Beta 1-adrenergic receptor-directed autoimmunity as a cause of dilated cardiomyopathy in rats

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Abstract

Progressive cardiac dilatation and pump failure of unknown etiology has been termed idiopathic dilated cardiomyopathy (DCM). During recent years a large body of data has accumulated indicating that functionally active antibodies or autoantibodies being able to recognize and to stimulate the cardiac β1-adrenergic receptor (anti-β1-AR) may play an important role in the initiation and/or clinical course of DCM. Recent experiments in rats even point towards a cause-and-effect relation between stimulatory anti-β1-AR antibodies and DCM. Immunization of rats against the second extracellular loop of the human β1-adrenergic receptor (100% sequence-identity between human and rat) resulted in both development of stimulatory anti-β1-AR antibodies and development of progressive cardiac dilatation and dysfunction. Isogenic transfer of stimulatory anti-β1-AR from cardiomyopathic into healthy inbred animals reproduced the disease, hence providing conclusive proof for a β1-receptor-directed autoimmune attack as a possible cause of cardiomyopathy. This kind of cardiomyopathy is now referred to as anti-β1-AR-induced dilated immune-cardiomyopathy (DiCM).

The following article reviews recent evidence obtained from experimental animal-models implying a significant role of the cardiac β1-adrenergic receptor as a pathophysiologically and clinically relevant autoantigen also in human DCM.© 2006 Elsevier Ireland Ltd. All rights reserved.

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1. Introduction

Cardiac disease characterized by progressive dilatation and loss of contractile function in the absence of coronary artery disease has been termed idiopathic dilated cardiomyopathy (DCM) [1]. In younger adults, DCM still represents one of the main causes for severe heart failure and subsequent heart transplantation [2]. Genetic disorders [3] and a limited number of cardiotoxic substances (i.e., alcohol, anthracyclines) account for about one third of the cases. The etiology of the remaining 60–70% is, however, poorly understood.

Because alterations in both humoral and cellular immunity are frequently present in patients with DCM [4–7], induction of the disease has been claimed to be associated with abnormal or misled immune responses to non-inflammatory (i.e., toxic) or inflammatory cardiac tissue injury (i.e., caused by cardiotropic viruses, bacteria, or parasites) [8–10]. As a surrogate of this immunologic response a substantial number of cardiomyopathic patients develop cross-reacting antibodies and/or autoantibodies to a wide panel of cardiac antigens, including membrane proteins (i.e., G protein-coupled receptors) [4,11,12], mitochondrial proteins (i.e., adenine nucleotide translocator) [13], and/or myocyte structural proteins (i.e., actin, tubulin, myosin, and troponin) [9,14,15]. The clinical relevance of each of these cardiac (auto-)antibodies is, however, far from clear. Low titers of autoantibodies to several housekeeping antigens can also be detected in the healthy population as a part of the
natural immunologic repertoire [10]. In addition, under physiological conditions, at least the intracellularly localized cardiac antigens are not easily accessible for circulating antibodies.

From a theoretical point of view, the possible pathophysiologic (and thus clinical) relevance of a specific autoantibody is mainly determined by two factors, (a) the accessibility, and (b) the functional relevance of the target [16]. Thus, it seems conceivable that autoantibodies directed against myocyte surface β1-adrenergic receptors (β1-AR), which moreover have the potential to affect cardiac function by interaction with these “key regulators” of myocardial contractility and relaxation, may play an important role in the initiation and/or progression of cardiac dilatation and dysfunction [17,18].

This article will summarize previous and most recent experimental findings focusing on the cardiac β1-adrenergic receptor as an autoantigen in the pathogenesis of a newly defined DCM entity, now referred to as anti-β1-AR-induced dilated immune-cardiomyopathy (DiCM).

2. Structure and antigenicity of the cardiac β1-adrenergic receptor

The β1-adrenergic receptor (β1-AR), which constitutes about 70–80% of the cardiac β-AR complement, belongs to the superfamily of G protein-coupled membrane receptors (GPCR) [19]. GPCR’s transduce extracellular signals to an intracellular effector mechanism by means of interaction with a GTP-regulated heterotrimeric protein (G protein) [20].

The β1-AR consists of seven transmembrane (TM) α-helices, which are linked together by three extracellular (β1-ECi-III) and three intracellular loops (β1-ICI-III) and form a kind of hydrophobic “pocket” [21]. The aminoterminal head of the receptor molecule is located in the extracellular, the carboxyterminal tail in the intracellular space (Fig. 1). Activation of the β1-AR by its physiologic agonists adrenaline or noradrenaline triggers a signaling cascade leading to sequential activation of the stimulatory G protein Gs, adenylate cyclase (which katalyzes formation of cAMP), and the cAMP-dependent protein kinase (PKA) [16,22]. Activated PKA phosphorylates molecules involved in the regulation of sarcomplasmic Ca2+ concentration, thereby increasing myocyte inotropy and lusitropy [17,18,23].

In the case of the β2-adrenergic receptor (β2-AR), 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 [24,25]. On the other hand, the primary amino-acid composition of the second extracellular loop (ECII) allows for the formation of a β-hairpin in almost all GPCRs which dips down partly into the ligand binding site. Consequently, the instantaneous conformation of this loop may influence receptor–ligand interactions to some extent [21]. The ECII-hairpin contains conformation-stabilizing cysteines which form an intra-loop disulfide bridge (assumed to be localized at the top of the hairpin), and a second disulfide bridge with a conserved cysteine at the top of TM III, linking ECi with ECII [21] (see also Fig. 1). For the β2-AR it has been demonstrated that reduction or mutation of one or several of...
these cysteines – most notably those in β2-ECII(Cys184, Cys190/191), or Cys106 situated at the top of TM III – results in a significant reduction of agonist as well as antagonist affinities [26–28]. Subsequent in vitro experiments have confirmed that the extracellular disulfide bridges between conserved and non-conserved cysteines do in fact stabilize the “high affinity” state of the β2-AR [27], and that a reduction of the conformation-stabilizing intra- and inter-loop disulfide bridges also inactivates the β1-AR subtype [29]. Taken together, these data indicate that correct folding of one or both extracellular loops (ECI/II) is essential for the formation of the ligand binding pocket in both β1- and β2-AR subtypes. This might explain why antibodies or autoantibodies directed against these loops can (a) interfere with ligand binding, (b) alter receptor conformation, and thereby also (c) affect receptor activity [16,30].

The sequence of pathophysiologically events, however, which leads to the generation of functionally active anti-β1-AR in the human has not yet been clarified. Homologies between myocardite surface molecules such as membrane receptors and microbial determinants represent one possible mechanism for the elaboration of endogenous receptor autoantibodies by antigen mimicry [31]. Alternatively, potentially antigenic components of the cell surface or from the cytosol of the myocytes themselves, which are protected against the immune system under physiological conditions, may be accessible following myocyte damage. It may be hypothesized that such damage most likely occurs during ischemic or inflammatory myocyte injury leading to apoptosis and necrosis of myocardial cells. Subsequent liberation and presentation of myocardial autoantigens to the immune system may then engender an autoimmune response [7,9,10,16]. To serve as an autoantigen, (endogenous) myocyte membrane receptors must be degraded by proteolysis to small fragments (oligopeptides), and one or several of the generated fragments must be able to form a complex with one of the major histocompatibility complexes (MHCs) or human leucocyte antigen (HLA) class II molecules [31]. Via their membrane MHC class II molecules endosomes may then present receptor-derived antigenic peptide stretches (at least 10–12 amino-acids long) to T-cells [32]. In the worst case, the subsequent receptor-directed immune response results in perpetuation of myocyte injury involving either cellular (i.e., T-cell) or humoral (i.e., B-cell) immune reactions, or leads to co-activation of both, the innate and the adaptive immune system [14,33].

To further elucidate the antigenicity of the human β1-AR, the receptor protein has previously been analyzed for potential immunogenic amino-acid stretches based on the structural analysis of a human HLA class II molecule [34]. This analysis was performed using a homology scanning algorithm which compares short receptor-fragments with peptides known to be immunogenic under a mouse (!) MHC haplotype [31,35]. Not surprisingly, the analysis confirmed previous experimental data [36,37] inferring that the only β1-AR fragment which contains B- and T-cell epitopes, and is easily accessible to antibodies, is in fact the predicted second extracellular receptor loop (β1-ECII) [31]. In addition, the analysis revealed some possibly immunogenic amino-acid sequences within the first and third extracellular β1-AR loops. In the following, only one peptide corresponding to the first extracellular receptor loop (β1-ECI) was used successfully to immunize rabbits [32]. Significant anti-β1-ECI antibody titers, however, were obtained only after coupling of the ECI-peptide to bovine serum albumin, suggesting the absence of a T-cell epitope in the β1-ECI sequence. This is in clear contrast to β1-ECII peptides, which in the last decade have been widely used to generate large amounts of specific anti-β1-ECII antibodies in a variety of animals/animal-models — with or without utilizing carrier proteins, suggesting the presence of a T-cell epitope in the β1-ECII sequence (i.e., rabbit, mouse, rat) [30,38–43].

3. Stimulatory anti-β1-AR (auto-)antibodies are conformational

As already mentioned, the harmful potential of a specific (auto-)antibody depends on the accessibility and the functional relevance of its target. Therefore, (auto-)antibodies directed against cell surface adrenoreceptors which have the potential to influence cardiomyocyte function by modulating receptor activity, represent proper candidates for a pathophysiologically significant role in the development and course of cardiac dysfunction and failure [17,18,44]. In particular, autoantibodies targeting epitopes within the functionally relevant first and – probably even more important – second extracellular loops of the β1-adrenergic receptor (β1-ECII) are thought to play a pathogenic role in human DCM [12,30,44].

In previous work, we and others have independently demonstrated that (auto-)antibodies directed against β1-ECII preferentially recognize a native β1-AR conformation in different immunologic assays (i.e., enzyme-linked immunosassay and/or immunofluorescence using intact whole cells, immunoprecipitation experiments). Further, the same antibodies also affected receptor function, such as intracellular cAMP-production and/or cAMP-dependent protein kinase (PKA) activity [30,45]. In contrast, antibodies directed against the β1-aminoterminus or the intracellularly localized β1-carboxyterminus were not sensitive to denaturation of the receptor and did not affect receptor function [30,32,38]. In addition, we have shown that the functional effects of distinct anti-β1-ECII antibodies may differ considerably, although all of them were generated against the same small stretch of amino acids (i.e., those forming the β1-ECII loop) [30]. This suggests that anti-β1-ECII are “conformational” and act indeed as allosteric regulators of receptor activity; they may promote, reduce or stabilize conformational changes of the receptor similar to those induced by agonist or partial agonist ligands [16,22,24,30,46]. Because most anti-β1-ECII generated and/or described so far are polyclonal, one possible explanation...
for the observed functional diversity is that some receptor-antibodies may recognize the active, and others the inactive receptor conformation. In addition, the subtype and the source of the immunoglobulin (i.e., the species in which it was generated) may also influence its functional properties.

We have previously proposed to describe the different functional effects of anti-\(\beta_1\)-ECII according to a model [47] which assumes two receptor states, inactive (R) and active (R*) [30]. Agonists (A) can bind to both states, but induce and/or stabilize the active state, forming preferentially AR*. Antibodies (=immunoglobulins, I) can bind to the active (IR*) or inactive (IR) states, and can also do so in the presence of agonist. In our experiments, inhibitory anti-\(\beta_1\)-ECII (generated in rabbits or mice) [30] reduced the amount of active \(\beta_1\)-receptors expressed in CHW cells probably by stabilizing an inactive state IR. In the presence of agonist, they had a similar inhibitory effect, either because they reduced agonist binding (forming again IR), or because they returned the receptors to an inactive state even with agonist bound (AIR). These anti-\(\beta_1\)-ECII behaved as inverse agonistic allosteric regulators because they inhibited basal as well as stimulated receptor activities. In contrast, functionally active human or rat anti-\(\beta_1\)-ECII increased basal receptor activity, presumably by forming an active state (IR*) [30,41]. Again, they might have done so by preferentially binding to an active state R* (as suggested for antibodies against the \(\beta_2\)-AR [46]), or by inducing an active receptor conformation (IR*). In the presence of agonist, all our rat anti-\(\beta_1\)-ECII and the large majority of anti-\(\beta_1\)-ECII from human patients caused a further increase in receptor activity. However, some human anti-\(\beta_1\)-ECII were also able to decrease agonist-induced \(\beta_1\)-AR activity in vitro, comparable to the effect of a partial agonist [30]. According to our proposed model, purely activating anti-\(\beta_1\)-ECII may either induce a state AIR*, which is more active than the agonist-activated state AR*, or alternatively, induce more receptors to switch into the active state than even high agonist concentrations do [30].

4. Stimulatory anti-\(\beta_1\)-ECII (auto-)antibodies are pathogenic

According to Witebsky’s postulates indirect evidence for the autoimmune etiology of a disease requires (a) identification of the responsible “self-antigen”, and (b) induction of a corresponding self-directed immune response in an experimental animal, which subsequently should develop a similar disease [48,49]. Direct evidence, however, requires reproduction of the disease by transfer of homologous pathogenic antibodies or pathogenic autoreactive T-cells from one to another individual of the same species [49].

For almost two decades it has been well established that \(\beta_1\)-ECII represents a potent “self-antigen” [32,36,37]. However, it took about 10 more years of time until Matsui et al. in 1997 presented a first animal-model, in which they could demonstrate that rabbits after 12 months of immunization with a \(\beta_1\)-ECII-homologous peptide developed biventricular dilatation (as determined by histology), and an upregulation of total cardiac \(\beta_1\)-AR (which was not expected in the presence of stimulatory anti-\(\beta_1\)-ECII antibodies) [39]. Using a similar rabbit model, 4 years later the experiment was repeated by Iwata et al. [40]. In contrast to the results obtained by Matsui et al., total cardiac \(\beta_1\)-AR (predominantly \(\beta_1\)-AR) were significantly downregulated in anti-\(\beta_1\)-ECII-positive animals. Moreover, after 6 months of immunization the rabbits were found to develop LV-hypertrophy rather than LV-dilatation (as determined by echocardiography and histology) which was, however, no longer present after 12 months of immunization, perhaps indicating the transition into an early DCM-phenotype [40]. In the following time, (indirect) evidence for a pathogenic role of anti-\(\beta_1\)-ECII has been supported further by the fact that intraperitoneal injection of blood lymphocytes either from immunized anti-\(\beta_1\)-ECII-positive rabbits [50], or from anti-\(\beta_1\)-AR-positive DCM patients [51] into immunodeficient mice – in order to avoid the expected immune reaction against rabbit or human non-self proteins – may lead to an early stage of cardiac dilatation. Nonetheless, direct evidence for a cause-and-effect relation between anti-\(\beta_1\)-AR antibodies and DCM still remained to be established.

In order to clarify the pathogenetic potential of stimulatory anti-\(\beta_1\)-AR, we chose an experimental in vivo approach which met the Witebsky criteria for autoimmune diseases. We attempted to induce dilated cardiomyopathy by immunizing rats against \(\beta_1\)-ECII (100% sequence homology between human and rat; indirect evidence), and then to reproduce the disease in healthy rats of the same strain by isogenic transfer of the generated anti-\(\beta_1\)-ECII (direct evidence) [49]. In our study, we immunized inbred rats against \(\beta_1\)-ECII every month over a 15-month period. All immunized animals developed high titers of stimulatory anti-\(\beta_1\)-ECII-antibodies and then, after 9 months of immunization, progressive left ventricular dilatation and dysfunction (as determined by echocardiography (Fig. 2a), left heart catheterization, and histology) [41]. Subsequent isogenic transfer of anti-\(\beta_1\)-ECII antibodies from immunized into healthy rats of the same strain (in order to mimic autoantibodies) within 6–9 months also transferred the disease, hence providing the first direct evidence for a cause-and-effect relationship between activating anti-\(\beta_1\)-AR antibodies and dilated cardiomyopathy (Fig. 2b). The cardiomyopathic phenotype in our rats was characterized by (a) progressive LV dilatation and dysfunction, (b) a relative decrease in LV wall-thickness, and (c) selective downregulation of \(\beta_1\)-AR, a feature that is also seen in human DCM [23,52]. Recently, Larsson et al. [42] were able to reproduce the first part of our previous study, again by active immunization of rats with a \(\beta_1\)-ECII-peptide. In perfect agreement with our results they found a significant reduction in LV fractional shortening in anti-\(\beta_1\)-ECII-positive rats (as determined by echocardiography) and, at the molecular level, a significant increase in cardiac tissue \(\beta_1\)-adrenergic
receptor kinase mRNA (as determined by RT-PCR), which fits very well with the selective cardiac β₁-AR downregulation observed in our studies. These findings in conjunction with the previously demonstrated agonist-like short-term effects of our rat anti-β₁-ECII in vivo (modest increases in contractility and in blood pressure upon injection of stimulatory anti-β₁-ECII into naive control rats [41]) suggest that the induced and the transferred cardiomyopathic phenotypes have to be attributed mainly to the mild but sustained receptor activation by stimulatory anti-β₁-ECII. As a consequence, this type of anti-β₁-AR antibody-induced DiCM should now be categorized with other established receptor-directed autoimmune diseases, such as myasthenia gravis, Grave’s disease, and type B insulin-resistant diabetes mellitus [17,53].

5. Clinical and therapeutic perspectives

Although the above presented animal experiments strongly suggest a cause-and-effect relationship between functionally active anti-β₁-AR (auto-)antibodies and the initiation and/or course of dilated cardiomyopathy, the true clinical relevance of these antibodies in human heart disease remains to be determined. With respect to stimulatory anti-β₁-ECII autoantibodies, however, we have previously shown that the prevalence of such antibodies in healthy individuals was rather low (<1%), using an antibody-screening algorithm based on cell systems which present the human β₁-AR in its natural conformation [4]. In patients with a known cause of heart failure, by using the same screening strategy, we were able to exclude significant amounts of anti-β₁-AR in smaller patient-cohorts with valvular or hypertensive heart disease [54]. In contrast, we and others have detected significant amounts of stimulatory anti-β₁-ECII in patients with ischemic cardiomyopathy (ICM, about 10% prevalence), in Chagas cardiomyopathy (CCM, about 30% prevalence [8]), and, most notably, in DCM with a prevalence varying from 26% to 95% of the patients, depending on screening strategy [4,12,55,56]. Differences in the screening modalities for functionally active anti-β₁-AR

Fig. 2. Echocardiography of rat hearts. (a) Immunization experiment, month 15: representative M-Mode tracings of an anti-β₁-ECII antibody-positive animal (left panel), and a corresponding control animal (right panel); LVED/LVES, left ventricular end-diastolic/ end-systolic diameters [numbers in mm]. (b) Time course of echocardiographically determined LVED and LVES in the immunization (left panel) and serum transfer experiment (right panel). Error bars indicate mean±SEM; *P<0.05, **P<0.01, ***P<0.001 (ANOVA and Bonferroni post hoc test). (Figures adopted with permission from Jahns et al. [41]).
autoantibodies (which may comprise antibodies targeting β₁-ECII, β₁-ECI, or both epitopes [55]), most probably account for the relatively wide range of antibody-prevalences reported in the literature. One prerequisite for future clinical trials would thus be a standardized screening algorithm for functionally active (conformational) human anti-β₁-AR autoantibodies, utilizing cell systems which present the target receptor in its native conformation [16].

Recent clinical observations indicate that the presence of stimulatory anti-β₁-AR autoantibodies in patients with DCM might be associated with poorer left ventricular function [4], the occurrence of more severe ventricular arrhythmia [57], and a higher incidence of sudden cardiac death [56]. Concordant with these observations, in our recently accomplished 10-year follow-up study of the DCM collective described in 1999 [4], we found a higher prevalence of ventricular premature capture beats and Lown class III-IV arrhythmia in anti-β₁-ECII-positive compared with antibody-negative patients. In addition, analysis of the demographic data revealed that in our collective the presence of stimulatory anti-β₁-ECII was associated with an almost three-fold increase in all-cause and cardiovascular mortality-risk after adjustment for other prognostic factors [58]. These clinical data underscore the prognostic relevance of stimulatory anti-β₁-AR in DCM, and encourage further research in the evolving field of antibody-directed strategies as a therapeutic principle [17,53,59].

One (today generally accepted) pharmacologic strategy would be the use of beta-blocking agents in order to attenuate or even abolish the autoantibody-mediated stimulatory effects, at least if β-blockers can indeed prevent the antibody-induced activation of β₁-AR [17,30,60]. New therapeutic approaches include elimination of stimulatory anti-β₁-AR by non-selective or selective immunoabsorption [53,61], or direct targeting of the anti-β₁-ECII antibodies and/or the anti-β₁-ECII producing B-cells themselves (that is, induction of immune tolerance) [62]. In our rat model, prophylactic application of a newly developed β₁-ECII-homologous cyclopeptide (β₁-ECII-CP) 6 weeks after the active induction of stimulatory anti-β₁-ECII antibodies significantly reduced the amount of circulating anti-β₁-ECII, and effectively prevented development of cardiac dilatation and dysfunction [63]. The beneficial effects of the new cyclopeptide were similar to those achieved by prophylactic application of the cardioselective β-blocker bisoprolol. However, compared with bisoprolol, β₁-ECII-CP did reduce neither heart rate nor blood pressure, which might be advantageous in a clinical condition. Moreover, β₁-ECII-CP not only induced a rapid decrease in anti-β₁-ECII-titers, but — within a few months — also induced a complete cessation of anti-β₁-ECII antibody-production despite continued boosts of the animals with the β₁-ECII-immunogen (i.e., induced a kind of immunological anergy) [63]. Future research will show, whether this promising new experimental approach proves to be efficient also in the treatment of advanced anti-β₁-ECII-induced cardiomyopathy and heart failure.

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