Pathogenesis and therapy of autoimmunity-induced dilated cardiomyopathy

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1. ABSTRACT

Myocarditis and dilated cardiomyopathy can potentially originate from autoimmune responses. Although genetic predisposition, viral infection, molecular mimicry, and oxidative stress are potential contributing factors to dilated cardiomyopathy, the underlying mechanism (s) has not been fully elucidated. Autoantibodies (AABs) against cardiotropic targets such as β-adrenergic receptors, mitochondria proteins, myosin, tropomyocin and actin as well as structural proteins such as laminin and desmin may participate in the development of dilated cardiomyopathy. These autoantibodies disrupt cardiac excitation-contraction coupling and activate immune response to initiate tissue injury through complement and circulatory immunocomplexes (CICs). These antibodies are present prior to the onset of dilated cardiomyopathy and may be used to predict the deterioration of cardiac function. Depletion of these cardiac-specific antibodies by extracorporeal immunoabsorption has been considered as a new and effective approach in the treatment of autoimmunity-induced dilated cardiomyopathy. In order to better understand the pathogenesis and therapeutic remedy against this myopathy, the present review will summarize the manifestation and key signaling mechanisms involved in compromised cardiac contractile function during autoimmunity.

2. INTRODUCTION

Dilated cardiomyopathy (DCM), manifested as ventricular dilation and systolic dysfunction, is a leading cause of heart failure and a clinical indication for heart transplantation. The clinical manifestations of DCM encompass progressive development of heart failure, arrhythmia, thromboembolism, and sudden cardiac death (1;2). DCM is the most prevalent form of cardiomyopathy, when compared with hypertrophic and restrictive cardiomyopathies (3). Heart muscle of patients with DCM is usually damaged by atherosclerotic coronary artery disease. The prevalence of DCM is greatly enhanced in patients with diabetes mellitus, alcohol abuse, bacterial and viral infections, exposure to certain drugs and toxins, nutritional deficiencies, connective tissue diseases, hereditary disorders, and even pregnancy (1;3;4). In a recent survey, DCM was found to be the most prevalent form of cardiomyopathy in Africa and was present in nearly all age groups and regions. The survey revealed that DCM accounts for 10-17% of cardiac conditions upon autopsy examination and in 17-48% of patients hospitalized for heart failure (5). The prevalence of DCM appears to be much higher in men than women, with a male-to-female ratio of 2.6. Congestive heart failure (CHF) and arrhythmias are believed to be the leading causes of death in patients with DCM (1;3;4;6). Prescription drug therapy, including angiotensin converting enzyme (ACE) inhibitors,
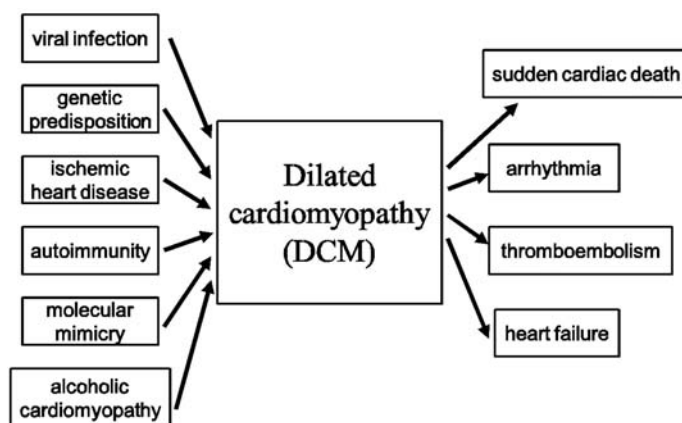
Figure 1. Different contributing factors for dilated cardiomyopathy (DCM) as well as common fates of DCM for the cardiovascular system

β-adrenergic blockers, and diuretics for DCM with complicated CHF, is often directed toward treating the underlying causes of DCM.

The etiology of DCM is multifactorial, involving many different clinical conditions leading to the phenotypic DCM. Genetic factors such as genetic mutations in sarcomeres, and cytoskeletal, nuclear and Ca^{2+}-regulatory proteins appear to play important roles in the etiology of idiopathic DCM (7). However, the precise mechanism of action responsible for the pathogenesis of DCM is still unclear and includes potential contributions from ischemic heart disease, viral infection, molecular mimicry, and alcoholic cardiomyopathy, in addition to a genetic predisposition. Figure 1 summarizes various contributing factors in the onset and development of DCM. Numerous epigenetic triggers, including inflammation, hypertension, chronic alcohol intake and autoimmunity, may predispose the heart to DCM and heart failure and ultimately to cardiac death. Recent evidence has suggested that immune mediators may trigger the onset and progression of a wide variety of heart diseases, including myocarditis, DCM, and myocardial infarction, which may eventually lead to heart failure. It has been shown that both cellular and humoral components of the immune system may participate in pathological cardiac remodeling through extracellular matrix degradation, collagen deposition, cardiomyocyte hypertrophy, and apoptosis, all leading to cardiomyocyte injury and contractile dysfunction (3). Although autoimmunity has been speculated to play a role in the pathogenesis of DCM and myocarditis, the precise mechanism (s) of action behind autoimmunity-induced DCM is still undefined. The purpose of this review is to briefly summarize some of the recent findings regarding abnormalities of cardiac contractile elements under enhanced autoimmunity with an emphasis on cardiac excitation-contraction coupling.

3. INTRACELLULAR Ca^{2+} HANDLING IN NORMAL AND DCM HEARTS

Intracellular Ca^{2+} homeostasis and handling have been extensively studied over the past decades. Ca^{2+} is an essential signaling molecule for normal cardiac electromechanical activity and contractile function. An abrupt rise in cytosolic Ca^{2+} levels is the direct activator of myofilament cross-bridge linking and the initiation of contraction in cardiomyocytes. Cytosolic Ca^{2+} levels are tightly regulated during contraction and relaxation cycles. During phase 2 (the plateau phase) of the action potential, extracellular Ca^{2+} ions enter the cell through voltage-gated L-type Ca^{2+} channels located on the cell membrane, resulting in a relatively small Ca^{2+} influx. This subtle Ca^{2+} entrance triggers an approximately 1000-fold increase in intracellular (technically "cytosolic") Ca^{2+} levels through the release of sarcoplasmic reticulum (SR) Ca^{2+}, a process commonly defined as Ca^{2+}-induced Ca^{2+}-release (CICR). Elevated Ca^{2+} concentrations result in actin-myosin cross-bridge linking via the binding of Ca^{2+} to the Ca^{2+}-sensitizing protein troponin C. Such binding leads to displacement of tropomyosin, formation of myofilament cross-bridges and initiation of cardiac contraction. This increase in cytosolic Ca^{2+} concentration during a contraction is immediately followed by cytosolic Ca^{2+} removal, resulting in deactivation of the contractile machinery and myocardial relaxation. Cytosolic Ca^{2+} is pumped back into the SR mainly via the sarco (endo)plasmic reticulum Ca^{2+}-ATPase (SERCA). A portion of Ca^{2+} is expelled via the sarcolemmal Na^+/Ca^{2+} exchanger. The entire process of cardiac contraction and relaxation is defined as cardiac excitation-contraction coupling (8;9). Disruption of one or more of these physiological steps involved in cardiac excitation-contraction coupling may result in dysregulation of intracellular Ca^{2+} homeostasis and contractile dysfunction commonly seen in DCM and other forms of heart disease.

In a recent Japanese study, a group of 99 unrelated adult patients with DCM were screened for the following cardiac regulatory genes: myosin heavy chain (MHC), myosin-binding protein C (MYBPC3), regulatory and essential myosin light chains (MLC), actin, tropomyosin, troponin T, troponin I, troponin C, dystrophin and lamin A/C. A mutation (R820Q) in MYBPC3 was identified in one older DCM patient. In addition, dystrophin mutations were identified in 3 male patients (2 with exon

45–48 deletion and 1 with exon 48–52 deletion). The prevalence of the dystrophin mutation was found to be 4.4% (3 of 68 patients) in males with DCM. No mutations involving amino acid changes were identified in other genes (10). In addition, it was discovered that a mutation in the sarcomeric thin filament protein tropomyosin may also lead to DCM. Two distinct point mutations within α-tropomyosin, namely Glu40Lys and Glu54Lys, have been identified in patients with DCM. To better understand the role of these point mutations in cardiac morphology and function, transgenic mice with cardiac expression of mutant α-tropomyosin (Glu54Lys) were generated. These mice displayed reduced endogenous α-tropomysin levels, signs of heart failure and high mortality. Echocardiographic and functional analyses confirmed the dilated phenotype of the heart with decreased left ventricular fractional shortening and impaired systolic and diastolic functions. Real-time RT-PCR quantification revealed an increased expression of β-myosin heavy chain, brain natriuretic peptide, and skeletal actin and a decreased expression of SERCA and ryanodine receptors in these transgenic mice. The pathological and physiological phenotypes found in these Glu54Lys mutant mice are consistent with those seen in human DCM and heart failure (11). Other than the contribution of genetics to DCM, such as mutations in cardiac contractile or Ca^{2+} regulating proteins (7), autoimmunity has been demonstrated to trigger post-translational modification of key cardiac contractile or Ca^{2+} regulating proteins *en route* to myocarditis and DCM in a subset of patients with DCM (12). Enhanced oxidative stress resulting from inflammatory and viral myocarditis, for example, and may initiate oxidation of SERCA, an essential Ca^{2+} regulatory protein prone to oxidative modification (13). Moreover, compromised actin binding and myofilament Ca^{2+} sensitivity have been documented to play a role in reduced myocardial tension development in DCM (11). Using an experimental model of autoimmune myocarditis, Veeraveedu and colleagues (14) tested the effect of long-acting loop diuretic agent, which usually develop into DCM. Their studies revealed that the loop diuretics alleviate myocarditis-induced downregulation in SERCA protein expression. Given the essential role of the Ca^{2+}-ATPase pump SERCA in the regulation of cytosolic Ca^{2+} concentration in cardiomyocytes during excitation-contraction coupling, defective SERCA function/expression in DCM, cardiac hypertrophy and heart failure may result in compromised SR Ca^{2+} uptake.

4. AUTOANTIBODY, GENETIC HETEROGENEITY AND EXCITATION-CONTRACTION COUPLING

Abnormalities of the cellular and humoral immune systems, along with immunohistological changes characteristic of myocardial inflammation, are among the main hallmarks of cardiac dysfunction in DCM patients. The presence of inflammation and autoimmunity can be consolidated by the appearance of lymphocytes, mononuclear cells, cell adhesion molecules, and autoantibodies in patients with DCM (15;16). Appearance of autoantibodies against cardiac proteins is consistent with the experimental evidence that peripheral lymphocytes from DCM patients are capable of transferring myocardial

disease onto the immunodeficient SCID mice (17;18). These findings have recapitulated the notion of post-infectious autoimmunity as a major culprit in the onset of chronic myocardial dysfunction following a acute attack of myocarditis (19). Production of anti-myocardial autoantibodies (AABs) is one of the main autoimmune responses secondary to persistent inflammation with alterations in cellular immunity and complement activation. As a normal humoral immune response, most cardiomyopathic patients develop antibodies and/or autoantibodies against cardiac antigens of various origins including mitochondrial proteins, contractile proteins, cardiac $β_1$-adrenergic receptors, and sarcolemmal Na^+/K^+-ATPase. The pathogenic capacity of autoantibodies has been consolidated, and the initial clinical trials to remove such autoantibodies via immunoadsorption are promising. However, intracellular myocyte antigens are not easily accessible to the immune system under physiologic conditions (20) thus should not cause harm under physiological conditions.

In a recent study, Jahns and colleagues (21) examined the role of autoantibodies against cardiac $β_1$-adrenoceptors in the pathogenesis of DCM. Their results indicated that rats immunized with AABs against the second extracellular loop of cardiac $β_1$-receptors develop overt and progressive left ventricular dilatation and dysfunction, hallmarks of DCM. The role of anti-$β_1$-receptor AABs received further support from clinical studies in which anti-$β_1$-adrenergic receptor autoantibodies were found in patients with DCM with clinical manifestations of compromised heart function (22), severe ventricular arrhythmias (23), and a high incidence of sudden cardiac death (24). The $β_1$-adrenergic receptor belongs to the family of G protein-coupled receptors, where its stimulation by epinephrine or norepinephrine may trigger Gs-mediated activation of adenylate cyclase to produce cAMP and cAMP-dependent protein kinase (PKA) activation. Anti-$β_1$- adrenergic receptor autoantibodies may increase the concentration of intracellular cAMP and intracellular Ca^{2+}, a condition often leading to a transient hyper-performance of the heart followed by depressed heart function and heart failure. Anti-$β_1$-adrenergic receptor autoantibodies together with isoprenaline can reduce isoprenaline-induced positive inotropic effect. These data demonstrated that anti-$β_1$-AR autoantibody excites the β1-adrenergic receptors through protein kinase A (PKA). Activated PKA phosphorylates signaling molecules involved in the regulation of sarcoplasmic Ca^{2+} concentration, thereby increasing myocyte inotropicity and lusitropicity (20;25). Excitation-contraction coupling in the heart initiates membrane depolarization, leading to the opening of Ca^{2+} channels. Local elevation in cytosolic Ca^{2+} around SR Ca^{2+} channels (ryanodine receptors, RyRs) liberates more Ca^{2+} into the cytosol. Ca^{2+} sparks are produced upon activation of the RyRs. The synchronous activation of Ca^{2+} sparks leads to the global elevation of cytosolic Ca^{2+} in cardiomyocytes, triggering contraction. Anti-$β_1$-AR autoantibodies of patients with DCM can bind with mouse cardiomyocytes *in vitro* to enhance L-type Ca^{2+} current, intracellular Ca^{2+} concentration, automaticity, and transiently increased contractility in cardiomyocytes. A β-
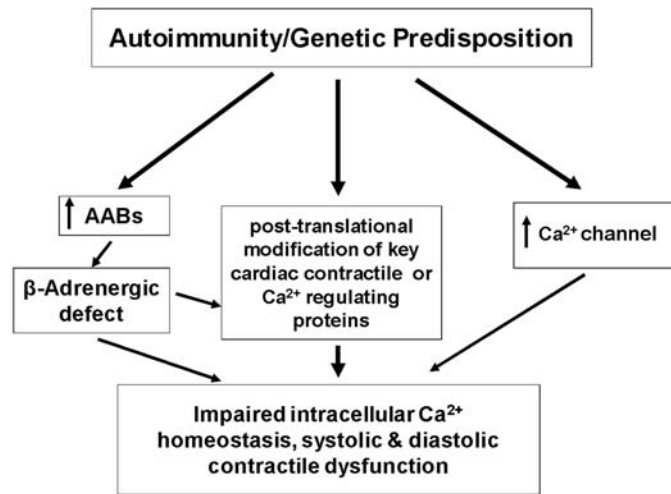
Figure 2. Potential mechanisms of action in autoimmunity-associated cardiac mechanical dysfunction. AAbs = autoantiboby.

adrenergic receptor antagonist such as metoprolol may antagonize these effects induced by the anti-β_1-adrenergic receptor AABs (26). Inducible transgenic mice with enhanced sarcolemmal L-type Ca^{2+} channel activity showed progressive myocyte necrosis leading to pump dysfunction and premature death. These detrimental effects were dramatically aggravated following acute stimulation of β-adrenergic receptors. Enhanced Ca^{2+} influx–induced cellular necrosis and cardiomyopathy were prevented with either L-type Ca^{2+} channel blockers or β-adrenergic receptor antagonists, suggesting a causal relationship among AABs, β-adrenergic receptors, cytosolic Ca^{2+} handling, and heart function in autoimmunity-induced heart dysfunction (27). In addition, AABs against other contractile proteins such as myosin, tropomyocin, and actin, as well as structural proteins such as laminin and desmin, have been documented in DCM (11;28-30). These data suggest possible involvement of AABs of a wide range of proteins in the autoimmune response in patients with DCM. In order to elucidate the full autoantibody repertoire involved in DCM, Buse and coworkers (28) performed an autoantibody screening test using ventricular and atrial proteomes as autoantigenic sources and subsequently tested the autoantibody-binding patterns of sera from dogs with DCM. Five potentially DCM-related autoantigens, including myosin heavy chain α isoform, α-cardiac actin, mitochondrial aconitate hydratase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and brain glycogen phosphorylase (GPBB) were identified (28). The contribution of AABs against β-adrenergic receptor and other proteins, as well as other cellular machineries to the cardiac mechanical dysfunction in autoimmune diseases, is depicted in Figure 2.

Mitochondrial ADP/ATP carriers are important self-antigens with high organ specificity, transporting ATP and regulating energy metabolism between mitochondrion and cytoplasm. Autoantibodies against the ADP/ATP carrier are highly sensitive and specific in DCM patients along with direct cardiomyocyte toxicity (Figure 3). The anti-ADP/ATP carrier autoantibodies from patients with DCM may enhance voltage-gated Ca^{2+} current, resulting in Ca^{2+} overload. Disturbing Ca^{2+} channel gating by AABs may attribute to AABs-induced onset of DCM (31). Using male Balb/c mice immunized against mitochondria ADP/ATP carrier peptides, Yuan and coworkers (32) established a model of autoimmune cardiomyopathy to evaluate the efficacy of immunotherapy with the anti-L3T4 monoclonal antibody (McAb). Their results indicated that antibodies against the ADP/ATP carrier were negative in the early treatment and the sham control groups, while they were positive in the mid-term-treated and cardiomyopathic groups with a transient decline in the mid-term-treated group. Interestingly, administration of anti-L3T4 McAb displayed effective protection against autoimmune cardiomyopathy induced by mitochondria ADP/ATP carrier peptides (32).

Viral infections frequently result in the production of autoantibodies. Using a model of autoimmune myocarditis induced in genetically susceptible mice infected with coxsackievirus B3, Rose and colleagues (33) found that the autoimmune sequelae of viral infections can be mimicked by immunization of the susceptible mice with murine cardiac myosin. A disease progress from a contained viral myocarditis to a pathogenic autoimmune response was achieved within hours after induction of infection in both murine models. These investigators observed production of key cytokines, including IL-1β and TNFα (33). It was suggested that these virus-triggered immune responses may occasionally progress to a pathogenic autoimmunity to form autoimmune disease.

As indicated previously, genetic predispositions attribute to autoimmunity-induced onset and progression of DCM phenotype. Genetic heterogeneity in DCM has been widely documented with many causative genes being identified. For example, mutations in cardiac actin (34),
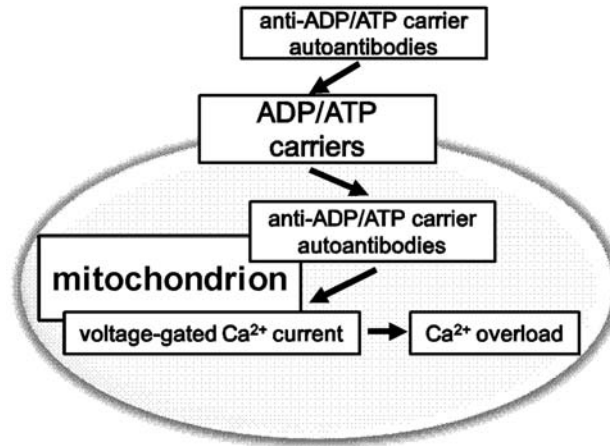
Figure 3. Role of mitochondrial ADP/ATP carriers as important self-antigens. Autoantibodies against ADP/ATP carrier possess highly sensitivity and specificity in DCM patients in addition to direct cardiomyocyte toxicity.

troponin T (35), β-myosin heavy chain (36;37), α-actinin (38), troponin I (4), and troponin C (39) have been associated with cardiac dysfunction resembling DCM. Furthermore, mutations in genes encoding sarcomeric proteins troponin C (TnC) and troponin T (TnT) were identified in five families with inherited DCM (39). The mutation of these proteins led to the altered affinity of cardiac troponin C (cTnC) for Ca^{2+} and changes in the ability of TnC to bind Ca^{2+}, which ultimately led to dysregulated intracellular Ca^{2+} homeostasis. Earlier evidence showed that heterogeneous excitation-contraction coupling may be one possible mechanism attributing to arrhythmic induction in patients with DCM (40). Stretches of relatively inactive myocardial regions can trigger the release of Ca^{2+} from myofilaments. A release of Ca^{2+} from such regions may occur in response to changes in sarcomere length, thus reducing the affinity of TnC for Ca^{2+} (40). Triggered contraction and Ca^{2+} waves as a result of the release of Ca^{2+} are likely to be accompanied by delayed afterdepolarization possibly associated with effects on the electrogenic Na^+/Ca^{2+} exchange or nonselective sarcolemmal channels. These effects may explain the pro-arrhythmic effect of the relevant genetic mutations of cardiac proteins in nonischemic heart failure (41).

5. POTENTIAL THERAPIES AGAINST AUTOIMMUNITY-INDUCED DCM

Autoimmune-induced DCM is a common form of cardiomyopathy in humans. Unfortunately, it's clinical diagnosis is rather difficult prior to a myopathic process. Myocarditis and DCM may be idiopathic, infectious, or autoimmune in nature, representing different stages of an organ-specific autoimmune disease in genetically predisposed individuals. In animal models, cell- or antibody-mediated autoimmune myocarditis/DCM can be induced by viral infection or immunization with heart-specific autoantigens or may develop spontaneously in genetically predisposed strains. Clinical diagnosis of autoimmune myocarditis/DCM requires the exclusion of viral genome on endomyocardial biopsies and the presence of serum heart-reactive autoantibodies. Cardiac-specific

and disease-specific antibodies of the IgG class are potential biomarkers for identifying populations at risk. Future work is warranted on the genetic basis of human autoimmune myocarditis/DCM to improve early diagnosis of autoimmune DCM (42).

Autoimmune mechanisms, in addition to genetic predisposition and viral infection, has been viewed as one of the major causes for DCM. Patients with heart failure due to DCM are usually treated with a conventional regimen against heart failure, including diuretics, angiotensin-converting enzyme (ACE) inhibitors, digitalis, β-blockers, vasodilators, aldosterone antagonists, and other non-pharmacological methods. The clinical management of myocarditis is dependent on the etiology of the illness. Treatment remedies that are currently under investigation include immunosuppressants, nonsteroidal antiinflammatory agents, immunoglobulins, immunomodulation, antiadrenergics, Ca^{2+}-channel blockers, nitric oxide inhibitors (e.g., aminoguanidine), and antivirals (43). Nonetheless, the mortality is still quite high in patients with DCM despite aggressive treatment (44). It is recommended that β-blockers should be initiated as soon as an acute decompensation phase resolves. While early detection of myocarditis seems to be essential to patients' prognosis, better efforts are required to distinguish viral from noninfectious autoimmune forms of the disease in order to guide appropriate treatment. Carvedilol may suppress expression of the cytokine IL-1 and matrix metalloproteinase 8 mRNA, in addition to reduction of CD_4 T-cell infiltrate and improvement of left ventricular ejection fraction in a Coxsackie virus-induced murine myocarditis model (45). However, its clinical efficacy remains to be confirmed.

Given the identification of autoantibodies against certain membrane receptors or pumps in DCM patients, immunoabsorption therapy in an effort to remove the excessive antibodies has been considered a new strategy to treat patients with DCM and heart failure (46). To evaluate the hemodynamic effects of immunoabsorption in DCM patients, immunoglobulin (Ig)G was substituted following immunoabsorption treatment to minimize the risk of

infection after IgG depletion. The results indicated unchanged hemodynamics and left ventricular ejection fraction throughout 3 months in the control group. On the contrary, cardiac index and stroke volume were significantly enhanced in the immunoabsorption group. Meanwhile, left ventricular ejection fraction was significantly improved in patients from the immunoabsorption group, indicating the beneficial role of immunoabsorption and subsequent IgG substitution in the management of DCM. A further study indicated that immunoabsorption not only improves hemodynamic indices but also modulates myocardial inflammation in DCM patients (47). A randomized study was performed in DCM patients to evaluate the immunohistological changes following immunoabsorption therapy and subsequent IgG substitution. In control patients, the number of lymphocytes (CD_3, CD_4 and CD_8) and the number of leukocyte common antigen (LCA)-positive cells in the hearts remained stable over 3 months. In addition, no changes in expression of HLA class II antigens were observed. In contrast, immunoabsorption therapy and subsequent IgG substitution triggered a drastic decrease in lymphocytes and LCA-positive cells in the myocardium during follow-up, which was accompanied by a significant decline in HLA class-II antigen expression (48). Immunoabsorption therapy involving larger, randomized, prospective, multicenter trials is warranted to consolidate its clinical effectiveness.

6. SUMMARY

Growing evidence from pathophysiological studies of AABs against cardiac membrane structures, receptor proteins, and intracellular antigens has indicated an essential role of AABs and autoimmunity in the onset and progression of DCM. A better understanding of the effect of AABs on cardiac electromechanical function will shed some light on the potential clinical application of new clinical therapies such as immunosuppression, immunomodulation, and antiviral therapy in patients with myocarditis and DCM.

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