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Update on coxsackievirus B3 myocarditis

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Abstract

Purpose of review—To present recent findings on the pathogenesis of coxsackievirus B3 (CVB3) myocarditis based on animal models, with a focus on the role of T helper (Th) immune responses in disease progression.

Recent findings—Acute CVB3 myocarditis is known to be increased by Th1 immune responses, but recent findings indicate that Th1-type immunity protects against acute myocarditis by reducing viral replication and prevents the progression to chronic myocarditis and dilated cardiomyopathy (DCM) by inhibiting Th2 responses. Th2 responses reduce acute myocarditis by inhibiting Th1 responses via regulatory T cells and anti-inflammatory cytokines, but can be deleterious when they induce acute cardiac remodeling leading to chronic myocarditis/DCM. Th2-skewed immune responses allow resistant strains of mice to progress from myocarditis to DCM. In contrast, Th17 responses are elevated during acute and chronic myocarditis and have been found to contribute to cardiac remodeling and DCM.

Summary—Recent data indicate that elevated Th2 and Th17 responses during acute CVB3 myocarditis are critical for the progression from myocarditis to DCM and heart failure because of their ability to induce cardiac remodeling. Th1 responses protect against CVB3 myocarditis by inhibiting Th2 responses and viral replication, but increase acute inflammation.

Keywords

myocarditis; autoimmune disease; coxsackievirus; T helper response

INTRODUCTION

Myocarditis is an autoimmune disease that leads to a significant minority of dilated cardiomyopathy (DCM) cases in the US [1–3]. From 4–20% of sudden cardiovascular deaths among young adults, the military, and athletes are due to myocarditis [4]. However, the true incidence and prevalence of myocarditis are unknown due to the lack of widely available, safe and accurate noninvasive diagnostic tests [5,6**]. Although most cases of suspected myocarditis are not linked to a specific cause [6**], viral infections like

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Conflicts of interest

Authors have nothing to disclose.

coxsackievirus B3 (CVB3) are the most commonly identified cause of myocarditis in developed countries [2,4]. Antiviral treatments with interferon (IFN)-β have been shown to reduce inflammation and DCM in animal models and patients [7,8] suggesting that viral infections are an important cause of myocarditis cases in patients.

Infections are also believed to induce or trigger autoimmunity [9–11]. CVB3 induces autoimmune myocarditis that progresses to DCM in susceptible strains of mice like A/J and BALB/c [12,13]. Resistant strains of mice like C57BL/6 develop acute CVB3 myocarditis, but do not progress to DCM. Myocarditis and DCM occur more frequently in men than women and in male than female mice [2,14**,15**]. A recent study of myocarditis/acute DCM patients found that myocardial recovery and transplant-free survival were significantly worse in men [14**]. Similar to findings in clinical biopsies of myocarditis patients [2], the primary infiltrate in mouse models of myocarditis consists of macrophages and neutrophils with lower levels of T cells, B cells, mast cells and dendritic cells [16–18]. Natural killer cells, CD8 T cells and γδT cells, important in antiviral defense, are present in the heart during the early cellular response in viral animal models of myocarditis [12,19]. Acute myocarditis is characterized by a predominantly T helper (Th)1 and Th17 response [19,20**,21,22]. However, only mice that respond to infection and/or self-antigen (i.e. damaged self) with a Th2 response, such as BALB/c and A/J strains, develop the chronic stage of myocarditis with fibrosis and DCM [23,24,25**].

This review focuses on recent findings in animal models of CVB3 myocarditis. There are essentially three animal models of myocarditis: an experimental autoimmune myocarditis (EAM) model induced by adjuvant and cardiac myosin, a hybrid-CVB3 model that closely resembles EAM that is induced using heart-passaged CVB3 and damaged heart proteins, and the classic CVB3 model that uses tissue culture-derived or purified virus. Each of these models contributes uniquely to our understanding of the pathogenesis of disease.

ANIMAL MODELS OF MYOCARDITIS

In order to appreciate recent findings, it is important to understand similarities and differences between the three primary models of autoimmune and CVB3 myocarditis in mice.

CVB3-only model

In 1974 Woodruff *et al*. originally described CVB3-induced myocarditis in mice [26]. CVB3 purified from tissue culture is the most common method used to induce acute myocarditis. In this model mice are infected intraperitoneally (ip) with purified virus or RNA from various CVB3 strains (i.e. Woodruff, H3, Nancy) after passage through HeLa cells. Viral replication peaks in the heart from day 5–7 post infection (pi) (10^7–10^9 plaque forming units/PFU/g heart) and causes severe necrosis but low levels of inflammation (5–10% of heart tissue inflamed) followed by heart failure with only around 30% of mice surviving to day 7 pi (Table 1) [13]. Disease is more severe in male than female mice [27–29], γδT cells are critical for disease induction [19,30**], and increased acute inflammation is associated with a predominant Th1 response- particularly in male mice [19]. The

importance of autoreactive T and B cells in this model indicates that autoimmunity is involved in disease pathology [9].

EAM

EAM is induced using cardiac myosin/cardiac peptides and adjuvants (i.e. inactivated *Mycobacterium* and/or Pertussis toxin) administered at day 0 and 7 [31]. Peak inflammation (30–60% of heart tissue inflamed) occurs around 10–14 days later at day 21 post inoculation (Table 1) [32**,33**]. Acute inflammation consists predominantly of macrophages and neutrophils with lower amounts of T and B cells, mast cells and dendritic cells [18,32**]. Disease develops in susceptible strains of mice (e.g. Th2 responders like BALB/c and A/J mice) and progresses to DCM around day 42 post inoculation when fibrosis and necrosis is observed [23,34]. A Th1 immune response has been shown to prevent acute and chronic EAM [34,35] while a Th17 response increases chronic fibrosis and DCM [22]. Thus, only Th2 responding strains develop EAM and progress to DCM [23]. 100% of susceptible wild type (WT) mouse strains survive to develop DCM.

Hybrid-CVB3 model

This model is induced by passaging CVB3 originally isolated from a patient (i.e. Nancy strain) through Vero cells and then through the heart [10]. Infectious virus obtained from the heart (i.e. heart-passaged) along with damaged self-tissue is injected ip into mice at day 0. All mouse strains (e.g. BALB/c, C57BL/6) develop acute myocarditis that peaks at day 10 pi (30–60% of heart tissue inflamed) and is comprised predominantly of macrophages and neutrophils with lower numbers of T and B cells, mast cells and dendritic cells (Table 1) [16], similar to EAM. Viral replication peaks around day 7 pi (10^5 PFU/g heart), declines by day 10 pi (10^3 PFU/g heart) and is cleared from the heart by day 14 pi [10,12]. 100% of WT mice survive acute myocarditis, but only susceptible Th2 responding strains of mice like BALB/c develop chronic DCM and fibrosis from day 35 pi [10,24,25**]. Necrosis is observed histologically in the heart during chronic myocarditis/DCM, but not during acute myocarditis. This hybrid-CVB3 model more closely resembles EAM than CVB3-only models (Table 1) [18,24,35]. Myocarditis and DCM are more severe in male mice [15**, 16,36*].

RECENT FINDINGS

Several review articles summarize current knowledge on the role of cytokines and Th responses in the pathogenesis of myocarditis (see 30**,32**,33**,37**,38**). This review adds understanding by highlighting the most recent findings on the role of Th responses in the pathogenesis of CVB3 myocarditis.

Th1 response

Recently Wiltshire *et al.* raised two very important questions: 1) does uncontrolled viral replication drive inflammation during CVB3 myocarditis, or 2) does virus trigger an inflammatory response via TLR activation, for example [39*]? The answer to these questions may lie in the model and/or viral strain being used to induce myocarditis. EAM is a clear example of inflammation being triggered by activation of TLR via cardiac myosin

and the inactivated *Mycobacterium* component of complete Freund's adjuvant. Viral replication in CVB3-only models, however, is closely associated with elevated cardiac inflammation (Table 2) [13,37**,40**]. In contrast, inflammation usually does not correlate with viral replication in the hybrid-CVB3 model [16,25**,41]. These differences in the role of virus as a "driver" vs. "trigger" of inflammation are highlighted by a number of recent studies that examined the role of Th1 responses in CVB3 myocarditis.

Wiltshire *et al.* identified a viral myocarditis susceptibility gene (*Vms1*) locus on murine Chromosome 3 by comparing susceptible strains of mice such as A/J to resistant strains like B10.A using a CVB3-only model [39*]. They found several IFN-related genes that determined susceptibility to infection (e.g. *Fpgt*, *H28*, *Tnni3k*). IFN-β has been shown previously to protect against viral myocarditis in animal models and patients [7,8], and recently several groups showed that protection by IFN-β is mediated via the transcription factor TRIF (25**,40**,42,43]. CVB3 limits a host antiviral response by repressing mRNA translation. Interestingly, activation of the translational suppressor eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) was found to inhibit IFN-β production by host cells [42]. Additionally, low-dose oral IFN-α administration in a CVB3-only model was found to reduce viral replication in the heart by increasing a Th1 response resulting in reduced acute myocarditis (Table 2) [44]. Although most studies using CVB3-only models find a strong association between the level of cardiac viral replication and myocarditis, studies by Yue *et al.* found that inhibiting the chemokines IFN-γ-induced protein-10 (IP-10/CXCL10) or monocyte chemotactive protein-1 (MCP-1/CCL2) reduced Th1/IFN-γ responses and myocarditis but had no significant effect on cardiac viral replication [45**,46]. These findings suggest that type I IFNs like IFN-α/β may be more important in preventing viral replication, at least in CVB3-only models, while IFN-γ increases inflammation. Interestingly, investigators using the Nancy strain of CVB3 observed 100% survival at day 7 pi in WT mice compared to around 20–30% survival using other CVB3 strains (25**,40**, 43,44,45**,46). Although TLR3 and TRIF deficient mice develop increased myocarditis and cardiac viral replication, only TRIF deficiency results in severe chronic myocarditis/DCM using the hybrid-CVB3 model [25**]. TRIF deficiency decreases IFN-β levels in the heart but not IFN-γ in contrast to TLR3 deficiency, which decreases IFN-γ but not IFN-β. This results in an IL-4-mediated Th2-driven response in TLR3 deficient mice and an IL-33-mediated Th2-driven response in TRIF deficient mice [25**]. An IL-33-skewed response causes more severe cardiac dysfunction than an IL-4 response, yet a Th2-predominant IL-4 or IL-33 response reverses resistance to DCM in C57BL/6 mice [25**]. Th2 cytokines are critical for the development of DCM because of their role in cardiac remodeling. These findings indicate that in the hybrid-CVB3 model both IFN-β and IFN-γ prevent viral replication. In all models, Th1-type immune responses protect against CVB3 myocarditis by reducing viral replication and inhibiting Th2 responses. Interestingly, the protective effect of mesenchymal stem cell (MSC) treatment on CVB3 myocarditis was found to require IFN-γ [47,48**], providing further evidence of a protective role for Th1 responses in myocarditis.

Th2 response

Although Th1 responses protect against viral replication and prevent chronic myocarditis/ DCM, they increase acute inflammation- particularly in males [16,19,28,29]. Th2 responses,

as transcriptional regulators of IFN-γ, inhibit acute inflammation. Likewise, Treg and IL-10 reduce CVB3 myocarditis [16,30**]. IFN-γ-producing γδT cells, important drivers of disease in CVB3-only models of myocarditis, have been found to inhibit Treg [30**]. Histamine receptor-1, important for mast cell activation, was shown to be protective in this myocarditis model by decreasing pathogenic Vγ4+ γδT cells and Th1 responses in the heart [49**]. In contrast, MSC-type cells inhibit myocarditis by increasing Treg, IL-10 and IFN-γ [47,48**]. Induction of tolerance by nasal cardiac myosin peptide treatment of mice with CVB3 myocarditis reduced disease by elevating IL-10 and Treg [50]. Administration of recombinant galectin-9, a ligand for the inhibitory receptor Tim-3 [16,51], was found to inhibit acute CVB3 myocarditis by increasing IL-4/Th2 cells, $Tim\text{-}3^{+}Gr1^{+}CD11b^{+}$ macrophages and IL-10 in the heart [52**]. And finally, administration of astragaloside IV (obtained from the therapeutic root *Astragalus membranaceus*) to mice with CVB3 myocarditis was found to reduce chronic myocarditis and DCM by downregulating TGF-β_1 and Smad signaling [53]. In summary, researchers have recently identified several agents/pathways that reduce acute CVB3 myocarditis by increasing Th2 and/or regulatory responses, such as nasal administration of cardiac myosin, histamine receptor-1 signaling, galectin-9 and astragaloside IV, but more research is needed to determine whether increasing these Th2-like responses protects or contributes to chronic myocarditis/DCM. If these Th2-type responses activate cardiac remodeling genes during acute CVB3 myocarditis, there is evidence that these gene changes could promote progression to chronic myocarditis and DCM (Table 2) [25**,36*].

Th17 response

In the past few years the role of IL-17 (i.e. IL-17A) in the pathogenesis of CVB3 myocarditis has been more clearly elucidated. IL-17 levels increase in the circulation and heart during the peak of CVB3 myocarditis (day 7–10 pi) [54,55]. Recently, IL-23 and STAT3, both important in IL-17 responses, as well as IL-17 were found to be significantly elevated in the heart during acute and chronic CVB3 myocarditis (day 7 to 42 pi) [56], suggesting a role for IL-17 in mediating disease. Th17-producing cells are also elevated in the spleen during CVB3 myocarditis (day 7 to 42 pi) compared to controls, representing around 5–14% of splenic $CD4^{+}$T cells [57,58]. Blocking IL-17 using neutralizing antibody in CVB3-only models improved survival, reduced acute myocarditis and viral replication in the heart, and increased COX-2, prostaglandin E2 and Treg [58–60]. In the hybrid-CVB3 model, treatment of mice for 2 weeks with low-dose inorganic mercury prior to administering CVB3/heart proteins did not alter the severity of acute myocarditis, but increased chronic fibrosis and DCM [61*]. This response was associated with elevated cardiac IL-17 levels during acute CVB3 myocarditis. Similar to this study, elevated IL-17 during the acute phase of myocarditis has been shown to increase chronic fibrosis and DCM in the EAM model [22]. Interestingly, blocking the tissue damage-associated protein high-mobility group box 1 (HMGB1) reduced EAM and suppressed Th17 responses [62], suggesting the possibility that damaged heart proteins released during viral infection may increase IL-17 levels in the heart. Recently, we examined whether IL-23, a cytokine important in promoting IL-17-mediated immunity, was required for acute inflammation using the hybrid-CVB3 model. We found that acute myocarditis was not altered in IL-12p40/IL-23 deficient mice (Figure 1). Viral replication and Th1/Th17-related cytokines

were also unchanged in knockout mice compared to WT controls (Figure 1), demonstrating that IL-23 is not critical for the induction of acute myocarditis in the hybrid-CVB3 model. More research is needed to examine the role of IL-23/IL-17 in the progression to chronic myocarditis/DCM using the various CVB3 models.

CONCLUSION

In this review we have examined recent data on the role of Th responses on the pathogenesis of CVB3 myocarditis. Th1 responses increase acute inflammation, yet this response reduces viral replication and protects against CVB3 myocarditis by increasing survival (in CVB3-only models) and by preventing progression to DCM (in the hybrid-CVB3 model). In contrast, Th2 responses reduce acute myocarditis by elevating regulatory T cells and anti-inflammatory cytokines, but can be detrimental by inducing acute cardiac remodeling leading to chronic myocarditis/DCM. If the immune response is deviated toward a Th2 response in resistant strains of mice, as occurs in TLR3 and TRIF deficient mice, resistance is overcome leading to DCM and heart failure. Th17 responses are also able to increase acute CVB3 myocarditis, but unlike Th1 responses, Th17 immunity contributes to cardiac remodeling leading to DCM and heart failure.

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KEY POINTS

- Myocarditis is an autoimmune disease that leads to DCM in susceptible individuals and mice
- Viral infections like CVB3 are the most commonly identified cause of myocarditis in developed countries and induce myocarditis and DCM in animal models
- Th1-type immune responses reduce CVB3 myocarditis and DCM by inhibiting viral replication and preventing Th2 responses
- Th2 responses reduce CVB3 myocarditis by inhibiting inflammation, but can promote progression to DCM by stimulating cardiac remodeling
- Th17 responses promote DCM and cardiac remodeling

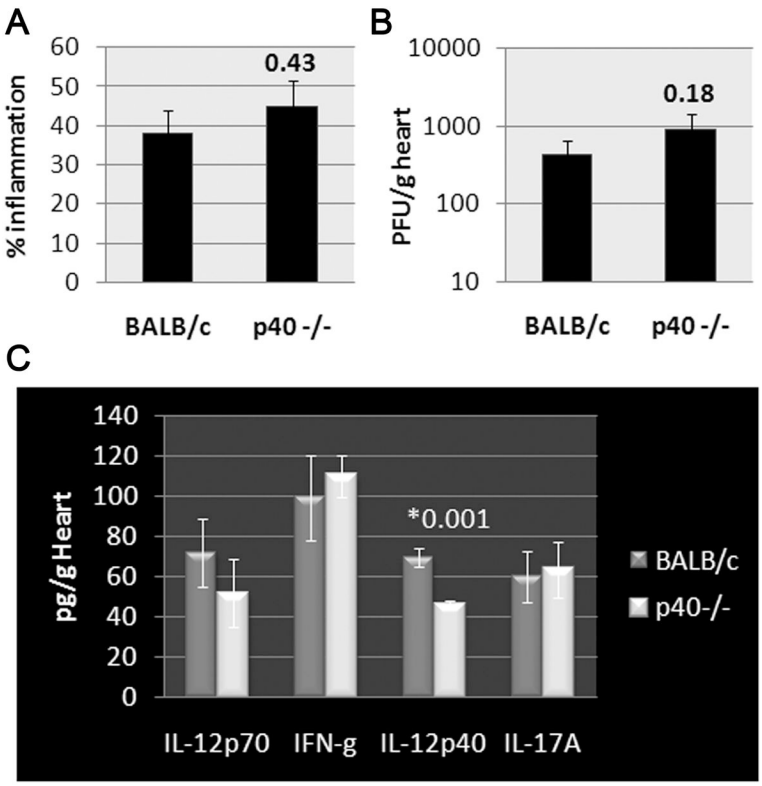

Figure 1. Role of IL-23/p40 in CVB3 myocarditis

Male WT BALB/c and IL-12p40 deficient (p40−/−) mice were inoculated ip with 10^3 PFU of heart-passaged CVB3+heart proteins on day 0 and A) myocarditis, B) viral replication, and C) cytokines assessed at day 10 pi. IL-23/p40 deficiency did not alter myocarditis, viral replication or cytokine levels in the heart, except for reducing IL-12/IL-23p40 levels. Data show the mean ±SEM from one of three experiments using 7–10 mice/group. *P* values were evaluated using the Student's *t* and/or Mann-Whitney rank tests.

Table 1

Animal models of myocarditis[a]

	CVB3-only model	Hybrid-CVB3 model	EAM
Survival	20–30% by day 7 pi	100% to day 90 pi	100% to day 90 pi
Viral replication d7 pi	10^7–10^9 PFU/g heart	10^5 PFU/g heart	0
Acute myocarditis	Peak @ day 7 pi	Peak @ day 10 pi	Peak @ day 21
Severity of myocarditis	5–10% inflammation	30–60% inflammation	30–60% inflammation
Key cell mediators	γδT & $CD8^+$ T cells	Macrophages	Macrophages
DCM	Few survive	Yes	Yes
Sex differences	Males > females	Males > females	Males > females

[a]Abbreviations: CVB3, coxsackievirus B3; DCM, dilated cardiomyopathy; EAM, experimental autoimmune myocarditis; PFU, plaque forming units; pi, post infection or post inoculation;

Table 2

Role of Th immune responses in animal models of myocarditis[a]

	CVB3-only model	Hybrid-CVB3 model	EAM
Role of IFN-α (Th1)	Inhibits viral replication	ND	ND
Role of IFN-β (Th1)	Inhibits viral replication	Inhibits viral replication Prevents DCM	ND
Role of IFN-γ (Th1)	Inhibits viral replication	Inhibits viral replication Prevents DCM	Prevents DCM
Role of IL-4 (Th2)	Prevents myocarditis	Increases DCM	Increases DCM
Role of IL-33 (Th2)	ND	Increases DCM	ND
Role of IL-23 (p40)	Increases myocarditis	No effect on myocarditis	Increases EAM
Role of IL-17 (Th17)	Increases myocarditis	ND	No effect on EAM Increases DCM

[a] Abbreviations: CVB3, coxsackievirus B3; DCM, dilated cardiomyopathy; EAM, experimental autoimmune myocarditis; IL, interleukin; ND, not done; Th, T helper type immune response; IFN, interferon