β₁-Adrenergic Receptor Function, Autoimmunity, and Pathogenesis of Dilated Cardiomyopathy

Roland Jahns*, Valérie Boivin, and Martin J. Lohse

Dilated cardiomyopathy (DCM) is a heart disease characterized by progressive depression of cardiac function and left ventricular dilatation of unknown etiology in the absence of coronary artery disease. Genetic causes and cardiotoxic substances account for about one third of the DCM cases, but the etiology of the remaining 60% to 70% is still unclear. Over the past two decades, evidence has accumulated continuously that functionally active antibodies or autoantibodies targeting cardiac β₁-adrenergic receptors (anti-β₁-AR antibodies) may play an important role in the initiation and/or clinical course of DCM. Recent experiments in rats indicate that such antibodies can actually cause DCM. This article reviews current knowledge and recent experimental and clinical findings focusing on the role of the β₁-adrenergic receptor as a self-antigen in the pathogenesis of DCM. (Trends Cardiovasc Med 2006;16:20–24) © 2006, Elsevier Inc.

Progressive cardiac dilatation and pump failure of unknown etiology and in the absence of coronary artery disease has been termed idiopathic dilated cardiomyopathy (DCM) (Richardson et al. 1996). Today, DCM represents one of the main causes of severe heart failure and disability in younger adults with an annual incidence of up to 100 patients and a prevalence of 300 to 400 patients per million (American Heart Association 2005, Centers for Disease Control and Prevention 1998). Mutations in genes encoding myocyte structural proteins and several cardiotoxic substances, including alcohol and anthracyclines, account for about one third of the cases (Morita et al. 2005). The etiology of the other two thirds, however, remains poorly understood. Patients with DCM often present alterations in both humoral and cellular immunity (Jahns et al. 1999b, Limas 1997, Luppi et al. 1998). Therefore, current theories on cardiac tissue injury in DCM focus on abnormal or misled immune responses to infections caused by cardiotropic viruses, bacteria, or parasites (Limas 1997, Rose 2001). In the context of their humoral immune response, a substantial number of cardiomyopathic patients develop cross-reacting antibodies and/or autoantibodies to various cardiac antigens, including membrane proteins (e.g., cell surface receptors (Jahns et al. 1999b, Magnusson et al. 1994), mitochondrial proteins (e.g., adenine nucleotide translocator [Schultheiss and Bolte 1985]), and myocyte structural proteins (e.g., actin, laminin, myosin, troponin [Caforio et al. 2002, Neumann et al. 1990, Okazaki et al. 2003]). In addition, genetic factors may contribute to the susceptibility to immunologic factors or to the phenotypic expression of the disease (Limas et al. 2004). Irrespective of whether development of DCM is primarily triggered by acute or chronic inflammatory or ischemic myocyte damage, or by abnormalities in the adaptive or innate immune system (Eriksson et al. 2003, Luppi et al. 1998), in both cases progressive cardiac tissue injury is thought to be mediated mainly by cytokines and/or heart-specific autoantibodies (Caforio et al. 2002, Eriksson et al. 2003, Limas 1997). The pathophysiologic importance of such heart-specific antibodies is, however, far from clear. Low titers of autoantibodies to certain housekeeping antigens are also found in healthy subjects as a part of the natural immunologic repertoire (Rose 2001). Moreover, under physiologic conditions at least the intracellularly localized myocyte antigens are not easily accessible for the immune system. From a pathophysiologic point of view, it seems thus very likely that the disease-inducing (i.e., harmful) potential of a specific autoantibody depends on two major factors: (a) the accessibility and (b) the functional importance of the respective target. Therefore, cell surface receptors, and in particular the contraction/relaxation-regulating cardiac β₁-adrenergic receptors (β₁-ARs), represent ideal candidates...
for pathophysiologically relevant antibodies (Freedman and Lefkowitz 2004, Limas 1997). The present article reviews current knowledge and recent experimental and clinical data focusing on the role of the \( \beta_1 \)-AR as an autoantigen in the pathogenesis of DCM.

**The Cardiac \( \beta_1 \)-AR**

The \( \beta_1 \)-AR belongs to the family of the G protein-coupled receptors and constitutes about 70% to 80% of the cardiac \( \beta \)-AR complement (Frielle et al. 1987). It has ligand-binding properties that clearly distinguish it from the other \( \beta \)-ARs (Hoffmann et al. 2004). The receptor molecule consists of seven transmembrane (TM) \( \alpha \)-helices with a general structure as revealed by x-ray crystallography of the “light-receptor” rhodopsin (Palczewski et al. 2000). The \( \alpha \)-helices form a hydrophobic pocket that spans the membrane lipid bilayer and serves as binding site for receptor ligands (Bywater 2005). The seven TM-helices are linked by three extracellular (\( \beta_1 \)-EC1-III) and three intracellular loops (\( \beta_1 \)-IC1-III). With their intracellular domains, \( \beta_1 \)-ARs couple to stimulatory G proteins (\( G_s \)) (Figure 1).

Stimulation of \( \beta_1 \)-AR by their physiologic ligands epinephrine or norepinephrine triggers a signaling cascade leading to sequential activation of \( G_s \), adenylate cyclase (which generates cAMP), and cAMP-dependent protein kinase (PKA). Activated PKA phosphorylates molecules involved in the regulation of sarcoplasmic Ca\(^{2+} \) concentration, thereby increasing myocyte inotropy and lusitropy (Freedman and Lefkowitz 2004, Lohse et al. 2003).

In the \( \beta_2 \)-AR subtype, amino acids in TM-helices III, V, and VI have been assigned an anchoring function for agonists, suggesting that the extracellular loops do not directly participate in ligand binding (Wieland et al. 1996). However, the second extracellular loop (\( \beta_2 \)-ECII) is predicted to form a \( \beta \)-hairpin, which dips down partly into the ligand-binding site and thus might in fact influence receptor–ligand interactions to some extent (Bywater 2005). The conformation of this hairpin is thought to be stabilized by cysteines situated in EC1 and ECII. In the case of \( \beta_2 \)-AR, it has been shown that reduction or mutation of one or several of these cysteines results indeed in a significant reduction of agonist and antagonist affinities (Dohlman et al. 1990, Noda et al. 1994). Thus, in \( \beta \)-AR correct folding of one or both extracellular loops (\( \beta_1 \)-ECIII) may be essential for correct formation of the ligand-binding pocket and might explain why antibodies directed against these loops can interfere with ligand binding, modulate receptor conformation, and thereby also modulate receptor activity (Jahns et al. 2000).

**Etiology of Autoantibodies Against Membrane Receptors**

Homologies between myocyte surface molecules such as surface membrane...
receptors and microbial determinants may represent one key mechanism for the generation of endogenous receptor autoantibodies by antigen mimicry (Hoebek et al. 1996). Chagas' heart disease, a slowly evolving inflammatory cardiomyopathy, is probably one of the best examples to illustrate this mechanism (Elies et al. 1996). The disease results from an infection with the protozoon Trypanosoma cruzi; molecular mimicry between the ribosomal PO protein of T. cruzi and a polyanionic stretch within the N-terminal half of β1-ECII results in cross-reacting antibodies in about 30% of the Chagas' patients (Ferrari et al. 1995). In contrast, receptor antibodies associated with DCM have been found to recognize mainly epitopes encompassing the cysteine residues situated in the C-terminal half of the cysteine residues associated with DCM have been found to recognize mainly epitopes encompassing the cysteine residues situated in the C-terminal half of the cysteine residues situated in the C-terminal half of β1-ECII (Wallukat et al. 1995). It has been speculated that recognition of this epitope might also originate from molecular mimicry between β1-ECII and a hitherto unidentified viral pathogen (Magnusson et al. 1996).

Another hypothesis for the etiology of endogenous receptor autoantibodies is that antigenic determinants from the surface or cytosols of the myocytes themselves, which are protected against the immune system under physiologic conditions, may become accessible after myocyte injury. Such injury most likely occurs during ischemic (acute myocardial infarction) or infectious heart disease (acute myocarditis) leading to myocyte apoptosis and/or necrosis (Caforio et al. 2002, Rose 2001). Subsequent liberation and presentation of myocardial self-antigens to the immune system may then induce an autoimmune response. In the worst case, this response might result in perpetuation of immune-mediated myocyte damage involving either cellular (i.e., T cell) or humoral (i.e., B cell) immune responses or coactivation of both the innate and the adaptive immune system (Eriksson et al. 2003, Rose 2001).

**The β1-AR as an Antigen**

To serve as an antigen, myocyte membrane receptors must be degraded to small oligopeptides, and one or more of the degradation products must be able to form a complex with one of the major histocompatibility complexes or HLA class II molecules of the host. More than a decade ago, the human β1-AR has been analyzed for potential immunogenic amino acid stretches accomplishing the requirements for a peptide to be complexed and presented to a T-cell receptor (Guillet et al. 1991). The analysis confirmed previous experimental data inferring that the only stretch of the β1-receptor molecule containing B- and T-cell epitopes and being accessible to antibodies is in fact the predicted β1-ECII loop (Hoebek et al. 1996). This might explain the successful use of β1-ECII peptides for the generation of anti-β1-ECII antibodies in various animal models with or without the use of carrier proteins and suggests the presence of a T-cell epitope in the ECII loop (Jahns et al. 1996, 2000, Matsui et al. 1997). Subsequently, several groups have independently demonstrated that almost all anti-β1-ECII antibodies preferentially recognize a native β1-AR conformation in a variety of immunologic assays (whole cell ELISA, immunoprecipitation, immunofluorescence), indicating that most anti-β1-ECII antibodies are "conformational" antibodies (Hoebek et al. 1996, Jahns et al. 2000). Moreover, functional tests revealed that the same antibodies were able to affect receptor function, such as intracellular cAMP production and/or cAMP-dependent PKA activation, suggesting that anti-β1-ECII antibodies may also act as allosteric regulators of receptor activity (Figure 1). A striking feature of such allosterically modulating anti-β1-AR antibodies is that they may promote, reduce, and/or stabilize conformational changes of the receptor similar to those induced by agonist or partial agonist ligands (Jahns et al. 2000, Vilardaga et al. 2005). Because these anti-β1-AR antibodies recognize a native receptor conformation, it seems conceivable that they may recognize different conformational states within the targeted receptor domain (i.e., some antibodies recognize and stabilize the active and others the inactive form).

**Pathogenic Impact of Stimulating Anti-β1-AR (Auto)antibodies**

Following Witebsky's postulates (Witebsky et al. 1957), indirect evidence for an autoimmune etiology of a disease requires identification of the corresponding self-antigen and induction of an analogous immune response in an experimental animal, which finally must also develop a similar disease. Direct evidence, however, requires reproduction of the disease by transfer of homologous pathogenic antibodies or pathogenic autoimmune T-cells from one to another animal of the same species (Rose and Bona 1993). For more than a decade, it has been accepted that β1-ECII represents a self-antigen (Magnusson et al. 1989). In 1997, Matsui et al. were able to show that rabbits immunized against β1-ECII develop a cardiomyopathic phenotype. Three years later, Omerevic et al. (2000) reported that intraperitoneal injection of blood lymphocytes from anti-β1-AR antibody-positive DCM patients into immunodeficient mice may lead to an early stage of cardiac dilatation. Nonetheless, direct evidence for a cause-and-effect relation between anti-β1-ECII antibodies and DCM still remained to be obtained.

To analyze the pathogenetic potential of anti-β1-AR antibodies, we chose an experimental in vivo approach that met the Witebsky criteria for direct evidence of autoimmune diseases. We induced DCM by immunizing inbred rats against β1-ECII (100% sequence homology between human and rat [indirect evidence]) and then reproduced the disease in healthy isogenic rats by transferring the "autoantibodies" (direct evidence) (Jahns et al. 2004). The cardiomyopathic phenotype in these rats was characterized by progressive left ventricular dilatation and dysfunction, a relative decrease in left ventricular wall thickness, and selective downregulation of β1-AR, a feature that is also seen in human DCM (Lohse et al. 2003). These results, in conjunction with the demonstrated agonist-like short-term effects of the antibodies in vivo (Jahns et al. 2004), suggest that both the induced and the transferred cardiomyopathic phenotypes have to be attributed mainly to the mild but sustained receptor activation achieved by stimulatory anti-β1-ECII antibodies. This hypothesis is supported by the large body of data available on the cardiotoxic effects of excessive and/or long-term β1-AR activation seen after genetic or pharmacologic manipulation (Engelhardt et al. 1999, Woodiuss et al. 2001). Therefore, anti-β1-AR-induced dilated immune cardio-
myopathy should now be regarded as a pathogenetic disease entity of its own, together with other established receptor-directed autoimmune diseases such as myasthenia gravis or Graves disease (Freedman and Lefkowitz 2004, Hershko and Naparstek 2005).

- Clinical Impact of Stimulating Anti-β1-AR Antibodies and Future Perspectives

The actual clinical importance of autoantibodies directed against cardiac antigens is difficult to assess because low titers of such antibodies can also be detected in the healthy population as a part of the natural immunologic repertoire (Rose 2001). However, regarding functionally active anti-β1-AR antibodies, we have previously shown that their prevalence was very low in healthy individuals (<1%), when a screening procedure based on cell systems presenting the respective target (e.g., the human β1-AR) in its natural conformation is used (Jahns et al. 1999b). By using the same screening modality, we could also exclude significant amounts of circulating anti-β1-AR antibodies in patients with heart failure due to chronic valvular or hypertensive heart disease (Jahns et al. 1999a). In contrast, the prevalence of stimulating anti-β1-AR antibodies was ~10% in ischemic and ~30% in dilated cardiomyopathy (Jahns et al. 1999b), which was significantly higher than in healthy collectives, but in the lower range of the values obtained for DCM patients in previous studies, reporting antibody prevalences from 33% up to 95% (Limas et al. 1992, Magnusson et al. 1994, Wallukat et al. 1995). It seems conceivable that differences in screening modalities aiming to detect functionally active anti-β1-AR antibodies, which may comprise autoantibodies directed against β1-ECII, β1-ECI, or against both epitopes, account for the wide range of anti-β1-AR antibody prevalences reported in the past. In this regard, it has been shown that of ELISA-defined human autoantibodies against β-AR, only a minor fraction seems to be able to bind to cell surface located native β1- or β2-AR. Only this fraction recognized (as determined by immunofluorescence) and activated (as determined by increases in cellular cAMP and/or PKA activity) human β1-AR expressed in the membrane of intact cells (Jahns et al. 1999b, 2000). We therefore advocate cell systems presenting the target in its natural conformation as an important tool in the screening for functionally relevant anti-β-AR autoantibodies.

Clinically, the presence of anti-β1-AR autoantibodies in DCM has been shown to be associated with a more depressed cardiac function (Jahns et al. 1999b), the occurrence of more severe ventricular arrhythmia (Chiale et al. 2001), and a higher incidence of sudden cardiac death (Iwata et al. 2001). Concordantly, in our DCM patients judged to be positive for anti-β1-ECII antibodies, we found a higher prevalence of ventricular arrhythmia (Lown class III-IV) compared with antibody-negative DCM patients during a follow-up period of more than 10 years (Störk et al. 2004). Moreover, multivariate analysis of the follow-up data revealed that, in DCM patients, the presence of activating anti-β1-ECII antibodies was independently associated with an almost three-fold increase in cardiovascular mortality risk (Störk et al. 2004). Taken together, the preliminary clinical data underscore the pathophysiologic relevance of functionally active anti-β1-AR antibodies in DCM and encourage further research in the evolving field of antibody-directed strategies as a therapeutic principle (Freedman and Lefkowitz 2004, Hershko and Naparstek 2005). In this regard, one aspect of the beneficial effects of β1-receptor blockade in DCM might be the pharmacologic neutralization of autoantibody-mediated stimulatory effects (Freedman and Lefkowitz 2004, Jahns et al. 2000), at least if β-blockers can indeed completely or largely prevent the antibody-induced activation of the receptors. Alternative therapeutic options would include elimination of the antibodies using cell systems presenting the target in its natural conformation. One prerequisite for an unequivocal interpretation of future clinical trials would be a standardized screening procedure for functionally active human anti-β1-AR antibodies using cell systems presenting the target receptor in its natural conformation.

In conclusion, although stimulating anti-β1-AR antibodies can clearly be pathogenic, the pathophysiologic sequence of events leading to their generation, their relative contribution to the pathogenesis of human DCM, and their relevance for prognosis and therapy still remain to be determined.

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References


