Semin Immunopathol (2014) 36:351–363
DOI 10.1007/s00281-014-0425-9

REVIEW

Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases

Gerd Wallukat · Ingolf Schimke

Received: 5 February 2014 / Accepted: 1 April 2014 / Published online: 29 April 2014

Abstract Agonistic autoantibodies (AABs) against G-protein-coupled receptor (GPCR) are present mainly in diseases of the cardiovascular system or in diseases associated with cardiovascular disturbances. The increasing knowledge about the role of autoantibodies against G-protein-coupled receptor (GPCR-AABs) as pathogenic drivers, the resulting development of strategies aimed at their removal or neutralization, and the evidenced patient benefit associated with such therapies have created the need for a summary of GPCR-AAB-associated diseases. Here, we summarize the present knowledge about GPCR-AABs in cardiovascular diseases. The identity of the GPCR-AABs and their prevalence in each of several specific cardiovascular diseases are documented. The structure of GPCR is also briefly discussed. Using this information, differences between classic agonists and GPCR-AABs in their GPCR binding and activation are presented and the resulting pathogenic consequences are discussed. Furthermore, treatment strategies that are currently under study, most of which are aimed at the removal and in vivo neutralization of GPCR-AABs, are indicated and their patient benefits discussed. In this context, immunoadsorption using peptides/proteins or aptamers as binders are introduced. The use of peptides or aptamers for in vivo neutralization of GPCR-AABs is also described. Particular attention is given to the GPCR-AABs directed against the adrenergic beta1-, beta2-, and α1-receptor as well as the muscarinic receptor M2, angiotensin II-angiotensin receptor type I, endothelin1 receptor type A, angiotensin (1–7) Mas-receptor, and 5-hydroxytryptamine receptor 4. Among the diseases associated with GPCR-AABs, special focus is given to idiopathic dilated cardiomyopathy, Chagas' cardiomyopathy, malignant and pulmonary hypertension, and kidney diseases. Relationships of GPCR-AABs are indicated to glaucoma, peripartum cardiomyopathy, myocarditis, pericarditis, preeclampsia, Alzheimer's disease, Sjörgren's syndrome, and metabolic syndrome after cancer chemotherapy.

Keywords G-protein-coupled receptors · Autoantibodies · Cardiomyopathy · Cardiovascular diseases · Hypertension · Treatment options

Introduction

Autoimmune processes are increasingly accepted as the origins or amplifiers of various diseases. Classic autoantibodies (AABs) induce regular immune responses leading to the destruction of the affected tissue. There is one additional class of AABs, however, that shows functional activity. These AABs target G-protein-coupled receptors (GPCRs), agonistically innervating their function [1], and are therefore referred to as agonist-like AABs.

The knowledge about autoantibodies against G-protein coupled receptor (GPCR-AABs)—particularly with regard to their pathogenic function in several cardiovascular diseases—has continuously increased over the last two decades [1–4]. Table 1 summarizes data of GPCR-AABs, their activity, their distribution, and their prevalence in patients with cardiovascular diseases and diseases associated with vascular complications.

The story of GPCR-AABs in cardiovascular diseases began in 1976, when Sterin-Borda et al. [5] discovered the agonistic activity of chagasic sera. In 1984, Borda

This article is a contribution to the special issue on B cell-mediated autoimmune diseases - Guest Editors: Thomas Winkler and Reinhard Voll

G. Wallukat (✉)
Max Delbrück Centrum für Molekulare Medizin, Berlin-Buch, Robert-Rössle-Straße 10, 13125 Berlin, Germany
e-mail: gwalluk@mdc-berlin.de

I. Schimke
Charité, Universitätsmedizin Berlin, Chariteplatz 1, 10117 Berlin, Germany
e-mail: ingolf.schimke@charite.de

Table 1 G-protein-coupled receptors targeted by functional autoantibodies

Receptor	Disease	Activity	Extracell. Loop	Prevalence (%)
β1-Adrenoceptor	Dilated cardiomyopathy	Agonistic	I.or II.	70–80
β1-Adrenoceptor	Peripartum cardiomyopathy	Agonistic	II.	n.d.
β1-Adrenoceptor	Myocarditis	Agonistic	II.	60
β1-Adrenoceptor	Chagas' cardiomyopathy	Agonistic	II.	100
β1-Adrenoceptor	Electric. Cardiac abnormalities	Agonistic	II.	n.d.
β1-Adrenoceptor	Ventricular tachycardia	Agonistic	II.	n.d.
β1-Adrenoceptor	Arrhythmia	Agonistic	II.	n.d.
β1-Adrenoceptor	Periodontitis	Agonistic	II.	n.d.
β2-Adrenoceptor	Allergic asthma	Inhibitory	III.	n.d.
β2-Adrenoceptor	Chagas' cardiomyopathy	Agonistic	II.	90
β2-Adrenoceptor	Open angle glaucoma	Agonistic	II.	90
β2-Adrenoceptor	Alzheimers' disease	Agonistic	I.	n.d.
β2-Adrenoceptor	Regional pain syndrome	Agonistic	II.	n.d.
β2-Adrenoceptor	Orthostatic hypotension	Agonistic	n.d.	n.d.
α1-Adrenoceptor	Refractory hypertension	Agonistic	I. or II.	44
α1-Adrenoceptor	Pulmonary hypertension	Agonistic	II.	n.d.
α1-Adrenoceptor	Diabetes mellitus type II	Agonistic	II.	n.d.
α1-Adrenoceptor	Alzheimers' disease	Agonistic	I. or II.	n.d.
α1-Adrenoceptor	Cancer after chemotherapy	Agonistic	II.	n.d.
Muscarinic M2	Chagas' disease	Agonistic	II.	80–100
Muscarinic M2	Dilated cardiomyopathy	Agonistic	II.	25–38
Muscarinic M2	Regional pain syndrome	Agonistic	II.	n.d.
Muscarinic M3	Sjörgren' syndrome	Agonistic	II.	n.d.
Muscarinic M3	Primary biliary cirrhosis	Agonistic	n.d.	n.d.
Muscarinic M3	Orthostatic hypotension	n.d.	n.d.	n.d.
Muscarinic M	Breast cancer	Agonistic	n.d.	n.d.
Angiotensin II AT1	Maligne hypertension	Agonistic	II.	14–33
Angiotensin II AT1	Preeclampsia	Agonistic	II.	90
Angiotensin II AT1	Vascular renal rejection	Agonistic	II.	100
Angiotensin 1–7 Mas	Cancer after chemotherapy	Agonistic	II.	n.d.
Endothelin 1 ETA	Pulmonary hypertension	Agonistic	II.	n.d.
Endothelin 1 ETA	Skleroderma	Agonistic	n.d.	n.d.
Serotoninergic 5HT4	Systemic lupus erythematosus	Agonistic	II..	n.d.

n.d. not determined

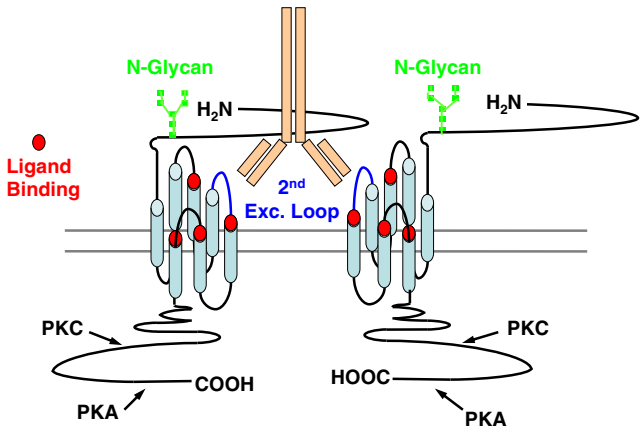
et al. [6] attributed this activity to circulating IgG, which targeted the beta-adrenergic receptors of the myocardium, thus modulating their activity. By this time, however, Venter et al. already (in 1980) described an autoantibody against the beta2-adrenergic receptor (beta2-AABs) in the sera of patients suffering from allergic asthma and rhinitis [7]. With respect to cardiomyopathies in western industrial countries, Wallukat and Wollenberger [8] first reported the existence of autoantibodies against the beta1-adrenergic receptor (beta1-AABs), which target the first (beta1(I)-AABs) or second extracellular loop of the receptor (beta1(II)-AABs) in patients with idiopathic dilated cardiomyopathy (DCM).

G-protein-coupled receptors

GPCRs are the largest superfamily in the human genome. Several hundred of receptors, including amine receptors, nucleotide receptors, olfactory receptors, and class A and B peptide-binding receptors belong to the GPCR superfamily. GPCRs are involved in the regulation of almost all of the body's systems, from sensory reception to the regulation of cell activity, movement, and death. A distinct number of neurotransmitters and hormones bind to GPCRs and internalize their information this way. Much of our recent knowledge about GPCRs has come from the work of Robert Lefkowitz

and Brian Kobilka, for which both were awarded the 2012 Nobel Prize for Chemistry.

For illustration of the GPCR structure and its communication with the related AABs, Fig. 1, reproduced from [9], depicts schematically the situation for the beta1-receptor and beta1(II)-AABs. The different epitopes on the second extracellular receptor loop targeted by beta1(II)-AABs present in DCM, Chagas' cardiomyopathy, and Peripartum cardiomyopathy are added. As an integral membrane protein, the receptor amino acid chain forms seven transmembrane regions, resulting in extracellular N-terminal and intracellular C-terminal domains as well as three extracellular and three intracellular loops this way forming the ligand-binding pocket which is detected by hypophobicity [10]. The extracellular domains are often glycosylated. Conserved cysteine, forming disulfide bridges are thought to provide receptor stabilization. Furthermore, as recently demonstrated [11], the disulfide bridges are involved in the regulation of the receptor's response to agonists and antagonists, which also explains older observations that oxidative processes interfere with agonistic receptor response in a biphasic way [12].



Epitops:	
Second Loop:	RAESDEARRCYNDPKCCDFYTN
DCM:	RAESDEARRCYNDPKCCDFYTN
Chagas' CM:	RAESDEARRCYNDPKCCDFYTN
Peripartum CM:	RAESDEARRCYNDPKCCDFYTN

Fig. 1 The human G-protein-coupled receptor targeted by the corresponding autoantibody. The N-terminal domain usually contains less than 50 amino acids and is located in the extracellular space, whereas the C-terminal part of the protein varies from 23 (muscarinergic2 receptor) to about 100 amino acids (beta2-adrenergic receptor). The transmembrane regions usually contain between 23 and 24 amino acids, limited by the helical secondary structure and the thickness of the hydrophobic lipid bilayer. The homology varies throughout the whole superfamily. However, the homology is higher (35 %; 90 %) in the transmembrane helices and functionally important side chains in the transmembrane helices, and the loops are strongly conserved between different vertebrate species. Agonists bind to a hydrophobic cave formed by the transmembrane helices. Indicated epitopes on the second extracellular loop are the targets of beta1-adrenergic receptor autoantibodies present in patients with idiopathic dilated cardiomyopathy (*DCM*), Chagas' cardiomyopathy (*Chagas' CM*), and peripartum cardiomyopathy (*Peripartum CM*). Reproduced from [9] with permission of Pabst Science Publishers, Lengerich

The intracellular part of the GPCRs contains the contact site for the hetero-trimeric G-proteins. Ligand receptor interaction results in a conformation shift that induces a cycle of G-protein activation and inactivation. This way, the G-protein modulates the activity of enzymes and ion channels, regulating the formation and the concentration of cytosolic second messengers that transfer extracellularly located signals into the cells. Prolonged receptor activation by physiologic or phamacologic ligands results in desensitization of the signal transduction.

Consequently, GPCRs play an important role in the regulation of signal transduction from the extracellular environment to the internal metabolic machinery. Disturbances in the regulation of these signal pathways can cause a shift of the metabolic balance and may induce pathologic conditions.

Autoantibodies against G-protein-coupled receptors

The extracellular domains of the receptor protein are targets of autoimmune recognition. After binding to receptors, GPCR-AABs can exert stimulatory, inhibitory, and synergistic effects on the receptors [5, 8, 13–15]. The stimulatory or agonistic AABs activate the receptors in a manner similar to that of their corresponding agonists. This leads to an activation of the receptor-mediated signal cascade. These agonistic AABs recognize epitopes localized on the first or second extracellular loops of the receptors. Inhibitory AABs, on the other hand, block the receptors and prevent their activation through the relevant agonists [16]. These AABs act via the third extracellular receptor loop. Synergistic AABs alone have no stimulatory or inhibitory activity. When combined with physiological or pharmacological agonists, however, their presence causes the agonists' effect to be significantly pronounced compared to the effect of the agonist alone.

How do agonist-like autoantibodies realize their effects?

The classic GPCR agonists and the GPCR-AABs have different binding sites (hydrophobic pockets and extracellular loops, respectively). Therefore, it is necessary to address how the agonistic GPCR-AABs realize their functional effects.

As frequently demonstrated [17–20], the agonistic responses of GPCR-AABs are closely connected with the stabilization of the active receptor conformation after AAB binding. This is clearly different from the binding of the classic agonists to the hydrophobic pockets. Based on our own investigation, we suggest that the GPCR-AABs stabilize the

active receptor conformation by dimerization. Dimerization is a frequently observed phenomenon in the activation of GPCRs, and a shift between the monomeric and dimeric state of the receptors is supposed to be important for the activity of the receptors [21]. In addition, the stimulation of cells with classic agonists shifts the receptor equilibrium from an inactive monomeric to an active dimeric receptor state, leading to an apparently bigger receptor size [22]. With regard to the GPCR-AABs, the bivalent nature of IgG would be ideally suited for receptor cross-linking and conformation stabilization.

To prove this hypothesis, we prepared bivalent $F(ab)_2$ fragments from affinity purified beta1-AABs isolated from sera of DCM patients. Despite the Fc fragment loss, the bivalent $F(ab)_2$ fragment retained its agonist-like activity, as demonstrated through our analysis of the beating frequency of cultured neonatal rat cardiomyocytes [19]. Likewise, the monovalent Fab fragments bind to the beta1-receptor. This was shown through the incubation of neonatal rat cardiomyocytes with the β-adrenergic agonist isoproterenol. It has been documented that myocardial cells indicate beta1-AAB binding in the form of a reduced response to isoproterenol. This was clearly visible as a lower level of cAMP accumulation [13] and—in our own experiments—as a reduced chronotropic response compared with that in response to isoprenaline treatment alone. Despite the obvious binding of the monovalent Fab fragment, however, any agonistic-like effect could not be observed. In contrast, when the bound Fab fragments were cross-linked with an anti-Fab antibody, cardiomyocytes responded with an agonistic effect similar to that of the intact beta1-AAB molecule. Using a human monoclonal beta2-AAB against the second extracellular receptor loop of the human adrenergic beta2-receptor for fragment preparation, we obtained comparable results.

To summarize, these data indicate that the monovalent Fab fragments themselves are able to bind to the receptors, but either the bridging of the monovalent Fab fragments or the use of structurally intact divalent GPCR-AABs for receptor dimerization seems to be obligatory for them to realize their agonist-like activity. Similar data were obtained for AABs against the muscarinic receptor M2 (M2-AABs). In the spontaneously beating cardiomyocyte model, M2-AABs induce a negative chronotropic effect, as do the muscarinic receptor M2 agonists acetylcholine and carbachol. Fab fragments prepared from monoclonal antibodies against the muscarinic receptor M2 bind to the receptors, but a negative chronotropic effect was only seen after the fragments were bridged with an anti-mouse Fab-specific antibody [18].

The hypothesis that agonist-like GPCR-AABs cross-link and stabilize the active receptor conformation was elegantly supported by the results of Elias [23]. This author isolated B cells dissected from endomyocardial biopsies from patients with chronic Chagas' disease. From these B cells, he isolated the DNA and rearranged the genes coding for the heavy- and light-chain variable regions of an antibody. By cloning the V-genes in bacteria and expressing them as recombinant Fab fragments, he designed a monovalent Fab fragment that consisted of a mouse part and a human part and contained the high-variable region. In this recombined Fab construct, the high-variable region is directed against the beta1-adrenergic receptor. Furthermore, this Fab construct contains a His-Tag for purification.

When added to cardiomyocytes, this monovalent Fab construct bound to the beta1-adrenergic receptor but did not increase the beating rate of the cells (Fig. 2a). When an antibody was added to bridge the mouse Fab fragments, a positive chronotropic response was clearly seen. Furthermore, the fact that this effect can be blocked by bisoprolol—a specific antagonist of the beta1-adrenergic receptor—shows that the above-mentioned data are clearly associated with the Fab/beta1-adrenoceptor interaction. All this has been supported by experiments using cross-linking of His-Tag-containing Fab constructs with an anti-His-Tag AAB to induce chronotropy (Fig. 2b).

Taking this information together, we propose that GPCR-AABs cross-link two receptors or a receptor dimer for active receptor stabilizing as a prerequisite of the AABs' agonist-like effect.

Staudt et al. [24] proposed an alternative mechanism for realization of the GPCR-AABs' agonistic-like activity. These authors observed that the beta1-AABs bind via their high-variable region to the receptor epitope but subsequently their Fc fragments cross-link to the cardiac Fc(gamma)-receptor IIa. In contrast to the evidence presented above, these authors did not observe any effects of the F(ab)2 fragment in their experiments.

Another line of inquiry—which must be discussed mainly because contrary observations could be explained this way—concerns whether any specific receptor environment is necessary for receptor epitope recognition by GPCR-AABs. For example, Lukitsch et al. [25] compared the effects of angiotensin II and AABs against the angiotensin II-angiotensin receptor type I (AT1-AABs) prepared from patients with kidney diseases on non-ischemic and ischemic arteries of healthy rats. In both kinds of arteries, angiotensin II caused pronounced vasoconstriction. In contrast, AT1-AABs induced the vasoconstriction only in ischemic arteries. The AT1-AAB-induced vasoconstriction was also seen in arteries prepared from rats with kidney transplantation several days before.

One other example of a lack of response was observed in the cardiomyocyte model using beta2-AABs prepared from the sera of patients with allergic asthma. These are inhibitory AABs, which realize their inhibitory effects only in cardiomyocytes cultured under stationary conditions. Under these conditions, the cells are partially under-supplied with oxygen. In so-called rocker cultures (slowly moved cultures)

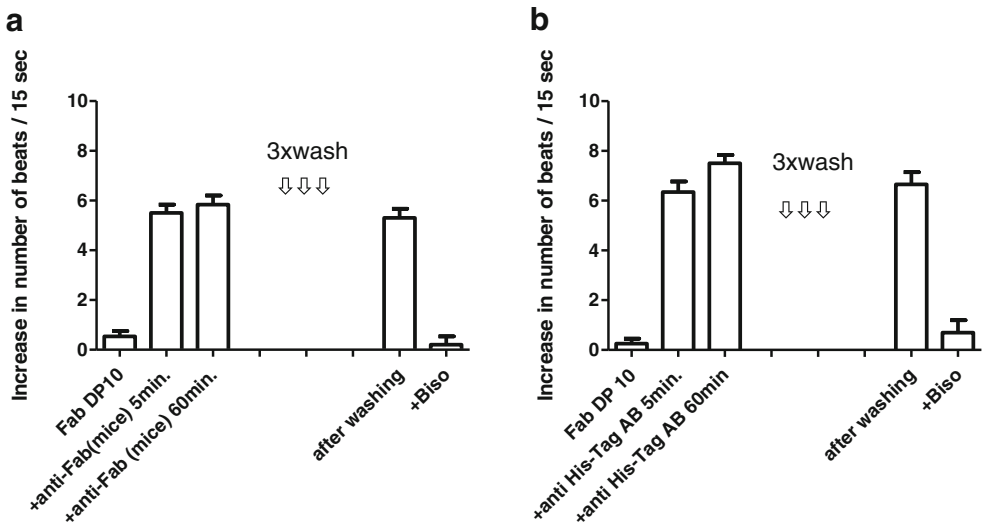


Fig. 2 Influence of the recombinant His-Tag containing Fab-DP10 fragment on cultured neonatal rat cardiomyocytes. **a** Fab-DP10 fragment recognize an epitope on the second extracellular loop of the beta1-adrenoceptor. The Fab-DP10 fragment did not increase the beating rate of the spontaneously beating rat cardiomyocytes. Addition of an anti-Fab (mice) antibody cross-linked receptor bound Fab-DP10s and stabilized on this way by receptor dimerization the active receptor conformation. This resulted in the elevation of the cardiomyocytes' beating rate. This effect was not diminished by washing. In contrast, bisoprolol antagonized the receptor binding of Fab-DP10 fragment–antibody complex. **b** The similar effect was observed when an anti-His-Tag antibody was used for cross-linking two Fab-DP10 fragments

in which the oxygen supply of the cardiomyocytes is optimal, however, the beta2-AABs were inactive. In case of addition of 3 mM L(+) lactate, the cells exhibited full activity against the beta2-AABs [26].

Based on the above examples, we hypothesize that specific metabolic conditions, such as those induced by hypoxia, ischemia, and/or inflammation, could be prerequisite to the realization of the full activity of GPCR-AABs. However, more data are necessary for confirmation of this hypothesis.

Pathophysiological consequences of the GPCR-AABs

Cell experiments, animal studies, and treatment studies concerning GPCR-AAB-positive patients have been demonstrated pathophysiological consequences of GPCR-AABs. Consequently, GPCR-AABs are understood as pathogenic drivers, mainly in cardiovascular diseases.

For beta1-AABs, it has been shown that the antibodies activate adenylate cyclase and thereby moderately elevate second messenger cyclic AMP [27]. Moreover, we observed that beta1-AABs, like the agonist isoproterenol, activate protein kinase A, which phosphorylates several phosphoproteins within the cells [28]. Furthermore, beta1-AABs can elongate the action potential duration and increase the L-type Ca^{++} (I_{Ca}) current from the extracellular into the cytosolic compartment [29].

The activation of receptors with classical agonists leads to fast inactivation and desensitization of the receptor-mediated response. Under these conditions, receptors are only marginally available to any new stimulation. Analyzing the same time of investigation, stimulation of receptors with agonistic GPCR-AABs leads to permanent stimulation without desensitization and internalization of the receptor. Under these conditions, AABs stimulate the receptor-mediated signal cascade permanently. Consequently, in the presence of GPCR-AABs, the receptor downregulation and cell protection mechanisms that regulate the effects of classic agonists do not function. This lack of tachyphylaxia, which has been observed for all agonistic GPCR-AABs, may play an important role in the pathogenesis of GPCR-AAB-associated diseases [30–32]. Only far beyond this time frame in case of long-lasting incubation with beta1-AABs, cardiomyocytes were sensitive for receptor downregulation which was visible by reduced receptor mRNA and protein levels [33]. Permanent beta1-receptor stimulation and consequent long-lasting activation of the receptor-associated signal cascades can cause Ca^{++} overload, the induction of apoptosis, and cell death [29, 34–36]. Moreover, GPCR-AABs are able to influence the maturation and the degranulation of cardiac mast cells [37].

Taken together, these findings constitute an increasing body of evidence suggesting that the antibody-induced loss of desensitization and internalization represents a general principle. These findings demonstrate that agonistic antibodies can induce permanent stimulation. Under these conditions, the cells lose their most important protection mechanism against agonist-mediated overstimulation.

The role of agonistic GPCRs as pathogenic drivers was substantiated in animal models. Matsui et al. immunized rabbits with peptides corresponding to the second extracellular loop of the beta1-adrenergic receptor. He observed that the

animals developed beta1-AABs, followed 12 months later by an enlargement of the left and right heart ventricles [38]. This could be prevented, however, if the animals were treated with antagonists of the beta1-adrenergic receptor [39]. Comparable results were observed by Jahns et al. [40], who immunized rats with a fusion protein of the beta1-adrenergic receptor. After 9 months, these animals developed severe symptoms of heart failure in the form of reduced pump function and dilated left ventricles. In a second set of experiments, beta1-AABs from the immunized and diseased animals were transferred to healthy inbred rats of the same strain. These animals likewise developed left ventricular dysfunction [41]. Such transfer experiments, which underline the pathogenic potency of the GPCR-AABs, were confirmed by others, Matsui et al. [42], who transferred IgG preparations and/or lymphocytes from cardiomyopathic rabbits into immunodeficient mice (SCID mice), which after 70 days, presented with elevated heart weights and heart weight/body weight ratios. This transfer also resulted in early-stage myocardial damage. Omerovic et al. [43] published a similar investigation in which lymphocytes from patients with DCM were injected into mice which then developed increased left ventricular dimension in diastole. This lymphocyte injection also caused diffuse fibrosis and induced early-stage heart dilatation.

Another strategy for proving the pathogenic role of GPCR-AABs, mainly that of the beta1-AABs, relies on animal studies designed to prevent the effects of beta1-AABs. Jahns et al. [44], for example, demonstrated that the development of symptoms was completely prevented when the immunized animals were treated with cyclized peptides corresponding to the second extracellular loop of the beta1-adrenergic receptor.

"Therapeutic peptides" have also been used in a mouse model of Chagas' disease [45]. Treatment of chagasic mice, which display acetylcholine receptor-related dysfunction, with peptides derived from the muscarinic receptor M2 prevented the typical dysfunctions observed in Chagas' mice, such as decrease in heart contractility, impaired response to carbachol, and significant reduction of acetylcholine receptor-binding sites.

Last but not least, the most persuasive arguments for beta1-AABs as pathogenic drivers can be derived from human studies, which have clearly shown that the removal of beta1-AABs from DCM patients' blood through immunoadsorption (IA) either stops disease progression or, in a considerable number of patients, causes disease regression [46, 47].

New therapeutic options

As mentioned above, the removal of GPCR-AABs appears to be a promising strategy for the treatment of GPCR-positive patients. The first study using IA to remove GPCR-AABs from patients' blood as a therapeutic option was published in 1996 for DCM patients [48]. The aim of the study was to create a bridge allowing patients with DCM who were positive for beta1-AABs to survive until heart transplantation. In this study, the removal of beta1-AABs by an unspecific whole IgG-binding immunoadsorber improved the impaired myocardial function of the patients and therefore could be used as a bridge enabling survival until heart transplantation. Several other studies have confirmed the benefit of IA in DCM patients [49–52]. In a case-controlled study with 34 patients, it was shown that the group treated with IA (17 patients) experienced long-lasting benefits. After IA, beta1-AABs were completely removed from the serum and did not return within 12 months following the treatment. Twelve months after IA, the treated group showed significant improvement in the left ventricular ejection fraction and the left ventricular end-diastolic diameter in systole compared to the IA-untreated control group [53]. Additionally, the treated patients presented with decreased serum markers of oxidative stress [54, 55]. Five years later, the survival rate was 82 % in the treated group but only 42 % in the controls without IA, although both groups had received a continuously optimized standard drug treatment [56]. The long-lasting benefits of IA for beta1-AAB-positive DCM patients have been frequently confirmed [57–59].

Due to the nonspecificity of the IgG adsorber for beta1-AABs, other GPCR-AABs such as M2-AABs which are sometimes present in DCM patients can also be removed from the blood; this can provide further benefits to the patients. In a study using the specific adsorber Coraffin® for selective beta1-AAB removal, however, the superiority of beta1-AAB removal in terms of the benefit to DCM patients was clearly evidenced [60]. Recently, Dandel et al. published a meta-analysis of patient outcomes 15 years after IA [46]. This study demonstrated that patients who underwent IA (64 %) had significantly better survival rates than DCM patients treated with standard medications (10 %). In that study, no significant differences were observed between patients treated with specific or unspecific IA, a result that once again suggests the importance of the beta1-AABs as pathogenic drivers in DCM. IA has been also suggested as a therapeutic option for patients with Chagas' cardiomyopathy [61, 62]. However, nearly all patients with Chagas' cardiomyopathy present with both beta1-AABs and M2-AABs [63], and both of these GPCR-AABs are thought to drive the disease. Consequently, adsorption of the whole IgG for parallel removal of both GPCR-AABs in chagasic patients should be superior to the selective removal of one of the two GPCR-AABs, but this remains to be confirmed.

More recently, it was shown that serum samples from patients with arterial pulmonary hypertension (PH) contain α1-AABs and AABs against the endothelin1 receptor type A (ETA-AABs) [64, 65]. In a pilot study, six patients with PH underwent IA using an unspecific column [47]. After IA, the

patients exhibited improved pulmonary and cardiac function as evidenced by reduced pulmonary resistance and diminished enlargement of the cardiac right atrium and ventricle.

IA was also applied for the treatment of patients with refractory hypertension and glaucoma. A certain fraction of patients with refractory hypertension present with α1-AABs, and these patients exhibit blood pressure reduction after IA [66]. Beta2-AABs were identified in 90 % of patients with open-angle glaucoma and were suggested to support the increase in intraocular pressure (IOP) that is typical of the disease. In a pilot study, five patients with this disease were treated with IA, and all responded with a reduction in IOP, indicating a possible role of the beta2-AABs in aqueous humor dynamics and IOP regulation [67].

In addition to the testing of GPCR-AAB-focused IA with already established columns, new binding materials were suggested and have already been tested for GPCR-AAB blood clearing [68, 69]. Recently, we suggested the application of aptamers as binders in apheresis technology [70]. Aptamers are synthetic, highly structured single- or double-stranded oligonucleotide ligands, which, when well selected, bind to their corresponding target molecules with high specificity but possess low immunogenicity and toxicity in their applications [71].

Presently, we are considering two aptamers whose characteristics may make them suitable for use in apheresis procedures aimed at GPCR-AAB removal.

The first of these two aptamers is a specific binder of beta1(II)-AABs, such as those associated with DCM, Chagas' cardiomyopathy, and peripartum cardiomyopathy [36, 72]. The second aptamer [73] binds all GPCR-AABs that have thus far been tested with—in our view—pathogenic potency in cardiovascular diseases. Non-GPCR-AABs did not bind to this aptamer.

Recently, we evidenced the potency of the beta1-AAB-specific aptamer in apheresis [74]. In this study, beta1-AAB positive SHR were treated with IA using the aptamer column which resulted in a complete removal of the AABs. As our second aptamer apparently binds to all pathogenic GPCR-AABs, its use in apheresis procedures should be decidedly superior. The first in vitro clearing experiments on GPCR-AAB-positive serum samples have been successful, supporting our position.

Yet, the high costs of apheresis and the problematic logistics of the procedure (patients must remain in apheresis centers for several days) limit the usefulness of apheresis treatment, so that in the future, probably only a minority of patients will profit from this treatment.

Recently, the use of aptamers for in vivo GPCR-AAB neutralization, specifically of beta1(II)-AABs, was described as a "hopeful avenue for future drug development to treat DCM" [3]. Preliminary results based on the treatment of beta1-AAB-positive rats with our beta1(II)-AAB-specific aptamer support this conclusion. However, as we have already reasoned with regard to apheresis, the wider spectrum of indications for our GPCR-AAB group-targeting aptamer is expected to make it the clear favorite for the treatment of GPCR-AAB-positive patients.

Before aptamers were introduced as potential neutralizers of beta1(II)-AABs, a cyclic peptide (COR-1®) corresponding to the second extracellular loop of the beta1-adrenergic receptor had already been suggested, based on successful animal experiments, for use in the neutralization of beta1-AABs in DCM patients [44]. This concept has recently passed through phase I testing [75] on its way to use in treating humans.

Autoantibodies against G-protein-coupled receptors in various diseases

Autoantibodies against beta-adrenergic receptors

As indicated in Table 1, beta1-AABs were found in the sera of 70 to 80 % of DCM patients. With somewhat variable frequency according to the patient's geographic origin, these beta1-AABs recognize the first or second extracellular receptor loops. They were measured using a bioassay [8, 63] that monitored the chronotropic activity of the beta1-AABs in neonatal cultured rat cardiomyocytes. With 70 % prevalence (personal information), the high beta1-AAB prevalence in DCM patients was confirmed through measurement using the FRET technique [76]. Other groups using ELISA techniques, however, found that only 30 to 60 % of DCM patients were serum positive for beta1-AABs [77–79].

Whereas nearly 30 % of symptomless chagasic patients present with beta1-AABs, nearly all patients in the subgroup suffering from cardiomyopathy, the most life-threatening complication of Chagas' disease, carry beta1-AABs [63]. Chagasic beta1-AABs recognize only the second extracellular loop of the beta1-adrenergic receptor and bind to an epitope localized more toward the N-terminus of this loop. This is in contrast to DCM patients, where the beta1-AABs target the cysteine-rich middle part of the second extracellular receptor loop [80]. Beta1-AABs were also found in patients with peripartum cardiomyopathy, primary electrical cardiac abnormalities, arrhythmias, ventricular tachycardia, and sudden death as well as in patients with myocarditis [81–84]. Heart patients with peridontitis presented with pronounced beta1-AAB titers [85, 86].

A few years ago, two other GPCR-AABs were found in Chagas' patients. These are beta2-AABs and M2-AABs [87–89], and their high prevalence in Chagas' patients was recently confirmed [63]. In contrast to the beta1- and M2-AABs that are mostly seen in patients with Chagas' cardiomyopathy, beta2-AABs targeting the second extracellular receptor loop are dominant in Chagas' patients suffering from mega syndromes in the gastrointestinal tract. GPCR-AAB

positivity in still symptomless chagasic patients could indicate their risk for the development of cardiomyopathy and/or gastrointestinal mega syndromes [63, 90]. As already described above, beta2-AABs were also found in patients with open-angle glaucoma [67].

Recently, beta2-AABs were also found in patients with Alzheimer's disease and in those with complex regional pain syndrome [91–93]. Whereas the beta2-AABs in patients with complex regional pain syndrome recognize the second extracellular receptor loop and belong to the IgG1 and IgG2 subclasses, the Alzheimer's beta2-AABs bind to the first extracellular loop and belong to the IgG1 subclass. The pathophysiological relevance of beta2-AAB positivity in these two diseases is still under study.

Autoantibodies against the α1-adrenoceptor

α1-AABs were first described in patients with malignant hypertension [94] (see also Table 1), but they are also present in patients with refractory hypertension, essential, and pulmonary hypertension [64–66, 91, 92, 95]. α1-AABs may be involved in the pathogenesis of hypertension due to their ability to influence the contractile state of the blood vessels [95] α1-AABs are also found in patients with Alzheimer's' disease [92] and in those with Diabetes mellitus type II [96], although their prevalence in these conditions is not accurately known.

A study in which α1-AABs were prepared from the sera of Alzheimer's patients and tested in a rat model demonstrated that these antibodies induce swelling of the endothelial cells of the brain vessels and induce a reduction in and disturbances of the blood flow in these areas [97, 98]. We mention this here as this vascular component has been found to be associated with GPCR-AABs in Alzheimer's disease.

Following chemotherapy, a number of cancer patients develop the metabolic syndrome characterized among others with high blood pressure and Diabetes mellitus. In a recently published case report [99], we described the detection of α1-AABs in a patient after chemotherapy.

Autoantibodies against the angiotensin II-angiotensin receptor type I

AT1-AABs were first identified in patients with preeclampsia [100]. In this pregnancy-associated disease, approximately 90 % of tested patients' sera were antibody positive. AT1-AABs target the second extracellular loop of the receptor and belong to the IgG3 subclass. In the pathogenesis of preeclampsia, AT1-AAB-dependent activation of the transcription factor NFκB, the tissue factor promoter AP-1 and NADPH oxidase could be important [101, 102]. In mesangial cells and trophoblasts, AT1-AABs induce the synthesis of the plasminogen activator inhibitor-I, which may contribute to hyper coagulation [2, 15, 103]. Furthermore, it has been shown that AT1-AABs activate cardiac mast cells and induce the adhesion of monocytes to endothelial cells [37, 104].

AT1-AABs were found in 33 % of patients with secondary malignant hypertension, in 18 % with renovascular diseases, and in 14 % with malignant essential hypertension [105].

AT1-AABs seem to play a pathogenic role in a subgroup of patients with allograft rejection that is in transplant recipients who experienced kidney rejection based on vasculopathy. These patients developed AT1-AABs [106]. Like the AT1-AABs in patients with malignant hypertension, the AT1-AABs of these kidney patients recognize two epitopes localized on the second extracellular loop. One of these two epitopes corresponds to the epitope identified in preeclamptic patients. Depending on the epitope targeted by the AT1-AABs, they belong to the IgG1 or IgG3 subclass. In cases of allograft rejection, it could be significant that AT1-AAB-dependent chronic kidney stimulation may induce inflammatory processes that promote the occlusion of the kidney vessels. The early identification of AT1-AABs in such cases and their removal or neutralization could therefore improve outcomes in these patients [106].

Autoantibodies against the muscarinic receptor M2

M2-AABs were first detected in patients with Chagas' disease [88, 89] and have now been found in patients with DCM [107, 108]. M2-AABs recognize an epitope on the second extracellular receptor loop [109]. The agonistic effect of M2-AABs produces negative chronotropy as demonstrated in cultured neonatal cardiomyocytes. ELISA results indicate that 36 to 38 % of DCM patients carry M2-AABs [107]. Nearly 30 % of the symptomless chagasic patients are positive for M2-AABs. Of patients with Chagas' cardiomyopathy and of those with gastrointestinal mega syndromes, nearly all carry M2-AABs [63].

Patients with complex regional pain syndrome are also positive for two different M2-AABs; one belongs to the IgG1 subclass, the other to the IgG3 subclass. The different kinds recognize different epitopes on the second extracellular receptor loop [93].

Muscarinic AABs were also found in patients with Sjörgren's syndrome, although these AABs are directed against the muscarinic M3 receptor. The effects induced by M3-AABs may contribute to the development of the disturbances in secretory functions that are typical of Sjörgren's syndrome [110–112]. Comparable M3-AABs were also detected in the sera of patients with primary biliary cirrhosis [113]. This rare autoimmune disease of the liver is often associated with Sjögren's syndrome. M3-AABs together with beta2-AABs have been found in patients with orthostatic hypotension. The AABs induce vasodilation and may cause or exacerbate the orthostatic hypotension [114].

AABs against muscarinic receptors were also described in patients with breast cancer. These AABs may promote the VEGF-A generation and neovascularization in the breast tumors via the muscarinic receptor [115, 116]. In a mice model, it has been shown that the AABs exert pro-tumor actions. The AABs are able to stimulate the tumor cell proliferation or the neovascularization of the tumor [117].

Autoantibodies against endothelin1 receptor type A

ETA-AABs are present in the sera of patients with pulmonary hypertension (PH) [47, 64, 65]. In patients with PH, the ETA-AABs recognize an epitope on the second extracellular loop. It seems that these AABs may be involved in the pathogenesis of PH because their removal by IA leads to an amelioration of the clinical symptoms. After IA, the pulmonary resistance of the patients was reduced and the dilatation of the right cardiac atrium and ventricle was significantly diminished [47]. Based on this observation, we postulated that ETA-AABs might play a central role—probably in combination with the α1-AABs mentioned above—in the development and progression of PH. ETA-AABs are also present in patients with scleroderma [118].

Autoantibodies against the angiotensin (1–7) Mas receptor

In the already cited case report [99] in which we analyzed patient blood following cancer chemotherapy for GPCRs, we described in addition to the presence α1-AABs the finding of autoantibodies against the angiotensin (1–7) Mas receptor (Mas-AABs). These AABs recognize an epitope on the second extracellular receptor loop of the receptor. In contrast to the α1 AABs, Mas-AABs exert a negative chronotropic response that can be blocked by a specific antagonist or can be neutralized by peptides corresponding to the second extracellular receptor loop. The stimulation of the receptor by Mas-AABs could as shown for angiotensin (1–7) reduce the effects of angiotensin II.

Autoantibodies against the 5-hydroxytryptamine receptor 4

Antibodies against the 5-hydroxytryptamine receptor 4 (5HT4-AABs) were found in the sera of patients with systemic lupus erythematosus. 5HT4-AABs recognize the second extracellular receptor loop [119]. It was discussed that these AABs may contribute to the development of the typical lupus congenital heart block found in lupus affected neonates [120].

Conclusion

Functional AABs against more and more GPCRs have been found in the sera of patients with different cardiovascular diseases. There is increasingly acceptance that these agonist-like AABs overdrive different signal cascades affecting negatively the cardiovascular system. In this context, it is most important that GPCR-AABs cross-link and stabilize the active receptor conformation of the GPCRs leading to a long-lasting activation without regulation by feedback mechanisms well known for the physiological classic GPCR agonists. Consequently, GPCR-AABs are seen as pathogenic drivers. Mainly not only for cardiomyopathy but also for hypertension, glaucoma, and kidney diseases, this role of GPCRs has been underlined in animal investigations and consolidated by the clearly demonstrated patient benefit of GPCR-AAB directed IA therapy. Dou to logistic and cost of IA, treatment strategies for in vivo neutralization of GPCR-AABs should be superior. Peptides derived from the receptor epitopes targeted by the GPCR-AABs and aptamers specifically selected for GPCR-AAB binding and neutralization are hopeful tools to realize such treatment strategy.

After successful animal experiments, this treatment concept is presently on its way to humans.

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