

# Autoimmune mediated G-protein receptor activation in cardiovascular and renal pathologies

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## Summary

Antibodies directed against G-protein coupled receptors (GPCR) can act as allosteric receptor agonists or antagonists. Prototypic disease for agonistic antibody action is a Graves disease of the thyroid gland where antibodies that stimulate G-protein coupled thyroid-stimulating hormone receptor (TSHR) were first described 50 years ago. Myasthenia gravis is the prototype for antagonistic autoimmune actions, where antibodies directed against the nicotinic acetylcholine receptor (AChR) cause blockade of neuromuscular junctions. Antibodies and

B-cells are increasingly recognised as major modulators of various cardiovascular and renal pathologies. We aim to critically review the notion that antibodies targeting other GPCRs may amplify or cause various cardiovascular and renal pathologies and summarise the current state of research, as well as perspectives in diagnostic and therapeutic strategies. In terms of targets we will focus on the  $\alpha_1$ -adrenergic receptor ( $\alpha_1$ AR), the  $\beta_1$ -adrenergic receptor ( $\beta_1$ AR), and the angiotensin II type I receptor (AT<sub>1</sub>R).

## Keywords

$\alpha_1$ -adrenergic receptor ( $\alpha_1$ AR),  $\beta_1$ -adrenergic receptor ( $\beta_1$ AR), angiotensin II type I receptor, autoantibodies

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## Receptor targets for antibodies against G-protein coupled receptors (GPCR)

### $\alpha_1$ -adrenergic receptor ( $\alpha_1$ AR)

Autoantibodies directed against an extracellular immunogenic epitope of the human  $\alpha_1$ AR expressed in the heart were first described almost two decades ago (1) and soon afterwards detected in patients with malignant hypertension (2). These findings were independently confirmed and extended in patients with primary hypertension. Autoantibodies exerted a positive chronotropic response on isolated rat neonatal cardiomyocytes, an effect that was blocked pharmacologically by  $\alpha_1$ AR-antagonists (3). In two thirds of cases, epitopes on the second extracellular loop were implicated in the functional assay (3). Immunization of rabbits with a peptide corresponding to the second extracellular loop of the human  $\alpha_1$ AR-induced polyclonal  $\alpha_1$ AR-antibody (Ab) production and made functional characterization feasible (4). In a very recent study, removal of  $\alpha_1$ AR-Abs with immunoadsorption lowered blood pressure in five subjects with severe hypertension, implicating their potential clinical relevance (4).

### $\beta_1$ -adrenergic receptor ( $\beta_1$ AR)

In the early 1990s, the human  $\beta_1$ AR was analyzed for potential immunogenic amino acid stretches accomplishing the requirements for a peptide to be complexed and presented to a T-cell receptor (5). The only stretch of the  $\beta_1$ AR molecule containing B- and T-cell epitopes which provides accessibility to antibodies was predicted in the second extracellular loop of the  $\beta_1$ AR (6). Involvement of  $\beta_1$ AR-Abs in heart failure received large attention and was demonstrated by several groups (7–9). Autoantibodies directed against  $\beta_1$ AR were then detected in a large fraction of patients with dilated cardiomyopathy (7–9), with lower frequency also in patients with ischaemic cardiomyopathy (7), whereas they do not appear to be associated with cardiomyopathies secondary to valvular or hypertensive disease (10). Interestingly, antibodies against the same small  $\beta_1$ AR-domain located at in the second extracellular loop display divergent allosteric effects, similar to anti-TSHR-Abs, ranging from inhibitory to agonist-promoting activities (11).

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**Table 1: Receptor targets and epitopes for autoantibodies directed against GPCR and association with clinical syndromes and proof of concept in animal model.**

Receptor	Epitope	Clinical entity	Proof in animal model
$\alpha_1$ -AR	2 <sup>nd</sup> loop (2) 1 <sup>st</sup> (FWAFGRAFCDVWA) and 2 <sup>nd</sup> loop (GWKEPVPPDERFCGITEEAGYAVFSSV) (3)  1 <sup>st</sup> (YWAFGR; GRVFCNI) and 2 <sup>nd</sup> loop (GWRQPA; APEDET; TICQIN; INEEPG; GYLFS) (4)	Malignant and secondary hypertension (2) Primary hypertension (3) Refractory hypertension (17) Refractory hypertension (4)	Immunized rats (18)
$\beta_1$ -AR	2 <sup>nd</sup> loop (HWWRAESDEARRCYNDPKCCDFVTNR) (19) 1 <sup>st</sup> (SFFCEL) and 2 <sup>nd</sup> loop (ARRCYND) (20)	Autonomic abnormalities (21) Dilated cardiomyopathy (22) Autoimmune myocarditis (23) $\beta_2$ -adrenergic receptor antibodies in myasthenia gravis (24) Chagas disease (25)	Immunized rabbits (26) Immunized rats (27)
AT <sub>1</sub> R	2 <sup>nd</sup> loop (AFYESQ) (12) 2 <sup>nd</sup> loop (AFHYESQ and ENTNIT) (13)	Preeclampsia (12) Renal allograft rejection (13)	Rat model of kidney transplantation (13) Rat transgenic model of preeclampsia (28) Mouse model of preeclampsia (29)

### Angiotensin II type I receptor (AT<sub>1</sub>R)

Agonistic antibodies against the AT<sub>1</sub>R (AT<sub>1</sub>R-Abs) were originally found in women with preeclampsia (12). The antibodies were oligoclonal (IgG3), developed with the syndrome but nonetheless regressed about four to six weeks after pregnancy (12). We reported the presence of AT<sub>1</sub>R-Abs in recipients of renal allografts who had severe vascular rejection and malignant hypertension, but who did not have anti-HLA antibodies (13). AT<sub>1</sub>R-Abs bind to and recognize epitopes on the second extracellular loop of the AT<sub>1</sub>R and belong to IgG1 and IgG3 subclass (12, 13). AT<sub>1</sub>R-Abs have also been associated with malignant hypertension (14). Evidence for presence of AT<sub>1</sub>R-Abs was provided by bioassay in neonatal rat cardiomyocytes, and independently confirmed in human trophoblast cells (15). Pregnancies complicated by preeclampsia and graft rejection bear some immunologic similarities (16). The described epitopes for AT<sub>1</sub>R-Abs isolated from transplant patients do not entirely coincide with those described in preeclampsia (Table 1), albeit both are located in the close proximity and on the second extracellular loop. The decision to seek and isolate AT<sub>1</sub>R-Abs was instigated by the serendipitous observation that the first patient we studied developed accelerated vascular rejection in a „zero-mismatch“ kidney. Rapid onset of malignant hypertension with seizures during the rejection process was so reminiscent of eclamptic crisis in pregnancy, a condition that she had developed two decades before transplantation.

### How do $\alpha_1$ AR-, $\beta_1$ AR-, and AT<sub>1</sub>R-Abs arise?

In order to serve as antigens, GPCRs must be degraded to small oligopeptides, and one or more degradation products must be able to form a complex with one of the HLA class II molecules. Antigenic determinants from targets, which are protected against the immune attack under physiologic conditions, may become accessible after injury to the target tissue. Subsequent liberation and presentation of target antigens to the immune system may

then induce an autoimmune response that is precipitated in the various conditions. It is not clear whether autoantibodies against GPCRs represent a primary mechanism inducing target damage or may arise secondary to the pre-existing tissue injury or viral infection. For instance, molecular mimicry could trigger the initial activation of autoreactive T cells and/or induce expansion of memory T-cell population (30). Anti-GPCR antibody-triggered perpetuation of immune mediated target tissue damage involving both humoral and cellular (antigen-specific T cells) could be the worst case scenario. Evidence for molecular mimicry is provided for generation of  $\beta_1$ AR-Abs, implicating involvement of *Trypanosoma cruzi* infection, perhaps the most common infectious cause of cardiomyopathy worldwide (31). *Trypanosoma cruzi* was demonstrated to induce a functional autoimmune response against the cardiac  $\beta_1$ AR (31). The presence of Parvovirus B19-specific antibodies directed against conformational VP2 epitopes seem to be implicated in the generation of AT<sub>1</sub>R-Abs in preeclamptic women (32, 33).

Due to the polymorphic nature of some  $\alpha_1$ AR,  $\beta_1$ AR, and AT<sub>1</sub>R sequences, the way of sensitization via transfusion or pregnancies may be similar to those of polymorphic anti-HLA-antibodies.  $\alpha_1$ AR-,  $\beta_1$ AR-, and AT<sub>1</sub>R-Abs may also arise secondary to immune activation associated with non-specific infections or local hypoxia, both, powerful stimulators of antibody production. Immune responses that are directed against persistent non-specific infectious agents, and not against autoantigens, can also induce tissue damage and thus possibly play a role in generation of anti-GPCR antibodies in general.

### Mechanisms of injury

Autoantibodies directed against  $\alpha_1$ AR,  $\beta_1$ AR, and AT<sub>1</sub>R induce wide, yet sometimes overlapping spectrum of cardiovascular and renal injuries. For this reason, it is important to better understand how Abs against GPCRs induce tissue or organ-restricted injuries in order to improve therapeutic approaches. Additional

conceptual difficulty is that these antibodies are directed against widely expressed autoantigens.

### Induction of signal transduction pathways

$\alpha_1$ AR-Ab induce PKC- $\alpha$  in cardiomyocytes and Erk 1/2 kinases in vascular smooth muscle cells, both potentially important in stimulated hypertension-induced organ damage, similar to the phenylephrine control (4).  $\alpha_1$ AR-Abs from patients with severe hypertension and polyclonal rabbit  $\alpha_1$ AR-Abs induce short-term cytosolic  $Ca^{2+}$  responses and increase transcription of the voltage-gated L-type  $Ca^{2+}$  channel pore subunit (34).

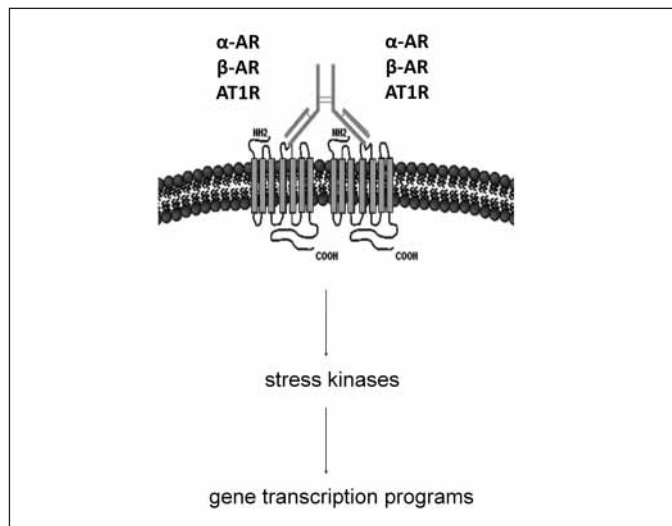
$\beta_1$ AR-Abs act as allosteric modulators of receptor activity, what means that they may promote, reduce and/or stabilize conformational changes of the receptor similar to those induced by agonist or partial agonist ligands (35). The particular signalling pathways responsible for  $\beta_1$ AR-Ab-mediated cardiomyocyte toxicity are complex and are more elaborately reviewed (36). Activation of the  $\beta_1$ AR requires a specific receptor conformation that is stabilized by agonist e.g.  $\beta_1$ AR-specific IgG. As shown in Figure 1, it appears that  $\beta_1$ AR dimerization may be involved, as suggested in some studies (37). Stimulation of  $\beta_1$ AR encites a cascade of activation processes including specific G-proteins, then adenylyl cyclase (which forms cAMP), then the cAMP-dependent protein kinase A and finally subsequent phosphorylation of molecules regulating sarcoplasmic  $Ca^{2+}$ , translating as increased cardiomyocyte inotropy, chronotropy and lusitropy (36).

AT<sub>1</sub>R-Abs exert direct effects on endothelial and vascular smooth muscle cells via induction of Erk 1/2 signal transduction cascade. Incubation of nuclear extracts of vascular smooth muscle cells with AT<sub>1</sub>R-Abs activated transcription factor activator protein 1 (AP-1) downstream from Erk 1/2 (13).

### Haemostasis and inflammation

AT<sub>1</sub>R-Abs may also act as an allosteric activator in a similar manner as a natural ligand for the AT<sub>1</sub>R, Ang II. AT<sub>1</sub>R-Abs derived from preeclamptic patients enhanced promoter activity of tissue factor (TF), an initiator of extrinsic coagulation pathway and a target gene for AP-1 and nuclear factor- $\kappa$ B (NF- $\kappa$ B) *in vitro* (38). Renal transplant biopsy specimens obtained during an AT<sub>1</sub>R-Ab mediated rejection episode and placentas of women with preeclampsia revealed intense diffuse TF staining in absence of complement activation (13, 38). TF mediates clotting abnormalities associated with hyperacute and xenograft rejection, as well as in antiphospholipid antibody syndrome (39, 40). AT<sub>1</sub>R-Abs may also contribute to long-term, structural changes in the arterial wall that promote clotting or/and luminal narrowing.

AT<sub>1</sub>R-Ab also increased DNA binding activity of NF- $\kappa$ B transcription factor and increased expression of NF- $\kappa$ B proinflammatory target genes such are chemokines MCP-1 and RANTES (13). In addition, AT<sub>1</sub>R-Ab-induced calcineurin-nuclear factor activating T-cell signalling pathway is involved in PAI-1 induction and trophoblast shallow invasion, as well as for increased sFLT-1 secretion in preeclampsia (41, 42).



**Figure 1: Working hypothesis on receptor dimerisation and down-stream effects after agonistic antibody (Ab)-mediated receptor activation.**

### Proofs of $\alpha_1$ AR-, $\beta_1$ AR-, and AT<sub>1</sub>R-Ab-related pathogenicity

Direct evidence that an autoimmune attack directed against the cardiac  $\beta_1$ AR plays a causal role in dilative cardiomyopathy has been provided according to Witebsky's postulates (27). Dilative cardiomyopathy was induced by immunizing inbred rats against  $\beta_1$ -extracellular loop epitopes with 100% sequence homology between human and rat and then the disease was reproduced in healthy isogenic rats by passive transfer of autoantibodies (27). Indirect evidence was provided earlier, as the intraperitoneal injection of blood lymphocytes from  $\beta_1$ AR-positive patients into immunodeficient mice lead to an early stage of cardiac dilatation (43).

Pregnant mice infused with either total IgG or affinity purified AT<sub>1</sub>R-Abs from preeclamptic women developed key-features of preeclampsia including hypertension, proteinuria, and placental abnormalities (29). Co-treatment with a selective AT<sub>1</sub>R-antagonist, or by an antibody neutralising seven-amino-acid epitope peptide achieved complete rescue of all preeclampsia-associated abnormalities (29).

Passive transfer of human IgG containing AT<sub>1</sub>R-Abs induced a transmural arteritis similar to human situation and led to increased blood pressure in otherwise non-rejecting and normotensive transplanted animals (13). These findings provided further evidence that AT<sub>1</sub>R-Abs may have a causative role. However, it remains unclear whether or not AT<sub>1</sub>R-Abs may initiate antibody-mediated rejection in a syngeneic context.

### Permissive factors of injury

Additional antigenic targets for  $\alpha_1$ AR,  $\beta_1$ AR, and AT<sub>1</sub>R autoantibodies can be generated on injured or activated target cells. Cytokine-mediated target cell activation may act as a danger sig-

nal and seem prerequisite for the induction of severe agonistic antibody related phenotypes. Lack or attenuated pathologies in allografts from living donors despite presence AT<sub>1</sub>R-Abs support this consideration. Initial injuries surrounding the organ transplantation process like cytokine storm during brain death or inflammation during ischaemia and reperfusion injury may lead to increased expression of target antigens for AT<sub>1</sub>R-Abs. The overall reactivity of the target cells to AT<sub>1</sub>R Abs may be thus increased. In heart transplantation, systemic upregulation of AT<sub>1</sub>R could be found in donors with spontaneous intracerebral haemorrhage that was associated with subsequent development of cardiac vasculopathy (44). In pregnant rats, ischemia and tumour necrosis factor (TNF)- $\alpha$  were shown to be important stimuli for AT<sub>1</sub>R-Ab generation (45). Although there are several polymorphisms described for AT<sub>1</sub>R, they have not been investigated in the context of autoimmune response. The most extensively studied A1166C polymorphism is associated with higher AT<sub>1</sub>R density on injured tissues and thus increased responsiveness to angiotensin II and various cardiovascular and renal pathologies (46).

### Diagnostic implications

Similar to many autoimmune diseases, antibodies against GPCRs may be diagnostically useful in certain clinical situations but it remains unclarified whether or not they would immediately represent an effector mechanism. The most significant barrier to the general acceptance of autoantibodies against  $\alpha_1$ AR,  $\beta_1$ AR, and AT<sub>1</sub>R as causative or modifying agent of various cardiovascular and renal pathologies is the lack of standardized assays to determine their presence. Major reason for these difficulties is the necessity of specific receptor conformations that enhance autoantibody-receptor target interaction, as well described in thyroid pathology (47). Currently available assays that measure autoantibodies against  $\alpha_{1A}$ AR,  $\beta_{1A}$ AR, and AT<sub>1</sub>R are not yet widely used routinely.

The most sophisticated findings come again from the  $\beta_1$ AR field and implicate use of the fluorescence resonance energy transfer (FRET) technique assay that detects antibody concentrations of 0.001 nM to 0.2 nM (48). FRET relies on energy transfer between two fluorochromes when these are close enough. The definition of a functionally active  $\beta_1$ AR is based on the signalling it mediates and the cystolic accumulation of cAMP, second messenger of many G-protein coupled receptors. In order to have a higher sensitivity and a better temporal resolution as with the ELISA method, Jahn's group fused the two fluorochromes used in FRET to Epac, a protein whose conformation changes due to the binding cAMP (49, 50). As a consequence, when the cells produce cAMP in response to the activation of the  $\beta_1$ AR by the autoantibodies, the Epac FRET signal gets lower (48).

These developments have not yet been translated to the field of antibody response against  $\alpha_1$ AR and AT<sub>1</sub>R. Detection of AT<sub>1</sub>R-Ab activity initially relied on the bioassay that measures the chronotropic responses to AT<sub>1</sub>R-IgG mediated stimulation of cultured cardiomyocytes coupled with receptor-specific antagonists. Dose-response relationship between AT<sub>1</sub>R-Ab concentration and the chronotropic response is linear (12). The time-

consuming setting bioassay precluded screening larger patient cohorts. A cell-based ELISA in collaboration with biotech partners for detection of AT<sub>1</sub>R-Abs in serum has been now validated and established (51). The ELISA currently has 100% specificity and 88% sensitivity as compared to bioassay. Interassay variability is 12% (51). Pretransplantation screening of recipients for AT<sub>1</sub>R-Abs may help to improve individual risk assessment and offer patients with AT<sub>1</sub>R-Abs preemptive specific treatment. Monitoring for AT<sub>1</sub>R-Abs in pregnant women may also prove valuable.

### Therapeutic implications

Strategies based on rapid and effective reduction of antibody titres using plasmapheresis or immunoadsorption known in therapies of severe autoimmune syndromes like myasthenia gravis are applicable in the area of other GPCR Ab-related pathologies. Influence of polyclonal lymphocyte depletion Abs, anti-CD20 antibody and IVIG treatments have not yet been studied in the context of cardiovascular and renal pathologies induced by GPCR Abs. These options clearly have potential for diseases such as dilatative cardiomyopathy, malignant hypertension, and renal allograft vascular rejection. For preeclampsia, antibody removal procedures have proved exceptionally difficult because of logistical problems. Nonetheless, identification of  $\alpha_1$ AR,  $\beta_1$ AR, and AT<sub>1</sub>R, as Ab targets offers also targeted pharmacologic inhibition with widely proved receptor blockers, and except in case of preeclampsia only few safety concerns. Immunoadsorption emerges as a potentially successful therapy for cardiomyopathy associated with  $\beta_1$ AR-Abs, as reported in non-randomised observational studies (52, 53). Another question is whether or not pharmacological blockade with  $\beta$ -blockers may modify natural course of cardiomyopathy, albeit they are surely mandatory in any patient with congestive heart failure. It is also currently unknown, whether or not  $\beta$ -blockers and antibody removal strategies may act in synergy.

The use of anti renin-angiotensin system drugs due to the concern of interference with renal allograft perfusion is still a matter of controversy in transplant nephrology. According to reported beneficial effects of blockade of RAS on early outcomes of renal transplants this view seems to be outdated (54, 55). None of the patients from the first study received ACE inhibitors or AT<sub>1</sub>R blockers prior to the rescue protocol. Interestingly AT<sub>1</sub>R-Ab-positive patients who received continuously AT<sub>1</sub>R blockers or angiotensin converting enzyme (ACE) inhibitors, together with intensified immunosuppression (depletional antibody induction, tacrolimus, MMF, and steroids), and who were recipients of living donor kidneys seemed not to be prone to develop AT<sub>1</sub>R-Ab-related pathology (56). Removal of AT<sub>1</sub>R-Abs by plasmapheresis in combination with pharmacologic AT<sub>1</sub>R blockade improved renal function and graft survival in AT<sub>1</sub>R-Ab positive patients (13).

We do not know whether or not therapeutic interventions could improve clinical outcomes after positive testing for individual agonistic antibodies against GPCRs. Well designed clinical studies will be necessary to test this hypothesis.

## Conclusions

The area of Abs directed against GPCRs continues to evolve in complexity and still raises many questions. Although the proof of pathogenicity is provided for  $\beta_1$ AR-Ab and AT<sub>1</sub>R-Ab in animal models, the permissive factors and better understanding how autoimmunity against the particular receptor results in an overt disease and particular organ-related pathology are required. In addition, the correct interpretation of the associative relationships derived from few clinical observational studies will require studies with well defined cohorts and more careful analysis in relation to different therapy rescue protocols. Antibodies directed against GPCRs are probably not an ultimate instigator of tissue injury in majority of cases. Severe “full blown” phenotypes may

develop in individuals at particular risk. The investigations of infectious or genetic factors that could be responsible for the differences in individual susceptibilities should be encouraged. Future studies should be addressed to explain whether or not GPCR-antibody related pathologies represent “true-clinical entities” or organ-specific autoimmune phenomena that become overt in the permissive hypoxic or inflammatory environment. In the near future anti-GPCR-Abs may also find application as biomarkers of ongoing immune response and herald the need for both receptor blockade and immunosuppression. Refined approaches considering the subtle mechanistic differences in the individual Ab responses directed against individual cardiovascular GPCRs may help to define patients at particular risk for irreversible and severe cardiovascular injuries and improve overall outcomes.

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