Angiotensin Receptor Agonistic Autoantibodies and Hypertension
Preeclampsia and Beyond

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Abstract: Hypertensive disorders are life-threatening diseases with high morbidity and mortality, affecting billions of individuals worldwide. A multitude of underlying conditions may contribute to hypertension, thus the need for a plethora of treatment options to identify the approach that best meets the needs of individual patients. A growing body of evidence indicates that (1) autoantibodies that bind to and activate the major angiotensin II type I (AT\textsubscript{1}) receptor exist in the circulation of patients with hypertensive disorders, (2) these autoantibodies contribute to disease pathophysiology, (3) antibody titers correlate to the severity of the disease, and (4) efforts to block or remove these pathogenic autoantibodies have therapeutic potential. These autoantibodies, termed AT\textsubscript{1} agonistic autoantibodies have been extensively characterized in preeclampsia, a life-threatening hypertensive condition of pregnancy. As reviewed here, these autoantibodies cause symptoms of preeclampsia when injected into pregnant mice. Somewhat surprisingly, these auto antibodies also appear in 3 animal models of preeclampsia. However, the occurrence of AT\textsubscript{1} agonistic autoantibodies is not restricted to pregnancy. These autoantibodies are prevalent among kidney transplant recipients who develop severe transplant rejection and malignant hypertension during the first week after transplantation. AT\textsubscript{1} agonistic autoantibodies are also highly abundant among a group of patients with essential hypertension that are refractory to standard therapy. More recently these autoantibodies have been seen in patients with the autoimmune disease, systemic sclerosis. These 3 examples extend the clinical impact of AT\textsubscript{1} agonistic autoantibodies beyond pregnancy. Research reviewed here raises the intriguing possibility that preeclampsia and other hypertensive conditions are autoimmune diseases characterized by the presence of pathogenic autoantibodies that activate the major angiotensin receptor, AT\textsubscript{1}. These pathogenic autoantibodies could serve as presymptomatic biomarkers and therapeutic targets, thereby providing improved medical management for these conditions. (Circ Res. 2013;113:78-87.)

Key Words: autoimmunity ■ hypertension ■ AT\textsubscript{1}-AA ■ preeclampsia

Hypertensive disorders are life-threatening diseases with high morbidity and mortality, affecting billions of individuals worldwide. The pathogenesis of essential hypertension is multifactorial, with different underlying mechanisms contributing to the disease. Because of disease heterogeneity, a variety of antihypertensive drugs are needed to tailor medical approaches to the specific needs of individual patients. Common areas of investigation for hypertension research include the vascular system, renal hemodynamics and renovascular hypertension, the endothelin system and the renin–angiotensin–aldosterone system. Here, we review evidence suggesting that some forms of hypertension may have an underlying autoimmune component.

Autoimmune diseases are relatively common (5% of the US population) and include well-known examples, such as Type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and celiac disease. It is worth noting that in each case the autoimmune nature of the disease was not originally obvious and only became apparent after extensive investigation. Research reviewed here suggests that hypertensive disorders may result from the presence of agonistic autoantibodies (AA) that are directed to a specific epitope on the second extracellular of loop of the angiotensin II type I receptor (AT\textsubscript{1}R). The classic example of receptor-activating autoantibodies and disease is Graves’ hyperthyroidism, in which autoantibodies activate the thyroid-stimulating hormone receptor resulting in overproduction of thyroid hormones.\textsuperscript{1,2} Other compelling examples come from the cardiovascular literature and include: (1) AA targeting the cardiac β\textsubscript{1}-adrenergic receptor, which are associated with dilated cardiomyopathy,\textsuperscript{3} (2) autoantibodies capable of activating α\textsubscript{2}-adrenergic receptors, associated with refractory hypertension,\textsuperscript{4,6} and (3) autoantibodies that activate the major angiotensin II receptor, associated with preeclampsia.\textsuperscript{7-9}
malignant hypertension, and renal allograft rejection.\textsuperscript{10–12} AT\textsubscript{1}-AAs are the focus of this review (Table).

### AT\textsubscript{1}-AA and Preeclampsia

Preeclampsia is a life-threatening hypertensive condition of pregnancy and a leading cause of maternal and neonatal morbidity and mortality.\textsuperscript{13,14} The condition generally appears during the third trimester and is also characterized by proteinuria, inflammation, and thrombosis. Preeclampsia affects \( \approx 7\% \) of pregnancies and accounts for 15% of premature births (180,000 in the United States). Current strategies for managing preeclampsia are inadequate and reflect a fundamental lack of understanding of the causes and pathogenesis of the disorder. Numerous studies during the past 14 years have shown that women with preeclampsia possess autoantibodies with the ability to bind and activate the major angiotensin receptor, AT\textsubscript{1}R.

### Early In Vitro Studies

AA to AT\textsubscript{1}R were initially described by Wallukat et al.\textsuperscript{7} In this seminal report, the authors described the use of a rat neonatal cardiomyocyte contraction assay to detect the presence of AT\textsubscript{1} agonistic autoantibodies, termed AT\textsubscript{1}-AA. Receptor specificity was shown pharmacologically and by immunohistochemistry and Western blotting. Peptide competition experiments were used to identify the precise epitope recognized by these autoantibodies, a 7-amino acid peptide sequence located on the second extracellular loop of the receptor. Subsequent database analysis revealed that this amino acid sequence corresponds to a highly antigenic region present on the coat protein of parvovirus B19, a common and relatively benign human pathogen. This finding raised the possibility that AT\textsubscript{1}-AA arise in part as a result of molecular mimicry. However, epidemiological studies rendered this explanation unlikely.\textsuperscript{15,16}

Although AT\textsubscript{1}-AA were initially detected by their ability to stimulate an increase in the beating rate of isolated neonatal rat cardiomyocytes, numerous additional early studies showed that AT\textsubscript{1}-AA could activate AT\textsubscript{1} receptors on a variety of cell types and provoke biological responses relevant to the pathophysiology of preeclampsia. Studies from multiple laboratories showed that AT\textsubscript{1}-AA may contribute to hypercoagulation by stimulating tissue factor production by vascular smooth muscle cells and monocytes,\textsuperscript{17} as well as plasminogen activator inhibitor-1 production from trophoblasts\textsuperscript{18} and mesangial cells.\textsuperscript{18} Other studies showed that immunoglobulin G (IgG) from women with preeclampsia, in contrast to IgG from normotensive pregnant women, contributes to the production of reactive oxygen species by stimulating nicotinamide adenine dinucleotide phosphate oxidase activity in vascular smooth muscle cells and human trophoblasts.\textsuperscript{19} Finally, antibody-mediated AT\textsubscript{1} receptor activation results in increased soluble Fms-like tyrosine kinase-1 (sFlt-1)\textsuperscript{20} and soluble endoglin (sEng)\textsuperscript{21} production from human trophoblasts and placental explants. In this way, these antibodies may contribute to the antiangiogenic state that is characteristic of preeclampsia.

### Antibody Transfer Experiments in Animals

Because these earlier studies were restricted to the use of cultured cells or tissue explants, they did not directly address the relevance of AT\textsubscript{1}-AA to hypertension and proteinuria, the defining clinical features of preeclampsia. For this reason, an in vivo experimental approach was needed to determine if these autoantibodies could cause clinical features of preeclampsia. Using a classical antibody transfer approach Zhou et al.\textsuperscript{22} showed that the introduction of these autoantibodies into pregnant mice resulted in hypertension, proteinuria, and a variety of other features of preeclampsia (Figure 1). Proteinuria was accompanied by a characteristic renal abnormality termed glomerular endotheliosis (endothelial cell swelling). These features appeared in pregnant mice after AT\textsubscript{1}-AA injection and were prevented by coinjection with losartan (an AT\textsubscript{1} antagonist) or a 7-amino acid epitope peptide that corresponds to a highly antigenic site on the second extracellular loop of the AT\textsubscript{1}R. These results indicate that hypertension, proteinuria, and renal pathology resulted from autoantibody-induced AT\textsubscript{1} receptor activation in pregnant mice.

AT\textsubscript{1}-AA injection into nonpregnant mice also results in hypertension but not the renal pathology observed in pregnant mice.\textsuperscript{23} These results indicate that the antiangiogenic action of
excessive sFlt-1 production by the placenta is detrimental to renal endothelial function, resulting in glomerular endotheliosis and proteinuria (discussed in more detail below). The hypertensive effects of AT$_1$-AA in the absence of pregnancy are consistent with the contribution of AT$_1$-AA to essential and malignant hypertension reported by others (see later sections).

Because AT$_1$-AA produce clinical features of preeclampsia when introduced into pregnant mice, it is likely that they contribute to symptoms of preeclampsia in the women from whom they were obtained. This view is supported by data showing that AT$_1$-AAs are highly prevalent in preeclampsia$_{7,8,15,22}$ and that antibody titers correlate to the severity of the disease.$^{22}$ Thus, in vitro and in vivo findings with IgG from patients’ sera suggest a pathophysiological role for these autoantibodies in preeclampsia and provide experimental support for the hypothesis that preeclampsia is an autoimmune condition characterized by the presence of disease-causing autoantibodies.

**Antibody-Induced Pathogenic Factors**

After the success of the initial antibody transfer experiments, this mouse model of preeclampsia was used to identify numerous antibody-induced factors that contribute to disease pathophysiology. These include the inflammatory cytokines tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6), the antiangiogenic factors sFlt-1 and sEng, the vasoconstrictor endothelin-1 (ET-1), and complement component C3a. In each case, it was possible to identify inhibitory strategies to block the action of these pathogenic mediators and achieve therapeutic benefit in the antibody-induced model of preeclampsia in pregnant mice. The results of these studies are summarized in Figure 2 and discussed below.

**Inflammatory Cytokines (TNA-$\alpha$ and IL-6)**

Circulating TNF-$\alpha$ levels are significantly elevated in women with preeclampsia and correlate with the level of AT$_1$-AA bioactivity.$^{21}$ Injection of IgG from women with preeclampsia (in contrast to IgG from normotensive pregnant women) into pregnant mice results in elevated levels of TNF-$\alpha$ characteristic of women with preeclampsia.$^{21,24}$ Coinjection of AT$_1$-AA with a TNF-$\alpha$-neutralizing antibody, or a soluble form of the TNF-$\alpha$ receptor, significantly attenuates key features of preeclampsia, including hypertension and proteinuria. Renal damage and placental abnormalities were also decreased by TNF-$\alpha$ blockade. TNF-$\alpha$ blockade also resulted in a reduction in sFlt-1, sEng, and IL-6, indicating that these biomolecules function downstream of TNF-$\alpha$ signaling.$^{21,22}$ Thus, TNF-$\alpha$ functions downstream of autoantibody-mediated AT$_1$R activation to promote clinical features of preeclampsia in pregnant mice.

IL-6 functions downstream of TNF-$\alpha$ signaling and contributes to increased ET-1 production in pregnant mice.$^{23}$ IL-6 blockade inhibits the appearance of preeclampsia features in autoantibody-injected pregnant mice. Extending the data to human studies, Zhou et al.$^{23}$ found that IL-6 was a key cytokine underlying autoantibody-mediated induction of ET-1 in human placental villous explants and that endothelial cells are a key source of ET-1. Overall, human and mouse studies show that AT$_1$-AA contributes to elevated ET-1 production and that increased TNF-$\alpha$/IL-6 signaling is a key mechanism underlying increased ET-1 production and subsequent maternal features of preeclampsia.

Direct evidence for the ability of inflammatory cytokines to contribute to clinical features of preeclampsia comes from experiments showing that the injection of TNA-$\alpha$, IL-$\alpha$, or IL-$\alpha$-17 into pregnant rats or mice causes hypertension, proteinuria, and other features of preeclampsia.

**Complement Component C3a**

Preeclampsia is associated with increased complement activation of undetermined causality. Use of the antibody transfer model of preeclampsia in pregnant mice has shown that autoantibody-mediated C3a receptor activation contributes to the pathogenesis of preeclampsia.$^{22}$ Hypertension and proteinuria in autoantibody-injected pregnant mice is significantly reduced by a complement C3a receptor-specific antagonist to interfere with C3a receptor signaling. Additional experiments showed that complement C3a receptor antagonism significantly attenuated autoantibody-induced sFlt-1 production, placental impairment, and intrauterine growth restriction. Human studies demonstrated that C3 deposition is significantly elevated in the placentas of preeclamptic patients compared with normotensive controls, and that complement C3a receptor activation is a key mechanism underlying autoantibody-induced sFlt-1 secretion and decreased angiogenesis in cultured human villous explants. Thus, complement component C3a signaling through its receptor contributes to autoantibody-induced features of preeclampsia in pregnant mice. These studies are consistent with earlier studies showing that complement C3a functions downstream of Ang II to mediate hypertension and renal malfunction.$^{20}$

**Antiangiogenic Factors (sFlt-1 and sEng)**

A soluble form of the vascular endothelial growth factor (VEGF) receptor-1 (also called sFlt-1) is elevated in the circulation of women with preeclampsia relative to normotensive pregnant women.$^{11,12}$ As a decoy receptor, sFlt-1 blocks VEGF-mediated signaling that is important for normal endothelial function and thereby contributes to hypertension and renal dysfunction. Siddiqui et al.$^{13}$ used the autoantibody-injection model of preeclampsia in pregnant mice to assess the therapeutic potential of recombinant VEGF$_{121}$, a relatively
Mechanistically, IL-6 functions downstream of TNF-α to induce ET-1 production in pregnant mice. Thus, either TNF-α blockade or IL-6 blockade reduced antibody-induced production of ET-1 and the associated hypertension (Figure 2).

Figure 2. Pathogenic mediators of angiotensin II type I receptor (AT1) agonistic autoantibodies (AT1-AA)–induced preeclampsia in pregnant mice. Therapeutic strategies based on blocking the detrimental effects of AT1-AA–induced pathogenic mediators are illustrated. ET-1 indicates endothelin-1; IL-6, interleukin-6; IUGR, intrauterine growth restriction; sEng, soluble endoglin; sFlt-1, soluble Fms-like tyrosine kinase-1; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; and 7-aa, 7-amino acid.

AT1-AAs Contribute to the Placental Abnormalities and Fetal Growth Restriction Associated With Preeclampsia

Preeclampsia is often associated with intrauterine growth restriction. Growth-restricted fetuses have a higher incidence of mortality and morbidity than fetuses of normal growth and are at increased risk for future development of metabolic disorders, such as hypertension, coronary heart disease, dyslipidemia, obesity, impaired glucose tolerance, Type 2 diabetes mellitus, and many other diseases. AT1-AAs are present in the cord blood of women with preeclampsia and retain the ability to activate AT1Rs. Using the antibody transfer model of preeclampsia in pregnant mice Irani et al showed that AT1-AAs cross the placenta and enter the fetal circulation where they are associated with small fetuses with renal and hepatic abnormalities. AT1-AAs also induce apoptosis in the placentas of pregnant mice, human villous explants, and human trophoblast cells in culture. Finally, autoantibody-induced intrauterine growth restriction and placental apoptosis are diminished by losartan or the autoantibody-neutralizing 7-amino acid epitope peptide. These studies highlight AT1-AA as a potential contributor to preeclampsia-associated intrauterine growth restriction and offer 2 possible underlying mechanisms: (1) a direct detrimental effect on fetal development by crossing the placenta and entering the fetal circulation and (2) fetal growth restriction secondary to AT1-AA–induced placental damage.

Experimental Induction of AT1-AA in Animal Models of Preeclampsia

As reviewed above, preeclampsia in humans is characterized by the presence of autoreactive antibodies, AT1-AA, that are able to induce numerous pathological factors that contribute to clinical features of preeclampsia when injected into pregnant mice (Figure 2). Somewhat surprisingly, these antibodies...
also appear in 3 animal models of preeclampsia that are reviewed below (Figure 3).

**Transgenic Rodents Engineered to Produce Excessive Angiotensin II During the Second Half of Pregnancy**

When dams (mice or rats) harboring the human angiotensinogen gene are mated with males carrying the human renin gene, the dams develop severe hypertension, proteinuria, and target organ damage that resembles preeclampsia. The features result from human renin produced by the placenta and released into the maternal circulation where it acts on human angiotensinogen produced and released from the maternal liver leading to increased circulating levels of Ang II during the second half of pregnancy. Working with the rat model, Dechend et al demonstrated that agonistic antibodies directed at the AT1 receptor develop during the second half of pregnancy along with other features of preeclampsia. Peptide competition experiments showed that the antibodies present in these pregnant rats are directed to the same epitope as the AT1-AA present in women with preeclampsia. The presence of these autoantibodies was initially shown by their chronotropic action in the neonatal cardiomyocyte assay and subsequently by antibody transfer experiments into pregnant rats.45 The introduction of these autoantibodies into pregnant rats stimulated features of preeclampsia, including hypertension and proteinuria. In this regard, the AT1-AA produced in transgenic pregnant rats with a modified renin-angiotensin system resembles the AT1-AA produced in women with preeclampsia.

**Surgically Induced Placental Ischemia**

Granger et al developed a rat model of preeclampsia based on placental ischemia resulting from surgical reduction in uterine perfusion pressure (RUPP). Such experimentally manipulated gravid rats develop hypertension, proteinuria, and other features of preeclampsia, including ET-1.52 Remarkably, RUPP rats also develop AT1-AA that contribute to pathophysiology in this animal model of preeclampsia, including hypertension and proteinuria. In this regard, the AT1-AA produced in transgenic pregnant rats with a modified renin-angiotensin system resembles the AT1-AA produced in women with preeclampsia.

**Infusion of Inflammatory Cytokines**

The Lamarca group has also shown that infusion of inflammatory cytokines (TNF-α, IL-6, or IL-17) into pregnant rats, but not virgin female rats, results in features of preeclampsia, including the production of AT1-AA.26–28

Thus, AT1-AA have appeared in animal models of preeclampsia associated with genetically induced hypertension, surgically induced placental ischemia, and cytokine infusion. The generation of these autoantibodies may be secondary to placental ischemia, vascular damage, and the increased maternal inflammatory response that is associated with preeclampsia. Experimental induction of uteroplacental ischemia and the infusion of inflammatory cytokines seem to be promising experimental models to study the relationship between preeclampsia and autoimmunity. The animal models of experimentally induced autoantibody production also represent a well-defined and experimentally pliable system for understanding the molecular mechanisms responsible for autoimmunity (Figure 3).

**The Clinical Impact of AT1-AA Outside of Pregnancy**

The occurrence of AT1-AA is not restricted to pregnancy. These autoantibodies are prevalent among kidney transplant recipients that develop severe transplant rejection and malignant hypertension during the first week after transplantation. AT1-AA are also highly abundant among a group of patients with essential hypertension that are refractory to standard therapy. More recent studies have identified these autoantibodies in patients with systemic sclerosis. These 3 examples extend the clinical impact of AT1-AA beyond pregnancy and are reviewed.
Acute Vascular Rejection Associated With Malignant Hypertension

Dragun et al. investigated a group of 16 kidney transplant recipients characterized by acute vascular rejection and malignant hypertension occurring during the first week after kidney transplantation. Four of these individuals also had seizures. The combination of vascular pathology, hypertension, and seizures prompted these investigators to consider the presence of AT1-AA, a pathogenic autoantibody previously observed in women with preeclampsia, a condition also associated with vascular lesions, hypertension, and seizures. The cardiomyocyte contraction assay was used to show that AT1-AA were present in all 16 patients with malignant hypertension and not in a group of patients characterized with acute vascular rejection in the absence of malignant hypertension. Retrospective analysis of historic sera taken from patients before transplantation revealed the presence of AT1-AA in all 16 patients presenting with acute vascular rejection and malignant hypertension during the week after transplantation. Characterization of the AT1-AA present in these 16 individual revealed IgG1 and IgG3 autoantibodies directed at 2 distinct sites on the second extracellular loop of the AT1 receptor. One site was identical to the 7-amino acid epitope observed in women with preeclampsia. Treatment of 7 antibody-positive patients with plasmapheresis, intravenous immune globulin and 100-mg losartan per day resulted in significantly improved allograft survival compared with that of patients receiving standard antirejection treatment. Subsequent analysis of 278 kidney transplant recipients performed at their center revealed a prevalence of AT1-AA of 3.6% (ie, 10 patients). These findings are consistent with an earlier report indicating the presence of AT1-AA in some patients with malignant hypertension and more recent reports showing AT1-AA association with essential hypertension (see following section). On the basis of these studies it was suggested that individuals being considered for a kidney transplant should be tested for the presence of AT1-AA, to allow for patient-specific posttransplant medical care for those testing positive. For this purpose, a high-throughput cell-based ELISA has recently been developed.

Essential Hypertension

In general, it is possible to divide the underlying causality for hypertension into 2 broad categories: renal and vascular. The well-known renin, angiotensin, aldosterone system spans both categories and is the most common system affected in hypertension. Because alterations in the renin–angiotensin–aldosterone system are common, ACE inhibitors and angiotensin receptor blockers (ARBs) are common therapeutic approaches. For some time has been apparent that pathogenic alterations in the renin–angiotensin–aldosterone system include the presence of autoantibodies capable of activating the major angiotensin receptor, AT1R. Liao et al. observed AT1-AA (which they term anti-AT1 receptor autoantibodies) in 43% of patients with refractory hypertension. The prevalence of AT1-AA among refractory hypertension was revised upward in more recent studies when autoantibody testing relied on using higher serum concentrations. In the latter case, an overall percentage of 59% of refractory hypertensive patients harbored AT1-AA, and among these patients the prevalence of AT1-AA increased with increasing blood pressure. The relationship of AT1-AA to systolic blood pressure ranged from 52% to 69%, with increasing blood pressure. Other studies from this group showed that these autoantibodies were able to activate AT1 receptors and initiate a chain of signaling events, resulting in the proliferation of vascular smooth muscle cells and vascular remodeling.

Weil et al. conducted a clinical trial to test the possibility that AT1-AA contribute to hypertension. The authors hypothesized that if AT1-AA contribute to hypertension, then AT1-AA–positive hypertensive patients would show a better response to ARBs than ACE inhibitors compared with AT1-AA–negative hypertensive patients. In a study involving 512 patients with roughly half on candesartan (an ARB) and half on imidapril (an ACE inhibitor), the results clearly showed that the ARB-based regimen is more effective in lowering blood pressure than an ACE inhibitor-based regimen in AT1-AA–positive patients. The results of this clinical trial highlight the contribution of AT1-AAs to high blood pressure in these patients with refractory hypertension and that treatment with ARBs alleviates the hypertensive effects of these pathogenic autoantibodies more effectively than ACE inhibitors.

Systemic Sclerosis

Systemic sclerosis (SSc) is a chronic systemic autoimmune disease characterized by fibrosis affecting the hands, arms, and face. Progressive forms of the disease affect large areas of the skin and one or more internal organs (kidneys, esophagus, heart, or lungs). Death occurs most often from pulmonary, cardiac, or renal complications, secondary to hypertension. ACE inhibitors, ET-1 receptor blockers, and ARBs reduce hypertension and alleviate some of the cardiac, renal, and pulmonary manifestations of SSc. On the basis of these clinical features, Riemekasten et al. were prompted to consider the possibility that receptor-activating autoantibodies were involved. To test this possibility, they developed a cell-based ELISA to detect antibodies to the AT1R and the ET-1 receptor, ETAR. Their results show that most patients with SSc possess antibodies to each of these receptors. Antibodies to each receptor are biologically active and induce receptor-directed ERK1/2 phosphorylation and increased TGF-β gene expression in human microvascular endothelial cells. Ang II and ET-1 induce collagen synthesis via target receptor stimulation in fibroblasts, features that could be attributed to the AT1R and ETAR receptor-activating autoantibodies in SSc. The biological effects of both autoantibodies were blocked by the respective receptor antagonists, providing additional evidence for antibody-mediated receptor activation. Higher antibody titers were associated with late complications, such as pulmonary hypertension, lung fibrosis, and digital ulcers. Thus, according to these authors, AT1R and ETAR AA may contribute to disease pathogenesis in SSc and link disease features, including autoimmunity, vascular pathology, and hypertension.

Mechanism of Autoantibody-Mediated AT1R Activation

A large body of biochemical evidence published in recent years indicates that G-protein–coupled receptors form homodimers, heterodimers, and possibly higher order oligomeric
structures.\textsuperscript{59–71} Agonist-induced dimerization has been shown for a number of G-protein–coupled receptors and in some cases is required for efficient signaling. Agonist-induced activation of the AT\textsubscript{1} receptor is accompanied by dimerization.\textsuperscript{72,73} Furthermore, the formation of stable covalently cross-linked AT\textsubscript{1} receptors is associated with enhanced signaling and prolonged activation.\textsuperscript{74} Because receptor-activating antibodies are bivalent, it is possible that they exert their agonistic effect by cross-linking and thereby stabilizing receptor homodimers. Thus, we hypothesize that autoantibody-induced AT\textsubscript{1} receptor activation is accompanied by receptor dimerization. Evidence in support of this view comes from studies of other receptor-activating autoantibodies showing that bivalency is required for antibody-induced activation of the \( \beta\text{,} \) adrenergic receptor\textsuperscript{75,76} and the M2 muscarinic receptor.\textsuperscript{77} In each of these cases, the original bivalent IgG autoantibody activated the receptor, the monovalent Fab fragments did not activate, whereas Fab fragments cross-linked by anti-Fab fragment antibody had restored receptor-activating ability. Another interesting feature of the AA to the \( \beta\text{,} \) adrenergic and M2 muscarinic receptors is their ability to promote a sustained receptor activation for many hours without the customary desensitization observed with natural ligands.\textsuperscript{75,77–80} These features have not yet been examined for AT\textsubscript{1}-AA. A thorough knowledge of the mechanism of antibody-induced receptor activation may provide further useful insights for the development of therapeutic strategies to block the action of the antibodies, thereby reducing the detrimental effects of excessive receptor activation.

Factors Contributing to Autoantibody Production

The immunologic basis for the loss of self-tolerance that allows antibodies to develop against a particular 7-amino acid epitope on the second extracellular loop of the AT\textsubscript{1}R is not understood. Molecular mimicry with a homologous sequence on human parvovirus has been considered for the origin of AT\textsubscript{1}-AA, but this view has not withstood the results of epidemiological data that failed to show a correlation between preeclampsia and prior parvovirus infection.\textsuperscript{15,16} Thus, if molecular mimicry does not account for the production of AT\textsubscript{1}-AA, then it is necessary to have loss of self-tolerance by the humoral (antibody-mediated) and cellular (T-cell mediated) arms of the immune system. A common mechanism for loss of self-tolerance is posttranslational modification, resulting in the creation of a neoantigen that is not recognized as self by the immune system. It is estimated that 50% to 90% of the proteins in the human body are posttranslationally modified.\textsuperscript{85–84} In the proper context, these modifications are necessary for the biological functions of a vast array of proteins and the effector function of the cells in which they reside. However, it is now clear that some posttranslational modifications can create new self-antigens (neoantigens) and, therefore, trigger specific antibody production under autoimmune conditions. It is possible that placental ischemia and the resulting tissue damage, inflammation, and syncytiotrophoblast shedding may create a favorable setting for autoimmunity. The fact that the placenta has the highest tissue level of AT\textsubscript{1}R may also contribute to enhanced immunogenicity in a setting of placental ischemia and oxidative stress. It is well known that a chronic inflammatory response activates the adaptive arm of the immune system and may create an environment that is permissive for autoimmunity. A role for helper T cells in this process should not be overlooked. Harrison et al\textsuperscript{85} have proposed a proinflammatory immune response on the basis of the production of neoantigens as a result of protein oxidation, nitrosylation, fragmentation, or posttranslational modification. These neoantigens are no longer recognized as self and are processed by antigen-presenting cells, such as dendritic cells, where peptide fragments are presented to T helper cells via major histocompatibility complex (MHC) class II molecules. T cells activated in this way are equipped to provide immunologic assistance to B cells producing AT\textsubscript{1}-AA. As discussed above, rodent models of preeclampsia that are based on placental ischemia (ie, the RUPP model)\textsuperscript{85–88} and a heightened inflammatory response (ie, infusion of proinflammatory cytokines, such as TNF-\( \alpha \), IL-6, and IL-17)\textsuperscript{26–28} are characterized by the presence of AT\textsubscript{1}-AA. These same conditions are associated with preeclampsia in humans (ie, placental ischemia and a heightened immunologic state) and are likely to contribute to AT\textsubscript{1}-AA production. Pregnant animal models in which surgical manipulation (ie, RUPP) or cytokine infusion (TNF-\( \alpha \), IL-6, and IL-17) result in production of AT\textsubscript{1}-AA represent ideal experimental systems to identify the underlying cause of autoantibody production.

Concluding Remarks

As reviewed here, a growing body of evidence indicates that (1) autoantibodies capable of activating AT\textsubscript{1}Rs exist in the circulation of patients with hypertensive disorders, (2) these autoantibodies contribute to disease pathophysiology, (3) antibody titers correlate to the severity of the disease, and (4) efforts to block or remove these pathogenic autoantibodies have therapeutic benefit. The production of these pathogenic autoantibodies most likely precedes the onset of clinical symptoms, a possibility that highlights the autoantibodies as potentially valuable presymptomatic biomarkers. This view was validated by retrospective analysis of serum samples obtained from renal dialysis patients before kidney transplantation.\textsuperscript{10} These studies indicated that knowledge of AT\textsubscript{1}-AA before kidney transplantation would influence patient-specific medical care at the time of transplantation. In view of the considerable evidence indicating that AT\textsubscript{1}-AAs contribute to disease, they are also likely to be important therapeutic targets in the management of hypertensive disease. In the coming years, we can expect to see continued development of improved immunologic and functional tests to detect and quantify these pathogenic autoantibodies. Preliminary evidence shows that the removal of these pathogenic antibodies by plasmapheresis or immunoadsorption along with receptor antagonism provides therapeutic benefit.\textsuperscript{10} It is encouraging to see initial promising results from these approaches for patients with antibody-mediated malignant hypertension and graft rejection. The results of clinical trials with patients with refractory hypertension show that AT\textsubscript{1}-AA–positive patients respond better to ARBs than ACE inhibitors,\textsuperscript{12} again illustrating the benefits of patient-specific medical treatment on the basis of identifying the specific underlying cause of the hypertension.

Research reviewed here raises the intriguing possibility that preeclampsia and other hypertensive conditions are
autoimmune diseases characterized by the presence of pathogenic autoantibodies that activate the major angiotensin receptor, AT R. These pathogenic autoantibodies could serve as presymptomatic biomarkers and therapeutic targets, thereby providing improved medical management for these conditions. For most autoimmune diseases, it has been possible to identify distinct polymorphic alleles of the MHC genes that are highly associated with the autoimmune condition. The tight linkage of specific MHC polymorphic genes and a particular autoimmune condition is presumed to result from the preferential ability of certain MHC class II complexes to present the specific antigenic peptide to the T-cell receptor complex. Conversely, for related reasons, certain MHC class II genes are highly excluded in certain autoimmune diseases. Thus, if preeclampsia and other hypertensive conditions have a significant autoimmune component it may be possible to identify distinct MHC polymorphisms that serve as genetic markers to identify individuals at increased risk for hypertension.

Disclosures

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