Autoantibodies in Heart Failure and Cardiac Dysfunction

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Abstract: Human heart failure is a disease with multifactorial causes, considerable morbidity, and high mortality. Several circulating autoantibodies, some of them being heart-specific, play a crucial role in the progression and induction of heart failure. However, the precise mechanisms on how these autoantibodies perpetuate or even induce an organ-specific autoimmune response are not yet fully understood. Also, it is being a matter of current research to elucidate a potential pathophysiological role of the innate immune system in generating auto-reactive antibodies. In this review, we will summarize the current available literature on circulating autoantibodies which are related to human heart failure. We will present clinical and animal studies that demonstrate the occurrence and pathophysiological relevance of several autoantibodies in heart failure, as well as point out biological mechanisms on molecular and cellular level. Finally, the beneficial therapeutic effects of numerous clinical studies that target the humoral arm of the immune system by using either intravenous immunoglobulins and/or immunoadsorption will be critically discussed. (Circ Res. 2012;110:145-158.)

Keywords: autoimmunity ■ autoantibody ■ myocardial dysfunction ■ heart failure ■ immunoadsorption

Background
Heart failure is the final clinical entity of many diverse disease causes and mechanisms. Among the modulators of disease progression, a dysregulation in the immune system of yet unknown reasons is believed to play a central role in disease progression. The immune system consists of a multicellular highly regulated and complex defense system that is characterized by a high interindividual variability in its response to injury and antigens. In its physiological condition, it is programmed to discriminate between self- and foreign constituents, hereby interacting and eliminating any structures that are recognized as foreign. This process can drift into a pathological situation in which self-tissue is attacked, resulting in auto-immune disease.

Circulating autoantibodies have been critically linked to heart failure. These autoantibodies are targeted against diverse self-antigens, which often are not restricted in their exposition to cardiac muscle. Their prevalence, mode of action, and potential therapeutic modulation are intensively investigated. Although a triggering injury to myocardium is believed to be the crucial initiating event, the genetic predisposition, environmental and epigenetic modulators, and other still unknown mechanisms are critical for development of the pathological antibody titers observed in peripheral blood and
the intensity of inflammation in myocardial structures. In a prospective study, Caforio et al showed that circulating antiheart autoantibodies may precede disease manifestation and are independent predictors of disease development.1

Clinical observations on a prognostic relevance of autoantibodies have prompted therapeutic trials focused on nonspecific removal of autoantibodies from the circulation via immunoadsorption. There are first reports on a beneficial outcome in patients treated by immunoadsorption. However, the presence of antiheart specific autoantibodies may not always be harmful because some antibodies seem to be protective in chronic heart failure. In the following we will discuss the pathophysiological role of autoimmunity in heart failure with focus on the humoral immune response.

Induction of an Immune Response to Auto-Antigens

The normal consequence of an adaptive immune response to foreign antigens in healthy individuals is the clearance of these foreign antigens from the body. Usually, clonal deletion by ubiquitous self-antigens and clonal inactivation by tissue-specific antigens presented in the absence of costimulatory signals induce self-tolerance, and there is no induction of an immune response to self-antigens. However, a defect in this selection process results in an adaptive immune response to self-antigens and damage of self-tissue. This injury leads to constant supply of new autoantigens, which induces persistent immune response. Hereby, autoreactive CD4+ T cell support is required, which are usually selected during their development in the thymus (Figure 1). Autoimmune diseases can be mediated by autoreactive antibodies by forming

Figure 1. T-cell selection in the thymus. Committed lymphoid progenitors arise in the bone marrow and migrate to the thymus. Early committed T cells lack expression of T-cell receptor (TCR), CD4, and CD8 and are termed double-negative (DN; no CD4 or CD8) thymocytes. DN thymocytes can be further subdivided into 4 stages of differentiation (DN1, DN4, CD4+CD25+; DN2, CD4+CD25-; DN3, CD4+CD25+; and DN4, CD4+CD25-). As cells progress through the DN2 to DN4 stages, they express the pre-TCR, which is composed of the nonrearranging pre-T chain and a rearranged TCR chain. Successful pre-TCR expression leads to substantial cell proliferation during the DN4 to double positive (DP) transition and replacement of the pre-TCR chain with a newly rearranged TCR α-chain, which yields a complete αβ-TCR. The αβ-TCR CD4+CD8+ DP thymocytes then interact with cortical epithelial cells that express a high density of myosin heavy chain (MHC) class I and class II molecules associated with self-peptides. The fate of the DP thymocytes depends on signaling that is mediated by interaction of the TCR with these self-peptide–MHC ligands. Too little signaling results in delayed apoptosis (death by neglect). Too much signaling can promote acute apoptosis (negative selection); this is most common in the medulla on encounter with strongly activating self-ligands on hematopoietic cells, particularly dendritic cells. The appropriate, intermediate level of TCR signaling initiates effective maturation (positive selection). Thymocytes that express TCRs that bind self-peptide–MHC-class–I complexes become CD8+ T cells, whereas those that express TCRs that bind self-peptide–MHC-class–II ligands become CD4+ T cells; these cells are then ready for export from the medulla to peripheral lymphoid sites. SP indicates single positive. Reprinted with permission from Germain R.N. et al.4
immune complexes, activating the complement system, binding to surface receptors, and influencing downstream signaling.5–9

The exact triggers for induction of an autoimmune response are not well known, but various human autoimmune diseases display a myosin heavy chain (MHC)-linked association (multiple sclerosis, Graves’ disease, myasthenia gravis, rheumatoid arthritis).10–14 Although, during the last years, it has been discovered that autoimmunity plays an important role in the pathogenesis of myocarditis and dilated cardiomyopathy (DCM) in genetically predisposed individuals, to our knowledge barely any of the available genetic studies in familial DCM has taken into account the autoimmune phenotype markers in the characterization of patients and relatives. In one study Caforio et al demonstrate that relatives of DCM patients show higher frequency of circulating autoantibodies, possibly predisposing them to developing DCM themselves.1 However, large genetic association studies with prevalence of cardiac autoantibodies are missing yet. We know from animal models of autoimmune myocarditis induced by viral infection or immunization with heart-specific autoantigens that there is a genetic predisposition for susceptibility.15

Cardiac autoimmunity can be triggered by autoantigens presented to the immune system following cardiac injury induced by endogenous or exogenous factors (such as viral infections). Molecular mimicry and cross-reactivity may play an important role in inducing an autoimmune response, especially in individuals with cardiotropic virus infections (Figure 2).16 The autoantibodies can hereby influence cardiac function by negative chronotropic and/or negative inotropic effects. Furthermore, they can induce apoptosis of cardiomyocytes and activate complement.16–19

**Link Between B-Cells and the Innate Immune System**

B-cells represent a crucial link between the innate and adaptive immune system. Besides the antigen-specific B-cell receptors, B-cells also express toll-like receptors (TLRs) on their cell surface. These receptors, being highly evolutionary conserved, are activated by both exogenous (bacterial or viral) or endogenous (cell-derived or ECM-derived) antigens, and thereby constitute an essential part of the innate immune system. TLR-signaling in B-cells is critically associated to B-cell activation and tolerance and to diverse pathological conditions, such as atherosclerosis, viral myocarditis, and septic cardiomyopathy (Figure 3).20,21 Stimulation of TLRs on B-cells leads to the activation of an intracellular signaling cascade in which the myeloid differentiation factor MyD88 and interleukin-1 receptor-associated kinase (IRAK) play a dominant role in generating autoreactive plasma cells.21 Signaling through the MyD88/IL-1R axis is linked to cardiac fibrosis during progression to heart failure.22 In coxsackievirus B3 (CVB3)-induced myocarditis in mice, the TLR-9 mediated activation of MyD88 with subsequent activation of TNF-α contributes to the development of acute myocarditis.23 Further, the severity of myosin-induced experimental autoimmune myocarditis has been shown to be dependent on TLR-7 triggered MyD88 activation.24 The role of TLR-signaling in human heart failure however is poorly understood. Two studies demonstrate that TLR-4 expression is upregulated in human heart failure.25,26 It has to be considered that short-term activation of TLRs located in myocardial tissue confers cytoprotection, whereas long-term activation results in upregulation of proinflammatory cytokines and recruitment of immunoreactive cells (such as neutrophils, monocytes, and dendritic cells) into the myocardium.20 Furthermore B cells can activate complement. Complement and complement receptors play an important role in both the CVB3-induced and in myosin-induced myocarditis.27–29

**β1-Adrenoceptor/M2-Receptor**

β1-adrenoceptors (β1-AR) play an important role in adrenergic regulation of myocardial contractility. They belong to the 7-transmembrane G-protein coupled receptors and stimulation by catecholamines activates the adrenoceptor-adenylylcyclase–protein kinase A cascade (PKA).30 Activation of β1-AR induces activation of adenylylcyclase, which in turn

![Figure 2. Overview on potential interactions in inducing an autoimmune response leading to cardiac damage.](http://circres.ahajournals.org/)

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leads to an increased synthesis of the second messenger cyclic adenosinemonophosphate (cAMP). PKA, which is activated by cAMP, phosphorylates L-Type Ca\(^{2+}\)-channels leading to an increased Ca\(^{2+}\)-influx from the extracellular milieu and Ca\(^{2+}\)-release from the sarcoplasmic reticulum. Overall, this β1-receptor mediated signaling cascade increases the contractility of the heart. There exists strong evidence that autoantibodies targeted against the β1-receptor play a pivotal role in human heart failure.

One of the first studies demonstrating that β-adrenergic receptor targeted autoantibodies exist in dilated cardiomyopathy (DCM) was reported by Limas et al.\(^3\) By use of a bio-assay measuring the binding of labeled \[^3\text{H}\] dihydroalprenolol to rat cardiomyocyte membranes they observed a competitive inhibition of \[^3\text{H}\] dihydroalprenolol on incubation with sera from DCM patients. This could not be observed with sera from ischemic/valvular heart disease patients and healthy controls. The inhibitory effect was mediated by β-adrenergic autoantibodies of the IgG class. Magnusson et al compared the sera of patients with DCM or ischemic heart disease (IHD) to healthy controls to test the antigenic determinants of the β1- and β2-AR with regard to the binding specificity to the second extracellular loop of the human β1- and β2-adrenergic receptors.\(^3\) They found antibodies directed to the antigenic β1-second extracellular loop in 31% of patients with DCM and only 12% of healthy controls. The functional consequences of β1-AR autoantibody binding have first been characterized on spontaneously beating myocytes of neonatal rat heart.\(^3\) Immunization of rabbits with a synthetic peptide corresponding to the second extracellular loop of β1-AR leads to production of IgG autoantibodies against this domain.\(^4\) As a consequence, these animals revealed a decreased density of β1-AR and increased expression of inhibitory G-proteins and G-protein receptors kinase 5, leading to reduced intracellular cAMP levels. Six months after immunization a left ventricular hypertrophy and contractile dysfunction was observed. The β1-AR desensitization and functional consequences could be prevented by using the selective β1-AR antagonist bisoprolol. The circulating levels of β1-AR autoantibodies were markedly increased by induction of experimental heart failure and cardiomyopathy in animal models.\(^5\) In recent years, blocking β1-AR with specific antagonists has been refined as a potential therapeutic strategy to effectively modulate the stimulating and uncoupling effects of β1-AR autoantibodies. Although several studies show an improved outcome of β-blockers in a clinical setting, more specific agents directed against the activating site of the autoantibodies are under development.\(^6,7\) Although β1-AR autoantibodies are associated with a high risk for progression and prevalence of heart failure, consistent information on their prevalence, their frequency of appearance, formation, and their kinetics in human blood is still lacking. The Etiology, Titre-Course, and Survival study is the largest clinical multicenter trial which will investigate the prevalence and kinetics of autoantibodies in different forms of heart failure until 2013.\(^8\) In the
prospective arm of this trial including 400 patients with myocarditis or myocardial infarction will be assessed for induction of β1-AR autoantibodies. The patients will be followed for cardiac remodeling, heart failure, arrhythmias and mortality. It is obvious that for treatment of the pathogenic autoantibodies, precise diagnostic assays are required, specifically to monitor treatment effects and to determine criteria for initiation of treatment. Nikolaev et al have established a highly sensitive detection method which measures β1-receptor-mediated increases in intracellular cAMP levels by fluorescence resonance energy transfer using a highly sensitive cAMP sensor, which will be used in the Etiology, Titre-Course, and Survival study.41

In other studies it has been shown that sera of patients with idiopathic DCM react with M2-muscarinic receptor peptide (36% to 39%) and β-AR peptide (31%), respectively.42–44 A synthetic peptide corresponding to the sequence 169 to 193 of the second extracellular loop of the human M2-receptor has been used as a capture antigen to screen sera of patients with idiopathic DCM and healthy blood donors by use of ELISA.42 Only 8% of healthy subjects revealed a signal corresponding to the M2-peptide, whereas 39% of DCM patients were positive for this peptide. Also, a highly significant coexistence has been found of anti-M2-receptor autoantibodies and anti-β1–AR autoantibodies in patient’s sera with idiopathic DCM.42 Antibodies directed against the sequence 169 to 193 of M2-receptor have been shown to exert a negative chronotropic and inotropic effect and inhibit adenylyl cyclase activity.45,46 Immunization of rabbits with peptides corresponding to the second extracellular loop of M2-receptor-induced right, but not left ventricular dilatation.47 On histological analysis, focal myofibrillar-lysis, loss of myofilaments, mitochondrial swelling and condensation, sarcoplasmic vacuolation, deposition of dense granules in the sarcoplasm, and the myofibrils were observed after immunization.48 Using specific adrenergic and muscarinic-blocking agents combined with an in vitro canine Purkinje fiber contractility assay Stavrakis et al studied the opposing effects and interactions of anti-β1–AR autoantibodies and anti-M2–receptor autoantibodies on contractility in vitro.48 Stavrakis et al showed that circulating anti-M2–receptor autoantibodies derived from sera of DCM patients compromised the agonistic inotropic effects of circulating anti-β-AR-1 autoantibodies, by binding on M2-receptors. The authors therefore propose that circulating activated autoantibodies to the muscarinic M2-receptor may reduce ventricular contractility and thereby promote heart failure in patients with cardiomyopathy.48 However, circulating anti-M2 or anti-β1–AR autoantibodies may not be cardiac-specific, which questions their exclusive role in heart failure.

Antimitochondrial Autoantibodies

There are numerous reports on specific antimitochondrial antibodies and their clinical relevance in a number of pathological disorders. The most relevant mitochondrial antigens M1, M2, and M7 are located in the inner mitochondrial membrane, whereas M3, M4, M5, M6, M8, and M9 are located in the outer mitochondrial membrane.49 Anti-M7 antibodies are detectable in patients with DCM and there are reports on their functional relevance.50 Thus, anti-M7 antibodies were observed in blood of 31% of DCM patients, 33% of hypertrophic cardiomyopathy patients, and 13% of acute myocarditis patients. In contrast, no anti-M7 antibodies could be found in control patients with other cardiac or immunologic disorders. These antibodies react either specifically with heart mitochondria (anti-M7 type a) or reveal cross-reactivity with antigenic determinants of pig kidney, beef pancreas, or rat lung (anti-M7 type b). Using immobilized submitochondrial particles derived from the myocardium, immunoadsorption (IA) of anti-M7 autoantibodies was able to abolish anti-M7 type a and type b activity. The authors therefore proposed cardio-specificity for both types of anti-M7 antibodies, although mitochondrial antigens are generally considered not to reveal tissue-specificity.51

The adenine-nucleotide transporter (ANT) is an ADP/ATP carrier located in the inner mitochondrial membrane.52 The existence of autoantibodies directed against ANT in dilated cardiomyopathy has been shown by Schultheiss et al in 1985.53 In their uncontrolled study, functionally active antibodies against ANT were observed in 94% of 18 patients with DCM.53 In contrast, in patients with coronary heart disease, suspected alcoholic heart disease or healthy blood donors no anti-ANT antibodies were observed. Antibody titers were inversely related to the clinical outcome of DCM. Epitope mapping revealed that the antigenic determinants of ANT are located in the C-terminal 146 amino acids, corresponding to the M2 and M3 hydrophilic region allocated to the mitochondrial matrix space.54 The amino acid sequence of ANT protein has, to some extent, homology to CVB3.55 Thus it was tempting to postulate a molecular mimicry of autoantibodies to ANT and CVB-infection, which is an established infectious agent causing myocarditis.56 In support of this hypothesis, immunization with CVB3 induces a marked production of ANT autoantibodies in mice, leading to specific alterations in cellular energy consumption and calcium homeostasis.57,58 The IL-17 producing CD4+ Th-cell subset (Th-17) is critically linked to the progression of inflammatory dilated cardiomyopathy.59 When circulating IL-17 is neutralized, anti-ANT autoantibody production is diminished in a CVB3 model of auto-immune myocarditis. It is hypothesized that the protective effect of IL-17 inhibition on cardiac inflammation is due to inhibition of CD19(+) B-lymphocyte proliferation and reduction of secretion of anti-ANT autoantibodies by these cells.60

Myosin

Neu et al described in 1987 that immunization of mice with cardiac, but not skeletal myosin, induces severe myocarditis accompanied by high titers of myosin autoantibodies in sera.61 In this study the authors also very elegantly showed that the genetic background of immunized mice determined the prevalence and severity of myocarditis and circulating levels of cardiac myosin autoantibodies. Genetic analysis provided some evidence that susceptibility to myocarditis was linked to the major histocompatibility complex genotype and other not well-defined genes. However a more refined analysis would have required genome wide association studies in inbred mice strains with and without susceptibility to antibody induced myocarditis. In humans and predominantly
after birth α-myosin heavy chain is strictly expressed in atrial cardiomyocytes, whereas the β-isofrm is expressed in both slow skeletal muscle and ventricular cardiomyocytes.65 66 Califorio et al provided some evidence that myosins may be detectable in blood of patients with DCM, as assessed by Western blot.65 Although this is intriguing to explain the prevalence of anti-α- and anti-β-myosin heavy chain antibodies in blood, more precise analyses are needed to definitively test for the circulating myosin heavy chains in heart failure. Only by the use of high sensitivity troponin assays it was possible to identify some marker positive patients with DCM. Antimyosin IgG autoantibodies are also detected in patients with myocarditis.64 ELISA and Western blotting analysis revealed that 42% patients with myocarditis exhibited autoantibodies against myosin. These antimyosin autoantibodies do not differentiate between myosin prepared from either cardiac or skeletal muscle, respectively, indicating the known tissue distribution of α- and β-myosin heavy chains. Anti-myosin autoantibodies can also be detected in experimental models of autoimmune myocarditis induced by CVB3 infection.65 In patients with DCM the presence of antimyosin autoantibodies was associated with deterioration of myocardial function.66 To investigate the functional effects of antimyosin antibodies, Warraich et al affinity purified antcardiac myosin autoantibodies of patients with DCM or IHD.67 When isolated cardiomyocytes were exposed to antcardiac myosin autoantibodies an altering of Ca2+-sensitivity of myofilaments and reduction of contractility of cardiomyocytes was observed. However, affinity-purified autoantibodies were not internalized by myocytes and had no effect on L-type Ca2+-currents. Because α- and β-myosin isoforms are both located in the sarcomer compartments, the mechanisms of antibody interaction and intracellular signal transduction are only poorly understood. A number of studies show that antcardiac myosin monoclonal antibodies lead to myocarditis in some mouse strains.68–70 However these observations do not resolve the molecular mechanism leading to heart failure. Li et al showed that antcardiac myosin autoantibodies cross-react with the β-AR, thereby specifically inducing cAMP-dependent PKA activity in heart cells.71 Therefore the activation of the β-AR dependent PKA signaling pathway might contribute to myocardial dysfunction and apoptosis of cardiomyocytes.72–73 In support of this, the addition of cardiac myosin, nonspecific anti-IgG or specific inhibitors of the β1-AR pathway inhibited the antcardiac myosin autoantibody-mediated PKA signaling. Li et al also showed that transfer of purified antcardiac myosin IgG from immunized rats results in myocardial IgG deposition and cardiomyopathy.74 In summary, molecular mimicry between cardiac myosin and the β1-AR is an attractive hypothesis to explain the antcardiac myosin autoantibody mediated induction of heart failure. The points raised so far are predominantly related to an autoantibody mediated humoral immune response. There are, however, also very convincing data on the role of the cellular and innate autoimmunity on myocardial function. Very recently Lv et al showed that α-myosin heavy chain (α-myosin–HC), although being located strictly intracellularly in atrial cardiomyocytes, acts as an autoantigen for CD4+ T-cells in myocarditis.74 The authors also show that α-myosin–HC transcripts are absent in murine and human thymus medullary epithelial cells, which are crucial for development of self-tolerance and autoimmunity.75 Transgenic expression of α-myosin–HC transcripts in medullary epithelial cells prevented myocarditis by inducing self-tolerance to α-cardiac–myosin-HC. Further α-myosin–HC specific Th1 CD4+ T-cell clones induced myocarditis after being transferred to DQ8+Rag−/− NOD mice, thereby demonstrating that spontaneous myocarditis is caused by loss of CD4+ T-cell tolerance to α-myosin–HC. The current literature on antimyosin autoantibodies thus proposes an imperfect selection process of (self-) antigens in the thymus during T-cell development, leading to generation of CD4+ Th-cells targeted against α-myosin. Molecular mimicry between α-myosin and β1-AR may then trigger the signal transduction of α-myosin autoantibody interaction intracellularly leading to β1-AR dependent functional impairment of cardiomyocytes.71–74

Cardiac Troponin I

Cardiac troponin is part of the regulatory complex of the thin filament of muscle fibers.76 It consists of the 3 subunits troponin C, T, and I, which interact in regulation of muscle contraction. Recently, it has been shown that mice deficient of programmed cell death-1 immune inhibitory coreceptor protein develop severe DCM followed by progressive heart failure in mice.77 In these mice, antibodies of IgG subtype could be detected on the surface of cardiomyocytes. These antibodies were specific for cardiac troponin I. Interestingly, administration of these anti-TnI-antibodies augmented the voltage-dependent L-type Ca2+-current of cardiomyocytes and induced ventricular dilatation and heart failure.78 These findings indicated that cardiac troponin I may not be strictly localized in the cytoplasm but may also be exposed on the cytoplasmic membrane, making it accessible for antigen–antibody interactions. Recently, we could show that immunization of mice with cardiac troponin I but not with cardiac troponin T induced severe inflammation in the myocardium followed by fibrosis and heart failure with increased mortality,79 although immunization with both troponin I and troponin T induced a strong cellular and humoral immune response. It is likely that differences in troponin expression in the cytoplasmic membrane may account for the observed differences. Elevated troponin I autoantibodies can also be detected in a virus-induced experimental auto-immune myocarditis model on infection with CVB3 virus.80 The antigenic determinant of the murine troponin I molecule that causes severe inflammation and fibrosis appears to be an 18-mer peptide of troponin I.81 Only mice immunized with residues 105 to 122 (referred to as peptide 9) of murine troponin I developed significant inflammation and fibrosis in the myocardium with increased expression of proinflammatory cytokines, chemokines, and chemokine receptors. This epitope of murine troponin I is located in the hydrophilic region of troponin I and comprises an α-helical structure, making it particularly susceptible to antigen–antibody interaction.

The clinical significance of circulating antitroponin autoantibodies as modulators of progression of DCM is not yet clear because there are conflicting results. Although I study
indicates an adverse effect of antitroponin autoantibodies on cardiac function and outcome in ischemic cardiomyopathy (ICM), other studies report on anti-Tnl antibody positive patients with very similar or even improved outcome as compared to TnI-antibody negative patients. Thus, it is still under debate whether in patients circulating antitroponin I autoantibodies modulate cardiac function or progression in heart failure.

Leuschner et al screened patients with DCM and ICM for the presence of troponin I autoantibodies and observed antitroponin I IgG antibody titer ≥1:160 in 7.0% of patients with DCM and 9.2% with ischemic cardiomyopathy. In this study the presence of anti-Tnl antibodies was associated with adverse remodeling post myocardial infarction. Patients with no elevated cardiac troponin I antibody titers showed an increase in left ventricular ejection fraction and stroke volume 6 to 9 months after acute myocardial infarction. In contrast, improvement of cardiac function and remodeling was not observed in patients with antitroponin I IgG antibody titer ≥1:160. Doesch et al investigated the prognostic value of circulating Tnl autoantibodies in plasma of patients with chronic heart failure. This study indicates that the presence of circulating Tnl autoantibodies in plasma is associated with an improved survival in patients with chronic DCM but not ICM. The authors thus propose a possible protective effect of circulating antitroponin I autoantibodies in DCM. However, further clinical and experimental studies are needed to finally elucidate the functional role of circulating Tnl autoantibodies and progression of DCM. In a prospective controlled study Halley et al examined the release of interferon-gamma and interleukin-10 (IL-10) from peripheral blood mononuclear cells on Tnl stimulation in 35 idiopathic DCM patients and 26 healthy controls. Although there was no difference in interferon-gamma secretion on Tnl stimulation in both groups, IL-10 secretion was significantly higher in the patients with DCM. Among DCM subjects, heightened IL-10 response to cardiac troponin I was associated with reduced systemic inflammation (reduced systemic levels of highsensitivity C-reactive protein) and lower prevalence of advanced diastolic dysfunction compared with those with normal IL-10 response to cardiac troponin I.

**Na-K–ATPase**

Impairment of sarcometal Na-K–ATPase activity in patients with idiopathic DCM has been reported previously. In a controlled study by Baba et al 100 patients with DCM and 100 age matched controls have been screened for anti-Na-K–ATPase autoantibodies using ELISA. Anti-Na–K-ATPase autoantibodies were detected in 26% of DCM patients and 2% of controls. Patients who were positive for anti-Na–K-ATPase autoantibodies showed more frequent premature ventricular complexes and nonsustained ventricular tachycardia. Na-K–ATPase activity has been measured in vitro in the presence of purified IgG from patients with and without anti-Na–K-ATPase autoantibodies. IgG from patients with anti-Na–K-ATPase autoantibodies lowered the activity of Na-K–ATPase in vitro, thereby demonstrating the biological activity of these autoantibodies. Further, Western blotting and radio-ligand binding analysis revealed that the Na-K–ATPase α subunit is bound by corresponding autoantibodies and that these autoantibodies exert an inhibitory effect. The authors therefore suggested that binding of Na-K–ATPase autoantibodies results in a conformational change leading to a low-affinity of the ATPase. They further hypothesize that the reduction of Na-K–ATPase activity due to corresponding antibody–antigen interactivity leads to abnormal intracellular Ca²⁺ handling and delayed afterdepolarizations via reverse-mode operation of the Na⁺/Ca²⁺ exchanger resulting from increased intracellular Na⁺ concentrations. Further, it has been shown that immunization of rabbits with sarcometal Na-K–ATPase results in myocardial hypertrophy due to left ventricular pressure overload and myocardial fibrosis. Immunoblotting showed that expression of the α3-isofrom of Na-K–ATPase was selectively reduced in myocardium in immunized animals. Taken together the presence of autoantibodies against Na-K–ATPase has been linked to clinical outcome of DCM. However, little is known on the specificity, role, and mechanism of Na-K–ATPase autoantibodies in other forms of heart failure.

**Other Heart Antibodies**

Latif et al screened sera of 45 patients with DCM and 43 patients with IHD for antihuman antibodies. Western blotting and 2-dimensional SDS-gel electrophoresis followed by N-terminal protein sequencing revealed that DCM patients, in contrast to IHD patients, showed significantly more frequently elevated autoantibodies. The most prevalent antigens were myosin light chain1, MHC, actin, troponymosin, and heat-shock protein 60 (hsp-60). Anti-hsp–60 autoantibodies were found in 85% of the DCM patients, whereas only 42% of patients with IHD were positive. Heat-shock proteins are highly conserved immunogenic molecules that are located intracellularly. They are part of the chaperone system, are involved in protein folding, and are upregulated in myocardial stress. In the study by Latif et al antmyosin autoantibodies were not the predominant autoantibodies in sera of DCM patients, as proposed by Caforio et al. 67% DCM patients were positive for antmyosin autoantibodies whereas 85% were positive for anti-hsp–60 autoantibodies. Also, Latif et al were not able to detect autoantibodies against β1-AR, ANT, or mitochondrial M7 antigen, neither in DMC nor in IHD patients. A difference that is explained by the use of the unfractionated myocardial homogenate in their studies. In CBV3-induced murine experimental auto-immune myocarditis antmyosin autoantibodies but not anti-hsp–60 autoantibodies are the predominant antibody fraction. The pathogenic role of anti-hsp–60 autoantibodies in the development of heart failure is still unknown. Very recently, Lin et al induced heart failure in rats by coronary ligation of left anterior descending artery. They could show that hsp-60 after induction of heart failure was translocated to the plasma membrane and, more importantly, it was also detectable on the cell-surface, thus being a potential target for antibodies or the innate immune system. Membrane hsp-60 correlated with increased apoptosis. The authors concluded that abnormal trafficking of hsp-60 to the cell surface may be an early trigger for myocyte loss and the progression of heart failure. Hsp-60 might also be pathologically significant by inducing cell lysis.
through activating complement. However, anti-hsp–60 autoantibodies are not cardiac-specific, and it remains to be elucidated whether anti-hsp–60 autoantibodies may serve as a diagnostic marker or as a specific target against heart failure. A number of other antigenic targets for autoantibodies have been described, but their pathogenic role is not understood (Table 1).

### Autoantibodies as a Clinical Target for Therapy of Heart Failure

Most therapeutic efforts target the humoral immune system by eliminating circulating autoantibodies by IA (Table 2, Figure 4). A number of these clinical studies have provided some encouraging results, but clearly more robust data and randomized trials are needed. One of the clinical pilot studies using IA strategy for treatment of DCM in patients was done by Wallukat et al: They first showed that IA removed circulating IgG3 antibodies targeted against /H92521-AR and later that IA may improve cardiac function in patients. 18 patients with DCM (NYHA: III–IV; LVES < = 30%) were randomly assigned either to IA with subsequent IgG substitution (IA/IgG) or control. IA/IgG patients showed significant improvement in hemodynamic parameters (cardiac index, stroke volume index, systemic vascular resistance) three months after therapy. However, this study did not report specifically on the changes of autoantibody levels following IA and was not adequately controlled and blinded. In a following retrospective study, patients with congestive heart failure (CHF) or DCM (NYHA class II–III; LVES < = 35%) who received IA therapy showed significantly decreased hospitalization rate compared to CHF- or DCM- controls during 3 years follow up.

In further studies, it has been shown that apart from improving cardiac hemodynamic parameters in DCM patients, IA influences the cellular component of the adaptive immune system. From 25 DCM patients (ejection fraction [EF] < 30%), 12 patients were randomized for IA therapy and subsequent IgG substitution at 1-month intervals until month

### Table 1. Overview of Autoantibodies Associated With Heart Failure and Cardiac Dysfunction

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Cardiomyopathy</th>
<th>Suggested Antigenic localization</th>
<th>Suggested Pathomechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-β1-receptor</td>
<td>DCM</td>
<td>Cell surface?</td>
<td>Negative ionotropic</td>
<td>Limas et al. 38</td>
</tr>
<tr>
<td>Anti-M2-receptor</td>
<td>IDCM</td>
<td>Second extracellular loop M2 (amino acids 163–169)</td>
<td>Negative ionotropic</td>
<td>Fu et al. 42, 43</td>
</tr>
<tr>
<td>Antimitochondrial M7</td>
<td>DCM acute myocarditis</td>
<td>Inner mitochondrial membrane</td>
<td>Unknown</td>
<td>Klein et al. 50</td>
</tr>
<tr>
<td>Adenine-nucleotide transporter</td>
<td>IDCM, DCM</td>
<td>C-terminal 146 amino acids allocated in the mitochondrial matrix</td>
<td>Alterations in energy metabolism</td>
<td>Schultze et al. 53, Manchado et al. 54</td>
</tr>
<tr>
<td>Myosin heavy chain alpha and beta</td>
<td>DCM</td>
<td>Alpha: atrial cardiomycocytes Beta</td>
<td>Negative ionotropic</td>
<td>Caforio et al. 63</td>
</tr>
<tr>
<td>Myosin heavy chain alpha</td>
<td>Myocarditis (mouse)</td>
<td>Intracellularly</td>
<td>Deterioration of self-tolerance during thymic selection processes</td>
<td>Lv et al. 74</td>
</tr>
<tr>
<td>Anti-troponin I</td>
<td>DCM (mouse)</td>
<td>Cell surface</td>
<td>Negative ionotropic</td>
<td>Okazaki et al. 78</td>
</tr>
<tr>
<td></td>
<td>Myocarditis/DCM (mouse)</td>
<td>Cell surface</td>
<td>Negative ionotropic</td>
<td>Goeser et al. 79</td>
</tr>
<tr>
<td></td>
<td>Myocarditis/DCM (mouse)</td>
<td>Amino acid residues 105–122</td>
<td>Negative ionotropic</td>
<td>Kaya et al. 81</td>
</tr>
<tr>
<td>Anti-Na-K-ATPase</td>
<td>IDCM (human)</td>
<td>Alpha3-subunit</td>
<td>Antiarrhythmic effect due to a conformational change of Na-K-ATPase from a high-affinity to low-affinity state.</td>
<td>Baba et al. 86</td>
</tr>
<tr>
<td></td>
<td>DCM (rabbit)</td>
<td>Sarcolemmal transmembrane</td>
<td>Cardiac hypertrophy</td>
<td>Baba et al. 87</td>
</tr>
<tr>
<td>Anti-hsp60</td>
<td>DCM/Ischemic heart disease (human)</td>
<td>Cell surface</td>
<td>Unknown</td>
<td>Latef et al. 90</td>
</tr>
<tr>
<td></td>
<td>Experimental coronary artery ligation (rat)/DCM/ICM (human)</td>
<td>Cell-surface</td>
<td>Abnormal trafficking of hsp-60 to the cell surface to trigger myocyte loss after LAD- ligation</td>
<td>Lin et al. 92</td>
</tr>
<tr>
<td>Antiserotonergic 5-HT4 receptor</td>
<td>Congenital heart block (human)</td>
<td>Cell surface</td>
<td>Unknown</td>
<td>Kamel et al. 95</td>
</tr>
<tr>
<td>Anti-SR-Ca2+/ATPase</td>
<td>Experimental myocarditis (mouse)</td>
<td>Cell surface</td>
<td>Metabolic interactions</td>
<td>Khaw et al. 96</td>
</tr>
<tr>
<td>Antiacetylcholine receptor</td>
<td>Bradycardia</td>
<td>Cell surface</td>
<td>Unknown</td>
<td>Goin et al. 94</td>
</tr>
<tr>
<td>Antilaminin</td>
<td>DCM/myocarditis</td>
<td>Extracellularly</td>
<td>Unknown</td>
<td>Wolf et al. 103</td>
</tr>
<tr>
<td>Antitroponymosin</td>
<td>DCM</td>
<td>Intracellularly</td>
<td>Unknown</td>
<td>Latef et al. 90</td>
</tr>
<tr>
<td>Antitau</td>
<td>DCM</td>
<td>Cell surface</td>
<td>Unknown</td>
<td>Latef et al. 90</td>
</tr>
<tr>
<td>Anti-myosin light chain-1</td>
<td>DCM</td>
<td></td>
<td>Unknown</td>
<td>Latef et al. 90</td>
</tr>
</tbody>
</table>

DCM indicates dilated cardiomyopathy; ICM, ischemic cardiomyopathy; hsp60, heat-shock protein 60; IDCM, idiopathic dilated cardiomyopathy; LAD, left anterior descending artery.
Table 2. Overview of Clinical Studies for Treatment and Their Clinical Outcome

<table>
<thead>
<tr>
<th>Autoantibody Targeted</th>
<th>Study Type</th>
<th>Type of Clinical Intervention</th>
<th>Patients and Cardiomyopathy</th>
<th>Clinical Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not specified</td>
<td>Prospective uncontrolled</td>
<td>Immunoabsorption</td>
<td>chronic DCM and NYHA class II–III CHF</td>
<td>Improvement in hemodynamic parameters</td>
<td>Cooper et al. 104</td>
</tr>
<tr>
<td>Not specified</td>
<td>Retrospective controlled</td>
<td>Immunoabsorption</td>
<td>DCM, left ventricular ejection fraction less than 35%, NYHA classes II–III</td>
<td>Improvement in hemodynamic parameters and reduction of morbidity</td>
<td>Knebel et al. 106</td>
</tr>
<tr>
<td>Not specified</td>
<td>Randomized uncontrolled 6 mo (Group 1: n=11 with four IA courses at monthly intervals. Group 2: n=11 with one IA course without repetition)</td>
<td>Immunoabsorption</td>
<td>DCM (left ventricular ejection fraction &lt;35%)</td>
<td>One course of IA treatment is comparable to multiple IA courses</td>
<td>Staudt et al. 107</td>
</tr>
<tr>
<td>Beta-1-AR-autoantibody</td>
<td>Prospective uncontrolled</td>
<td>Specific Immunoabsorption</td>
<td>DCM (NYHA III–IV, EF &lt;30%, stable medication)</td>
<td>The beneficial hemodynamic effects induced by IA are not directly associated with the removal of beta(1)AR autoantibodies</td>
<td>Mobini et al. 114</td>
</tr>
<tr>
<td>Not specified</td>
<td>Analysis of myocardial gene expression of the intermediate cytoskeletal filament desmin (n=6) 3 mo</td>
<td>Immunoabsorption</td>
<td>DCM (LVEF &lt;40%, NYHA II–III)</td>
<td>Myocardial desmin gene expression is significantly decreased upon IA therapy</td>
<td>Kallwellis-Opera et al. 115</td>
</tr>
<tr>
<td>Not specified</td>
<td>Prospective uncontrolled</td>
<td>Immunoabsorption</td>
<td>CHF (NYHA III and IV secondary due to chronic iDCM with EF &lt;35%)</td>
<td>Improvement in endothelial function and significant decrease of circulating microparticles</td>
<td>Bulut et al. 116</td>
</tr>
<tr>
<td>Not specified</td>
<td>Prospective randomized double blinded Not specified (n=20)</td>
<td>Intravenous immunoglobulins</td>
<td>chronic symptomatic CHF and LVEF of &lt;40%</td>
<td>Antiinflammatory effect on cytokine level which correlates to improved LVEF upon immunoglobulin treatment</td>
<td>Gullestad et al. 120</td>
</tr>
<tr>
<td>Not specified</td>
<td>Prospective double blinded placebo controlled (n=20) 24 wk</td>
<td>Intravenous immunoglobulins</td>
<td>Reduction of MIP-1alpha, MIP-1beta and IL-8 protein levels correlates with LVEF</td>
<td>Improvement of cardiac function by intravenous immunoglobulins is not due to neutralization of beta1AR autoantibodies</td>
<td>Damas et al. 129</td>
</tr>
<tr>
<td>Beta1-AR-auto-antibody</td>
<td>Prospective randomized plabeo controlled (CAD n=21) (DCM=12) 6 mo</td>
<td>Anti-idiotypic antibodies</td>
<td>CHF (NYHA functional class II/III)</td>
<td></td>
<td>Larsson et al. 130</td>
</tr>
</tbody>
</table>

DCM indicates dilated cardiomyopathy; IA, immunoadsorption; CAD, coronary artery disease; ICM, ischemic cardiomyopathy; IL, interleukin; CHF, congestive heart failure; EF, ejection fraction; MIP, monocyte inhibitory protein.

3. In this study a beneficial effect of IA/IgG therapy on myocardial inflammation was observed with decrease in the number of CD4+ and CD8+ T lymphocytes and total number of leukocytes in the myocardial biopsies. Further, IA/IgG treatment reduced the expression of HLA class II antigens. Differential expression of HLA antigens, a component of the major compatibility complex (MHC), has been critically linked to pathogenesis of DCM.112,113 Also, anti-β1–AR autoantibodies were significantly reduced in IA/IgG treated patients.111 Removal of anti-β1–AR IgG3 autoantibodies may not be linked to the beneficial hemodynamic outcome after IA therapy, because hemodynamic improvement after IA therapy was similar among patients positive and negative for β1-AR autoantibodies, as shown in an unblinded clinical study including 22 DCM patients (NYHA III–IV, EF <30%) by Mobini et al114 Opara et al showed in a case-control study with 6 DCM patients (LVEF <40%, NYHA II–III) that IA affects gene expression of the type III intermediate filament protein desmin, in that expression levels of desmin in endomyocardial biopsies were significantly decreased in patients after IA therapy.115 The authors suggest that production of cardiac autoantibodies is linked to DCM-associated changes in myocardial gene expression and that removal of these antibodies by IA therapy may modulate myocardial gene expression. Another study by Bulut et al shows the effect of IA on the concentration of endothelial microparticles (eMP) in blood of 13 patients with advanced CHF (NYHA III and IV) secondary due to chronic iDCM (EF <35%).116 Microparticles, which include exosomes, microvesicles, apoptotic bodies, and apoptotic microparticles, are small (0.1–1 μm), membranous vesicles that can contain DNA, RNA, miRNA, intracellular proteins, and express
extracellular surface markers from the parental cells. Elevated levels of eMP are found in patients with heart failure. Bulut et al reported reduced levels of circulating eMP in DCM patients after IA treatment. However, a causal relationship between IA treatment and decreased eMP levels was not proven in this study and the authors therefore cannot rule out confounding (and yet unknown) factors that might have caused the observed changes in endothelial function after IA. Taken together IA therapy holds the potential for clinical care of DCM or heart failure, yet larger controlled trials are urgently needed.

Immunoglobulin mixtures as clinically available in IVIG preparations are composed of diverse immunoactive immunoglobulins, mostly of IgG subtype, made from human plasma of a batch of healthy donors. In a double-blinded controlled study with 40 patients with chronic symptomatic CHF and LVEF of $<40\%$ IVIG, but not placebo treatment, changed the balance between inflammatory and anti-inflammatory cytokines in chronic heart failure in humans, which correlated with improved hemodynamic cardiac parameters. The potential of IVIG to prevent CHF and to reduce proinflammatory cytokines has been shown in an experimental model of virus induced autoimmune myocarditis in experimental Chagas disease and in human DCM. IVIG preparations are believed to bind to and neutralize circulating autoantibodies of diverse specificity.

IVIG consist of natural polyreactive antibodies that exhibit anti-idiotypicity and autoantibody neutralizing capacity. It has been reported that IVIG treatment has influence on cytokine balance and downregulates monocyte inhibitory protein (MIP)-1α, MIP-1β, and IL-8 protein levels in chronic heart failure. Whether IVIG treatment modulates circulating autoantibodies that are related to heart failure is still a matter of speculation because a direct proof for such an interaction is still lacking. In a prospective randomized placebo controlled study by Larsson et al rather questions the hypothesis that IVIG treatment neutralizes or modulates circulating autoantibodies because beneficial outcome of IVIG treatment in DCM was not due to neutralization of anti-β1-AR autoantibodies but was dependent on not yet clearly specified mechanisms. However, in other autoimmune diseases IVIG were capable of neutralizing autoantibodies against factor VIII, DNA, platelet, glycoproteins, cardioliopin, and cytoplasmic antineutrophil autoantibodies. Furthermore, animal studies propose that IVIG is capable of downregulating specific autoreactive B-cells.

The molecular and cellular mechanism on how IVIG are able to reduce or inhibit autoimmune responses is a matter of current research. A minor population of IVIG belonging to the IgG crystallizable fragments with glycans terminating in alpha2,6 sialic acids specifically bind to the lectin dendritic-cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN) on myeloid associated cells. Binding of sFC on DC-SIGN stimulates the very recently discovered DC-SIGN–Th2-pathway. The IVIG stimulated DC-SIGN–Th2-pathway leads to an upregulation of FgammaRIIB receptors on effector macrophages. FgammaRIIB receptors are transmembrane proteins that play a major role in autoimmunity and infection. Activation of FgammaRIIB receptors increases the activation threshold for an immune response by inhibiting the functions of activating Fgamma receptors. Further, FgammaRIIB is able to inhibit autoreactive B-cells function by decreasing antibody production. Application of IVIG thus may inhibit effector macrophages by activation of the DC-SIGN–Th2-pathway that leads to cross-linking FgammaRIIB receptors after stimulation with antigen-antibody complexes and subsequent proinflammatory cytokine production. However further experimental studies are needed to further elucidate the molecular action pattern of IVIG. In summary IVIG treatment reflects, besides immunoadsorption, a promising novel therapeutic option in order to treat heart failure with a pathological autoimmune mechanistic cause. However, it needs to be evaluated whether IVIG influences circulating autoaggressive autoantibodies in heart failure in any way. Further experimental or clinical data on such potential mechanisms are necessary in order to better...
understand a possible interaction between IVIG and autoantibodies in heart failure.

Future Directions

There should be more focused research on studying the role of antibodies and autoimmunity in the pathogenesis of not only myocarditis but also in the development of dilated cardiomyopathy even its role in postinfarct remodeling. Genetic studies in patients should be performed to identify genetic association for prevalence of cardiac autoantibodies and for susceptibility for autoimmune induced cardiomyopathy. Immunoabsorption, immunosuppression, and/or immunomodulation may be beneficial in some of these patients and cardiac-specific autoantibodies, which are shown to be disease-specific, could be used as biomarkers for identifying them and their relatives at risk. However, to get these therapeutic approaches established, more controlled, blinded, multecnter clinical studies are needed.

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Disclosures

Dr Katus developed the troponin T assay and holds a patent on this assay jointly with Roche Diagnostics. The remaining authors report no conflicts.

References


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