Chapter 17

Immunosuppressive Minimization Strategies in Kidney Transplantation

Jorge Andrade-Sierra,
Pedro Alejandro Vazquez-Galvan,
Hernesto Hernandez-Reyes,
Lydia A. Mercado-Jáuregui,
Jonathan S. Chávez-Iñiguez,
Eduardo González-Espinoza,
Benjamin Gómez-Navarro and
José I. Cerrillos-Gutiérrez

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77292

Abstract

The long-term graft survival in renal transplantation results is still controversial, the toxicity and adverse reactions of the immunosuppressive drugs are implicated, as well as cellular and humoral antigen-specific immune mechanisms; therefore, different strategies for adapting immunosuppression are used to reduce the complications associated with the use of these drugs. Calcineurin inhibitors (CNI) require an adequate dose-dependent concentration leading to the appearance of drug-related adverse reactions. The variability in the required dose of CNI leads to minimization strategies that do not result in a higher acute rejection (AR) incidence when compared to other immunosuppressive agents. Early steroid withdrawal is another strategy, although with an increase in AR, but without an impact on the function and survival of the renal graft. The reduction of mycophenolate mofetil to 1.5 g/day seems to be a therapeutic option, decreasing the infectious, hematological and gastrointestinal adverse reactions. Finally, alemtuzumab, bortezomib, belatacept and cellular therapies are in the search for the new treatments, whose premise is the induction of donor-specific nonresponse in the context of operational tolerance or mixed chimerism. The use of adapted and adequate immunosuppression has led to variable results and some are very encouraging; however, they must be validated with experimental studies.

Keywords: renal transplantation, immunosuppressive minimization, acute rejection

1. Introduction

Renal transplantation (RT) is currently considered the best therapeutic option for renal replacement therapy in patients with end-stage renal disease (ESRD), with controversial results related to long-term graft survival [1–3]. Several factors can contribute to loss of the renal graft over time, which may be nonimmunological in nature, such as chronic nephrotoxicity due to drugs used for transplantation maintenance [particularly calcineurin inhibitors (CNI) tacrolimus (TAC) and cyclosporin] or for the side effects of immunosuppression when corticosteroids are involved, such as: infections, neoplasms, dyslipidemia, hypertension, cardiovascular disease, and new-onset diabetes mellitus (NODAT) that can lead to high mortality in patients with a functional graft [4–6]. Other conditions that induce long-term graft loss are the antigen-specific humoral and cellular immune mechanisms that contribute to an increase in the number and severity of episodes of acute rejection (AR), inducing chronic alloimmune damage [5–14]. These damage mechanisms raise the awareness that there must be a balance in posttransplantation immunosuppression; however, the new and powerful immunosuppressive drugs used today, and the alarming loss of kidney grafts, particularly due to the side effects of immunosuppression, have motivated transplant centers globally to try to minimize, suspend, or change the immunosuppressive maintenance drugs to try and further reduce the complications associated to them [15–39].

2. Minimizing immunosuppression with calcineurin inhibitors in kidney transplantation

The introduction of CNI has achieved exceptional short-term results in recent years in the field of allograft transplants, especially by reducing the rate of AR episodes, reaching, in the last 20 years, an overall graft survival of more than 90% in the first year [39]. However, the attention now focuses on the search for better long-term outcomes with strategies that sustain a low AR rate along with a decrease in the side effects of immunosuppression. The immunosuppressants have three effects: the therapeutic effect (rejection of suppression), unwanted consequences related to immunosuppression (infections, neoplasms, metabolic and hemodynamic disorders), and the nonimmune toxicity to tissues [40]. The nonimmune toxicity is immunosuppressive agent-specific and is related to the mechanism of action of the drug, since they target-specific molecules with certain functions in nonimmune tissues, conditioning progressive tissue damage, and gradual kidney graft failure. This, coupled with the death of the patient with a functional graft, encourages the new concept of focusing on nonimmune factors that intervene in the long term, evoking enthusiasm for strategies to minimize the side effects of CNIs.

3. Pharmacodynamics and nonimmune toxicity of the calcineurin inhibitors

In the classification of immunosuppressants, small molecules are included (from which the immunophilin-binding drugs are derived, such as CNIs, mechanism target of rapamycin

(mTOR) inhibitor (imTOR), nucleotide blocking agents, and antimetabolites); the protein-depleting and nonlymphocyte-depleting agents (monoclonal and polyclonal antibodies), the intravenous immunoglobulin, and corticosteroids [40]. The effects of CNI are proportional to the serum concentration levels, since this depends on the saturation dose of its targets [40], which makes the dosage and the control of serum levels important in maintaining the balance between the desired immunosuppressant effect and the unwanted toxicity.

Cyclosporin A (CsA) is a fungal origin polypeptide (derived from *Tolypocladium inflatum),* composed of 11 amino acids, with a molecular weight of 1203 Da, which interacts by binding to its cytoplasmic receptor (cyclophilin); a protein from the family of immunophilins, forming a complex that binds to the calcineurin, inhibiting its normal phosphatase action on regulatory nuclear proteins (nuclear factor -KB and activator protein 1), preventing the cytokine production (IL-2), and eventually the T lymphocyte activation [41]. The adverse reactions to CsA, related to the serum concentration of the drug, include: nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, and tremor [42]; and, less frequently, hemolytic uremic syndrome, and NODAT [40].

In 2004, a longitudinal cohort where 888 renal biopsies were collected from 99 patients who were in immunosuppressive treatment with CsA for 10 years after renal transplantation, was evaluated; finding arteriolar hyalinosis as the most sensitive marker for nephrotoxicity due to CsA [43]. Another CNI introduced in the mid-1990s, that was initially called FK506 and is currently known as TAC, is a macrolide isolated from the fungi *Streptomyces tsukubaensis* that possesses suppressive effects similar to CsA (cell-mediated and humoral immune responses) [41]. The TAC binds to a protein called FKBP12 (binding protein of FK506–12) and a complex that inhibits the phosphatase activity of calcineurin, preventing the activation of the T cell, and selectively affecting the transcription of IL-2 and other cytokines. The adverse reactions are similar to those of CsA but with less incidence of hypertension, hyperlipidemia, hirsutism, and gingival hyperplasia; however, the incidence of NODAT and nephrotoxicity is higher [44].

The mechanism through which nephrotoxicity occurs is explained by the endothelial dysfunction associated with reduced production of local vasodilators (nitric oxide and prostaglandins) and increased production of vasoconstrictors (endothelin and thromboxane) [45].

The determination of the serum levels of the CNI is part of the management of immunosuppression in transplant recipients, due to the variability between patients (and the intra-patient variability). The inter-individual variability with TAC is explained by polymorphisms in genes that encode transporter proteins and enzymes that metabolize the drug. The TAC is metabolized in the intestine, liver, and kidney by cytochrome P450 (CYP) 3A4 and 3A5. Inter-individual differences in CYP3A activity are the most important determinants of variability in TAC metabolism. Polymorphisms in the CYP3A5 gene explain 40–50% of the variability in the TAC dose requirement to maintain adequate serum levels: the most studied one is the single nucleotide polymorphism CYP3A5*3. This allele causes a reduced enzymatic activity associating with the need to reduce the administered dose of TAC. On the other hand, when CYP3A5 is expressed, a dose of about 50% higher is required [46, 47]. To a lesser extent, the CYP3A4 genotype with impact on the determination of doses in transplant patients receiving TAC has also been identified. Individuals carrying the CYP3A4 * 1B allele reported up to a 35% dose

reduction in order to achieve a therapeutic concentration. Similarly, it has been identified that the CYP3A4 * 22 variant reduces the enzymatic activity of CYP3A4, associated with a lower dose requirement. On the other hand, there are ethnic considerations that participate in allelic variability since Caucasian patients are commonly carriers of the CYP3A5 * 3 allele [46].

4. Minimization strategies of immunosuppression with calcineurin inhibitors

Given the nonimmunological toxic effects of CNI, two general strategies to reduce CNI are proposed: *de novo* minimization, where maintenance immunosuppression with CNI is sought immediately after transplantation at low doses subsequent to a powerful induction; and the second strategy, selective minimization, in which a class of immunosuppressants is avoided, showing a reduction of the undesired effects related to the drug. The Symphony study evaluated 1645 patients divided into four groups: (1) Standard dose of CsA, mycophenolate mofetil (MMF), and prednisone (PDN); (2) Low dose of CsA with induction therapy with daclizumab; (3) Low dose of TAC with induction with daclizumab; (4) Low dose of sirolimus (SRL) with induction with daclizumab. The primary aim was to reduce the nephrotoxicity using a low dose of CNI and SRL and to secondarily reduce the side effects, at the same time as maintaining the efficacy in terms of avoiding acute rejection, improving the overall survival of the patient and the graft. Their results at 1 and 3 years showed a better glomerular filtration rate (GFR) and graft survival in the group with low dose of TAC, as well as a low AR rate, compared with the SRL group [42, 48].

On the other hand, avoiding CNIs is the complete omission of these drugs from the maintenance immunosuppression regimen, while minimization schemes use reduced doses of CNI in order to avoid their nephrotoxicity [49]. Larso et al., compared regimens without CNI (SRL, MMF and PDN) and with CNI (TAC, MMF and PDN), in RT recipients, with similar results at 12 months in patient survival (98% SRL, 96% TAC, $p = 0.42$) and graft survival (94% SRL, 92% TAC, $p = 0.95$), as well as in the incidence of AR between both groups [50]. The regimens without CNI were also evaluated in the ORION51 study, which compared the efficacy of three schemes; (1) SRL + TAC with discontinuation of CNI at 13 weeks; (2) SRL + MMF; and (3) TAC + MMF. The SRL + MMF group presented more AR events (32.8%) compared to SRL + TAC (17.4%) and TAC + MMF (12.3%); however, the graft and patient survival were similar and there was the presence of hyperlipidemia in the group treated with SRL and NODAT in the group with TAC (**Table 1**) [51].

The BENEFIT study [52] compared two regimens (an intensive and a less intensive dose) with betalacept (selective T cell co-stimulation blocker) *versus* CsA in patients with living donor RT with standard criteria; finding better renal function with belatacept regimens (GFR of 65, 63, and 50 ml/min, respectively) but with a lower AR rate with CsA (22, 17 and 7%, respectively).

On the other hand, Weir et al. [53], who evaluated the efficacy and safety of the combination of MMF and SRL *versus* MMF and a CNI (TAC or CsA) at 24 months, found that the GFR was higher in the MMF/SRL regimen, with a similar AR rate in both groups.

Finally, a meta-analysis and systematic reviews related to these strategies have recently been published, with the aim of preventing nephrotoxicity and graft loss by a nonimmune character.

http://dx.doi.org/10.5772/intechopen.77292

Immunosuppressor	Used dose in renal transplant	Adverse reactions	Minimization strategy
Tacrolimus	0.1–0.15 mg/kg/day divided in two doses	Nephrotoxicity, tremor, headache, dizziness, gingival hyperplasia, hypertension, carbohydrate intolerance, increased risk of infections and neoplasms.	Dose reduction; with lower nephrotoxicity without a higher acute rejection rate.
Cyclosporine	5–8 mg/kg/day divided in two doses	Nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, lymphoproliferative disorders associated to EBV, Kaposi sarcoma, TMA, HUS.	Dose reduction; improves the glomerular filtration rate and graft survival when compared to mTOR inhibitor.
Mycophenolate mofetil	2 g/day orally divided in two doses	Gastrointestinal: abdominal pain, nausea, vomits, diarrhea. Hematological: anemia, leukopenia, thrombocytopenia, increased risk of infections (especially viral), neoplasms.	Dose reduction not less than 1.5 g/day can decrease the gastrointestinal, hematological and infectious adverse reactions without an acute rejection rate increase.
Prednisone	0.5–1 mg/kg, orally divided in two doses with a taper of 5–10 mg/day indefinitely	Susceptibility to infections, obesity, osteonecrosis (avascular necrosis), hyperglycemia, hypertension, dyslipidemia, peptic ulcer, cushinoid features, long-term myopathy, osteoporosis, atherosclerosis, skin atrophy, cataracts.	Steroid withdrawal; better graft survival, lower risk of mortality, decrease in graft dysfunction with an improved metabolic and hemodynamic profile, although with contradictory results that may involve higher acute rejection rates proved by biopsy without affecting the long-term graft survival.
Alemtuzumab	30 mg/kg intravenously unique pre-transplant induction dose	Predisposition to severe infections (bacterial, viral, fungal) and increased risk of neoplasms.	Used as pre-transplant induction therapy allowing early steroid taper, CNI decrease or change to mTOR with a reduction in AR episodes in the first posttransplant year, without differences in graft survival in patients with low immunological risk and conventional therapy.
Bortezomib	1.3 mg/m^2 intravenously on day 1, 4, 8 and 11 for two cycles	Peripheral sensory neuropathies. Hematologic: anemia, leukopenia, thrombocytopenia Nausea, diarrhea, weakness.	Used as desensitization therapy, severe humoral rejection treatment, allowing an immunosuppression restart at an adjusted dose, being considered an immunosuppression minimization strategy.
Belatacept	10 mg/kg intravenously on posttransplant days 1, 5, 14 and then every 4 weeks indefinitely	Greater predisposition to lymphoproliferative disorders not associated to EBV, herpes virus and tuberculosis infections.	Adjuvant treatment with MMF and prednisone maintains a CNI-free immunosuppression with an increase in acute rejection in the first 6 months (posttransplant) but better long-term renal graft function compared with CsA.

Immunosuppressor	Used dose in renal transplant	Adverse reactions	Minimization strategy
Belimumab	Initial dose:120 mg intravenously, then 400 mg IV every 2 weeks indefinitely	Infusion related (bradycardia, myalgias, rash, urticarial, hypotension), depression, insomnia, nausea, diarrhea, bronchitis, pharyngitis, increased risk of viral infections.	Is in experimental phase, it can induce immunologic tolerance or mixed chimerism as an immunosuppression reduction strategy.

TMA, thrombotic microangiopathy; EBV, Epstein-Barr Virus; HUS, hemolytic uremic syndrome; mTOR, mechanistic target of rapamycin; CNI, calcineurin inhibitors; CsA, cyclosporine [50, 51, 53, 60, 61, 95, 97, 100, 101, 103].

Table 1. Immunosuppression in renal transplant, adverse reactions and minimization strategies.

Sawinski et al. [54], evaluated 88 clinical trials regarding CNI reduction strategies associated with MMF or imTOR in a meta-analysis (minimization of the CNI without suspending it, conversion to another immunosuppressant "imTOR," and withdrawal of the CNI in the post-transplant period or never used in the RT); finding the best results with the strategies in which the CNI was minimized, especially in the first 6 months without stopping it, with a lower incidence of AR (RR 0.80, 95% CI 0.68–0.95), graft loss (RR 0.71 95% CI 0.56–0.9), and better graft function and no differences in mortality (RR 0.87, 95% CI 0.66–1.15), compared to standard regimens with CNI. Nevertheless, it is important to mention that the majority of studies were based on CsA induction with basiliximab, thus more research is needed to determine the role with other immunosuppressants (TAC and thymoglobulin) and their doses, with the aforementioned strategies. Finally, a systematic review of 83 studies that included a total of 16,156 patients with a removing sample (RR 2.54; CI 95%: 1.56–4.12) or an avoiding sample (RR 2.16; CI 95%: 0.85–5.49) of CNI from the immunosuppression maintenance regimen, was associated to AR without a difference in graft loss (RR 0.96; CI 95%: 0.79–1.16), and with a lower incidence of hypertension in the CNI-abstained groups (RR 0.82, CI 95%: 0.71–0.95) [55].

5. Strategies for removing steroids from immunosuppression in kidney transplantation

Another tempting strategy for reduction of posttransplant immunosuppression is to withdraw or avoid the use of corticosteroids because of the numerous side effects, with the purpose of improving quality of life and reducing cardiovascular mortality.

This intervention has increased from 5 to 35% since the year 2000 until today, in RT recipients in the USA. Historically, the removal of steroids has been associated with the risk of precipitating AR [56, 57]; however, long-term safety in terms of patient and graft survival has been satisfactory with early steroid withdrawal (ESW); as Rizzaari shows [58] in a 10 year follow-up of 1241 RT recipients with graft survival, showing similar death in living donor RT recipients with maintained with steroids (79 *vs.* 73%) and with even an better survival in deceased donor RT (80 *vs.* 67%) with a report in their survival analyses free of AR, similar between the

groups with and without corticosteroids. Lopez Soler et al. [59], similarly reported in a cohort undergoing ESW with a 10-year follow-up that showed better graft survival ($p = 0.023$), lower risk of mortality (0.23, $p \leq 0.011$), and less graft failure (0.57, $p = 0.026$).

Similar to minimization of the CNI, numerous meta-analyses have been published regarding a population undergoing ESW, both in the adult and pediatric populations, concluding, in some, a higher rate of AR, especially of mild characteristics, without greater impact in the function or graft survival, and with satisfactory results in the metabolic and hemodynamic profile, reducing cardiovascular morbidity and mortality (**Table 1**) [19, 20, 60, 61].

Knight et al. [19], evaluated a meta-analysis of 34 clinical trials with 5637 patients in regimens that included withdrawal or nonuse of steroids at any time of the transplantation, and found that the withdrawal of steroids was associated with a higher incidence of AR (RR 1.56; 95% CI 1.31–1.87), but with a lower incidence of hypertension (RR 0.64; 95% CI 0.50–0.83), diabetes (RR 0.90; 95% CI 0.85–0.94), and hypercholesterolemia (RR0.96; 95% CI 0.67–0.87); concluding that AR had no impact on function or survival of the grafts because it was considered mild.

Zhang et al. [60], in a meta-analysis of 13 clinical trials in 3520 patients with ESW after transplantation found a higher incidence of AR; but when the trials that used TAC were exclusively analyzed, the statistical significance was lost and only remained in those that used CsA. Studies that involve corticosteroid withdrawal associated with TAC in the immunosuppression regimen, document the development of borderline changes in AR, especially in the early stage of transplantation, without impact on function or survival of the graft [23, 62].

The current use of immunosuppression induction with anti-DC25 antibodies (basiliximab) or lymphocyte depletion (thymoglobulin) combined with MMF and TAC has favored that the minimization or elimination of the use of posttransplant steroids be safe with cell type AR rates comparable to those that maintain the use of the posttransplant steroid [16, 17, 20–27, 29–31, 58, 60, 63–67].

The good results from minimization or suspension of some immunosuppressants are encouraging because one of the main associated causes with poor long-term kidney graft survival is directly or indirectly related to the side effects of immunosuppressants that cause long-term complications and even a higher cardiovascular mortality [4, 11, 43, 68–71].

Experience with this intervention in our transplant center has shown satisfactory short-term (12 months) results with similar AR rates in immunosuppression with and without steroids, with lower glucose levels, lipids, and better blood pressure parameters, which leads to less use of antihypertensive and lipid-lowering drugs in the group without steroids [17, 23]. Nonetheless, despite the acceptable results found with these strategies the community dedicated to transplantation is concerned about what happens with these long-term immunosuppression strategies, especially since presently one of the main causes of graft loss is the chronic antibody-mediated rejection mainly associated to sub-immunosuppression.

Nonetheless, the sub-immunosuppression generated by minimization strategies or suspension of an immunosuppressant in the posttransplant context causes uncertainty regarding the formation of antihuman leukocyte antigen (HLA) antibodies [donor-specific antibodies

(DSA) or nondonor-specific antibodies (NDSA)] over time, with an increased risk of antibody-mediated AR and graft loss. Some studies show results in the incidence of DSA with immunosuppression regimens considered less potent, which could cause the appearance of humoral AR and long-term graft loss [33–37]. Kreijveld et al. [72] showed that the reduction or removal of TAC from immunosuppression in the posttransplant period does not generate antibodies and does not even predict the development of AR. As for steroids, the mechanism of suppression of antibodies by the B lymphocyte with the use of these drugs has created the idea that avoiding or removing steroids in the posttransplant period favors the appearance of antibodies against the major histocompatibility complex (MHC) and against other renal donor antigens. Even so, information related to the formation of DSA with the minimization or suspension of steroids posttransplantation is scarce [73–75].

6. Antihuman leukocyte antigen antibodies in kidney recipients with steroid withdrawal

One of the leading causes of long-term graft loss is interstitial fibrosis and tubular atrophy (IFTA) and the appearance of DSA posttransplant, which seems to play an important role in graft dysfunction.

The B lymphocyte antibody suppression combined with the use of steroids has created the idea that avoiding or removing these drugs in the posttransplant period may induce the appearance of antibodies. Unfortunately, there is not sufficient evidence to uphold that the withdrawal of steroids contributes to the increase in production of antibodies or if it is associated to a higher rejection rate and chronic graft dysfunction, that being with steroids in the immunosuppression regimen. Delgado et al. [73], in a retrospective study of 43 kidney recipients during posttransplant antibody monitoring, showed that patients with steroid withdrawal did not develop DSA compared to the steroid maintained group posttransplantation. Drugs such as MMF and thymoglobulin, in addition to interacting with T lymphocytes, inhibit the formation of B lymphocyte antibodies, so it is possible that the immunosuppression regimens that use these drugs provide greater safety even when steroids are avoided or suspended after the transplantation. Furthermore, the avoidance or withdrawal of steroids may enhance the myelosuppressive effect of MMF, since steroids induce greater activity of the hepatic enzyme uridine diphosphate-glucoronosyltransferase that degrades MMF [76]. In addition, steroids induce the cytochrome P450 isoenzyme 34ª responsible for the metabolism of TAC, and so avoiding these drugs would favor the increase of TAC serum levels, thereby increasing their immunosuppressive effect [77]. These possible mechanisms suggest that the appearance of DSA induced by the suspension of steroids after transplantation could be no different than in the immunosuppression schemes maintained by these drugs.

In a clinical trial recently performed at our center with living donor kidney transplant recipients who underwent protocolized biopsies every 3 months, it was found that the presence of AR was no different between patients who had an early steroid removal compared to those in which the drugs were sustained. However, the suspension of steroids has generated uncertainty about the risk of developing DSA posttransplant, over the course of time. Due to this

concern, our team recently conducted a prospective cohort of 77 patients with low immunological risk (data not yet published) where findings revealed that the presence of cellular AR was a predictor for the formation of DSA against class II antigens, coinciding with the results of other authors [78]. There is currently little scientific evidence in which the absence of steroids in the posttransplant period may generate a greater presence of posttransplant DSA. Delgado et al. [73], observed that in a retrospective study of 43 kidney recipients during posttransplant antibody monitoring, patients with steroid suspension did not develop DSA compared to the group with maintained steroids. On the other hand, de Kort et al. [79] recently showed that in a population with steroids suspended using lymphocyte-depleting immunosuppressive induction (alentuzumab) and monotherapy with TAC, there was an increased risk for the development of DSA from an early posttransplant stage. Our study (data not yet published) also showed a higher incidence of DSA in patients with immunosuppression therapy without steroids appearing from a very early stage of the transplantation (<12 months). Unlike the study by de Kort et al. [79], 97% of our population undergoing steroid withdrawal used nonlymphocyte-depleting antibodies (basiliximab) with a double immunosuppression maintenance regimen based on MMF and TAC.

The immunoglobulin subclasses (IgG1/IgG3) capable of binding and activating the classical complement pathway (C1q) can predict the presence of antibody-mediated AR even with phenotypes of more severe damage (extensive microvascular inflammation and increased C4d deposition) and risk of kidney graft loss [80–83]. Undoubtedly, the measurement of antibody subclasses in patients subjected to a sub-immunosuppression state with minimization schemes or suspension of immunosuppression should be considered in order to discern whether the presence of these antibodies, according to the ability to fix complement, can generate chronic damage and lower the survival of the grafts. Finally, the benefits obtained from the nonsteroid schemes in the posttransplant stages in the lipid, metabolic, and blood pressure profiles, in our previously reported experience, should be considered for its possible risk of activating the immune system [17, 23].

7. Minimization strategies of mycophenolate mofetil in renal transplantation

Mycophenolate mofetil (MMF) has been established as the leading immunosuppressive regimen in most clinical trials and in almost 100% of the renal transplant centers in the world. With the initial use of CsA a daily dose of MMF was established at 2000 mg, while now, since the immunosuppressant regimen has changed to TAC significantly improving graft survival, the dose of MMF has not been established [84].

The MMF is an antiproliferative drug that requires de-esterification in gastrointestinal tissue for its absorption, thus releasing mycophenolic acid (MPA) that is freely absorbed and needs a pH > 5.5 to facilitate absorption in the small intestine. The most common use of MMF is still the prevention of AR in renal, pulmonary, cardiac, and hepatic organs, in adjunct with other immunosuppressive agents, which has shown to reduce AR by 20–40% in RT compared with azathioprine (AZA).

CsA and TAC have a different influence on enterohepatic circulation and the metabolism of MPA. The TAC increases serum levels of MMF and therefore exposure of the metabolite in the blood circulation in patients undergoing this immunosuppression regimen when compared to CsA, while the decrease in MMF dosage combined with TAC has not yet been well studied and no conclusive results have been established [85].

Clinical trials have tried to establish the MMF dosage. Doria et al. [86], included 901 patients with *de novo* RT, assigning three study groups with a MMF dose of <2000, =2000, and >2000 mg with thymoglobulin and an alemtuzumab-based induction, and no significant differences were found at 1 year follow-up regarding AR and graft loss; but they did find an increase, though not significant, in hematological complications related to leukopenia, anemia, and greater gastrointestinal disorders in patients with MMF doses of 2000 and >2000 mg.

These side effects have also motivated the establishment of adjusted dosing for certain populations. There are several controversies about whether reducing MMF dose modifies graft survival. Ji et al. [87], evaluated 128 patients with a low immunological risk at 12 months of follow-up, using immunological induction with basiliximab, methylprednisolone bolus (MPD) and TAC with a dosage of 0.1 mg/kg/day divided into two doses, PDN at 1 mg/kg/day at dose reduction, and MMF in different doses: = 500, <1500, and >1500 mg; finding, in the low dosage groups (=500 and <1500 mg), an increased number of cases of AR, renal graft dysfunction, and C4d deposition in follow-up biopsies, while the conventional dose group of MMF ≥ 1500 mg did not present any representative difference. Therefore, it is suggested that the dose should be individualized to the demographic characteristics of each population, under an integral evaluation of weight and height, and likewise that immunosuppression should not be reduced to doses less than 1 g of MMF per day nor suspension of the antimetabolite, since it jeopardizes the survival of the graft.

The side effects of MMF are divided into those due to gastrointestinal disease where diarrhea is the main manifestation with a frequency of up to 40–50% and in severe cases has been attributed as a cause of histologically inflammatory colitis type lesions similar to Crohn's disease.

Within the hematological side effects attributed to the drug there is leukopenia with or without neutropenia that can be potentiated by the use of other, concomitant drugs (Valganciclovir, trimethoprim with sulfamethoxazole, etc.) during the early period of RT. Other attributable side effects are hypogammaglobulinemia and severe anemia, especially in the first posttransplant months. The MMF has been associated with pneumonia due to pneumocystitis jirovecci, cytomegalovirus (CMV) disease, reactivation of Chagas disease, infection with Epstein-Barr virus (EBV), and risk of malignancy. On the other hand, patients with solid organ transplantation with hepatitis C seem to have better long-term outcomes with MMF therapy [88]. There is a strong association between the concentration of MPA, the pharmacological effects, and inter-individual variability between the MPA within the area under the curve (MPA AUC) estimated as the concentration of MMF after systemic elimination, enterohepatic recirculation, and the concentration before the dose (C 0) [89].

Two analysis tools have been used for the measurement of MPA plasma levels: high performance liquid chromatography (HPLC) and enzyme multiplied immunoassay technique (EMIT). The EMIT is less specific in the measurement of MPA than HPLC: the concentrations of MPA that are

obtained by the EMIT method are typically higher than those of HPLC. The overestimation of the MPA concentration by the use of EMIT is approximately 24–35%. The degree of overestimation varies depending on the patients' characteristics, the time elapsed since the transplantation, and time of the blood sampling. However, in pediatric RT recipients the EMIT assay showed a diagnostic efficacy comparable to HPLC to assess the risk of AR, leaving EMIT as an acceptable monitoring tool for MPA. Therefore, either HPLC or EMIT can be used, although HPLC is a more specific analytical tool for the accurate assessment of MPA and metabolites [90, 91].

This clinical data supports the need for therapeutic monitoring of MPA. However, this could result in higher costs and time since the precise measurement of MPA AUC 0–12 h requires multiple blood samples during the dosing interval, which can be expensive and clinically impractical [91].

It is well established that in RT recipients, MMF reduces the risk of AR and improves graft survival; nonetheless, the side effects that include diarrhea in up to 37.3%, hematological alterations (leukopenia, anemia, thrombocytopenia), and an increase in the incidence of infections in 23–25% during the first year of transplantation, make it necessary to reduce the dose of MMF. Such side effects can be avoided by individualizing immunosuppression in patients, and other studies have demonstrated that the minimization strategies of immunosuppression must be adjusted according to the race, gender, and anthropometric characteristics at each transplant center [92].

8. New strategies for minimization of immunosuppressive therapy in kidney transplantation

8.1. Alemtuzumab

New strategies in the minimization of immunosuppression involve the use of alemtuzumab (humanized monoclonal antibody that targets CD52 on lymphocytes) used as a reduction strategy in doses of CNI and immunosuppression without steroids [93, 94].

Chan et al. [95], reported in 82 patients treated with alemtuzumab (TAC as monotherapy) *versus* 42 patients with daclizumab, TAC, and MMF; all with ESR, with results of a low AR incidence at 6 months posttransplant, and without differences in the survival of the graft or in its function, confirming the minimization of immunosuppression as a therapeutic strategy with this drug (**Table 1**).

In 3-year posttransplant follow-up studies, alemtuzumab combined with ESR has shown reduction in AR episodes in patients with low immunological risk compared to basiliximab-based induction, while the presentation of AR was similar in those patients with high immunological risk in whom immunosuppressive induction was compared with thymoglobulin. The main advantage of the use of alemtuzumab as a strategy to reduce immunosuppression is found in the availability to reduce the used dosage of CNI and the subsequent conversion to maintenance immunosuppression based on imTOR, whose main objective is to avoid chronic nephrotoxicity and improve graft survival and long-term function (**Table 1**) [96, 97].

The therapeutic effect of alemtuzumab is not different from the immunological induction with thymoglobulin in the areas of AR incidence, delayed graft function, CMV infection, development of NODAT, and use of granulocyte colony stimulant [98].

8.2. Proteasome inhibitors

Proteasome nonselective inhibitors prevent the antibody-mediated AR of the graft. However, adverse effects outweigh the benefits by limiting their application in clinical practice. Up till now, the inhibition of immunoproteasomes is effective in experimental models in the context of autoimmune diseases being used for several weeks of treatment, without significant side effects. The ONX 0914, a selective proteasome inhibitor (B5i) of the LMP7 subunit, prevents chronic rejection in allogenic kidneys transplanted in rodents. The selective inhibition of immunoproteasomes by ONX 0914 and bortezomib reduces the number of plasma cells and B lymphocytes, and suppresses the formation of donor-specific antibodies in transplanted organs.

In renal grafts, T lymphocyte, B lymphocyte, and macrophage infiltration is reduced, as well as the complement deposit, interferon-γ, interleukin-17, and IgG [99].

Several series of cases have shown the efficacy of bortezomib in reversing the severe antibody-mediated rejection, establishing the maintenance therapy in posttransplant patients, and has even been used as a desensitization treatment in recipients with a positive cross test considered highly sensitized with satisfactory results; formulating guidelines to establish strategies for adjusting immunosuppression in long-term RT recipients. However, there are contradictory results. The BORTEJECT study [100] used bortezomib as a treatment for late antibody-mediated AR in 44 patients, with a follow- up of 3 years, with immunosuppression based on imTOR or CNI, with MMF 1–2 g/day and PDN, without finding a significant difference in the incidence of AR and renal function compared with placebo. Therefore, studies that evaluate the use of the drug in the induction and maintenance of immunosuppression are necessary to allow the minimization or optimization of the therapy used in selected cases (**Table 1**).

8.3. Belatacept

Belatacept (CTLA-4 Ig fusion protein) is a new drug with a mechanism of action that allows CNI-free maintained immunosuppression. Clinical studies show a higher incidence of T cell-mediated AR in the first 6 months after transplantation, but show better long-term renal graft function. Likewise, the use of belatacept shows a lower incidence of DSA formation and less graft damage compared to the use of CsA. The most relevant adverse reactions include: herpes virus infections, tuberculosis, and a higher frequency of posttransplant lymphoproliferative disorders.

Belatacept has not yet been compared to a TAC/MMF-based regimen, considered the immunosuppression maintenance standard in RT.

The current immunosuppressive treatment, far from being perfect, has contributed to the overall improvement of the renal graft and patient survival, which contributes to overcoming the barrier for the development of new therapeutic agents. Consequently, most of the

new drugs have failed in the course of transplantation, including janus kinase inhibitors (tofacitinib), sphingosine-1-phosphate receptor modulator (FTY720, fingolimod), protein kinase C inhibitor (sotrastaurin, AEB), inhibitors of adhesion anti-LFA-1 molecules (efalizumab), anti-ICAM-1, and the first generation of anti-CD40-ligand. Most of the current treatments, still in research and focused on the immunology of the transplant, are biological or cell-based treatments. The blocking of co-stimulation with the purpose to prevent T cell activation remains a point of interest. The ASKP1240, an anti-CD40 monoclonal antibody, has recently been tested in immunosuppression minimization regimens based on CNI dose reduction or suspension, compared to a control group based on standard dose TAC, finding higher AR and infection rates in the group treated with anti-CD40, so the future of this drug remains uncertain. More recently, CFZ533, a fully humanized monoclonal antibody has shown efficacy in primates, and clinical research is being initiated in humans (**Table 1**) [101].

8.4. Belimumab

Belimumab (human monoclonal antibody that inhibits B cell activating factor), approved for treatment in systemic lupus erythematosus (SLE), is in use in early-stage clinical studies for the prevention of antibody-mediated RA, as well as in patients sensitized with low titers of anti-donor-specific antibodies.

Cellular therapies represent an innovative therapeutic objective for the maintenance of long-term renal graft, and thus avoidance of adverse reactions related to the maintenance of immunosuppression. The premise of cell therapy is the induction of donor-specific nonresponse in the context of operational tolerance or mixed chimerism (**Table 1**) [100].

A single center study evaluated the autologous use of mesenchymal progenitor cells instead of the antibody-based induction with schemes based on low and high doses of CNI, comparing induction with basiliximab and standard maintenance with MMF-CNI. The induction of autologous mesenchymal progenitor cells resulted in a lower AR rate, a decrease in opportunistic infections, and better renal graft function 1 year after transplantation, concluding with conventional immunosuppressive maintenance [102].

Another study was carried out in hematopoietic progenitor cells transplant with HLA concordant kidney donors in adjunct with total lymphoid radiation and thymoglobulin, which resulted in persistent mixed chimerism with stable renal graft function and removal of all immunosuppressants in 50% of the patients (**Table 1**) [103].

The most recently used strategies include the use of a product based on hematopoietic stem cells "facilitating cells" co-administered with nonmyeloablative reconditioning in living donor kidney graft recipients, reaching, in five out of eight patients, a satisfactory donor-chimerism with successful immunosuppression maintenance withdrawal without evidence of AR or graft-versus-host disease [104].

The results of the previous studies, although very encouraging, should be validated in larger multi-centric controlled and randomized studies, from the safety-efficacy and cost–benefit points of view, compared with conventional immunosuppression therapy.

Conflict of interest

There are no conflicts of interest to report.

Author details

Jorge Andrade-Sierra[1,2,3]*, Pedro Alejandro Vazquez-Galvan[2], Hernesto Hernandez-Reyes[2], Lydia A. Mercado-Jáuregui[3], Jonathan S. Chávez-Iñiguez[4], Eduardo González-Espinoza[2], Benjamin Gómez-Navarro[2] and José I. Cerrillos-Gutiérrez[2]

*Address all correspondence to: jorg_andrade@hotmail.com

1 Department of Physiology, University Health Sciences Center, University of Guadalajara, Guadalajara, Jalisco, Mexico

2 Department of Nephrology and Organ Transplant Unit, Specialties Hospital, National Occidental Medical Centre, Mexican Social Security Institute (CMNO IMSS), Guadalajara, Jalisco, Mexico

3 Department of Internal Medicine, Civil Hospital of Guadalajara Dr. Juan I. Menchaca, Guadalajara, Jalisco, Mexico

4 Department of Nephrology, Civil Hospital of Guadalajara "Fray Antonio Alcalde", Guadalajara, Jalisco, Mexico

References

[1] Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. American Journal of Transplantation. 2004;**4**(3):378-383

[2] Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: A critical reappraisal. American Journal of Transplantation. 2011;**11**(3):450-462

[3] Meier-Kriesche HU, Schold JD, Kaplan B. Long-term renal allograft survival: Have we made significant progress or is it time to rethink our analytic and therapeutic strategies? American Journal of Transplantation. 2004;**4**(8):1289-1295

[4] Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term survival in renal transplant recipients with graft function. Kidney International. 2000;**57**(1):307-313

[5] Matas AJ, Humar A, Gillingham KJ, et al. Five preventable causes of kidney graft loss in the 1990s: A single-center analysis. Kidney International. 2002;**62**(2):704-714

[6] Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. The New England Journal of Medicine. 2003; **349**(24):2326-2333

[7] Terasaki PI. Humoral theory of transplantation. American Journal of Transplantation. 2003;**3**(6):665-673

[8] Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. Transplantation. 2012;**93**(8):751-756

[9] Terasaki PI, Cai J. Humoral theory of transplantation: Further evidence. Current Opinion in Immunology. 2005;**17**(5):541-545

[10] Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. Nature Reviews Immunology. 2005;**5**(10):807-817

[11] Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Rates and determinants of progression to graft failure in kidney allograft recipients with de novo donor-specific antibody. American Journal of Transplantation. 2015;**15**(11):2921-2930

[12] Sellares J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: The dominant role of antibody-mediated rejection and nonadherence. American Journal of Transplantation 2012;**12**(2):388-399

[13] Nankivell BJ, Kuypers DR. Diagnosis and prevention of chronic kidney allograft loss. Lancet. 2011;**378**(9800):1428-1437

[14] Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. Transplantation. 2010;**90**(1):68-74

[15] Cantarovich D, Hodemon-Corne B, Trebern-Launay K, Giral M, Foucher Y, Dantan E. Early steroid withdrawal compared with steroid avoidance correlates with graft failure among kidney transplant recipients with an history of diabetes. Transplantation Proceedings. 2013;**45**(4):1497-1502

[16] Woodle ES, Peddi VR, Tomlanovich S, Mulgaonkar S, Kuo PC, Investigators TS. A prospective, randomized, multicenter study evaluating early corticosteroid withdrawal with thymoglobulin in living-donor kidney transplantation. Clinical Transplantation. 2010;**24**(1): 73-83

[17] Andrade-Sierra J, Rojas-Campos E, Cardona-Munoz E, et al. Early steroid withdrawal in a renal transplant cohort treated with tacrolimus, mycophenolate mofetil and basiliximab. Nefrologia: Publicacion Oficial de la Sociedad Espanola Nefrologia. 2014;**34**(2):216-222

[18] Rike AH, Mogilishetty G, Alloway RR, et al. Cardiovascular risk, cardiovascular events, and metabolic syndrome in renal transplantation: Comparison of early steroid withdrawal and chronic steroids. Clinical Transplantation. 2008;**22**(2):229-235

[19] Knight SR, Morris PJ. Steroid avoidance or withdrawal after renal transplantation increases the risk of acute rejection but decreases cardiovascular risk: A meta-analysis. Transplantation. 2010;**89**(1):1-14

[20] Pascual J, Quereda C, Zamora J, Hernandez D, Spanish Group for Evidence-Based Medicine in Renal T. Updated metaanalysis of steroid withdrawal in renal transplant patients on calcineurin inhibitor and mycophenolate mofetil. Transplantation Proceedings. 2005;**37**(9):3746-3748

[21] Gonzalez-Molina M, Gentil MA, Burgos D, et al. Effect of long-term steroid withdrawal in renal transplant recipients: A retrospective cohort study. NDT Plus. 2010; **3**(Suppl_2):ii32-ii36

[22] Afaneh C, Cheng E, Aull MJ, et al. Renal allograft outcomes following early corticosteroid withdrawal in Hispanic transplant recipients. Clinical Transplantation. 2013; **27**(6):E611-E618

[23] Andrade-Sierra J, Rojas-Campos E, Cardona-Munoz E, et al. Early steroid withdrawal in recipients of a kidney transplant from a living donor: Experience of a single Mexican center. Transplantation Proceedings. 2016;**48**(1):42-49

[24] Iwamoto H, Hama K, Konno O, et al. Early steroid withdrawal in adult kidney transplantation at a single center. Transplantation Proceedings. 2012;**44**(1):179-181

[25] Lee YJ, Kim B, Lee JE, et al. Randomized trial of cyclosporine and tacrolimus therapy with steroid withdrawal in living-donor renal transplantation: 5-year follow-up. Transplant International. 2010;**23**(2):147-154

[26] Woodle ES, Alloway RR, Hanaway MJ, et al. Early corticosteroid withdrawal under modern immunosuppression in renal transplantation: Multivariate analysis of risk factors for acute rejection. Transplantation Proceedings. 2005;**37**(2):798-799

[27] Cantarovich D, Rostaing L, Kamar N, et al. Early corticosteroid avoidance in kidney transplant recipients receiving ATG-F induction: 5-year actual results of a prospective and randomized study. American Journal of Transplantation. 2014;**14**(11):2556-2564

[28] Nehus E, Liu C, Hooper DK, Macaluso M, Kim MO. Clinical practice of steroid avoidance in pediatric kidney transplantation. American Journal of Transplantation. 2015; **15**(8):2203-2210

[29] Woodle ES, Fujisawa Corticosteroid Withdrawal Study G. A prospective, randomized, multicenter, double-blind study of early corticosteroid cessation versus long-term maintenance of corticosteroid therapy with tacrolimus and mycophenolate mofetil in primary renal transplant recipients: One year report. Transplantation Proceedings 2005; **37**(2):804-808

[30] Teraoka S, Sato S, Sekijima M, et al. Comparative study of clinical outcome in kidney transplantation between early steroid withdrawal protocol using basiliximab, calcineurin inhibitor, and mycophenolate mofetil and triple regimen consisting of calcineurin inhibitor, mycophenolate mofetil, and steroid. Transplantation Proceedings. 2005; **37**(2):791-794

[31] Nagib AM, Abbas MH, Abu-Elmagd MM, et al. Long-term study of steroid avoidance in renal transplant patients: A single-center experience. Transplantation Proceedings. 2015;**47**(4):1099-1104

[32] Novosel MK, Bistrup C. Discontinuation of steroids in ABO-incompatible renal transplantation. Transplant International. 2016;**29**(4):464-470

[33] Kamar N, Del Bello A, Congy-Jolivet N, et al. Incidence of donor-specific antibodies in kidney transplant patients following conversion to an everolimus-based calcineurin inhibitor-free regimen. Clinical Transplantation. 2013;**27**(3):455-462

[34] Thierry A, Le Meur Y, Ecotiere L, et al. Minimization of maintenance immunosuppressive therapy after renal transplantation comparing cyclosporine A/azathioprine or cyclosporine A/mycophenolate mofetil bitherapy to cyclosporine A monotherapy: A 10-year postrandomization follow-up study. Transplant International. 2016;**29**(1):23-33

[35] Liefeldt L, Brakemeier S, Glander P, et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. American Journal of Transplantation. 2012;**12**(5):1192-1198

[36] Croze LE, Tetaz R, Roustit M, et al. Conversion to mammalian target of rapamycin inhibitors increases risk of de novo donor-specific antibodies. Transplant International. 2014;**27**(8):775-783

[37] de Sandes-Freitas TV, Felipe CR, Campos EF, et al. Subclinical lesions and donor-specific antibodies in kidney transplant recipients receiving tacrolimus-based immunosuppressive regimen followed by early conversion to sirolimus. Transplantation. 2015; **99**(11):2372-2381

[38] Augustine JJ, Hricik DE. Minimization of immunosuppression in kidney transplantation. Current Opinion in Nephrology and Hypertension. 2007;**16**(6):535-541

[39] Golshayan D, Pascual M. Minimization of calcineurin inhibitors to improve long-term outcomes in kidney transplantation. Transplant Immunology. 2008;**20**(1-2):21-28

[40] Halloran PF. Immunosuppressive drugs for kidney transplantation. The New England journal of medicine. 2004;**351**(26):2715-2729

[41] Danovich GM. Handbook of Kidney Transplatation. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2010. pp. 77-100

[42] Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. The New England Journal of Medicine. 2007;**357**(25):2562-2575

[43] Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: Longitudinal assessment by protocol histology. Transplantation. 2004;**78**(4):557-565

[44] Srinivas TR, Meier-Kriesche HU. Minimizing immunosuppression, an alternative approach to reducing side effects: Objectives and interim result. Clinical Journal of the American Society of Nephrology. 2008;**3**(Suppl 2):S101-S116

[45] Haroon N, Singh A, Bhat ZY. Tacrolimus toxicity with minimal clinical manifestations: A case report and literature review. American Journal of Therapeutics. 2016;**23**(2):e631-e634

[46] Tang JT, Andrews LM, van Gelder T, et al. Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: Recent developments and ethnic considerations. Expert Opinion on Drug Metabolism and Toxicology. 2016;**12**(5):555-565

[47] Thervet E, Loriot MA, Barbier S, et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clinical Pharmacology and Therapeutics. 2010;**87**(6):721-726

[48] Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the symphony study: Observational results 3 years after transplantation. American Journal of Transplantation. 2009;**9**(8):1876-1885

[49] Issa N, Kukla A, Ibrahim HN. Calcineurin inhibitor nephrotoxicity: A review and perspective of the evidence. American Journal of Nephrology. 2013;**37**(6):602-612

[50] Larson TS, Dean PG, Stegall MD, et al. Complete avoidance of calcineurin inhibitors in renal transplantation: A randomized trial comparing sirolimus and tacrolimus. American Journal of Transplantation. 2006;**6**(3):514-522

[51] Flechner SM, Glyda M, Cockfield S, et al. The ORION study: Comparison of two sirolimus-based regimens versus tacrolimus and mycophenolate mofetil in renal allograft recipients. American Journal of Transplantation. 2011;**11**(8):1633-1644

[52] Vincenti F, Charpentier B, Vanrenterghem Y, et al. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). American Journal of Transplantation. 2010;**10**(3):535-546

[53] Weir MR, Mulgaonkar S, Chan L, et al. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: A randomized, controlled spare-the-nephron trial. Kidney International. 2011;**79**(8):897-907

[54] Sawinski D, Trofe-Clark J, Leas B, et al. Calcineurin inhibitor minimization, conversion, withdrawal, and avoidance strategies in renal transplantation: A systematic review and meta-analysis. American Journal of Transplantation. 2016;**16**(7):2117-2138

[55] Karpe KM, Talaulikar GS, Walters GD. Calcineurin inhibitor withdrawal or tapering for kidney transplant recipients. Cochrane Database of Systematic Reviews. 2017;**7**:CD006750

[56] Matas AJ. Minimization of steroids in kidney transplantation. Transplant International. 2009;**22**(1):38-48

[57] Vincenti F. Immunosuppression minimization: Current and future trends in transplant immunosuppression. The Journal of the American Society of Nephrology. 2003; **14**(7):1940-1948

[58] Rizzari MD, Suszynski TM, Gillingham KJ, et al. Ten-year outcome after rapid discontinuation of prednisone in adult primary kidney transplantation. Clinical Journal of the American Society of Nephrology. 2012;**7**(3):494-503

[59] Lopez-Soler RI, Chan R, Martinolich J, et al. Early steroid withdrawal results in improved patient and graft survival and lower risk of post-transplant cardiovascular risk profiles: A single-center 10-year experience. Clinical Transplantation. 2017;**31**(2):e12878

[60] Zhang X, Huang H, Han S, Fu S, Wang L. Is it safe to withdraw steroids within seven days of renal transplantation? Clinical Transplantation. 2013;**27**(1):1-8

[61] Pascual J, Quereda C, Zamora J, Hernandez D, Spanish Group for Evidence-Based Medicine in Renal T. Steroid withdrawal in renal transplant patients on triple therapy with a calcineurin inhibitor and mycophenolate mofetil: A meta-analysis of randomized, controlled trials. Transplantation. 2004;**78**(10):1548-1556

[62] Gaber AO, Moore LW, Alloway RR, et al. Acute rejection characteristics from a prospective, randomized, double-blind, placebo-controlled multicenter trial of early corticosteroid withdrawal. Transplantation. 2013;**95**(4):573-579

[63] ter Meulen CG, van Riemsdijk I, Hene RJ, et al. Steroid-withdrawal at 3 days after renal transplantation with anti-IL-2 receptor alpha therapy: A prospective, randomized, multicenter study. American Journal of Transplantation. 2004;**4**(5):803-810

[64] Rostaing L, Cantarovich D, Mourad G, et al. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. Transplantation. 2005;**79**(7):807-814

[65] Laftavi MR, Stephan R, Stefanick B, et al. Randomized prospective trial of early steroid withdrawal compared with low-dose steroids in renal transplant recipients using serial protocol biopsies to assess efficacy and safety. Surgery. 2005;**137**(3):364-371

[66] Andrade-Sierra J. Effect of early steroid withdrawal (ESW) in a Mexican kidney transplants recipients (KTR) cohort, treated with tacrolimus (TAC) and mycophenolate mophetil (MMF). The Journal of the American Society of Nephrology. 2010;**21**(TH-PO988):337A

[67] Aull MJ, Dadhania D, Afaneh C, et al. Early corticosteroid withdrawal in recipients of renal allografts: A single-center report of ethnically diverse recipients and recipients of marginal deceased-donor kidneys. Transplantation. 2012;**94**(8):837-844

[68] Vlaminck H, Maes B, Evers G, et al. Prospective study on late consequences of subclinical non-compliance with immunosuppressive therapy in renal transplant patients. American Journal of Transplantation. 2004;**4**(9):1509-1513

[69] Butler JA, Roderick P, Mullee M, Mason JC, Peveler RC. Frequency and impact of nonadherence to immunosuppressants after renal transplantation: A systematic review. Transplantation. 2004;**77**(5):769-776

[70] Sharif A, Baboolal K. Complications associated with new-onset diabetes after kidney transplantation. Nature Reviews Nephrology. 2012;**8**(1):34-42

[71] Budde K, Matz M, Durr M, Glander P. Biomarkers of over-immunosuppression. Clinical Pharmacology and Therapeutics. 2011;**90**(2):316-322

[72] Kreijveld E, Hilbrands LB, van Berkel Y, Joosten I, Allebes W. The presence of donor-specific human leukocyte antigen antibodies does not preclude successful withdrawal of tacrolimus in stable renal transplant recipients. Transplantation. 2007;**84**(9):1092-1096

[73] Delgado JC, Fuller A, Ozawa M, et al. No occurrence of de novo HLA antibodies in patients with early corticosteroid withdrawal in a 5-year prospective randomized study. Transplantation. 2009;**87**(4):546-548

[74] Lachmann N, Terasaki PI, Schonemann C. Donor-specific HLA antibodies in chronic renal allograft rejection: A prospective trial with a four-year follow-up. Clinical Transplants. 2006;**13**(1):171-199

[75] Li L, Chaudhuri A, Chen A, et al. Efficacy and safety of thymoglobulin induction as an alternative approach for steroid-free maintenance immunosuppression in pediatric renal transplantation. Transplantation. 2010;**90**(12):1516-1520

[76] Schuetz EG, Hazelton GA, Hall J, Watkins PB, Klaassen CD, Guzelian PS. Induction of digitoxigenin monodigitoxoside UDP-glucuronosyltransferase activity by glucocorticoids and other inducers of cytochrome P-450p in primary monolayer cultures of adult rat hepatocytes and in human liver. The Journal of Biological Chemistry. 1986; **261**(18):8270-8275

[77] Hesselink DA, Ngyuen H, Wabbijn M, et al. Tacrolimus dose requirement in renal transplant recipients is significantly higher when used in combination with corticosteroids. British Journal of Clinical Pharmacology. 2003;**56**(3):327-330

[78] Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. American Journal of Transplantation. 2012;**12**(5):1157-1167

[79] de Kort H, Willicombe M, Brookes P, et al. Microcirculation inflammation associates with outcome in renal transplant patients with de novo donor-specific antibodies. American Journal of Transplantation. 2013;**13**(2):485-492

[80] Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. The New England Journal of Medicine. 2013;**369**(13):1215-1226

[81] Arnold ML, Ntokou IS, Doxiadis II, Spriewald BM, Boletis JN, Iniotaki AG. Donor-specific HLA antibodies: Evaluating the risk for graft loss in renal transplant recipients with isotype switch from complement fixing IgG1/IgG3 to noncomplement fixing IgG2/IgG4 anti-HLA alloantibodies. Transplant International. 2014;**27**(3):253-261

[82] Filippone EJ, Farber JL. Humoral immunity in renal transplantation: Epitopes, Cw and DP, and complement-activating capability--an update. Clinical Transplantation. 2015;**29**(4):279-287

[83] Everly MJ, Rebellato LM, Haisch CE, et al. Impact of IgM and IgG3 anti-HLA alloantibodies in primary renal allograft recipients. Transplantation. 2014;**97**(5):494-501

[84] Meier-Kriesche HU, Li S, Gruessner RW, et al. Immunosuppression: Evolution in practice and trends, 1994-2004. American Journal of Transplantation. 2006;**6**(5 Pt 2):1111-1131

[85] Park JM, Lake KD, Cibrik DM. Impact of changing from cyclosporine to tacrolimus on pharmacokinetics of mycophenolic acid in renal transplant recipients with diabetes. Therapeutic Drug Monitoring. 2008;**30**(5):591-596

[86] Doria C, Greenstein S, Narayanan M, et al. Association of mycophenolic acid dose with efficacy and safety events in kidney transplant patients receiving tacrolimus: An analysis of the mycophenolic acid observational Renal transplant registry. Clinical Transplantation. 2012;**26**(6):E602-E611

[87] Ji SM, Xie KN, Chen JS, et al. Retrospective evaluation of the effect of mycophenolate mofetil dosage on survival of kidney grafts based on biopsy results. Transplantation Proceedings. 2014;**46**(10):3383-3389

[88] Staatz CE, Tett SE. Pharmacology and toxicology of mycophenolate in organ transplant recipients: An update. Archives of Toxicology. 2014;**88**(7):1351-1389

[89] Oellerich M, Shipkova M, Schutz E, et al. Pharmacokinetic and metabolic investigations of mycophenolic acid in pediatric patients after renal transplantation: Implications for therapeutic drug monitoring. German study group on mycophenolate mofetil therapy in pediatric renal transplant recipients. Therapeutic Drug Monitoring. 2000;**22**(1):20-26

[90] Pawinski T, Hale M, Korecka M, Fitzsimmons WE, Shaw LM. Limited sampling strategy for the estimation of mycophenolic acid area under the curve in adult renal transplant patients treated with concomitant tacrolimus. Clinical Chemistry. 2002;**48**(9):1497-1504

[91] Jeong H, Kaplan B. Therapeutic monitoring of mycophenolate mofetil. Clinical Journal of the American Society of Nephrology. 2007;**2**(1):184-191

[92] Tornatore KM, Meaney CJ, Wilding GE, et al. Influence of sex and race on mycophenolic acid pharmacokinetics in stable African American and Caucasian renal transplant recipients. Clinical Pharmacokinetics. 2015;**54**(4):423-434

[93] Hanaway MJ, Woodle ES, Mulgaonkar S, et al. Alemtuzumab induction in renal transplantation. The New England Journal of Medicine. 2011;**364**(20):1909-1919

[94] Zachariah M, Nader ND, Brar J, et al. Alemtuzumab and minimization immunotherapy in kidney transplantation: Long-term results of comparison with rabbit anti-thymocyte globulin and standard triple maintenance therapy. Transplantation Proceedings. 2014; **46**(1):94-100

[95] Chan K, Taube D, Roufosse C, et al. Kidney transplantation with minimized maintenance: Alemtuzumab induction with tacrolimus monotherapy: An open label, randomized trial. Transplantation. 2011;**92**(7):774-780

[96] Friend PJ. Alemtuzumab induction therapy in solid organ transplantation. Transplantation Research. 2013;**2**(Suppl 1):S5

[97] Haynes R, Baigent C, Harden P, et al. Campath, calcineurin inhibitor reduction and chronic allograft nephropathy (3C) study: Background, rationale, and study protocol. Transplantation Research. 2013;**2**(1):7

[98] Zheng J, Song W. Alemtuzumab versus antithymocyte globulin induction therapies in kidney transplantation patients: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2017;**96**(28):e7151

[99] Li J, Basler M, Alvarez G, Brunner T, Kirk CJ, Groettrup M. Immunoproteasome inhibition prevents chronic antibody-mediated allograft rejection in renal transplantation. Kidney International. 2018;**93**(3):670-680

[100] Eskandary F, Regele H, Baumann L, et al. A randomized trial of Bortezomib in late antibody-mediated kidney transplant rejection. The Journal of the American Society of Nephrology. 2018;**29**(2):591-605

[101] Lim MA, Kohli J, Bloom RD. Immunosuppression for kidney transplantation: Where are we now and where are we going? Transplantation Reviews (Orlando, Fla.). 2017; **31**(1):10-17

[102] Tan J, Wu W, Xu X, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: A randomized controlled trial. Journal of the American Medical Association. 2012;**307**(11):1169-1177

[103] Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and withdrawal of immunosuppressive drugs in patients given kidney and hematopoietic cell transplants. American Journal of Transplantation. 2012;**12**(5):1133-1145

[104] Leventhal J, Abecassis M, Miller J, et al. Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation. Science Translational Medicine. 2012;**4**(124):124ra128