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Non-HLA-antibodies targeting Angiotensin type 1 receptor and antibody mediated rejection

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ABSTRACT

Antibody-mediated mechanisms directed against non-HLA related targets may exert negative impact on allograft function and survival. Angiotensin type 1 receptor (AT_1R) emerges as a functional target for non-HLA allo- and autoantibodies (AT_1R-Abs) comprising of IgG1 and IgG3 subclasses. Proof of concept for pathophysiologic relevance of AT_1R-Abs in antibody mediated rejection (AMR) in renal transplants was provided by passive transfer studies in animal model and therapeutic rescue of patients. Although AT_1R-Abs may belong to complement fixing IgG subclasses, C4d positivity in renal transplant biopsies was not frequently detected implicating complement independent mechanisms of injury. AT_1R-Abs exert direct effects on endothelial and vascular smooth muscle cells by induction of Erk1/2 signaling and increased DNA binding of transcription factors associated with pro-inflammatory and pro-coagulatory responses.

Establishment of enzyme-linked immunosorbent assay employing extracts of cells overexpressing AT_1R in its native conformation was instrumental for recent studies in independent cohorts. Assessing the AT_1R-Ab-status along with the HLA-antibodies may help to identify patients at particular risk for irreversible acute or chronic allograft injuries and improve overall outcomes. This review summarizes the current state of research in AT_1R biology, development in diagnostic strategies, discusses recent clinical studies, and provides perspectives on further refinements in understanding AT_1R-Ab-actions.

1. Introduction

Putative pathogenic antibodies that are not directed against the HLA system were considered in recipients who rejected HLA-identical kidneys more than three decades ago [1]. Confirmation for broad clinical relevance of non-HLA-related humoral immunity was in comparison rather recent [2]. Despite obvious detrimental effects on allograft survival, identification and characterization of target antigens for non-HLA antibodies remained difficult. In our "proof of concept" study several years ago, we have identified Ang type 1 receptor (AT_1R) as an antibody target in 16 recipients of renal allografts who had severe vascular rejection and malignant hypertension, but who did not have anti-HLA antibodies [3]. AT_1R-antibodies (Abs) were initially detected by means of the bioassay that measures the chronotropic response to AT_1R-IgG mediated stimulation of cultured cardiomyocytes. Passive transfer of AT_1 R-Abs isolated from affected renal transplant patients into low-responder allogeneic rat transplant induced similar vascular rejection phenotype as observed in their transplant biopsies stressing importance of AT_1R-Abs as an effector mechanism of rejection [3].

AT_1R-Abs were further characterized as IgG1 and IgG3 subclass antibodies which recognize conformational antigens contained in the second extracellular loop of the AT_1R capable of initiation of signal transduction in endothelial and vascular smooth muscle cells. These findings provided further evidence that AT_1R-Abs may have a causative role [3].

Similar to other non-HLA targets [4], the most significant barrier to the general acceptance of AT_1R-Abs as mediators of allograft rejection was the lack of standardized assay to determine their presence. Paying attention to the conformational nature of the target antigen for AT_1R-Abs, we have established and introduced a diagnostic tool which can provide quantitative antibody binding

Abbreviations: AT_1R, Angiotensin type 1 receptor; AMR, antibody mediated rejection; Abs, antibodies; GPCRs, G-protein-coupled receptors; β_2-AR, β_2 adrenergic receptor; AGTR1, β_1-AR avian, AT_1R gene; Ang II, angiotensin II; DSA, donor specific antibodies.

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assessment and is feasible for routine clinical use. Newly gathered data based on the use of a solid-phase assay confirmed clinical relevance of AT_1R-Abs in renal transplantation and data in other transplant settings is accumulating. In parallel, the past few years were dominated by remarkable advances in the structural biology and molecular pharmacology of G-protein-coupled receptors (GPCRs) which may be applicable to AT_1R [5]. In this review we first discuss complexity of GPCR structure and AT_1R-signaling in general, as well as biology of AT_1R-Abs in particular, in order to introduce complexity of antibody mediated AT_1R activation. We then discuss translation of new biological concepts into diagnostic development and refinement in understanding of mechanisms of AT_1R-Abs actions. Finally, we address some of the challenges for the future research and clinical application in various transplant settings.

2. AT_1R/ AT_1R-Abs and multiple facets of GPCR biology

2.1. Recent advances in GPCR biology

GPCRs are the largest family of membrane proteins and mediate most cellular reponses to hormones, neurotransmitters, ions, photons and other stimuli. All GPCRs including the AT_1R are characterized by the presence of seven transmembrane spanning α-helical segments separated by alternating intracellular and extracellular regions. GPCRs in vertebrates are divided on the basis of their sequence and structural similarities into five families [6]: rhodopsin (family A), secretin (family B), glutamate (family C), adhesion and Frizzled/Taste2. The rhodopsin family is by far the largest and most diverse of these families, and members are characterized by conserved sequence motifs that imply shared structural features and activation mechanisms. Solving the first crystal structures of ligand activated GPCRs including the structures of opsin and an active form of rhodopsin [7,8], the human A_{2A} adenosine receptor [9], the human β_2 adrenergic receptor (β_2-AR), and the avian β_1-AR [10–12] was a major break-through in the field. The elucidation of the crystal structures finally provided the opportunity to understand how protein structure dictates the unique functional properties of these complex signaling molecules. GPCRs are now considered as inherently flexible proteins that are able to exhibit a spectrum of conformations depending on the presence of bound ligand [13,14]. The relationship between conformational states and the rate of the interconversion between them dictate the biologic net effect upon ligand-receptor interaction [5].

2.2. AT_1R gene and structure

AT_1R belongs to type A family of GPCRs with similar structures to rhodopsin and exhibits an extracellular, glycosylated region connected to the seven transmembrane α-helices linked by three intracellular and three extracellular loops [15]. The human gene for AT_1R is located on chromosome 3. The single copy human AT_1R contains 4 exons (the entire coding region is found in exon 4). Alternative splicing of exons 1, 2, and 3 onto exon 4 yields four main transcripts with markedly different rates of translation, indicating that mRNA processing may play an important role in determining the level of AT_1R expression [16]. AT_1R gene (AGTR1) has 14 described polymorphisms, and some of them act as "gain" or "loss" of function mutations implicated in receptor activation [17,18]. However, they have not been investigated in the context of alloimmune response. The most extensively studied A1166C polymorphism is associated with increased responsiveness to angiotensin II and various cardiovascular and renal pathologies [19–24].

2.3. Recognition units on AT_1R including epitopes for AT_1R-Abs

AT_1R-Abs recognize and bind to epitopes on the second extracellular loop of the AT_1R [25] (Fig. 1). The second extracellular loop of the AT_1R recognized by AT_1R-Abs has been recently identified as a pivotal structural element in generating different conformational states of the AT_1R [13].

In contrast, Ang II (natural agonist) and different pharmacologic blockers (inverse agonists) bind to different amino-acids contained in transmembrane regions (Fig. 1). For example, the most frequently used pharmacologic AT_1R-blocker worldwide – valsartan – interacts with Ser105 and Ser109 in TM3 which are critical for induction of inverse agonism and induction of less active conformation [26]. The other also very frequently used AT_1R-blocker, candesartan, interacts with Lys199 (TM5) and His256 (TM6) in the receptor [27].

AT_1R-Abs have also been associated with systemic sclerosis, preeclampsia and malignant hypertension [25,28,29]. The described epitopes for AT_1R-Abs isolated from transplant patients do not entirely coincide with those described in preeclampsia, while binding epitopes in systemic sclerosis and malignant hypertension have not been identified yet.

3. Assays for detection of AT_1R-Abs

Detection of AT_1R-Abs initially relied on the bioassay that measures the chronotropic response to AT_1R-IgG mediated stimulation of cultured neonatal cardiomyocytes and its response to receptor-specific antagonists. Dose–response relationship between AT_1R-Ab concentrations and the chronotropic response is linear [25]. The time and labour consuming setting of the bioassay in rat neonatal cardiomyocytes precluded larger studies and emerged development of an alternative assay feasible for clinical routine. A sandwich ELISA for detection of AT_1R-Abs in serum has been now validated and established for testing transplant recipients and patients with autoimmune diseases [28,30]. The ELISA currently has 100% specificity and 88% sensitivity as compared to cardiomyocyte bioassay. Interassay variability is 12% [30]. The microtiter 96-well polystyrene plates are coated with extracts isolated from Chinese hamster ovary cells overexpressing the human AT_1R in its native configuration. Maintenance of conformational epitopes is provided by addition of appropriate buffers. Binding of AT_1R-Abs contained in patient's serum to AT_1R immobilized at plates is detected according to sandwich ELISA principle upon washing procedures

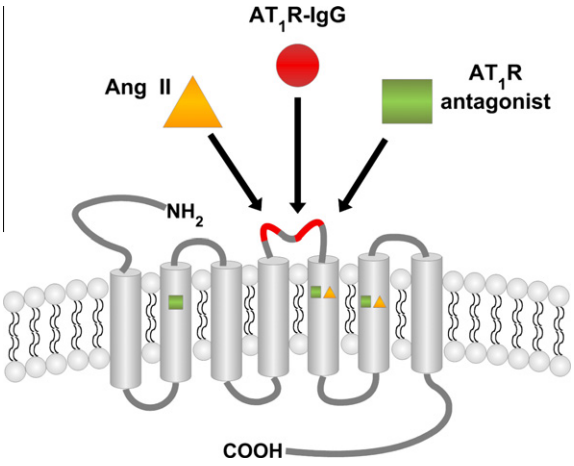

Fig. 1. AT_1R as a multifunctional target GPCR. Various agonists or antagonists may simultaneously bind to the AT_1R and induce functionally relevant conformational changes.