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Review Article

Diagnostic Challenges and Immunological Barriers in Kidney Transplantation

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ABSTRACT

The presence of donor specific antibodies and HLA-incompatibility between waiting list patients and potential donors make transplantation programs worldwide complicated and stand as a major barrier for a quick access for organ transplantation. Especially in kidney transplantation, the effector HLA antibodies are the most important reason for graft failures and antibody mediated rejection. At the same time, the demand for kidney transplantation is increasing remarkably worldwide, due to the continuously Raising number of patients with end stage renal disease. For these patients, hemodialysis was regularly considered as an intermediate step only and the ideal goal is to find a suitable kidney as early as possible. However, in case of HLA-incompatibility, the waiting time for an organ is too long and often accompanied with dialysis associated multiple diseases, including physical, social and psychological complications, especially in patients with additional limiting factors, like ABO incompatibility or multi-morbidity. This article provides an overview of the current diagnostic tools for an accurate testing, identification and analysis of HLA-antibodies and addresses the remaining concerns related to their clinical and immunological relevance. It reviews the increasing role of different technologies and approaches to overcome the immunological barriers in kidney transplantation, including the extracorporeal removal of clinically relevant antibodies. In addition, the review suggests avenues for future research on overcoming immunological challenges and inducing immune tolerance in kidney transplantation.

Keywords: Immunohematology; HLA antibodies; Immunogenetics; ABO Incompatibility; Transplantation immunology; Immune tolerance; Plasma exchange; C1q; Paired exchanged; T regulatory cells

INTRODUCTION

Since the introduction of Hemodialysis (HD) several decades ago, considerable developments and many improvements were achieved in treating patients with End-Stage Renal Disease (ESRD). Nowadays, the use of new procedures and innovative intra- and extracorporeal blood filtration systems allows the achievement of a proficient and better-tolerated renal replacement therapy [1,2]. Nevertheless, despite all efforts and successes in offering treatment with modern and high-quality HD, Renal Transplantation (RTX) remains superior to all kind of replacement treatments, which are still resulting in significant lower survival rates than RTX [3,4]. Beside higher mortality rate caused by HD related complications, kidney replacement therapies might be associated with more costs, which affect national social insurance systems economically. More importantly, till today kidney replacement therapies are stressful and not able to ensure a normal life style without enormous impairments and significant limitations for affected patients [3,5]. For the outlined reasons, RTX is considered as the best alternative for HD in patients suffering from ESRD. Consequently, the demand for kidney transplantation is increasing remarkably worldwide and HD is often considered as an intermediate target and optimal treatment of patients with ESRD is an early transplantation of a compatible kidney. However, immunological barriers, mainly

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Human Leukocyte Antibodies (HLA), hinder often fast kidney transplantation and must be pondered in each RTX procedure. After discovering the HLA molecules 1958, several studies in the following years resulted and indicated that HLA antigens might be responsible for immunological response [6-8]. It was shown that the most promising and successful RTX is that with absence of antibodies against HLA of the potential kidney donors, known as Donor Specific Antibodies (DSA) [9]. In this situation, RTX is easy to achieve, fast accessible and the outcome of RTX is regularly very convincing. In contrast, the presence of DSA and HLA-incompatibility between waiting list patients and potential donors make transplantation programs worldwide rather complicated, as the effector DSAs are the most important reason for RTX failures and Antibody Mediated Rejections (AMR) in renal transplanted patients [10-12]. In case of HLA-incompatibility, the waiting time for suitable organ is often too long and associated with multiple diseases, including physical, social and psychological complications, especially in patients with additional limiting factors, like ABO incompatibility or multi-morbidity [13,14]. Patients affected under those conditions are on a serious risk to die earlier as the mortality rate is unacceptable high in this group of patients [15-17]. This makes the HLA-incompatibility as one of the most challenging aspects for any kidney transplant program. Therefore, beside surgical technique and sufficient transplant nephrologist team with a suitable pre- and post-transplant care, accurate and professionally operating histocompatibility laboratory is the crucial part of each transplant program in overcoming immunological barriers and reducing significant transplant risks. The same might apply for Immunohematology and transfusion medicine in case of equipment-based technical removal of involved HLA-antibodies, which needs sufficient and special expertise and know-how. This knowledge should include, technical skills, apheresis experiences, a proper understanding of the development's nature and physiological kinetics of effector HLA-antibodies along with their intra- and extracellular behavior. In addition, a better consideration of the underlying immunological mechanisms of immune competent HLA-antibodies and the exact exploration of their clinical significance and ability in inducing AMR through activation the complement cascade. In this article, we report about our experience in HLA testing using current diagnostic tools, focus on the identification and analysis of HLA-antibodies and address the remaining challenges and concerns related to their diagnostic and clinical relevance. Furthermore, we address the increasing role of different technologies and approaches, including the extracorporeal removal of clinically relevant antibodies in overcoming major immunological barriers in kidney transplantation based in our own experience as a large kidney transplant center and based on available literature data. Finally, we suggest avenues for future research on overcoming immunological barriers by inducing immune tolerance in kidney transplantation.

LITERATURE REVIEW

Assays for detection of HLA antibodies

The review of published data about RTX showed that the outcome and successful rate of transplanted patients with HLA antibodies directed against donor tissue are considerably lower than transplanted patients without HLA antibodies, especially if crossmatch results are positive between donors and recipients [9,18-22]. This is the reason, why it is of the utmost important and essential for each transplant center to have a competent and state of the art histocompatibility diagnostic laboratory. To support these critical responsibilities and obligations of the HLA-lab, innovative and advanced technologies were developed in the recent years like Flow Cytometry for Crossmatch Testing (FCXM), Enzyme-Linked Immunosorbent Assay (ELISA) and the beads array for Luminex© (LMX) platform for detection of antibodies [23-27]. The last listed technology is however considered to be the most sensitive technique for the identification of HLA-antibodies introduced so far [28-30]. It is a solid phase fluorescent test and detects antibodies that bind direct to natural or purified recombinant HLA-antigens. In a first step (screening), the beads are bound, on its surface, with a large number of class 1 or class 2 molecules derived from lymphoblastoid cell lines (natural antigens). In case of the presence of HLA antibodies in patient's serum, the Luminex screening test provides positive result only, without the exact identification of the HLA-antibodies. For the identification and differentiation of HLA antibodies, beads with single HLA molecules are used. These single antigen beads, known as SAB, are produced per a recombinant technology, which is flexible and enough sufficient to produce the most clinically relevant HLA antigens. For this reason, the beads are the core of the Luminex technology and provide a real advantage to detect and identified accurately complex mixtures and large number of HLA antibodies. This allows a successful transplantation even in the presence of DSA that have less clinical relevance, e.g. Cw antibodies. Hence, beads array-SAB test is rapidly increased and now widely used in many transplant centers worldwide. In this very specific test, HLA-IgG antibodies against well-defined HLA-antigens are detected using a fluorescent anti-IgG detection antibody. The intensity of the fluorescence reflects the strength of HLA antibodies and is measured using two color laser technology and high-speed camera. The test is semi quantitative and the results are given as Mean Fluorescence Intensity (MFI). However, despite the widespread use of this innovative LMX platform the clinical relevance of HLA-antibodies detected by beads array particularly those with weak, moderate and in some cases even with strong MFIs is controversial and remains until now a major theme and a matter of unsatisfied debate. To date there are no consensuses, quantitative levels or standard cutoffs as a surrogate for clinical relevant HLA-antibodies detected by LMX technology, which can be uniformly and globally used [31-33]. Cutoffs varied significantly from one transplant center to other and each transplant facility defines its own threshold in accordance with its individual transplant experiences and procedures for finding and dealing with clinically relevant antibodies. Our transplant center considers MFI>600 as a positive for DSA, other centers push the barrier to 1000 or even 2000 MFIs [34,35]. With this unconvinced reality in mind, some renowned transplant organizations, like European Federation of Immunogenetics (EFI), remains recommending the "historical" Complement Dependent Cytotoxicity Test (CDC) for crossmatch testing or to use a technique for crossmatch that is equivalent to CDC or superior in sensitivity [36]. However, CDC is old methodology and extensive technique, requires complement and viable target cells, detects non-HLA antibodies and depends

totally on subjective assessment. Furthermore, CDC has not the desired sensitivity and thus it is not able to identify all complement binding or clinical relevant HLA-antibodies, especially those with low or moderate titers [37,38]. As a consequence, many transplant centers, including ours, are still combining high sensitive assays, like Luminex technology and the less sensitive CDC test in the diagnostic and evaluation of clinical relevant HLA-antibodies [39-42]. Regardless which methodology is used, performing of the HLA tests and interpretation of the results of old and new technologies need in all cases, advanced special technical skills and advanced expertise in transplantation immunology, immunogenetics and histocompatibility? Some HLA tests performed by combined old proven methods and new highly sensitive diagnostic tools can be very challenging and too complex. Thus, HLA labs should be cautious with interpretations and conclusions. For example, positive cross match results performed by new sensitive tests should not routinely ignore as "overly sensitive", when the less sensitive CDC crossmatch is negative. In generally the use of existing HLA-antibodies detecting sensitive and non-sensitive technologies in a combination might reduce the risk to overlook clinically relevant HLA antibodies. Nonetheless, it is advisable always to keep in mind that even the use of all HLA antibody test combinations cannot allow the prediction of AMR caused by effector HLA-antibodies with complete certainly. Rather, the experience showed us that HLA-antibodies differ significantly in their strength and ability to fix complement and thus in causing transplant rejection, even if they have the same specificity [43-45]. This should be a motivation and one more reason for us to look for more satisfactory alternative using complement binding technology with more complement fixing ability than the one observed by the less sensitive CDC assay.

For this reason Chen et al. developed a beads array-C1q test to detect complement binding antibodies, which mainly caused AMR by activation the complement cascade via classical pathway [46]. It has been postulated that fixing first component (C1) of the complement by clinical significant HLA-antibodies leads to activation of Membrane Attack Complex (MAC) through a formation of the terminal lytic fractions of the complement system C5b to C9. The MAC activation ends in cell lysis and tissue damage of transplanted organ. This means that HLA-antibodies detected by beads array-C1q might have more clinical relevance than those detected by other techniques. This assumption is apparently reported by different published studies, which found that C1q assay has a better correlation with rejection and clinical outcome in kidney transplantation than CDC [47-51]. However, other reported scientific analysis showed that C1 component may have only an indirect effect through inducing C3 fragment deposition, which blocks the binding of IgG detection elements by coating the surface of the beads resulting in false negative results [52,53]. Furthermore, a recently published study found that C1q binds weakly to low number of IgGs and this binding ability correlated positively with increasing density of IgG antibodies [54]. Hence, a negative C1q assay result does not exclude the presence of clinical relevant and complement-binding IgG HLA-antibodies, especially in the case of low titer HLA antibodies. On the other hand, a positive result resulting in C1q-fixing does not automatically mean the activation of MAC or tissue destruction of donated organ [55]. Recently the European Society for Blood and Marrow Transplantation (EBMT) issued consensus guidelines, which recommend the performing of C1q and even require it as a mandatory test in the setting of haploidentical bone marrow transplantation in case of moderate or strong positive anti-HLA DSA [56]. Even so, in our opinion it still remains unclear, whether C1q test can be established as a standard test in the pre-transplant diagnostic or not. Especially, under the impression of above described controversial and unsatisfied aspects, further extensive studies and investigations are needed to clarify and better evaluate the role of C1q test in HLA-diagnostic.

Renal transplantation through immunological hurdles

In recent years extensive efforts have been made and different options were discussed and identified with the view to transplant HLA-sensitized patients with ESRD early and prevent long-term dialysis associated complications [57-60]. One of the debated choices is the transplantation of donated organ through preformed anti-HLA DSA by suppressing the immune response using sufficient high dose immunosuppressant and by appropriate apparatus-based removal of effector DSA, which depends on different invasive techniques in a process called desensitization. In principle, there are existing different methods of desensitization, which based on the removing of the effector anti-HLA DSA using different technical methodologies or by inhibiting the building of DSA in targeting T and B cells (Table 1).

Table 1: Methods of desensitization to overcome immunological barriers in RTX.

Method	Mechanism	Frequency
Immunoadsorption	Removal of HLA antibodies	High
Therapeutic Plasmaphersis	Removal of HLA antibodies	High
Drug (Rituximab)	Anti-B cell effects by blocking of CD20	High
Drug (IVIGg)	Inhibition of C3b and C4b and neutralization of C3a and C5a and HLA-antibodies, competing for activating Fc-Rs	High
Drug (eculizumab)	Inhibition of complement cascade by binding of C5A	Medium
Drug (bortezemib)	Plasma cell inhibitors	Medium
Splenectomy	Prevention of HLA-building by removal of antibodies secreting B and plasma cells	Low

The desensitization as a "routine" therapeutic option is extensive to implement, especially in small transplant centers and it presents not insignificant challenges to be matched. In addition, it is not free of risks and side effects for the patients [61,62]. This raised the rational question, particularly in living RTX, whether it is a preferred solution to carry out RTX through the crossmatch or HLA-antibody barriers or whether it is more favorable and safer alternative to keep the patients on the waiting list till finding a compatible donor. This dilemma was faithfully and evidently addressed in a pioneering scientific study published by Montgomery and his research group already in 2011 [63]. Montgomery analyzed in a retrospective study the survival of renal-transplanted patients with preformed DSA, who underwent invasive desensitization procedures prior to living

kidney transplantation. Eight years after transplantation 81% of these patients were survived, compared with only 49% of patients were survived in a control group who were placed on the waiting list for compatible deceased RTX, regardless of whether they were transplanted during the observation period or not. After publishing this imperative study, different removal approaches and desensitization programs were established and increasingly used to overcome immunological hurdles in different parts of the world [63-66]. Besides these described methodologies, a new strategy of desensitization was proposed that relies on the IgG endopeptidase and its cleavage ability of DSA, by introducing the IgG degrading enzyme of Streptococcus pyogenes (IdeS) intravenously [67]. This study included 25 highly sensitized patients and all patients displayed near or complete reduction of DSA levels at 6, 24 hours and even few more days in some patients. According to this report, this significant reduction or elimination of DSA led to the transplantation of 24 out of 25 patients with HLA-incompatibility. However, the introduction of IdeS in vivo showed some adverse events include serious infectious complications that mainly in response to treatment, e.g. persistent myalgia and parvovirus viremia. We think that a better managing or avoiding the side effects of IdeS might result in a better acceptance of this technique in the transplantation medicine in the future. However, further investigations in a larger cohort prior to execution of IdeS desensitization in routine use are needed. Despite described challenges and unclear questions, the remarkable findings and exciting results in all above listed pioneering studies inspired and encouraged transplant staff to develop and use further invasive tools and noninvasive strategies to overcome immunological barriers in the field of organ transplantation, like Kidney Pair Exchange (KPE) and centralized Virtual Crossmatch (VC), marking a new era in kidney transplantation and resulting in a significant reduction of waiting time for transplantation [68-73]. One of the successful noninvasive strategies for overcoming immunological barriers is also the Acceptable Mismatch (AM) program, which was established in Europe 1988 to better facilitate kidney transplantation in multi-sensitized patients. According to this program deceased donor kidneys are mandatorily directed "pulled in" toward highly sensitized patients with cPRA>85% in the absent of DSA [74-76]. Unfortunately, despite all described appreciated efforts and creative methodologies, all discovered and currently existing noninvasive procedures can help only one part of HLA-immunized high-risk patients. The other part still requires additional invasive procedures and specific extensive treatments to overcome immunological barriers, especially the immunopathological effects of involved HLA-Antibodies [77]. Thus, it has become clear that desensitization as an invasive technique is now one of the most effective methods for facilitating sensitized patients with ESRD in overcoming immunological barriers prior to transplantation [78]. Using desensitization, high titer HLA-antibodies can be depleted or significantly reduced before transplantation. However, avoiding or a partial reduction of immunological obstacles and the evaluation of their remaining residual immunological risks for patients require staffs' outstanding knowledge and distinctive expertise in the nature and immune pathological mechanisms of involved HLA-antibodies. It requires moreover a close interdisciplinary teamwork and cooperation between the HLA-laboratory, blood bank staff on one side and the clinicians and transplant coordinators on the other side. The coordination and the sufficient support of whole transplant team are very important for every desensitization program, since the outcome of such elaborate and complex technique depends heavily on the motivation, the experiences and communication of all involved parties. Regularly training, meetings and consultations are critical and should be obligatory for the participating medical staff members. The clinicians need to inform the HLA laboratory about the patient's history, including underlying diseases and all possible sensitizing events, like previous transplants, blood transfusions, gravidities or miscarriages, recent viral infections, vaccinations, immunoglobulin applications or any other drugs might interfere with the results of HLA testing, e.g. Rituximab or Immunoglobulins. The transplant coordinators need to keep the communication between all involved parties and serve patients, lab and the clinical side accurate and timely with all transplant relevant logistics and information. The lab itself needs to perform adequate and appropriate screening and crossmatch tests of current sera and it should always take in consideration the previous HLA results and possible sensitization events as well, e.g. previous grafts. Furthermore, it is obligatory for the HLA-lab to be able to provide exact determination, differentiation and characterization of detected HLA-antibodies along with the antibody strength and titer as well as risk assessment and clinical relevance of all identified antibodies.

Experience of king faisal specialist hospital and research centre Jeddah

At our transplant center in King Faisal Specialist Hospital & Research Centre-Jeddah, we use the sensitive LMX platform for the detection of HLA-antibodies, which we described above and we use the same technology for the HLA typing as well. The HLA typing is performed by using Sequence Specific Oligonucleotide revers (SSOr) as low and intermediate resolution for A, B, C, DRB1, DRB345, DQA1/DQB1 and DP. For performing the HLA-crossmatch between patient's serum and donor's lymphocytes, we used the flow cytometry crossmatch method. Both T and B cell IgG FCXM are performed by Becton Dickinson (BD) FACSCanto II flow cytometer, where our cut-offs for positive MFI and for positive crossmatch are determined based on normal human studies. In addition to these highly sensitive methods, CDC test is used as a supporting tool for complicated and questionable results. Furthermore, we perform C1q test despite the ongoing debated discussion about the relevance of this assay, anticipating a better differentiation between clinical relevant HLA-antibodies binding to the complement from those with less clinical significance. Indeed, C1q test supports us in selected and specific cases and we found in one case no any signs of rejections with HLA-antibodies with high MFI score and positive flow cross match but negative C1q test [79]. In this case, we observed an immediately function of transplanted kidney, despite high DSA titer of 10,360 MFI for the allelic mismatched antigen HLA-B*51 and positive flow crossmatch for both T (+209 MCS) and B (+224 MCS) cell IgG flow crossmatch. We defined a cutoff off 1000 MFI as positive result for DSA and 30 MSC and 21 MSC for B and T cell cross match respectively. The transplanted organ was functioned promptly and the patient is without any signs of AMR, the donated kidney is still continues

to function well long months after transplantation. It is to be stated, that no any modifications have been made in the standard immunosuppressive protocol used at our transplant Centre (Induction: Basiliximab and Methylprednisolone; Maintenance: Mycophenolate mofetil, Prednisone and Tacrolimus).

DISCUSSION

This remarkable finding highlights the need to a better understanding the role of detected strong HLA antibodies illustrated as MFI by LMX assay. It confirms also the aforementioned appeal for further more detailed studies and additional data in the controversial role of C1q in the detection of clinical relevance in patients with moderate and high DSA titers in the setting of renal transplantation. Furthermore, our report and similar other studies suggest that the standardization of cutoffs using only the LMX assay, which now used somewhat everywhere, are potentially misdirected and may not be suitable to achieve the desired degree of harmonization and standardization in the HLA-antibody tests [80-84]. A satisfactory answering all these inquiries in the coming years, we will be able to predict the risk of DSA more accurately and most likely we will be more effective in reducing the waiting time for HLA-sensitized HD patients by transplanting them despite high titer antibodies detected by LMX technology in early stage of dialysis. The other strategy which we followed at our hospital to shorten the waiting time and reduce the HD related mortality is the establishing of apparatuses-based preoperative desensitization, for the reduction of high titer patient's DSA. At our facility, we use Therapeutic Plasma Exchange (TPE) for desensitization in recipients with DSA titer >4000 MFI. The plasma to be removed is 1.5 of patient's Total Plasma Volume (TPV) and the procedure is performed every other day. The TPV of the patient is calculated based on the dry weight of the patient before transplantation. IVIG or Anti-Thymocyte Globulin (ATG) is infused immediately or maximum within 2 h after each TPE. Our experience showed that using this extensive procedure is worthy, as we observed that adequate, results of desensitization can be achieved by sufficient removal of recipient's DSA. Using this methodology, fifteen kidney transplantations were carried out successfully in multi-sensitized high-risk patients, who are previously considered as "non-transplantable" cases.

These results encouraged us to start the transplantation with ABO incompatible (ABOi) kidney organs in order to achieve further shorting of the waiting time of our HD patients. In addition, here, we use TPE for the reduction of preformed ABO blood group antibodies and keep the ABO antibodies at the time of transplantation under a certain threshold titer of 1:8 using coombs gel centrifugation card test. Our first experience is very encouraging and the first 21 transplanted ABOi organs, functioned immediately and all the patients were discharged on time with normal renal parameters [79]. Using this extensive therapeutic concept, organ rejection can be avoided, even if blood group antibodies rise to titer above 1:8 after transplantation. This phenomenon is referred to accommodation process, which is currently incompletely understood [85,86]. However, the situation of ABO antibodies is pathologically different from the presence of HLA-antibodies, which have no or less ability to accommodate. Thus, the (re)-development and boosting of anti-HLA DSA bear often risk for donated organ after transplantation and is potentially associated with transplant acute or chronic rejection.

Furthermore, to encounter the obstacles associated with highly sensitized patient, we established a paired exchange program at our center to increase donor pools and accessibility to our transplant service. In total, we transplanted 23 patients using this program, among them 3-way paired exchange. All above listed technologies and clinically relevant results demonstrate the important role of these extensive procedures in overcoming immunological barriers and reducing the number of HD patients in the waiting list significantly.

Outlook and future perspective

Besides the above described apparatus-based overcoming of immunological barriers, future efforts are needed in kidney transplantation. Especially, the development of long-term immune tolerance between recipient immune system and transplanted kidney remains one of the most unsatisfied aspects in kidney transplantation. It would not be impossible such thoughts to be implemented and turned from "myth" to reality, as immunological tolerance can be definitively achieved in allogeneic Human Stem Cell transplantation (HSC) [87-89]. We know that successful HSC transplanted patients do not need long term immunosuppressive therapies, like it is the case in RTX. All systemic immunosuppressive drugs can be completely discontinued in most successful HSC transplanted patients within a year after HSC. In contrast, patients with RTX require lifelong immune suppressive medication with serious side effects and the disappointing and frustrating long-term outcome of RTX remains until now an unresolved problem. These serious immunological obstacles could not be satisfactorily improved despite all efforts over the last decade. Consequently, it is not surprising and entirely logical that numerous clinical attempts have been made using the HSC-model to optimize the outcome, achieve immune tolerance in RTX and thus avoid lifelong immunosuppressant [90-95]. However, the most of published therapeutic trials to achieve immune tolerance are extensive and may be too toxic to perform in solid organ transplantation, because the most of them depends on inducing hematopoietic chimerism in recipient's circulation to achieve immune tolerance using risky ablative conditioning program. Furthermore, very close donor–recipient HLA-matching or even HLA-identical donor-recipient combinations are needed to have successful and complication free mixed chimerism, which is a challenge that is not trivial to be matched. Also other clinical experiments, e.g. with mesenchymal stem cells, remain correspondingly to HSC associated with unsatisfied outcome [96-98]. It might be therefore more favorable to think more about other therapeutic strategies to facilitate comparable immunological tolerance in RTX without allogeneic HSC.

The developmentally learnt systemic immune tolerance of human in both HSC and RTX depends strongly on the balance between regulatory T cells (Tregs) and allospecific effector T cells (Teffs). Despite, the substantial differences in the underlying immunological pathological mechanisms between RTX and HSC, the inspiring development of tolerance in HSC should be sufficient grounds for us to develop similar novel therapeutic

alternatives in RTX. Thus, HSC might represent a suitable model system in humans to better understand immune tolerance in solid organ transplantation. Indeed, other methods for promoting tolerance have been clinically validated in the settings of liver, lung and heart transplantation and some published reports showed encouraging and significant developments in these fields [99-103]. However, trials in the field of RTX remain without achieving the eagerly awaited breakthrough. High hope is now placed in tolerance inducing by Tregs, which have aroused clearly increasing interest among immunology scientists and transplantation experts in the last few years [103-105]. Polyclonal Tregs are initially discovered and identified in chimeric patients transplanted with HLA-mismatched HSC and their presence strongly correlates to induction and maintenance of immune tolerance, making the therapy with Tregs as a real alternative treatment. In contrast to chimeric induction by HSC, the therapy with Tregs is less invasive and does not aggressively manipulate blood and the immune system. Beside the isolation of polyclonal Tregs by the transplant laboratories, the focus might be the isolation of purified antigen-specific Tregs which are in generally more effective than polyclonal Tregs, as yet there are no standardized protocols for efficient, Good Manufacturing Practice (GMP)-compliant generation of large numbers of antigen-specific Tregs [106]. Nevertheless, in a previous clinical trial, we have demonstrated that the isolation of Tregs (CD4+ CD25+ FOXP3) can be performed safe in agreement with GMP-regulations for application in HSC regimens [107,108]. Other groups were able to implement protocols for GMP-compliant expansion of polyclonal regulatory T cells and this approach could make even banking of HLA-typed polyclonal regulatory T cells for off-the-shelf use in RTX inducing immune tolerance a reality in the near or middle future [109]. Those principles are currently put into practice and interestingly a new published study has shown that regulatory T cells are able to decrease the anti-HLA DSA levels significantly in the allografts of murine [110]. Furthermore, a recent clinical report found a strong association between the outcome of graft and regulatory T cells [108]. More interestingly, we are looking with large portion of optimism for the outcome of the clinical trial "The ONE Study", which uses Tregs in living kidney transplantation. This ONE Study, which includes multi kidney transplant centers in different countries in the world, has newly published encouraging and important results [111]. The data demonstrated for the first time, that the therapy with Tregs is achievable and safe in the kidney transplant setting. Furthermore, the study showed that the recipients with Tregs, had less infectious complications, but similar rejection rates in the first year of observation, compared with recipients without Tregs. This and other ongoing studies might give us a justified hope in achieving breakthrough in inducing immune tolerance in kidney transplantation.

CONCLUSION

Major developments were made in the field of HLA diagnostics to ensure accurate laboratory results prior and post kidney transplantation and different technologies were successfully established to allow the transplantation of donated kidney organ across immunological barriers at an early stage of dialysis avoiding its related complications. These achievements turned out to influence positively and significantly the outcome of transplanted patients. However, the era of modern laboratory diagnostic and new invasive and extensive therapeutic approaches could not completely prevent serious immunological complications and HLA incompatibility remains until now the major immunological risk of graft rejection. Innovative strategies using cell-based therapies, like regulatory T cells, might minimize the risk of HLA-antibodies by inducing immune tolerance and preventing a pathogenic immune response against the graft. This makes the realization of immune tolerance and maintaining short and long-term immunological homeostasis in kidney transplantation achievable and no longer rule out in the future.

REFERENCES

1. Klinkmann H. Historical overview of renal failure therapy--a homage to Nils Alwall. Contrib Nephrol. 1990;78:1-23.
2. Garzotto F, Zanella M, Ronco C. The evolution of pediatric continuous renal replacement therapy. Nephron Clin Pract. 2014;127(1-4):172-175.
3. Yoo KD, Kim CT, Kim MH, Noh J, Kim G, Kim H, et al. Superior outcomes of kidney transplantation compared with dialysis: an optimal matched analysis of a national population-based cohort study between 2005 and 2008 in Korea. Medicine. 2016;95(33):e4352.
4. Prasad N, Vardhan H, Baburaj VP, Bhadauria D, Gupta A, Sharma RK, et al. Do the outcomes of living donor renal allograft recipients differ with peritoneal dialysis and hemodialysis as a bridge renal replacement therapy?. Saudi J Kidney Dis Transpl. 2014;25(6):1202.
5. Held PJ, McCormick F, Ojo A, Roberts JP. A cost-benefit analysis of government compensation of kidney donors. Am J Transplant. 2016;16(3):877-885.
6. Dausset J. Iso-leuko-antibodies. Acta Haematol. 1958;20(1–4):156-166.
7. Dausset J, Colombani J, Feingold N, Rapaport F. A Leukocyte Group System and Its Relations with Histocompatibility. Nouv Rev Fr Hematol. 1965;5:17-22.
8. Dausset J, Hors J. HL-A and kidney transplantation. Nat New Biol. 1972;238(83):150-152.
9. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med. 1969;280(14):735-739.
10. Zhang R. Donor-specific antibodies in kidney transplant recipients. Clin J Am Soc Nephrol. 2018;13(1):182-192.
11. Everly MJ, Rebellato LM, Haisch CE, Briley KP, Bolin P, Kendrick WT, et al. Impact of IgM and IgG3 anti-HLA alloantibodies in primary renal allograft recipients. Transplantation. 2014;97(5):494-501.
12. Valenzuela NM, Reed EF. Antibodies in transplantation: the effects of HLA and non-HLA antibody binding and mechanisms of injury. Methods Mol Biol. 2013;1034:41-70.
13. Masutani K, Tsuchimoto A, Kurihara K, Okabe Y, Kitada H, Okumi M, et al. Histological analysis in ABO-compatible and ABO-incompatible kidney transplantation by performance of 3-and 12-month protocol biopsies. Transplantation. 2017;101(6):1416-1422.
14. McKercher CM, Venn AJ, Blizzard L, Nelson MR, Palmer AJ, Ashby MA, et al. Psychosocial factors in adults with chronic kidney disease: characteristics of pilot participants in the Tasmanian Chronic Kidney Disease study. BMC Nephrol. 2013;14:1-9.

15. Morath C, Zeier M, Döhler B, Opelz G, Süsal C. ABO-incompatible kidney transplantation. Front Immunol. 2017;8:234.

16. Choi BH, Han DJ. Ongoing higher infection rate in ABO-incompatible kidney transplant recipient: is it a serious problem? A single-center experience. Ann Surg Treat Res. 2016;91(1):37-44.

17. Joshi VD. Quality of life in end stage renal disease patients. World J Nephrol. 2014;3(4):308.

18. Santos C, Costa R, Malheiro J, Pedroso S, Almeida M, Martins LS, et al. Kidney transplantation across a positive crossmatch: a single-center experience. Transplant Proc. 2014;46(6):1705-1709.

19. Yoo PS, Bonnel A, Kamoun M, Levine MH. Clinical outcomes among renal transplant recipients with pre-transplant weakly reactive donor-specific antibodies. Clin Transplant. 2014;28(1):127-133.

20. Matsuda Y, Sarwal MM. Unraveling the role of allo-antibodies and transplant injury. Front Immunol. 2016;7:432.

21. Zecher D, Bach C, Staudner C, Böger CA, Bergler T, Banas B, et al. Characteristics of donor-specific anti-HLA antibodies and outcome in renal transplant patients treated with a standardized induction regimen. Nephrol Dial Transplant. 2017;32(4):730-737.

22. Olausson M, Mjörnstedt L, Norden G, Rydberg L, Mölne J, Bäckman L, et al. Successful combined partial auxiliary liver and kidney transplantation in highly sensitized cross-match positive recipients. Am J Transplant. 2007;7(1):130-136.

23. Kaufman A, de Souza Pontes LF, Marques MT, Sampaio JC, Porto LC, de Moraes Souza ER. Analysis of AHG-PRA and ELISA-PRA in kidney transplant patients with acute rejection episodes. Transpl Immunol. 2003;11(2):175-178.

24. Lachmann N, Todorova K, Schulze H, Schönemann C. Luminex® and its applications for solid organ transplantation, hematopoietic stem cell transplantation, and transfusion. Transfus Med Hemother. 2013;40(3):182-189.

25. Picascia A, Infante T, Napoli C. Luminex and antibody detection in kidney transplantation. Clin Exp Nephrol. 2012;16:373-381.

26. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. Am J Transplant. 2013;13(7):1859-1870.

27. Mathur A, Thapa S, Jagannathan L. Luminex-Based Donor-Specific antibody crossmatching for renal transplant: A 3-Year experience in South India. Glob J Transfus Med. 2018;3(1):34.

28. Süsal C, Opelz G, Morath C. Role and value of Luminex®-detected HLA antibodies before and after kidney transplantation. Transfus Med Hemother. 2013;40(3):190-195.

29. Pérez-Flores I, Santiago JL, Calvo-Romero N, Barrientos-Guzmán A, Sánchez-Fructuoso AI. Different impact of pretransplant anti-HLA antibodies detected by Luminex in highly sensitized renal transplanted patients. Biomed Res Int. 2013;2013:738404.

30. Haarberg KM, Tambur AR. Detection of donor-specific antibodies in kidney transplantation. Br Med Bull. 2014;110(1):23-34.

31. Hoshino J, Everly MJ, Kaneku H, Ubara Y, Takaichi K, Terasaki PI. Impact of the presence and duration of donor-specific antibodies on renal function. Transplant Proc. 2014;46(1):75-80.

32. Cervelli C, Pisani F, Aureli A, Azzarone R, Scimitarra M, Battistoni C, et al. For anti-HLA-specific donor antibodies detection by flow cytometry cytotoxic crossmatches comparison of methods. Transplant Proc. 2013;45(7):2761-2764.

33. Wu P, Jin J, Everly MJ, Lin C, Terasaki PI, Chen J. Impact of alloantibody strength in crossmatch negative DSA positive kidney transplantation. Clin Biochem. 2013;46(15):1389-1393.

34. Sullivan HC, Liwski RS, Bray RA, Gebel HM. The road to HLA antibody evaluation: do not rely on MFI. Am J Transplant. 2017;17(6):1455-1461.

35. Frischknecht L, Deng Y, Wehmeier C, de Rougemont O, Villard J, Ferrari-Lacraz S, et al. The impact of pre-transplant donor specific antibodies on the outcome of kidney transplantation–Data from the Swiss transplant cohort study. Front Immunol. 2022;13:1005790.

36. Standards for histocompatibility and immunogenetics Testing, European Federation of Immunogenetics. Version 8.0, accepted by the EFI Executive Committee. 2019.

37. Na GH, Kim EY, Hong TH, You YK, Kim DG. Effects of Preoperative Positive Cross-Match and HLA Mismatching on Early Acute Cellular Rejection and Graft Survival in Living Donor Liver Transplantation. Ann Transplant. 2015;20:553-560.

38. Meneghini M, Melilli E, Martorell J, Revuelta I, Rigol-Monzó E, Manonelles A, et al. Combining sensitive crossmatch assays with donor/recipient human leukocyte antigen eplet matching predicts living-donor kidney transplant outcome. Kidney Int Rep. 2018;3(4):926-938.

39. Keeshan BC, O'Connor MJ, Lin KY, Monos DS, Lind CT, Mascio CE, et al. Clinical Importance of Flow Cytometry Crossmatch in the Context of Complement-Dependent Cytotoxicity Crossmatch Results Following Heart Transplantation. J Heart Lung Transplant. 2014;33(4):S84.

40. Eng HS, Bennett G, Bardy P, Coghlan P, Russ GR, Coates PT. Clinical significance of anti-HLA antibodies detected by Luminex®: Enhancing the interpretation of CDC-BXM and important post-transplantation monitoring tools. Hum Immunol. 2009;70(8):595-599.

41. Katalinić N, Starčević A, Mavrinac M, Balen S. Complement-dependent cytotoxicity and Luminex technology for human leucocyte antigen antibody detection in kidney transplant candidates exposed to different sensitizing events. Clin Kidney J. 2017;10(6):852-858.

42. Tait BD. Detection of HLA antibodies in organ transplant recipients–triumphs and challenges of the solid phase bead assay. Front Immunol. 2016;7:570.

43. Dada Sr A, Habhab W, Zabani N, Elamein FE, Fahmy A, Al Sayed A, Al Baz N, Bokhari A. Lbp20: Relevance of Strong Positive Donor Specific Antibodies and Flow Crossmatch While C1q Test Negative in Renal Transplantation. Hum Immunol. 2015;76(4):226.

44. Zecher D, Bach C, Staudner C, Böger CA, Bergler T, Banas B, et al. Characteristics of donor-specific anti-HLA antibodies and outcome in renal transplant patients treated with a standardized induction regimen. Nephrol Dial Transplant. 2017;32(4):730-737.

45. Süsal C, Döhler B, Ruhenstroth A, Morath C, Slavcev A, Fehr T, et al. Donor-specific antibodies require preactivated immune system to harm renal transplant. EBioMedicine. 2016;9:366-371.

46. Vimal M, Chacko MP, Basu G, Daniel D. Correlation of pretransplant donor-specific antibody assay using luminex crossmatch with graft outcome in renal transplant patients. Indian J Nephrol. 2017;27(5):347-352.

47. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol. 2011;72(10):849-858.

48. Thammanichanond D, Wiwattanathum P, Mongkolsuk T, Kantachuvesiri S, Worawichawong S, Vallipakorn SA, et al. Role of pretransplant complement-fixing donor-specific antibodies identified by C1q assay in kidney transplantation. Transplant Proc. 2016;48(3):756-760.

49. Guidicelli G, Guerville F, Lepreux S, Wiebe C, Thaunat O, Dubois V, et al. Non-complement–binding de novo donor-specific anti-HLA antibodies and kidney allograft survival. J Am Soc Nephrol. 2016;27(2):615-625.

50. Thammanichanond D, Mongkolsuk T, Rattanasiri S, Kantachuvesiri S, Worawichawong S, Jirasiritham S, et al. Significance of C1q-fixing donor-specific antibodies after kidney transplantation. Transplant Proc. 2014;46(2):368-371.

51. Tyler JL, Gaspari J, Fisher C, Mowery C, Domen R, Kadry Z, et al. DSA and C1q assay in kidney post-transplant monitoring. Hum Immunol. 2015;76:50.

52. Arreola-Guerra JM, Castelán N, de Santiago A, Arvizu A, Gonzalez-Tableros N, López M, et al. C1Q assay results in complement-dependent cytotoxicity crossmatch negative renal transplant candidates with donor-specific antibodies: high specificity but low sensitivity when predicting flow crossmatch. J Transplant. 2016;2016:2106028.

53. Crespo M, Torio A, Mas V, Redondo D, Pérez-Sáez MJ, Mir M, et al. Clinical relevance of pretransplant anti-HLA donor-specific antibodies: does C1q-fixation matter?. Transpl Immunol. 2013;29(1-4):28-33.

54. Schwaiger E, Wahrmann M, Bond G, Eskandary F, Böhmig GA. Complement component C3 activation: the leading cause of the prozone phenomenon affecting HLA antibody detection on single-antigen beads. Transplantation. 2014;97(12):1279-1285.

55. Navas A, Molina J, Agüera ML, Guler I, Jurado A, Rodríguez-Benot A, et al. Characterization of the C1q-binding ability and the IgG1-4 subclass profile of preformed anti-HLA antibodies by solid-phase assays. Front Immunol. 2019;10:1712.

56. Danobeitia JS, Djamali A, Fernandez LA. The role of complement in the pathogenesis of renal ischemia-reperfusion injury and fibrosis. Fibrogenesis Tissue Repair. 2014;7(1):1-16.

57. Ciurea SO, Cao K, Fernandez-Vina M, Kongtim P, Malki MA, Fuchs E, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus guidelines for the detection and treatment of donor-specific anti-HLA antibodies (DSA) in haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. 2018;53(5):521-534.

58. Garcia-Garcia G, Jha V, Tao Li PK, Garcia-Garcia G, Couser WG, Erk T, et al. Chronic kidney disease (CKD) in disadvantaged populations. Clin Kidney J. 2015;8(1):3-6.

59. Haller MC, Kammer M, Oberbauer R. Dialysis vintage and outcomes in renal transplantation. Nephrol Dial Transplant. 2019;34(4):555-560.

60. Knight RJ, Teeter LD, Graviss EA, Patel SJ, DeVos JM, Moore LW, et al. Barriers to preemptive renal transplantation: a single center questionnaire study. Transplantation. 2015;99(3):576-579.

61. Garbaini J, Rao P, Lal D, Caltabiano MJ, Shi PA, Shaz BH. Current patterns of use in therapeutic apheresis: a metropolitan center experience. Transfusion. 2014;54(7):1899-1900.

62. Cid J, Carbassé G, Andreu B, Baltanás A, Garcia-Carulla A, Lozano M. Efficacy and safety of plasma exchange: an 11-year single-center experience of 2730 procedures in 317 patients. Transfus Apher Sci. 2014;51(2):209-214.

63. Kauke T, Klimaschewski S, Schoenermarck U, Fischereder M, Dick A, Guba M, et al. Outcome after desensitization in HLA or ABO-incompatible kidney transplant recipients: a single center experience. PLoS One. 2016;11(1):e0146075.

64. Montgomery RA, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, et al. Desensitization in HLA-incompatible kidney recipients and survival. N Engl J Med. 2011;365(4):318-326.

65. Malvezzi P, Jouve T, Noble J, Rostaing L. Desensitization in the Setting of HLA-Incompatible Kidney Transplant. Exp Clin Transplant. 2018;16(4):367-375.

66. Gabardi S, Townsend K, Martin ST, Chandraker A. Evaluating the impact of pre-transplant desensitization utilizing a plasmapheresis and low-dose intravenous immunoglobulin protocol on BK viremia in renal transplant recipients. Transpl Infect Dis. 2013;15(4):361-368.

67. Abu Jawdeh BG, Cuffy MC, Alloway RR, Shields AR, Woodle ES. Desensitization in kidney transplantation: review and future perspectives. Clin Transplant. 2014;28(4):494-507.

68. Jordan SC, Lorant T, Choi J, Kjellman C, Winstedt L, Bengtsson M, et al. IgG endopeptidase in highly sensitized patients undergoing transplantation. N Engl J Med. 2017;377(5):442-453.

69. Aull MJ, Kapur S. Kidney paired donation and its potential impact on transplantation. Surg Clin North Am. 2013;93(6):1407-1421.

70. Blumberg JM, Gritsch HA, Reed EF, Cecka JM, Lipshutz GS, Danovitch GM, et al. Kidney paired donation in the presence of donor-specific antibodies. Kidney Int. 2013;84(5):1009-1016.

71. Hunter J, Heetun M, Sinha S. Finding the perfect match: the living donor paired exchange system. Br J Hosp Med (Lond). 2014;75(4):202-206.

72. Ferrari P, Weimar W, Johnson RJ, Lim WH, Tinckam KJ. Kidney paired donation: principles, protocols and programs. Nephrol Dial Transplant. 2015;30(8):1276-1285.

73. Johnson CP, Schiller JJ, Zhu YR, Hariharan S, Roza AM, Cronin DC, et al. Renal transplantation with final allocation based on the virtual crossmatch. Am J Transplant. 2016;16(5):1503-1515.

74. Piazza A, Ozzella G, Poggi E, Caputo D, Manfreda A, Adorno D. Virtual crossmatch in kidney transplantation. Transplant Proc. 2014;46(7):2195-2198.

75. Claas FH, De Meester J, Witvliet MD, Smits JM, Persijn GG, Doxiadis II. Acceptable HLA mismatches for highly immunized patients. Rev Immunogenet. 1999;1(3):351-358.

76. Maggiore U, Oberbauer R, Pascual J, Viklicky O, Dudley C, Budde K, et al. Strategies to increase the donor pool and access to kidney transplantation: an international perspective. Nephrol Dial Transplant. 2015;30(2):217-222.

77. Do Nguyen H, Wong G, Howard K, Claas FH, Craig JC, Fidler S, et al. Modeling the benefits and costs of integrating an acceptable HLA mismatch allocation model for highly sensitized patients. Transplantation. 2014;97(7):769-774.

78. Ford S, Summers S, Cantwell L, Mulley W. Hidden perils in a highly sensitized kidney transplant recipient. Nephrology. 2012;17:9-11.

79. Lim WH, Chapman JR, Coates PT, Lewis JR, Russ GR, Watson N, et al. HLA-DQ mismatches and rejection in kidney transplant recipients. Clin J Am Soc Nephrol. 2016;11(5):875-883.

80. Jolly EC, Key T, Rasheed H, Morgan H, Butler A, Pritchard N, et al. Preformed donor HLA-DP-specific antibodies mediate acute and chronic antibody-mediated rejection following renal transplantation. Am J Transplant. 2012;12(10):2845-2848.

81. Dada A, Lafi A, Mousali R, Rahym S, Zabani N, Alsuraihi O, et al. Implementation of Abo Incompatible Renal Transplantation-A Single-Center Experience. InHLA 2019;93(5):251-252.

82. Park WY, Kang SS, Park SB, Park UJ, Kim HT, Cho WH, et al. Comparison of clinical outcomes between ABO-compatible and ABO-incompatible spousal donor kidney transplantation. Kidney Res Clin Pract. 2016;35(1):50-54.

83. Dörje C, Mjøen G, Strøm EH, Holdaas H, Jenssen T, Øyen O, et al. One-year protocol biopsies from ABO-incompatible renal allografts compared with a matched cohort of ABO-compatible allografts. Clin Transplant. 2015;29(3):268-276.

84. Tay J, Daly A, Jamani K, Labelle L, Savoie L, Stewart D, et al. Patient eligibility for hematopoietic stem cell transplantation: a review of patient-associated variables. Bone Marrow Transplant. 2019;54(3):368-382.

85. Muraro PA, Martin R, Mancardi GL, Nicholas R, Sormani MP, Saccardi R. Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. Nat Rev Neurol. 2017;13(7):391-405.

86. Passweg JR, Halter J, Bucher C, Gerull S, Heim D, Rovó A, et al. Hematopoietic stem cell transplantation: a review and recommendations for follow-up care for the general practitioner. Swiss Med Wkly. 2012;7(41):w13696.

87. Leventhal JR, Ildstad ST. Tolerance induction in HLA disparate living donor kidney transplantation by facilitating cell-enriched donor stem cell Infusion: the importance of durable chimerism. Hum Immunol. 2018;79(5):272-276.

88. Massart A, Ghisdal L, Abramowicz M, Abramowicz D. Operational tolerance in kidney transplantation and associated biomarkers. Clin Exp Immunol. 2017;189(2):138-157.

89. Yolcu ES, Leventhal JR, Ildstad ST. Facilitating cells in tolerance induction for kidney transplantation. Curr Opin Organ Transplant. 2015;20(1):57-63.

90. Leventhal J, Miller J, Abecassis M, Tollerud DJ, Ildstad ST. Evolving Approaches of Hematopoietic Stem Cell–Based Therapies to Induce Tolerance to Organ Transplants: The Long Road to Tolerance. Clin Pharmacol Ther. 2013;93(1):36-45.

91. Leventhal J, Abecassis M, Miller J, Gallon L, Ravindra K, Tollerud DJ, et al. Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation. Sci Transl Med. 2012;4(124):124ra28.

92. Mahr B, Granofszky N, Muckenhuber M, Wekerle T. Transplantation tolerance through hematopoietic chimerism: progress and challenges for clinical translation. Front Immunol. 2017;8:1762.

93. Imberti B, Monti M, Casiraghi F. Pluripotent stem cells and tolerance induction in organ transplantation. Current opinion in organ transplantation. 2015;20(1):86-93.

94. Guo K, Ikehara S, Meng X. Mesenchymal stem cells for inducing tolerance in organ transplantation. Front Cell Dev Biol. 2014;2:8.

95. Casiraghi F, Remuzzi G, Perico N. Mesenchymal stromal cells to promote kidney transplantation tolerance. Curr Opin Organ Transplant. 2014;19(1):47-53.

96. Casiraghi F, Perico N, Remuzzi G. Mesenchymal stromal cells to promote solid organ transplantation tolerance. Curr Opin Organ Transplant. 2013;18(1):51-58.

97. Cunningham EC, Sharland AF, Bishop GA. Liver transplant tolerance and its application to the clinic: can we exploit the high dose effect?. Clin Dev Immunol. 2013;2013: 419692.

98. İnal A. Immunology of liver transplantation. Exp Clin Transplant. 2014;12:5-10.

99. Zhuang J, Wang Y, Du Z, Wang S. Protein F-induced immune tolerance in liver transplantation in rats. Mol Biol Rep. 2014;41:3425-3429.

100. Jiang X, Nicolls MR. Working toward immune tolerance in lung transplantation. J Clin Invest. 2014;124(3):967-970.

101. Lu X, Yang X, Zhou Q, Wang Y, Feng S, Jin J, et al. Cardiac allograft tolerance induced by isogeneic CD4+ CD25+ regulatory T cells. Exp Clin Transplant. 2014;12(2):133-138.

102. Pierini A, Nishikii H, Baker J, Kimura T, Kwon HS, Pan Y, et al. Foxp3+ regulatory T cells maintain the bone marrow microenvironment for B cell lymphopoiesis. Nat Commun. 2017;8(1):15068.

103. Ashton-Chess J, Dugast E, Colvin RB, Giral M, Foucher Y, Moreau A, et al. Regulatory, effector, and cytotoxic T cell profiles in long-term kidney transplant patients. J Am Soc Nephrol. 2009;20(5):1113-1122.

104. Fan H, Cao P, Game DS, Dazzi F, Liu Z, Jiang S. Regulatory T cell therapy for the induction of clinical organ transplantation tolerance. Semin Immunol. 2011;23(6): 453-461.

105. Sagoo P, Lombardi G, Lechler RI. Relevance of regulatory T cell promotion of donor-specific tolerance in solid organ transplantation. Front Immunol. 2012;3:184.

106. Hoffmann P, Boeld TJ, Eder R, Albrecht J, Doser K, Piseshka B, et al. Isolation of CD4+ CD25+ regulatory T cells for clinical trials. Biol Blood Marrow Transplant. 2006;12(3):267-274.

107. Dada A, Rothe G, Schmitz G, Klouche M. Quality Assessment and Regulatory Aspects of Autologous and Allogeneic Peripheral Stem Cell Preparation. Laboratoriums Medizin. 2002;26(7-8):408-417.

108. Chandran S, Tang Q, Sarwal M, Laszik ZG, Putnam AL, Lee K, et al. Polyclonal regulatory T cell therapy for control of inflammation in kidney transplants. Am J Transplant. 2017;17(11):2945-2954.

109. Liao T, Xue Y, Zhao D, Li S, Liu M, Chen J, et al. In vivo attenuation of antibody-mediated acute renal allograft rejection by ex vivo TGF-β-induced CD4+ Foxp3+ regulatory T cells. Front Immunol. 2017;8:1334.

110. Krajewska M, Kościelska-Kasprzak K, Kamińska D, Żabińska M, Myszka-Kozłowska M, Gomułkiewicz A, et al. Kidney transplant outcome is associated with regulatory T cell population and gene expression early after transplantation. J Immunol Res. 2019;2019: 7452019.

111. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet. 2020;395(10237):1627-1639.