

Activating autoantibodies against the AT1-receptor in vascular disease

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Activating autoantibodies to the angiotensin II type I receptor (AT1-AA) have been described in different vascular diseases: preeclampsia, non HLA-mediated transplant rejection and systemic sclerosis. All three diseases are characterised by severe obliterative, inflammatory vasculopathy with a prominent autoimmune background. AT1-AA were described 12 years ago in preeclampsia. Preeclampsia is a common, pregnancy-induced disorder, consisting of hypertension and proteinuria in the last trimester. The condition is one of the leading causes for maternal and perinatal morbidity and mortality. An observation of a patient with transplant rejection without HLA antibodies and a preeclampsia in her medical history, led to the discovery of AT1-AA in these patients. Until then we relied on a subjective, time consuming bioassay to detect AT1-AA. The establishment and validation of a cell-based ELISA facilitated research on AT1-AA immensely. Recently, AT1-AA were found in patients with systemic sclerosis.

Key words: AT1-receptor, AT1-AA, preeclampsia, transplant rejection, systemic sclerosis

Aktivierende Autoantikörper gegen den AT1-Rezeptor bei Gefäßerkrankungen

Aktivierende Autoantikörper gegen den Angiotensin-II-Typ-1-Rezeptor (AT1-AA) wurden bei verschiedenen Gefäßerkrankungen beschrieben: Präeklampsie, nicht-HLA-vermittelte Transplantatabstoßung und systemische Sklerose. Alle drei Erkrankungen sind durch eine schwere obliterative, entzündliche Vasculopathie mit deutlichem autoimmunem Hintergrund charakterisiert. Die AT1-AA wurden vor 12 Jahren bei Präeklampsie beschrieben. Bei der Präeklampsie handelt es sich um eine häufige, durch eine Schwangerschaft induzierte Störung bestehend aus Hypertonie und Proteinurie im letzten Drittel der Schwangerschaft. Dieses Krankheitsbild stellt eine der wichtigsten Ursachen für Morbidität und Mortalität von Mutter und Kind dar. Die Beobachtung einer Patientin mit Transplantatabstoßung ohne HLA-Antikörper und mit einer Präeklampsie in der Anamnese führte zur Entdeckung von AT1-AA in diesen Patienten. Bis zu diesem Zeitpunkt musste man einen subjektiven, zeitaufwendigen Bioassay durchführen, um AT1-AA zu entdecken. Mit der Einführung und Validierung eines zellbasierten ELISA wurden die Forschungen zu AT1-AA wesentlich erleichtert. Vor kurzem konnten AT1-AA bei Patienten mit systemischer Sklerose nachgewiesen werden.

Schlüsselwörter: AT1-Rezeptor, AT1-Autoantikörper, Präeklampsie, Transplantatabstoßung, systemische Sklerose

1. AT1-AA in preeclampsia

1.1 Molecular mechanism in preeclampsia

Activating autoantibodies against the AT1-receptor (AT1-AA) were first described in preeclampsia (1). Preeclampsia is a major complication of pregnancy characterized by hypertension and proteinuria developing in the second half of the pregnancy, affecting 3-10% of all pregnancies (2). Preeclampsia is a major

healthcare problem worldwide and represents a major cause of maternal and neonatal morbidity and mortality (2).

It is defined as an increase in blood pressure appearing after 20 weeks gestation (>140/90 mm Hg at two readings, 6 h apart) accompanied by newly developed proteinuria (>300 mg/l) in a previously normotensive woman (3). Moreover, evidence has been presented that mothers and children who have undergone a

preeclamptic pregnancy suffer from an increased long-term cardiovascular risk (4).

The pathogenesis is unknown but is likely to be multifactorial. Thus, preeclampsia has been called the "disease of theories" (5). Preeclampsia can be categorized as maternal and placental, although many cases are a mix of the two (6). In the maternal type, the clinical symptoms develop due to abnormal maternal response to pregnancy (such as excess inflammation in women with preexisting obesity or diabetes), but with a normal placenta. In placental preeclampsia a poor placentation process with a dysfunctional placenta causes the maternal symptoms (5).

A 3-stage model of preeclampsia is suggested. (6). Dysregulated immunological factors (stage 1) underlying a defect placentation with reduced invasion of fetal extravillous trophoblast cells and reduced remodelling of maternal uteroplacental spiral arteries (stage 2) are initial pathophysiological events. Preeclampsia is related to a failure of the extravillous trophoblasts to invade and remodel the maternal decidual (endometrium of pregnancy) spiral arteries. An unfavorable uteroplacental circulation ensues, with enhanced oxidative and generation of one or several circulating factors. The excess concentration of the circulating factor causes an excessive maternal inflammatory response the endothelial cell dysfunction (leading to the clinical signs of hypertension and proteinuria stage 3) (6).

Multiple circulating factors have been postulated to cause the maternal syndrome, including trophoblast fragments, a soluble VEGF receptor termed soluble fms-like tyrosine kinase 1 (sFlt1) (7), or soluble endoglin (sEng) (8) and AT1-AA (1).

Some investigators view the fetus as an allograft. Interaction between decidual leukocytes and invading cytotrophoblast cells is essential for normal trophoblast invasion and development (9). Immune maladaptation may cause shallow invasion of spiral arteries by endovascular cytotrophoblast cells. According to this hypothesis immune maladaptation may, via an inappropriate decidual release of Th1 cytokines, proteolytic enzymes, and free radical species, cause both shallow invasion of spiral arteries by endovascular cytotrophoblast cells and systemic endothelial cell dysfunction (10). Several explanations for maternal immune disturbances have been proposed, one of which invokes factors of paternal origin. Various autoantibodies have been described in preeclampsia. The induction of antibodies by pregnancy indicates that the maternal immune system is confronted with paternal HLA-A and HLA-B antigens of the child (11). Possibly, the tolerance originally induced by soluble HLA-A and HLA-B antigens or translated sperm mRNA encoding for paternal HLA spreads to

epitopes of non-classical HLA antigens expressed on the trophoblast (6).

1.2 Characterization of AT1-AA

The observations of AT1-AA stem from an observation in a single patient (1). However, that observation occurred not solely by chance, but coupled the known knowledge of autoimmunity and tolerance in pregnancy, the observed changes in the renin-angiotensin system during pregnancy, and the remarkable alterations observed in these regulatory systems in preeclamptic, compared to normal pregnant women. Serum from preeclamptic women contains an IgG autoantibody that reacts with the AT1 receptor (AT1-AA) in a stimulatory fashion. For a long period we relied on a bioassay for AT1-AA consisting of spontaneously beating neonatal rat cardiomyocytes (1). AT1-AA increases the spontaneous beating rate of neonatal rat cardiomyocytes. With the help of the bioassay, the exact binding site of AT1-AA to the AT1-receptor have been identified (the peptide AFHYESQ), corresponding to the second extracellular loop of the AT1 receptor. Subsequently the binding could be confirmed by confocal-microscopy and co-immunoprecipitation (12). Thus the binding of AT1-AA to the receptor were shown functionally and by biochemical methods. Meanwhile several groups have confirmed the presence of AT-AA in preeclamptic patients. Recently, Siddiqui et al showed that the titer of AT1-AA correlates with the severity of the disease and that there was a strong correlation between AT1-AA activity and sFlt-1 in severe preeclamptic patients (13). They used a different bioassay in their studies. They constructed a CHO cell line, which was stably transfected with the AT1-receptor and a 4xNFAT-driven luciferase construct (13).

1.3 Signal transduction of AT1-AA

Several groups, including our own, demonstrated signaling events of AT1-AA in-vitro, showing that several features of preeclampsia could be explained by the ability of AT1-AA to activate vascular cells and trophoblasts. AT1-AA induce intracellular ROS through activation of the NADPH oxidase (14). Important downstream signal transduction targets included by AT1-AA are Protein kinase C- α (PKC α), extracellular-related kinase (ERK) 1/2 and the transcription factors NF- κ B and AP-1 (12). Xia et al. also showed that AT1-AA activate the calcineurin-nuclear factor of activated T-cells, a NF-AT-dependent pathway (15). They followed this report with another study showing that the antibodies can induce calcium signaling, and stim-

ulate mesangial cells to produce interleukin-6 and PAI-1 (16).

1.4 AT1-AA in animal models

In an artificial rat model of preeclampsia, the group of Lamarca and Granger were the first to show that placental ischemia is an important stimulus for AT1-AA production (17). They developed an animal model where uterine perfusion pressure was reduced surgically (RUPP model). The RUPP model shows an exaggerated inflammatory reaction with elevations of TNF- α in the circulation and in the uteroplacental unit (17). AT1-AA are present in the RUPP model, by contrast, AT1-AA were not detected in normal pregnant rats. Moreover, they have shown that chronic infusion of TNF- α in pregnant rats induced AT1-AA. B-lymphocyte cell depletion by chronic administration of CD20 blockade reduced the hypertension and suppressed AT1-AA production in the RUPP model secondary to placental ischemia (18). These results would suggest that immune mechanisms, stimulated in response to placental ischemia may play an important role in AT1-AA production. We could confirm these results in a transgenic rat model for preeclampsia, where uteroplacental ischemia, exaggerated inflammation, disturbed vascular remodeling and AT1-AA are present (19). Walther et al followed up on these studies and could show that AT1-AA are not specific for preeclampsia (20). They found AT1-AA in pregnant patients with an abnormal uterine artery Doppler flow and increased resistance index. A pathological Doppler finding indicates impaired placentation in the context of uteroplacental ischemia. In such a patient cohort, AT1-AA were found in patients who had preeclampsia, but also in normotensive women with intrauterine growth restricted fetuses and in pregnant women with uncomplicated pregnancies (20). Analogous to the animal data, these results also suggest that AT1-AA are secondary to uteroplacental hypoxia and/or ischemia.

1.5 AT1-AA and anti-angiogenesis

The group of Kellems and Xia provided evidence that converge the antiangiogenic potential in preeclampsia with the AT1-AA findings (21). They found that IgG from women with preeclampsia stimulates the synthesis and secretion of sFlt-1 via AT1 receptor activation in human placental villous explants and human trophoblast cells. The autoantibody-induced sFlt-1 secretion resulted in inhibition of endothelial cell migration and capillary tube formation in vitro. In a follow up paper, the same group could show that the AT1-AA-me-

diated decreased angiogenesis in human placenta villous explants was attenuated by TNF- α -neutralizing antibodies and hemin, which ameliorated soluble Endoglin (sEng) and sFlt-1 induction (22). These data show that heme oxygenase-1 is a key underlying mechanism in AT1-AA-mediated TNF- α induction, which is upstream of sEng and sFlt-1 secretion. To follow-up the functional interaction between AT1-AA and sFlt-1, the group investigated the effect of recombinant VEGF121 on AT1-AA induced features of preeclampsia (23). Continuous infusion of VEGF121 in pregnant mice reduced AT1-AA-induced hypertension, proteinuria and placental impairment. A different interaction between two vasoactive systems has been reported by Lamarca et al. (24). She could show that the effects of AT1-AA are endothelin-1-dependent. AT1-AA induced endothelin-1 in renal cortices and in the placenta in pregnant rats. Co-treatment with an Endothelin-A receptor antagonist reduced AT1-AA induced hypertension (24).

1.6 Passive transfer of AT1-AA

Following Witebsky's postulates, indirect evidence for an autoimmune etiology of a disease requires a corresponding selfantigen and an analogous immune response, induced in an experimental animal, which will develop a similar disease (25). Direct evidence, however, requires induction of the disease by transfer of homologous pathogenic antibodies. These passive transfer experiments by Zhou et al can be judged as "proof-of-principle". Their results indicate that preeclampsia may be a pregnancy-induced autoimmune disease in which key features of the disease result from autoantibody-induced angiotensin receptor activation (26). They isolated AT1-AA from patients directed against the second extracellular loop. Injection with either total IgG or affinity-purified AT1-AA from women with preeclampsia in pregnant mice induced those key features of preeclampsia, including hypertension, proteinuria and glomerular endotheliosis. These features were prevented by co-injection with an AT1 receptor antagonist or by an antibody neutralizing seven-amino-acid epitope peptide. Using the same autoantibody-induced animal model of preeclampsia, Irani et al showed that AT1-As cross the mouse placenta, enter fetal circulation, and lead to small fetuses with organ growth retardation (27). Earlier we had shown that AT1-AA are present in the newborn from preeclamptic mothers (28).

Most recently Lamarca et al confirmed that AT1-AA infusion increased blood pressure in pregnant rats (24). We used a different approach and generated and purified activating antibodies against the AT1-receptor (AT1-AB)

by immunizing rabbits against the AFHYESQ epitope of the second extracellular loop, which is the binding epitope of endogenous AT1-AA from patients with preeclampsia (29). We were able to detect AT1-AB both by ELISA and a functional bioassay. AT1-AB activated PKC α and pERK 1/2. We then passively transferred AT1-AB into pregnant rats, alone or combined with Angiotensin II (Ang II). Passive transfer of AT1-AB alone or Ang II in high dosages infused alone did not induce a preeclampsia-like syndrome in pregnant rats. However, the combination (AT1-AB plus Ang II) induced hypertension, proteinuria, intrauterine growth retardation, and arteriosclerosis in the uteroplacental unit. We next performed gene-array profiling of the uteroplacental unit and found that hypoxia-inducible factor 1 α (HIF-1 α) was up-regulated by Ang II plus AT1-AB, which we then confirmed by Western blotting in villous explants. Furthermore, endothelin-1 was upregulated in endothelial cells by Ang II plus AT1-AB. We show that AT1-AB induces Ang II sensitivity. This mechanistic study supports the existence of an "autoimmune-activating receptor" that could contribute to Ang II sensitivity and possibly to preeclampsia. A dysregulated renin-angiotensin system in preeclampsia has been reported since 30 years, however Ang II levels are not increased in preeclampsia (30). However, pregnant women who subsequently develop preeclampsia are highly sensitive to infused Ang II, whereas pregnant women without preeclampsia are resistant (31). The increased Ang II sensitivity in preeclamptic patients persists postpartum and may be one reason for the augmented cardiovascular risk later in life (32).

2. AT1-AA in transplantation medicine

2.1 Clinical importance of AT1-AA

Analogous to the preeclampsia study, the work on kidney rejection stems from a serendipitous observation in a single patient who received a zero HLA-A, -B, -DR-mismatched allograft and developed accelerated vascular rejection with an obliterative and inflammatory vasculopathy, refractory to steroids and antilymphocyte antibody preparation (33). This patient suffered from severe vascular pathologic condition including hypertensive crisis accompanied by seizures. She also had a history of preeclampsia. Consequently Dragun et al. studied 33 kidney-transplant recipients who had refractory vascular rejection. 16 patients of this group had AT1-AA (33). These 16 patients suffered from malignant hypertension, and no anti-HLA antibodies could be detected. These AT1-AAs could be classified as IgG1 and IgG3 subclass

antibodies that bind to two different epitopes on the second extracellular loop of the AT1-receptor. One binding epitope is identical to the one identified in preeclampsia. Renal biopsies showed obliterative vasculopathy and endarteritis with fibrinoid changes and necrosis. Graft survival of the patient cohort which was AT1-AA +/HLA- was poorer compared to AT1-AA-/HLA+, indicating the functional relevance of the AT1-AA. Although resistant to steroids and antilymphocyte antibody preparation, treatment with an AT1-Receptorblocker and plasmapheresis prolonged graft survival substantially. The signal transduction induced by these AT1-AA was similar to the one observed earlier in preeclampsia: they induced phosphorylation of ERK 1/2 kinase and increased the DNA-binding activity of the transcription factors AP-1 and NF-kB. Passive transfer of isolated AT1-AA into a rat transplantation model induced hypertension and evidence of endarteritis and intravascular inflammatory cells in kidney vessels.

2.2 A cell based ELISA for the detection of AT1-AA

In spite of the known problems with this technique, including subjective aspects, expense, and time expenditure, the bioassay has remained the method of choice to detect AT1-AA. Analogous to other autoantibodies, it was difficult to develop an ELISA based assay. Finally, the biotechnology company Celltrend introduced a cell-based ELISA to detect AT1-AA. Recently the first study was published, where the Celltrend-ELISA was used. Reinsmoen et al found a strong association of AT1-AA and AMR in antibody-mediated rejection (34). The aim of this study was to evaluate in patients with transplant rejection, whose sera had no donor human leukocyte antigen (HLA)-specific antibody. Pretransplant sera and sera obtained at the time of acute rejection were tested from 97 recipients. High-binding AT1-AA were found only in antibody-mediated rejection and not in the cell-mediated rejection. High levels of AT1-AA were found in 33% (32/97) of pretransplant sera; however, there was no association of AT1-AA levels with different causes of end-stage renal disease. The conclusion of this study is that measuring AT1-AA status together with the donor human leukocyte antigen (HLA)-specific antibodies is important and provides additional information to determine the immunologic risk for recipients. The antibody status of recipients to HLA and AT1-AA may be valuable since it will help to understand the pathologic process involved to determine the immunologic risk for recipients and to aid treatment options of presumed rejection. To understand the importance of AT1-AA in antibody-mediat-

ed rejection, one retrospective cohort of 279 patients, risk stratified by complement-dependent cytotoxicity crossmatches, and a prospective cohort of 154 patients were investigated in a follow up study (35). The incidence of early antibody-mediated rejection because of non-donor-specific human leukocyte antigens, such as AT1-AA was 2% and stable in both cohorts. Earlier, Scornik et al. reported that post-transplant antibodies to AT1-AA did not correlate to C4d-positive rejection (36). While the influence of immunosuppression on AT-AA is not investigated yet, this study suggests that testing for AT1-AA may be more relevant in the subset of patients with malignant hypertension.

3. AT1-AA in systemic sclerosis

3.1 Prognostic relevance of AT1-AA

Another large study, where the Celltrend cell-based ELISA was used to detect AT1-AA, has been reported by Riemekasten et al. (37). She investigated a cohort of systemic sclerosis. A triad of vasculopathy, autoimmunity and fibrosis is a characteristic feature of systemic sclerosis. Thus systemic sclerosis represents another disease with the hallmark of severe obliterative, inflammatory vasculopathy with a prominent autoimmune background, similar to with allograft rejection and preeclampsia. Higher levels of AT1-AA were associated with more severe disease manifestations and predicted systemic sclerosis-related mortality. The authors conclude that AT1-AA may be involved in the disease pathogenesis and that AT-1AA maybe the first suitable biomarkers for risk assessment of disease progression in systemic sclerosis. (This part is reviewed in a separate book chapter by Riemekasten et al.)

3.2 Future aspects of AT1-AA

The existence of AT1-AA is exciting, but progress in the pathophysiological understanding has hampered until recently by the fact that detection still relied on a difficult and cumbersome bioassay. However, recent immunoassay attempts are successful for the AT1-AA in the transplant rejection and systemic sclerosis AT1-AA, which have two binding sites on the 2nd extracellular loop on the AT1 receptor. Attempts to establish an ELISA for preeclampsia have been difficult, but appear to be promising (CellTrend, Luckenwalde, personal communication). Thus, confirmatory studies in large populations are ongoing. So far it is unclear, how AT1-AA develop. The specific stimulus for the production of the AT1-AA remains unknown. Until now the AT1-AAs

known are generally of the IgG class requiring T-cell help. T-cell self-tolerance may be broken by an infectious inflammatory trigger, generating different cryptic T-cell epitopes (38). AT1-AA may be the result of molecular mimicry or crossreactivity with microbial antigens. This is the case in myasthenia gravis and Chagas' disease, where autoantibodies directed against G-protein receptors are involved in the disease process (39). This molecular mimicry could trigger activation of autoreactive and memory T cells. Protein alignment suggests that the binding site for AT1-AAs is highly homologous to the capsid protein VP2 of parvovirus B19 (40). We detected significantly more AT1-AA in women with an immune response corresponding with parvovirus B19 infection corresponding with a distant viral infection associated with virus elimination. A human monoclonal immunoglobulin G antibody against parvovirus B19 VP2-protein showed a positive reaction in the AT1-AA bioassay, which could be blocked by an AT1 receptor blocker. Currently, no specific treatment for these vascular diseases (preeclampsia, systemic sclerosis, non HLA-based transplant rejection) is available. If AT1-AAs play a causal role, blocking or removal of the autoantibodies may be beneficial. AT1-Receptor blockers may not be sufficient to completely block AT1-AA binding to the AT1-receptor, in pregnancy they are contraindicated. Removal of the AT1-AA by immunoadsorption or blocking with the specific 7-amino acid epitope peptide, which confers binding to the AT1 receptor, may be valuable therapeutic options in the near future.

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