Immunosuppression, Compliance, and Tolerance After Orthotopic Liver Transplantation: State of the Art

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Abstract

Orthotopic liver transplantation is the treatment of choice for several otherwise irreversible forms of acute and chronic liver diseases. Early implemented immunosuppressant regimens have had disappointing results with high rejection rates. However, new drugs have reduced the daily immunosuppression requirements, thereby improving graft and patient survival as well as kidney function. Liver rejection is a T-cell-driven immune response and is the active target of immunosuppressive agents. Immunosuppressants can be divided into pharmacological or biological drugs: the gold standard is the calcineurin inhibitors, steroids, mycophenolate mofetil, and mechanistic target of rapamycin inhibitors. Compliance with these agents is essential, although they can increase the risk of infections and neoplastic diseases. In some patients, graft tolerance can be achieved. Graft tolerance is defined as the absence of acute and chronic rejection in a graft, with normal function and histology in an immunosuppression-free, fully immunocompetent host, usually as the final result of a successful attempt at immunosuppression withdrawal. The occurrence of immunosuppressive-related complications has led to new protocols aimed at protecting renal function and preventing de novo cancer and dysmetabolic syndrome. The backbone of immunosuppression remains calcineurin inhibitors in association with other drugs, mainly over the short-term period. To avoid rejection and the side effects on renal dysfunction, de novo cancer, and cardiovascular syndrome, optimal long-term immunosuppressive therapy should be tailored in liver transplant recipients.

Key words: *Immune response, Rejection, Renal dysfunction*

Introduction

Since the first human liver transplantation, performed in 1963 by T. E. Starzl in Denver, Colorado,[1] orthotopic liver transplant (OLT) has been considered an experimental procedure up to the 1980s. Today, it is regarded as the treatment of choice for a number of otherwise irreversible forms of acute and chronic liver diseases.[2]

Following the success shown with regimens for kidney transplantation, early OLT immunosuppressant cocktails were based on azathioprine, corticosteroids, and antithymocyte globulins (ATGs). The results with these were disappointing with high rejection rates.[3] However, with the introduction of cyclosporine, a new immunosuppressant in the early 1980s, rapid and significant improvements in survival were shown.[4]

Eventually, with the discovery of tacrolimus in the 1990s, outcomes of liver transplant recipients dramatically changed, with increased long-term graft and patient survival rates.[5,6] Moreover, several later introduced new drugs (such as mycophenolate mofetil [MMF] or everolimus) (Figure 1) reduced the daily requirement of immunosuppression drugs among liver transplant recipients, improving their kidney function.

Immunosuppressive Therapy After Orthotopic Liver Transplant: State of the Art

Apart from hyperacute rejection, T-cell activation acts as the starter of the rejection cascade, with the key to controlling the rejection represented by immunosuppressive drugs. As stated earlier, the first regimens were characterized by high rejection rates, lower graft survival, and lower patient survival. Presently, there are different classes of immunosuppressive drugs that target the mechanisms of action focused on T-cell activation. Immunosuppressive agents can be divided into pharmacological or biological types (Table 1 and Table 2).

The corticosteroids are the first class of hormones and have lymphocytolytic effects.[7] They interact with the immune system at various levels, reducing the number and size of lymphoid cells and inhibiting the production of inflammatory mediators such as platelet-activating factor, leukotrienes, and prostaglandins. Moreover, they inhibit monocyte and neutrophil chemotaxis and produce lympho- and neutropenia, not through direct cytotoxicity but by altering the diffusion of these cell populations. Corticosteroids are common components of combined immunosuppressant regimens and are also administered as intravenous boluses to treat acute rejection events. Glucocorticoids, particularly when used for long periods, have several side effects: glucose intolerance, hypertension, osteoporosis, muscle mass reduction, weight gain with central obesity, moon facies, striae rubrae, psychosis, cataract, glaucoma, and even iatrogenic Cushing syndrome.[8,9]

Cyclosporine, introduced in the 1980s,[4] reduced the rates of rejection from 15% (reported by various groups) to 2% to 5%,[10] validating calcineurin inhibitors (CNIs) as the backbone of immunosuppression. Tacrolimus (FK506), first used in clinical practice in the 1990s,[11-13] and cyclosporine
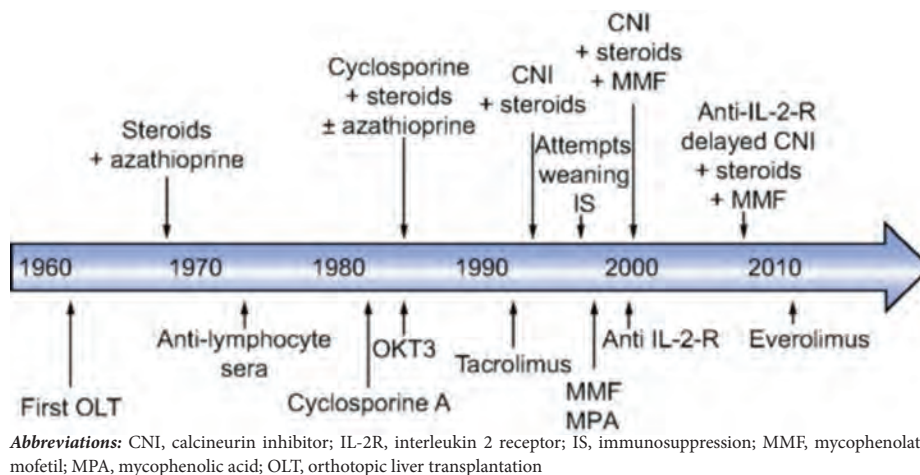
Figure 1. Evolutions of Immunosuppressant Therapy

Abbreviations: CNI, calcineurin inhibitor; IL-2R, interleukin 2 receptor; IS, immunosuppression; MMF, mycophenolate mofetil; MPA, mycophenolic acid; OLT, orthotopic liver transplantation

Table 1. Pharmacological Immunosuppressive Agents	
Class	Mechanism of Action
Corticosteroids	Inhibit cytokine transcription by antigen-presenting cells, broad spectrum of effects
Calcineurin inhibitors (cyclosporine, tacrolimus)	Inhibition of signal 2 transduction
Antimetabolites (azathioprine, mycophenolate)	Inhibition of purine and DNA synthesis and prevention of T-cell proliferation
mTOR inhibitors (sirolimus, everolimus)	Inhibition of signal 3 transduction and prevention of T-cell proliferation

Abbreviations: mTOR, mechanistic target of rapamycin

Table 2. Biological Immunosuppressive Agents	
Class	Mechanism of Action
T-cell-depleting agents (anti-CD3 monoclonal OKT3)	Interference with signal 1
T-cell-depleting agents (ATG/ALG horse and rabbit)	Interference with signals 1-3
T-cell-depleting agents (anti-CD52, alemtuzumab)	Depletion of thymocytes, T cells, B cells, monocytes
Non-T cell-depleting agents (anti-IL-2 receptors, basiliximab, daclizumab)	Inhibition of T-cell proliferation and signal 3
Non-T-cell-depleting agents (belatacept)	Inhibition of signal 2

Abbreviations: ALG, antilymphocyte globulin; ATG, antithymocyte globulin; IL-2, interleukin 2

are CNIs, a serine-threonine phosphatase involved in the activation of various transcription factors. With activated T lymphocytes, the inhibition of calcineurin blocks the transcription of various cytokines, including interleukin 2, which plays a fundamental role in activating the immune response. Tacrolimus is more potent than cyclosporine in suppressing the immune response. The selected administered dose is based on drug levels in the blood, which need to be monitored at regular intervals. Both drugs are metabolized in the liver by the P450 IIIA cytochrome system, allowing reactions with other drugs to increase (erythromycin, fluconazole, verapamil, cimetidine) or reduce (phenobarbital, phenytoin, carbamazepine) cyclosporine or tacrolimus levels in the blood.

These drugs also have multiple side effects. Their nephrotoxicity is due to dose-dependent damage to the renal tubule as well as vasa-spastic effects on the renal artery. Other side effects include arterial hypertension, glucose intolerance, and neurological symptoms (tremor), whereas cyclosporine also causes gingival hyperplasia and hirsutism.[14]

Another immunosuppressant, rapamycin,[15,16] shares the same targets as tacrolimus, but it acts during a later phase of lymphocyte activation. It can cause bone marrow suppression, so white blood cell counts must be closely monitored. Rapamycin also interferes with lipid metabolism, and signs of dyslipidemia are a common side effect.[17-19]

Antimetabolites such as MMF[20] and azathioprine work by different mechanisms of action. The first one inhibits the proliferation of activated T lymphocytes by blocking purine metabolism, and it can cause diarrhea, its main side effect,[21] whereas azathioprine, a derivative of mercaptopurine, acts along with MMF by adding an antimetabolite effect. It is metabolized by the enzyme xanthine oxidase, which is the molecular target of gout medication, allopurinol. Coadministration of the 2 drugs can cause serious azathioprine toxicity with severe bone marrow suppression.

Finally, the last group of pharmacological agents are mechanistic target of rapamycin inhibitors, such as everolimus and sirolimus, inhibiting the transduction of interleukin 2 and preventing T-cell proliferation.[17,18]

Immunosuppressive biological agents are immunoglobulins directed against the lymphocytes (antilymphocyte globulin), immunoglobulins directed against thymocytes (ATG), monoclonal antibodies against T lymphocytes (OKT3, alemtuzumab), and non-T-cell depleting agents (basiliximab, belatacept). They are used in many centers to induce immunosuppression and to treat acute rejection events that are unresponsive to boluses of corticosteroids.[22,23] Still, all immunosuppressant drugs increase the risk of all types of infections (bacterial, viral, fungal) and of several neoplastic diseases, such as hematological diseases (posttransplant lymphoproliferative disease) and solid tumors.

Compliance and Tolerance After Orthotopic Liver Transplant: How to Achieve It?
Major histocompatibility complex antigens remain the most important alloantigens in graft rejection since discovery of their transplant relevance in the late 1960s/early 1970s.[24] Liver rejection is a T-cell-driven immune response that predominantly targets bile ducts.

The liver is a tolerogenic organ, and its microanatomy, cellular composition, and cytokine microenvironment contribute to easier acceptance of this graft compared with other solid-organ transplants. Preservation and reperfusion injury can contribute to the breaking of tolerance and triggering of immune-mediated injury. Immunosuppression weaning is achieved in 20%[25] of selected transplant patients, but hepatitis C virus (HCV) eradication is recommended in recipients with HCV positivity before attempting immunosuppression weaning.

The backbone of immunosuppression after OLT remains CNIs. The current acute and chronic rejection rates are 10% to 40% and 5%, respectively.[25] Medium-term and long-term complications of immunosuppression are significant concerns; these complications include renal, metabolic, and cardiovascular diseases and de novo cancer. The presently used renal function-sparing regimens include immunosuppression that combine low-dose CNI with anti-interleukin 2 antibodies, mycophenolic acid prodrugs, or everolimus.

In 1992, microchimerism in OLT recipients was reported[26,27] in Pittsburgh, Pennsylvania. Since 1995, there has been increasing evidence that OLT recipients who cease to take immunosuppressive drugs may maintain allograft function, suggesting that tolerance is often present. Tolerance is generally characterized by the absence of acute and chronic rejection. Through a prospective trial of complete drug weaning, it was shown that withdrawal of immunosuppression after OLT is possible, allowing graft survival (with normal function and histology) to be achieved in an immunosuppression-free recipient. In subsequent trials from other institutions, complete drug weaning was safely accomplished in up to 20% of OLT recipients and even

in a handful of living-related kidney transplant recipients who had been drug-free for as long as 30 years.

"Acquired tolerance" is the specific failure of the host's immunological response, and "operational tolerance" is the absence of acute and chronic rejection in a graft, with normal function and histology in an immunosuppression-free, fully immunocompetent host, usually as the final result of a successful attempt at immunosuppression withdrawal. The tolerance, however, also includes minimal adverse effects, apart from rejection or recurrence, such as de novo malignancies or renal function.

In 2013, Wimmer and colleagues[28] studied de novo malignancies as a major cause of late death after liver transplant. The study tried to determine whether the use of cyclosporine versus tacrolimus affects long-term tumor incidence when considering potential confounders. When target tacrolimus levels are reduced, the risk for de novo malignancies may be reduced. Although yet to be determined in prospective trials, tacrolimus-based immunosuppression should be discussed, especially in older male patients.

In a study from Sterneck and colleagues,[29] liver transplant patients were randomized at 4 weeks to start everolimus and discontinue CNI or continue their current CNI-based regimen; the primary endpoint was adjusted estimated glomerular filtration rate, confirmed by biopsy-proved acute rejection during core study. Everolimus-based, CNI-free immunosuppression is feasible after liver transplant, and patients can have sustained preservation of renal function for at least 3 years. This beneficial effect on renal function continues to be evident after 3 years.

In 2014, Ganschow and colleagues[30] analyzed the role of everolimus in liver transplant, providing an overview of the efficacy and safety of everolimus-based regimens for de novo and maintenance settings and "special" populations. These special populations included patients with hepatocellular carcinoma (HCC) recurrence, those who were HCV-positive, and pediatric transplant recipients. In this study, introducing everolimus at 30 days posttransplant in combination with reduced-dose tacrolimus (exposure reduced by 39%) had comparable efficacy (composite efficacy failure rate of treated acute rejection biopsy-proven, graft loss, or death) and achieved superior renal function versus standard exposure tacrolimus as early as 1 month and maintained over 2 years.

Xing and colleagues[31] evaluated the efficacy and safety of using basiliximab in place of a corticosteroid for immunosuppression following liver transplant for HCC: in patients who met the Milan criteria, basiliximab was associated with a better 5-year overall survival rate than with steroid therapy (88.9% vs 57.4%, respectively; *P* = .022). These findings provided further evidence of the negative impact of steroids as a part of immunosuppression therapy following liver transplant for HCC.

In a multicenter randomized trial, the role of sirolimus was investigated in OLT candidates with HCC.[32] Recurrence-free survival and overall survival benefits were present in the first 3 to 5 years, especially in low-risk patients, but not beyond 5 years. This trial provided the first high-level evidence base for selecting immunosuppression in OLT recipients with HCC.

Uhlmann and colleagues[33] studied the long-term efficacy and safety of conversion from a CNI-based immunosuppressive regimen to sirolimus monotherapy in liver transplant recipients with renal dysfunction. This type of immunosuppression conversion resulted in stabilization of renal function (in 75% to 85% of cases) and blood pressure, without increased risk of rejection.

In a randomized trial, the long-term outcomes with the use of tacrolimus[34] were evaluated in which triple therapy was compared versus monotherapy after transplant for HCV cirrhosis. A long-term immunosuppression regimen with tacrolimus, azathioprine, and short-term prednisolone in liver transplant recipients with HCV cirrhosis resulted in slower progression to severe fibrosis and less portal hypertension and decompensation compared with tacrolimus alone. Severe fibrosis was assessed by collagen proportionate area and Ishak stage.[35]

Rabbit ATG induction is increasingly used in liver transplant in conjunction with steroid-free protocols to delay the initiation of CNIs. A single-center retrospective study[36] demonstrated that ATG-based induction could be safely used in adult OLT recipients with excellent survival for patients with HCV and HCC. Overall, this induction therapy demonstrated low rejection rates without any increase in immunosuppression-related side effects.

Uemura and colleagues[37] compared standard cortico-steroid induction, ATG, or daclizumab induction for liver transplant, with a particular interest in patients with HCV. Induction with ATG appeared to be preferentially used in patients with renal dysfunction, improving renal function after liver transplant. Thus, ATG induction can be used for patients with renal dysfunction in non-HCV diseases. Daclizumab induction achieved satisfactory short-term

and long-term outcomes in liver transplant recipients with all liver diseases, including HCV.

A literature review published in 2013 discussed the results of immunosuppressive studies, taking into account current strategies for immunosuppression in liver transplant recipients, including the design of protocols targeting a more individualized approach to reduce risk factors such as renal failure, cardiovascular complications, and malignancies.[38]

In 2015, the DIAMOND Study,[39] a 24-week multicenter randomized trial, investigated the effect of different once-daily, prolonged-release tacrolimus regimens on renal function after de novo liver transplant. In this 3-arm analyses, the study suggested that early posttransplant tacrolimus exposure is critical for preserving renal function over the long term.

The safety and feasibility of daily tacrolimus were also confirmed by another report.[40] Early conversion to once-daily tacrolimus during liver transplant hospitalization resulted in a 26.2% dose increase during the first 2 weeks after conversion. Adverse events after conversion were scarce, and all patients had normal liver function.

Thorat and colleagues had a similar conclusion,[41] reporting that tacrolimus can be safely converted from the twice-daily to the once-daily formulation for most stable liver transplant recipients, although acute rejection may occur in a minority of patients during conversion and should be carefully monitored.

In a recent retrospective study involving the European Liver Transplant Registry,[42] which analyzed up to 8 years of data between 2008 and 2016, the prolonged-release tacrolimus-based immunosuppression seemed to improve long-term outcomes in liver transplant recipients more than immediate-release tacrolimus-based immunosuppression. A previous study by the same group[43] concluded that prolonged-release tacrolimus-based immunosuppression could improve long-term outcomes in liver transplant recipients compared with immediate-release tacrolimus. Furthermore, use of the immediate-release formulation was a significant predictor of long-term graft loss and patient mortality. Importantly, these findings confirmed that prolonged-release tacrolimus continues to provide ongoing benefits for graft and patient survival beyond 3 years posttransplant.

O'Leary and colleagues[44] also studied the correlation of donor-specific anti-HLA antibodies with clinical outcomes in patients after OLT and did not establish a link. Although a further study with larger numbers of patients is needed to identify clinically significant thresholds, there is an association of high mean fluorescence intensity donor-specific antibodies with chronic rejection after OLT.

The ability to produce a state of tolerance after transplant would obviate long-term immunosuppression. To date, studies have shown that many subsets of regulatory T cells (Tregs) control immune responses to foreign and alloantigens.[45] The identification of Tregs has resulted in major advances in our understanding of the immunology of rejection and the development of transplant tolerance. Although no clinical trials are currently using Tregs for chronic graft dysfunction, several experimental models have demonstrated the ability of Tregs to prevent manifestations of chronic graft failure.[46,47] An important role for Tregs in the promotion of tolerance has also been shown in human renal and liver transplant, and this supports the use of Treg-based therapies to induce tolerance in the clinical setting.

Conclusions

On December 23, 1954, the team of Joseph E. Murray at Peter Bent Brigham Hospital in Boston, Massachusetts, performed the first successful solid-organ transplant. In 1990, Dr. Murray became a Nobel laureate for that historic surgery. The kidney transplant donor was an identical twin, and the scientists were correct in predicting that the organ could work without any immunosuppressive treatment. Other kidney transplant procedures between identical twins were performed with success and prolonged survival. Tissue typing and immune system research were beginning, and rejection remained the Achille's heel of transplant for several years. The introduction of steroids and azathioprine allowed the first series of human transplant with deceased or living donors by suppressing the human body's immune system reaction. However, steroids and other drugs used in those years had severe side effects, and many patients died with overwhelming infections. The real game changer was the introduction in 1979 of cyclosporine A and, 10 years later, tacrolimus (then called FK506). In other words, the new CNIs.

The gold standard of immunosuppression remains CNIs, mainly in the short-term period, in association with steroids and/or MMF or mechanistic target of rapamycin inhibitors (everolimus, Rapamune). In 2004, it was shown[48] that basiliximab, in a tacrolimus-based immunosuppressive regimen, effectively reduced acute cellular rejection and increased acute cell rejection-free survival after OLT. The occurrence of post-OLT immunosuppression

related complications has led to new protocols aimed at protecting renal function and preventing de novo cancer and dysmetabolic syndrome. Calcineurin inhibitor-sparing protocols with induction therapy (ATG, daclizumab, rituximab, basiliximab) are now well-established immuno-suppressive approaches, thereby minimizing CNI doses and possibly avoiding steroids. Studies on once-daily "prolonged-release tacrolimus" are encouraging,[49] with lower trough levels and better graft and patient survival than standard twice-daily tacrolimus dosage. The optimal long-term immunosuppressive therapy should be tailored and adjusted based on the diagnosis among liver transplant recipients. Finally, induction therapy with CNI-sparing protocols can avoid the side effects on renal dysfunction, de novo cancer, and cardiovascular syndrome.

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