Relevant effects of beta₁-adrenoceptor autoantibodies in chronic heart failure

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1. ABSTRACT

Patients suffering from chronic heart failure (CHF) caused or promoted by autoantibodies against cardiac β₁-adrenergic receptors (β₁AR) could benefit from specific therapies aimed at tolerance induction, removal or neutralisation of β₁AR autoantibodies, provided the patients can be selected for these therapies by reliable detection and quantitation of β₁AR autoantibodies in their circulation and by a valid assessment of the autoantibodies’s putative cardio-pathogenic potential. Here, we discuss the current state of knowledge regarding the effects of CHF-associated (auto)antibodies on β₁AR function and β₁AR-mediated signal transduction and discuss the presumed role of these effects in the development and progression of CHF. Identification of disease-relevant functional autoantibody effects and their specific assessment in medical diagnostic will be a prerequisite for the implementation of novel specific therapies not only for CHF caused or promoted by β₁AR autoantibodies but also for most other diseases involving autoantibodies that target G-protein coupled receptors.

2. INTRODUCTION

Autoantibodies against cardiac β₁-adrenergic receptors (β₁AR) cause or promote chronic heart failure (CHF) in the context of several aetiologies (1). CHF-patients could benefit from a variety of therapeutic approaches aimed at tolerance induction, removal or neutralisation of β₁AR autoantibodies (2-12) (see also in this issue: Ungerer et al. and the other contribution by Jahns-Boivin et al.). Such specific therapies may not always be curative but they can certainly provide a significant extension of the time interval between the onset of CHF and surgical interventions such as heart transplantation and ventricular assist devices (13). However, clinical implementation of specific therapies requires (i) reliable detection and quantitation of β₁AR autoantibodies in human serum and (ii) valid assessment of their putative cardio-pathogenic potential (14).

To date, reliable detection of β₁AR autoantibodies is based on functional readouts or measurements of IgG-binding to β₁AR presented on intact native cells (15). A solid phase IgG binding assay using as antigenic target native isolated cell membranes of β₁AR overexpressing cells coated onto microtitre plates is commercially available (CellTrend, Luckenwalde, Germany) and has been CE-certified for medical diagnostic. While poorly correlated with functional readouts or IgG-binding assays using native β₁AR presented on intact cells, this assay detects a similar fraction of positive individuals among DCM-patients (own unpublished observations). Further clinical epidemiological studies will be required to determine sensitivity and specificity of this assay regarding chronic heart failure and other diseases possibly associated with β₁AR autoantibodies. Assays using immobilised linear peptide mimics of the presumed epitopes in first and second extracellular loops of the receptor (16, 17) as antigenic targets (commercially offered e. g. by CUSABIO, Wuhan, PR China) clearly have insufficient sensitivity, because CHF-relevant β₁AR autoantibodies apparently target a
conformational epitope that is not or only inadequately represented by linear peptide mimics (1, 15, 18-24). The same hesitation applies to assays based on denatured cells or tissues (25). However, there may be ways to reconstitute the conformational epitope targeted by potentially cardio-pathogenic β1AR autoantibodies from composite or circular peptides (26).

However, IgG-β1AR binding to the appropriate target epitope alone is most probably not a sufficient companion diagnostic for specific therapy, because several studies show that only β1AR autoantibodies affecting receptor function play a role in CHF (18, 27) and related electrical cardiac abnormalities (28-30). Therefore, a staged diagnostic strategy is heralded. First, autoantibody-positive patients should be detected by IgG-binding to the native β1AR; second, the cardio-pathogenic potential of the β1AR-autoantibodies thus detected should be characterised by their pathogenic functional effects; third, response to specific therapy and possible reappearance of the autoantibodies in the circulation can then be monitored by again assessing IgG-binding to the native β1AR. While there exist several valid IgG-binding tests for diagnostic screening and therapy monitoring of β1AR-autoantibodies (15, 20, 23, 24, 31), the middle step of the diagnostic cascade remains ill defined. Currently, it is unclear which functional effects of β1AR-autoantibodies bring damage to the heart in CHF-pathogenesis. It is even less clear, how putative cardio-pathogenic functional effects of β1AR-autoantibodies should be assessed in the setting of health care or controlled clinical studies (14).

3. PUTATIVE CARDIO-PATHOGENIC EFFECTS OF β1AR AUTOANTIBODYES ON CELL SIGNALLING

Induction of CHF or related electrical abnormalities in various rodent models by active or passive immunisation against the β1AR is invariably associated with stimulation of β1-adrenergic signalling (27, 32-35). Moreover, clinical epidemiology suggests that only such β1AR autoantibodies that stimulate the receptor are associated with a poorer outcome of CHF and a higher incidence of related electrical abnormalities in humans (18, 27-30, 36-39).

At the molecular level, β1AR autoantibodies associated with human CHF induce/stabilise an active conformation of the β1AR-molecule (19, 40), which suggests targeting of a conformational epitope associated with the activation state of the receptor. In support of this notion, presentation of the auto-epitope is strongly biased by a common genetic polymorphism (β1AR<sup>389Gly/Ser</sup>) that alters baseline activity of the receptor (22). It has also been observed that β1AR autoantibodies inhibit the binding of radio ligands to the receptor (16, 41, 42). However, this property is not stringently associated with receptor stimulation (19).

Rather the opposite seems to be the case, namely that inhibition of ligand binding is associated with antibodies that target and stabilise the inactive conformation of the receptor (43). Therefore, inhibition of radioligand binding is probably not a relevant readout with respect to cardio-pathogenesis.

In addition to activating the receptor, CHF-associated human autoantibodies can also interfere with receptor cycling (40, 44, 45) and desensitization (46), which possibly entails either blunting or sensitization of the receptor for endogenous catecholamines (19, 40). Interestingly, the capability of β1AR autoantibodies to interfere with receptor cycling is independent from their capability to activate the receptor (40). So far, it is unclear how the distinct impact of the autoantibodies on receptor trafficking is related to cardio-pathogenesis (1).

Moreover, the susceptibility of the target for the pathogenic effect of a given set of circulating β1AR autoantibodies is modulated by a variety of confounding factors. These encompass the haplotype of the β1AR expressed in the target cell or tissue, since the overall effect of the autoantibodies is strongly influenced by a common genetic polymorphism (β1AR<sup>389Gly/Ser</sup>) affecting baseline activity of the receptor (22). In addition, β1AR autoantibodies are prone to cross-react with other subtypes of β-adrenergic receptors also expressed on the target cell or to coexist with autoantibodies targeting other β-adrenergic receptor subtypes. Variable combinations of cross-reactivity and co-expression of receptor subtypes will crucially determine the final outcome of the interaction between autoantibody and target cell. Such a mechanism has been demonstrated for the profibrotic effect of autoantibodies against endothelin receptors and angiotensin receptors in systemic scleroderma. In this disease, the level and balance of receptor subtype expression on monocytes crucially determines the impact of stimulatory autoantibodies targeting these receptors on the induction and secretion of the profibrotic effector molecule chemokine ligand 18 (CCL18) by these cells (47). Similar mechanisms may play a role in CHF induced by β1AR autoantibodies. For instance, it has been shown that pulmonary complications in CHF-patients positive for β1AR autoantibodies are more frequent when the autoantibodies cross-react with β2AR. Moreover, induction of CHF in rodents by active immunisation against the β1AR can be modulated by simultaneous immunisation against the β2AR and the balance of expression of β1/β2 receptor subtypes in heart and lung tissues becomes altered by co-immunisation (34, 48, 49). Along the same lines, the stimulatory effect of CHF-associated β1AR autoantibodies can be masked by the coincidence of stimulatory autoantibodies against muscarinic acetylcholine receptors (50). And similar
cross-reactions of stimulatory GPCR-autoantibodies with β₃AR and muscarinic acetylcholine receptors have been correlated to the incidence of rhythmic abnormalities in CHF (51-53).

Downstream of the receptor the autoantibodies also exert multiple effects on cell signalling. On the one hand, they can induce coupling to the stimulatory G-protein and thereby stimulate cAMP and cAMP-dependent signalling cascades (18, 19, 32, 40, 45, 54-56). On the other hand they can promote stimulation of the ERK1/2 pathway (45, 57), which is most probably mediated by recruitment of β₁-arrestin to the receptor (58). Systematic studies of monoclonal β₁AR-antibodies suggest that the two pathways are independently triggered via distinct epitopes of the receptor (59). Moreover, activation of the ERK1/2 pathway may also involve simultaneous recruitment of the β₂AR (45) and/or the β₁AR (34, 49).

4. TOXIC EFFECTS OF β₁AR AUTOANTIBODIES ON CARDIAC CELLS

The historical hallmark of β₁AR autoantibodies derived from CHF-patients is a positive chronotropic and dromotropic effect on isolated cardiomyocytes or atrial preparations (46, 60-62). For a long time it has been assumed that continuous exposure to such agonistic antibodies would lead to desensitisation and ultimately down-regulation of cardiac β₁AR-signalling, which is a common hallmark of CHF (63-65). Alterations of cardiac signal transduction compatible with this putative pathogenic mechanism have indeed been observed in rodents undergoing left ventricular dysfunction following active immunisation against the β₁AR or isogenic transfer of induced β₁AR antibodies (32, 33, 66, 67). However, receptor desensitisation has not been observed upon passive immunisation of rodents with agonistic β₁AR monoclonal antibodies (45, 68). Similarly, in vitro-exposure of primary cardiomyocytes or other cellular reporter systems of β₁-adrenergic signal transduction to agonistic β₁AR autoantibodies derived from human CHF-patients failed to induce receptor desensitisation or gradual attenuation of cAMP-accumulation over time (40, 45, 46, 55). In conclusion, mechanisms other than desensitisation of β₁AR signalling may be involved in cardio-pathogenesis driven by β₁AR autoantibodies in humans.

One such alternative mechanism is direct cardiomyocyte toxicity, which is suggested by several independent observations: Induction of CHF in rodents by active immunisation with β₁AR fusion proteins or passive immunisation with monoclonal β₁AR antibodies is accompanied by an increase in cardiomyocyte apoptosis and endoplasmic stress response (68-70). Similarly, β₁AR autoantibodies derived from CHF patients induce apoptosis in primary adult cardiomyocytes (71). Other putatively toxic effects of CHF-derived β₁AR autoantibodies encompass alterations of cardiac L-type calcium channels leading to calcium overload and apoptosis (72, 73) or restriction of the lateral mobility of the β₁AR through simultaneous interactions of the autoantibodies with the Fcγ receptor IIa (74). It has also been demonstrated that β₁AR autoantibodies induce homo-dimerisation of cardiomyocyte β₁AR (75), which is known to affect cardiac signalling efficacy and contractility (76, 77).

Most notably, the cardiomyocyte may not even be the only and primary target cell of cardiac autoimmune-pathogenesis directed at the β₁AR. A recent study demonstrates convincingly that passive immunisation of mice with an agonistic β₁AR monoclonal antibody leads to a CHF-compatible phenotype of cardiac dilation and fibrosis, which is induced through the stimulation of cardiac fibroblasts. On the one hand, the agonistic β₁AR monoclonal antibody stimulates fibroblast growth via cAMP and ERK1/2-signalling. Furthermore, conditioned medium of cultures of primary cardiac fibroblast treated with the agonistic β₁AR monoclonal antibody induce apoptosis of cardiomyocytes. Specific inhibition of the pathways involved in these effects abolishes induction of CHF by the antibodies. These findings could indicate that cardiomyocyte toxicity of β₁AR autoantibodies possibly has an indirect component mediated by increased proliferation and altered cytokine secretion of the cardiac fibroblast compartment (45). However, it has still to be demonstrated that human CHF-associated β₁AR autoantibodies have such an effect on human adult cardiac fibroblasts.

Based on the above observations, the following modifications of the current paradigm seem indicated: (i) cardio-pathogenesis of agonistic β₁AR autoantibodies in humans seems to be executed by a variety of mechanisms not necessarily encompassing desensitisation of cardiac β₁AR signalling, (ii) susceptibility to β₁AR autoantibodies depends on receptor haplotype and is modulated by cross reactions with other β₁AR subtypes or even other GPCR an their relative expression levels in the target tissues, (iii) the cardiomyocyte may not be the only target cell of autoimmune-pathogenesis, (iv) positive chronotropic and dromotropic effects on cardiomyocyte contraction - hitherto the hallmark of potentially cardio-pathogenic β₁AR autoantibodies - could be an epiphenomenon not necessarily involved in their pathogenic action(s).

5. ASSESSMENT OF THE CARDIO-PATHOGENIC POTENTIAL OF β₁AR AUTOANTIBODIES IN CLINICAL SETTINGS

Over four decades, the impact of autoantibodies on cardiac autonomous regulation has been determined
by stimulatory or depressive effects of isolated IgG on the contractility of rodent-derived Langendorff hearts, isolated atria or primary cardiomyocytes, and the various signalling pathways involved have been distinguished by the addition of specific receptor blockers (5, 17, 30, 46, 60, 61, 78-82). More recently, spontaneously beating, embryonic cardiomyocytes derived from induced human adult precursor cells (iPC) have been introduced as a more versatile and better standardised biological reporter system (40) allowing adaptation of the test to a high-throughput format (24, 83). However, the diagnostic practicability of cardiomyocyte contractility assays is still severely compromised by prohibitive cost and unsatisfactory standardisation. Even more importantly, the pathogenic relevance of autoantibody effects on cardiomyocyte contraction must be critically discussed in the light of recent data demonstrating in a passive immunisation model that most if not all cardio-pathogenic effects of an agonistic β1,AR monoclonal antibody have been transduced by cardiac fibroblasts (45). Thus, cardiomyocyte contraction is possibly not the only readout that should be taken into account with regard to the cardio-pathogenic potential of human β1,AR autoantibodies, besides being impractical for the diagnostic application in a clinical setting. It should also be noted that the action of the autoantibodies on cardiomyocyte contraction is also determined by their impact on receptor cycling and by common polymorphisms of the receptor (22, 40). Therefore it can only be assessed in the context of endogenous pulsatile catecholamine stimulation and the haplotype of the receptor present in the patient.

A variety of genetically engineered reporter cell systems have been created that allow a more direct and specific assessment of the effects of human autoantibodies on cell signalling that are thought to constitute the root cause of cardio-pathogenesis. These encompass cells overexpressing human β1,AR furnished with a bio-fluorescent sensor that reports on the activation-associated conformational switch of the receptor molecule by a change in intramolecular fluorescence resonance energy transfer (FRET), thereby allowing a direct measurement of the molecular activation of the receptor by autoantibodies derived from CHF-patients (40). Cells overexpressing bio-fluorescent human β1,AR have also been used to quantitatively assess the impact of the autoantibodies on receptor cycling by total internal fluorescence reflection microscopy (TIRF) (40). Another direct assay of cell signalling effects uses a cell line overexpressing human β1,AR together with a cytosolic FRET-reporter of cAMP concentration. This system has already been evaluated in a large cohort of patients. It enables a clear distinction of agonistic and non-stimulating β1,AR autoantibodies and even discriminates between strong and weak agonistic β1,AR autoantibodies (55). While all these cell line-based systems may be suitable for a specific and detailed investigation of putative cardiac-pathogenic mechanisms of β1,AR-autoantibodies at a cellular level, they are clearly unsuited for routine clinical diagnostic. This is mostly due to the fact that the analytical readouts (TIRF, FRET) have to be obtained (i) by the use of highly specialised laser scanning microscopes that are usually not available in diagnostic laboratories or (ii) by customised micro-titre plate readers equipped with multichannel fluorescence detectors of FRET (84) that are not even commercially available or not certified for clinical diagnostics.

Given the recent observation that - at least in passively immunised mice - alterations of the secretory phenotype of cardiac fibroblasts could be one of the most relevant cardio-pathogenic mechanisms of β1,AR-autoantibodies, one should consider addressing this effect in a diagnostic manner. Such an approach seems feasible since primary culture of human fibroblasts is a longstanding routine procedure and the assessment of alterations of the secretory phenotype of primary fibroblast cultures by mass spectroscopy proteomics is an established high-throughput method frequently applied in clinical studies (85).

6. CONCLUSIONS AND OUTLOOK

During the past five years, the method base for a reliable and valid detection of potentially cardio-pathogenic β1,AR-autoantibodies in human blood specimen has significantly advanced. Today, β1,AR-autoantibody-positive patients may become detectable in a clinical setting and the possibility emerges that autoantibody-titres can be monitored during the course of the disease (2, 8, 10, 15, 20). Thus, the way towards a clinical implication of existing and emerging specific therapies directed against β1,AR-autoantibodies is being paved in principle. However, animal studies as well as epidemiological data strongly suggest that only β1,AR-autoantibodies that stimulate the receptor are associated with CHF. The established IgG-binding assays cannot distinguish such stimulatory β1,AR-autoantibodies from those that just bind to the receptor without altering its activity state or those that block receptor activation. These assays are also unable to detect cross-reactions with other β1AR-subtypes or other GPCR or the modulating effect of genetic polymorphisms of the β1,AR expressed in the heart of the patient. Therefore, additional confirmatory diagnostic tests will be required that allow to judge the cardio-pathogenic potential of β1,AR-autoantibodies by their functional effects including their effects on the proliferation and cytokine-production of cardiac fibroblasts. Such predictive diagnostics will be particularly meaningful, when specific therapeutic concepts are to be applied in a preventive manner to healthy human individuals positive for β1,AR-autoantibodies, although prevalence of GPCR autoantibodies in the general (healthy) population is very low (~5 %) (1, 7, 18, 75, 86-88).
It seems obvious that prognostic tests addressing the cardio-pathogenic potential of β₁AR-autoantibodies must directly assess the impact of the autoantibodies on the function and signalling of those cardiac cells (or cell-types) presumed to be causally involved in CHF development and progression. However, currently it is not entirely clear which of the many functional effects of the autoantibodies are causally associated with CHF, and it is even less clear how the relevant effects can possibly be assessed in a clinical setting. Therefore, future research efforts should be directed at evaluating the various known functional effects of the autoantibodies in longitudinal studies and to develop diagnostic tests for those effects that exhibit a strong correlation with the onset and progression of CHF.

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