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Activating Autoantibodies and Cardiovascular Disease

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Stimulating antibodies against G-protein-coupled receptors, including the β 1- and β 2-adrenergic receptors, the α 1-adrenergic receptor, and the angiotensin II AT1 receptor, have been described, as well as activating antibodies directed at the platelet-derived growth factor receptor tyrosine kinase. Their existence and actions appear to be established. Lacking are mechanistic studies of receptor activation and translational studies to document receptor-stimulating antibodies as worthwhile therapeutic targets.

Autoimmune thyroid disease is the most established example of stimulating autoantibodies initiating and sustaining a human disease. In Grave's disease, an interaction between genetic and environmental factors results in an autoimmune response to the thyroid-stimulating hormone receptor (TSHR), resulting in the production of TSHR-specific T cells and TSHR autoantibodies (3). Autoantibodies that bind to the TSHR can be stimulating, blocking, or neutral. An infusion of plasma from Graves' disease patients into a normal individual was dramatic and absolute proof regarding the role of anti-TSHR antibodies in inducing human hyperthyroidism. The experiment was also one of the first demonstrations of antibody transfer causing an autoimmune disease. Over 50 years of intense research has elucidated this example of agonistic autoantibodies that is very well established and that should serve as a model for pursuing other putative stimulating autoantibodies, which will be outlined below.

Beta-Adrenergic Receptor (β 1-AR, β 2-AR)

Wallukat and Wollenberger observed that the spontaneously beating cardiomyocytes of newborn rats exposed in culture to the gamma globulin fraction from asthmatics inhibited the positive chronotropic response of the β 2AR agonist clenbuterol, whereas the gamma globulin fraction of patients with cardiomyopathy increased the beating rate (33). The latter effect was selectively inhibited by propranolol. The effects of both the asthmatic and cardiomyopathic gamma globulin fractions were abolished by immunoprecipitation with antihuman gamma globulin and antihuman IgG. Wallukat and Wollenberger first suggested that the effects were mediated by autoantibodies. Magnusson et al. extended these findings by observing that the specific target for autoantibodies with stimulatory activity in patients with dilative cardiomyopathy was

the second extracellular loop of the β 1AR (18). Chiale et al. confirmed the finding that the autoantibodies bind to the second extracellular loop of the β 1AR and also identified agonistic autoantibodies directed at the β 2AR (2). The authors suggested that the autoantibodies were responsible for ventricular arrhythmias.

Magnusson et al. used synthetic peptide sequences to study autoantibodies directed at β AR further (19). They used peptide sequences corresponding to the second extracellular loop of the β 1AR and β 2AR as antigens in an enzyme immunoassay to screen sera from patients with dilated cardiomyopathy, ischemic heart disease, or healthy blood donors. All 13 patients with dilative cardiomyopathy harbored a serum component that was recognized by the β 1AR peptide sequence. None of the ischemic heart disease control patients and only four of the healthy controls had serum that recognized the β 1AR peptide sequence. Affinity-purified autoantibodies from these patients inhibited radioligand binding to the β 1AR in C6 rat glioma cells. In immunoblot experiments, the authors showed that the autoantibodies recognized the receptor protein and found that they bound in situ to human myocardium. Jahns et al. provided the first direct evidence that autoimmunity involving the cardiac β 1AR could precipitate a dilated cardiomyopathy (15). They first immunized inbred rats against the second extracellular β 1AR loop sequence. The rats produced anti- β 1AR autoantibodies that stimulated the receptor. Furthermore, 9 mo later, a progressively severe left ventricular dilatation developed, accompanied by impaired ventricular function. Jahns et al. then transferred sera from β 1AR-stimulating autoantibody-positive animals to healthy autoantibody-negative animals every month. The recipient rats also developed cardiomyopathy within a similar time frame.

The antibodies can be extracted with immunoadsorption, a state-of-affairs that caused Dörffle et al. to treat nine patients positive for β 1AR-stimulating

autoantibodies with an immunoabsorber for IgG (8). Cardiac output increased, systemic blood pressure decreased, pulmonary artery pressure decreased, systemic vascular resistance fell, and filling pressures were reduced in their patients. A clinical study testing the utility of a fluorescence-based functional assay to detect stimulating β 1AR antibodies is under way (6). Large-scale randomized treatment trials have not been reported, although smaller randomized studies on immunoabsorption and nonspecific immunoglobulin treatments appear encouraging (26). Since beta-blocking drugs seem to be effective even in patients with agonistic autoantibodies, the future of such treatments is uncertain.

Alpha-1 Adrenergic Receptor

Fu et al. reported on 15 patients with malignant hypertension that harbored an autoantibody against the alpha-1 adrenergic receptor (α 1AR) (11). This autoantibody caused neonatal cardiomyocytes to beat more rapidly; prazosin blocked the effect. The authors raised the possibility that antibodies activating the α 1AR could be responsible for malignant hypertension. These observations were later extended to a larger patient series. Luther et al. mapped the epitope to the first extracellular loop of the α 1AR (17). Liao et al. reported similar observations in Chinese patients that were identified by a specific ELISA (16). Zhou et al. developed an experimental animal model (44). They immunized Wistar rats with synthetic peptides corresponding to first extracellular loop of the α 1AR. The animals developed α 1AR-activating antibodies that elevated free Ca^{2+} in isolated cardiomyocytes from adult rats. The immunized rats did not develop hypertension. However, cardiac hypertrophy was observed, collagen deposition in cardiac interstitium was increased, and the mRNA for c-jun and matrix metalloproteinase (MMP)-2 was increased.

We observed that 41 of 81 patients with refractory hypertension had α 1AR autoantibodies (36). Immunoabsorption appeared to reduce blood pressure in individual patients. We immunized rabbits to generate α 1AR-stimulating antibodies. We purified autoantibodies from both patients and rabbits with affinity chromatography and characterized the autoantibodies by epitope mapping and surface plasmon resonance measurements. We found that the genes encoding phospholipase A2 and L-type calcium channels were upregulated in cardiomyocytes and vascular smooth muscle cells after stimulation with both purified autoantibody sets. We demonstrated that patient-derived α 1AR-stimulating autoantibodies and rabbit-derived α 1AR autoantibodies caused protein kinase C- α activation and stimulated extracellular-related kinase (EK1/2) phosphorylation. Finally, we showed that

the autoantibodies could increase intracellular Ca^{2+} in cardiomyocytes and induce mesentery artery segment contraction. We next immunized Lewis rats with the second extracellular-loop peptides of the human α 1AR and maintained the animals for 1 year (38). We monitored blood pressure in these rats telemetrically and performed echocardiography. At 12 mo, the left ventricles of immunized rats had greater wall thickness than control rats. The fractional shortening and $\text{dp/dt}(\text{max})$ were normal. However, a decreased E/A ratio in immunized rats indicated a diastolic dysfunction. We made direct invasive hemodynamic measurements and found that left ventricular end-diastolic pressures were increased, whereas $\text{dp/dt}(\text{min})$ was decreased. The cardiomyocyte diameters were increased. However, telemetric blood pressure values and heart rates were not different.

We next performed a small but exacting patient-oriented study in our Clinical Research Center on five patients with refractory hypertension who harbored autoantibodies directed at the α 1AR (23). Before immune adsorption, supine blood pressure was 169/88 mmHg. After completion of the immune adsorption protocol, blood pressure was reduced in four patients and increased in patient (group average: 146/78 mmHg; $P = \text{not significant}$). Resting heart rate was 59 beats/min before immune adsorption and 59 beats/min after immune adsorption. The phenylephrine and nitroprusside-induced heart rate responses were identical before and after immune adsorption. That observation excludes the possibility that altered vascular responses were masked by opposing changes in baroreflex heart rate regulation. Our study did not exclude the possibility that agonistic α 1AR autoantibodies chronically affect cardiovascular structure and function in some patients with resistant hypertension. However, we strongly suggest that experimental findings cannot be simply extrapolated to patients with endogenously produced antibodies and that controlled clinical trials are required.

Angiotensin II Receptor

Preeclampsia is a catastrophic illness of women in late pregnancy of unknown cause; however, immune mechanisms and the renin-angiotensin system are both implicated. We managed a woman with malignant hypertension who had experienced a preeclamptic pregnancy 20 years earlier. This patient had circulating antibodies that stimulated the angiotensin (Ang) II receptor (AT1R) (FIGURE 1). Thereupon, we began searching for AT1R-stimulating autoantibodies in preeclamptic patients. We used a simple but extremely sensitive bioassay and found such antibodies in 25 preeclamptic patients

but not in 12 normotensive pregnant women or 10 pregnant patients with essential hypertension (32). Immunoglobulin isolated from the preeclamptic patients stimulated the AT1R, whereas immunoglobulin from controls did not. The autoantibody titers decreased after delivery. We then used affinity-column purification and found that we were dealing with IgG. We synthesized peptides and found a sequence corresponding to sites on the AT1 receptor's second extracellular loop that abolished the stimulatory effect. Western blotting with purified patient IgG and a commercially obtained AT1R-stimulating autoantibody produced similar bands. Furthermore, confocal microscopy of vascular smooth muscle cells showed colocalization of purified patient IgG and AT1R-stimulating autoantibody. The protein kinase C inhibitor calphostin prevented the stimulatory effect. Our results suggested that preeclamptic patients develop stimulatory autoantibodies against the second extracellular AT1R loop. The effect appeared to be protein kinase C mediated. We next obtained IgG AT1R-stimulating autoantibodies from preeclamptic patients and purified the antibodies with anti-human IgG columns (4). We then transfected AT1R-overexpressing Chinese

hamster ovary cells with tissue factor (TF) promoter constructs that were coupled to a luciferase reporter gene and exposed these cells to Ang II and to autoantibodies. Ang II and AT1R autoantibodies both activated ERK1/2, activator protein-1 (AP-1), TF promoter-transfected vascular smooth muscle cells, and Chinese hamster ovary cells. However, the activation required the presence of the AP-1 binding site. We found that placentas from preeclamptic women stained strongly for TF, whereas control placentas showed far less staining. We documented autoantibody specificity by coimmunoprecipitating the AT1R receptor with autoantibodies. We then demonstrated TF expression in vascular smooth muscle cells exposed to either Ang II or the agonistic autoantibodies. All these effects were blocked by losartan. Nonspecific IgG or IgG from nonpreeclamptic pregnant women had a negligible effect. Walther et al. confirmed our clinical observations and found that, in the third trimester, the AT1R-stimulating autoantibodies were present in 89% of patients with manifest preeclampsia, 86% of those with manifest intrauterine growth retardation (IUGR), and even in some apparently healthy pregnant women at term with a history of abnormal

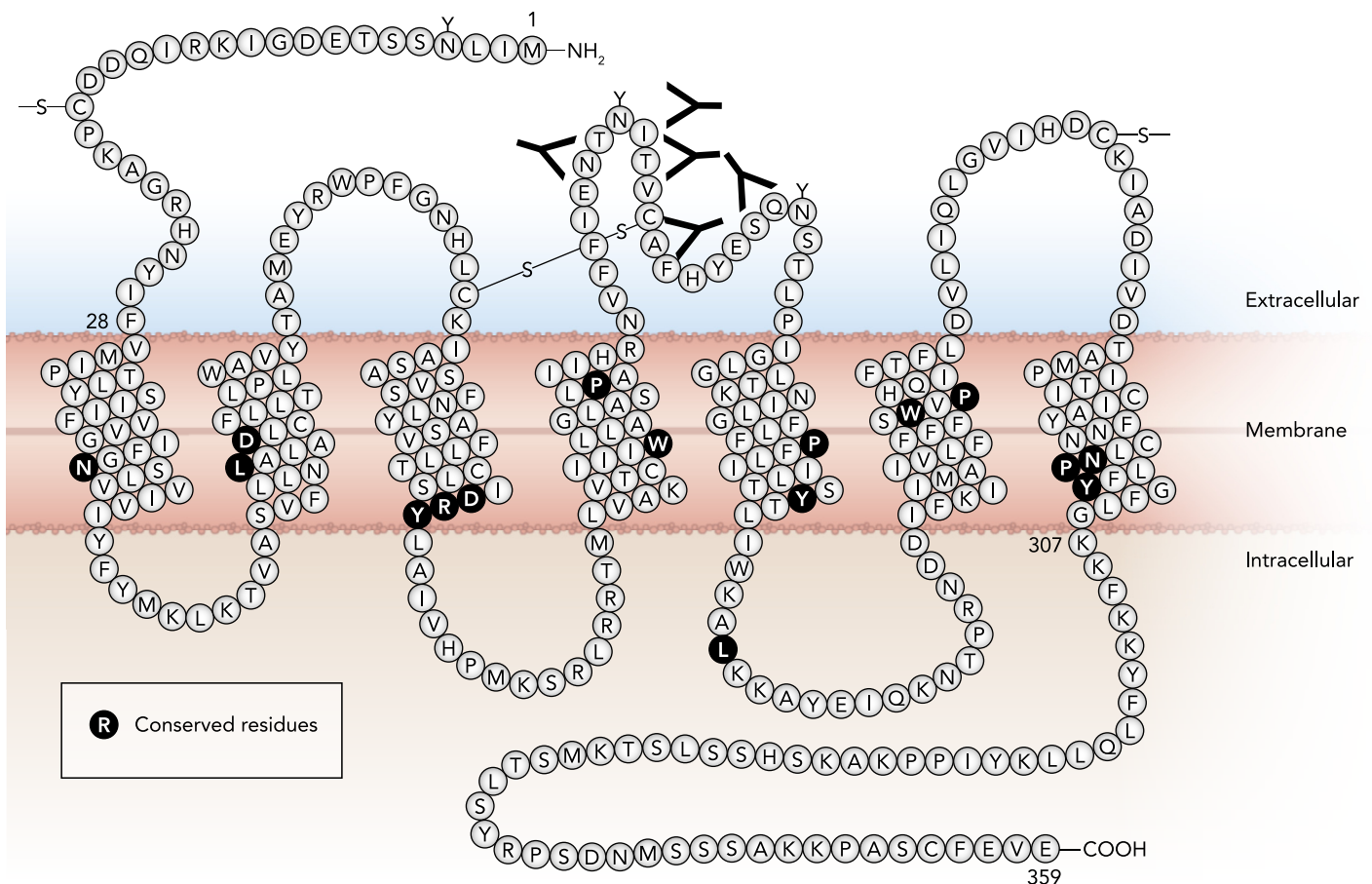


Figure 1. Amino-acid sequence structure of AT1R

Antibody binding sites on the second extracellular loop of AT1R are identified. Receptor diagram is from Ref. 5.

uterine perfusion in the second trimester (34). These data provided further mechanistic insights that could be relevant to preeclampsia.

Xia et al. have taken these observations further (39). They initially studied 38 pregnant patients, 20 of whom had severe preeclampsia. They also used a bioassay to identify AT1R-stimulating autoantibodies and found that the autoantibodies were present in the preeclamptic patients. The autoantibodies stimulated the production of plasminogen activator-inhibitor-1 from human trophoblasts and diminished trophoblast invasion into Matrigel. The epitope they discovered was identical to ours. The group then showed that the AT1R-stimulating autoantibodies caused mobilization of intracellular Ca^{2+} , resulting in the activation of the transcription factor, nuclear factor of activated T cells (NFAT) (31).

An additional very attractive mechanism that could be responsible for preeclampsia is elaboration of the soluble *fms*-like tyrosine kinase (sFlt-1). sFlt-1 is a soluble splice variant of a vascular endothelial growth factor (VEGF) receptor. The sFlt-1 interferes with placental growth factor and VEGF, thereby impairing placental development. The Xia laboratory speculated that Ang II might lead to increased sFlt-1 expression in pregnancy (41). They first found that infusion of Ang II significantly increased circulating levels of sFlt-1 in pregnant mice. They also observed that Ang II stimulated sFlt-1 production dose- and time-dependently in human villous explants and in cultured trophoblasts. This observation supported the idea that trophoblasts are the primary source of sFlt-1 during pregnancy. In vitro and in vivo studies with losartan, calcineurin-specific small-interfering RNA, and an NFAT inhibitor demonstrated that Ang II-mediated sFlt-1 release occurred via AT1R activation and calcineurin signaling, respectively. Subsequent observations by the same investigators indicated that AT1R-stimulating autoantibodies also led to sFlt-1 production, linking these putative pathogenic mechanisms (40). Notably, Stepan et al. found that preeclamptic women harboring AT1R-stimulating autoantibodies also featured circulating sFlt-1 values, but they could not find a correlation between the two (27).

The Xia laboratory next showed that hypertension, proteinuria, glomerular endotheliosis, placental abnormalities, and small fetal size, namely IUGR, appeared in pregnant mice after injection with either total IgG or affinity-purified AT1R-stimulating autoantibodies from women with preeclampsia (43). These preeclampsia-like features were prevented by losartan or by an antibody neutralizing the epitope on AT1R. Their studies indicate that preeclampsia may be a pregnancy-induced autoimmune disease and that autoantibody-induced

AT1R activation plays a major role. The group then capitalized on this model further and showed that AT1R autoantibodies crossed the mouse placenta, entered fetal circulation, and led to small fetuses with IUGR (13). The antibodies also induced apoptosis in the placentas of pregnant mice, human villous explants, and human trophoblast cells. Finally, losartan or an autoantibody-neutralizing peptide diminished autoantibody-induced IUGR and placental apoptosis. The studies underscored two possible underlying mechanisms by which autoantibodies could act, namely a direct detrimental effect on fetal development by crossing the placenta and entering fetal circulation, and an indirect effect through AT1R stimulation-induced placental damage.

The Xia laboratory also conducted further clinical studies (24). They developed a sensitive, high-throughput, luciferase bioassay to detect AT1R autoantibody levels and studied large numbers of preeclamptic patients and normal pregnant women. They found that 95% of the preeclamptic women harbored the antibodies. They also reported that antibody levels were correlated with systolic blood pressure, proteinuria, and sFlt-1 levels. Returning to their mouse model, the group next demonstrated that AT1R-stimulating autoantibodies mediated tumor necrosis factor- α (TNF- α) induction by overcoming its negative regulator, heme oxygenase-1 (14). The autoantibodies also induced both soluble endoglin, another preeclampsia-associated molecule, and sFlt-1 secretion from human villous explants. The investigators have since suggested that TNF- α induction by autoantibodies is particularly mechanistically important (42). The group has also drawn attention to autoantibody-mediated complement activation and complement C3a receptor signaling as a key mechanism underlying the pathogenesis of the disease (35). Recently, they demonstrated that reduced circulating aldosterone levels in preeclamptic women are associated with AT1R-stimulating autoantibodies and the presence of sFlt-1 (25). The observations from patients were then documented in their animal model. The findings could point to a novel mechanism explaining reduced aldosterone levels in patients with preeclampsia.

The binding site of the stimulating AT1R autoantibodies on the AT1R is distant from the receptor's Ang II ligand binding site. One possible mechanism how the antibodies might work is an alteration of the AT1R's conformation, making it more sensitive to Ang II. Preeclamptic women are known to be hypersensitive to Ang II compared with normal pregnant women or nonpregnant women. We immunized rabbits against the AFHYESQ epitope of the second extracellular loop, which is the binding epitope of endogenous activating autoantibodies against

the AT1R, from patients with preeclampsia and generated and purified activating antibodies (37). Passive transfer of AT1R-stimulating antibodies or Ang II alone did not induce a preeclampsia-like syndrome in pregnant rats. However, the combination of AT1R-stimulating antibodies plus Ang II induced hypertension, proteinuria, intrauterine growth retardation, and arteriolosclerosis in the uteroplacental unit.

Stepan et al. raised the possibility that parvovirus B19 could be the cause for autoimmunity against the AT1R (28). We therefore performed a prospective, nested, case-control study of 30 preeclamptic women and 30 normotensive pregnant women controls (12). We measured AT1R-stimulating autoantibodies, sFlt-1, and serum immunoglobulin G against parvovirus B19 proteins. Autoantibodies were present in most of the preeclamptic patients but not in the controls. Late-onset preeclampsia improved prediction. We did not find an interaction between sFlt-1 and the autoantibodies. Immunoglobulin G against parvovirus B19 proteins was similarly distributed between preeclamptic patients and controls.

The reduced uterine perfusion pressure (RUPP) pregnant rat model resembles human preeclampsia. Hypertension in pregnant rats with RUPP features elevated inflammatory cytokines, agonistic autoantibodies to the AT1R, and CD4⁺ T cells. Novotny et al. tested the notion that AT1R-stimulating antibodies influence T-cell-induced blood pressure increases in this model (20). They treated pregnant RUPP rats with losartan or rituximab, which destroys B lymphocytes and thereby decreases autoantibodies. Novotny et al. (20) magnetically isolated CD4⁺ T splenocytes on *day 19* of gestation from control RUPP and normal pregnant rats and then injected them into a new group of normal pregnant rats at *day 13* of gestation. Circulating AT1R-stimulating autoantibodies increased in normal-pregnant rats receiving RUPP CD4⁺ T cells but decreased in normal-pregnant rats receiving RUPP CD4⁺ T cells chronically treated with rituximab. Hypertension that developed in normal-pregnant rats receiving RUPP CD4⁺ T cells was attenuated by losartan and with B-cell depletion. The authors concluded that one mechanism of hypertension in response to CD4⁺ T lymphocytes activated during placental ischemia is via AT1 receptor activation, potentially via AT1R-stimulating autoantibodies during pregnancy. These findings are particularly interesting since, in Graves' disease, not only TSHR-stimulating autoantibodies but also TSHR-specific T cells are important mechanistically (3).

The same laboratory recently reported on the effect of interleukin-17 (IL-17) on AT1R-stimulating autoantibody production in pregnancy (7). The

investigators studied four groups of pregnant rats, normal-pregnant controls, IL-17 infusion, reactive oxygen species scavenger-treated (Tempol), and IL-17 plus Tempol. The data indicated that IL-17 causes placental oxidative stress, which then serves as stimulus for AT1R-stimulating autoantibody production that may play an important role in mediating IL-17-induced hypertension during pregnancy.

There are no effective treatments for preeclampsia other than delivery of the fetus and placenta. AT1R blockers and angiotensin-converting enzyme inhibitors are not an option because these drugs lead to faulty fetal renal development. Immunoabsorption or plasmapheresis would appear to be therapeutic possibilities, although no convincing trials of this treatment have been reported. Thadhani et al. recently reported a pilot study of extracorporeal removal of sFlt-1 from preeclamptic patients (30). The apheresis columns employed by the investigators adsorb circulating proteins on the basis of electrostatic interactions. The extent to which they may have eliminated AT1R-stimulating autoantibodies, in addition to sFlt-1, is unknown.

Although much work has been done on AT1R-stimulating autoantibodies in preeclampsia, the same antibodies have also been investigated in other conditions. Dragun et al. studied 33 renal transplant recipients who all had refractory vascular rejection (9). Thirteen patients had donor-specific anti-HLA antibodies, whereas 20 patients did not. Sixteen patients had malignant hypertension with no anti-HLA antibodies. The remaining 17 patients, although hypertensive, did not have malignant hypertension. Dragun et al. hypothesized that AT1R-stimulating autoantibodies might be involved. AT1R-stimulating autoantibodies were detected in serum from all 16 patients with malignant hypertension and without anti-HLA antibodies, but in no other patients. The investigators identified both subclass IgG1 and IgG3 antibodies that bound to two different epitopes on the second extracellular loop of the AT1R. Antibody removal with plasmapheresis appeared to benefit these patients.

Similar autoantibodies have been identified in patients with systemic sclerosis. Riemekasten et al. investigated serum samples from 478 patients with systemic sclerosis, 372 healthy subjects, and 311 control-disease subjects (21). All were tested for autoantibodies against AT1R and the endothelin-1 type A receptor by solid phase assay. Both autoantibodies were highly prevalent in the patients with systemic sclerosis. Both autoantibodies exerted biological effects, since they induced ERK1/2 phosphorylation and increased transforming growth factor- β gene expression in endothelial cells that could be blocked with specific receptor antagonists. Systemic

sclerosis is particularly interesting because of an earlier report implicating a different stimulating antibody in this condition, directed at the platelet-derived growth factor receptor (PDGFR). Baroni et al. investigated serum from 46 scleroderma patients and 75 control persons, including patients with other autoimmune diseases, for stimulatory autoantibodies to the PDGFR (1). They incubated mouse-embryonic fibroblasts carrying inactive copies of PDGFR- α or - β chains or the same cells expressing PDGFR- α or - β chains with purified IgG from the subjects and controls and then measured reactive oxygen species production. Stimulatory antibodies were found in all their patients but not in controls. The autoantibodies initiated tyrosine phosphorylation and reactive oxygen production.

Rossitto et al. recently described elevated AT1R-stimulating autoantibodies in patients with aldosterone-producing adenomas, compared with patients with micronodular adrenal hyperplasia (22). After a captopril challenge test, plasma aldosterone concentration fell more in AT1R-stimulating autoantibody-positive patients than in patients who did not harbor

these autoantibodies. The authors suggested an agonistic role for these autoantibodies in patients with functionally active adrenal adenomas.

Perspectives

Stimulating autoantibodies against G-protein-coupled receptors (and receptor tyrosine kinases) exist and may contribute to disease (FIGURE 2). In the example of β 1AR-stimulating autoantibodies, a convincing study was performed in animal models that completed the requirements of Koch's postulates (15). The clinical relevance of the findings can be argued, since autoantibody removal might be no more effective than judicious β 1AR blockade. Several groups have independently reported the existence of α 1AR-stimulating autoantibodies. We could not produce an animal model in any way approaching the model produced for the β 1AR by Jahns et al. (15). Furthermore, our human experiments on antibody removal did not inspire our confidence (23). Independent laboratories have also reported the existence of AT1R-stimulating antibodies. Most studies have focused on preeclampsia, a

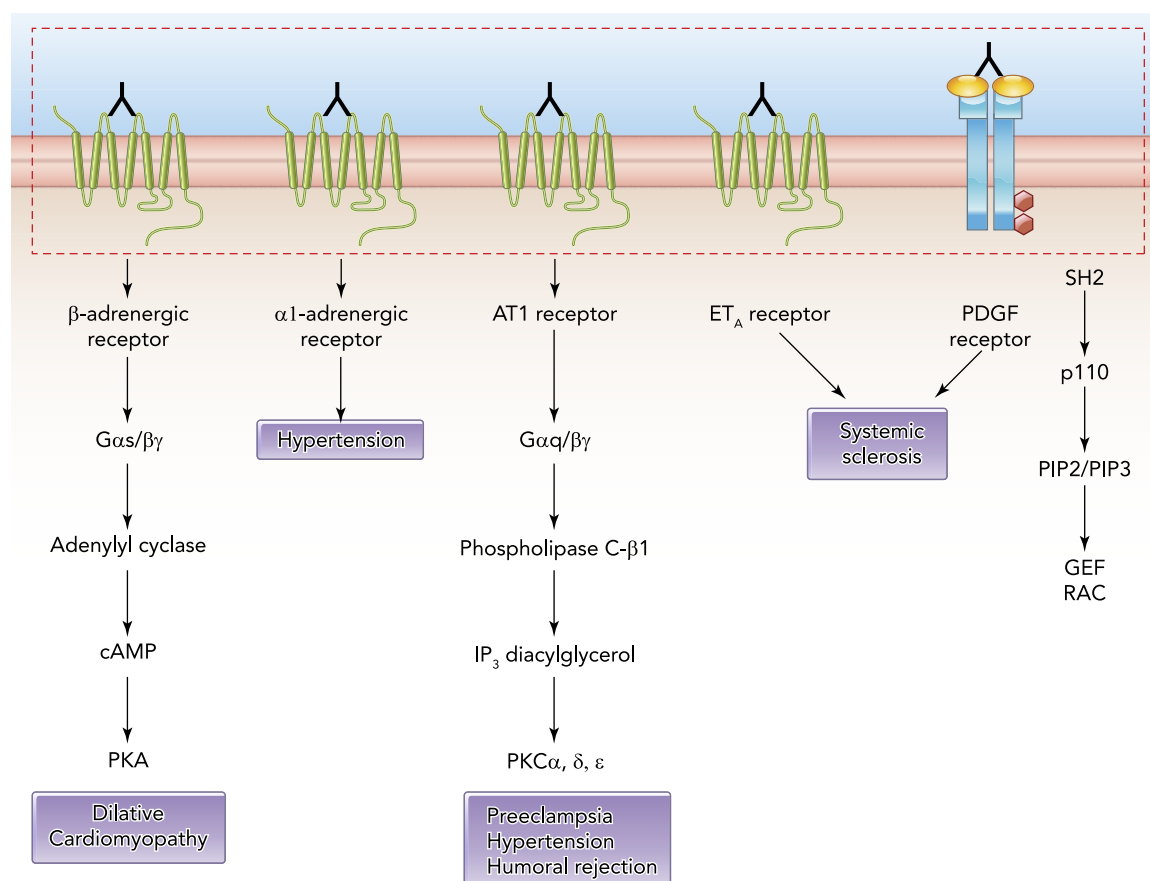


Figure 2. β 1AR, α 1AR, AT1R, and ET_{AR} are all important cardiovascular G-protein-coupled receptors that regulate cardiac function and blood pressure via activation by their respective ligands (Ang II, ET-1, and norepinephrine, respectively)

Activating antibodies involving these receptors have been implicated in various cardiovascular disorders. Such antibodies are also implicated in activating the PDGF receptor tyrosine kinase. Additional evidence reviewed here supports a contributing role for these autoantibodies in disease pathogenesis. Missing (within the red-hatched lines) are detailed data on how the receptor activation actually proceeds.

devastating disease that always produces two patients. There are still no effective treatments, although here removal of autoantibodies, sFlt-1, and soluble endoglin may offer alternatives, since these treatments are now relatively safe. However, convincing clinical trials must be performed. Importantly, much basic research remains to be done to determine the relevance of these findings.

Although the epitope binding sites of activating autoantibodies are largely known, still missing is very basic receptor research elucidating how antibody binding may alter receptor conformation to actually activate the receptors. Crystallography data have mainly focused on receptors occupied in their transmembrane domains by their low molecular weight ligands. However, G-protein-coupled receptors also are able to bind larger peptide molecules. For instance, Fillion et al. (10) recently used a unique chemoselective photoaffinity labeling strategy to directly identify 38 discrete ligand/receptor contact residues that form the extracellular peptide-binding site of an activated AT1R. They then used the dataset in homology modeling to guide the positioning of Ang II peptide within several crystal structure templates. They found that the C-X-C chemokine receptor type 4 (CXCR4) accommodated the results. In the resulting receptor structure, a β -hairpin fold in *extracellular loop 2*, in conjunction with two extracellular disulfide bridges, appeared to open and shape the entrance of the ligand-binding site. The bound Ang II adopted a vertical binding mode, allowing concomitant contacts across the extracellular surface and deep within the transmembrane domain core of the receptor. With their technology, Fillion et al. (10) may be in a position to study the interaction between autoantibodies, which interestingly bind the second extracellular loop, and the AT1R. Furthermore, participation of innate immunity, aside from preliminary studies of CD4⁺ T cells and IL17 production, has not been studied in sufficient detail. Finally, the underlying antigens leading to the autoantibody formation also remain largely unclear. Thus skepticism remains a healthy mindset (29). ■

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References

1. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Avvedimento EV, Gabrielli A. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 354: 2667–2676, 2006.
2. Chiale PA, Rosenbaum MB, Elizari MV, Hjalmarson A, Magnusson Y, Wallukat G, Hoebeke J. High prevalence of antibodies against beta 1- and beta 2-adrenoceptors in patients with primary electrical cardiac abnormalities. *J Am Coll Cardiol* 26: 864–869, 1995.

3. Davies TF, Ando T, Lin RY, Tomer Y, Latif R. Thyrotropin receptor-associated diseases: from adenomata to Graves' disease. *J Clin Invest* 115: 1972–1983, 2005.
4. Dechend R, Homuth V, Wallukat G, Kreuzer J, Park JK, Theuer J, Juepner A, Gulba DC, Mackman N, Haller H, Luft FC. AT(1) receptor agonistic antibodies from preeclamptic patients cause vascular cells to express tissue factor. *Circulation* 101: 2382–2387, 2000.
5. De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger TH. International Union of Pharmacology. XXII. The angiotensin receptors. *Pharmacol Rev* 52: 415–472, 2000.
6. Deubner N, Berliner D, Schlipp A, Gelbrich G, Caforio AL, Felix SB, Fu M, Katus H, Angermann CE, Lohse MJ, Ertl G, Störk S, Jahns R. Etiology, Titre-Course, and Survival-Study Group. 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: 753–762, 2010.
7. Dhillon P, Wallace K, Herse F, Scott J, Wallukat G, Heath J, Mosely J, Martin JN Jr, Dechend R, LaMarca B. IL-17-mediated oxidative stress is an important stimulator of AT1-AA and hypertension during pregnancy. *Am J Physiol Regul Integr Comp Physiol* 303: R353–R358, 2012.
8. Dörffel WV, Felix SB, Wallukat G, Brehme S, Bestvater K, Hofmann T, Kleber FX, Baumann G, Reinke P. Short-term hemodynamic effects of immunoadsorption in dilated cardiomyopathy. *Circulation* 95: 1994–1997, 1997.
9. Dragun D, Müller DN, Bräsen JH, Fritsche L, Nieminen-Kelhä M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schönemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 352: 558–569, 2005.
10. Fillion D, Cabana J, Guillemette G, Leduc R, Lavigne P, Escher E. Structure of the human AT1 receptor bound to angiotensin II from multiple chemoselective photoprobe contacts reveals a unique peptide binding mode. *J Biol Chem* 288: 8187–8197, 2013.
11. Fu ML, Herlitz H, Wallukat G, Hilme E, Hedner T, Hoebeke J, Hjalmarson A. Functional autoimmune epitope on alpha 1-adrenergic receptors in patients with malignant hypertension. *Lancet* 344: 1660–1663, 1994.
12. Herse F, Verlohren S, Wenzel K, Pape J, Muller DN, Modrow S, Wallukat G, Luft FC, Redman CW, Dechend R. Prevalence of agonistic autoantibodies against the angiotensin II type 1 receptor and soluble fms-like tyrosine kinase 1 in a gestational age-matched case study. *Hypertension* 53: 393–398, 2009.
13. Irani RA, Zhang Y, Blackwell SC, Zhou CC, Ramin SM, Kellems RE, Xia Y. The detrimental role of angiotensin receptor agonistic autoantibodies in intrauterine growth restriction seen in preeclampsia. *J Exp Med* 206: 2809–2822, 2009.
14. Irani RA, Zhang Y, Zhou CC, Blackwell SC, Hicks MJ, Ramin SM, Kellems RE, Xia Y. Autoantibody-mediated angiotensin receptor activation contributes to preeclampsia through tumor necrosis factor-alpha signaling. *Hypertension* 55: 1246–1253, 2010.
15. Jahns R, Boivin V, Hein L, Triebel S, Angermann CE, Ertl G, Lohse MJ. Direct evidence for a beta 1-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J Clin Invest* 113: 1419–1429, 2004.
16. Liao YH, Wei YM, Wang M, Wang ZH, Yuan HT, Cheng LX. Autoantibodies against AT1-receptor and alpha1-adrenergic receptor in patients with hypertension. *Hypertens Res* 25: 641–646, 2002.
17. Luther HP, Homuth V, Wallukat G. Alpha 1-adrenergic receptor antibodies in patients with primary hypertension. *Hypertension* 29: 678–682, 1997.
18. Magnusson Y, Marullo S, Hoyer S, Waagstein F, Andersson B, Vahlne A, Guillet JG, Strosberg AD, Hjalmarson A, Hoebeke J. Mapping of a functional autoimmune epitope on the beta 1-adrenergic receptor in patients with idiopathic dilated cardiomyopathy. *J Clin Invest* 86: 1658–1663, 1990.
19. Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation* 89: 2760–2767, 1994.

20. Novotny SR, Wallace K, Heath J, Moseley J, Dhillon P, Weimer A, Wallukat G, Herse F, Wenzel K, Martin JN Jr, Dechend R, Lamarca B. Activating autoantibodies to the angiotensin II type I receptor play an important role in mediating hypertension in response to adoptive transfer of CD4⁺ T lymphocytes from placental ischemic rats. *Am J Physiol Regul Integr Comp Physiol* 302: R1197–R1201, 2012.
21. Riemekasten G, Philippe A, Näther M, Slowinski T, Müller DN, Heidecke H, Matucci-Cerinic M, Czirjak L, Lukitsch I, Becker M, Kill A, van Laar JM, Catar R, Luft FC, Burmester GR, Hegner B, Dragun D. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis* 270: 530–536, 2011.
22. Rossitto G, Regolisti G, Rossi E, Negro A, Nicoli D, Casali B, Toniato A, Caroccia B, Seccia TM, Walther T, Rossi GP. Elevation of angiotensin-II type-1-receptor autoantibodies titer in primary aldosteronism as a result of aldosterone-producing adenoma. *Hypertension* 59: e6–e7526–533, 2013.
23. Schroeder C, Stabroth C, Luft FC, Jordan J. Adrenergic cardiovascular control before and after removal of stimulatory α -1 adrenoreceptor antibodies. *Hypertension* 59: e6–e7526–533, 2012.
24. Siddiqui AH, Irani RA, Blackwell SC, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with disease severity. *Hypertension* 55: 386–393, 2010.
25. Siddiqui AH, Irani RA, Zhang W, Wang W, Blackwell SC, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody-mediated soluble fms-like tyrosine kinase-1 induction contributes to impaired adrenal vasculature and decreased aldosterone production in preeclampsia. *Hypertension* 61: 472–479, 2013.
26. Staudt A, Schäper F, Stangl V, Plagemann A, Böhm M, Merkel K, Wallukat G, Wernecke KD, Stangl K, Baumann G, Felix SB. Immunohistological changes in dilated cardiomyopathy induced by immunoadsorption therapy and subsequent immunoglobulin substitution. *Circulation* 103: 2681–2686, 2001.
27. Stepan H, Faber R, Wessel N, Wallukat G, Schultheiss HP, Walther T. Relation between circulating angiotensin II type 1 receptor agonistic autoantibodies and soluble fms-like tyrosine kinase 1 in the pathogenesis of preeclampsia. *J Clin Endocrinol Metab* 91: 2424–2427, 2006.
28. Stepan H, Wallukat G, Schultheiss HP, Faber R, Walther T. Is parvovirus B19 the cause for autoimmunity against the angiotensin II type receptor? *J Reprod Immunol* 73: 130–134, 2007.
29. Stepan H, Walther T. Questionable role of the angiotensin II receptor subtype 1 autoantibody in the pathogenesis of preeclampsia. *Hypertension* 50: e3, 2007.
30. Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, Jank A, Kribs A, Cornely OA, Kreyssig C, Hemphill L, Rigby AC, Khedkar S, Lindner TH, Mallmann P, Stepan H, Karumanchi SA, Benzing T. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. *Circulation* 124: 940–950, 2011.
31. Thway TM, Shlykov SG, Day MC, Sanborn BM, Gilstrap 3rd LC, Xia Y, Kellems RE. Antibodies from preeclamptic patients stimulate increased intracellular Ca^{2+} mobilization through angiotensin receptor activation. *Circulation* 110: 1612–1619, 2004.
32. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jüpner A, Baur E, Nissen E, Vetter K, Neichel D, Dudenhausen JW, Haller H, Luft FC. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest* 103: 945–952, 1999.
33. Wallukat G, Wollenberger A. Effects of 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: S634–S639, 1987.
34. Walther T, Wallukat G, Jank A, Bartel S, Schultheiss HP, Faber R, Stepan H. Angiotensin II type 1 receptor agonistic antibodies reflect fundamental alterations in the uteroplacental vasculature. *Hypertension* 46: 1275–1279, 2005.
35. Wang W, Irani RA, Zhang Y, Ramin SM, Blackwell SC, Tao L, Kellems RE, Xia Y. Autoantibody-mediated complement C3a receptor activation contributes to the pathogenesis of preeclampsia. *Hypertension* 60: 712–721, 2012.
36. Wenzel K, Haase H, Wallukat G, Derer W, Bartel S, Homuth V, Herse F, Hubner N, Schulz H, Janczkowski M, Lindschau C, Schroeder C, Verlohren S, Morano I, Muller DN, Luft FC, Dietz R, Dechend R, Karczewski P. Potential relevance of alpha(1)-adrenergic receptor autoantibodies in refractory hypertension. *PLoS One* 3: e3742, 2008.
37. Wenzel K, Rajakumar A, Haase H, Geusens N, Hubner N, Schulz H, Brewer J, Roberts L, Hubel CA, Herse F, Hering L, Qadri F, Lindschau C, Wallukat G, Pijnenborg R, Heidecke H, Riemekasten G, Luft FC, Muller DN, Lamarca B, Dechend R. Angiotensin II type 1 receptor antibodies and increased angiotensin II sensitivity in pregnant rats. *Hypertension* 58: 77–84, 2011.
38. Wenzel K, Wallukat G, Qadri F, Hubner N, Schulz H, Hummel O, Herse F, Heuser A, Fischer R, Heidecke H, Luft FC, Muller DN, Dietz R, Dechend R. Alpha1A-adrenergic receptor-directed autoimmunity induces left ventricular damage and diastolic dysfunction in rats. *PLoS One* 5: e9409, 2010.
39. Xia Y, Wen H, Bobst S, Day MC, Kellems RE. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells. *J Soc Gynecol Invest* 10: 82–93, 2003.
40. Zhou CC, Ahmad S, Mi T, Abbasi S, Xia L, Day MC, Ramin SM, Ahmed A, Kellems RE, Xia Y. Autoantibody from women with preeclampsia induces soluble Fms-like tyrosine kinase-1 production via angiotensin type 1 receptor and calcineurin/nuclear factor of activated T-cells signaling. *Hypertension* 51: 1010–1019, 2008.
41. Zhou CC, Ahmad S, Mi T, Xia L, Abbasi S, Hewett PW, Sun C, Ahmed A, Kellems RE, Xia Y. Angiotensin II induces soluble fms-like tyrosine kinase-1 release via calcineurin signaling pathway in pregnancy. *Circ Res* 100: 88–95, 2007.
42. Zhou CC, Irani RA, Zhang Y, Blackwell SC, Mi T, Wen J, Shelat H, Geng YJ, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody-mediated tumor necrosis factor-alpha induction contributes to increased soluble endoglin production in preeclampsia. *Circulation* 121: 436–444, 2010.
43. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, Hicks MJ, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. *Nat Med* 14: 855–862, 2008.
44. Zhou Z, Liao YH, Wei Y, Wei F, Wang B, Li L, Wang M, Liu K. Cardiac remodeling after long-term stimulation by antibodies against the alpha1-adrenergic receptor in rats. *Clin Immunol* 114: 164–173, 2005.