Update on Immunosuppression in Liver Transplantation

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Abstract

The standard therapy for decompensated end-stage chronic liver disease of any etiology and acute fulminant hepatic failure is liver transplantation (LT). Advances in immunosuppressive therapy decreased the rates of acute and chronic rejections. Thus, graft and patient survivals have significantly improved. However, long-term adverse effects of prolonged use of immunosuppressive agents such as malignancies, opportunistic infections, metabolic disorders, and other organ toxicities have now become a major concern. Consequently, alternative approaches are needed to deescalate the customary drugs and their side effects. Therapy must be individualized and additional preventive measures should be taken by patients with particular risk factors or predisposed to certain adverse effects. Current opinion favors a combination of agents with different mechanism of actions and toxicity profiles. Corticosteroids are employed in immediate and early postoperative period. Although they have a pronounced side effect profile, calcineurin inhibitors (CNIs) are still the backbone of early and late phase immunosuppressive regimens because of their proved efficacy. Antimetabolites are frequent choices for steroid and/or CNI-sparing strategies. Studies also have established a role for mammalian target of rapamycin (mTOR) inhibitors in specific groups of recipients. Biologic agents are a hot topic of interest and made their way into current strategies for induction. Agents extrapolated from other transplantation or immunologic experience are being evaluated.

Keywords: Adverse effects, Immunosuppression, Liver transplantation.

Euroasian Journal of Hepato-Gastroenterology (2019): 10.5005/jp-journals-10018-1301

Introduction

Liver transplantation (LT) is offered as the ultimate curative therapy for end-stage liver disease of any etiology as well as for acute liver failure cases. Since the first successful orthotopic LT in 1967,[1] it has reached to approximately 25,000 cases per year worldwide.[2] Mostly due to the increased efficacy of immunosuppressive regimens, the 1-year survival has improved significantly (Fig. 1). In a retrospective long-term survival analysis of 1,11,568 patients who underwent LT between 1987 and 2016, the 1-year survival prolonged significantly
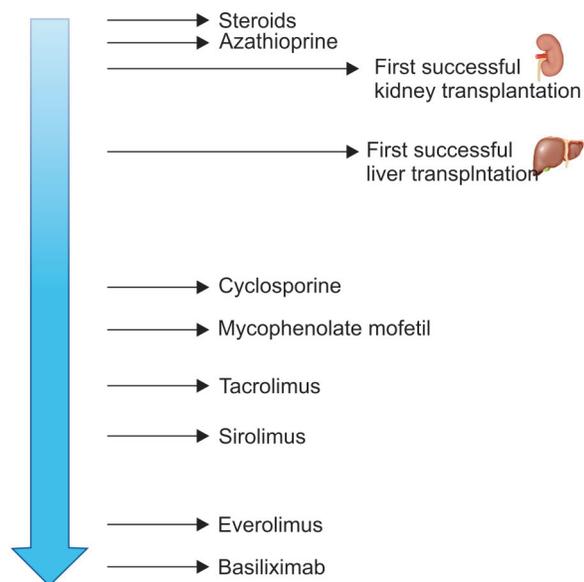
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Fig. 1: Timeline of drug discovery illustrating the progression of immunosuppressive therapy in liver transplantation (Timeline of Historical Events and Significant Milestones. Retrieved March 06, 2018, from https://organdonor.gov/about/facts-terms/history.html)

to 66% in 1986 and ultimately to 92% in 2015. Only 1.7% of patients died of rejection and graft failure.[3]

Long-term side effects of these drugs such as malignancies, opportunistic infections, metabolic disorders, and organ toxicities have now become a major clinical concern. The improvement in 1-year overall survival in the above-mentioned study was not seen when looked at their long-term survival. There has been an increased risk of premature mortality from infections, cancer, kidney, and liver diseases.[4] In patients who have survived more than 1 year, the two leading causes of death were malignancy and infection, seen in 16.4% and 10.5% of patients, respectively.[3] Additionally, LT recipients face other organ toxicities. Renal dysfunction is the most common toxicity with stage 4 to 5 chronic renal disease occurring in nearly one fifth of recipients surviving 5 years post-LT.[5]

Corticosteroids and antimetabolites, with the addition of calcineurin inhibitors (CNIs) a decade later, were the first immunosuppressive agents used among LT recipients.[1] They are currently among the most common choices for antirejection.[6] As mentioned above, prolonged utilization of these drugs has significant drawbacks.

Table 1: Major studies for the immunosuppressive agents in liver transplantation with dosages and outcomes

| Study	Drug	Dose	Year	Result
Busuttil[62]	Tacrolimus	Titrated for a trough level of 0.2–5 ng/mL (max 0.6 mg/kg)	1994	Decreased rejection rate
Schlitt et al.[63]	Mycophenolate mofetil	Stepwise to 1,000 × 2 mg (on week 4)	2001	Increased rejection rate. Decreased renal impairment, metabolic syndrome and uric acid
Benitez et al.[46]	Antithymocyte globulin	9 mg/kg × 1 (on day 0)	2004	Increased rejection rate
Neuhaus et al.[48]	Basiliximab	40 mg × 2 (on days 0 and 4)	2010	Decreased rejection rate
Levitsky et al.[55]	Alemtuzumab	30 mg × 1 (on day 0)	2011	Decreased rejection rates, increased infectious complications, lower incidence of new-onset hypertension
Klintmalm et al.[52]	Belatacept	Various schemes	2014	Increased rejection rate, posttransplant lymphoproliferative disease, progressive multifocal leukoencephalopathy, graft loss, and death
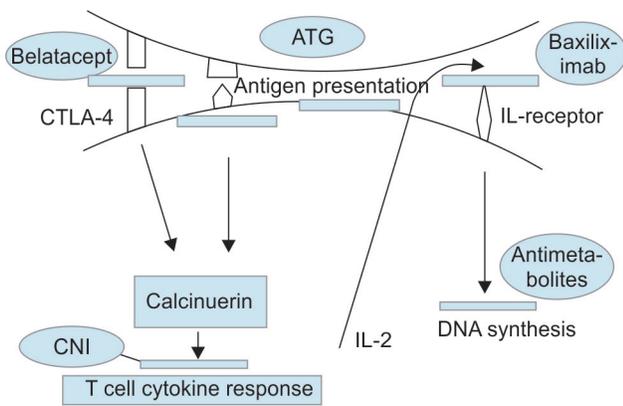
Fig. 2: The mechanisms of actions of various immunosuppressive agents. ATG, antithymocyte globulin; CNI, calcineurin inhibitors

Consequently, there is ongoing search for effective immunosuppressive regimens with acceptable side effects (Table 1). Newer protocols contain combinations of drugs with different modes of action and toxicity profiles (Fig. 2). The two prominent approaches are as follows: the upfront strategy in which the immunosuppressive drugs were chosen with respect to patient's pretransplant and/or intraoperative risk factors, and the downstream strategy in which a standard therapy is given and changed according to its toxicity and efficacy during the course of therapy.[7]

MAINTENANCE THERAPY

Calcineurin Inhibitors

The mechanism of action is the inhibition of intracellular small molecules, cyclophilin, for cyclosporine A (CsA) and FK-binding protein for tacrolimus (TAC) and thus preventing the activation of calcineurin molecule. Subsequently, transcription of important cytokines like interleukin-2 (IL-2) that are essential for T-lymphocyte activation is inhibited.[6,8] They have significantly improved the survival of short-term grafts. Current data show that the risk of acute rejection in the first posttransplant year is approximately 13%.[9] Side effect profiles of CsA and TAC are comparable. They both cause acute and chronic renal failure, neurotoxicity, metabolic dysregulation, and vasculopathy. Nearly one fifth of patients have chronic kidney disease at the end of their fifth post-LT year.[5] One of the proposed mechanisms of nephrotoxicity is the reversible renal vasoconstriction causing an irreversible tubulointerstitial fibrosis.[5] Tacrolimus is more diabetogenic since it inhibits insulin secretion, whereas CsA frequently causes hypertension and hyperlipidemia. These are particularly relevant especially in patients who had pretransplant nonalcoholic steatohepatitis (NASH) and who possibly are susceptible to metabolic dysregulation. Furthermore, the metabolic side effects of CNIs may cause a predisposition to NASH in the graft liver. Cyclosporine A is known to cause endothelial dysfunction and hypertension, so cardiovascular disease is also a major adverse effect. In a retrospective study about cardiovascular outcomes of post-LT patients, TAC treatment was found to be associated with lower risk of cardiovascular events compared to other immunosuppressive therapies except CsA-based regimens ($p < 0.001$).[10] Another point to keep in mind is that both CNIs use p-glycoprotein and are metabolized mostly in liver by cytochrome enzymes; therefore, they have significant drug interactions.[11]

Currently, TAC has mostly replaced CsA as the primary choice in post-LT rejection prophylaxis.[6] Evidence suggests that TAC is the main immunosuppressive agent in LT patients.[12] A meta-analysis comparing two CNIs concluded that TAC reduces 1-year mortality, rate of rejection, length of steroid-resistant rejection periods, and graft loss.[13] Data from two large studies show that overall patient survivals under CNI immunosuppression are within a range of 81–84%, 70–72%, and 57–68% at 1, 5, and 10 years post-LT, respectively.[14,15] To increase patient adherence, a prolonged-release (TAC-PR) and extended-release (TAC-ER) once-daily formulation of TAC was developed, with similar efficacy to immediate release twice-daily formula.[16,17]

Antimetabolites

Azathioprine (AZA) and mycophenolate mofetil (MMF) are the two antimetabolites interfering with nucleic acid synthesis, thus diminishing proliferative response of T and B lymphocytes. Antimetabolites were components of the earliest immunosuppressive regimens.[1] They are less efficient than CNIs and are frequently used to deescalate or discontinue CNIs. Azathioprine has a predominant myelotoxicity and hepatotoxicity. Mycophenolate mofetil also causes significant diarrhea in nearly one third of the patients and also predisposes to opportunistic viral infections.[18]

Randomized-controlled studies comparing AZA and MMF showed that they have similar effects on graft and patient survivals.[19] Mycophenolate mofetil has become the mostly used

antimetabolite agent.[6] In practice, it is combined with a lower dose CNI, yielding a similar efficacy with a decreased risk of toxicity compared to CNI monotherapy.[8,20,21] None of the studies with antimetabolites in LT recipients showed a significant renal toxicity. Enteric-coated MMF can be used to subside the gastrointestinal side effects with similar efficacy.[22]

Mammalian Target of Rapamycin Inhibitors

Everolimus (EVR) and sirolimus (SRL) interfere with immune-promotion of IL-2 and IL-5 cytokines by inhibiting downstream signaling of mTOR molecule.[23] Everolimus is a derivative of SRL, with an extra hydroxyethyl group at position 40, and it is more lipophilic.[24] Apart from its immunosuppressive effects, mTOR inhibitors (mTORi) are known to have antiproliferative effects, reducing the risk of posttransplant recurrence and *de novo* malignancies.[25] Pronounced side effects of mTORi are dose-dependent dyslipidemia, thrombocytopenia, anemia, leukopenia, oral sores, hypertension,[26,27] hindered epithelial regeneration, and fluid retention. Patients treated with mTORi, particularly patients who received LT for NASH, should be counseled on appropriate preventive lifestyle changes.[28] This group of drugs was also been implicated in early hepatic artery thrombosis (HAT) when given in the early post-LT period, resulting in graft loss and patient death.[29] However, several other trials evaluated the safety of mTORi 30 days post-LT and did not find an increased risk of HAT.[27,30]

In contrast to CNI's promoting carcinogenesis,[31] experimental and clinical trials showed that mTORi have antiproliferative effects.[32] Posttransplant hepatocellular carcinoma (HCC) recurrence is shown to be reduced by mTORi.[33] A prospective randomized phase III study evaluated HCC recurrence and survival parameters of LT recipients with the respect to their immunosuppressive maintenance regimens. Patients who were followed with regimens including SRL showed better recurrence free survival and overall survival at 3 and 5 years, respectively. However, more than 5 years' outcomes were not significantly different.[34] Another recent trial evaluated the impact of EVR among HCC LT recipients. Patients who were within the University of California San. Francisco (UCSF) criteria were enrolled into two arms and followed up for a median of 46 months post-LT. The first arm of 37 recipients was treated with TAC and EVR and the second arm of 29 patients was treated with TAC monotherapy. The 1-, 3-, and 4-year overall survival rates in the first arm were 94.9, 86.5, and 86.5%, respectively, while the 1-, 3-, and 4-year overall survival rates in the second group of patients were 82.8, 69.0, and 62.1%, respectively. For HCC recurrence, four patients (10.8%) of the EVR and TAC arm had extrahepatic recurrences, whereas from the TAC monotherapy arm seven patients (24.1%) had the evidence of recurrence.[35]

Mammalian TORi may have a role in preserving renal function in post-LT period since they provide a CNI-sparing opportunity. A prospective multicenter study randomized the patients into two groups, with a follow-up of 4–12 months following LT. The first group received MMF + SRL, while the other group received MMF plus CNI. The first group had significantly improved renal function from baseline but had a higher incidence of acute rejection (12% vs 4%).[36] Another multicenter prospective study compared EVR with low-dose TAC to TAC monotherapy, yielding a better preservation of renal function and decreased rates of rejection.[37]

Three studies of EVR use in LT were conducted. H2304 study consisted of three arms: the first arm with EVR and low-dose TAC, the second arm with EVR and TAC stopped after 4 months, and the third arm with conventional TAC regimen. The second arm, TAC elimination group, was terminated because of increased rejection rates (19.5 vs 6.5 and 9.5%).[37–39] In the RESCUE trial, conversion to EVR from CNI group experienced similar rate of rejection episodes compared to the standard dose CNI group.[40] Lastly, PROTECT study reported that a high percentage of patients discontinued the study due to the drug's side effects (49.5% in EVR group and 38.2% in the control CNI group) but with similar long-term rejection episodes and better renal function in the CNI-free EVR-based group.[30,41,42] With current evidence, mTORi can be considered among recipients especially the ones with renal impairment, pretransplant HCC, and post-LT *de novo* neoplasms.

INDUCTION THERAPY

Antithymocyte Globulin

Antithymocyte globulin (ATG) includes antibodies against multiple T-lymphocyte surface antigens. It has immunomodulatory activity and causes a polyclonal depletion of lymphocytes.[43]

Antithymocyte globulin has an established role in prophylaxis and treatment of rejection in solid organ recipients, especially in cases of high immunologic risk.[44] Among LT recipients, possibilities of an ATG induction and a deescalated use of CNIs were investigated. A scheduled ATG induction followed later with CNI administration was compared with a conventional early CNI regimen. The ATG group had preserved kidney functions and yielded a lower rate of opportunistic infections.[45] A randomized controlled study grouped the LT recipients into two arms: the first arm with a customary TAC and steroid regimen and the second arm with an ATG induction followed by low-dose TAC. The primary end point was to decrease the dose of TAC at 12 months following LT, in the absence of any rejection episode. Acute rejection was higher in the ATG group (52% vs 25%) and none of the patients in this arm reached the primary end point. For these reasons, the trial was prematurely terminated.[46] Although ATG is not used routinely, it may be considered as an induction agent among selected patients with a high immunologic risk or renal compromise.

Clinically significant side effects of ATG may include cytokine release, lymphopenia, opportunistic infections, posttransplant lymphoproliferative disease, and other malignancies.[44]

Interleukin-2 Antibodies

Interleukin-2 and its receptor CD25 play a paramount role in proliferation and activation of T lymphocytes and triggering cellular immune response. Daclizumab and basiliximab are humanized and chimeric CD25 monoclonal antibodies, respectively. They act on a receptor site expressed on activated T lymphocytes, selectively inhibiting their proliferation. The IL2 receptor antibodies (IL2RAs) are used among renal transplant recipients as part of induction regimen and it is also known to decrease the rate of acute cellular rejection (ACR) in LT patients.[47]

An earlier placebo-controlled study with basiliximab, containing 381 patients, showed statistically significant benefit within the basiliximab group when compared to the placebo group, for both biopsy-proven acute rejection during first 6 months (35% vs 43%) and problem-free post-LT survival during 12 months (39% vs 30%).[48] Another study comparing the perioperative renal outcomes between basiliximab and CNI among LT recipients did not find any difference in ACR rates, renal functions, and overall survival.[49] A more recent trial with 114 patients treated with basiliximab revealed 25.4% cellular rejection, which is consistent with literature.[50]

A meta-analysis concluded that IL2RA use was associated with preserved kidney function, decreased rates of steroid-resistant

acute rejections, and lower incidence of posttransplant diabetes mellitus. No difference was found concerning patient and graft survival.[47] Interleukin-2 RAs can be a reasonable adjunct to maintain regimens as an induction strategy with its different toxicity profile and decreased ACR rates.

CD28 Antibodies

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or CD28 is essential for antigen presenting cell and T-lymphocyte costimulatory interaction and initiation of adaptive immune response. Anti-CD28 inhibitor, belatacept, is frequently used in renal transplantation, and its use in LT is currently not recommended.[51] A multicenter phase 2 trial investigated the use of belatacept in LT. In this study, 260 patients were enrolled into five arms:

- Basiliximab with high-dose belatacept and MMF,
- High-dose belatacept and MMF,
- Low-dose belatacept and MMF,
- TAC and MMF, and
- only TAC.

Steroids were included in all groups. The primary end point was a composite of acute rejection, graft loss, and death by 6 months. The proportion of patients meeting the primary end point was statistically higher in the belatacept groups (42–48%) when compared to the other groups that include TAC (15–38%). The highest number of graft and patient loss was seen in the low-dose belatacept group. By months 12, the percentage of patients surviving with a functional graft was 90, 83, 67, 93, and 88 in the respective groups. The study had to be eventually discontinued due to the higher incidence of graft and patient loss.[52]

CD52 Antibodies

Alemtuzumab (Campath-1H) is a humanized antibody to CD52 which is found in B and T lymphocytes and also in other cells of the immune system. CD52 antigen has a proposed mechanism of T-cell modulation. Alemtuzumab induces a long-term depletion of CD4 T cells that can last for 2–3 years and is used as an induction agent in solid organ transplantations.[53]

The experience regarding alemtuzumab use as an induction agent in LT is limited. One study reported a decreased acute rejection rate and lower nephrotoxicity in hepatitis C virus (HCV)-negative patients treated with alemtuzumab.[54] A more recent retrospective study also showed decreased rejection but an increased risk of infection.[55] Significant increase in infection rates was observed among LT patients treated with alemtuzumab, particularly herpes simplex virus;[56] and historically, caution was advised among HCV-positive recipients.[57] Other clinically important side effects of alemtuzumab include cytokine storm and autoimmune diseases.[53]

Long-term Immunosuppression

Compared to other organs, the liver allograft has the advantage of demonstrating lower rates of acute and chronic rejection, a resistance to antibody-mediated rejection as well as a higher likelihood of developing spontaneous tolerance. Over time, alloreactivity decreases and patients may achieve "operational tolerance" defined as successful immunosuppressive drug cessation with stable graft function and no evidence of rejection.[58,59] A multicenter study included 98 stable patients who were at least 3 years post-LT and were considered stable with the following major characteristics: (i) absence of graft rejection during the previous year, (ii) no history of autoimmune liver disease, and (iii) transaminase levels and alkaline phosphatase lower than 1.5 times of the upper laboratory limit. The immunosuppressive treatments tapered and discontinued at 6–9 months, and patients were followed up for 3 years after the cessation of the therapy. Fifty-seven patients (58.1%) subsequently developed clinically and/or biopsy-proven rejection and had to restart immunosuppressive therapy. The remaining 41 patients successfully remained off immunosuppression. Successful discontinuation of immunosuppressive therapy without a rejection episode was found to be associated with older age of recipient and male gender.[60]

There is no definitive evidence as to how to guide long-term immunosuppression for LT recipients. A cross-sectional study among clinicians with a six-question survey can enlighten our current medical practice. Seventeen clinicians from 15 different centers participated in the survey. The results revealed that no center reported total discontinuation of therapy, while 40% of the centers reported no minimization of therapy. Nearly half of the centers (47%) reported using long-term monotherapy for stable patients, but the timeline of weaning to monotherapy varied widely among the centers (from 3 months to 10 years). This survey suggests that transplantation centers are worried about graft rejection, perhaps in the light of the previous weaning studies and thus do not perform immunosuppression discontinuation.[61]

In our routine practice, each patient receives an individualized combination therapy, which may be minimized without being totally discontinued. Intravenous methylprednisolone is administered from postoperative day 1–5 in tapering doses and then 30 mg per oral (PO) is administered by postoperative day 6. On discharge, patients generally remained on prednisone 20 mg PO daily for 1 month. After a problem-free 1-month, the dosage was tapered by 5 mg at a time over 2 months to the maintenance of 5 mg daily dose. At 1-year following LT, patients may be candidates for discontinuation of corticosteroids depending on their clinical progress and primary liver disease etiology. Among LT recipients with native disease of autoimmunity, i.e., autoimmune cirrhosis, primary sclerosing cholangitis (PSC), history of inflammatory bowel disease, the maintenance 5 mg dose of prednisone can be considered.

If baseline serum creatinine is more than 2 mg/dL, basiliximab 20 mg may be given on postoperative day 0 and postoperative day 4; and in this case, TAC is held until postoperative day 10 or until serum creatinine improves to under 2 mg/dL. If the baseline serum creatinine is less than 2 mg/dL, TAC 1 mg PO twice daily is started on postoperative day 1, with subsequent adjustments based on a target trough level of 8–10 ng/mL during the first 3 months and target trough level of 5–8 ng/mL afterward. After the first-year trough levels, 4–5 ng/mL may be considered as adequate. Mycophenolate mofetil 1,000 mg PO twice daily is started postoperatively, decreased to 500 mg twice daily at 2 months, and may be discontinued after 3 months depending on clinical stability of the patient. For patients who do not tolerate TAC due to nephrotoxicity or neurotoxicity, mTORi is a choice if suitable. Everolimus can be administered PO twice daily on postoperative day 30, targeting a trough level similar to TAC.

Conclusion

Over the 50 past years since the first successful LT, rejection rates and graft survivals have now improved to an acceptable range. Advances in the immunosuppressive treatments are the cornerstone for this

improvement. Nonetheless, immunosuppression is a double-edged sword. The side effects of the prolonged immunosuppression are one of the limitations of long-term survival among LT recipients and consequently has become a concern for the clinicians. A tailored therapy with patient-specific preventive measures are crucial in successful management of a posttransplant patient. A reasonable approach is to include various agents with diverse side effect profiles and deescalate the drugs when feasible. Multiple trials have studied this approach and nevertheless, CNI-containing regimens are still preferred for their documented efficacy in preventing rejection. In today's perspective, CNIs are the backbones of rejection prophylaxis, with MMF and corticosteroids considered as the essential adjuncts to CNI. Mammalian TORi has an established role in specific patients. Further studies are needed for the application of biologic treatments as an induction. In our opinion, these agents should currently be reserved for specific indications and more clinical trials are concluded.

Author Contributions

Interpretation of findings and drafting the article (Burcak E Tasdogan), literature search and final editing (Michelle Ma), literature search and editing (Cem Simsek), revising the article for important intellectual content, and drafted the initial and final manuscript (Behnam Saberi, Ahmet Gurakar).

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