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Angiotensin II type I receptor antibodies in thoracic transplantation☆

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ABSTRACT

Angiotensin II type I receptor (AT1R) is a critical player in regulating vasoconstriction, blood pressure, sodium retention. Even though AT1R has limited polymorphism, AT1R antibodies have been detected in several diseases. The role of AT1R antibodies in transplantation is first reported in kidney transplant, and then identified in heart and lung transplants. Mechanical circulatory support devices (MCS) can also stimulate production of AT1R antibodies. AT1R antibodies may negatively impact graft or patient survival through mechanisms independent of the classical complement activation.

1. Introduction

Despite the improvement of patient management and advancement of immunosuppression regimens, antibody mediated rejection (AMR) remains a leading cause of allograft rejection and graft loss [1]. Initial focus has been on the role of HLA specific antibodies in AMR. However, many patients who experience allograft dysfunction and show histological characteristics of AMR on biopsy, do not have donor HLA specific antibodies [2]. Even though the identity of many non-HLA antibodies remains elusive, antibodies to angiotensin II type I receptor (AT1R) have been shown to be associated with graft dysfunction or rejection.

AT1R is the central component of the renin-angiotensin system. AT1R is expressed in many tissues and organs including heart and vasculature. AT1R belongs to the super family of G protein coupled receptors. AT1R, upon binding with its native ligand angiotensin II, activates various downstream signaling molecules. The classical AT1R signaling pathway is through recruiting heterotrimeric G proteins $G\alpha_{q/11}$, $G\alpha_{12/13}$, and $G_{\beta\gamma}$ to promote vasoconstriction and hypertension [3]. AT1R can also transduce signals through β-arrestin, which stimulates cell growth and promotes cell survival via the extracellular signal-regulated kinase (ERK) and Ca^{2+} pathways [3–5]. The activation of the AT1R signaling is controlled at several levels. AT1R's ligand angiotensin II is a cleaved product of protein angiotensinogen. Renin, a protease that catalyzes the rate-limiting step in this cleavage process, converts angiotensinogen to the inactive peptide angiotensin I. Then angiotensin converting enzyme (ACE) changes angiotensin I to the active form angiotensin II [6].

The presence of antibody against AT1R has been shown to be associated with several diseases caused by dysregulation of the immune system. AT1R antibodies have been found in patients with systemic sclerosis which features vasculopathy and tissue fibrosis [7]. AT1R antibodies from these patients can stimulate phosphorylation of ERK and expression of transforming growth factor β in endothelial cells [7]. However, the association of AT1R antibodies with systemic sclerosis disease manifestation, such as ischemic digital ulcers is still under debate [7–9]. In addition, it is suggested that AT1R antibody can cause preeclampsia. In a rodent preeclampsia model, infusion of AT1R antibody has been shown to increase blood pressure and activate natural killer (NK) cells [10]. The role of AT1R antibody in transplantation is first reported by Dragun et al. in a kidney transplant cohort with malignant hypertension [11]. Since then, studies on the role of AT1R antibody in transplantation have been expanded to other organ transplants. The contribution of AT1R antibody on kidney transplantation is covered by other reviews in this same issue. This review will focus on the role on AT1R antibody in heart and lung transplantation.

Abbreviations: AMR, antibody mediated rejection; AT1R, angiotensin II type I receptor; CCL18, chemokine ligand 18; CMR, cellular mediated rejection; ERK, extracellular signal–regulated kinase; ETAR, endothelin receptors type A; IL-8, interleukin 8; MCS, mechanical circulatory support devices; Neu5GC, sialic acid N-glycolylneuraminic acid; NK cells, natural killer cells; p-S6K, phosphorylated S6 kinase; p-S6RP, phosphorylated S6 ribosomal protein; UTR, untranslated region; vWF, von Willebrand factor

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2. Sensitization of AT1R

Unlike alleles of the HLA system which are highly polymorphic, AT1R only has limited polymorphism. Only a few rare variants that show difference in the amino acid sequence of the AT1R protein, have been identified in patients with renal tubular dysgenesis [12]. The most characterized polymorphism of the AT1R gene, is A1166C which has a nucleotide substitution in 3′ untranslated region (UTR). This polymorphism, however, only affects the expression level of the AT1R protein but does not change the amino acid sequence in the mature protein. Therefore, AT1R antibodies identified in patients with auto immune diseases or transplants are likely to be auto antibodies in most situations. It is suggested that AT1R antibodies recognize the second extracellular loop of AT1R which might include homologous glycated structures of sialic acid N-glycolylneuraminic acid (Neu5GC) [13,14]. It has been shown that autoantibodies can be stimulated by excessive antigens released from dying cells [15]. The transcription of the AT1R mRNA and the expression of the AT1R protein on the surface of cells can be upregulated under inflammation which commonly happens in organ transplantation [16]. AT1R is expressed in the vasculature of the recipient and its expression is significantly increased following renal transplantation in a rat kidney transplant model [17]. AT1R is also expressed at higher level in patients with AMR in kidney transplant patients, and higher levels of AT1R antibody one-year posttransplant are associated with subsequent graft failures [18]. These findings suggest that abnormal high expression of AT1R may stimulate production of auto antibody against AT1R.

Of interest, higher levels of AT1R antibodies were also observed in patients implanted with mechanical circulatory support devices (MCS). MCS is used as bridge-to-transplant for patients with advanced heart failure who are otherwise too sick to receive a heart transplant, or as destination therapy. Our group and others found that implantation of MCS significantly increased the sensitization of AT1R [19,20]. It has been shown that MCS can significantly increase shear stress and shear rate systemically. This increased stress can dislodge the von Willebrand factor (vWF) from the cell surface and cleave it into smaller peptides [21]. It is conceivable that sheer stress created by MCS can clip off the second extracellular loop of the AT1R protein from the cell surface, thereby generating neoantigens, which in turn stimulate the production of AT1R antibody. Another possibility is that MCS increases shedding of AT1R proteins. Excess release of AT1R proteins in blood then stimulates the production of AT1R antibody. Consistent with this hypothesis, it has been shown that pressure overload increases the release of AT1R containing exosome in the blood using a transverse aortic constriction mouse model [22].

3. Impact of AT1R antibody on graft function

3.1. Signaling activated by AT1R antibody

Since AT1R has limited polymorphism, and it is likely that most AT1R antibodies are against auto antigen, AT1R antibodies will not only directly target the graft, but can also bind AT1R expressed on other organs of the recipient and have a global effect on the recipient. Ligation of AT1R by antibodies can activate various downstream signals. It has been shown that AT1R antibodies isolated from kidney transplant patients with malignant hypertension can activate ERK signaling [11]. We found that the levels of AT1R antibodies post MCS implantation distributed into two distinct groups [20]. The levels of the first group were centered around 10–15 U/ml, and the second group had AT1R antibody reading ≥ 40 U/ml at which the AT1R antibody assay becomes saturated. We did not find that the presence of the saturated levels of AT1R antibodies was associated with increased blood pressure in patients implanted with MCS, which suggests that AT1R antibodies identified in this MCS cohort may not act as an agonist of AT1R signaling regulating blood pressure. Nevertheless, we found that MCS patients with the saturated level of AT1R antibody had lower short-term survival compared with patients with non-saturated levels of AT1R antibody [21]. These results suggest that binding of AT1R antibodies can affect the AT1R signaling regulating functions other than blood pressure. It has been shown that AT1R antibodies derived from systemic sclerosis patients can upregulate the expression of chemokine ligand 18 (CCL18) and proinflammatory interleukin 8 (IL-8), and activate microvascular endothelial cells [23,24]. Activation of AT1R signaling promotes development of atherosclerotic plaques in an animal model, and inhibition of AT1R signaling reverses plaque progression by decreasing the inflammatory properties of the plaque [25,26]. Given that activation of AT1R signaling exacerbates inflammation, AT1R antibody may stimulate inflammation, and thereby impact the patient survival post-MCS implantation.

4. Impact of AT1R antibody in heart and lung transplant

The endothelium lies at the interface between the allograft and the recipient blood, and is directly targeted by antibodies produced by the recipient immune system. AT1R is expressed in human vascular endothelial cells [27]. Antibody binding to the endothelium of the graft can potentially activate the classical complement pathway or recruit NK cells or neutrophils through the Fc fragment. However, the presence of AT1R antibodies is not associated with positive C4d staining in the biopsy, a hallmark of complement activation, even if AT1R antibodies belong to the isotypes of IgG 1 and IgG3 [11]. Nevertheless, AT1R antibodies can still negatively impact the graft outcome. In heart transplantation, pre-transplant AT1R antibodies are associated with micro-vasculopathy [28]. Higher levels of AT1R antibodies were also detected in patients with cellular mediated rejection (CMR) or AMR. Similar to the findings reported in kidney transplant [29], AT1R antibodies can substantiate the impact of donor specific HLA antibodies on the allograft in heart transplant. Patients with both AT1R antibodies and donor HLA specific antibodies lose graft at a higher rate than patients with donor HLA specific antibodies alone [30,31].

The role of AT1R antibody has also been investigated in lung transplant. AT1R signaling is involved in the regulation of inflammation, proliferation and fibrosis in pulmonary diseases and pulmonary arterial hypertension [32]. Inhibition of AT1R signaling has been used to treat inflammatory lung disease [33]. Elevated levels of AT1R antibodies were detected in patients with systemic sclerosis-associated pulmonary arterial hypertension, and antibodies isolated from these patients could increase cytosolic Ca^{2+} concentration in endothelial cells [33]. Higher levels of AT1R antibodies were also detected in patients with cystic fibrosis that is commonly associated with recurrent infections [34,35]. In a case study, a patient with cystic fibrosis underwent lung transplantation [36]. The patient developed a severe pulmonary hypertension perioperatively and experienced deterioration in hemodynamics which led to patient's expiration day 5 post-transplant. Only AT1R and endothelin receptors type A (ETAR) antibodies were detected pre- and post-transplant. No donor HLA specific antibodies were detected throughout the course. C4d staining was negative on the biopsy, but staining for phosphorylated S6 kinase (p-S6K) and phosphorylated S6 ribosomal protein (p-S6RP) on capillary endothelia cells were positive [36]. P-S6K and p-S6RP have been used as biomarkers of cardiac AMR [37].The role of AT1R antibody in lung transplant is further substantiated by a 3-center study [38]. Reinsmoen et al. has shown that the presence of strong or moderate binding levels of AT1R antibodies is associated with AMR [38]. Recipients with AT1R antibodies also trend lower freedom from ACR compared with recipients without AT1R antibodies, but the difference did not reach statistical significance. Patients with AT1R antibodies pre-transplant have a higher risk to develop de novo donor specific HLA antibody. Patients with pretransplant HLA antibodies have a higher risk to develop de novo donor HLA specific antibodies posttransplant (hazard ratio 1.69). The presence of both HLA antibodies and strong AT1R

Table 1
Literature summary: role of AT1R antibody in heart and lung transplant.

Study	Organ	Number of patients	Cutoff of AT1R assay	Major findings
Urban et al. [19]	Heart	69	Positive: > 17 U/ml	63% of patients who were negative for AT1R antibody became positive after Heart Mate II implantation. The positivity of AT1R antibody was not associated with survival, ACR, and AMR
Zhang et al. [20]	Heart	88	Positive: ≥ 40 U/ml	MCS implantation induced AT1R antibody production. Patients with AT1R antibody level ≥40 U/ml had lower 18-month survival
Hiemann et al. [28]	Heart	30	Positive: > 16.5 U/ml	Elevated AT1R antibody pre-transplantation was associated with microvasculopathy
Reinsmoen et al. [30]	Heart	200	Positive: ≥17 U/ml, ≥12 U/ml	AT1R antibody alone pre-transplant did not increase a risk of AMR or ACR, but the combination of AT1R antibody and de novo HLA donor specific antibodies had synergistic effect on AMR and ACR
Reinsmoen et al. [38]	Lung	162	Positive: ≥17 U/ml	The presence of AT1R antibodies was associated with AMR. AT1R antibody substantiated the effect of pretransplant HLA on the development of de novo donor specific antibodies post-transplant
Cozzi et al. [36]	Lung	1	Positive: > 10 U/ml	This case study described one lung transplant patient with cystic fibrosis. The patient developed hypertension and AMR, but only AT1R and ETAR antibodies were detected in the patient

antibodies increased hazard ratio to 2.38, suggesting a double hit model by AT1R antibodies and HLA antibodies [38].

5. Conclusion

The presence of AT1R antibodies has been shown to be associated with poor outcome in heart and lung transplant (Table 1). Development of AT1R antibody predisposes patients to generate de novo donor specific HLA antibodies. Production of AT1R antibody could be stimulated by MCS implantation. Because the AT1R gene has limited polymorphism, most AT1R antibodies detected in transplant patients may react with self AT1R antigen. It is suggested that AT1R antibodies target the epitope in the second extracellular loop of AT1R receptor. However, it is yet to be investigated if all AT1R antibodies identified in different transplant cohorts recognize the same epitope and if these AT1R antibodies have same capacity to activate AT1R signaling.

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