CVB Infection and Mechanisms of Viral Cardiomyopathy

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Abstract Coxsackievirus infection has been demonstrated to be a cause of acute and fulminant viral myocarditis and has been associated with dilated cardiomyopathy. While considerable attention has focused on the role of the cellular and humoral, antigen-specific immune system in viral myocarditis, the interaction between the virus and the infected host myocyte is also important. Coxsackievirus has a relative tropism for the heart that is in part mediated by relatively high levels of the coxsackievirus and adenovirus receptor (CAR) on the cardiac myocyte. Once within the myocyte, coxsackievirus produces proteases, such as protease 2A, that have an important role in viral replication, but can also affect host cell proteins such as dystrophin. Cleavage of dystrophin may have a role in release of the virus from the myocyte since viral infection is increased in the absence of dystrophin. In addition to the direct effect of viral proteins on cardiac myocytes, there is now evidence that the cardiac myocyte has a potent innate immune defense against coxsackieviral infection. Suppressors of cytokine signaling (SOCS) can inhibit an interferon-independent mechanism within the cardiac myocyte. In summary, the interaction between coxsackievirus and the infected myocyte has a significant role in the pathogenesis of viral myocarditis and the susceptibility to viral infection.

1 Evidence for CVB Infection in Myocarditis

1.1 Coxsackievirus Infection is Associated with Myocarditis and Dilated Cardiomyopathy in Humans

The association of acute myocarditis with coxsackievirus infection in humans was identified as early as the mid-1950s (Dalldorf 1955; Fechner et al. 1963; Kibrick and Benirschke 1958). Reports of isolation of coxsackievirus from the heart or pericardial fluid of patients with acute myocarditis date back to the mid-1960s (Sun 1966; Sun and Smith 1966), with numerous reports since then that have isolated virus from the heart or pericardial fluid or demonstrated the presence of viral proteins in diseased heart tissue (Grist and Bell 1969; Lerner and Wilson 1973; Li et al. 2000; Sainani et al. 1975; Sutinen et al. 1971; Windorfer and Sitzmann 1971; Burch et al. 1968). According to World Health Organization surveys from many different countries, 34.6 per 1,000 of all coxsackie B virus infections are associated with cardiovascular disease (Gerzen et al. 1972).

In addition to the clear association between enteroviral infection and acute myocarditis, it has been shown that dilated cardiomyopathy can also be a sequela of viral myocarditis (For review, see Martino et al. 1994). Attempts to isolate virus from the myocardium of patients with chronic forms of dilated cardiomyopathy have been unsuccessful.

The studies described above indicate that in cases of acute myocarditis, Koch's first postulate (the organism must be regularly found in the lesions of the disease) and second postulate (the organism must be isolated in pure culture) are met. It is

also clear that in a subset of patients with dilated cardiomyopathy, the first postulate has been met, but the second is lacking. These reports establish associations between viral infection and heart disease, but, of themselves, do not establish a clear cause-effect relationship.

1.2 Mouse Models of CVB-Mediated Myocarditis

In 1969, Wilson et al. (Wilson et al. 1969) found that acute infection with CVB3 in weanling Swiss mice was followed by marked fibrosis, and dystrophic mineralization in the heart, and microscopic myocardial hypertrophy, which persisted for at least 6 months. Coxsackieviral infection of mice has, subsequently, been used widely to study the acute effects of viral infection on the myocardium. Following inoculation of mice with coxsackievirus, the virus can be consistently isolated from the heart. Therefore, the murine model of viral myocarditis fulfils Koch's postulates three (inoculation of such a pure culture of organisms into a host should initiate the disease) and four (the organisms must be recovered once again from the lesions of the host) for the acute phase of viral heart disease.

2 Overview of Mechanisms by Which CVB3 Can Cause Myocarditis

2.1 Immune Mechanisms of Myocarditis

One of the more prominent features of coxsackieviral infection of the heart is the marked cellular inflammation that is present at 6-7 days after infection and onward. This has appropriately led to a large number of studies on activation of the humoral and cellular immune response in viral myocarditis. Both antigen-specific and autoimmune mechanisms have been implicated in the immune-mediated effects on the heart. The immune response can be beneficial by limiting viral replication and can also be detrimental if directed against heart antigens or by killing potentially viable cells that are near infected cells. Immune mechanisms involved in viral myocarditis are discussed in considerable detail in the chapter by N.R. Rose, this volume.

2.2 Interactions Between the Cardiac Myocyte and Virus in Myocarditis

In addition to the important host cellular and humoral immune response, attention has been directed to the interaction between the infected cell and the virus. There

are two important considerations that will be emphasized below. First, since the virus is able to infect the cardiac myocyte, it is important to understand mechanisms by which coxsackievirus can directly affect the cardiac myocyte. Second, there are innate, antigen-independent immune defense mechanisms within the cardiac myocyte that can inhibit the pathogenesis of viral myocarditis. When these mechanisms are altered, they can affect susceptibility to viral infection in the heart. The major focus of the mechanisms that will be described in this chapter will be on the earlier stages of viral infection, days 2-5 after inoculation, prior to the onset of a significant cellular immune response.

3 Evidence that Coxsackievirus Can Cause a Direct Myocytopathic Effect

3.1 Coxsackievirus Infects the Heart

3.1.1 Coxsackievirus Infects Cardiac Cells in Culture and in the Infected Rodent Heart

Cell culture experiments have demonstrated that CVB3 can infect human and rodent cardiac cells. For example, coxsackievirus is able to infect human embryonic fibroblasts and myocytes and can establish a carrier-state infection in culture (Kandolf et al. 1985). In addition, coxsackievirus can infect isolated neonatal rat and mouse cardiac myocytes and isolated adult mouse myocytes (Yajima et al. 2006; Yasukawa et al. 2001). In addition to the evidence that coxsackievirus can infect cardiac myocytes and fibroblasts, there is evidence that enteroviruses can also infect endothelial cells (Tracy et al. 2000) and smooth muscle cells in culture (Godeny et al. 1986). These cultured cell data indicate that several cardiac cell types have the potential to be infected by coxsackievirus.

Supporting the infection of myocytes in culture is the identification of CVB3 genome and capsid proteins in cardiac myocytes in the murine model of myocarditis (Badorff et al. 1999, 2000b; Kandolf et al. 1987). It is likely that there are other cells such as fibroblasts or endothelial cells are infected in the intact heart as well (Klingel et al. 1998).

3.1.2 Coxsackievirus Can Infect Cardiac Myocytes in the Human Heart

In addition to these experimental models that demonstrate direct infection of cardiac cells with coxsackievirus, there is considerable evidence that coxsackievirus can directly infect the intact human heart as well as be determined by isolation of virus from the heart and identification of viral genomes in the heart, as described above. However, these experiments do not identify which cell types are infected.

Demonstration of infection of cardiac myocytes in humans has been accomplished using immunohistochemical staining for enteroviral-specific capsid proteins that could be identified within the cardiac myocyte in patients with fulminant and acute myocarditis and occasionally in patients with chronic cardiomyopathy (Kandolf and Hofschneider 1989; Lee et al. 2006; Li et al. 2000). In addition, in situ hybridization for viral RNA in patients with acute myocarditis and in a subset of patients with dilated cardiomyopathy is consistent with this finding since viral genomes can be identified in the cardiac myocyte (Hohenadl et al. 1991; Kandolf et al. 1987). While these data demonstrate that coxsackievirus can infect cardiac myocytes, the exact incidence of enteroviral infection in myocarditis and dilated cardiomyopathy is not clear and likely varies with the overall incidence of coxsackieviral infection in the population.

The capability of coxsackievirus to directly infect cells in the heart suggests the possibility that coxsackievirus could cause a direct cytopathic effect on the cells acutely and perhaps in the setting of a chronic, persistent infection and that this may have a role in the pathogenesis of viral myocarditis.

4 There Is a Cause-Effect Relationship Between Viral Infection in the Heart and Myocyte Injury

4.1 Evidence of Direct Virus-Induced Myocyte Damage in Viral-Mediated Cardiomyopathy

While it is generally accepted that there is myocardial damage in viral myocarditis, it is less clear whether the damage is from activation of the immune response or the direct effects of viral infection. While it is likely that activation of the immune response contributes to the myocyte damage in viral myocarditis, there is also considerable evidence that virus can directly injure infected myocardial cells. For example, extensive cardiac damage has been demonstrated following infection of mice with severe combined immunodeficiency (Chow et al. 1992) and athymic mice (Hashimoto and Komatsu 1978; Hashimoto et al. 1983), where the usual cellular immune response is genetically reduced or absent. In addition, evidence of direct myocyte-mediated myocardial injury was identified histologically in infected cardiac myocytes of immunocompetent mice (McManus et al. 1993). Using Evans blue dye, it was subsequently demonstrated that during early time points after infection, there is clear evidence of myocyte membrane disruption in the infected cells (Badorff et al. 1999; Lee et al. 2000; Yajima et al. 2006). There is a high correlation between the cells that are positive for Evans blue dye and those that are infected with coxsackievirus as determined by immunofluorescent staining for coxsackievirus (Yajima et al. 2006). Disruption of membrane proteins is not limited to infected murine cells, but has also been demonstrated in humans in the setting of fulminant myocarditis (Lee et al. 2006).

4.2 Evidence of Persistence of the Enteroviral Genome

In addition to the experiments described above in acute viral heart disease, evidence suggests that persistence of the viral genome can contribute to the evolution of ongoing heart disease, though there is less direct evidence that low-level expression of viral genomes in the cardiac myocyte can induce a direct myocytopathic effect and cardiomyopathy (Klingel et al. 1992; Kyu et al. 1992). The data obtained from coxsackievirus-infected murine models imply that myocardial damage in CVB3-infected mice can occur in two phases: an acute phase with prominent virus replication and cellular infiltration. Second, there can be a chronic phase characterized by progressive myocardial disease that may be associated with low-level persistence of viral genomes and progressive cardiomyopathy (Klingel et al. 1992; Kyu et al. 1992).

4.3 Low-Level Expression of Coxsackieviral Genome in Cardiac Myocytes Causes Dilated Cardiomyopathy

While there is an association between the presence of enteroviral genomes and cardiomyopathy, it is more difficult to establish a cause-effect relationship between low-level expression of viral genomes and the induction of cardiomyopathy. To accomplish this goal, infectious recombinant coxsackievirus B3 (CVB3) cDNA was mutated at the autocatalytic cleavage site in the VP0 capsid protein from the amino acids asparagine and serine to lysine and alanine, thus preventing formation of infectious virus progeny (Wessely et al. 1998a). This was initially tested in cultured neonatal ventricular myocytes transfected with the CVB3-mutated cDNA copy of the viral genome, and found to induce a cytopathic effect in transfected myocytes (Wessely et al. 1998a).

To determine whether a similar pathogenic effect could also be observed in the intact heart, transgenic mice were generated that expressed the replication-restricted CVB3 cDNA mutant exclusively in the heart, using the cardiac myocyte-specific myosin light chain-2v (MLC-2v) promoter. This allowed for low-level expression of coxsackieviral genomes in the cardiac myocyte without formation of infectious virions, thus preventing a productive viral replication cycle. In addition, the MLC-2v promoter directs expression in the heart at day 8.5 of embryogenesis (Lee et al. 1992), thus preventing activation of a potent immune response against viral antigens (Wessely et al. 1998b). As expected, heart muscle-specific expression of the CVB3 mutant resulted in the synthesis of viral plus- and minus-strand RNA without formation of infectious viral progeny. Histopathologic analysis of transgenic hearts revealed typical morphologic features of myocardial interstitial fibrosis, hypertrophy, and degeneration of myocytes, thus resembling dilated cardiomyopathy in humans. This occurred in the absence of viral neutralizing antibodies. Analysis of isolated myocytes from

transgenic mice demonstrated that there is defective excitation-contraction coupling and a decrease in the magnitude of isolated cell shortening. These findings demonstrate that restricted replication of enteroviral genomes in the heart can induce cardiomyopathy with characteristics that are typical of dilated cardiomyopathy in humans (Wessely et al. 1998b).

5 Significant Steps in Viral Replication and Pathogenesis in the Cardiac Myocyte

The data above demonstrate that coxsackievirus can infect cardiac myocytes and can induce a cytopathic effect. Much of the knowledge about the steps involved in viral replication come from experiments in non-myocytes and is discussed elsewhere in this volume. However, some of the steps are of particular relevance to the heart. These include the following points.

1. The virus attaches to the target cell by binding to its receptor, the coxsackievirus and adenovirus receptor (CAR). CAR is expressed at high levels in the cardiac myocyte.
2. Once the virus enters the cell and the viral RNA is translated, the viral monocistronic polyprotein is cleaved by the coxsackieviral proteases creating the nonstructural proteins required for viral RNA replication and efficient translation of the viral RNA. The viral proteases can also cleave a small subset of host proteins, a process that facilitates viral replication and can cause a direct cytopathic effect.
3. Replication of the virus positive-strand RNA through a negative-strand intermediate is accompanied by formation of double-stranded RNA that is thought to have a role in induction of the host-cell innate immune response.
4. Once the replicated, viral positive-strand viral genome is encapsidated and released from the cell during a process that is associated with disruption of the cell membrane and cleavage of dystrophin by a viral protease, a myocyte-specific protein, the replicated virus is released from the infected cell to allow secondary infection of other cells (Racaniello 2007). Alterations in these processes could affect how efficiently a virus can replicate in the heart and how susceptible the host is to the induction of viral myocarditis.

5.1 Binding and Entry of Virus into the Cardiac Myocyte

The first step of viral infection and replication is attachment of the virus to the target cell. The CAR has been shown to be the major receptor for coxsackievirus. Elegant studies have defined the role for CAR in cells, but less is known regarding its role in the heart (Bergelson et al. 1997, 1998; Carson 2001; Coyne and Bergelson 2006).

One of the likely explanations for the tropism of coxsackievirus for the heart is the relatively high level of expression of the CAR on the cardiac myocyte (Bergelson et al. 1997, 1998; Kashimura et al. 2004). CAR is a membrane adhesion molecule that has two immunoglobulin domains in the extracellular region: a transmembrane domain and an intracellular domain that includes a PDZ-binding motif. CAR is expressed throughout the sarcolemma of the cardiac myocyte during embryonic growth (Kashimura et al. 2004) and is required for normal embryogenesis. This is demonstrated by embryonic lethality in the global cardiac knockout of the CAR gene (Asher et al. 2005; Chen et al. 2006; Dorner et al. 2005). As the heart reaches its fully developed state, CAR localizes predominantly to the intercalated disc where its function is not clearly known. In addition, as the heart ages the level of CAR expression in the heart decreases, but continues to be expressed (Kashimura et al. 2004). CAR expression is increased in heart failure and autoimmune myocarditis (Fechner et al. 2003; Poller et al. 2002). The susceptibility of the heart to viral infection is thought to correlate with the level of CAR expression (Poller et al. 2002).

5.2 Role of Viral Proteases in Viral Replication and Pathogenesis

As described previously, viral proteases have an essential role in cis-cleavage of the monocistronic viral polyprotein. In addition to cleavage of the viral polypeptide, enteroviral proteases can cleave host-cell proteins in trans at highly specific proteolytic sites. The enteroviroviral protease 3C can cleave the TATA-binding protein (Clark et al. 1993), the poly(A)-binding protein (Kuyumcu-Martinez et al. 2002), and the poly(ADP-ribose) polymerase (PARP), a nuclear protein involved in DNA repair and apoptosis (Barco et al. 2000). Enteroviral protease 2A has been shown to cleave host proteins involved in translation, such as eukaryotic initiation factors 4G (eIF4G) -1 (Lamphear et al. 1993) and −2 (Gradi et al. 1998), and the polyadenylate binding protein (PABP) (Kerekatte et al. 1999). In addition, it was discovered that protease 2A could cleave the cytoskeletal proteins dystrophin (Badorff et al. 1999) and cytokeratin-8 (Seipelt et al. 2000), a process that facilitates release of virus from the cell (Xiong et al. 2002) (see below). Both eIF4G-1 and −2 are part of the translation initiation complex that is required for efficient translation of host-cell-capped mRNA, which includes the majority of eukaryotic mRNA. Enteroviral-mediated cleavage of eIF4G results in inhibition of host-cell, cap-dependent translation in favor of viral, IRES-mediated protein synthesis (Lamphear et al. 1995). It is possible that cleavage of eIF4G has a cytopathic effect on the cardiac myocyte since inhibition of its cleavage in HeLa cells inhibits the coxsackievirus-mediated cytopathic effect (Zhao et al. 2003). However, the importance of cleavage of eIF4G in cardiac myocytes has not yet been thoroughly evaluated. The role of protease 2A in the cardiac myocyte and the importance of the dystrophin-glycoprotein complex in viral heart disease will be discussed in more detail in the following section.

5.3 Dystrophin-Glycoprotein Complex, Protease 2A, and Sarcolemmal Membrane Stability

5.3.1 Dystrophin-Glycoprotein Complex in Cardiomyopathy

The dystrophin-glycoprotein complex is important for maintaining myocyte sarcolemmal membrane integrity. Dystrophin is a subsarcolemmal rod-shaped protein that stabilizes the sarcolemma by attaching the actin-cytoskeleton to the extracellular matrix through the dystrophin-associated glycoprotein complex (Durbeej and Campbell 2002). Abnormalities in dystrophin cause Duchenne and Becker muscular dystrophy. While the most visible phenotype of these muscular dystrophies is a severe skeletal myopathy, most patients develop a dilated cardiomyopathy by the early to mid teens that is thought to contribute to their short life span (Towbin 1998). If dystrophin is expressed at relatively normal levels in the skeletal muscle but is decreased in the cardiac muscle, the patients develop a cardiomyopathy without a significant skeletal myopathy. This occurs in patients with X-linked dilated cardiomyopathy that is associated with an inherited abnormality in dystrophin (Muntoni et al. 1993; Towbin et al. 1993). Abnormalities in other members of the dystrophin-glycoprotein complex can also cause dilated cardiomyopathy (Towbin 1998; Tsubata et al. 2000; van der Kooi et al. 1998).

5.3.2 Coxsackievirus B3-Derived Protease 2A Directly Cleaves Dystrophin

Given the importance of the dystrophin-glycoprotein complex in hereditary cardiomyopathy, the effect of coxsackievirus infection on the dystrophin-glycoprotein complex was assessed. A neural network algorithm that allowed identification of potential enteroviral protease 2A cleavage sites identified two putative cleavage sites within the dystrophin molecule (see http://www.cbs.dtu.dk/services/NetPicoRNA/). One of the putative protease 2A cleavage sites is in the hinge-3 region of the murine dystrophin molecule, a region previously shown to be accessible to proteases (Blom et al. 1996; Koenig and Kunkel 1990). There is evidence of a similar putative cleavage site in human dystrophin.

The hinge-3 region of dystrophin lies between the actin-binding sites and the β-dystroglycan anchoring motif of dystrophin (Koenig and Kunkel 1990). Cleavage at this site would be predicted to disconnect the (actin-binding) N-terminal region from the sarcolemma where the C-terminal fragment binds β-dystroglycan.

When purified coxsackieviral protease 2A was added to protein extracts from neonatal rat cardiac myocytes or adult human heart, there was time-dependent cleavage of dystrophin of both rat and human dystrophin, but the cleavage of human dystrophin appeared to be less efficient than that of rodent dystrophin (Badorff et al. 1999). It was later demonstrated that the cleavage of dystrophin occurred through a direct cleavage of the dystrophin protein by enteroviral protease

2A rather than through secondary activation of another protease and that cleavage occurred at the predicted protease 2A-cleavage site in the hinge-3 region of dystrophin (Badorff et al. 2000a).

5.3.3 Disruption of Dystrophin, the Dystrophin-Glycoprotein Complex, and Sarcolemmal Membrane Integrity in the CVB-Infected Heart

In addition to the ability of protease 2A to cleave dystrophin in vitro, dystrophin is also proteolytically cleaved and its sarcolemmal localization is disrupted in the intact heart of mice that are infected with CVB3. Additionally, the sarcolemmal integrity in these cells is impaired, as determined by Evans blue dye tracer uptake, in a manner similar to that observed in muscular dystrophy (Badorff et al. 1999). Since genetic dystrophin deficiency leads to a loss of dystrophin-associated glycoproteins such as the sarcoglycans (Matsumura et al. 1993), it was shown that dystrophin cleavage during coxsackievirus B3 infection similarly affects the sarcoglycans (Lee et al. 2000).

In analogy to findings from patients with dilated cardiomyopathy due to Duchenne muscular dystrophy, it is possible that the cleavage of dystrophin with its subsequent biochemical, morphological, and functional disruption of the dystrophin-glycoprotein complex during coxsackievirus B3 infection initiates a cascade of events that contributes to the pathogenesis of dilated cardiomyopathy (Badorff et al. 1999) (Fig. 1). In addition, it is also possible that cleavage of dystrophin is an important step in viral replication, facilitating viral release from the infected cell by weakening the cell membrane and allowing infection of the adjacent cardiac myocytes.

5.3.4 Dystrophin Deficiency Increases Susceptibility to Virus-Mediated Cardiomyopathy Via an Increase in Membrane Permeability

In the experiments described earlier, it was demonstrated that CVB protease 2A increases cell membrane permeability as assessed by Evans blue dye staining. This is associated with cleavage of dystrophin. Therefore, it was hypothesized that dystrophin deficiency might increase susceptibility to viral infection by decreasing myocyte membrane stability, allowing the virus to exit from the cell more efficiently and infecting adjacent cells. In CVB3-infected, dystrophin-deficient, mdx mice there was significantly more Evans blue dye uptake when compared to the infected, dystrophin-competent mice. In addition, there was also a nearly 100-fold increase of virus titer in the infected, dystrophin-deficient hearts when compared to the infected, dystrophin-competent, wild type mice (Xiong et al. 2002) (Fig. 2).

When cardiac sections were stained for coxsackievirus, in the wild type mice there was bright staining for coxsackievirus in the rare Evans blue dye-positive cells. In the hearts of dystrophin-deficient mice infected with coxsackievirus, however, the majority of Evans blue dye-positive cells contained only remnants of viral

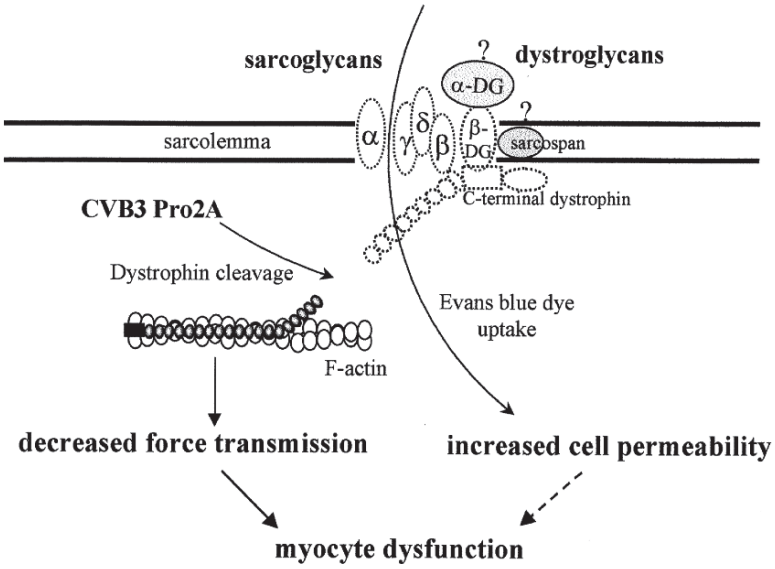

Fig. 1 Schematic of the pathogenic role of the sarcoglycan complex in the acquired enteroviral cardiomyopathy that demonstrates the loss of the carboxyl-terminal dystrophin, β-dystroglycan (DG), and α-, β-, γ-, and δ-sarcoglycans in the sarcolemmal membrane (From Lee et al. 2000)

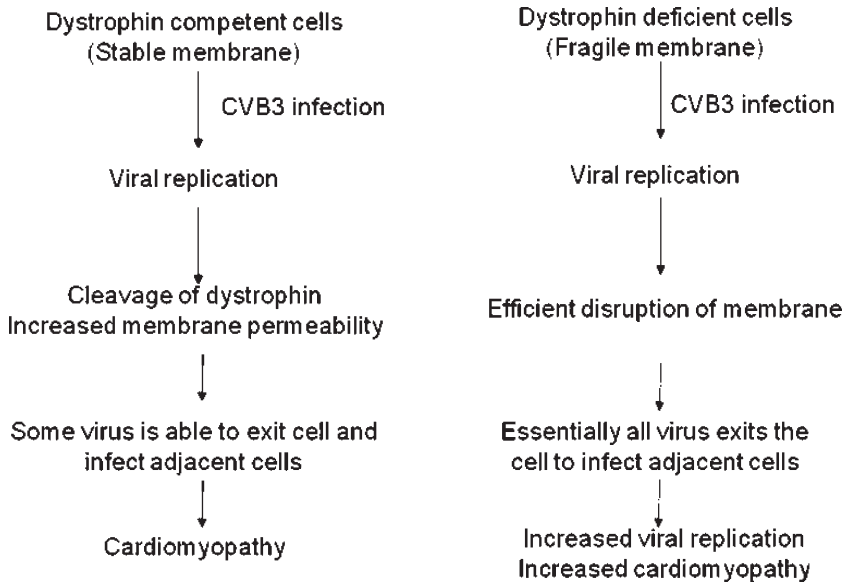

Fig. 2 Summary of mechanisms by which dystrophin deficiency induces more cardiomyopathy compared to dystrophin-competent myocytes

proteins. Membrane disruption was rarely observed in uninfected mdx mice. This suggested that the absence of dystrophin increases membrane permeability and release of virus from the myocyte. A similar phenomenon was observed in cultured cells where dystrophin expression decreased release of virus from the cells (Xiong et al. 2002).

Enteroviruses are typically released from the cell by disruption of the cell membrane or cell lysis. Because dystrophin provides mechanical support to the sarcolemma, and the absence of dystrophin results in increased susceptibility to sarcomere rupture, the virus exits from the infected cell more efficiently in the absence of dystrophin. This allows more rapid propagation of the virus to adjacent myocytes, resulting in higher viral titers and greater cardiomyopathy. In the presence of dystrophin, the rate and extent of viral release may be slowed by the requirement that protease 2A cleave dystrophin or that there are other viral-mediated mechanisms that lead to membrane disruption. This would result in lower viral titers than are seen in the absence of dystrophin. While there are undoubtedly several mechanisms by which dystrophin deficiency causes cardiomyopathy in individuals with Duchenne muscular dystrophy or X-linked dilated cardiomyopathy, the data in the dystrophin-deficient mouse hearts support the notion that the absence of dystrophin in patients with muscular dystrophies involving the dystrophin-glycoprotein complex increases the susceptibility of such individuals to virally mediated cardiomyopathy and that this could contribute to the development of cardiomyopathy in these patients. The increased susceptibility may be broadly applicable to viruses that are capable of infecting cardiac myocytes and are released from the cell by disruption of the cell membrane. It remains to be seen whether people that develop myocarditis with coxsackieviral infection have genetic mutations in the dystrophin-glycoprotein complex proteins that contribute to an increase in cell membrane permeability and thus an increase in susceptibility to viral infection.

5.3.5 Viral Protease 2A Alone in the Heart is Sufficient to Induce Cardiomyopathy

The data described in the preceding section demonstrate that coxsackieviral infection of cardiac myocytes can cause a direct myocytopathic effect. Furthermore, expression of a full-length, replication-defective coxsackieviral genome in the cardiac myocyte is able to cause a cardiomyopathy that appears to be largely independent of the cellular immune response. However, until recently, it was not clear whether expression of coxsackieviral protease 2A alone was sufficient to induce a dilated cardiomyopathy independent of the other viral proteins and viral RNA. Therefore, a transgenic mouse was generated that expressed protease 2A only in the adult cardiac myocyte. Furthermore, the transgenic expression construct was designed to only express protease 2A in an inducible manner following administration of tamoxifen (Xiong et al. 2007).

Within 22 days after induction of protease 2A in the adult heart, the mice developed severe cardiomyopathy. In addition, there was an increase in the number of

Evans blue dye-positive myocytes in the hearts of the mice that expressed protease 2A. This was associated with disruption of dystrophin localization to the sarcolemma in a subset of myocytes. A similar Evans blue dye staining was not observed in mice that had cardiomyopathy due to absence of the muscle LIM protein (MLP), a different model of cardiomyopathy (Xiong et al. 2007). This study demonstrated that the presence of coxsackieviral protease 2A in the cardiac myocyte in the absence of other viral proteins or RNA is sufficient to induce cardiomyopathy with disruption of the sarcolemmal membrane and loss of localization of dystrophin in the intact heart. These findings, however, do not exclude the possibility that protease 2A may also affect other myocyte proteins in addition to dystrophin and may also contribute to the development of cardiomyopathy. Nevertheless, the findings make a strong case for the potential for protease inhibitors in the treatment of coxsackieviral-mediated cardiomyopathy. Protease inhibitors would likely inhibit protease-mediated cytopathic effects in the cardiac myocyte and also inhibit viral replication. A peptide based on the protease 2A dystrophin-cleavage site has been shown to be able to inhibit protease 2A (Badorff et al. 2000a).

6 Innate Immune Response Within the Heart and Cardiac Myocyte

Host defense mechanisms can be divided into two broad categories: adaptive and innate. Adaptive immune responses to coxsackievirus infection are discussed in the chapter by N.R. Rose, this volume. Although more effective, the onset of the adaptive immune response is slow (several days). The innate immune response includes the activation of natural killer cells and the production of cytokines. Interruption of the innate immune response in all cells, including circulating cells, can affect susceptibility to coxsackieviral infection. For example, global knockout of myeloid differentiation factor-88 (MyD88) decreases susceptibility to coxsackieviral myocarditis (Fuse et al. 2005). Recently, it has been demonstrated that the cardiac myocyte has innate defense mechanisms and that there are some unique characteristics compared to circulating immune cells and other tissues such as the liver. Alteration of these cardiac myocyte innate defense mechanisms can have a profound effect on the host susceptibility to coxsackieviral infection.

6.1 Endogenous Interferon Has Little Direct Effect on Early Viral Infection of the Heart

Interferons were the first identified cytokines that play a central role in host defense against invasive viruses (Isaacs and Lindemann 1957). Interferons of α and β subtype are referred to as type I interferons. The only type II interferon is interferon-γ.

Interferons exert their effect by binding to specific receptors in the cell membrane that subsequently activate intracellular signaling through Janus kinase (JAK) and signal transducers and activators of transcription (STAT) signaling. It has been shown that both type I and type II interferons can inhibit CVB replication in cultured cells and administration of interferon-α/β can ameliorate CVB-induced myocarditis in mice (Fairweather et al. 2004; Karupiah et al. 1993; Matsumori et al. 1988; Wang et al. 2007). In order to determine the effect of interferon receptor-mediated signaling on CVB replication in the heart, mice lacking either type I or type II receptor were infected with CVB3. CVB3 infection in type I receptor-deficient mice lead to a marked increase in viral replication in the liver and a marked increase in mortality in CVB3-infected mice. However, there was no significant increase of viral RNA in the heart of the type I interferon receptor-deficient mice (Fig. 3). These findings show that the presence of type I interferon receptor signaling is required to prevent high-level viral replication in noncardiac organs such as the liver, but that there is no significant effect on early viral replication in the heart of mice infected with CVB3. In contrast, the absence of the type II interferon signaling did not have a significant effect on mortality and resulted in only a mild increase of the viral titers in heart and liver (Wessely et al. 2001). These results demonstrate that endogenous type I interferons have little effect on viral replication in the heart, but it does not exclude the possibility that exogenous administration of interferon could have a beneficial effect on the heart and other organs (Wang et al. 2007).

6.2 JAK-STAT and SOCS Signaling Within the Cardiac Myocyte and Their Role in Susceptibility to Coxsackieviral Infection

A family of transmembrane receptors have been identified that transduce their signals to the nucleus via activation of the JAK/STAT pathway. These receptors include the interferon (interferon) receptors, gp130-associated receptors such as the receptors for leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), interleukin-6 (IL-6), and the erythropoietin receptor. Ligand binding leads to phosphorylation of JAK1 and JAK2 (Muller et al. 1994). Activated JAKs then phosphorylate the intracellular region of the receptor molecules, which then allow phosphorylation of STAT1 or STAT3 (Greenlund et al. 1994; Heim et al. 1995). Activated STAT1 or STAT3 dimers translocate to the nucleus, where they stimulate expression of a panel of genes. The suppressor of cytokine signaling (SOCS) proteins, also known as JAB (JAK binding protein) and SSI (STAT inducible STAT inhibitor) are upregulated when JAK-signaling is activated.

Upregulation of the SOCS family of proteins has been shown to be a counter-regulatory mechanism (Nicholson et al. 1999). SOCS-mediated counter-regulatory mechanisms can inhibit overactivation of the cytokine signaling cascade (Alexander et al. 1999; Endo et al. 1997; Marine et al. 1999; Naka et al. 1997). SOCS1 is essential for inhibition of interferon signaling, but it can also inhibit most JAK-mediated signaling cascades. Alternatively, endogenous SOCS3 was originally

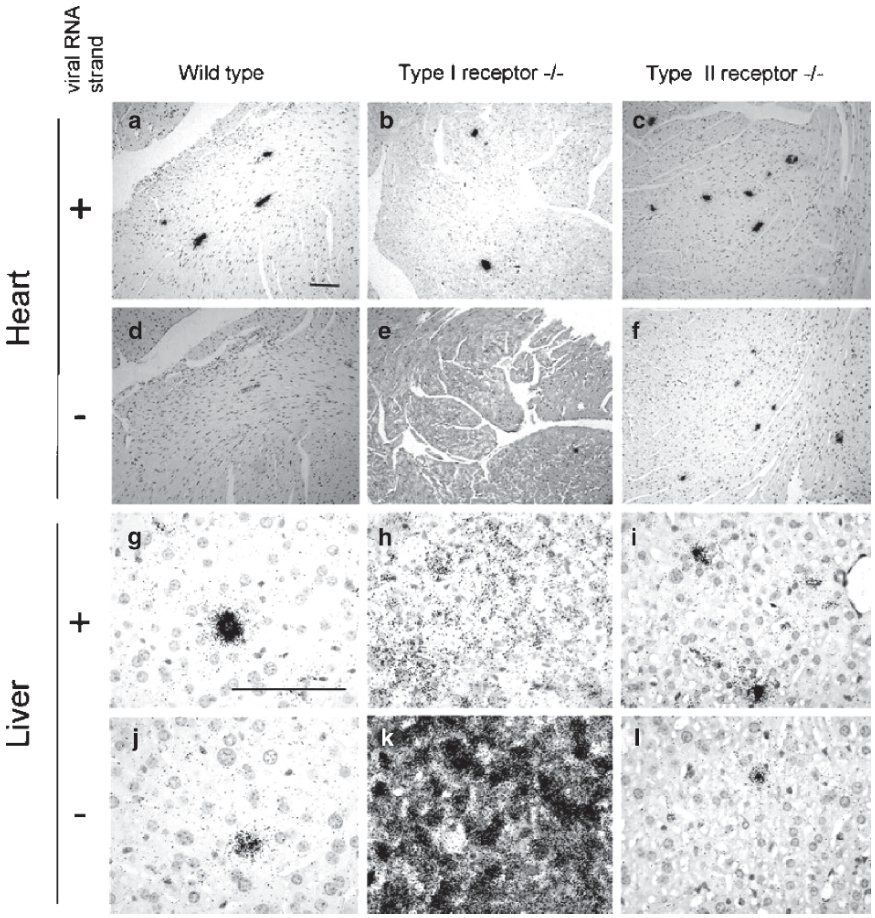

Fig. 3 In situ hybridization in the heart and liver for coxsackievirus-infected wild type, type I interferon receptor deficient, and type II interferon receptor deficient mice 3 days after inoculation. RNA was probed for both positive-strand (+) and negative-strand (-) viral RNA

thought to act primarily as a negative regulator of gp130 signaling. While it has been implicated in other JAK-STAT signaling cascades, it has little or no effect on type I or type II interferon signaling.

6.3 gp130 Signaling in the Heart

gp130 signaling has been shown to have a role in cardiomyocyte survival and induction of ventricular hypertrophy with pressure overload (Hirota et al. 1999). Its stimulation activates STAT3 and SOCS3 (Hirota et al. 1999; Yasukawa et al. 2003).

6.4 Role of SOCS in Viral Myocarditis

Given the potential importance of JAK-STAT signaling in the pathogenesis of coxsackieviral myocarditis, transgenic mice that expressed SOCS1 only in the heart were infected with CVB3. Consistent with the fact that SOCS1 inhibits JAK signaling stimulated by a variety of cytokines (Naka et al. 1997; Yasukawa et al. 2000), both STAT1 and STAT3 activation and induction of interferon-responsive genes by CVB3 infection were totally inhibited in the SOCS1 transgenic mouse hearts, indicating that SOCS1 transgenic mice are resistant to stimulation by interferons and gp130-activating cytokines. In addition, CVB3-infected SOCS1 transgenic mice had significantly earlier mortality when compared with their wild type littermates. This was associated with a marked increase in myocyte damage as assessed by an increase in the percent area of myocardial injury than that observed in wild type littermates (Fig. 4). The virus titer in the heart in SOCS1 transgenic mice on the 4th and 5th days after CVB3 infection was also nearly 100-fold greater when compared to the wild type littermates at day 7 after infection. The viral titer in the liver was not elevated in the transgenic mice, showing the cardiac specificity for the effect (Yasukawa et al. 2003). These results demonstrate that there is a potent innate defense mechanism that is within the cardiac myocyte that is inhibited by SOCS expression.

Since SOCS3 inhibits gp130 signaling but has no significant effect on interferon signaling and interferon antiviral effects in the cardiac myocyte, transgenic mice that expressed SOCS3 only in the cardiac myocyte were generated and infected

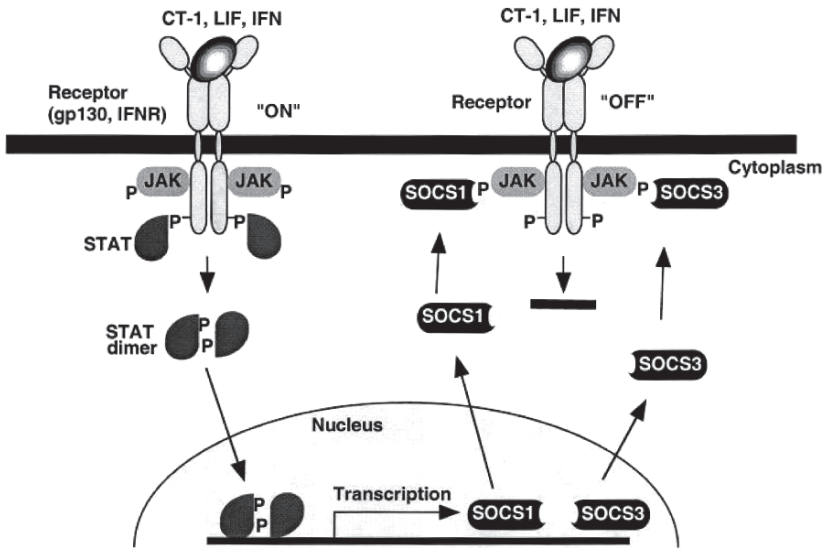

Fig. 4 Schematic of JAK-STAT signaling and negative feedback regulation by suppressors of cytokine signaling (SOCS)-1 and SOCS3

with CVB3 and found to have essentially the same marked increase in CVB3-mediated cytopathic effects that were observed with SOCS1 expression (Yajima et al. 2006). Consistent with the fact that SOCS3 did not inhibit interferon effects in the myocyte but inhibited gp130-signaling and inhibited a potent cardiotrophin-1-mediated cytoprotective effect against CVB3 infection, it was subsequently shown that at least part of the increased susceptibility to coxsackieviral infection seen with SOCS expression could be replicated by cardiac-specific knockout of gp130, indicating that at least part of the increase in susceptibility in the SOCS3 transgenic mice could be accounted for by inhibition of gp130.

Knockout of interferon receptors in the CVB3 infected mouse had little or no effect on replication of virus in the heart (Wessely et al. 2001), and SOCS3 expression could not inhibit interferon signaling and antiviral effects but markedly increased susceptibility to coxsackieviral infection, both of which strengthen the argument that endogenously produced interferon has little or no antiviral effect in the cardiac myocyte during the early phases of viral replication. Nevertheless, there is a potent innate defense mechanism within the adult cardiac myocyte that is inhibited by SOCS1 and SOCS3. Additional work is ongoing to identify the predominant pathway or pathways that are inhibited by SOCS3. Immunoblots demonstrate that inhibition of gp130 signaling by knockout of the gp130 molecule or the expression of SOCS3 leads to a decrease in the amount of dystrophin-glycoprotein complex proteins that are expressed in the heart (Yajima et al. 2006), thus associating the gp130 signaling cascade with the importance of the dystrophin-glycoprotein complex in the pathogenesis of coxsackieviral mediated myocarditis and cardiomyopathy.

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