[Frontiers In Bioscience, Landmark, 23, 2146-2156, June 1, 2018]

Relevant effects of beta$_1$-adrenoceptor autoantibodies in chronic heart failure

Valerie Boivin-Jahns[1,2], Roland Jahns[2,3], Fritz Boege[4]

[1]Institute for Pharmacology and Toxicology, University of Wuerzburg, Germany, [2]Comprehensive Heart Failure Center (CHFC), University and University Hospital of Wuerzburg, Germany, [3]Interdisciplinary Bank of Biomaterials and Data Wuerzburg (IBDW), University and University Hospital of Wuerzburg, Germany, [4]Institute of Clinical Chemistry and Laboratory Diagnostics, University and University Hospital, Duesseldorf, Germany

TABLE OF CONTENTS

1. ABSTRACT

Patients suffering from chronic heart failure (CHF) caused or promoted by autoantibodies against cardiac β_1-adrenergic receptors (β_1AR) could benefit from specific therapies aimed at tolerance induction, removal or neutralisation of β_1AR autoantibodies, provided the patients can be selected for these therapies by reliable detection and quantitation of β_1AR 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 β_1AR function and β_1AR-mediated signal tranduction 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 prerequesite for the implementation of novel specific therapies not only for CHF caused or promoted by β_1AR autoantibodies but alos for most other diseases involving autoantibodies that target G-protein coupled receptors.

2. INTRODUCTION

Autoantibodies against cardiac β_1-adrenergic receptors (β_1AR) 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 β_1AR 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 β_1AR autoantibodies in human serum and (ii) valid assessment of their putative cardio-pathogenic potential (14).

To date, reliable detection of β_1AR 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 β_1AR 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 β_1AR 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 β_1AR 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 β_1AR 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 β_1AR autoantibodies from composite or circular peptides (26).

However, IgG-β_1AR binding to the appropriate target epitope alone is most probably not a sufficient companion diagnostic for specific therapy, because several studies show that only β_1AR 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 β_1AR; second, the cardio-pathogenic potential of the β_1AR- 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 β_1AR. While there exist several valid IgG-binding tests for diagnostic screening and therapy monitoring of β_1AR- autoantibodies (15, 20, 23, 24, 31), the middle step of the diagnostic cascade remains ill defined. Currently, it is unclear which functional effects of β_1AR- autoantibodies bring damage to the heart in CHF-pathogenesis. It is even less clear, how putative cardio-pathogenic functional effects of β_1AR- autoantibodies should be assessed in the setting of health care or controlled clinical studies (14).

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

Induction of CHF or related electrical abnormalities in various rodent models by active or passive immunisation against the β_1AR is invariantly associated with stimulation of β_1-adrenergic signalling (27, 32-35). Moreover, clinical epidemiology suggests that only such β_1AR 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, β_1AR autoantibodies associated with human CHF induce/stabilise an active conformation of the β_1AR-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 (β_1AR$^{389Gly/Ser}$) that alters baseline activity of the receptor (22). It has also been observed that β_1AR 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 β_1AR 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 β_1AR autoantibodies is modulated by a variety of confounding factors. These encompass the haplotype of the β_1AR expressed in the target cell or tissue, since the overall effect of the autoantibodies is strongly influenced by a common genetic polymorphism (β_1AR$^{389Gly/Ser}$) affecting baseline activity of the receptor (22). In addition, β_1AR 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 β_1AR autoantibodies. For instance, it has been shown that pulmonary complications in CHF-patients positive for β_1AR autoantibodies are more frequent when the autoantibodies cross-react with β_3AR. Moreover, induction of CHF in rodents by active immunisation against the β_1AR can be modulated by simultaneous immunisation against the β_3AR and the balance of expression of β_1/β_3 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 β_1AR autoantibodies can be masked by the coincidence of stimulatory autoantibodies against muscarinic acetylcholine receptors (50). And similar

cross-reactions of stimulatory GPCR-autoantibodies with β_1AR 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 β-arrestin to the receptor (58). Systematic studies of monoclonal β_1AR-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 β_2AR (45) and/or the β_3AR (34, 49).

4. TOXIC EFFECTS OF β1AR AUTOANTIBODIES ON CARDIAC CELLS

The historical hallmark of β_1AR 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 β_1AR-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 β_1AR or isogenic transfer of induced β_1AR antibodies (32, 33, 66, 67). However, receptor desensitisation has not been observed upon passive immunisation of rodents with agonistic β_1AR monoclonal antibodies (45, 68). Similarly, *in vitro*-exposure of primary cardiomyocytes or other cellular reporter systems of β_1-adrenergic signal transduction to agonistic β_1AR 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 β_1AR signalling may be involved in cardio-pathogenesis driven by β_1AR 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 β_1AR fusion proteins or passive immunisation with monoclonal β_1AR antibodies is accompanied by an increase in cardiomyocyte apoptosis and endoplasmic stress response (68-70). Similarly, β_1AR autoantibodies derived from CHF patients induce apoptosis in primary adult cardiomyocytes (71). Other putatively toxic effects of CHF-derived β_1AR 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 β_1AR through simultaneous interactions of the autoantibodies with the Fcγ receptor IIa (74). It has also been demonstrated that β_1AR autoantibodies induce homo-dimerisation of cardiomyocyte β_1AR (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 β_1AR. A recent study demonstrates convincingly that passive immunisation of mice with an agonistic β_1AR 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 β_1AR monoclonal antibody stimulates fibroblast growth *via* cAMP and ERK1/2-signalling. Furthermore, conditioned medium of cultures of primary cardiac fibroblast treated with the agonistic β_1AR 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 β_1AR 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 β_1AR 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 β_1AR autoantibodies in humans seems to be executed by a variety of mechanisms not necessarily encompassing desensitisation of cardiac β_1AR signalling, (ii) susceptibility to β_1AR autoantibodies depends on receptor haplotype and is modulated by cross reactions with other βAR 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 chrono- and dromotropic effects on cardiomyocyte contraction - hitherto the hallmark of potentially cardio-pathogenic β_1AR 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 β_1AR 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 β_1AR 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 β_1AR furnished with a bio-fluorescent sensor that reports on the activation-associated conformational switch of the receptor molecule by a change in intra-molecular 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 β_1AR 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 β_1AR 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 β_1AR autoantibodies and even discriminates between strong and weak agonistic β_1AR autoantibodies (55). While all these cell line-based systems may be suitable for a specific and detailed investigation of putative cardio-pathogenic mechanisms of β_1AR-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 β_1AR-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 β_1AR-autoantibodies in human blood specimen has significantly advanced. Today, β_1AR-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 β_1AR-autoantibodies is being paved in principle. However, animal studies as well as epidemiological data strongly suggest that only β_1AR-autoantibodies that stimulate the receptor are associated with CHF. The established IgG-binding assays cannot distinguish such stimulatory β_1AR-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 βAR-subtypes or other GPCR or the modulating effect of genetic polymorphisms of the β_1AR expressed in the heart of the patient. Therefore, additional confirmatory diagnostic tests will be required that allow to judge the cardio-pathogenic potential of β_1AR-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 β_1AR-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 β_1AR-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.

7. REFERENCES

1. B. Bornholz, D. Roggenbuck, R. Jahns and F. Boege: Diagnostic and therapeutic aspects of beta-adrenergic receptor autoantibodies in human heart disease. *Autoimmunity Rev* 13, 954-962 (2014)
DOI: 10.1016/j.autrev.2014.08.021

2. G. Munch, V. Boivin-Jahns, H. P. Holthoff, K. Adler, M. Lappo, S. Truol, H. Degen, N. Steiger, M. J. Lohse, R. Jahns and M. Ungerer: Administration of the cyclic peptide COR-1 in humans (phase I study): *ex vivo* measurements of anti-beta1-adrenergic receptor antibody neutralization and of immune parameters. *Eur J Heart Fail*, 14(11), 1230-1239 (2012)
DOI: 10.1093/eurjhf/hfs118

3. A. Haberland, G. Wallukat, S. Berg, A. M. Schulz, E. J. Freyse, R. Vetter, E. Salzsieder, J. Muller, R. Kreutz and I. Schimke: Neutralization of pathogenic beta1-receptor autoantibodies by aptamers *in vivo*: the first successful proof of principle in spontaneously hypertensive rats. *Mol Cell Biochem*, 393(1-2), 177-180 (2014)
DOI: 10.1007/s11010-014-2057-8

4. A. Haberland, G. Wallukat and I. Schimke: Aptamer binding and neutralization of beta1-adrenoceptor autoantibodies: basics and a vision of its future in cardiomyopathy treatment. *Trends Cardiovasc Med*, 21(6), 177-82 (2011)
DOI: 10.1016/j.tcm.2012.05.006

5. A. Haberland, G. Wallukat, C. Dahmen, A. Kage and I. Schimke: Aptamer neutralization of beta1-adrenoceptor autoantibodies isolated from patients with cardiomyopathies. *Circ Res*, 109(9), 986-92 (2011)
DOI: 10.1161/CIRCRESAHA.111.253849

6. M. Dandel, A. Englert, G. Wallukat, A. Riese, C. Knosalla, J. Stein and R. Hetzer: Immunoadsorption can improve cardiac function in transplant candidates with non-ischemic dilated cardiomyopathy associated with diabetes mellitus. *Atheroscler Suppl*, 18, 124-33 (2015)
DOI:10.1016/j.atherosclerosissup.2015.02.023

7. P. A. Patel and A. F. Hernandez: Targeting anti-beta-1-adrenergic receptor antibodies for dilated cardiomyopathy. *Eur J Heart Fail*, 15(7), 724-9 (2013)
DOI: 10.1093/eurjhf/hft065

8. V. Boivin, N. Beyersdorf, D. Palm, V. O. Nikolaev, A. Schlipp, J. Muller, D. Schmidt, V. Kocoski, T. Kerkau, T. Hunig, G. Ertl, M. J. Lohse and R. Jahns: Novel Receptor-Derived Cyclopeptides to Treat Heart Failure Caused by Anti-beta1-Adrenoceptor Antibodies in a Human-Analogous Rat Model. *PloS One*, 10(2), e0117589 (2015)
DOI: 10.1371/journal.pone.0117589

9. S. B. Felix, A. Staudt, M. Landsberger, Y. Grosse, V. Stangl, T. Spielhagen, G. Wallukat, K. D. Wernecke, G. Baumann and K. Stangl: Removal of cardiodepressant antibodies in dilated cardiomyopathy by immunoadsorption. *J Am Coll Cardiol*, 39(4), 646-52 (2002)
DOI: 10.1016/S0735-1097(01)01794-6

10. I. P. Nnane, A. H. Plotnikov, G. Peters, M. Johnson, C. Kojak, A. Vutikullird, J. Ariyawansa, R. De Vries and B. E. Davies: Pharmacokinetics and Safety of Single Intravenous Doses of JNJ-54452840, an Anti-beta1-Adrenergic Receptor Antibody Cyclopeptide, in Healthy Male Japanese and Caucasian Participants. *Clin Pharmacokinet*, 55(2), 225-36 (2016)
DOI: 10.1007/s40262-015-0309-8

11. A. Haberland, M. Holtzhauer, A. Schlichtiger, S. Bartel, I. Schimke, J. Muller, M. Dandel, P. B. Luppa and G. Wallukat: Aptamer BC 007 - A broad spectrum neutralizer of pathogenic autoantibodies against G-protein-coupled receptors. *Eur J Pharmacol*, 789, 37-45 (2016)
DOI: 10.1016/j.ejphar.2016.06.061

12. W. Ronspeck, R. Brinckmann, R. Egner, F. Gebauer, D. Winkler, P. Jekow, G. Wallukat, J. Muller and R. Kunze: Peptide based adsorbers for therapeutic immunoadsorption. *Ther Apher Dial*, 7(1), 91-7 (2003)
DOI: 10.1046/j.1526-0968.2003.00017.x

13. M. Dandel, G. Wallukat, A. Englert, H. B. Lehmkuhl, C. Knosalla and R. Hetzer: Long-term benefits of immunoadsorption in beta(1)-adrenoceptor autoantibody-positive transplant candidates with dilated cardiomyopathy. *Eur J Heart Fail*, 14(12), 1374-88 (2012)
DOI: 10.1093/eurjhf/hfs123

14. N. Deubner, D. Berliner, A. Schlipp, G. Gelbrich, A. L. Caforio, S. B. Felix, M. Fu, H. Katus, C. E. Angermann, M. J. Lohse, G. Ertl, S. Stork and R. Jahns: Cardiac beta1-adrenoceptor autoantibodies in human heart disease: rationale and design of the Etiology, Titre-Course, and Survival (ETiCS) Study. *Eur J Heart Fail*, 12(7), 753-62 (2010)
DOI: 10.1093/eurjhf/hfq072

15. B. Bornholz, B. Hanzen, Y. Reinke, S. B. Felix, R. Jahns, I. Schimke, G. Wallukat and F. Boege: Detection of DCM-associated beta1-adrenergic receptor autoantibodies requires functional readouts or native human beta1-receptors as targets. *Int J Cardiol*, 202, 728-30 (2016)
DOI: 10.1016/j.ijcard.2015.10.068

16. Y. Magnusson, S. Marullo, S. Hoyer, F. Waagstein, B. Andersson, A. Vahlne, J. G. Guillet, A. D. Strosberg, A. Hjalmarson and J. Hoebeke: Mapping of a functional autoimmune epitope on the beta 1-adrenergic receptor in patients with idiopathic dilated cardiomyopathy. *J Clin Invest*, 86(5), 1658-63 (1990)
DOI: 10.1172/JCI114888

17. G. Wallukat, A. Wollenberger, R. Morwinski and H. F. Pitschner: Anti-beta 1-adrenoceptor autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops. *J Mol Cell Cardiol*, 27(1), 397-406 (1995)
DOI: 10.1016/S0022-2828(08)80036-3

18. R. Jahns, V. Boivin, C. Siegmund, G. Inselmann, M. J. Lohse and F. Boege: Autoantibodies activating human beta1-adrenergic receptors are associated with reduced cardiac function in chronic heart failure. *Circulation*, 99(5), 649-54 (1999)
DOI: 10.1161/01.CIR.99.5.649

19. R. Jahns, V. Boivin, T. Krapf, G. Wallukat, F. Boege and M. J. Lohse: Modulation of beta(1)-adrenoceptor activity by domain-specific antibodies and-heart failure-associated autoantibodies. *J Am Coll Cardiol*, 36(4), 1280-1287 (2000)
DOI: 10.1016/S0735-1097(00)00881-0

20. B. Bornholz, T. Benninghaus, Y. Reinke, S. B. Felix, D. Roggenbuck, V. Jahns-Boivin, R. Jahns and F. Boege: A standardised FACS assay based on native, receptor transfected cells for the clinical diagnosis and monitoring of beta1-adrenergic receptor autoantibodies in human heart disease. *Clin Chem Lab Med*, 54(4), 683-91 (2016)
DOI: 10.1515/cclm-2015-0603

21. R. Jahns and F. Boege: Questionable validity of petide-based ELISA strategies in the diagnostics of cadrdiopathogenic autoantibodies that activate G-protein-coupled receptors. *Cardiology*, 131, 149-150 (2015)
DOI: 10.1159/000376546

22. B. Bornholz, B. Hanzen, Y. Reinke, S. B. Felix and F. Boege: Impact of common beta-adrenergic receptor polymorphisms on the interaction with agonistic autoantibodies in dilated cardiomyopathy. *Int J Cardiol*, 214, 83-85 (2016)
DOI: 10.1016/j.ijcard.2016.03.032

23. F. Boege, R. Westenfeld and R. Jahns: beta1AAb Determined by Peptide ELISA: A Signal in the Noise? *J Am Coll Cardiol*, 70(6), 807-808 (2017)
DOI: 10.1016/j.jacc.2017.03.617

24. K. Wenzel, S. Schulze-Rothe, J. Muller, G. Wallukat and A. Haberland: Difference between beta1-adrenoceptor autoantibodies of human and animal origin-Limitations detecting beta1-adrenoceptor autoantibodies using peptide based ELISA technology. *PLoS One*, 13(2), e0192615 (2018)
DOI: 10.1371/journal.pone.0192615

25. H. P. Holthoff, S. Zeibig, V. Boivin, J. Bauer, M. J. Lohse, S. Kaab, S. Clauss, R. Jahns, A. Schlipp, G. Munch and M. Ungerer: Detection of Anti beta1-AR Auto-Antibodies in Heart Failure by a Cell-Based Competition ELISA. *Circ Res*, 111(6), 675-84 (2012)
DOI: 10.1161/CIRCRESAHA.112.272682

26. R. Bibilashvili, M. V. Sidorova, A. S. Molokoedov, D. Bespalova Zh, E. B. Bocharov, E. E. Efremov, T. V. Sharf, M. M. Rogova, N. A. Mironova, K. A. Zykov and S. P. Golitsyn: (Novel conformational peptide antigen, which simulates an immunodominant epitope of the

2nd extracellular loop of beta1-adrenoceptor. Computer simulation, synthesis, spatial structure). *Bioorg Khim*, 39(6), 658-70 (2013)

27. S. Stork, V. Boivin, R. Horf, L. Hein, M. J. Lohse, C. E. Angermann and R. Jahns: Stimulating autoantibodies directed against the cardiac beta1-adrenergic receptor predict increased mortality in idiopathic cardiomyopathy. *Am Heart J*, 152(4), 697-704 (2006) DOI: 10.1016/j.ahj.2006.05.004

28. P. A. Chiale, I. Ferrari, E. Mahler, M. A. Vallazza, M. V. Elizari, M. B. Rosenbaum and M. J. Levin: Differential profile and biochemical effects of antiautonomic membrane receptor antibodies in ventricular arrhythmias and sinus node dysfunction. *Circulation*, 103(13), 1765-1771 (2001) DOI: 10.1161/01.CIR.103.13.1765

29. P. A. Chiale, M. B. Rosenbaum, M. V. Elizari, A. Hjalmarson, Y. Magnusson, G. Wallukat and J. Hoebeke: High prevalence of antibodies against beta 1- and beta 2-adrenoceptors in patients with primary electrical cardiac abnormalities. *J Am Coll Cardiol*, 26(4), 864-9 (1995) DOI: 10.1016/0735-1097(95)00262-2

30. S. Stavrakis, X. Yu, E. Patterson, S. Huang, S. R. Hamlett, L. Chalmers, R. Pappy, M. W. Cunningham, S. A. Morshed, T. F. Davies, R. Lazzara and D. C. Kem: Activating autoantibodies to the beta-1 adrenergic and m2 muscarinic receptors facilitate atrial fibrillation in patients with Graves' hyperthyroidism. *J Am Coll Cardiol*, 54(14), 1309-16 (2009) DOI: 10.1016/j.jacc.2009.07.015

31. A. Y. Shevelev, M. V. Kostiukevich, E. E. Efremov, T. N. Vlasik, N. A. Mironova, K. A. Zykov, N. M. Kashirina, I. B. Kuznetsova, T. V. Sharf, E. N. Mamochkina, L. N. Lipatova, M. M. Peklo, P. N. Rutkevich, E. V. Yanushevskaya, I. N. Rybalkin, O. V. Stukalova, T. A. Malkina, M. M. Belyaeva, T. V. Kuznetsova, G. A. Tkachev, L. V. Zinchenko, E. M. Gupalo, O. Y. Agapova, T. V. Yureneva-Tkhorzhevskaya, A. V. Rvacheva, M. V. Sidorova, A. S. Sadgyan, S. N. Tereshchenko and S. P. Golitsyn: (Detection of Autoantibodies Against the 1-Adrenergic Receptor in the Sera of Patients via the Competitive cell-Based Enzyme Linked Immunosorbent Assay). *Kardiologiia*, 56(11), 61-70 (2016) DOI: 10.18565/cardio.2016.11.61-70

32. R. Jahns, V. Boivin, L. Hein, S. Triebel, C. E. Angermann, G. Ertl and M. J. Lohse: Direct evidence for a beta(1)-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J Clin Invest*, 113(10), 1419-1429 (2004) DOI: 10.1172/JCI200420149

33. L. Buvall, E. Bollano, J. Chen, W. Shultze and M. Fu: Phenotype of early cardiomyopathic changes induced by active immunization of rats with a synthetic peptide corresponding to the second extracellular loop of the human beta-adrenergic receptor. *Clin Exp Immunol*, 143(2), 209-15 (2006) DOI: 10.1111/j.1365-2249.2005.02986.x

34. E. Montaudon, L. Dubreil, V. Lalanne, M. Vermot Des Roches, G. Toumaniantz, M. Fusellier, J. C. Desfontis, L. Martignat and M. Y. Mallem: Cardiac effects of long-term active immunization with the second extracellular loop of human beta1- and/or beta3-adrenoceptors in Lewis rats. *Pharmacol Res*, 100, 210-9 (2015) DOI: 10.1016/j.phrs.2015.08.006

35. H. Li, B. J. Scherlag, D. C. Kem, A. Benbrook, L. Zhang, B. Huang, M. W. Cunningham, R. Lazzara and X. Yu: Atrial tachyarrhythmias induced by the combined effects of beta1/2-adrenergic autoantibodies and thyroid hormone in the rabbit. *J Cardiovasc Transl Res*, 7(6), 581-9 (2014) DOI: 10.1007/s12265-014-9573-5

36. P. A. Chiale and I. Ferrari: Autoantibodies in Chagas' cardiomyopathy and arrhythmias. *Autoimmunity*, 34(3), 205-10 (2001) DOI: 10.3109/08916930109007386

37. M. U. Yalcin, K. M. Gurses, D. Kocyigit, S. A. Kesikli, M. Dural, B. Evranos, H. Yorgun, L. Sahiner, E. B. Kaya, M. A. Oto, D. Guc, K. Aytemir and N. Ozer: Cardiac Autoantibody Levels Predict Recurrence Following Cryoballoon-Based Pulmonary Vein Isolation in Paroxysmal Atrial Fibrillation Patients. *J Cardiovasc Electrophysiol*, 26(6), 615-21 (2015) DOI: 10.1111/jce.12665

38. M. Iwata, T. Yoshikawa, A. Baba, T. Anzai, H. Mitamura and S. Ogawa: Autoantibodies against the second extracellular loop of beta(1)-adrenergic receptors predict ventricular tachycardia and sudden death in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*, 37(2),

418-424 (2001)
DOI: 10.1016/S0735-1097(00)01109-8

39. P. A. Chiale, H. A. Garro, J. Schmidberg, R. A. Sanchez, R. S. Acunzo, M. Lago, G. Levy and M. Levin: Inappropriate sinus tachycardia may be related to an immunologic disorder involving cardiac beta adrenergic receptors. *Heart Rhythm*, 3(10), 1182-1186 (2006)
DOI: 10.1016/j.hrthm.2006.06.011

40. B. Bornholz, S. Weidtkamp-Peters, S. Schmitmeier, C. A. Seidel, L. R. Herda, S. B. Felix, H. Lemoine, J. Hescheler, F. Nguemo, C. Schafer, M. O. Christensen, C. Mielke and F. Boege: Impact of human autoantibodies on beta1-adrenergic receptor conformation, activity, and internalization. *Cardiovasc Res*, 97(3), 472-80 (2013)
DOI: 10.1093/cvr/cvs350

41. C. J. Limas, I. F. Goldenberg and C. Limas: Autoantibodies against beta-adrenoceptors in human idiopathic dilated cardiomyopathy. *Circ Res*, 64(1), 97-103 (1989)
DOI: 10.1161/01.RES.64.1.97

42. L. R. Herda, S. B. Felix and F. Boege: Drug-like actions of autoantibodies against receptors of the autonomous nervous system and their impact on human heart function. *Br J Pharmacol*, 166(3), 847-57 (2012)
DOI: 10.1111/j.1476-5381.2012.01828.x

43. M. Soave, G. Cseke, C. J. Hutchings, A. J. H. Brown, J. Woolard and S. J. Hill: A monoclonal antibody raised against a thermo-stabilised beta1-adrenoceptor interacts with extracellular loop 2 and acts as a negative allosteric modulator of a sub-set of beta1-adrenoceptors expressed in stable cell lines. *Biochem Pharmacol*, 147, 38-54 (2018)
DOI: 10.1016/j.bcp.2017.10.015

44. C. J. Limas, I. F. Goldenberg and C. Limas: Effect of antireceptor antibodies in dilated cardiomyopathy on the cycling of cardiac beta receptors. *Am Heart J*, 122(1 Pt 1), 108-14 (1991)
DOI: 10.1016/0002-8703(91)90766-B

45. T. Lv, Y. Du, N. Cao, S. Zhang, Y. Gong, Y. Bai, W. Wang and H. Liu: Proliferation in cardiac fibroblasts induced by beta1-adrenoceptor autoantibody and the underlying mechanisms. *Sci Rep*, 6, 32430 (2016)
DOI: 10.1038/srep32430

46. Y. Magnusson, G. Wallukat, F. Waagstein, A. Hjalmarson and J. Hoebeke: Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation*, 89(6), 2760-7 (1994)
DOI: 10.1161/01.CIR.89.6.2760

47. J. Rademacher, A. Kill, K. Mattat, D. Dragun, E. Siegert, J. Gunther and G. Riemekasten: Monocytic Angiotensin and Endothelin Receptor Imbalance Modulate Secretion of the Profibrotic Chemokine Ligand 18. *J Rheumatol*, 43(3), 587-91 (2016)
DOI: 10.3899/jrheum.150474

48. L. Vanhoutte, C. Guilbaud, R. Martherus, C. Bouzin, B. Gallez, C. Dessy, J. L. Balligand, S. Moniotte and O. Feron: MRI Assessment of Cardiomyopathy Induced by beta1-Adrenoreceptor Autoantibodies and Protection Through beta3-Adrenoreceptor Overexpression. *Sci Rep*, 7, 43951 (2017)
DOI: 10.1038/srep43951

49. G. Miao, Z. Chen, X. Fang, M. Liu, G. Hao, H. An, Z. Zhang, L. Lu, J. Zhang and L. Zhang: Relationship between the Autoantibody and Expression of beta3-Adrenoceptor in Lung and Heart. *PloS one*, 8(7), e68747 (2013)
DOI: 10.1371/journal.pone.0068747

50. S. Stavrakis, D. C. Kem, E. Patterson, P. Lozano, S. Huang, B. Szabo, M. W. Cunningham, R. Lazzara and X. Yu: Opposing cardiac effects of autoantibody activation of beta-adrenergic and M2 muscarinic receptors in cardiac-related diseases. *International Journal of Cardiology*, 148(3), 331-6 (2011)
DOI: 10.1016/j.ijcard.2009.11.025

51. X. Yu, E. Patterson, S. Stavrakis, S. Huang, I. De Aos, S. Hamlett, M. W. Cunningham, R. Lazarra and D. C. Kem: Development of cardiomyopathy and atrial tachyarrhythmias associated with activating autoantibodies to beta-adrenergic and muscarinic receptors. *J Am Soc Hypertens: JASH*, 3(2), 133-40 (2009)
DOI: 10.1016/j.jash.2008.10.004

52. M. U. Yalcin, K. M. Gurses, D. Kocyigit, S. A. Kesikli, A. H. Ates, B. Evranos, H. Yorgun, M. L. Sahiner, E. B. Kaya, M. A. Oto, D. Guc, N. Ozer and K. Aytemir: Elevated M2-muscarinic and beta1-adrenergic receptor autoantibody levels are associated with paroxysmal atrial fibrillation. *Clin Res Cardiol*, 104(3), 226-33 (2015)
DOI: 10.1007/s00392-014-0776-1

53. A. Baba, T. Yoshikawa, Y. Fukuda, T. Sugiyama, M. Shimada, M. Akaishi, K. Tsuchimoto, S. Ogawa and M. Fu: Autoantibodies against M2-muscarinic acetylcholine receptors: new upstream targets in atrial fibrillation in patients with dilated cardiomyopathy. *Eur Heart J*, 25(13), 1108-15 (2004)
DOI: 10.1016/j.ehj.2004.05.012

54. C. J. Limas, I. F. Goldenberg and C. Limas: Influence of anti-beta-receptor antibodies on cardiac adenylate cyclase in patients with idiopathic dilated cardiomyopathy. *Am Heart J*, 119(6), 1322-8 (1990)
DOI: 10.1016/S0002-8703(05)80182-6

55. V. O. Nikolaev, V. Boivin, S. Stork, C. E. Angermann, G. Ertl, M. J. Lohse and R. Jahns: A novel fluorescence method for the rapid detection of functional beta1-adrenergic receptor autoantibodies in heart failure. *J Am Coll Cardiol*, 50(5), 423-31 (2007)
DOI: 10.1016/j.jacc.2007.03.051

56. Y. Du, L. Yan, H. Du, L. Wang, F. Ding, L. Quan, X. Cheng, K. Song and H. Liu: beta(1) -adrenergic receptor autoantibodies from heart failure patients enhanced TNF-alpha secretion in RAW264.7. macrophages in a largely PKA-dependent fashion. *J Cell Biochem*, 113(10), 3218-28 (2012)
DOI: 10.1002/jcb.24198

57. A. S. Tutor, P. Penela and F. Mayor, Jr.: Anti-beta1-adrenergic receptor autoantibodies are potent stimulators of the ERK1/2 pathway in cardiac cells. *Cardiovasc Res*, 76(1), 51-60 (2007)
DOI: 10.1016/j.cardiores.2007.05.022

58. M. Azzi, P. G. Charest, S. Angers, G. Rousseau, T. Kohout, M. Bouvier and G. Pineyro: Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci U S A*, 100(20), 11406-11 (2003)
DOI: 10.1073/pnas.1936664100

59. C. J. Hutchings, G. Cseke, G. Osborne, J. Woolard, A. Zhukov, M. Koglin, A. Jazayeri, J. Pandya-Pathak, C. J. Langmead, S. J. Hill, M. Weir and F. H. Marshall: Monoclonal anti-beta 1-adrenergic receptor antibodies activate G protein signaling in the absence of beta-arrestin recruitment. *MAbs*, 6(1), 246-61 (2014)
DOI: 10.4161/mabs.27226

60. L. Sterin-Borda, P. M. Cossio, M. F. Gimeno, A. L. Gimeno, C. Diez, R. P. Laguens, P. C. Meckert and R. M. Arana: Effect of chagasic sera on the rat isolated atrial preparation: immunological, morphological and function aspects. *Cardiovasc Res*, 10(6), 613-22 (1976)
DOI: 10.1093/cvr/10.6.613

61. G. Wallukat and A. Wollenberger: Effects of the serum gamma globulin fraction of patients with allergic asthma and dilated cardiomyopathy on chronotropic beta adrenoceptor function in cultured neonatal rat heart myocytes. *Biomed Biochim Acta*, 46(8-9), S634-9 (1987)

62. M. B. Rosenbaum, P. A. Chiale, D. Schejtman, M. Levin and M. V. Elizari: Antibodies to beta-adrenergic receptors disclosing agonist-like properties in idiopathic dilated cardiomyopathy and Chagas' heart disease. *J Cardiovasc Electrophysiol*, 5(4), 367-75 (1994)
DOI: 10.1111/j.1540-8167.1994.tb01174.x

63. M. R. Bristow: beta-adrenergic receptor blockade in chronic heart failure. *Circulation*, 101(5), 558-69 (2000)
DOI: 10.1161/01.CIR.101.5.558

64. M. R. Bristow, R. Gingurg, W. Minobe, R. S. Cubiciotti, W. S. Sageman, K. Lurie, M. E. Billingham, D. E. Harrison and E. B. Stinson: Decreased catecholamine sensitivity and beta-adrenergic receptor density in failing human hearts. *N. Engl. J. Med.*, 307, 205-211 (1982)
DOI: 10.1056/NEJM198207223070401

65. N. J. Freedman and R. J. Lefkowitz: Anti-beta(1)-adrenergic receptor antibodies and heart failure: causation, not just correlation. *J Clin Invest*, 113(10), 1379-1382 (2004)
DOI: 10.1172/JCI21748

66. M. Iwata, T. Yoshikawa, A. Baba, T. Anzai, I. Nakamura, Y. Wainai, T. Takahashi and S. Ogawa: Autoimmunity against the second extracellular loop of beta(1)-adrenergic receptors induces beta-adrenergic receptor desensitization and myocardial hypertrophy *in vivo*. *Circ Res*, 88(6), 578-86 (2001)
DOI: 10.1161/01.RES.88.6.578

67. Y. Fukuda, S. Miyoshi, K. Tanimoto, K. Oota, K. Fujikura, M. Iwata, A. Baba, Y. Hagiwara, T. Yoshikawa, H. Mitamura and S. Ogawa: Autoimmunity against the second extracellular loop of beta(1)-adrenergic receptors induces early afterdepolarization and decreases in K-channel density in

rabbits. *J Am Coll Cardiol*, 43(6), 1090-100 (2004)
DOI: 10.1016/j.jacc.2003.09.057

68. J. Liu, W. Mao, C. Iwai, S. Fukuoka, R. Vulapalli, H. Huang, T. Wang, V. K. Sharma, S. S. Sheu, M. Fu and C. S. Liang: Adoptive passive transfer of rabbit beta1-adrenoceptor peptide immune cardiomyopathy into the Rag2-/- mouse: participation of the ER stress. *J Mol Cell Cardiol*, 44(2), 304-14 (2008)
DOI: 10.1016/j.yjmcc.2007.11.007

69. D. Jane-wit, C. Z. Altuntas, J. M. Johnson, S. Yong, P. J. Wickley, P. Clark, Q. Wang, Z. B. Popovic, M. S. Penn, D. S. Damron, D. M. Perez and V. K. Tuohy: Beta 1-adrenergic receptor autoantibodies mediate dilated cardiomyopathy by agonistically inducing cardiomyocyte apoptosis. *Circulation*, 116(4), 399-410 (2007)
DOI:10.1161/CIRCULATIONAHA.106.683193

70. R. Jahns, V. Boivin, V. Schwarzbach, G. Ertl and M. J. Lohse: Pathological autoantibodies in cardiomyopathy. *Autoimmunity*, 41(6), 454-61 (2008)
DOI: 10.1080/08916930802031603

71. Y. Staudt, R. Mobini, M. Fu, S. B. Felix, J. P. Kuhn and A. Staudt: beta(1)-Adrenoceptor antibodies induce apoptosis in adult isolated cardiomyocytes. *Eur J Pharmacol*, 466(1-2), 1-6 (2003)
DOI: 10.1016/S0014-2999(03)01431-6

72. C. Del Corsso, A. C. de Carvalho, H. F. Martino and W. A. Varanda: Sera from patients with idiopathic dilated cardiomyopathy decrease ICa in cardiomyocytes isolated from rabbits. *Am J Physiol*, 287(5), H1928-36 (2004)
DOI: 10.1152/ajpheart.00044.2004

73. T. Christ, S. Schindelhauer, E. Wettwer, G. Wallukat and U. Ravens: Interaction between autoantibodies against the beta1-adrenoceptor and isoprenaline in enhancing L-type Ca2+ current in rat ventricular myocytes. *J Mol Cell Cardiol*, 41(4), 716-23 (2006)
DOI: 10.1016/j.yjmcc.2006.06.011

74. A. Staudt, P. Eichler, C. Trimpert, S. B. Felix and A. Greinacher: Fc(gamma) receptors IIa on cardiomyocytes and their potential functional relevance in dilated cardiomyopathy. *J Am Coll Cardiol*, 49(16), 1684-92 (2007)
DOI: 10.1016/j.jacc.2006.11.051

75. G. Wallukat and I. Schimke: Agonistic autoantibodies directed against G-protein-coupled receptors and their relationship to cardiovascular diseases. *Semin Immunopathol*, 36(3), 351-63 (2014)
DOI: 10.1007/s00281-014-0425-9

76. C. Lavoie, J. F. Mercier, A. Salahpour, D. Umapathy, A. Breit, L. R. Villeneuve, W. Z. Zhu, R. P. Xiao, E. G. Lakatta, M. Bouvier and T. E. Hebert: Beta 1/beta 2-adrenergic receptor heterodimerization regulates beta 2-adrenergic receptor internalization and ERK signaling efficacy. *J Biol Chem*, 277(38), 35402-10 (2002)
DOI: 10.1074/jbc.M204163200

77. W. Z. Zhu, K. Chakir, S. Zhang, D. Yang, C. Lavoie, M. Bouvier, T. E. Hebert, E. G. Lakatta, H. Cheng and R. P. Xiao: Heterodimerization of beta1- and beta2-adrenergic receptor subtypes optimizes beta-adrenergic modulation of cardiac contractility. *Circ Res*, 97(3), 244-51 (2005)
DOI: 10.1161/01.RES.0000176764.38934.86

78. M. L. Fu: Anti-peptide antibodies against an autoimmune epitope on human muscarinic receptor mimic functional autoantibodies against the same epitope in patients with idiopathic dilated cardiomyopathy. *Eur Heart J*, 16 Suppl O, 89-91 (1995)
DOI: 10.1093/eurheartj/16.suppl_O.89

79. T. Skomedal, M. L. Fu, A. Hjalmarson, J. Hoebeke, I. G. Schiander and J. B. Osnes: Anti-M2 muscarinic receptor antibodies inhibit beta-adrenoceptor-mediated inotropic response in rat myocardium. *European Journal of Pharmacology*, 333(2-3), 169-75 (1997)
DOI: 10.1093/eurheartj/16.suppl_O.89

80. G. Wallukat and E. Nisson: Anti beta(1)-adrenoceptor autoantibodies analyzed in spontaneously beating neonatal rat heart myocyte cultures - Comparison of methods. *In vitro Cellular & Developmental Biology-Animal*, 37(3), 175-176 (2001)
DOI: 10.1290/1071-2690(2001)037<0175:AAAAIS>2.0.CO;2

81. G. Wallukat, S. G. Munoz Saravia, A. Haberland, S. Bartel, R. Araujo, G. Valda, D. Duchen, I. Diaz Ramirez, A. C. Borges and I. Schimke: Distinct patterns of autoantibodies against G-protein-coupled receptors in Chagas' cardiomyopathy and megacolon.

Their potential impact for early risk assessment in asymptomatic Chagas' patients. *J Am Coll Cardiol*, 55(5), 463-8 (2010)
DOI: 10.1016/j.jacc.2009.06.064

82. L. G. Eckerle, S. B. Felix and L. R. Herda: Measurement of antibody effects on cellular function of isolated cardiomyocytes. *J Vis Exp*(73), e4237 (2013)
DOI: 10.3791/4237

83. S. Joshi-Barr, A. Haberland, S. Bartel, J. Muller, T. Choi and G. Wallukat: High throughput bioassay for beta1-adrenoceptor autoantibody detection. *Int J Cardiol*, 219, 98-104 (2016)
DOI: 10.1016/j.ijcard.2016.06.002

84. T. Pfeifer, C. Schmidt, F. Boege, B. Bornholz, V. Nikolaev and H. Lemoine: Comparison of beta-adrenoceptor signalling by agonists and antagonists by the use of FRET-based assays with new multichannel fluorescence detectors. *N-S Arch Pharmacol*, 388, S7-S7 (2015)

85. D. M. Waldera-Lupa, F. Kalfalah, K. Safferling, P. Boukamp, G. Poschmann, E. Volpi, C. Gotz-Rosch, F. Bernerd, L. Haag, U. Huebenthal, E. Fritsche, F. Boege, N. Grabe, J. Tigges, K. Stuhler and J. Krutmann: Characterization of Skin aging Associated Secreted Proteins (SAASP) Produced by Dermal Fibroblasts Isolated from Intrinsically Aged Human Skin. *J Invest Dermatol* (2015)
DOI: 10.1038/jid.2015.120

86. H. R. Liu, R. R. Zhao, J. M. Zhi, B. W. Wu and M. L. Fu: Screening of serum autoantibodies to cardiac beta1-adrenoceptors and M2-muscarinic acetylcholine receptors in 408 healthy subjects of varying ages. *Autoimmunity*, 29(1), 43-51 (1999)
DOI: 10.3109/08916939908995971

87. R. Jahns, V. Boivin, C. Siegmund, F. Boege, M. J. Lohse and G. Inselmann: Activating beta-1-adrenoceptor antibodies are not associated with cardiomyopathies secondary to valvular or hypertensive heart disease. *J Am Coll Cardiol*, 34(5), 1545-1551 (1999)
DOI: 10.1016/S0735-1097(99)00381-2

88. F. C. Luft: Activating autoantibodies and cardiovascular disease. *Physiology (Bethesda)*, 28(4), 254-61 (2013)
DOI: 10.1152/physiol.00014.2013

Key Words: Autoantibodies, Chronic Heart Failure, Beta$_1$-Adrenergic Receptor, Signal Transduction, autoimmunity, Review

Send correspondence to: Fritz Boege, Institute of Clinical Chemistry and Laboratory Diagnostics, University Hospital, Moorenstrabe 5, 40225 Duesseldorf, Germany, Tel: 49-211-8118290, Fax 49-211-8118021, E-mail: boege@med.uni-duesseldorf.de