

# The role of angiotensin II type 1 receptor-activating antibodies in renal allograft vascular rejection

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**Abstract** Acute rejection with vascular involvement remains a challenging problem in renal allotransplantation. Fibrinoid necrosis of the arteries with secondary thrombotic occlusions is C4d negative in 50% of cases and has the worst prognosis among all allograft vascular lesions. Nonhuman leukocyte antigen (HLA) non-complement-fixing antibodies reacting to artery-specific antigens have been speculated to be responsible for causing severe vascular injury. We recently reported the presence of agonistic antibodies against the angiotensin II type 1 receptor (AT<sub>1</sub>R-AA) in 16 recipients of renal allografts who had severe vascular rejection and malignant hypertension but who did not have anti-HLA antibodies. AT<sub>1</sub>R-AA stimulate AT<sub>1</sub>R and induce mediators of inflammation and thrombosis. Removal of AT<sub>1</sub>R-AA by plasmapheresis in combination with pharmacologic AT<sub>1</sub>R blockade leads to improved renal function and graft survival in AT<sub>1</sub>R-AA-positive patients. We have shown that the analysis of the subtle diagnostic and mechanistic differences may help to identify patients at particular risk and improve outcome of rejections with vascular pathology.

**Keywords** Vascular rejection · Non-HLA antibodies · Alloantibodies · Angiotensin II · AT<sub>1</sub> receptor · Kidney transplantation

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## Introduction

Improvements in immunosuppressive modalities that were designed to target T-cell-mediated immune response and prevent destruction of tubular epithelia have resulted in improved overall allograft survival [1]. On the other hand, a rejection with vascular involvement remains a diagnostic and therapeutic challenge. When acute vascular rejection occurs in organ transplants, the process resists conventional treatment approaches and frequently leads to allograft loss [2]. The morphologic features include a wide variety of vascular lesions in the allograft, including thrombosis, fibrinoid necrosis of the arteries, and endarteritis [3, 4]. The association of antidonor humoral reactivity against human leukocyte antigens (HLA) and vascular rejection has been documented in clinical studies [5]. Donor-specific anti-HLA alloantibodies initiate rejection through complement-mediated and antibody-dependent cell-mediated cytotoxicity [6]. The diffuse staining of the complement degradation product C4d affecting the surface of peritubular capillaries is generally regarded as a marker for HLA antibody-mediated alloresponse and is associated with inferior graft survival [7]. Nevertheless, 40–50% of rejections with severe vascular changes such as fibrinoid necrosis are C4d negative, implicating involvement of non-HLA and/or non-complement-fixing antibodies [8]. Characterization of non-HLA antibodies remains very poor; many appear to be autoantibodies. Most of the efforts in the past were focused on antiendothelial antibodies (AECA) [9]. However, the existence of a common polymorphic non-HLA antigen system in endothelial cells could not be confirmed by biochemical identification of the relevant antigens [10]. Obviously, clinical, etiological, and histopathological heterogeneity of vascular allograft lesions emphasizes the need to better recognize the subtle differ-

ences between affected patients and to implement individualized treatment strategies.

### Clinicopathological features of AT<sub>1</sub>R-AA-positive vascular rejections

We reported the presence of agonistic antibodies against the angiotensin II type 1 receptor (AT<sub>1</sub>R-AA) in 16 recipients of renal allografts who had severe vascular rejection and malignant hypertension but who did not have donor-specific anti-HLA antibodies [11]. AT<sub>1</sub>R-AA have also been associated with preeclampsia and malignant hypertension [12, 13]. Pregnancies complicated by preeclampsia and graft rejection bear some immunologic similarities [14]. The decision to seek and isolate AT<sub>1</sub>R-AA was instigated by the observation that the first patient we studied developed vascular rejection with transmural arteritis refractory to steroids and antilymphocyte antibody preparations and later fibrinoid necrosis with allograft infarction in a “zero-mismatch” kidney. As this patient developed malignant hypertension with seizures during the rejection process, the clinical picture was so reminiscent of eclamptic crisis in pregnancy, a condition that she had developed two decades before transplantation, that we started to prospectively look for patients with similar clinical features. We detected a further 15 patients primarily based on severe vascular pathology, absence of donor-specific antibodies, hypertensive crisis, and lack of response to steroids or antilymphocyte preparations. Apart from arterial changes, we also noticed tubulitis and interstitial infiltrates characteristic for acute cellular rejection. AT<sub>1</sub>R-AA rejections had significantly shorter allograft survival and more severe histology irrespective of the treatment compared with donor-specific anti-HLA-antibody-associated rejections [11]. Clinicopathological features of AT<sub>1</sub>R-AA-related process in patients were noticed between days 5 and 14 posttransplant, with minor interindividual differences concerning temporal occurrence of maximal allograft injury and increase in blood pressure. Most patients (13/16) did not have hypertension before vascular rejection occurred, implying that the posttransplantation hypertension was most likely secondary to rejection.

Causes of end-stage renal disease (ESRD) were not primarily attributed to hypertensive nephrosclerosis but, instead, to a variety of tubulointerstitial or glomerular diseases. The frequency of AT<sub>1</sub>R-AA-positive rejections was equal between female and male renal transplant recipients. AT<sub>1</sub>R-AA -related vascular rejections occurred during the first week after transplantation. As retrospective analysis of historic sera from our patients obtained before transplantation showed positivity for AT<sub>1</sub>R-AA, preformed and not de-novo-produced AT<sub>1</sub>R-AA were likely responsi-

ble for vascular rejection. The crude prevalence of the rejection episodes associated with AT<sub>1</sub>R-AA during 4 years of observation in our center was 3.6% (ten among 278 kidney transplantations performed).

In our initial study, seven of 16 patients with AT<sub>1</sub>R-AA were treated with a combination of plasmapheresis, intravenous immune globulin infusions, and the AT<sub>1</sub>R-blocker losartan. This combination treatment led to improved renal function and graft survival compared with the outcomes amongst patients with AT<sub>1</sub>R-AA who received standard treatment for humoral rejection [11]. Transplant nephrologists are perhaps the only remaining clinicians skeptical about the use of anti renin-angiotensin-system drugs. However, we believe our findings, even with their limitations, are highly suggestive. The small number of patients in our initial study may limit the degree to which the results can be generalized. However, the finding that patients with preformed AT<sub>1</sub>R-AA develop severe vascular rejection that can be treated with specific therapy stands as a novel observation.

### Effectors of AT<sub>1</sub>R-AA-mediated vascular rejection

AT<sub>1</sub>R-AA seem to act via mechanisms distinct from those in patients with HLA antibodies, as C4d was detected in biopsy specimens from only five of our 16 patients. We raised and confirmed the hypothesis that AT<sub>1</sub>R-AA may act in similar manner as a natural agonist for the AT<sub>1</sub>R angiotensin II and exert direct effects on endothelial and vascular smooth-muscle cells via induction of Erk 1/2 signal transduction cascade. AT<sub>1</sub>R-AA incubation of nuclear extracts of vascular smooth-muscle cells activated transcription factor activator protein 1 (AP-1) downstream from Erk 1/2. AT<sub>1</sub>R-AA also increased DNA binding activity of nuclear factor- $\kappa$ B (NF- $\kappa$ B) transcription factor and increased expression of NF- $\kappa$ B proinflammatory target genes, such are chemokines MCP-1 and RANTES. Although we have documented that AT<sub>1</sub>R-AA belong to complement-fixing IgG1 and IgG3 antibodies, our findings suggest that genes regulated by AT<sub>1</sub>R-triggered transcription factors and not complement-directed cytotoxicity act as an effector pathway of the vascular injury. The illustrative example is that AT<sub>1</sub>R-AA enhanced promoter activity of tissue factor, an initiator of extrinsic coagulation pathway and a target gene for AP-1 and NF- $\kappa$ B in vitro. Tissue factor mediates clotting abnormalities associated with hyperacute and xenograft rejection, as well as in antiphospholipid antibody syndrome [15]. Accordingly, renal transplant biopsy specimens obtained during an AT<sub>1</sub>R-AA-mediated rejection episode revealed intense diffuse Tissue Factor staining of epithelial, endothelial, and mesangial cells in absence of complement activation.

Binding of AT<sub>1</sub>R-AA to AT<sub>1</sub>R expressed on target cells is a critical step for activating the downstream cascade and inducing damage to the allograft. However, we have not yet proven whether AT<sub>1</sub>R-AA function only through procoagulatory and chemotactic activity or whether they also act by means of innate and specific immune responses and increased vascular reactivity. Our current working hypothesis is that factors surrounding the organ transplantation process may lead to increased expression of AT<sub>1</sub>R and thereby affect the overall reactivity of the vascular cells to AT<sub>1</sub>R-AA (Fig. 1). Passive transfer of human IgG containing AT<sub>1</sub>R-AA induced a transmural arteritis similar to human situations and led to increased blood pressure in otherwise nonrejecting and normotensive transplanted animals. These findings provided further evidence that AT<sub>1</sub>R-AA may have a causative role. Similar to stimulation of the AT<sub>1</sub>R by its natural ligand angiotensin II [16], agonistic receptor activation mediated by AT<sub>1</sub>R-AA could play a key role in the initiation and amplification of pathobiological events that lead to transplant vasculitis and hypertension.

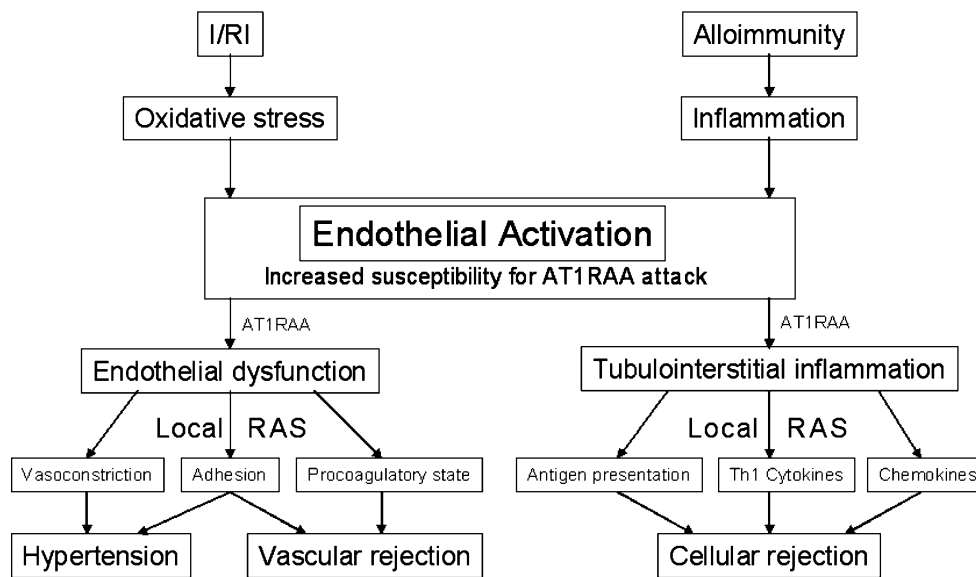
**Perspectives for pediatric nephrology**

Obliterative vasculopathy features arterial and arteriolar thickening that is beyond the transplant setting common for a range of clinical conditions affecting the kidney in pediatric patients. Future studies are aimed at elucidating whether AT<sub>1</sub>R-AA may be associated with malignant hypertension and atypical hemolytic uremic syndrome (HUS). These conditions are similar to preeclampsia and severe vascular rejection. They all show fibrinoid necrosis and are associated with progressive renal failure. These similarities among proliferative renal arteriopathies could implicate a common pathophysiologic mechanism. Our current working hypothesis is that AT<sub>1</sub>R-AA may be centrally involved in the pathogenesis of vasculopathies refractory to conventional therapies.

**Conclusion**

We provide a novel concept in the pathogenesis of accelerated vascular rejection processes where autoanti-

**Permissive Factors**



**AT<sub>1</sub>RAA mediated clinical sequelae**

**Fig. 1** Proposed model for angiotensin II type 1 receptor (AT<sub>1</sub>R-AA) action that integrates the putative multiple actions of AT<sub>1</sub>R-AA in the complex pathobiological process that occurs in the development of vascular rejection in the transplant kidney. Oxidative stress and inflammatory response are central to ischemia/reperfusion injury (I/Ri) and alloantigen-triggered immune response after transplantation. These permissive factors may alter homeostatic balance maintained by endothelium and/or change biochemical and biophysical characteristics of antibody binding to the target receptor and thereby increase

susceptibility for AT<sub>1</sub>R-AA attack. AT<sub>1</sub>R-AA may mediate endothelial dysfunction, resulting in vasoconstrictive arterial response, increase adhesion and transmigration of effector cells, or mediate procoagulatory state, all involved in pathogenesis of hypertension and vascular rejection. On the other hand, AT<sub>1</sub>R-AA may act on tubular epithelia and renal interstitial cells, amplifying local inflammation and resulting in augmented antigen presentation by local nonprofessional antigen-presenting cells and/or increase production of Th1 cytokines and inflammatory chemokines, all involved in pathogenesis of cellular rejection

body-triggered receptor activation is linked to a severe vascular pathology in the situation of allogeneic transplantation. Our future studies are designed to explain whether or not AT<sub>1</sub>R-AA-related pathology represents a “true rejection” or an autoimmune phenomenon that becomes overt in the permissive allogeneic environment. At present, we believe that pretransplantation testing of both adult and pediatric recipients for AT<sub>1</sub>R-AA may help to improve individual risk assessment offered to patients with AT<sub>1</sub>R-AA preemptive specific treatment.

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