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Transplantation Genetics: The Importance of non-HLA Antibodies, Genetic Insights and Future Perspectives

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Abstract: Solid organ transplantation is an effective and life-saving treatment method for patients with end-stage organ failure. Immunological evaluation is of great importance in the achievement of transplantation, and the determination of HLA incompatibility forms the basis of immunological transplantation success. The presence of donor HLA-related antibodies is known to be asssociated with graft loss and decreased survival rates. However, recent studies have shown clinical and experimental findings related to the important role of non-HLA antibodies in the antibody-mediated rejection and chronic rejection process. The mechanisms triggering auto-antibody production in organ transplantation are an important area of research. These mechanisms stimulate auto-antibody production in the patient causing the expression of organ-origin autoantigens such as ischaemia-reperfusion damage, surgical trauma, alloimmune responses, soluble antigens, extracellular vesicles and apoptotic bodies. In patients with a risk of non-HLA antibody-mediated rejection, there is an urgent need to determine the recipient immunological phenotypes, both for the development of targetted treatments and to increase both graft and patient survival. The aim of this review was to evaluate non-HLA antibody types and their effects on transplantation. ©2024 NTMS.

Keywords: non-HLA Antibodies; Solid Organ Transplantation; Transplantation Genetics.

1. Introduction

The detection of foreign structures in donor cells by the adaptive immune system of the patient constitutes the main immunological obstruction in organ transplantations. Human leukocyte antigens (HLA), which are located on the p arm of the 6th chromosome and encoded in the HLA complex, are accepted as the most important alloantigens in transplantation [1]. The determination of HLA incompatibility forms the basis of immunological transplantation success. The widespread clinical use of anti-HLA antibodies significantly increases transplantation success.

The presence of donor HLA-related antibodies is known to be associated with decreased survival rates and graft loss. It has been suggested that the transplantation outcome is affected synergistically by non-HLA antibodies together with the harmful effects of HLA antibodies. The damage caused to the transplantation process by HLA antibodies may cause the emergence of neo-antigens, and this can result in the production of antibodies against non-HLA antigens. However, the presence of non-HLA antibodies can increase the risk of the patient developing HLA-specific antibodies. All these findings emphasize the importance of determining the immunological risk of the patient by classifying non-HLA and HLA antibodies [2].

Despite improvements in immunosuppression regimens and the optimisation of patient management,

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antibody-mediated rejection remains a major impediment to long-term survival [3]. The identification of HLA-specific antibodies against donor HLA class I and II antigens has become a priority at this stage. With commercially developed tests and techniques in this field, there is a greater level of knowledge about the specificity, power, and function of these antibodies. From the correlation of antibody information obtained from clinical transplantation outcomes, it has been reported that patients with antibodies to donor HLA are faced with antibody-mediated rejection and worse transplantation outcomes [4]. Despite the histopathological findings of allograft dysfunction and antibody-mediated rejection during biopsy, antibodies specific to donor HLA have not been determined [4.5]. For example, in heart transplantations, non-detection of donor-specific HLA antibodies that cannot be detected in peripheral blood has prioritised the research of non-HLA antibodies, many of which are expressed by the vascular endothelium and often emerge after stress or transplant injury [5]. Although the determination of non-HLA antibodies is difficult, antibodies to non-HLA antigens have been shown to be related with transplant dysfunction or transplant rejection [6]. To date, the focus in clinical transplantation has been alloimmunity associated with HLA. However, the presence of antibody -mediated humoral rejection developing after kidney transplantation from siblings with compatible HLA shows the importance of non-HLA antigens of alloimmunity [7]. Terasaki et al. [8] reported that only 18% of kidney allograft losses may be associated with non-HLA independent immunological factors compared to 38% due to HLA mismatches [8]. Opelz et al. was reported the importance of the non-HLA alloimmunity response in transplantation success [9]. When the effect on the long-term results has been analyzed of the pre-transplant panel reactive antibody (PRA) levels of patients undergoing kidney transplantation from sibling donors fully compatible in HLA-A, HLA-B, and HLA-DR loci, the probability of incompatibility in other HLA loci has been reported (<3%, especially DQ and DP). From the findings obtained, the most noteworthy is that the PRA effect only becomes evident after the first year post-transplantation. Therefore, it has been concluded that non-HLA immunity has a stronger role than previously thought and non-HLA alloimmunity is usually associated with chronic allograft loss [9].

The development of antibodies against non-HLA autoantigens after organ transplantation is related with rejection and long-term survival. Although there is a lack of data about non-HLA antibodies, there are strong clinical and experimental findings that the antibodies determined play an important role in antibody-mediated acute and chronic rejection processes[9].

The mechanisms triggering autoantibody production in organ transplantation is an important area of research. Auto-antibody production in the patient is stimulated causing the expression of organ-origin autoantigens such as ischaemia-reperfusion damage, surgical trauma, alloimmune responses, soluble antigens, extracellular vesicles and apoptotic bodies. Th17 cells regulate autoantibody production, supporting the proliferation and maturation of autoreactive B cells in ectopic tertiary lymphoid tissue. Autoantibody-mediated graft damage can trigger alloimmunity and can cause the development of donor-specific HLA antibodies. In patients with a risk of non-HLA antibody-mediated rejection, there is an urgent need to determine the recipient immunological phenotypes, both for the development of targetted treatments and to increase both graft and patient survival [2].

The mechanisms of antibody formation

Non-HLA antibodies can be directed to auto or alloantigens, may be present before transplantation or may form *de novo* after tranplantation. There are thought to be various triggering events for autoantibody formation which can cause self-tolerance loss step by step. Inflammation can cause an increase in antigen expression and varying antigen processing and proteolysis [10-13]. In addition, post-translational modification, oxidative stress, and apoptosis may cause the formation of neoantigens [10]. Finally, the close relationship of infection agents and to their own peptides may cause cross-activation of autoreactive B and T cells [14]. By playing an important role in the selection of B cells directed against alloantigens, follicular helper T cells (Tfh) prevent the formation of autoreactive B cell clones. The irregularity of Tfh cells may cause humoral autoimmunity by causing impairment in B cell selection. Factors causing impairments in the eradication of immature autoreactive B cells through increased Th17 formation against allograft and/or donor HLA may further facilitate self-tolerance loss [15,16] (Figure 1).

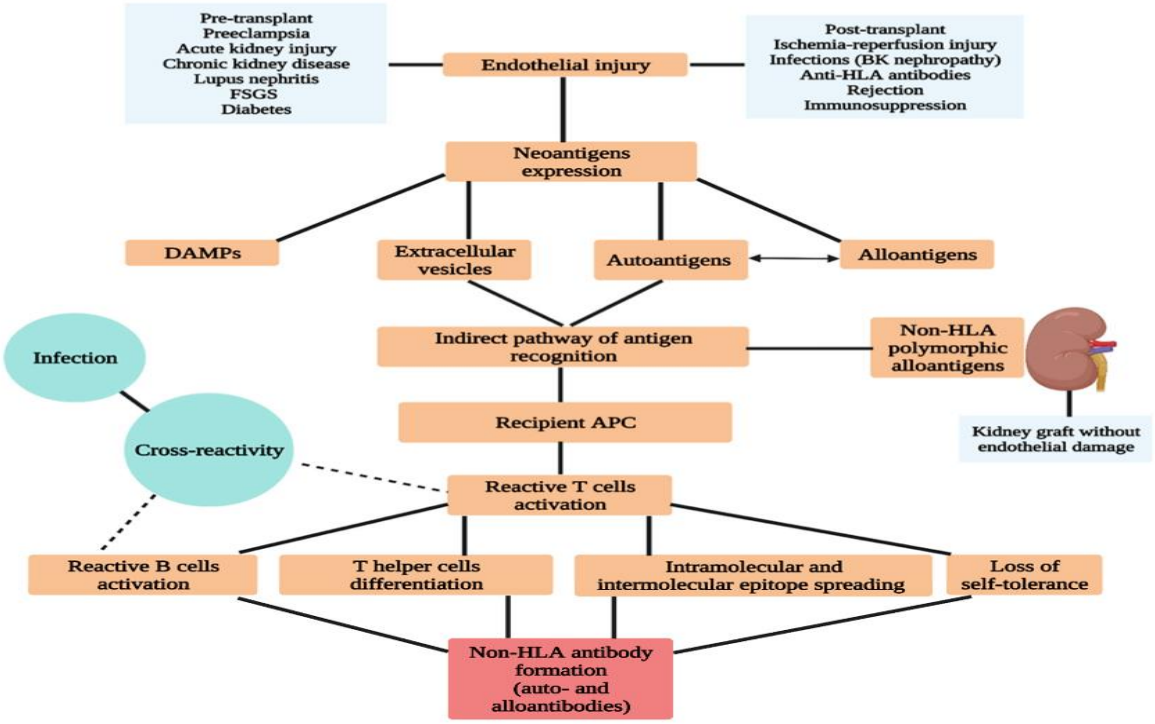

Figure 1: The mechanism of non-HLA antibody formation. FSGS: focal segmental glomerular sclerosis, BK: BK virus, HLA: human leukocyte antigen, DAMPs: Damaging molecular patterns, APC: antigen presenting cells [73].

The mechanism of non-HLA mediated graft damage

There is thought to be a synergistic effect of HLA and non-HLA antibodies. While HLA antibodies can reveal endothelial damage causing autoantibody formation and subsequent autoantigen exposure, the inflammatory response triggered by non-HLA antibodies can make the allograft more vulnerable to alloimmune response by increasing HLA expression [17]. This hypothesis is supported by several studies showing that patients with HLA and non-HLA antibodies have lower graft survival rates than patients with only one of these [18]. However, there are also studies in literature stating that in patients with both HLA and non-HLA antibodies, there is no relationship between these antibodies and graft survival [19]. Most autoantigens have been determined not only in the kidneys, but also in most other solid organs of the body [20]. To create a response, these antibodies are activated with highly specific mechanisms such as HLA antibody binding or ischaemia-reperfusion damage. Non-HLA antibodies do not directly cause major graft damage, because hyperacute rejection stimulated by these antibodies rarely occurs [21]. Several hypotheses have been suggested to explain non-HLA antibody-mediated graft damage, but the mechanisms have not been fully clarified (Figure 2).

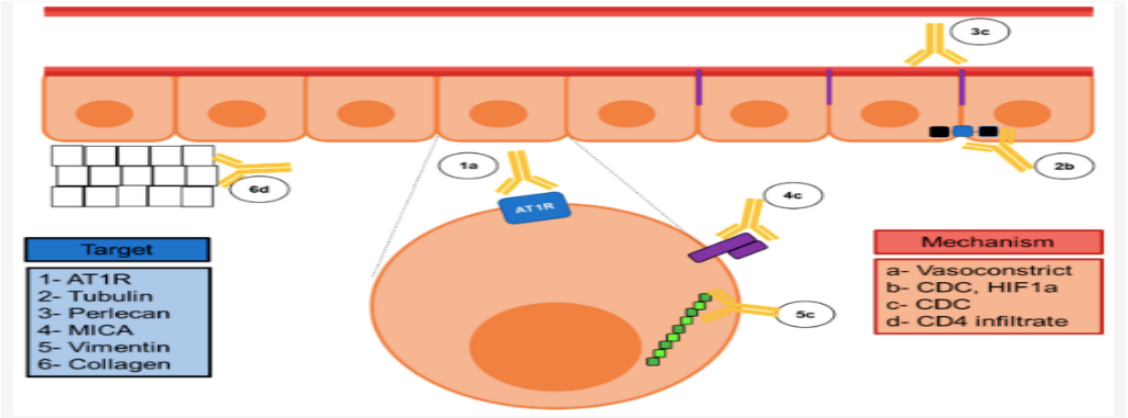

Figure 2: The mechanisms of non-HLA antibodies in solid organ transplantation. The structures containing blood vessels (red) and endothelial lining of cells (orange), extracellular matrix (collagen) and intercellular gap connections (black and blue) are shown. It is indicated how the antibodies are linked to intracellular components (eg.tubulin) and different membrane receptors with a single cell. The antibody targets are listed in the blue box, and the mechanisms associated with the target in the red box. The corresponding targets and mechanisms are defined in the white squares. Abbreviations: AT1R: Angiotensin 1 receptor, MICA: MHC class I-related chain gene A, CDC: Complement dependent cytotoxicity, HIF-1α = Hypoxia-inducible Factor 1 alpha [74].

Types of non-HLA Antibodies

Antibodies to MICA

Major histocompatibility complex (MHC) class I-related chain A (MICA) antigen is a polymorphic glycoprotein, encoded by the gene known as MHC class I chain within the HLA complex on the 6th chromosome. While the MICA molecule is expressed in monocyte, dendritic cells, endothelial and epithelial. It is shown that not found in T and B lymphocytes [22]. MHC class I chain-related gene A belongs to the HLA gene family. MICA protein has a structure similar to HLA class I, but is not associated with β2 microglobulin on the cell surface and shown that cannot bind to peptides [23]. Just like HLA molecules, they may carry different recipient and donor MICA alleles. The donor may develop antibodies against donor-specific MICA alleles [24]. The effect of MICA antibody on transplantation pathogenesis has been reported in kidney transplantations [19]. Patients with donor-specific MICA antibody are at higher risk of antibody-mediated rejection [5]. MICA is located in the interface between the allograft and the recipient blood and cannot be determined in silent endothelial cells directly targetted by the immune response. MICA can be stimulated by stress factors or cytokines such as TNF-α [25]. Previous studies have shown that MICA expression in tumour cells leads to the activation of NK cells mediated by the interaction of MICA/NKG2D and this causes cytotoxic protein and IFN-γ expression [23] (Figure 3).

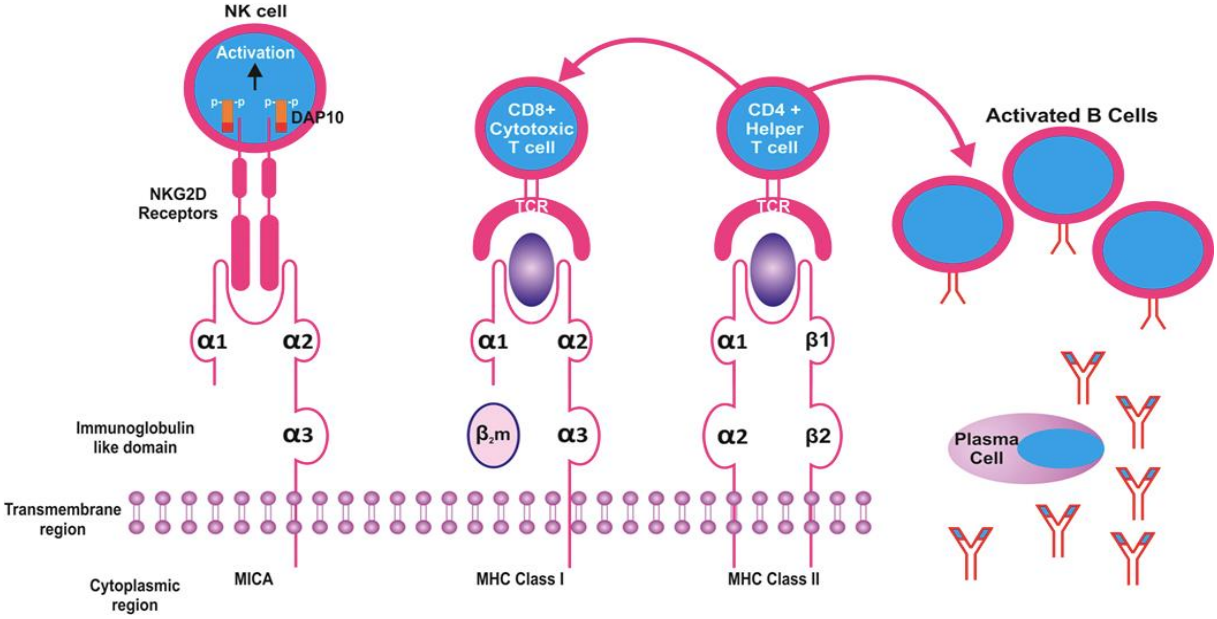

Figure 3: Stractural Similarities Between Major Histocompatibility Complex (MHC) Class I and II Molecules with MICA. The latter is equivalent to the heavy chain of MHC class I molecule without the β2 microglobulin. While the MHC I and II present peptides to CD8 and CD4 cells, respectively, the MICA recognizes NKG2D receptors on the surface of natural killer (NK) cells [22].

Antibodies against G Protein-Coupled Receptors (GPCRs)

AT1R (angiotensin II receptor type I) and ETAR (endothelin-1 type A receptor) belong to the GPCR family of the seven-transmembrane domain. As AT1R and ETAR are expressed on the cell surface and because of the extracellular regions where antibodies can be reached, the development of antibodies against these may be possible. Some antibodies such as AT1R have been reported to play a role in the pathophysiology of pre-eclampsia in pregnancy and autoimmune diseases such as systemic sclerosis [26-28]. There are several potential mechanisms to explain how these antibodies could develop in patients without any autoimmune disease. One of the possible causes may be immune suppression or the underlying inflammatory process itself causing the condition that cannot be tolerated. Another reason may be the separation of proteins such as Von Willebrand factor to smaller peptides, triggered by procedures such as dialysis [29]. Just as shear stress may cause AT1R to break from the cell surface and thus be exposed to neo-antigens, it may also disrupt the extracellular cycle of AT1R. The severity of damage of AT1R antibodies can be affected by the level of different AT1R isotypes expressed in the allograft. The effect in clinical findings of anti-AT1R antibodies has been defined in a group of kidney transplantation recipients with malignant hypertension [30]. This shows that AT1R antibodies such as AT1R binding with angiotensin II can promote vasoconstriction, H_2O intake, and sodium confinement, and can increase blood pressure [31]. Just like HLA antibodies, AT1R antibodies have a negative effect on the survival of transplanted organs. Although the presence of AT1R antibodies is associated with antibody-mediated rejection in kidney transplantations, they are not related with cellular-mediated rejection [32]. By creating a synergy with HLA antibodies, AT1R antibodies create a predisposition to transplantation rejection. The presence of AT1R antibodies which are

strongly bound and HLA class II donor-specific antibodies may be a reason for the acceleration of kidney transplantation rejection, hypertensive encephalopathy, and shorter survival following transplantation [32,33]. In addition, the harmful effect of AT1R antibodies on the graft does not require complement system activation. Reinsmoen et al. [32] reported that CD4 positivity was determined in the biopsy of only 1 of 6 patients with strong AT1R antibodies and the graft rejection was antibody mediated. In parallel with that study, Fuss et al. [34] reported that in cases with acute antibody-mediated rejection with CD4 negativity proven in biopsy according to Banff 2013, AT1R antibodies were determined in 11 cases, unlike donor-specific HLA antibodies. AT1R also plays an important role in glucose metabolism [35]. It has been suggested that increased expression of AT1R is associated with an increased risk of diabetic nephropathy and that AT1R blockage is effective in the treatment of diabetic nephropathy. However, there is a need for further studies in this field (Figure 4).

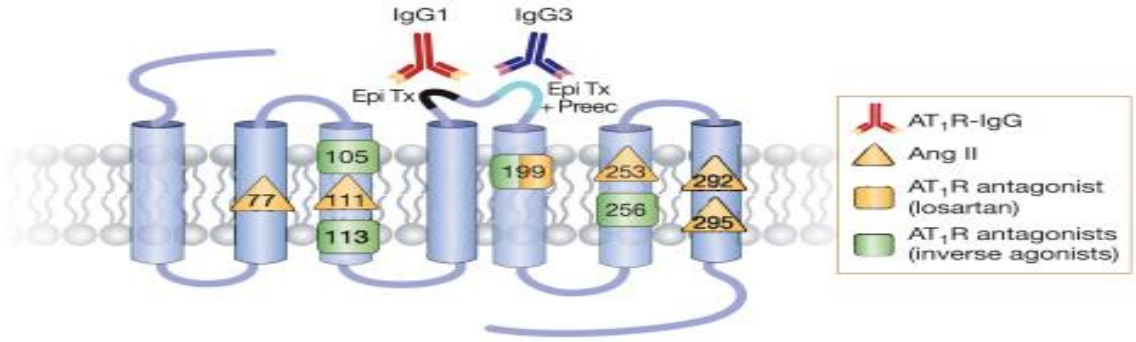

Figure 4: Key amino acids for the binding of receptor modulators are shown. The receptor is bound to Ang II, an orthosteric ligand, angiotensin II, transmembranes 2, 3, 6, and 7. AT1R antibodies as allosteric ligands are bound to the extracellular cycle 2 (ECL2) on 2 different epitopes (Epi) related to EpiTx and EpiTx+Preec acute graft rejection. Losartan is bound to the Angiotensin II orthosteric domain within transmembrane 5 (TM5). Other receptor antagonists are bound to receptors on transmembrane 3, 5, and 6 (TM3, 5, and 6). Preec: pre-eclampsia; Tx: transplantation [30].

Antibodies to Vimentin

Vimentin is a cytoskeletal type III intermediate filament protein, which is expressed in T cells, endothelial cells, neutrophils, fibroblasts, thrombocytes, smooth muscle cells, epithelial cells [36]. Vimentin is a molecule generally found within the cell. However, by being expressed on the cell surface in conditions such as endothelial damage or apoptosis, it becomes an immunogenic auto-antigen. This stimulates the formation of anti-vimentin auto-antibody [37]. Antibodies developing against vimentin play a role in the pathogenesis of these autoimmune diseases. Antibodies to vimentin have been associated with an increased risk of cardiovascular disease in some populations, but the mechanism of this relationship has not been fully clarified.

Vimentin, which is a sub-unit of the intermediate filament is important for the stabilisation of cytoplasm as a cytoskeletal component. It has been reported that it is expressed by macrophages, endothelial cells, vascular smooth muscle cells, active thrombocytes, apoptotic T cells and neutrophils [38]. While expression is increased with proinflammatory cytokine TNF-α, it is inhibited by anti-inflammatory cytokine IL-10. This suggests that vimentin could be important in immune response [39]. Vimentin antibodies have been determined in the pre-transplant serum of patients with kidney failure [40]. In a previous study, it was reported that IgM increased antibody titers against vimentin every year compared to pre-transplant titers. However, no difference was found between patients with interstitial fibrous and tubular atrophy and the kidney recipient control group, although IgG vimentin antibody was determined at a high level in the patients with interstitial fibrous and tubular atrophy. These results suggest that IgG antibodies in patients with interstitial fibrous and tubular atrophy could be related to the diseases pathogenesis, but there is no effect of IgM antibodies [41].

Anti-Endothelin A Receptor Antibodies

Endothelin A receptor (ETAR) is a transmembrane G-protein-related receptor, which plays a role in providing the balance of blood pressure and sodium and is encoded by the ENDRA gene on the 4th chromosome [42]. ETAR can be found in immune cells and is expressed primarily in vascular endothelial cells, mesengial cells, tubular epithelial cells and vascular smooth muscle cells [43]. Anti-ETAR antibodies are IgG1 subtype autoantibodies and show a similarity to the mechanism of formation of anti-AT1R antibodies [44]. Previous studies have reported that anti-ETAR antibodies are associated with ABMR, vascular rejection, and deteriorating graft function and graft loss following kidney transplantation [45,46].

Anti-ARHGDIB (Rho Guanine Nucleotide Exchange Factor 2) Antibodies

ARHGDIB is an intracellular GTP-binding protein, which is expressed in different tissues and organs and

plays a role in several cellular activities. Expression in kidney graft is affected in normal or pathological conditions. In a kidney graft when abnormality is not determined histologically, ARHGDIB expression is seen less in the endothelial cells in interlobular arteries, peritubular capillaries and glomerular capillaries. In contrast, in a kidney graft with acute tubular necrosis, ARHGDIB expression has been determined to be greater in the endothelial cells in interlobular arteries, peritubular capillaries and glomerular capillaries [47]. In a large-scale cohort study, antibodies to ARHGDIB were determined to have a significant effect on graft loss independently of anti-HLA DSA [47]. Endothelial damage triggered by ischaemia-reperfusion damage has been reported to trigger ARHGDIB expression and autoantibody formation [47]. ARHGDIB is considered to be a minor histocompatibility antigen, we examined the relation between antibody levels and potentially immunizing events, but found no link with repeat transplantation, female sex, pregnancies, or potentially confounding factors such as diabetes type 1, or several primary renal diseases [47]. In another study, anti-ARHGDIB antibodies determined in anti-HLA DSA positive patients were reported to be associated with an increase in graft damage [48]. However, there are also other studies in literature stating the opposite. Betjes et al. reported that increased anti-ARGHDIB auto-antibody expression in patients with chronic ABMR was not related to graft survival [49].

Anti-PECR (Peroxisomal Trans-2-Enoyl-CoA Reductase) Antibodies

PECR is a trans-2-enoil-CoA reductase specific to peroxisomal NADPH, which catalyses the reduction of trans-2-enoil-CoA with chain length varying between 6:1 and 16:1 [50]. PECR expression increases due to graft damage in kidney transplantation. It has been reported to be associated with the development of glomerulopathy and biopsy-proven ABMR [51].

Anti-PRKCZ (Protein Kinase C Zeta Type) Antibodies

PRKCZ is a type of protein kinase C, associated with proliferation, apoptosis, survival, and inflammation. Over-expression of PRKCZ has been reported in an ischaemia-reperfusion study [52]. In a study of paediatric renal transplant patients, although it was thought that graft rejection was related to antibodies to PRKCZ, it was concluded that there is insufficent data on this subject yet [53].

Anti-Agrin Antibodies

Agrin is a heparan sulfate proteoglycan, which is expressed at a high level in glomerular basal membranes. The C-terminal fragment (CAF), which is a fragmentation product of agrin, has been utilized as a potential biomarker for graft function in patients with renal transplantation. CAF has been reported to be related to creatinin, cystatin C, estimated glomerular filtration rate, and both early period and delayed graft function following kidney transplantation [54,55]. In another study, CAF was utilized as a risk factor for proteinuria and kidney graft loss in patients with transplant glomerulopathy [56].

Anti-Myosin Antibodies

Myosins are a series protein family which bind to the actin cytoskeleton and transport protein through ATP hydrolysis. The human and rat thymus does not express myosin heavy chain proteins and therefore, CD4+ T cells cannot be selected as negative for myosin in the maturation process [57]. As a result of this mechanism, it may be associated with autoimmune myocarditis, which is frequently associated with autoantibodies [57]. The presence of myosin antibodies has been associated to antibody-mediated rejection in heart transplants and the development of chronic allograft vasculopathy [58]. Although many single nucleotide polymorphisms of cardiac myosin have been identified, it is still not clearly known whether the myosin antibodies determined in patients are donor-specific or not.

Anti-Perlecan/LG-3 Antibodies

Perlecan is a heparan sulfate proteoglycan, which is found in vascular and epithelial basal membranes. Perlecan is an extracellular matrix proteoglycan, and an important component of the endothelial basal membrane, functioning as a barrier between the blood in circulation and the surrounding tissues. The CAF of perlecan contains LG3 regions (lamina-like globular areas) with high immunogenic properties [59]. In a rat transplant model, it has been shown to function as a co-receptor for fibroblast growth factor 2 to be able to stimulate cell proliferation [60]. Studies have also shown that vascular damage caused the release of apoptotic vesicle-like vesicles, and these were trriggered by the production of LG3 antibodies, which are the CAF of perlecan [59]. In kidney transplant patient, a high level of LG3 antibodies in both the pre- and post-transplant periods has been reported to be associated with acute vascular rejection [18]. Anti-LG3 antibodies are one of the factors increasing ischaemia-reperfusion damage, and it has been reported that these antibodies could be related with immune-mediated vascular rejection and delayed graft function within 1 year of transplantation [61]. Previous studies have also shown that memory B cells against LG3 are independent of T cells for the production of anti-LG3 antibodies, but the help of T cells is necessary for the production of anti-LG3 antibodies. T lymphocytes play an important role in anti-LG3 antibody production. This theory is supported by the decreased antibody level at the end of immunosuppressive treatment applied with calcineurin inhibitors [62].

Collagen

Late- term kidney allograft loss associated with chronic allograft nephropathy is one of the main problems threatening the long-term success of kidney transplantation. Transplant glomerulonephropathy is characterised by glomerular basal membrane

duplication and has been associated with chronic renal allograft rejection. The development of antibodies against collagen IV and fibronectin has been reported in kidney transplantation patients diagnosed with transplant glomerulonephropathy [63]. IFN-γ and IL-17 are expressed in these patients, and an increase has been determined in CD4+T cells specific to collagen IV and fibronectin, and a decrease in the cytokine IL-10 level. This indicates the role of collagen IV in the pathogenesis of chronic rejection [63].

HY Antigens

Proteins encoded in the Y chromosome have a sequence of similarity at the rate of 90% to homologs in the X chromosome. For example, RSP4Y protein (40S ribosomal protein S4, Y isoform 1) in the Y chromosome shows a difference with a change in 19 amino acids when compared to RSP4X variant which is based on a single nucleotide polymorphism [64]. Following the identification of HY-specific alloimmune T cells in transplants with gender mismatch, HY alloantibodies have also been determined to have been observed in individuals with acute allograft damage [65]. In a retrospective cohort study, long-term negative effects were reported of kidney transplantations made between different genders [66].

Other non-HLA Antibodies

The endothelium covers the interface between the graft and the recipient tissue. The first target of the antigens expressed by these cells is the recipient immune system. Antibodies against 4 non-HLA endothelial antigens have been determined in the serum of renal transplantation patients; endoglin, FMS-like tyrosine kinase-3 ligand (FLT-3) EDIL3, and ICAM4. Endoglin is a membrane glycoprotein expressed primarily in the vascular endothelium, which regulates angiogenesis and revascularisation [67]. FLT-3 is a tyrosine kinase that regulates cell differentiation, survival, and proliferation [68]. FLT-3 signal activation is thought to promote multiple myeloma angiogenesis [69]. EDIL3 is expressed by endothelial cells and is associated with the extracellular matrix. EDIL3 expression inhibits leukocyte-endothelial adhesion [70]. ICAM4 is known as a Landsteiner-Wiener blood group antigen. It is a one-way transmembrane protein and provides leukocyte binding by entering into interaction with integrin [71]. The presence of these antibodies has been associated with post-transplant donor-specific HLA antibodies, antibody-mediated glomerulopathy, and early transplant glomerulopathy [72] (Figure 5).

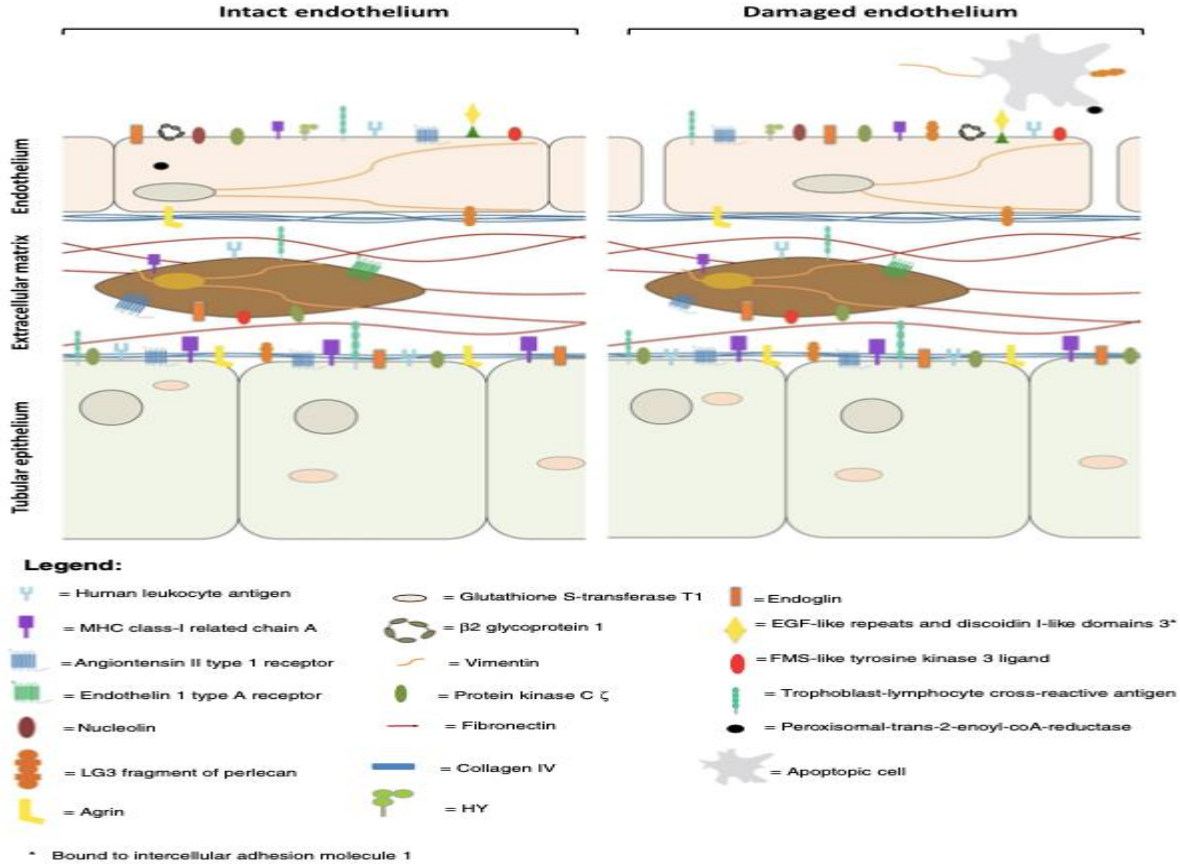

Figure 5: Localization of non-HLA antigen targets in the peritubular capillary in the quiescent state following endothelial damage [66].

Suggestions and Future Perspectives

Antibodies play a crucial role in transplantation, influencing both the success and potential complications of the procedure. The personalized characterization of antibodies is crucial in various fields such as biotechnology, biomedical research, and drug development. The fundamental steps of the characterization process include the binding properties to specific antigens, the impact of immunoglobulin structure on biological activity, functional activities such as cellular immune stimulation, post-administration immunogenicity and immune response, stabilization, post-translational modifications, and cross-reactivity. This process plays a critical role in the development of therapeutic antibodies and provides essential evaluation in clinical applications.

The evaluation of immunological risk in kidney transplantation is based on the evaluation of alloimmunity and anti-HLA antibodies. ABMR due to anti-HLA antibodies is evaluated with several different clinical tests. However, recent studies in this field have shown that immunological risk evaluation only focussed on anti-HLA donor-specific antibodies is insufficient. Moreover, in addition to the evaluation of non-HLA antibodies in respect of the immunological risk, it can also be of benefit in terms of graft survival, preventing graft rejection, and case management. There is increasing importance of non-HLA antibodies in addition to the classic HLA antibodies in renal transplantation. Many non-HLA antibodies have been identified in kidney transplantation patients, and with proteomic approaches, more potential antigen targets have become detectable. However, the full clinical importance of non-HLA antibodies in renal transplantation is prevented because of extremely heterogenic study designs with differences in test methods, immunsuppresive regimens, and result measurements. When the great differences in antibody incidence reported even in the same tests and the technical difficulties of the existing non-HLA antibody tests are taken into consideration, it can be seen to be important that efforts continue to be made on the subject of developing reliable and sensitive diagnostic tests. Moreover, the establishment of an antibody panel instead of a single antibody will be able to provide information about the role of non-HLA antibodies as an aid to defining the rejection and graft survival risk profiles. Non-HLA antibodies can be co-produced in certain types of transplants. Further studies in this area should be increased. The development and widespread use in clinical practice of a new Luminex-based test which will test more than one non-HLA antibody at the same time, will be extremely important in the success of graft survival.

2. Conclusions

Antibodies play a crucial role in transplantation, influencing both the success and potential complications of the procedure. The personalized characterization of antibodies is crucial in various fields such as biotechnology, biomedical research, and drug development. The fundamental steps of the characterization process include the binding properties to specific antigens, the impact of immunoglobulin structure on biological activity, functional activities such as cellular immune stimulation, postadministration immunogenicity and immune response, stabilization, post-translational modifications, and cross-reactivity. This process plays a critical role in the development of therapeutic antibodies and provides essential evaluation in clinical applications.

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Limitations of the Study

The full clinical importance of non-HLA antibodies in renal transplantation is prevented because of extremely heterogenic study designs with differences in test methods, immunsuppresive regimens, and result

measurements. When the great differences in antibody incidence reported even in the same tests and the technical difficulties of the existing non-HLA antibody tests are taken into consideration, it can be seen to be important that efforts continue to be made on the subject of developing reliable and sensitive diagnostic tests. Moreover, the establishment of an antibody panel instead of a single antibody will be able to provide information about the role of non-HLA antibodies as an aid to defining the rejection and graft survival risk profiles. Non-HLA antibodies can be co-produced in certain types of transplants. Further studies in this area should be increased. The development and widespread use in clinical practice of a new Luminex-based test which will test more than one non-HLA antibody at the same time, will be extremely important in the success of graft survival.

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Author Contributions
MA: Conceived and designed the study, collected data and wrote the manuscript. EB, NA: Contributed to study design, supervised the work. All authors have read and approved the final version of the manuscript.

Ethical Approval
None.

Data sharing statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent to participate
None.

Informed Statement
None.

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