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Review article

Complement and complement regulatory protein in allogeneic and xenogeneic kidney transplantation

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ABSTRACT

Kidney transplantation is the most optimal treatment for patients with end-stage renal disease, offering significant improvements in patient outcomes over dialysis. However, the potential for immune rejection, where the recipient's immune system attacks the transplanted kidney, can compromise transplant success. The complement system, a key component of the immune response, plays a crucial role in both acute and chronic rejection, including T-cell- and antibody-mediated rejection. Understanding and controlling the complement system is essential for managing rejection and enhancing graft survival and overall success of kidney transplantation. In allogeneic transplantation, complement activation through various pathways contributes to graft damage and failure. Recent advancements in genetic engineering enable the development of transgenic pigs expressing human complement regulatory proteins, which display potential for reducing rejection in xenotransplantation. Despite these advances, the complex mechanisms of complement activation and regulation are not fully understood, necessitating further research. This review examines the role of the complement system in kidney transplantation, explores the latest developments in complement regulatory strategies, and discusses potential therapeutic approaches to improve transplant outcomes.

1. Introduction

Kidney transplantation is the most effective treatment for end-stage renal disease, offering improved quality of life and survival compared to dialysis [1–4]. However, the success of transplantation is sometimes hindered by rejection, where the immune system of the recipient attacks the transplanted organ [5,6]. The complement system, a crucial component of immunity, plays a significant role in this rejection process. Understanding the dynamics of complement activation and regulation in allogeneic and xenogeneic kidney transplantation is essential for enhancing graft survival and improving transplantation outcomes.

In allogeneic kidney transplantation, where the donor and recipient are of the same species, the complement system contributes to both acute and chronic rejection. The complement system is primarily involved in antibody-mediated rejection through the classical pathway [7]; it also plays a role in T cell-mediated rejection [8,9]. Acute rejection is characterized by a rapid immune response, involving the activation of complement pathways and the formation of membrane attack complexes (MACs) that damage the graft. In contrast, chronic rejection involves long-term immune responses and fibrosis, with complement components playing a subtle, yet persistent role [9]. In kidney xenotransplantation, in which the donor and recipient are of different species, more pronounced immune responses occur, particularly in antibody-mediated rejection [10]. Preformed natural antibodies against xenogeneic antigens can trigger hyperacute rejection, a rapid and severe immune response mediated by the complement system. Advances in genetic engineering result in the development of transgenic pigs expressing human complement regulatory proteins (CRPs), such as membrane cofactor protein (MCP, also known as CD46), decayaccelerating factor (DAF, also known as CD55). These developments are expected to mitigate these responses and extend graft survival.

Despite these advances, the precise mechanisms of complement

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activation and regulation in the transplant setting remain incompletely understood. Further research is needed to elucidate these pathways and develop targeted therapies to effectively control complement-mediated damage. This review provides a comprehensive overview of the current understanding of complement and complement regulatory factors in kidney transplantation, with a specific focus on xenotransplantation, highlighting recent advancements and future directions for improving transplantation outcomes.

2. Complement and complement regulatory factors

The complement system, a critical component of the immune response that identifies and eliminates pathogens [11–14], is composed of soluble proteins that are present in the blood and CRPs on the surface of cells. These proteins are activated in a specific sequence to facilitate pathogen removal [15]. These proteins are primarily synthesized in the liver and also in the renal tubular epithelial cells of the kidneys [16,17]. This process is known as the "complement cascade" and is activated through three main pathways: the classical, lectin, and alternative pathways (Fig. 1). The classical pathway is initiated when an antibody binds to a pathogen [18]. The lectin pathway is activated when mannose-binding lectin or ficolins recognize sugar chains on the surface of pathogens [19]. The alternative pathway is initiated when C3 spontaneously splits on the pathogen surface. CRPs regulate and inhibit the activity of the complement system to prevent damage to host cells and tissues [20,21] (Table 1). These factors ensure that the complement cascade is directed towards pathogens and altered cells, avoiding unnecessary tissue inflammation and damage. DAF is a membrane-bound glycoprotein that inhibits the activation of complement components such as C3b and C5b by blocking the formation of C3 and C5 convertases, thus interrupting the complement cascade on cell surface [22-24]. MCP is another membrane-bound glycoprotein that binds to the complement components C3b and C4b, and inactivates them together with Factor I as a cofactor [25-27]. DAF and MCP are human genes that have been transferred into pigs for xenotransplantation, which will be discussed later in this review. Overall, CRPs are crucial for

Alternative pathway Classical pathway Lectin pathway C1 MASP C3 → C3b C4 C4 C.2 C3 C4h C2h C4b C2b C3bBb C4bC2b СЗа C3b C3bBbC3b C4bC2bC3b C5 C5a C5b C5b-C9 Terminal complement complex

Fig. 1. The complement cascade.

Table 1

	Th	e role	and	function	of	the co	ompl	lement	regu	latorv	proteins
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	Role	Function	References
Factor H	Inhibition of the alternative pathway	Binds to C3b and promotes its inactivation, thereby inhibiting the complement cascade	[28–30]
C4 Binding Protein	Regulation of the classical and lectin pathways	Inactivates C4b and controls the progression of the complement cascade	[31,32]
Decay- Accelerating Factor	Interruption of the complement cascade on cell surfaces	Promotes the dissociation of C2s from C4bC2a and Bb from C3bBb, thereby preventing the formation of these C3 convertases	[23–25,33,34,35–41]
Membrane Cofactor Protein	Inactivation of complement components	Binds to C3b and C4b and inactivates them in conjunction with Factor I	[26,27,42,43,44]
CD59	Protection of host cells	Binds to C8 and C9 components, preventing the formation of the membrane attack complex on host cells and protecting them from lysis	[45,46,47]
Clusterin	Cell protection	Binds to C5b-7 in the fluid phase, inhibiting membrane-bound membrane attack complex formation	[48]
Complement Receptor 1	Removal and regulation of complement	Binds to C3b and C4b, aiding in the inactivation of complement components and removing immune complexes containing complement	[49,50]
Vitronectin	Cell protection	components Binds to C5b-7 in the fluid phase, inhibiting membrane-bound membrane attack complex formation	[51,52]

maintaining the balance of the immune response, ensuring its efficiency against pathogens while preventing collateral damage to host tissues.

3. Complement in antibody-mediated rejection of allogeneic kidney transplantation

Antibody-mediated rejection (AMR) could occur if the recipient has donor specific anti-HLA antibodies (DSAs) or anti-blood group antibodies in the blood [42]. DSAs are produced as a result of sensitization through blood transfusions, pregnancies, or transplants [53]. DSAs found in the blood before kidney transplantation are referred to as preexisting or preformed DSAs, while newly produced DSAs after the transplantation are referred to as *de novo* DSAs. Whether pre-existing or *de novo*, DSAs pose a risk for AMR as they particularly target the blood vessels of the transplanted organ. In this process, the complement system plays a crucial role [7]. The classical pathway is specifically activated when DSA binds to the HLA antigens or other endothelial antigens of the transplanted organ. This activation eventually promotes the cleavage of C3 and C5, producing inflammatory complement breakdown products, such as C3a and C5a, which are potent inflammatory inducers that promote the attraction and activation of leukocytes, thus increasing the permeability of blood vessels. Activation of the complement system also plays a role in enhancing antibody-dependent cellular cytotoxicity. When complement components such as C3b bind to antibody-coated target cells, immune cells such as NK cells, macrophages, and neutrophils recognize and destroy these target cells. Complement activation markers, especially C4d deposition in peritubular capillaries, are evaluated in biopsy samples as diagnostic indicators of AMR. In ABOincompatible kidney transplantation, similar immune responses are triggered by anti-blood group antibodies. Although ABO-incompatible kidney transplantation has shown very good outcomes with the use of rituximab and plasma exchange [54,55], many challenges remain in DSA-positive kidney transplantation.

DSA-positive kidney transplantation carries a high risk of developing AMR and graft loss if performed without treatment. Owing to the absence of effective treatments for AMR, performing desensitization therapy using immunoglobulins or plasmapheresis before transplantation is crucial as a preventive measure [56–58]. Desensitization strategies that inhibit complement system activation, such as eculizumab (a monoclonal antibody anti-human C5), are being explored. A study showed that eculizumab significantly reduced AMR incidence within the first three months post-transplant (7.7 % vs. 41.2 %) and lowered transplant glomerulopathy at one year [59]. However, no differences in long-term graft survival or chronic AMR incidence were observed after two years [60]. Further studies suggested eculizumab might not improve long-term outcomes in crossmatch-positive transplants, though it might reduce early AMR, particularly grade I [61,62]. While the potential benefits of eculizumab for the AMR prevention and treatment have been demonstrated, further investigation is necessary. DSAs, which bind to C1q or C3d, carry a higher risk of graft loss and are linked to lower graft survival rates [63-66]. C1 inhibitor (C1-INH), part of the serpin family, deactivates C1r and C1s and regulates the classical complement pathway. In a Phase I/II placebo-controlled trial for AMR prevention, a reduction in C1q-binding HLA antibodies was observed [67]. However, a Phase IIb randomized, double-blind, placebocontrolled study for treatment showed no significant difference in day 20 pathology or graft survival [68]. It is suggested that the C1-INH dose might have been insufficient. A study in a non-human primate model found that Cp40 (a C3 inhibitor) could prevent early antibody-mediated rejection and significantly prolong graft survival in the presence of high levels of donor-specific antibodies [69]. While eculizumab is used alongside desensitization therapies like IVIG and plasmapheresis, C3 inhibitors are effective even without desensitization.

4. Complement in T-cell mediated rejection of allogeneic kidney transplantation

While the complement system is mainly associated with AMR, it may also play direct or indirect roles in T cell-mediated rejection. A study analyzing allograft TCMR biopsies reported that the mRNA levels of complement-related genes such as C1S, C3, CFB, CFH, CR1, and SERP-ING1 were significantly elevated compared to those in the control group. Moreover, approximately 75 % of the analyzed complement-related genes were associated with cold ischemia time and inflammatory markers, indicating that complements played a significant role in transplant pathology, partly driven by cold ischemia time [70].

An important consideration in understanding the role of complement in TCMR is that C3 is produced by transplanted kidneys, and its gene expression increases during TCMR [71]. The locally synthesized complement component C3 modulated rejection of renal allografts *in vivo* and regulated T-cell responses *in vivo* and *in vitro* [8]. In this study, wildtype kidney transplants showed rapid rejection within 12.5 days, while C3-deficient grafts functioned for over 100 days. Experiments with C4null donor kidneys revealed no link between C4 and graft survival. Therefore, the alternative pathway was regarded as the main pathway involved in TCMR. DAF regulates T cell differentiation by modulating IL-12 secretion from APCs. This IL-12 secretion stimulates a greater number of responding T cells to differentiate into IFN-y-producing effector cells [72]. Additionally, downregulation of DAF in dendritic cells increases the C3a and C5a production [33]. We previously reported that the expressions of C3, C3aR, C5aR, Factor B, C9, and C1q, but not C4 and C5, increased during rejection in a rat kidney transplant model [9]. We also observed that CD59 and the rodent-specific complement regulator complement receptor 1-related gene/protein-y (Crry) were downregulated by rejection. Furthermore, the administration of anti-Crry and anti-CD59 mAbs decreased graft survival. We also investigated the association between MCP and TCMR in human transplanted kidneys and revealed that higher MCP expression levels were correlated with better serum creatinine levels and significantly higher 5-year survival rates.

Despite the limited complement role in TCMR, the complement system activation may be an important factor in graft damage pathophysiology. Therefore, therapeutic strategies targeting the complement system might be useful in managing TCMR under certain circumstances.

5. Complement in xenogeneic kidney transplantation

5.1. Immunological background

The complement system plays an important role in xenotransplantation. After the incompatibility of the complement system reaction between pigs and humans was reported [10], species-specific carbohydrate antigens were identified [73], and further research on other immune responses was conducted. In xenotransplantation, xenoantigens, such as α -Gal (Gala1,3Gal: GGTA1) and Neu5Gc (Hanganutziu-Deicher antigen: CMAH), are the main factors that cause hyperacute rejection [74].

First, α -Gal is a carbohydrate antigen expressed on the cell surface of all mammals, especially in pigs. However, it is not expressed in Old World monkeys and humans. Therefore, humans have many natural antibodies against α -Gal [75]. In 2005, kidneys from α -Gal knockout pigs were transplanted into baboons using a tolerance induction protocol that included thymectomy, splenectomy, and anti-CD154 monoclonal antibodies to suppress T cell secondary stimulation, and the baboons survived for up to 83 days [76].

Next, humans do not produce Neu5Gc, however, most mammals have this carbohydrate on their cell surfaces. The human immune system recognizes Neu5Gc as a foreign antigen and produces antibodies against it [34]. When these antibodies bind to the foreign antigens, the complement system is activated, leading to the formation of MAC and cell destruction. Protecting porcine xenografts from human complement attack is thought to be difficult because they express porcine CRPs instead of human CRPs.

Moreover, the Sda antigen is a glycan antigen generated by the action of β 4GALNT2 [74]. Its antigenicity is noted because pigs are (+), while the monkeys used in the experiment are (-). Since there are also Sda (-) individuals in humans, it is the subject of knockout. There is also a theory that this antigenicity is a glycan antigen formed by the difference in function of β 4GalNT2 between pigs and humans [77].

5.2. Insights from allogeneic transplantation to xenotransplantation: ABO blood-type incompatibility

The findings from kidney allografts are applicable to xenografts. For example, ABO blood-type incompatible kidney transplantation, which was previously a major barrier due to allogeneic carbohydrate antigens, has been almost overcome in clinical practice by using rituximab and plasma exchange to prevent AMR caused by blood type carbohydrate antigens and antibodies [54,55]. The expression of human CRPs likely contributes to this success, potentially making rejection relatively easier

to avoid. However, in xenotransplantation, the expression of xenogeneic carbohydrate antigens, such as Gal, is much higher, leading to initial attempts to knock out these antigens to prevent rejection. Additionally, even if Gal or CMAH is knocked out, the other antigens remain. Therefore, the expression of human CRPs is the second clinical step to prevent rejection in xenotransplantation, and it is expected that they must be expressed in sufficient amounts [78]. It is also noteworthy that the alternative complement pathway is not usually activated in antigenantibody reactions in allogeneic transplantation but is activated in xenotransplantation. Reports have shown that the perfusion of human blood into pig hearts rapidly depletes natural antibodies and reduces the activity of the classical complement pathway while mainly activating the alternative complement pathway [79]. Similarly, in the case of kidney xenotransplantation, the alternative pathway may also be activated. This can be controlled by the genetic expression of human CRPs. As a result, to overcome human complement attack in xenotransplantation, a genetic engineering approach has been employed that includes knockout of the three major xenoantigens (α-Gal, Neu5Gc, Sda: b4GALNT2) along with gene transfer of human CRPs into the xenotransplant.

5.3. Insights from allogeneic transplantation to xenotransplantation: Relationship between DSA and SLA, and innate immune cells

We also discussed DSA in the allogeneic transplantation section. Not only carbohydrate antigens but also de novo protein antibodies are problematic in xenotransplantation. It is worth noting that some humans have antibodies against swine leukocyte antigens (SLAs). In a study examining the sera of 52 patients waiting for organ transplants, 14 patients were found to have antibodies that reacted to 18 different SLA class I (SLA-I) proteins. These antibodies included IgM, IgG, or both, and some reacted only to specific SLA alleles, while others recognized multiple SLA-I proteins [80]. Therefore, pigs with multiple gene knockouts, including SLA-I, have been created and described to effectively reduce human antibody binding. However, it also described that combining SLA-I knockouts with other genetic modifications did not necessarily further suppress complement activation [81]. In allogeneic kidney transplantation, DSAs, especially against HLA-DQ, are known to cause transplant glomerulopathy and reduce long-term graft survival. Compared with recipients without DSAs, recipients with de novo DQ-DSA alone or with DQ-DSA plus other DSAs had higher acute rejection rates, higher risk of graft loss, and lower 5-year graft survival rates [82]. Similarly, a study examining serum from four rhesus macaques that survived more than 300 days after porcine kidney xenotransplantation revealed that all recipients had DQ-SLA and transplant glomerulopathy [83]. These results suggest that, as in allotransplantation, de novo DQ-SLA may be a barrier to improving longterm outcomes in xenotransplantation. In response, the knockdown of MHC class II transactivator is being applied for SLA-II [84], or knockout of SLA-class II genes is being studied.

On the other hand, if AMR occurs in allogeneic transplants, NK cells and macrophages are activated in association with complement deposition in allogeneic kidney transplants. In the case of xenotransplantation, it has been demonstrated that human macrophages can phagocytose pig cells even in the absence of antibody or complement opsonization, indicating that innate immunity represents a significant immunological obstacle to xenotransplantation [85]. Studies are being conducted to control this reaction by expressing molecules such as CD47 in xenografts [86]. Our studies have also reported the usefulness of recombinant expression of HLA-E, CD200, CD177, and TIGIT in controlling NK cells and macrophages *in vitro* studies [87–90]. However, research on the control of these innate immune cells, especially neutrophils, is still incomplete, although studies are underway to explore their regulation by CD31, and CLSP-D [91–94].

We will describe the features of each CRP that has been genetically introduced. First, human DAF is a phosphatidylinositol-anchored

protein. DAF consists of four short consensus repeats (SCRs), a Ser/Thrrich (ST) region, and a glycosylphosphatidylinositol anchor. It facilitates the dissociation of C3 convertases, C4b2a and C3Bb. SCR2 and SCR3 play a role in the dissociation of C4b2a, while SCR2 to SCR4 contribute to the dissociation of C3bBb [35-37,95]. It exists in both membranebound and soluble forms. The former is expressed in various cell types, particularly within the vascular space, where it prevents unwanted complement activation, and in other tissues such as the dermis and muscular layers of the intestine [38]. Furthermore, DAF binds to CD97 on T cells via SCR1 and regulates T cell activation and differentiation into type 1 regulatory T cells [39]. Experiments with porcine endothelial and fibroblast cells expressing different DAF variants (delta-SCR1-DAF, delta-SCR2-DAF, delta-SCR3-DAF, and delta-SCR4-DAF) assessed their effect on human NK cell-mediated cytolysis. The results showed that DAF variants lacking the SCR2 and SCR3 regions were unable to inhibit NK cell activity, whereas a variant lacking the SCR4 region was effective in regulating complement but did not reduce NK cell-mediated cytolysis. These results highlight that DAF directly suppresses the cytotoxic function of NK cells via the SCR2-4 region, independent of its role in complement regulation [40].

MCP, in turn, is a single-chain glycoprotein composed of four SCRs, an ST region, and different cytoplasmic tails. MCP acts as a cofactor for factor I in the serum and controls the irreversible reaction that degrades C3b and C4b into iC3b and C4d, respectively, components of the C3 convertase that forms on the same membrane [25–27]. SCR2–4 are involved in the binding and degradation of both enzymes, and the N-glycans of SCR2 and SCR4 are necessary for their function [41]. MCP, which is also involved in regulating T cell activation, functions as a key regulator of both $CD4^+$ and $CD8^+$ T cell immunity by mediating nutrient influx and enhancing fatty acid synthesis [43]. CD59 (Membrane-Attack Complex Inhibition Factor: MACIF, Homologous Restriction Factor: HRF or Protectin) binds C8 and C9 to inhibit the formation of MAC [44].

Next, we looked at these CRPs in preclinical studies. As evidenced by several key studies, great progress has been made in extending xenograft survival through gene transfer of human CRP into pigs. Cozzi et al. transplanted kidneys from human DAF transgenic pigs into bilaterally nephrectomized cynomolgus monkeys that had undergone splenectomy and were immunosuppressed with cyclosporine A, cyclophosphamide, and steroids. This approach resulted in the longest reported survival time of 78 days, demonstrating the importance of transgenic human CRP in xenotransplantation [45]. About 20 years later, Kim et al. reported survival of more than 1 year in xenotransplantation using α -Gal knockout/DAF transgenic pigs. They investigated the role of CD4+ T cells in kidney xenotransplantation from pigs to rhesus monkeys and found that CD4+ T cell depletion significantly improved graft survival, with some animals surviving for over 400 days. They also observed that pretransplant selection of recipients with low levels of anti-pig antibodies combined with selective CD4⁺ T cell depletion was essential to achieve long-term xenograft survival [96].

Meanwhile, the efficacy of human MCP transgenic animals has also been widely reported. In 2001, the effect of splenectomy and immunoadsorption (IA) in transplanting human MCP transgenic pig kidneys into baboons was investigated. The mean survival time in the control group was 3–7 days, while splenectomy and IA extended this period to 13–15 days [97]. Other studies reported that human MCP transgenic pig kidneys exhibited the ability to suppress complement-mediated rejection when transplanted into baboons, significantly prolonging graft survival [98].

In summary, in recent studies with typical genetically modified pigs, DAF is the most commonly transgenic complement regulator, followed by MCP, which is often co-transgenic with DAF. A study in which Yucatan miniature swine were gene-edited at 69 sites to knock out three carbohydrate antigens (α Gal, Neu5Gc, Sd(a)), express seven human transgenes including DAF and MCP, and inactivate porcine endogenous retroviruses (PERV) reported prolonged graft survival (up to 758 days) [99]. Immunosuppressive induction therapy included B- and T-

lymphocyte depletion, followed by maintenance therapy with anti-CD154 antibodies and mycophenolate mofetil. In addition, the regimen included a short course of tacrolimus and steroids to manage the immune response of the primate recipients. In the same study, expression of the human transgenes effectively inhibited complement activation, controlled blood clotting, and contributed to a reduction in graft rejection. CD59 is a small molecule with limited species-specific diversity, and the use of human CD59 is considered unnecessary, as porcine CD59 may function adequately [100]. CD59-transgenic pigs are less frequently used.

In addition to CRP transgenics, other genetic modifications have been reported, such as knockout (KO) of Growth Hormone Receptor (GHR) or PERV, or transgenics of CD47, Thrombomodulin (THBD), Tumor Necrosis Factor Alpha-induced protein 3 (TNFAIP3), Heme Oxygenase-1 (HMOX1), and Endothelial Protein C receptor (EPCR). The pigs used in the experiment that reported long-term survival of 758 days had knockout of three glycan synthesis genes, insertion of transgenic constructs containing seven human genes (MCP, DAF, THBD, EPCR, CD47, TNFAIP3, HMOX1), and KO of PERV [99]. In 2022, a genetically modified pig heart was transplanted into a 57-year-old man with nonischemic cardiomyopathy who was dependent on veno-arterial extracorporeal membrane oxygenation and had no access to standard treatment. The pig was genetically modified to knock out three major xenoglycoantigens and GHR, introduce seven human transgenes, including DAF, MCP, CD46, THBD, EPCR, TNFAIP3, and HMOX1, and inactivate PERV [101]. Over the past approximately 20 years, studies of pig kidney xenotransplantation in nonhuman primates and brain-dead humans [102] have advanced our understanding of the immune response to xenotransplantation, but the optimal immunosuppressive regimen for live human recipients remains unclear. However, in 2021, it was demonstrated that eculizumab, an anti-C5 monoclonal antibody, could prevent thrombotic microangiopathy (TMA) in pig-to-brain-dead human xenotransplantation [103]. In summary, kidneys from pigs genetically modified with 10 genes, including DAF and MCP, were transplanted into three brain-dead humans, two of whom received eculizumab as part of the immunosuppressive regimen. In the subject who did not receive eculizumab, TMA and MAC deposition was observed in the transplanted kidney immediately after transplantation. In contrast, in the two subjects who received eculizumab, although MAC deposition was observed in subject 3, the immune response was reduced, likely due to insufficient therapeutic blood concentration of eculizumab. In this subject, TMA was not detected by blood tests.

6. Conclusion

In kidney transplantation, the classical pathway of the complement cascade is primarily involved, contributing to antibody- and T cellmediated rejection. Complement regulators are essential to mitigate these effects by inhibiting different steps of the complement cascade. Enhancing the expression and function of these regulators may increase graft survival and improve transplant outcomes.

In kidney xenotransplantation, hyperacute rejection is more prominent because natural antibodies against donor antigens are already formed. In addition, the alternative pathway also becomes significantly involved. This requires a strong and targeted complement inhibition strategy. To address this challenge, the use of genetically modified porcine organs for human CRPs, such as DAF, MCP, and CD59, is investigated. These approaches aim to prevent hyperacute rejection and allow for xenotransplant adaptation.

Overall, regulation of the complement system is essential for the success of allogeneic and xenogeneic kidney transplantation. Future studies should focus on optimizing the use of complement inhibitors and regulators to improve graft acceptance and longevity. Better understanding of complement-mediated immune responses and developing targeted therapies may improve transplant outcomes for patients with end-stage renal disease waiting for a kidney transplantation.

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Author contributions

YK and SM were responsible for conceptualization, investigation, methodology, project administration, supervision, writing original draft and review, and editing. The other author contributed to resources, validation, data curation. All authors were involved in the preparation of the article and approved the final submitted version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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