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REVIEW

Drug-like actions of autoantibodies against receptors of the autonomous nervous system and their impact on human heart function

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Antibodies against cholinergic and adrenergic receptors (adrenoceptors) are frequent in serum of patients with chronic heart failure. Their prevalence is associated with Chagas' disease, idiopathic dilated cardiomyopathy (DCM), and ischaemic heart disease. Among the epitopes targeted are first and second extracellular loops of the β-adrenergic (β-adrenoceptor) and M2 muscarinic receptor. β_1-adrenoceptor autoantibodies affect radioligand binding and cardiomyocyte function similar to agonists. Corresponding rodent immunizations induce symptoms compatible with chronic heart failure that are reversible upon removal of the antibodies, transferable via the serum and abrogated by adrenergic antagonists. In DCM patients, prevalence and stimulatory efficacy of β_1-adrenoceptor autoantibodies are correlated to the decline in cardiac function, ventricular arrhythmia and higher incidence of cardiac death. In conclusion, such autoantibodies seem to cause or promote chronic human left ventricular dysfunction by acting on their receptor targets in a drug-like fashion. However, the pharmacology of this interaction is poorly understood. It is unclear how the autoantibodies trigger changes in receptor activity and second messenger coupling and how that is related to the pathogenesis and severity of the associated diseases. Here, we summarize the available evidence regarding these issues and discuss these findings in the light of recent knowledge about the conformational activation of the human β_2-adrenoceptor and the properties of *bona fide* cardiopathogenic autoantibodies derived from immune-adsorption therapy of DCM patients. These considerations might contribute to the conception of therapy regimen aimed at counteracting or neutralizing cardiopathogenic receptor autoantibodies.

Abbreviations

β-adrenoceptor, β-adrenergic receptor; β_1-adrenoceptor, β-adrenergic receptor type 1; β_2-adrenoceptor, β-adrenergic receptor type 2; Fc, invariable domain of IgG; G-protein, heterotrimeric GTPase protein; Gi, G-protein inhibiting adenylate cyclase; Gs, G-protein stimulating adenylate cyclase; m2AChR, muscarininic actylcholine receptor type 2; TSH-R, receptor of the thyroid-stimulating hormone

Humoral receptor autoimmunity and chronic heart disease

At least three human diseases are most certainly caused by autoantibodies that bind to the receptors of neuroendocrine transmitters and alter their function. Grave's disease (*M. Basedow*), a pathologically enhanced growth and endocrine function of the thyroid gland is caused by autoantibodies that stimulate the receptor of the thyroid-stimulating hormone (TSH-R). *Myasthenia gravis*, an intermittent weakness of skeletal muscles, is caused by autoantibodies blocking the

nicotinic acetylcholine receptor at the neuromuscular endplate. *Autoimmune autonomic ganglionopathy* is an idiopathic acquired disorder of the autonomic nervous system associated with antibodies blocking the ganglionic nicotinic acetylcholine receptor found in sympathetic, parasympathetic and enteric ganglia.

Over the past two decades, various renal and cardiovascular pathologies have been added to this list, which are associated with humoral autoimmunity against G-protein coupled receptors involved in autonomous vegetative regulation. These encompass malignant (Fu *et al.*, 1994), primary (Luther *et al.*, 1997) or refractory hypertension (Wenzel *et al.*, 2008) associated with humoral autoimmunity against α_1-adrenergic receptors (α_1-adrenoceptors), and preeclampsia (Wallukat *et al.*, 1999) or renal allograft rejection (Dragun *et al.*, 2005) associated with the occurrence of autoantibodies against angiotensin receptors. It has been demonstrated that immunization of rodents against these receptors leads to alterations in the regulation of blood pressure and kidney function, which may entail cardiac failure as a secondary complication (Dragun *et al.*, 2009). Further experimental data also suggest a blood pressure-independent effect of these autoantibodies on cardiac remodelling (Zhou *et al.*, 2005). Autoantibodies against β-adrenoceptors and muscarinic acetylcholine receptors are thought to be a primary cause of chronic heart failure and a causal factor in the pathogenesis of dilated cardiomyopathy (DCM). Although chronic heart failure can be a consequence of hypertension induced by autoantibodies against α_1-adrenoceptors (Fu *et al.*, 1994; Luther *et al.*, 1997; Wenzel *et al.*, 2008), primary forms such as DCM seem to be caused by humoral autoimmunity against heart-specific antigens. Our review will focus on the latter entity.

DCM denominates a disease characterized by a chronic decline in cardiac function and progressive ventricular dilation and dysfunction due to non-ischaemic myocardial damage. A DCM subgroup of about 30% is addressed as 'idiopathic' because its origin remains unclear despite efforts at reclassification (Maron *et al.*, 2006). Data accumulated over the past three decades strongly suggest that at least a fraction of this DCM subgroup could represent a later stage of a heart-specific autoimmune disease triggered by viral (Yoshikawa *et al.*, 2009) or protozoic (Cunha-Neto *et al.*, 2006) infections or possibly induced by autoimmunization with heart-specific antigens in genetically predisposed individuals (Caforio *et al.*, 2008b). Humoral autoimmunity seems to play a crucial role in DCM, as heart-reactive autoantibodies found in patients and relatives from familial and non-familial pedigrees predict disease development among healthy relatives (Caforio *et al.*, 2008a). Some of these antibodies have functional effects on cardiac myocytes *in vitro* and in animal models (Caforio *et al.*, 2005) and possibly in a DCM subset characterized by clinical responsiveness to extracorporeal antibody elimination (Felix *et al.*, 2002). Moreover, chronic cardiac dysfunction can be induced in rodents by peritoneal injection of immune competent B-lymphocytes from DCM patients (Omerovic *et al.*, 2000).

Among the cardiac autoantibodies that are pathognomonic and possibly pathogenic in DCM, those that modulate the function of receptors transducing the regulation of cardiac contraction frequency and force by the autonomous nervous system seem to play a particularly prominent role. Antibodies stimulating cholinergic and β-adrenoceptor signalling are frequently found in serum of patients with chronic heart failure. The epitopes most frequently targeted by such autoantibodies are the first and second extracellular loops of the β_1- and β_2-adrenoceptors (Magnusson *et al.*, 1990) and the M2 muscarinic acetylcholine receptor (m2AChR) (Fu *et al.*, 1993). In the case of the human β_1-adrenoceptor the second extracellular loop is the only extracellular receptor domain capable of inducing antibody production with pharmacological effects on the receptor (Tate *et al.*, 1994). This may be also true for the β_2-adrenoceptor and m2AChR, given their structural similarity to the β_1-adrenoceptor. Such autoantibodies are associated with cardiomyopathy evolving in the course of Chagas' disease (Sterin-Borda *et al.*, 1976; Rosenbaum *et al.*, 1994; Hernandez *et al.*, 2003; Labovsky *et al.*, 2007; Munoz-Saravia *et al.*, 2010), DCM (Magnusson *et al.*, 1990; 1994; Fu *et al.*, 1993; Rosenbaum *et al.*, 1994; Matsui *et al.*, 1995; Wallukat *et al.*, 1995; Jahns *et al.*, 1999b; Staudt *et al.*, 2001), congestive heart failure (Zhang *et al.*, 2002), ischaemic heart disease (Jahns *et al.*, 1999b), atrial tachyarrhythmia (Baba *et al.*, 2004; Chiale and Ferrari, 2001; Del Corsso *et al.*, 2004; Stavrakis *et al.*, 2009; 2011; Yu *et al.*, 2009), but not cardiomyopathies of other aetiology (Magnusson *et al.*, 1996; Jahns *et al.*, 1999a). In DCM patients, prevalence (Jahns *et al.*, 1999b) and cAMP stimulatory efficacy (Nikolaev *et al.*, 2007) of β_1-adrenoceptor autoantibodies are correlated to reduced cardiac function (Jahns *et al.*, 1999b), increased mortality (Stork *et al.*, 2006), severe ventricular arrhythmia (Chiale *et al.*, 2001), and higher incidence of sudden cardiac death (Iwata *et al.*, 2001). Interestingly, atrial fibrillation in Graves' disease is also associated with the occurrence of autoantibodies stimulating cholinergic and β-adrenoceptors and these autoantibodies are distinct from the ones causing hyperthyroidism through stimulation of the TSH-R (Stavrakis *et al.*, 2009). This observation suggests that syndromes associated with autoantibodies against β-adrenergic and cholinergic receptors can cross the borders of organ-specific aetiologies. On the other hand, low levels of autoantibodies against β_1-adrenoceptor, β_2-adrenoceptor and m2AChR are also present in the bloodstream of many healthy individuals (Liu *et al.*, 1999) and are thought to be a part of the natural immunologic repertoire (Fraser and Venter, 1984; Rose, 2001; Jahns *et al.*, 2006b). This raises the question of whether such autoantibodies can indeed cause heart failure, or are just an epiphenomenon.

Evidence that stimulatory β_1-adrenoceptor autoantibodies cause heart failure

In Chagas' disease immune responses to the C-terminal end of the ribosomal P2β protein of *T. cruzi* give rise to antibodies cross-reacting with first and second extracellular loops of human β_1-adrenoceptor, β_2-adrenoceptor and m2AChR and trigger sustained humoral autoimmunity against these receptors (Lopez Bergami *et al.*, 2005). What triggers receptor autoimmunization in DCM is unclear. Autoantibodies associated with DCM seem to be directed against a different

portion of the second extracellular receptor loop than those associated with Chagas' disease (Magnusson *et al.*, 1996). Various viral and microbial candidate proteins have been proposed that could trigger a bystander effect analogous to the one triggered by ribosomal P2β protein of *T. cruzi* in Chagas' disease (Levin and Hoebeke, 2008), but firm evidence of such a causative microbial immunogen is still lacking.

IgG autoantibodies from Chagas' patients increase cellular cAMP (Sterin-Borda *et al.*, 1976; Rosenbaum *et al.*, 1994) and impair L-type Ca^{2+} currents (Hernandez *et al.*, 2003) in isolated cardiomyocytes, indicating that they can promote receptor coupling to stimulatory (G_s) as well as inhibitory (G_i) G-proteins, consistent with a varied spectrum of agonist-like actions on β_1-adrenoceptor, β_2-adrenoceptor and m2AChR. The clinical syndromes of chronic Chagas' disease developing with a latency of several decades after *T. cruzi* infection can be predicted from the cross-reactivity patterns of these receptor-stimulating autoantibodies: The development of cardiomyopathy is associated with the induction of autoantibodies against β_1-adrenoceptor and m2AChR, whereas the development of mega-colon is associated with the induction of autoantibodies against β_2-adrenoceptor and m2AChR (Wallukat *et al.*, 2010). These observations also reported from animal models (Mijares *et al.*, 1996b; Silvina Lo Presti *et al.*, 2008) suggest that Chagas' cardiomyopathy could be caused or at least promoted by the continuous action of autoantibodies activating the β_1-adrenoceptor, whereas vagal dysfunctions associated with the disease (Davila *et al.*, 2005) seem to be linked to the occurrence of autoantibodies activating the m2AChR. The role of autoantibodies stimulating the β_2-adrenoceptor that are also present in many of these patients remains unclear.

Direct immunization of rodents with peptides or fusion proteins representing sequences of the second extracellular loop of the β_1-adrenoceptor have been demonstrated to induce left ventricular dilation and dysfunction (Jahns *et al.*, 2004; Buvall *et al.*, 2006) among other effects compatible with chronic cardiac dysfunction (Matsui *et al.*, 1999; Omerovic *et al.*, 2000; Fukuda *et al.*, 2004; Jane-wit *et al.*, 2007; Zuo *et al.*, 2011). These effects were associated with the induction of cAMP-stimulatory β_1-adrenoceptor autoantibodies and clinical signs consistent with chronic stimulation and desensitization of β_1-adrenoceptor signalling in the heart (Jahns *et al.*, 2004); they were reversible upon removal of the antibodies (Matsui *et al.*, 2006b), transferable via serum transfusions (Jahns *et al.*, 2004; Matsui *et al.*, 2006a; Jane-wit *et al.*, 2007; Liu *et al.*, 2008) and at least partially abrogated by β_1-adrenoceptor antagonists (Matsui *et al.*, 2000). These findings support the assumption that autoantibodies stimulating G_s-coupling of the β_1-adrenoceptor are a probable cause or decisive pathogenic cofactor of chronic heart failure. This assumption implies that there should be symptoms of increased heart rate or contractility during the early phase of immunization, which has not been observed. Moreover, there is evidence that receptor antibodies induced by the immunization of rodents act in a different fashion on receptor activity than human autoantibodies associated with DCM or Chagas' disease (Jahns *et al.*, 2000).

Despite these inconsistencies, the paradigm of Chagas' cardiomyopathy in conjunction with the available results of experimental immunizations build a reasonably strong case for autoantibodies against the second extracellular loop domain of the human β_1-adrenoceptor as a specific pathogen in chronic left ventricular dysfunction. These autoantibodies appear to somehow stimulate the receptor and thereby cause left ventricular failure in immunized animals. Currently, the pathogenic effect is blamed on inappropriate ino- and chronotropism leading to down-regulation and desentization of the cardiac β_1-adrenoceptor (Jahns *et al.*, 2004; 2006a). However, it has also been shown that such autoantibodies stimulate apoptosis (Staudt *et al.*, 2003; Jane-wit *et al.*, 2007) and stress responses of the endoplasmic reticulum (Liu *et al.*, 2008) in isolated cardiomyocytes, suggesting that the pathogenic mechanism may also involve direct myocardial cytotoxicity. The high incidence of stimulatory β_1-adrenoceptor autoantibodies in DCM (Magnusson *et al.*, 1994; Jahns *et al.*, 1999b; Staudt *et al.*, 2001; Nikolaev *et al.*, 2007) and Chagas' disease (Labovsky *et al.*, 2007) and the predictive value of β_1-adrenoceptor autoantibodies for the development of Chagas' cardiomyopathy (Wallukat *et al.*, 2010) supports the hypothesis that these autoantibodies can trigger chronic left ventricular dysfunction not only in immunized rodents but also in human patients (Jahns *et al.*, 2006b).

Role of cardiostimulatory versus -depressant autoantibodies

In contrast to β_1-adrenoceptor autoantibodies, the role of β_2-adrenoceptor or m2AChR autoantibodies in the pathogenesis of cardiomyopathy remains somewhat enigmatic. These autoantibodies are also frequently found in association with DCM (Fu *et al.*, 1993; Magnusson *et al.*, 1996) and Chagas' cardiomyopathy (Wallukat *et al.*, 2010). However, they are expected to have the opposite effect to β_1-adrenoceptor autoantibodies, that is, to act cardiodepressive via G_i-mediated inhibition of adenylate cyclase (Higgins *et al.*, 1973) and impairment of L-type Ca^{2+} currents (He *et al.*, 2005). Interestingly, the latter effect was found to dominate in some studies of IgG samples from Chagas' patients (Hernandez *et al.*, 2003). Moreover, haemodynamic improvement following extracorporeal removal of *bona fide* cardiopathogenic IgG from DCM patients is poorly correlated to the extracorporeal removal of cardiostimulatory autoantibodies (Felix *et al.*, 2002; Wallukat *et al.*, 2002; Mobini *et al.*, 2003; Dorffel *et al.*, 2004; Kallwellis-Opara *et al.*, 2007) or their subsequent reappearance (Felix *et al.*, 2002). The parameter most closely related to the extent and time course of haemodynamic response to immune absorption therapy is the effect of eluated autoantibodies on L-type Ca^{2+} currents and cardiomyocyte contraction (Felix *et al.*, 2002; Trimpert *et al.*, 2010). However, these cardiodepressant effects may not solely be caused by stimulation of G_i-coupled cardiac receptors (e.g. m2AChR or β_2-adrenoceptor). They may also be exerted through interactions of the Fc part of cardiac autoantibodies with Fc receptors that have been associated with the development of many human autoimmune diseases (Takai, 2002). Fcγ receptors IIa recently discovered on cardiomyocytes may be involved in the negative inotropic effects of cardiac antibodies obtained from DCM patients (Staudt *et al.*, 2007). After autoantibody-binding to the respective myocardial

antigen via the F(ab)$_2$ part and cross-linking via the Fc part to Fcγ receptors IIa, these receptors then may induce an activating signal via the FC receptor's cytoplasmic domain, thereby possibly triggering a cardiodepressive effect (see Figure 1I). This novel mechanism is independent of the cardiac antigen specifically targeted by these antibodies. It seems particularly relevant for the response to immune-adsorption therapy, as patients with a polymorphism of the Fcγ receptor IIa that is associated with low affinity to the Fc fragment of antibodies exhibit significantly greater improvement in left ventricular function upon extracorporeal IgG elimination (Staudt *et al.*, 2010).

It is unclear whether cardiodepressant autoantibodies acting via stimulation of the m2AChR or possibly the β_2-adrenoceptor also play a causal role in cardiomyopathy. Data of immunization experiments with the second extracellular loop of the β_2-adrenoceptor are not available. Immunizations against the second extracellular loop of the m2AChR had inconclusive results, as symptoms consistent with cardiomyopathy were only inducible by combined immunization with peptides corresponding to the second loop of the β_1-adrenoceptor (Matsui *et al.*, 1999). However, m2AChR autoantibodies arising from such immunizations directly induce fibrillation in isolated atria (Hong *et al.*, 2009), and this finding is consistent with the increased incidence of m2AChR autoantibodies in patients suffering from atrial fibrillation in conjunction with DCM (Baba *et al.*, 2004) or Graves' hyper-thyreoidism (Stavrakis *et al.*, 2009). In a recent study the combined impact of autoantibodies against β_1-adrenoceptor, β_2-adrenoceptor and m2AChR retrieved from patients with cardiomyopathy and/or atrial tachyarrhythmias on isolated canine Purkinje fibre contractility was addressed in a systematic manner (Stavrakis *et al.*, 2011). This study revealed that in most samples the positive inotropic effects of β_1-adrenoceptor autoantibodies were negatively modulated by coincident β_2-adrenoceptor and m2AChR autoantibodies, prompting the conclusion that β_1-selective antagonists routinely used in these clinical conditions may place the patients at a disadvantage due to the unopposed muscarinic effect of m2AChR autoantibodies and the possible unmasking of G_i-signalling by β_2-adrenoceptor autoantibodies. Both effects could blunt the contractile response of the failing heart mandating an adjustment of medication to the individual 'mix' of receptor autoantibodies present in a given patient. In principle, the same reasoning applies to the treatment of DCM with cyclic peptides specifically neutralizing β_1-adrenoceptor autoantibodies (proposed by Jahns *et al.*, 2006a; 2010), while it should not play a role in therapy regimen employing extracorporeal elimination of all IgG.

In summary, the available data are consistent with a model where stimulatory autoantibodies directed against the β_1-adrenoceptor cause chronic heart failure through continuous inappropriate ino- and chronotropism and/or cytotoxic effects on cardiomyocytes. It seems conceivable that neutralization of these autoantibodies by peptides could prevent or postpone clinical manifestations of the disease as demonstrated in immunized rodents (Jahns *et al.*, 2006a; 2010). However, the clinical phenotype of the full-blown disease seems to be influenced more significantly by the simultaneous occurrence of cardiodepressant autoantibodies. In this respect autoantibodies acting through the m2AChR and possibly also the β_2-adrenoceptor seem to promote the incidence of atrial tachy-arrhythmia and may indicate the selection of appropriate receptor-directed medication, whereas cardiodepressant effects due to simultaneous interaction of IgG autoantibodies with specific myocardial antigens and Fcγ receptors IIa seem to be a relevant criterion for the prediction of the haemodynamic response to immune-adsorption therapy, irrespective of which myocardial antigen is specifically targeted.

What happens at the level of the receptor?

It is frequently proposed that stimulatory autoantibodies against second extracellular loops of adrenergic and cholinergic receptors are allosteric receptor agonists (Jahns *et al.*, 2006b). This hypothesis is mainly based on the longstanding observation that human autoantibodies and antibodies raised in rodents against second extracellular loops of the human β_1-adrenoceptor or m2AChR decrease affinity and maximal capacity of equilibrium radioligand binding to the receptor in a dose-dependent fashion (Magnusson *et al.*, 1990; Fu *et al.*, 1993; Jahns *et al.*, 2000). This observation suggests a classical non-competitive type of interaction typical for allosteric receptor modulation (Kenakin, 2004; Kenakin, 2003). However, given the close position of the targeted epitope relative to the binding site (Cherezov *et al.*, 2007), this observation could also be interpreted in terms of a steric hindrance of ligand access to the ligand binding pocket. The impact of human β_1-adrenoceptor autoantibodies on ligand binding is frequently associated with moderately increased cAMP stimulation through the otherwise unliganded receptor (Magnusson *et al.*, 1994; Jahns *et al.*, 2000; Nikolaev *et al.*, 2007). However, this weak agonist-like activity intrinsic to the autoantibodies is notably different from that of classical agonists: The autoantibodies have a weaker chrono- and inotropic potency and are less prone to induce receptor desensitization and down regulation of β-adrenergic signal transduction (Christ *et al.*, 2006); they have a higher potency to trigger apoptosis (Staudt *et al.*, 2003; Jane-wit *et al.*, 2007); they induce stress responses of the endoplasmic reticulum (Liu *et al.*, 2008); they stimulate the ERK1/2 pathway through a different intracellular signal cascade (Tutor *et al.*, 2007). Moreover, maximal cAMP stimulation by a classical agonist can be potentiated or attenuated when a stimulatory autoantibody is bound at the same time (Jahns *et al.*, 2000). Taken together, these observations suggest that the autoantibodies can have three distinct effects on the receptor: (i) they modulate the binding of true ligands; (ii) they activate *per se* various effector pathways downstream of the receptor; (iii) they modulate the receptor's disposition and response to simultaneous agonist binding in a varied manner.

One possible mechanism to explain these pleiotropic effects is that the antibodies induce or stabilize changes in receptor conformation that mimic or modulate the ones induced or stabilized by true agonistic ligands (see Figure 1B). At least for β_1-adrenoceptor autoantibodies it is known that they bind to conformational epitopes (Jahns *et al.*, 1999b; 2000) and therefore have the potential to alter receptor

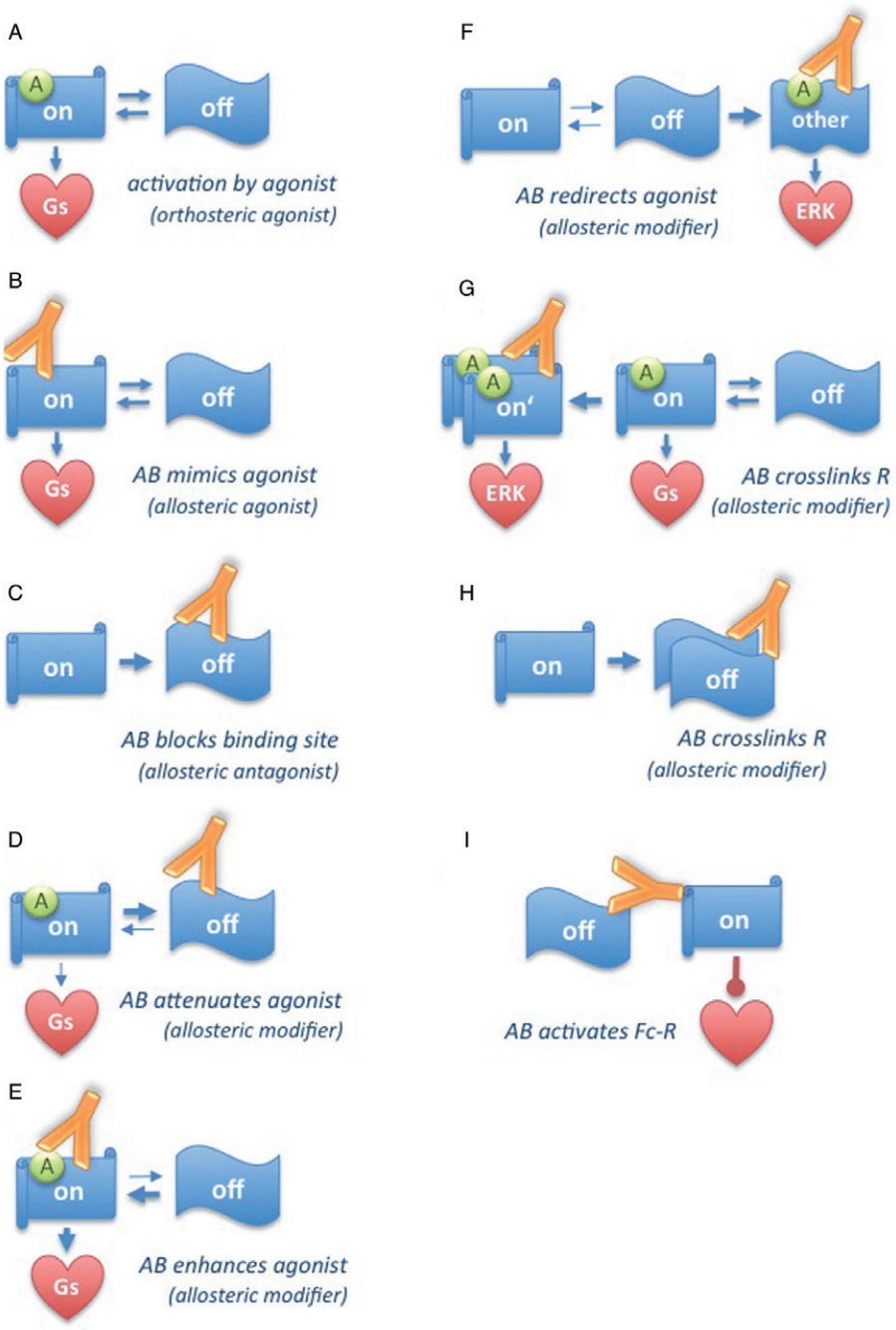

Figure 1

Modes of modulation of receptor conformation and function by autoantibodies. G-protein coupled receptors idle between various conformations with different abilities to couple to specific signalling pathways; orthosteric agonists act by stabilizing receptor conformations linked to one specific signalling pathway (A). Autoantibodies can act as direct allosteric agonists by mimicing agonists (B) or act as direct allosteric antagonists by blocking the agonist's binding site (C). Autoantibodies can act as allosteric modulators by promoting inactive (D) or active (E) conformations of the unliganded receptor thereby enhancing or attenuating subsequent agonist actions. Autoantibodies can act as allosteric modulators by inducing alternative receptor conformations that predispose for coupling to other signalling pathways (F). Autoantibodies can also modify agonist action by promoting receptor polymerization, which may enhance, redirect (G) or attenuate agonist action (H) depending on which receptor is targeted. Autoantibodies can activate as yet unknown cardiodepressive signalling pathways by cross-linking G-protein coupled receptors with Fc-receptors (I).

conformation and function. They exert these putative effects through established interactions with a receptor domain, which, based on the known structure of the β_2-adrenoceptor (Cherezov *et al.*, 2007), is not a part of the ligand binding pocket, but forms a separate, extracellular helix (Cherezov *et al.*, 2007). This helix can reach down into the ligand-binding pocket and touch the ligand; disulfide bonds crucial for keeping the entire helix out of the binding pocket (Cherezov *et al.*, 2007) and thus ensuring proper ligand binding (Dohlman *et al.*, 1990; Bywater, 2005) are located within the very epitopes targeted by the antibodies (Magnusson *et al.*, 1990). Thus, interference with this domain's conformation and relative position within the receptor molecule seems a plausible mechanism by which the autoantibodies could hinder the access of ligands to the binding pocked and/or induce distortions of the ligand binding pocket that alter its ligand binding properties and/or mimic the effects of orthosteric agonist binding (Cherezov *et al.*, 2007). Along the same lines it is conceivable that the autoantibodies block the ligand-binding site or stabilize the inactive receptor conformation, thus acting as allosteric antagonists (Figure 1C) or attenuators (Figure 1D). However, up to now, it has not been demonstrated that autoantibodies against second extracellular loops of the β_1-adrenoceptor, β_2-adrenoceptor or m2AChR are indeed capable of triggering changes in the conformation of these receptors; consequently it is not known whether such putative conformation changes could have any resemblance to the ones known to be triggered by true agonistic ligands when they occupy the ligand binding pocket (Cherezov *et al.*, 2007).

Another possible mechanism of autoantibody action is the stabilization of transient default states of the receptor. It has been suggested that receptors can exist in (or even idle between) distinct states with different abilities of G-protein interaction. Certain receptor states are selected by agonists to promote particular receptor/G-protein combinations with different abilities to stimulate particular effectors (Figure 1A). This mechanism for instance plays a role in the pleiotropic response of the β_2-adrenoceptor to the various β-agonists used in the treatment of obstructive lung disease (Swift *et al.*, 2007). Moreover, receptors can regulate various cellular functions by direct recruitment, activation and scaffolding of cytoplasmic signalling complexes via β-arrestins (Lefkowitz and Shenoy, 2005), a mechanism playing a role in the divergent effects of various agonistic and inverse agonistic β-adrenergic ligands on cAMP- versus MAP-kinase signalling (Azzi *et al.*, 2003). There are some indications that β_1-adrenoceptor autoantibodies could exert their effects by changing the spectrum of receptor states. There is evidence that they can influence the stability of transient conformational states of the β_1-adrenoceptor induced by true agonists (Hoebeke, 1996; Mijares *et al.*, 1996a; Jane-wit *et al.*, 2007). Based on these observations it is conceivable that the autoantibodies act as enhancers or attenuators of normal agonist action by stabilizing active or inactive intermediates of receptor conformations involved in normal signalling (Figure 1D, E). They could also redirect agonist action to alternative downstream signalling pathways, as they promote or stabilize transient conformations of the unliganded receptor, which then possibly pre-dispose to its selection by an agonist for the promotion of a particular set of downstream events, while the receptor would be selected for another set of downstream events when no autoantibody is bound (Figure 1F). Such hypothetical mechanisms could explain how autoantibodies can, at the same time, increase basal and decrease maximal receptor activity (Jahns *et al.*, 2000) and how they can possibly alter the receptor's balance of signalling into distinct downstream pathways (Tutor *et al.*, 2007).

The third possible mechanism is that the autoantibodies have an impact on receptor di-/ oligomerization. It is known that heterodimerization between β_1-adrenoceptor and β_2-adrenoceptor plays a crucial role in the regulation of cardiac contractility (Zhu *et al.*, 2005) as well as receptor internalization and the activation of β-arrestin-dependent effector systems such as ERK1/2 (Lavoie *et al.*, 2002). The m2AChR on the other hand has been demonstrated to exist as a constitutive homotetramer (Pisterzi *et al.*, 2010) but the relevance of m2AChR oligomerization for receptor function is unclear (reviewed in Milligan, 2008; Smith and Milligan, 2010). Given their divalent binding domain, IgG autoantibodies are ideally suited to induce or redirect receptor polymerization and thereby modulate downstream signalling (Figure 1G, H). On the other hand they can form bulky receptor adducts that at high titers (where IgG concentration is in excess to receptor density) could lead to inhibition of receptor oligomerization or hetero-dimerization. The autoantibodies could thereby exert some or even all of their functional effects without the need to interfere with the activation associated intra-molecular conformation switch of the receptor. However, smoking gun experiments demonstrating the impact of autoantibodies on receptor oligomerization (or the absence of such effects) are not available to date.

Diagnostic issues

It is not clear how autoantibodies against receptors of the autonomous nervous system can be reliably measured in a clinical setting. Measurements of the impact of isolated IgG on Ca^{2+}-transients or the contraction amplitude and frequency of isolated mammalian cardiomyocytes (Wallukat and Nisson, 2001) or cardiac fibres (Stavrakis *et al.*, 2011) seems to be the most valid and unbiased diagnostic approach, as it detects the net outcome of pleiotropic autoantibody actions in an analytical setting with a high resemblance of the situation in the patient. It has been demonstrated that such assays have a high power to predict the development of cardiomyopathy in asymptomatic Chagas' patients (Wallukat *et al.*, 2010). However, these bio-assays are difficult to standardize and for a number of other technical reasons are unsuitable as a routine clinical diagnostic test. The pillars of autoantibody detection in clinical studies have been solid phase immune assays based on peptide analogues of the crucial epitopes targeted in the β_1-adrenoceptor (Magnusson *et al.*, 1990) and the m2AChR (Fu *et al.*, 1993). However, a number of considerations caution against the use of peptide binding as the sole criterion of autoantibody detection. First of all, these assays cannot discriminate between autoantibodies that stimulate the receptor and those that merely bind or even block the receptor. There is increasing evidence that this distinction is crucial, as it seems to be not the mere presence

of the antibodies but their impact on receptor function that is related to cardiopathogenesis (Stork *et al.*, 2006; Nikolaev *et al.*, 2007). Secondly, there are huge discrepancies between the abilities of human β_1-adrenoceptor autoantibodies to bind to peptide analogues as compared with binding to native receptors presented on the cell surface (Jahns *et al.*, 1999a; Labovsky *et al.*, 2007). Moreover, the autoantibody epitope is lost upon denaturation of the receptor and recovered upon its re-naturation (Jahns *et al.*, 1999a). These observations suggest that the autoantibodies target a labile conformational epitope that is poorly represented by synthetic receptor analogues and difficult to preserve outside the living cell. Consequently, various live cell-based assays have been developed (Labovsky *et al.*, 2007; Nikolaev *et al.*, 2007) that are currently evaluated in clinical studies (Deubner *et al.*, 2010) but have as yet not been made available for routine clinical diagnostics.

Therapeutic issues

Currently, β_1-adrenoceptor antagonists and angiotensin converting enzyme inhibitors or angiotensin receptor blockers are considered equally effective first line therapeutics of DCM. There is, however, some debate as to which of the two strategies should be employed first (Funck-Brentano *et al.*, 2011). It has been proposed that the effectiveness of β_1-adrenoceptor blockade is to some extent due to the disruption of receptor stimulation by autoantibodies (Magnusson *et al.*, 1996) and there are some recent indications that therapy responses to β-blockers are indeed correlated with the presence of β_1-adrenoceptor autoantibodies (Nagatomo *et al.*, 2009). However, in many DCM patients, the positive inotropic effects of β_1-adrenoceptor autoantibodies are negatively modulated by coincident β_2-adrenoceptor and m2AChR autoantibodies so that β_1-selective antagonists will unmask unfavourable muscarinic effects of m2AChR autoantibodies and/or G_i-signalling by β_2-adrenoceptor autoantibodies (Stavrakis *et al.*, 2011).

Similar concerns apply to therapy approaches aiming at the specific removal of β_1-adrenoceptor autoantibodies by adsorption to synthetic β_1-adrenoceptor analogues (Wallukat *et al.*, 2002; Mobini *et al.*, 2003) or their specific neutralization by the systemic application of such analogues that has been demonstrated *in vitro* (Haberland *et al.*, 2011) or in immunized rodents (Jahns *et al.*, 2010) but as yet has not been tested in patients. Given the imperfect representation of the autoantibody epitope by synthetic analogues it is to be expected that only a subgroup of antibodies will be targeted by such procedures. Moreover, selective removal or blockade of β_1-adrenoceptor autoantibodies could elicit negative effects due to the unmasking of m2AChR- and/or β_2-adrenoceptor autoantibodies. This concern is clearly supported by the observation that β_1-adrenoceptor antagonists are not counteracting all the adverse effects of cardio depressant autoantibodies, as end-stage DCM patients subjected to total unselective IgG exchange benefit from positive haemodynamic effects that are additive to those of a preceding therapy with β_1-adrenoceptor antagonists (Felix *et al.*, 2002). However, it is unclear whether these additional beneficial effects are due to the removal of autoantibodies targeting autonomous transmitter receptors or of autoantibodies targeting other myocardial antigens. It is moreover unclear whether these effects – if related to the removal of receptor autoantibodies – rely on the disruption of receptor signalling by the autoantibodies or abolishment of cardiodepressant effects delivered through simultaneous interactions of the autoantibodies with Fc-receptors (Staudt *et al.*, 2007).

Summary, conclusion and outlook

Over the last decade, humoral autoimmunity against β-adrenergic and cholinergic receptors has developed from a curious coincidence to a probable cause of chronic heart failure. Various therapeutic concepts of targeting this pathogenic process in a causal manner show promising results in the treatment of end-stage DCM. However, our knowledge about how the autoantibodies alter receptor function and how these effects possibly contribute to the pathogenesis and the clinical phenotype of chronic heart failure is still very limited. As a consequence, it is not known, which features of the autoantibodies should be assessed to indicate and control antibody-directed therapy. Peptide-directed binding assays have an insufficient sensitivity and specificity, because the autoantibodies target a conformational epitope that is only exposed when the receptor has its native conformation and is properly embedded in the cell membrane. On the other hand, the assessment of selected autoantibody effects on receptor function seems an insufficient criterion, given the pleotropic effects of β_1-adrenoceptor autoantibodies on various functions of various receptors with opposing biological functions. Moreover, it is not clear to what extent the phenotypes of chronic heart failure are determined by the individual contributions of cardiostimulating autoantibodies promoting Gs-coupling of β_1-adrenoceptor and cardiodepressive autoantibodies actin through Fcγ receptors IIa or promoting G_i-coupling of β_2-adrenoceptor and m2AChR. Currently, the measurement of the impact of isolated IgG on Ca^{2+}-transients or the contraction of isolated mammalian cardiomyocytes seems the most valid and unbiased diagnostic approach. More practical diagnostic assays could be designed, if more information were available regarding the molecular action of the autoantibodies at the level of the receptor. The current lack of such specific and practical diagnostic criteria and tools is particularly unfortunate, as these autoantibodies seem to constitute a part of the natural immunologic repertoire and therefore are also present in the bloodstream of many healthy individuals, which may or may not develop an autoimmune cardiomyopathy later on.

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Conflict of interest

We state that there are no conflicts of interest to disclose by any of the authors.

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