acetone at −20°C for 10 min, followed by immunofluorescent staining with primary antibodies for 1 h. The following antibodies were used: T cells, CD3 polyclonal antibody (R&D Systems, Minneapolis, MN); macrophages, F4/80 (eBioscience, San Diego, CA); and from NK cells, CD94 (Biolegend, San Diego, CA). This was followed by incubation with Alexa Flour 488 or Texas red conjugated secondary antibodies (Molecular Probes, Carlsbad, CA) or Alexa Flour 594 phalloidin for 30 min (Molecular Probes). Subsequently, those sections were incubated with 4,6-diamidino-2-phenylindole (DAPI; Roche, Indianapolis, IN) for 5 min. Sections were mounted with prolonged gold antifade reagent (Molecular Probes) and visualized using the Olympus BX51 microscopy system.

Measurement of serum cardiac troponin I levels. Concentrations of cardiac troponin I in the serum of naive and EMCV-infected TLR3<sup>+/+</sup> and TLR3<sup>−/−</sup> mice were measured with commercially available kits (Life Diagnostics, West Chester, PA).

Myocardial cytokine and chemokine expression. Mice were killed on days 0, 3, and 5 following EMCV infection, and total RNA was extracted from the hearts as previously described (4). The level of gene expression for tumor necrosis factor (TNF), IL-1β, IL-6, interferon-β (IFN-β), RANTES (regulated on activation of normal T-cells expressed and -secreted chemokine), and interferon-inducible protein 10 (IP-10) was determined with a multiprobe RNaSe protection assay system, according to the manufacturer’s suggestions (RiboQuant; Pharmingen, San Diego, CA). Signals were quantified using Image-QuanT software and normalized to L32 (Molecular Dynamics, Sunnyvale, CA).

TNF immunohistochemistry. To localize the cellular source of TNF expression, we harvested hearts from TLR3<sup>+/+</sup> and TLR3<sup>−/−</sup> mice at 0, 3, and 5 days after EMCV infection. Frozen hearts were sectioned and immunostained for 1 h at room temperature using an antibody against mouse TNF or α-actin (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA). This was followed by incubation with Alexa Flour 488 secondary antibody (1:1,000; Molecular Probes) or Alexa Flour 594 phalloidin for 30 min (1:1,000; Molecular Probes). Subsequently, sections were incubated with DAPI (1:1,000; Roche) for 5 min. Sections were imaged with a confocal microscope (Olympus Fluoview).

TNF administration. Recombinant human TNF produced in Escherichia coli by recombinant DNA technology was purchased from R&D Systems. TNF (2 µg) was dissolved in 0.1 ml of PBS and injected intravenously to TLR3<sup>−/−</sup> mice 5 h after EMCV infection (50 pfu/mouse). TLR3<sup>+/+</sup> mice treated with PBS (0.1 ml) served as controls. This dose of TNF has been shown to increase survival of TLR3<sup>−/−</sup> mice when administered 6 h after EMCV infection (27).

Statistical analysis. All values are expressed as means ± SE. A Kaplan-Meier log-rank analysis with a post hoc Holm-Sidak test for pairwise comparisons (7 and 14 days) was used to compare survival curves among TLR3<sup>+/+</sup>, TLR3<sup>−/−</sup>, and TLR3<sup>+/+</sup> mice. A two-way ANOVA was used to test for differences in EMCV-induced cytokine and chemokine expression, as well as viral load. Where appropriate, post hoc testing was performed using Fisher’s protected least significant difference (PLSD) test to detect mean differences between groups. Cardiac histopathological scores were examined using the Mann-Whitney test. Significant differences were considered to exist at P < 0.05.

RESULTS

TLR3<sup>−/−</sup> mice have decreased survival following EMCV infection. To test whether TLR3 is functionally important during infection with EMCV, we infected TLR3<sup>−/−</sup> mice (n = 17) and TLR3<sup>+/+</sup> mice (littermates; n = 17) with a myocar-}

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mice. That is, 80% of EMCV-infected TLR3−/− mice died within 4 days of inoculation. Beyond the tenth day of infection, the survival curves for the TLR3+/+ and TLR3−/− mice continued to diverge. That is, whereas the survival curve for the TLR3+/+ mice showed no further increase in mortality beyond day 10, there was a continued progressive decrease in survival in TLR3−/− mice from day 10 to day 14. Kaplan-Meier analysis showed that there was a significant ($P < 0.0001$) difference in survival between EMCV-infected TLR3−/− and TLR3+/+ mice. When post hoc analysis was confined to the first 7 days after EMCV infection, a significant survival difference ($P < 0.0001$) was observed between TLR3−/− and TLR3+/+ mice. A gene-dose effect was noted when TLR3+/− mice were challenged with EMCV. That is, the mortality of TLR3−/− mice ($n = 15$) at day 7 was significantly greater ($P < 0.05$) than in TLR3+/− mice, but it was only 50% of that in TLR3−/− mice. Post hoc analysis at 14 days after EMCV infection revealed a significant survival difference ($P < 0.0001$) only between EMCV-infected TLR3−/− and TLR3+/+ mice. These data clearly demonstrate that TLR3 is important in host defense against EMCV and that in the absence of TLR3, infection is associated with rapid and increased mortality.

**Myocardial EMCV replication.** To investigate whether the observed differences in survival in the TLR3−/− mice were secondary to differences in viral replication, we performed RT-PCR of EMCV mRNA and obtained viral titers from the hearts of EMCV-infected mice. As shown in Fig. 2, A and B, EMCV genomic RNA levels were significantly higher ($P < 0.05$) in the hearts of TLR3−/− mice on days 3 and 5 compared with TLR3+/+ mice. Figure 2C shows that on days 3 and 5, the viral titers in the hearts of TLR3−/− mice were significantly higher ($P < 0.05$) compared with TLR3+/+ mice. That is, viral titers were ~1,000- and ~100-fold higher in the hearts of EMCV-infected TLR3−/− mice at 3 and 5 days, respectively. EMCV titers were also significantly higher in the livers of TLR3−/− mice at day 3, but no differences were detected at day 5 after infection (Fig. 2D). Notably, viral titers measured on day 5 in the hearts of TLR3−/− mice were almost 100-fold higher than in the livers of these mice. Together, these data suggest that the TLR3−/− mice are incapable of eliminating replicating EMCV as efficiently as TLR3+/+ mice, leading to increased mortality.

**Myocardial histopathology.** To further investigate the mechanism for increased early mortality in the TLR3−/− mice, we assessed cardiac histopathology in TLR3+/+ and TLR3−/− mice at days 3 and 5. Histological examination of TLR3+/+ and TLR3−/− mouse hearts before infection revealed no evi-
dence of inflammation or ventricular fibrosis (data not shown). On day 3 after infection, mononuclear cells were present in the myocardium of TLR3+/+ and TLR3−/− mice. Although there was some variability among mice in each group, quantification of cellular infiltration revealed an overall reduction in TLR3−/− mice (P = 0.04; Fig. 3, A and B). Although by day 5 after infection the cellular infiltration was not robust in either group, it was still suppressed in TLR3−/− mice compared with that in TLR3+/+ mice (0.74 ± 0.11 vs. 1.4 ± 0.19; n = 7; P = 0.03). The modest (<25%) cellular infiltration noted at 3 and 5 days after EMCV infection is consistent with previous reports in the literature (27). When cardiac sections from TLR3+/+ and TLR3−/− mice were stained for the presence of T cells (CD3+), macrophages (F4/80), or NK cells (CD94+), there was a paucity of cells that stained positive for these markers (data not shown). These data suggested that the increased early mortality (day 3) and enhanced viral replication in TLR3−/− mice were not likely to be secondary to differences in the type of cells infiltrating the myocardium. We then measured serum cardiac troponin levels as a marker of direct EMCV-induced myocardial injury. Serum levels of cardiac troponin I in TLR3−/− mice were significantly higher than those of TLR3+/+ mice at day 3 (Fig. 3C), suggesting that significant direct virus-induced myocardial injury had indeed occurred in TLR3−/− mice in the phase of impaired cellular infiltration. These findings are similar to those seen in EMCV-infected TNF−/− mice, in which lower inflammatory scores were associated with higher viral titers and creatine kinase levels (27).

Myocardial TNF, IL-1β, IFN-β, and chemokine expression. We infected TLR3+/+ and TLR3−/− mice with EMCV and measured cytokine mRNA expression in the heart. Figure 4A shows that compared with that at day 0, TNF, IL-1β, and IL-6 mRNA expression increased significantly at 3 and 5 days after EMCV infection in the TLR3+/+ mice. The onset of expression of cardiac TNF and IL-1β mRNA in the TLR3−/− mice was different from that observed in the TLR3+/+ mice. That is, expression of cardiac TNF and IL-1β mRNA in the TLR3−/− mice did not occur until day 5 of infection. As shown by the representative RPA in Fig. 4A and the group data summarized in Fig. 4, B and C, the levels of TNF, IL-1β, and IL-6 mRNA were each significantly decreased in the TLR3−/− mice at 3 days after EMCV infection compared with TLR3+/+ mice. When TNF expression was compared within EMCV-infected TLR3−/− mice, a significant increase in cardiac TNF expression was noted in these mice at 5 days after infection (day 0 vs. day 5; P < 0.05). Immunohistochemical analysis for TNF in heart sections from EMCV-infected TLR3+/+ and TLR3−/− mice also revealed that the onset of expression of cardiac TNF protein in the TLR3−/− mice was different from that observed in the TLR3+/+ mice (Fig. 5). More importantly, dual staining for TNF and α-actin (merged image) demonstrated that the differences in TNF expression are due, at least in part, to altered TNF expression in cardiac myocytes.

TLR3-mediated signaling is known to stimulate the production of the antiviral cytokine IFN-β and a variety of chemokines, which may aid with viral clearance (3, 6). Figure 6A shows the kinetics of mRNA transcript expression for IFN-β, RANTES, and IP-10 in TLR3+/+ and TLR3−/− mice. Notably, myocardial IFN-β expression was not impaired in the TLR3−/− mice. In fact, at 3 days after infection, the IFN-β response was significantly augmented (Fig. 6B; P < 0.05) compared with TLR3+/+ mice. The level of expression of RANTES and IP-10 was significantly augmented (Fig. 6, C and D; P < 0.05) in the TLR3+/+ mice compared with the TLR3−/− mice at day 3 after infection. In contrast, mRNA expression of macrophage inflammatory protein (MIP)-2, MIP-1α, and MIP-1β in the heart did not differ between the two groups after EMCV infection (data not shown).
Effect of exogenous TNF administration on survival of EMCV-infected TLR3−/− mice. The delay in TNF expression in the hearts of TLR3−/− mice is of interest given that Wada et al. (27) have shown that TNF−/− mice have decreased survival and increased viral replication in the heart following EMCV infection. Because a significant increase in cardiac TNF expression was observed within 3 days after infection of EMCV in TLR3+/+ mice, TLR3−/− mice were treated with TNF (2 μg) 36 h after EMCV challenge. As shown in Fig. 7, TNF administration did not alter survival significantly (at 7 and 14 days) in EMCV-infected TLR3−/− mice compared with diluent-treated TLR3+/+ mice or untreated TLR3−/− mice. However, the survival curve for TNF-treated TLR3−/− mice was shifted to the right compared with untreated TLR3−/− mice. That is, TNF treatment delayed but did not completely prevent death in TLR3−/− mice. We speculate that the difference in response to treatment between EMCV-infected TNF−/− and TLR3−/− mice is related to a more globally...
abnormal innate response in TLR3−/− mice. That is, whereas TNF deficiency is the only defect in TNF−/− mice, TLR3−/− mice have abnormal production of TNF, IL-1β, IL-6, and the chemokines RANTES and IP-10.

DISCUSSION

This study, in which we compared TLR3+/+ and TLR3−/− mice, constitutes the initial demonstration that TLR3-mediated signaling plays a role in protecting the heart against EMCV-mediated injury. In the absence of TLR3, TLR3−/− mice show markedly increased mortality after infection with EMCV, increased viral replication in the heart, and increased myocardial injury as demonstrated by higher serum troponin I levels. Histopathological analysis revealed that myocardial cellular infiltration in TLR3−/− mice was suppressed at days 3 and 5 after infection compared with TLR3+/+ mice. In contrast, EMCV-infected TLR3+/+ mice showed lower viral titers in the heart and significantly improved survival. Although the precise mechanisms for the increased susceptibility to virus infection in TLR3−/− mice have yet to be completely defined, we have provided evidence for an altered early antiviral response to EMCV infection in cardiac myocytes (Fig. 5). In the context of a dsRNA-induced TLR3-mediated antiviral response, the expression of TNF, IL-1β, IL-6, IP-10, and RANTES was blunted and delayed in the hearts of TLR3−/− mice compared with TLR3+/+ mice. The reduced early cytokine expression in the hearts of TLR3−/− mice was temporally associated with the onset of death and an increased viral load in the hearts of TLR3−/− mice. However, it should be noted that 80% of EMCV-infected TLR3−/− mice died within 4 days of inoculation and therefore could not be included when the cytokine responses in the heart at day 5 were evaluated. Consequently, the data presented in Figs. 4 and 6 may overestimate the actual cytokine/chemokine response induced in hearts of EMCV-infected TLR3−/− mice. Wada et al. (27) reported that mice with targeted disruption of the TNF gene (TNF−/−) had increased mortality after infection with EMCV and that exogenous administration of TNF prevented the increase in virus-induced mortality in the TNF−/− mice. Similarly, the acute administration of IL-6 at the time of EMCV infection resulted in enhanced viral clearance and decreased myocardial necrosis (11). Interestingly, transgenic mice with cardiac overexpres-

Fig. 6. Effect of TLR3 deficiency on cardiac interferon (IFN)-β, RANTES (regulated on activation of normal T-expressed and -secreted chemokine), and interferon-inducible protein 10 (IP-10) mRNA expression after EMCV infection. A: cardiac IFN-β, RANTES, and IP-10 mRNA expression was assessed using RPA. IFN-β (B), RANTES (C), and IP-10 mRNA levels (D) were normalized to L32. *P < 0.05, TLR3+/+ vs. TLR3−/− mice.

Fig. 7. Effects of intravenous administration of TNF on survival of TLR3−/− mice infected with EMCV (50 pfu). A Kaplan-Meier log-rank analysis with a post hoc Holm-Sidak test for pairwise comparisons (7 and 14 days) was used to compare survival curves between PBS-treated TLR3+/+ mice (n = 10), TNF-treated TLR3−/− mice (n = 13), and untreated TLR3−/− mice (n = 17).
sion of IL-6 had accelerated tissue injury and decreased viral clearance following EMCV infection (26). In that study, the negative effects of IL-6 overexpression were linked to decreased TNF production, because exogenous TNF administration reversed the phenotype induced by EMCV. The observation that exogenous administration of TNF after EMCV infection did not improve the survival of TLR3⁻/⁻ mice suggests that other innate mediators also are involved in the antiviral response. We speculate that the difference in response to treatment between EMCV-infected TNF⁻/⁻ and TLR3⁻/⁻ mice may be related to a more globally abnormal innate response in TLR3⁻/⁻ mice. That is, whereas TNF deficiency is the only defect in TNF⁻/⁻ mice, TLR3⁻/⁻ mice also have abnormal production of antiviral molecules such as IL-1β, IL-6, and the chemokines RANTES and IP-10. Although at the present time it is not possible to completely discern whether the cardioprotection afforded in this model results from TLR3 signaling (cytokine production) in the heart or in immune cells, the data presented strongly suggest that loss of TLR3 signaling allowed EMCV to multiply unchecked, thereby increasing its cytopathic effects in cardiac myocytes and ultimately leading to increased mortality in TLR3⁻/⁻ mice. 

Pattern recognition receptors, such as TLRs, discriminate molecular patterns expressed by pathogens and facilitate differential recognition of pathogens and microbial products (19). This innate response provides a critical, rapid defense mechanism that acts before the maturation of acquired immunity. Once triggered by microbial antigens, most TLR signal transduction is mediated by the adaptor protein termed myeloid differentiation factor 88 (MyD88) (1). However, TLR3 and TLR4 utilize a unique adapter molecule called Toll-interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-β (TRIF), which upon recruitment to the receptor complex leads to activation of the NF-κB-responsive element and the IFN-β promoter, leading to the production proinflammatory cytokines and IFN-α/β (32). These effector proteins of the innate immune system have been shown to possess important antibacterial and antiviral properties that help contain pathogens at the site of entry. A number of studies have demonstrated the involvement of TLRs in host defense against bacterial infections. For example, TLR2- and TLR4-deficient mice are highly susceptible to infection with Staphylococcus aureus and gram-negative bacteria, respectively (25, 29). The role of TLRs in viral infections has only recently been appreciated. Several different viruses that infect mammals, including cytomegalovirus (TLR3/TLR9), respiratory syncytial virus (RSV; TLR4), and West Nile virus (TLR3) have been shown to interact with TLRs (13, 24, 28). Interestingly, TLR3⁻/⁻ mice are more resistant to lethality following West Nile virus infection because these mice have reduced viral entry into the brain. The observation in the present study that TLR3 deficiency blunted rather than abrogated the inflammatory response (TNF, IL-1β, and IL-6) suggests that other TLRs or TLR-independent pathways may contribute to EMCV-induced cytokine production in the heart. Moreover, the finding that impairment of TLR3 signaling did not have a dramatic effect on the expression of IFN-β was somewhat surprising. Cardiac IFN-β mRNA expression was in fact enhanced in the hearts of TLR3⁻/⁻ mice compared with TLR3⁺/⁺ mice at day 3 after infection. We considered that the IFN-β response in hearts of TLR3⁻/⁻ mice might result from the activation of the TLR4→TRIF signaling pathway, perhaps through recognition of a viral structural protein, as has been reported for RSV. Interestingly, Moran et al. (20) have shown that activation of mitogen-activated protein kinases by EMCV in macrophages is associated with the interaction between a structural protein of the virion and macrophage cell surface receptor. Lund et al. (17), using TLR7⁻/⁻ mice, have provided evidence that TLR7 is required for the recognition of viral ssRNA and that the secretion of type I interferon depends on the functional expression of MyD88 and TLR7. Thus recognition of EMCV ssRNA by TLR7 also may contribute to the enhanced INF-β response measured in TLR3⁻/⁻ mice. Finally, other molecules responsible for TLR3-independent recognition of dsRNA and viruses have been recently described. Yoneyama et al. (33) reported that the cytoplasmic protein retinoic acid-inducible gene I (RIG-I) participates in the recognition of virus nucleic acid inside the cell. Indeed, fibroblasts derived from RIG-I-deficient mice are unable to produce type I interferon and inflammatory cytokines after infection with Newcastle disease virus, Sendai virus, or vesicular stomatitis virus (12). 

The finding that impairment of TLR3 signaling did not have a dramatic effect on the expression of IFN-β is more intriguing given that administration of IFN-α or -β has been shown to have a beneficial effect on viral myocarditis in the early stages of disease. Moreover, IFN-β⁻/⁻ mice have increased mortality (~65%) and develop severe myocarditis when infected with CVB3 (5). Interestingly, Wessely et al. (30) reported that type I IFN signaling played an essential role in preventing early CVB3 replication in the liver but had little effect on early viral replication in the heart. In interferon receptor-I knockout mice, early death was not associated with higher CVB3 titers in the heart but rather with increased amounts of virus in the liver. Our data are in agreement with these findings, since by day 5 after infection, viral loads in the liver were similar between the two groups but viral load was significantly higher (~100-fold) in the hearts of TLR3⁻/⁻ mice despite higher IFN-β mRNA expression.

In conclusion, the findings of the present study establish the importance of TLR3 signaling in protecting the heart during viral infection and further support the importance of the antiviral effects of TNF and IL-6 in the early stages of disease. As suggested by this study, mice lacking functional TLR3 appear unable to control the proliferation of EMCV, which subsequently leads to increased cytopathogenic effects in cardiac myocytes and early death. Whether strategies directed at enhancing the TLR3→TRIF-mediated signaling in the heart will ameliorate virus-induced cardiac myocyte damage is currently being investigated.

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