Cardiovascular Research (2010) **85**, 347–356
doi:10.1093/cvr/cvp225

SPOTLIGHT REVIEW

Protein degradation systems in viral myocarditis leading to dilated cardiomyopathy

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Received 2 May 2009; revised 9 June 2009; accepted 25 June 2009; online publish-ahead-of-print 3 July 2009

Time for primary review: 25 days

The primary intracellular protein degradation systems, including the ubiquitin-proteasome and the lysosome pathways, have been emerging as central regulators of viral infectivity, inflammation, and viral pathogenicity. Viral myocarditis is an inflammatory disease of the myocardium caused by virus infection in the heart. The disease progression of viral myocarditis occurs in three distinct stages: acute viral infection, immune cell infiltration, and cardiac remodelling. Growing evidence suggests a crucial role for host proteolytic machineries in the regulation of the pathogenesis and progression of viral myocarditis in all three stages. Cardiotropic viruses evolve different strategies to subvert host protein degradation systems to achieve successful viral replication. In addition, these proteolytic systems play important roles in the activation of innate and adaptive immune responses during viral infection. Recent evidence also suggests a key role for the ubiquitin-proteasome and lysosome systems as the primary effectors of protein quality control in the regulation of cardiac remodelling. This review summarizes the recent advances in understanding the direct interaction between cardiotropic viruses and host proteolytic systems, with an emphasis on coxsackievirus B3, one of the primary aetiological agents causing viral myocarditis, and highlights possible roles of the host degradation systems in the pathogenesis of viral myocarditis and its progression to dilated cardiomyopathy.

Keywords Viral myocarditis • Dilated cardiomyopathy • Coxsackievirus • Proteasome • Immunoproteasome • Autophagy • Autophagosome • Proteasome activators

This article is part of the Spotlight Issue on: The Role of the Ubiquitin-Proteasome Pathway in Cardiovascular Disease

1. Introduction

Intracellular mechanisms of protein degradation play a vital role in many physiological cellular processes, including disposal of damaged, misfolded proteins, and unwanted components, post-translational protein modification, degradation of extraneous intracellular matter to basic amino acids for re-use, and also the maintenance of many homeostatic functions.[1–4] Alongside the evolutionary development of this vital cellular processes, certain viruses have developed mechanisms which usurp the functions or mechanisms of protein degradation to prevent viral clearance or facilitate their own replication.[5–8] The balance of host anti-viral responses and virus-mediated pro-viral mechanisms using host machinery determines the outcome of infection.

Viral myocarditis is the inflammation of the myocardium caused by virus infection, and may be associated with acute heart failure and the development of dilated cardiomyopathy (DCM)—a major cause of morbidity and mortality worldwide.[9–11] Recent *in vitro* and *in vivo* findings suggest that host proteolytic systems play key roles in controlling viral infectivity, immunity, and pathogenesis during the onset and progression of viral myocarditis.[12–17] This review will cover the role of the primary intracellular protein degradation pathways, the ubiquitin-proteasome system (UPS) and autophagy, in regulating the pathogenesis of viral myocarditis leading to DCM.

2. Intracellular protein degradation pathways

2.1 The UPS

The UPS catalyses the rapid degradation of abnormal proteins and short-lived regulatory proteins controlling a variety of fundamental cellular processes.[3,4] In addition, the functional significance of the

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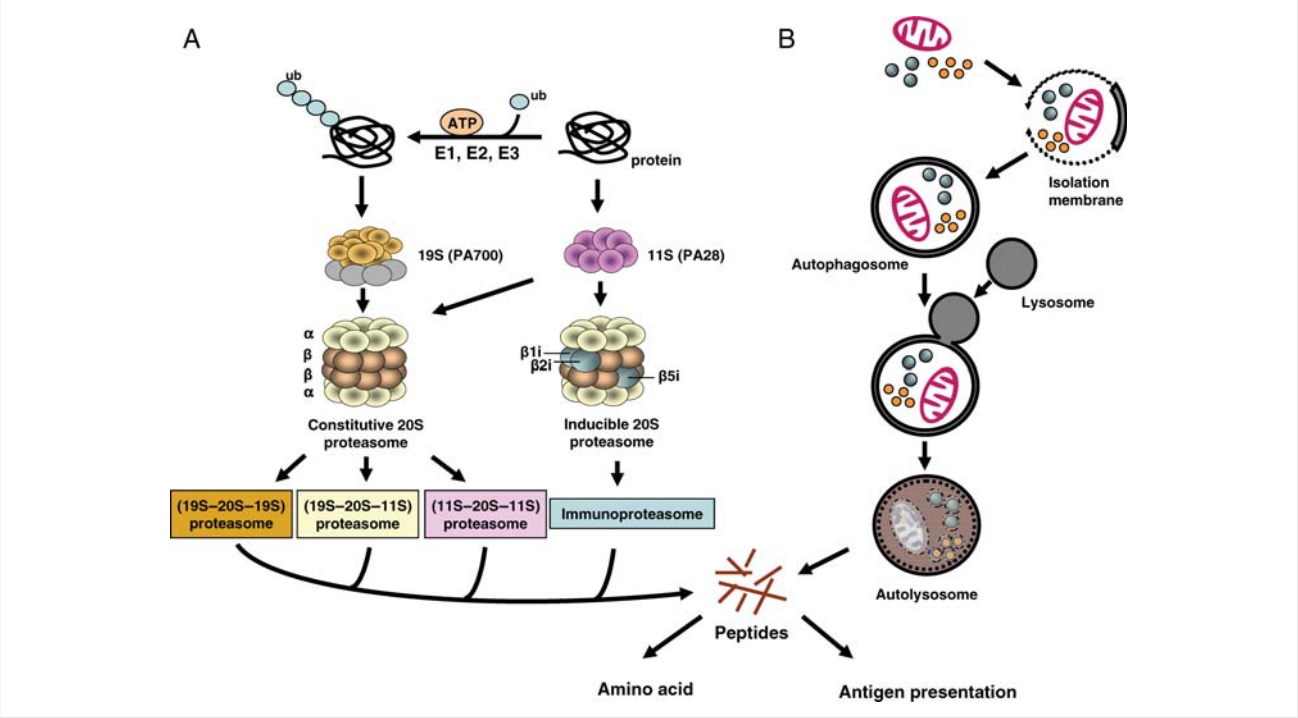

Figure 1 Primary intracellular protein degradation systems in eukaryotes. (*A*) The ubiquitin-proteasome system. The 20S proteasome is latent and present in cells in two forms, constitutive proteasome and immunoproteasome, which differ in the composition of three β-catalytic subunits. There are at least two classes of proteasome activators. The 19S (PA700) activator binds to the constitutive 20S proteasome to form the 26S (19S–20S) or 30S (19S–20S–19S) proteasome, which is primarily responsible for the degradation of ubiquitinated proteins in an ATP-dependent manner. Proteasome activator 11S (PA28) binds to either the constitutive or the immunoproteasome and facilitates ATP- and ubiquitin-independent protein degradation. In addition, 19S can also form a hybrid proteasome (19S–20S–11S) with 20S proteasome and 11S, which enhances the proteolytic efficiency of antigen processing. For ubiquitin (ub)-dependent proteolysis, substrates are first covalently attached to multiple ubiquitin moieties via the action of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) in an ATP-dependent manner. (*B*) The autophagy pathway. A portion of the cytoplasm including organelles is sequestrated by the autophagic isolation membrane, which results in the formation of an autophagosome. The outer membrane of the autophagosome eventually fuses with the lysosome to degrade its contents.

UPS in the regulation of viral infectivity, inflammation and immunity, and viral pathogenicity has been increasingly recognized.[6,7,18–20] Impairment of this system has been implicated in the pathogenesis of various diseases, including cancer, inflammatory, neurodegenerative, and cardiovascular diseases.[21–23]

The 20S proteasome is a large multicatalytic protease, consisting of two outer (α-subunits) and two inner (β-subunits) rings (*Figure 1A*). In cells, the 20S proteasome is latent and requires activation for its proteolytic function. At least two classes of proteasome activators have been identified to bind to the 20S core and enhance its catalytic function.[24] As illustrated in *Figure 1A*, the 19S (or PA700) activator attaches to the outer α-rings of the 20S proteasome to form the 26S (19S-20S) or 30S (19S-20S-19S) proteasome, which is primarily responsible for the degradation of ubiquitinated proteins.[25,26] The majority of intracellular proteins are degraded via the 26S proteasome after polyubiquitination. Alternatively, proteasome activator 11S (or PA28, REG) binds to 20S core and initiates the proteasomal degradation in an ATP- and ubiquitin-independent manner.[27]

Among the three isoforms of PA28, PA28α and PA28β form heteroheptamers that are constitutively expressed and widely distributed in various tissues and its expression can be induced by interferon-γ (IFN-γ), whereas PA28γ forms homoheptamers whose expression is not responsive to IFN-γ.[24] The best-known function of the PA28α/β complex is to process antigens for major histocompatibility complex (MHC) class I presentation via attaching with and activating both 20S constitutive proteasome and 20S immunoproteasome.[28,29] Immunoproteasomes have proteolytic specificity distinct from constitutive proteasomes, through the presence of three unique, IFN-γ-inducible β subunits: β1i (LMP2); β2i (MECL-1); and β5i (LMP7). The biological functions of PA28γ have not been fully characterized. Recent studies support a role of PA28γ in the regulation of apoptosis, cell cycle progression, and viral pathogenesis.[27] However, identified intracellular protein substrates for this pathway are limited.

During ubiquitin-dependent protein degradation, substrates are first covalently attached to multiple ubiquitin moieties through three sequential enzymatic reactions catalysed by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) in an ATP-dependent manner.[3,4] Then the polyubiquitinated substrate containing at least four ubiquitin molecules is

quickly recruited to the proteasome for degradation, and ubiquitin is recycled by deubiquitinating enzymes (DUBs).[3,4]

Independent of proteasomal degradation, protein ubiquitination, including monoubiquitination and, in some cases, polyubiquitination (e.g. lysine 63-linked polyubiquitination), has emerged as one of the most common protein modification mechanisms regulating protein function, such as endocytosis, gene regulation, chromatin remodelling and virus budding without targeting it for degradation.[30,31]

2.2 Autophagy

Another major intracellular pathway is autophagy which primarily disposes of long-lived cytoplasmic proteins and damaged organelles.[1,2,32] Autophagy is a general degradation process that involves the sequestration of regions of the cytoplasm by double-membrane vesicles, termed autophagosomes. They are derived from the elongation of crescent-shaped double-membrane structures known as the isolation membranes. This sequestration is followed by fusion with a lysosome and enzymatic digestion of the sequestered materials (*Figure 1B*). Recent studies have indicated that autophagy not only participates in the maintenance of cellular homeostasis, but also plays a crucial role in both innate and adaptive immune responses in response to environmental stress.[33,34] In addition, increasing evidence suggests that autophagy, the host defense machinery, can be subverted by some pathogens to facilitate their own replication.[8,35] Abnormalities in autophagy have been associated with cancer, infectious, and cardiovascular diseases.[36–38]

Autophagy is a highly conserved cellular mechanism in eukaryotes. The formation and maturation of the autophagosome requires the activation of two ubiquitin-like molecules, the microtubule-associated protein light-chain (LC3) or autophagy-related homolog 8 (ATG8) in yeast, and ATG12, by the activating enzyme ATG7.[2,32] This process is tightly regulated by various kinases, phosphatases, and small GTPases.[2]

The interaction between the UPS and the autophagy has been increasingly recognized. Recent evidence suggests that autophagy may play a compensatory role when the UPS function is damaged, and vice versa.[39] Inhibition of the UPS pathway has been shown to activate autophagy-mediated protein degradation,[40] and suppression of autophagy leading to the accumulation of ubiquitinated proteins in the cytosol.[41]

3. Viral myocarditis leading to the development of DCM

3.1 Myocarditis and its primary causative viral agents

Myocarditis is a non-ischaemic inflammatory disease of the myocardium, most often caused by a virus infection, with 5–15% of patients who have a viral infection developing myocarditis some time during the course of their illness.[9–11] Endomyocardial biopsy examination demonstrates that up to 60% of patients with myocarditis and DCM are virus-positive in the heart.[42–44] Numerous viruses have been associated with myocarditis and DCM, which include enteroviruses, adenoviruses, influenza viruses, human immunodeficiency virus-1 (HIV-1), parvovirus, cytomegalovirus, herpesviruses, and hepatitis C virus.[45] Among them, the most prevalent and extensively studied viruses are the enteroviruses, in particular coxsackievirus type B3 (CVB3).[45]

It is estimated that about 10–20% of patients with histological evidence of myocarditis will develop chronic disease eventually progression to DCM, a common cause of heart failure.[46] In North America, viral myocarditis and its sequela DCM account for approximately 20% of heart failure and sudden death in children and youth.[46,47] Despite much research and a large prospective clinical trial with immunosuppressive treatments, current management of active myocarditis is still mainly based on supportive therapy for systolic dysfunction.[48]

CVB3, an enterovirus in the *Picornaviridae* family, is a non-enveloped virus that contains a single-strand positive polarity RNA genome.[10] Identified coxsackievirus receptors include the coxsackievirus and adenovirus receptor and the decay accelerating factor co-receptor. Recent studies have suggested that the viral cardiotropism is due, at least in part, to a preferential accumulation of the coxsackievirus and adenovirus receptors on cardiomyocytes.[49] Following entry into the cell via these receptors, the positive-strand RNA directs synthesis of a polyprotein via a cap-independent, internal ribosome entry site (IRES)-mediated mechanism.[10] This polyprotein is subsequently processed into individual structural (VP1, VP2, VP3, and VP4) and non-structural (2A, 2B, 2C, 3A, 3B, 3C, and 3D) proteins following cleavage by viral protease 2A and 3C.[10] Viral RNA-dependent RNA polymerase 3D ($3D^{pol}$) then synthesizes negative-strand viral RNA intermediate that serves as a template for transcription of multiple progeny genomes (*Figure 2A*). In addition to polyprotein cleavage, viral proteases are also known to cleave multiple host proteins essential for the maintenance of cellular architecture, protein translation, transcription, and cell signalling.[10,50,51]

3.2 Pathogenesis of viral myocarditis: a tri-phasic disease

It is generally accepted that viral myocarditis is a triphasic disease that occurs in three distinct stages: acute viral infection, inflammatory cell infiltration, and myocardial remodelling.[52] Acute viremic stage is characterized by early cardiomyocyte damage associated with prominent viral replication in the absence of significant host immune responses. Accumulating evidence has demonstrated that virus can directly injure infected cardiomyocytes, contributing significantly to the pathogenesis of viral myocarditis.[10] *In situ* hybridization of viral RNA demonstrates a co-localization of viral genome with the damaged cardiomyocytes.[53] Recently, viral proteases are recognized as an important pathogenic mechanism. Cardiac-restricted overexpression of enteroviral protease 2A is sufficient to induce cardiomyopathy, probably through the cleavage of dystrophin, resulting in the detachment of the cardiomyocyte cytoskeleton from the external basement membrane, thereby disrupting myocyte integrity which not only reduces myocyte contractility, but also induces cell death.[50,51]

The second stage of infection is featured by inflammatory cell infiltration which results in further damage to the myocardium.

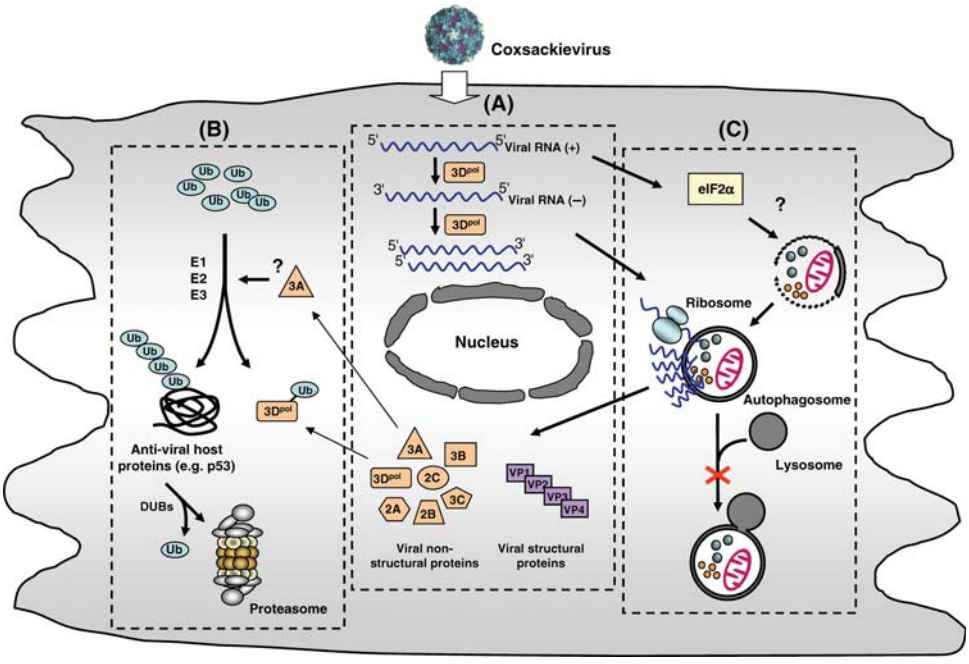

Figure 2 Interaction between coxsackievirus and the host proteolytic systems. (*A*) Following entry into the cell, the positive-strand coxsackieviral RNA directs synthesis of a polyprotein via the host translational machinery. This polyprotein is subsequently processed into individual structural (VP1–VP4) and non-structural (2A, 2B, 2C, 3A, 3B, 3C, and 3D) proteins following cleavage by viral proteases 2A and 3C. Viral RNA-dependent RNA polymerase 3D ($3D^{pol}$) then synthesizes negative-strand viral RNA intermediate which serves as a template for transcription of multiple progeny genomes. (*B*) Coxsackievirus infection facilitates protein ubiquitination, which subsequently increases host antiviral protein degradation (e.g. p53) by the proteasome and/or viral protein modification (e.g. viral $3D^{pol}$) by ubiquitination. Viral protein 3A likely promotes protein ubiquitination by recruiting host ubiquitin ligases to the viral replication complex. Degradation of host anti-viral proteins and ubiquitin-mediated modification of viral protein represent strategies that CVB3 evolves to promote its infectivity. DUBs, deubiquitinating enzymes. (*C*) Coxsackievirus infection induces the formation of cellular autophagosome without promoting protein degradation by the lysosome, probably through the activation of eIF2α. The host double-membrane autophagosomes may serve as sites for viral RNA synthesis by recruiting the polyribosomes and assembling the viral replication complex.

In response to viral infection, the initial host immune response is elicited by natural killer cells and macrophages, causing profound cytokine production (tumour necrosis factor-α, interleukin-1, and interleukin-2, and IFN-γ) and inflammatory cell infiltration of the myocardium.[10] The secondary immune response is carried out by the antigen-specific T-lymphocytes and antibody-producing B-cells.[10] Although the immune response plays a critical role in host defense mechanism by clearing viral particles and infected cardiomyocytes, exaggerated or persistent immune response, as well as autoimmune response elicited by exposure to cardiac-antigens (e.g. cardiac myosin and troponin I), can be detrimental to the cardiomyocytes, causing further damage to the heart.[54] Both innate and adaptive immune responses have been implicated in the immune-mediated damage of the heart, which determine the severity of myocarditis and the progression to end-stage DCM.[54]

The third stage consists of fibrotic reparation and cardiac dilatation in the presence or absence of low-level persistent viral genomes. It has been well documented that cardiac damages inflicted upon myocytes during the previous stages lead to ongoing necrosis of cardiac muscle, reparative cardiac fibrosis, as well as cardiac remodelling—the molecular and cellular restructuring of the myocardium and interstitium, eventually resulting in cardiac dilatation and loss of contractile function of the heart.[10,11,46,55]

Recent *in vitro* and *in vivo* evidence indicates that the host proteolytic systems may participate in all three stages of viral myocarditis.[12–17] The interplays between the viruses and the host protein degradation system for each phase are reviewed below.

4. The direct interaction between cardiotropic viruses and the host protein degradation systems

At the onset and during the progression of viral myocarditis, there is a constant interplay between the virus and the host. Cardiomyocytes and the host immune system attempt to limit viral replication or to induce apoptosis to clear the invaded pathogen, whereas virus wants to inhibit anti-viral host mechanisms or even usurp the host intracellular machinery to facilitate viral replication and promote host cell survival.

Emerging evidence suggests that the replication of CVB3 and many other cardiotropic viruses requires the function of host protein degradation systems. Inhibition of either the UPS or

autophagy effectively inhibits coxsackieviral replication and viral progeny release, highlighting the importance of host protein degradation systems in the intracellular virus lifecycle.[13,14,56,57]

Recent *in vitro* experiments demonstrate that inhibition of proteasome by proteasome inhibitor MG132 or lactacystin markedly reduces CVB3 replication and viral progeny production without inhibiting the proteolytic activities of viral proteases, suggesting that the UPS may be utilized during CVB3 infection to control host protein proteolysis and promote viral infectivity.[14,57] In addition, pyrrolidine dithiocarbamate, an antioxidant,[58] and curcumin, a natural compound derived from turmeric,[59] have recently been reported to potently inhibit CVB3 replication, probably through inhibition of host protein degradation. These studies reinforce the importance of the UPS in the regulation of CVB3 replication.

Destablization of host intracellular proteins that perturb efficient virus infection is an important mechanism evolved by viruses to optimize progression through the viral lifecycle. The tumour suppressor gene p53 has been implicated as a host anti-viral factor by directly interfering with viral replication and/or through promoting host cell apoptosis.[13,60,61] As a result, several viruses have evolved different strategies to inactivate p53.[60,61] For example, adenovirus gene products, E1B 55K and E4orf6, have been reported to promote the proteasomal degradation of p53.[61] It has recently been demonstrated that CVB3 infection facilitates ubiquitin-dependent proteolysis of cyclin D1,[62] p53,[62] and β-catenin,[63] preventing the host-induced apoptosis of infected cells, thereby ensuring sufficient time for viral replication and viral progeny assembly. Degradation of these intracellular proteins may represent an evolved strategy that CVB3 utilizes to promote infectivity by attenuating their inhibitory effects on virus replication. Moreover, studies have suggested that degradation of excess viral proteins may be required by some viruses to achieve optimal replication efficiency.[64,65] However, potential degradation of coxsackieviral proteins during replication and its role in viral infectivity have not been explored.

Independent of proteasome-mediated degradation, viruses also utilize host ubiquitination machinery for post-translational modification of viral proteins. For example, monoubiquitination of the HIV-1 Gag polyproteins is required for virus packaging and release.[66] In addition, ubiquitination of HIV-1 Tat protein has been shown to enhance its transactivation activities.[67] Interestingly, CVB3 polymerase $3D^{pol}$ was also reported to be modified during infection by ubiquitination.[57] Although the role of its ubiquitination in the regulation of virus replication remains to be determined, such observations raise the possibility that the UPS may, in part, regulate CVB3 replication through ubiquitination of the viral protein 3D.[57]

In addition to ubiquitin-mediated proteolysis, ubiquitin-independent degradation also plays a role in controlling viral infectivity and virus-mediated pathogenesis.[27] Recently, it has also been found that the proteasome activator PA28γ undergoes SUMOylation and changes sub-nuclear localization upon CVB3 infection (unpublished data from the authors' laboratory). SUMOylation is a post-translational modification involved in various cellular processes, such as subcellular localization, transcription regulation, and protein stability.[68] Further investigations through protein overexpression and siRNA knockdown of PA28γ suggest that subversion of PA28γ function by CVB3 enhances viral replication by delaying apoptosis (unpublished data from the author's laboratory).

It has been reported that CVB3 infection results in an increased accumulation of ubiquitin protein conjugates and a subsequent decrease in free ubiquitin without apparent changes in proteasome activities.[13,57] The underlying mechanisms by which CVB3 facilitates protein ubiquitination remain elusive. It is speculated that CVB3 may modulate ubiquitination by directly encoding its own ubiquitinating and deubiquitinating enzymes, or by redirecting the host UPS to new targets.[6] Interestingly, the non-structural coxsackieviral 3A protein carries a ubiquitin-related signature motif PPXY (P, proline; X, any amino acid; Y, tyrosine), a consensus binding site for WW domain-containing E3 ligases.[69] This raises the possibility that 3A viral protein may promote recruitment of host ubiquitin ligases to the viral replication complex to increase protein ubiquitination. Future studies to define the ubiquitination profile using proteomic techniques will help us understand the mechanisms by which CVB3 modulates the ubiquitin conjugation process.

Aside from the UPS, CVB3 also utilizes host autophagy machinery to facilitate its own replication. Autophagy is considered both an innate and adaptive host defense mechanism for clearing intracellular pathogens.[33,34] However, some viruses can subvert this host anti-viral machinery to promote their own replication.[70,71] CVB3 infection induces massive intracellular membrane reorganization. Electron and confocal microscopy confirms the presence of perinuclear autophagosome vesicles in infected cells.[56] The underlying mechanisms of CVB3-induced autophagosome formation have not been fully elucidated. CVB3-induced phosphorylation of eIF2α may contribute, at least in part, to this process.[56] In addition, a recent study on CVB4 suggests a mechanism of calpain activation in the induction of autophagosome formation.[72] Blockage of autophagy formation effectively inhibits viral replication, whereas induction of autophagosome formation or prevention of autophagosome-lysosome fusion promotes viral replication, suggesting that the autophagosome is an important intracellular component required for CVB3 replication.[56] The autophagosome likely serves as a docking site for viral replication machinery, as replication of positive-strand RNA viruses requires an intracellular membrane surface to assemble their replication complexes.[56]

Figure 2 summarizes the direct interaction between the UPS/autophagy systems and the intracellular lifecycle of coxsackieviruses. The overlap between the UPS and the autophagy has been recognized.[39] However, the interactive role of these two major protein degradation pathways in regulating viral infection has not been explored and warrants further studies.

5. The protein degradation system and virus immunity

As alluded to earlier, viral myocarditis has been considered an immune-mediated disease of the heart, and both innate and adaptive host responses contribute significantly to the myocardial injury and progression to DCM.[10,46,54]

The significance of the protein degradation systems in host immune responses has been well recognized.[18,28,29,34,73] The UPS participates in the development of inflammatory and autoimmune diseases via multiple mechanisms, including regulation of cytokine production and MHC-mediated antigen presentation. The majority of peptide antigens presented on MHC I molecules are generated by the UPS. The immunoproteasome is believed to be the major proteasome pathway involved in such process. Immunoproteasome formation and subsequent antigen presentation influence the adaptive immune response to the infectious agent.

In an attempt to identify the susceptible host factors that affect the progression of CVB3-induced myocarditis, Szalay *et al.*[17] reported that myocarditis-susceptible mice express elevated levels of immunoproteasome β subunits LMP2, LMP7, and MECL-1 in virus-infected heart, similar to the results from a previous microarray study.[74] This increased expression of proteins results in enhanced formation of immunoproteasomes and altered proteolytic activities of the proteasome, which are accompanied by upregulation of genes involved in MHC I antigen presentation and augmented immune cell infiltration. This study suggests that enhanced immunoproteasome expression in cardiomyocytes affects the generation of antigenic peptides and subsequent T lymphocyte-mediated immune responses, contributing to elevated immunopathology and the pathogenesis of myocarditis. Increased production of IFN-γ during ongoing myocarditis is probably involved in such modulation. IFN-γ is a key regulatory cytokine for proteasome-mediated MHC antigen presentation. It induces the expression of proteasome activator PA28α and β, as well as the three inducible β subunits of the immunoproteasome, leading to increased peptide production for MHC class I presentation.[28,29]

However, the function of immunoproteasome in the heart does not appear to be limited to immune modulation. Recent study demonstrates that it also plays an important role in normal cardiac development and in cardioprotection in response to ischaemic preconditioning by regulating the degradation of damaged proteins.[75]

Autophagy participates in immune responses by enhancing MHC class II presentation of cytosolic antigens to CD4 T cells and regulating T lymphocyte homeostasis.[33,34] Several lines of evidence have suggested that autophagy may be implicated in the development of autoimmune diseases aside from its best characterized function in the clearance of pathogens.[76] However, the role of autophagy in viral myocarditis remains to be established. Whether it serves as a mechanism contributing to the pathogenesis and progression of viral myocarditis requires future investigation.

Through its ability to activate the NFκB pathway, the UPS has been associated with several inflammatory and autoimmune diseases, such as systemic lupus erythematosus, allergy, and asthma, psoriasis, rheumatoid arthritis, as well as myocardial infarction/reperfusion injury.[77] The NFκB is a key transcriptional regulator of multiple genes involved in inflammation, and its activation is mediated by the UPS through regulating the stability of IκB, an inhibitor of NFκB.[78] Proteasome inhibition markedly reduces the production of multiple inflammatory mediators and leukocyte adhesion molecules, providing a potential therapeutic option for inflammatory and autoimmune diseases. It has been reported that UPS dysfunction contributes to the inflammatory injury of the myocardium in both myocardial infarction/reperfusion model[79,80] and ischaemic diabetic model.[81] Inhibition of proteasome attenuates such injury, probably through the inhibition of NFκB activation.[79–81]

Coxsackievirus infection has been demonstrated to induce NFκB activation in cells.[82] To explore the potential value of proteasome inhibition in viral myocarditis, Gao *et al.*[13] reported that application of proteasome inhibitor reduces cardiac damages and improves the outcome of CVB3 infection at the inflammatory stage of this disease, probably through moderating the host immune response. It has further been shown that CVB3 infection of mice promotes the abnormal accumulation of protein-ubiquitin conjugates without significant alteration in core proteasome activities.[13] Several enzymes involved in protein ubiquitination/deubiquitinating are upregulated in CVB3-infected heart, suggesting that increased inflammatory cytokines following CVB3 infection may stimulate protein ubiquitination by upregulating ubiquitin enzymes in an autocrine or paracrine manner.[13] Recent studies suggest that cytokines are key modulators for protein ubiquitination.[83,84] These studies provide a novel mechanism of coxsackievirus pathogenesis, and suggests that the manipulation of the UPS may provide a viable therapeutic option against viral myocarditis.[13]

6. The protein degradation system and cardiac remodelling leading to DCM

Along with chaperones that protect proteins against stress-induced misfolding and aggregation (see other review articles in this Spotlight issue for the details), the UPS and autophagy have emerging as central effectors of protein quality control in maintaining myocyte function and homeostasis through the control of protein turnover.[22,38,39]

Increased accumulation of ubiquitin–protein conjugates has commonly been observed in end-stage human heart diseases, including in cardiomyopathies.[12,16] Data from failing human hearts and animal models of myocardial infarction have shown that enhanced protein ubiquitination and damaged proteasome function may account for this aberrant accumulation. The UPS function is demonstrated to be impaired in various models of heart disease, including desmin-related DCM,[85] pressure-overload hypertrophy,[86] and myocardial infarction/reperfusion injury.[87] Similar to the cardiac remodelling process of other aetiologies, UPS impairment during the cardiac remodelling stage of virus-induced DCM may be caused by oxidative stress-induced modification and inactivation of the proteasome,[87] increased proteasomal load due to an excess production of abnormal protein products,[39] and the formation of protein aggregates.[88] Other mechanisms that contribute to UPS dysfunction include post-translational modifications, such as phosphorylation, to the cardiac 20S proteasome.[89]

Although the consequence of impaired UPS function in cardiac remodelling remains unclear, immunohistochemical staining

demonstrates the co-localization of protein–ubiquitin conjugates with cells undergoing autophagic cell death, indicating a role for the protein degradation systems in the cardiomyocyte death in failing hearts.[15] A recent report suggests that the elevated expression of p53 in human DCM which is associated with UPS impairment may activate downstream apoptosis effectors and contribute to cardiomyocyte loss.[12] Furthermore, the UPS is known to be responsible for the dynamic turnover of sarcomeric proteins, including troponin, myosin, actin, and tropomyosin. As such, UPS impairment may result in sarcomeric disorganization and contribute to a reduction in cardiac output.[90]

Recent studies suggest that under baseline conditions, autophagy represents an important cytoprotective mechanism for maintaining normal cardiomyocyte function and structure. For example, cardiac-specific knockdown of ATG5 in adult mice causes cardiac hypertrophy, left ventricular dilatation, and contractile dysfunction.[41] However, increased activation of autophagy under stress may be detrimental to the heart by inducing cell death.[38,91] In response to cardiac ischaemia/reperfusion injury, autophagy is activated in the mouse myocardium, and disruption of autophagy attenuates cardiomyocytes autophagy and myocardial damage.[92] Furthermore, it has been shown that pressure-overload triggers cardiomyocyte autophagy in mice and disruption of autophagy decreases cardiomyocyte autophagy and pathological remodelling.[93]

Despite the recognized importance of the UPS and autophagy in the regulation of cardiac homeostasis and function, the underlying mechanisms through which these systems contribute to cardiac remodelling remain poorly understood. The current literature on the role of the UPS in different heart disease settings is still controversial. Multiple studies suggest that proteasome inhibition may be beneficial in several experimental animal models, including pressure-overload hypertrophy[94–96] and myocardial infarction/reperfusion injury.[97,98] Further studies are required to address the function and regulation of these systems in DCM and to explore the specific mechanisms involved.

7. Manipulation of protein degradation systems in developing therapeutics

On the basis of the current knowledge of the role of the UPS/autophagy in viral myocarditis progression to DCM (summarized in *Figure 3*), we postulate that temporally targeted blockage of protein proteolytic systems may be beneficial during the acute viral infection and inflammatory stages of myocarditis, when the functions of these systems are utilized to promote viral replication and to induce immune-mediated pathogenesis. However, prolonged inhibition of protein degradation, especially during late

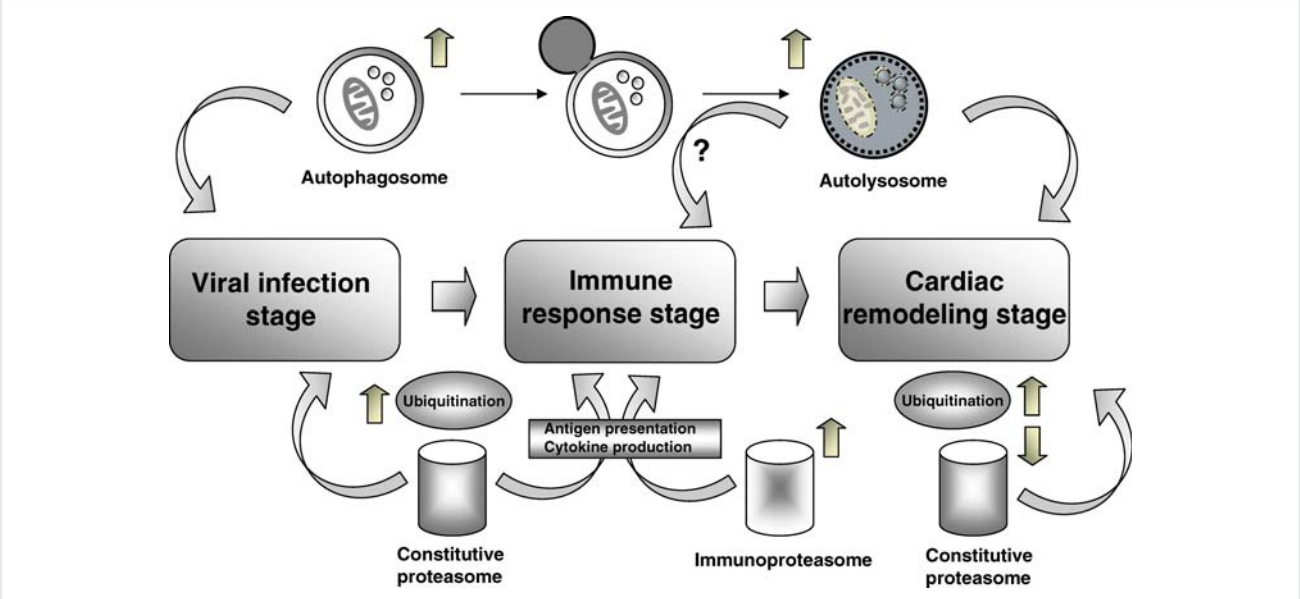

Figure 3 The role of the protein degradation systems in viral myocarditis leading to dilated cardiomyopathy. Viral myocarditis consists of three stages: viral infection, immune response, and cardiac remodelling progression to dilated cardiomyopathy. (*A*) During the viral infection stage, virus evolves different strategies to utilize the host UPS and the autophagy machinery to facilitate its replication (see *Figure 2* for the possible mechanisms). (*B*) At the immune response stage, virus infection induces the formation of immunoproteasome to increase MHC class I antigen presentation. Meanwhile, production of pro-inflammatory cytokines is enhanced, partially through UPS-mediated NFκB activation. Autophagy may also contribute to immune-mediated pathogenesis by modulating MHC class II antigen presentation. (*C*) Pathological cardiac remodelling leads to dilated cardiomyopathy. Increased accumulation of abnormal ubiquitin-protein conjugates/aggregates and elevated oxidative stress lead to the eventual impairment of UPS function, subsequently result in abnormal regulation of contractile apparatus expression and also trigger apoptosis and autophagic cell death. As a result of myocyte loss and decreased contractile property, the left ventricle of the heart begins to dilate to compensate for impaired cardiac function.

cardiac remodelling stage, where protein degradation impairment has already developed, may exacerbate myocardial damage resulting in heart failure. This is consistent with recent clinical reports that long-term treatment with proteasome inhibitor in cancer patients increases the incidence of cardiac failure.[99,100]

8. Conclusions and future directions

Studies have begun to unravel the biological significance of the protein degradation systems in the pathogenesis of viral myocarditis and its progression to DCM. Cardiotropic viruses, in particular CVB3, adapt to utilize pre-existing host cellular machineries to facilitate their own replication while evading host clearance mechanisms. Meanwhile, virus-mediated modulation of the UPS also contributes, at least in part, to the pathogenesis and progression of myocarditis by participating in the regulation of the host inflammatory response and autoimmune responses and subsequent cardiac muscle remodelling.

Therapeutic manipulation of host protein degradation systems in viral myocarditis appears attractive, but is complicated by the complex interactions between the host and the virus throughout disease progression. Elucidating the precise functional and regulatory mechanisms of protein degradation system at each disease stage of viral myocarditis will guide future drug treatment strategies. System-like approaches, such as ubiquitomics, degradomics, and RNAi screens, will be necessary to identify more specific modulators and targets of the protein degradation systems in viral myocarditis and DCM pathogenesis.

Conflict of interest: none declared.

Funding

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) (H.L.); and the Heart and Stroke Foundation of British Columbia and Yukon (H.L.). H.L. is a Michael Smith Foundation for Health Research (MSFHR) Scholar. J.W. and B.W. are the recipients of Studentships from the MSFHR, the CIHR, and the Heart and Stroke Foundation of Canada.

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