Liver Transplant: A Primer

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Abstract

Liver transplant has been accepted as a successful therapeutic option for patients with end-stage liver disease. Patient and graft survival has incrementally increased over the past 2 decades, mainly because of immunosuppressive regimens.

However, the nonspecific nature of immunosuppressive agents is associated with an increased risk of development of opportunistic infections, renal impairment, metabolic derangements, neurotoxicity, de novo malignancies, and recurrence of the primary disease.

Immunosuppressive regimen pharmacologic classes include calcineurin inhibitors, anti-metabolites, mTOR inhibitors, steroids, and antibody-based therapies. These agents affect T-cell–dependent B-cell activation, and target different sites in the T-cell activation cascade by inhibiting T-cell activation or causing T-cell depletion.

The goals of immunosuppression in solid-organ transplant are to prevent allograft rejection as well as optimize allograft function, prolong patient survival, and improve patient quality of life. Therefore, it is essential to carefully select the immunosuppressive regimen that will result in significant improvements in long-term liver transplant patients' survival and quality of life.

Key words: *Liver transplantation, Immunosuppressive, Rejection*

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Introduction

Liver transplant is the treatment of choice for selected patients with end-stage liver disease. By using selective immunosuppressive agents, the survival and quality of life for patients after liver transplant has markedly improved during the last decade, 1-year graft survival rates now exceed 80% (1-3).

In general, the goals of immunosuppression in solid-organ transplant are to prevent allograft rejection, as well as optimize allograft function, prolong patient survival, and improve patient quality of life. The emphasis of immunosuppressive therapy has shifted from preventing rejection, to balancing acceptable rates of rejection with tolerable adverse effects of the immunosuppressive agents (4).

There is no universal immunosuppressive protocol for all patients. Selection of immuno-suppressive agents avoids or minimizes adverse effects (5). For instance, patients with renal insufficiency may experience worsening renal function with particular immunosuppressive agents. Because of the well-known renal toxicity associated with calcineurin inhibitors, patients with renal impairment should be considered for calcineurin inhibitor-free or sparing regimens (6, 7). Additionally, studies have shown that calcineurin inhibitors can directly promote tumor growth, whereas sirolimus may have potential antitumor properties (8, 9). Moreover, because both corticosteroid and calcineurin inhibitor-based regimens have been associated with development of diabetes, select patients may be considered for alternative immunosuppressive agents (10).

Overview of immunosuppressive agents for liver transplant

There are several immunosuppressant pharmacologic classes (Table 1). Immunosuppressive

Table 1. Immunosuppressant pharmacologic classes.

Pharmacologic classes	Agents
Calcineurin inhibitors	cyclosporine, tacrolimus
mTOR inhibitors	sirolimus
Antiprolifratives	azathioprine, mycophenolate mofetil
Monoclonal antibodies	anti-CD3 monoclonal antibody uromonab-CD3, alemtuzumab anti-CD52 monoclonal antibody anti-IL-2 receptor monoclonal antibody basiliximab, daclizumab
Corticosteroids	prednisone, hydrocortisone
Polyclonal anti-T-cell antibodies	Anti-thymocyte globulin (ATG) Anti-lymphocyte globulin (ALG)

therapy can be divided into 2 phases, induction and maintenance. Induction agents are potent medications used at the time of transplant, and generally consist of monoclonal or polyclonal antibodies. Maintenance therapy is administered after transplant, usually for the life of the transplanted organ. Maintenance immunosuppressive therapy often consists of a multiple-drug regimen composed of agents that act simultaneously at different levels of the immune cascade (4, 11).

Historically, induction therapy was depicted as the use of monoclonal antibodies like anti-CD3 (Muromonab CD3) or polyclonal antibodies like horse (equine) antithymocyte globulin. The goal of induction therapy is to minimize the risk of rejection or avoid associated toxicity, specifically during the early posttransplant period (12). Because interleukin-2 receptor antagonists (basiliximab, daclizumab) are better tolerated than any T-cell depleting agent, their use has increased over the past few years (13, 14).

The overall use of induction immunosuppression for liver transplant recipients has increased just in the past decade. The rate of induction immunosuppression for liver recipients has increased steadily since 1997, when it was 7%, compared to 21% during 2003 and 2004 (15). The trend of induction antibody selection continues to favor the class of anti–IL-2 receptor monoclonal antibodies (basiliximab and daclizumab), which make up a total of 11% of overall use of this class of immunosuppressive agents. These agents prevent rejection by blocking IL-2-mediated T-cell activation, not by depleting T cells, have minimal adverse effects, and are well tolerated (15).

Induction therapy with T-cell–depleting agents is usually initiated immediately after transplant and continued for 7 to 14 days, whereas induction with interleukin-2–receptor antagonists is administered at the time of transplant, and once again within the first week after transplant. Induction strategies allow for a delay in initiating calcineurin blockers for a fixed number of days or until the serum creatinine reaches a desired level. Furthermore, induction therapy also allow clinicians to minimize calcineurin blocker exposure (reduced mg/kg daily dosage and level; calcineurin sparing) during the immediate postoperative period. During this phase, the antiproliferatives (azathioprine, mycophenolate) and corticosteroids are usually coadministered with an induction agent. Toward the end of the induction therapy, or when the desired serum creatinine is reached, the second immunosuppressive phase with maintenance therapy can be initiated. This is done by either adding a calcineurin blocker or increasing the existing calcineurin blocker dosage, and titrating it to the desired level.

Maintenance therapy is administered after transplant to preserve graft function, usually for the life of the transplanted organ. Current maintenance immunosuppressive therapy in liver transplant primarily consists of a tacrolimus-based immunosuppression regimen (tacrolimus, mycophenolic acid, and prednisone) whereas a cyclosporine-based immunosuppressive regimen (cyclosporine, azathioprine or mycophenolic acid, prednisone) was primarily used until the late 1990s.

As patients achieve adequate liver function and remain free from T-cell–mediated acute rejection beyond 6 months posttransplant, maintenance therapy can be tailored or reduced, based on patient specific needs or altered owing to an adverse effect. Additional options for maintenance therapy include double drug therapy (tacrolimus or cyclosporine plus prednisone; tacrolimus or cyclosporine plus mycophenolic acid; mycophenolic acid plus prednisone), or in some cases monotherapy (primarily with tacrolimus). Developing renal failure after liver transplant is a factor of poor prognosis associated with a high mortality ranging from 44% to 50% (16, 17). Select patients with renal impairment should be considered for calcineurin inhibitor-free or sparing regimens (6, 7).

Glucocorticoids: Glucocorticoids are used during standard initial immunosuppression in patients undergoing liver transplant. Corticosteroids have a wide range of immunosuppressive properties including their effects on antigen presentation by dendritic cells, inducing a decrease in circulating CD4+ T cells, and inhibition of IL-1–dependent

lymphocyte activation by decreasing IL-1 transcription (5, 18). Glucocorticoids are associated with major adverse effects, which are shown on Table 2 (19).

The main concern with corticosteroid use is accelerating hepatitis C virus (20). However, this has not been evident with low, gradually tapered dosages (21). Recent studies suggest that low-dose steroids can be used effectively early, and, with gradual tapering, be used without increasing fibrosis related to hepatitis C virus reinfection (22).

Steroid-free regimens seemed beneficial in terms of lowering hypertension, reducing cholesterol, lowering the risk of *cytomegalovirus* infection, and are associated with reduction of hepatitis C virus recurrence (23-26). Other investigators also have demonstrated that steroid-free regimens have shown advantages in terms of de novo diabetes mellitus development, *cytomegalovirus* infection, hepatitis C virus recurrence, and cholesterol levels (27). Most liver transplant centers either rapidly taper or completely avoid glucocorticoids (28).

Calcineurin inhibitors: The calcineurin inhibitors are currently the cornerstone of most immunosuppressive regimens to prevent graft rejection. Cyclosporine and tacrolimus inhibit IL-2–mediated T-cell activation and lymphocyte proliferation by binding with their respective intracellular binding proteins, cyclophilin, or FK binding receptors. This drug-protein complex then inhibits the phosphatase activity of calcineurin, resulting in decreased IL-2 transcription (4, 5). Both cyclosporine and tacrolimus are metabolized principally by the cytochrome P450 system in the liver and have much potential for drug-drug interactions. Concomitant use of drugs (eg, fluconazole, diltiazem) or foods (eg, grapefruit) that induce or inhibit the P450 system can significantly alter the levels of calcineurin inhibitors requiring careful monitoring of drug levels (Table 3) (19).

Calcineurin inhibitors have a wide range of toxicities, many of which are dose-dependent (Table 2) (2, 29, 30). The neurologic effects from calcineurin inhibitors are usually dose-dependant and range from mild symptoms such as tremor, paresthesia, and headache, to severe symptoms such as agitation, seizure confusion, hallucinations, or overt psychosis, and leukoencephalopathy (31-34). The onset of neurologic symptoms in these patients is unrelated to serum levels of creatinine (35, 36).

Nephrotoxicity requires careful management. Cyclosporine and tacrolimus produce a dosage-related, reversible, renal, calcium-mediated vasoconstriction that particularly affects the afferent arterioles. Cyclosporine/tacrolimus-induced renal vasoconstriction may manifest itself clinically as delayed renal recovery in hepatorenal patients or as transient elevations in serum creatinine that is difficult to differentiate from other causes of renal dysfunction. Management of this type of nephrotoxicity often includes calcineurin inhibitor dose reduction or the addition of renal protective agents such as calcium channel blockers. Chronic, interstitial fibrosis is frequently seen in native kidneys of patients receiving chronic calcineurin inhibitor therapy. Thrombotic microangiopathy is

Table 2. Immunosuppressive mechanisms of action and selected adverse effects.

Agent	Mechanism of action	Selected adverse effects
Prednisone	Suppresses leukocyte, macrophage, and cytotoxic T-cell activity. Decreases cytokines, prostaglandins, and leukotrienes	Hypertension, dyslipidemia, glucose intolerance, bone cell abnormalities, peptic ulcers, adrenal suppression, psychiatric disorders, infection, cataract, osteoporosis, cushingoid features, Na+ retention; insomnia; avascular necrosis, poor wound healing
Cyclosporine	Inactivates calcineurin, decreases IL-2 production. Inhibits T-cell activation	Hypertension, renal insufficiency, neurotoxicity (hallucination, confusion, seizure, neuropathy), hyperlipidemia, hirsutism, gingival hyperplasia, insulin resistance
Tacrolimus	Inactivates calcineurin. Decreases IL-2 production. Inhibits T-cell activation	Hypertension, renal insufficiency, insulin resistance, neuropathy, diarrhea, hyperlipidemia
Azathioprine	Inhibits adenosine and guanine production. Inhibits DNA and RNA synthesis in rapidly proliferating T cells	Leukopenia, anemia, thrombocytopenia, pancreatitis, hepatitis, cholestasis
Mycophenolate mofetil	Selectively inhibits production of inosine monophosphate dehydrogenase, thereby inhibiting de novo purine synthesis; prevents T- and B-cell proliferation	Leukopenia, anemia, thrombocytopenia, gastrointestinal adverse effects (diarrhea, heartburn), PML, lymphomas
Sirolimus	Inhibits mTOR (target of rapamycin) T-cell replication	Leucopenia, thrombocytopenia, dyslipidemia, hepatic artery, prevents thrombosis, proteinuria, peripheral edema, pulmonary toxicity; Interstitial pneumonitis, BOOP, delayed wound, healin

Abbreviations: BOOP, bronchiolitis obliterans with organizing pneumonia; PML, progressive multifocal leukoencephalopathy.

Table 3. Cytochrome P450 3A4 inhibitors/inducers.

Inhibitors (immediate onset)			
Antibiotics	Antifungal	Calcium blockers	Others
erythromycin	fluconazole	diltiazem	amiodarone
clarithromycin	itraconazole	verapamil	cannabinoids
norfloxacin	ketoconazole		cimetidine
metronidazole	voriconazole		grapefruit
Inducers (slow, time-dependant process)			
Antiepileptic agents	Antituberculars	Others	
Carbamazepine	Rifampin	Dexamethasone	
Phenytoin	Isoniazid	Griseofulvin	
Phenobarb/primidone		St John's Wart	

uncommon, yet a distinct form of CNI-induced vascular toxicity. This syndrome is similar to hemolytic uremic syndrome and is associated with poor renal function, unless aggressive medical interventions are undertaken. Current management strategies may include switching immunosuppressive maintenance therapy to target of rapamycin (TOR) inhibitors with mycophenolic acid or monotherapy with either TOR inhibitor or mycophenolic acid (37).

Another important feature of calcineurin inhibitors is their interaction with transforming growth factor-b, a cytokine that augments fibrosis development and promotes tumor cell invasiveness (38). Transforming growth factor-b transcription is increased with calcineurin inhibitor use, which is of concern, given the possibility of hepatocellular carcinoma recurrence or the emergence of posttransplant lymphoproliferative disorder. The results of retrospective studies have suggested that the introduction of cyclosporine as an immunosuppressive agent in the 1980s was associated with an increased incidence of posttransplant lymphoproliferative disorder (39-40). More-recent reports of solid-organ transplant suggest the use of tacrolimus instead of cyclosporine is associated with a 2- to 5-fold increase in the risk of developing posttransplant lymphoproliferative disorder; however, the incidence is still quite rare (41).

Another potential attribute to calcineurin inhibitors is the development of hepatitis C virus reinfection. Firpi and associates suggested tacrolimus may be associated more with hepatitis C virus reinfection than cyclosporine (42). Moreover, cyclosporine shows some antiviral effects in vitro (43, 44). However, the results of a recent meta-analysis did not confirm the differential effect on hepatitis C virus reinfection of the different calcineurin inhibitors (45).

Antimetabolites: Azathioprine and mycophenolic acid (mycophenolic acids; mycophenolate mofetil, or enteric-coated mycophenolic acid) prevent the expansion of activated T cells and B cells, which induce immune-mediated injury. Azathioprine, a purine analogue, is metabolized in the liver to its active compound, 6-mercaptopurine, which inhibits adenosine and guanine production, thereby inhibiting DNA and RNA synthesis in rapidly proliferating T cells. Mycophenolic acids are potent noncompetitive inhibitors of inosine monophosphate dehydrogenase, an enzyme necessary for the synthesis of guanine, a purine nucleotide. Inhibition of the inosine monophosphate dehydrogenase pathway results in selective blockade of T and B lymphocyte proliferation, thereby offering a unique opportunity to reduce both the acute and chronic rejection processes (46).

The major advantage in using this group of immunosuppressant agents is their lack of impact on renal function. The predominant adverse effects of mycophenolic acid are related to gastrointestinal disorders and bone-marrow suppression. Diarrhea is the most-common dose-limiting adverse effect. Clinicians often change mycophenolic acid dosing from twice daily to 4 times a day to reduce the gastrointestinal adverse effects. Pancreatitis can occur in patients taking azathioprine (47). Other potential associations include *cytomegalovirus*, herpes simplex virus, and candida infections, and rarely, progressive multifocal leukoencephalopathy (48-53). However, with the implementation of ganciclovir-based antiviral prophylaxis and triazole (ie, fluconazole) antifungal prophylaxis, these infections are commonly prevented. The use of mycophenolate mofetil in pregnant patients increased risks of spontaneous abortions during the first trimester and serious congenital malformations also have been reported (www.fda.gov).

Recent studies suggest that the mycophenolic acid may be superior to azathioprine, and the use of mycophenolic acid has replaced azathioprine in many centers (54). The major advantage of mycophenolic acid over azathioprine is that they exhibit a relatively selective effect on the activation of lymphocytes. Mycophenolic acid acts by selectively affecting the de novo pathway for purine synthesis, whereas azathioprine is nonselective. This specificity is believed to reduce the adverse effects of mycophenolic acid compared with azathioprine,

while maintaining superior efficacy (46, 55). Mycophenolic acid also may have antiviral activity against hepatitis C virus (56-61). Because of the immunosuppressive potency of mycophenolic acid, it can help in avoiding, or at least reducing, calcineurin inhibitor-related nephrotoxicity. The ability of mycophenolic acid to facilitate sparing of other immunosuppressive agents, particularly in cyclosporine-related nephrotoxicity, is promising and allowing secure calcineurin inhibitor dose minimizing to better preserve renal function, which allows cyclosporine reduction dosages (54, 62-64).

M-TOR inhibitors: Sirolimus exerts its immunosuppressive effect by inhibiting mTOR (mammalian target of rapamycin). Inhibition of mTOR causes arrest of the G1 to S phase of the cell replication cycle and diminishes intracellular signaling distal to the IL-2 receptor and prevents T-cell replication. In addition, because mTOR regulates cell growth and angiogenesis, mTOR inhibition may have a role in treating or modulating growth of various cancers including hepatocellular carcinoma (65, 66). Sirolimus has been shown to block vascular endothelial growth factor, which has the potential to help patients with underlying malignancy (67). Data suggest a potential survival benefit with sirolimus-based therapy in patients undergoing liver transplant for end-stage liver disease and concomitant malignancy (68). The negative effect of sirolimus on primary tumor growth and the proliferation of metastatic foci have been demonstrated in rodent models of hepatocellular carcinoma (69, 70). Several retrospective studies have shown sirolimus-based immunosuppression regimens appear to have a beneficial effect on tumor recurrence and survival with an acceptable rate of rejection and toxicity in recipients with hepatocellular carcinoma (71, 72). Sirolimus is metabolized by the cytochrome P450 system and is prone to similar drug interactions as calcineurin inhibitors (4).

Major adverse effects of mTOR inhibitors are listed in Table 2 (73-77). Hepatic arterial thrombosis has been reported, especially in patients who received sirolimus in the weeks immediately after liver transplant, possibly related to a loading dose or center specific factors (75). Several studies have noted problems with wound-healing in patients who received sirolimus) (76).

Polyclonal antibodies: Polyclonal antibodies, including anti-thymocyte and anti-lymphocyte globulins have been used since the early days of liver transplant. They are prepared by inoculating rabbits or horses with human lymphocytes or thymocytes (18). Polyclonal antibodies are occasionally used as induction therapy in liver transplant. In 2007, approximately 10.8% of transplant centers use these agents for induction therapy (78).

The main mechanism of action of polyclonal antibodies is lymphocyte depletion from the circulating pool through complement-mediated cell lysis and uptake by the reticulo-endothelial system of opsonized T-cells. Lymphocyte depletion is believed to play a role in preparing the recipient's immune system to adapt and recognize the transplanted organ as self and prevent destruction of the allograft. In addition, they also may cause partial T-cell activation and blockade of T-cell proliferation (79, 80).

One of the most-common adverse effects of these polyclonal antibodies, affecting 80% of patients, is a "first-dose reaction" and a febrile episode due to pyrogen release from the massive destruction of lymphocytes (80-81). Other adverse effects include thrombocytopenia, anemia, *cytomegalovirus* infection, posttransplant lymphoproliferative disorder, rashes, serum sickness, and anaphylaxis (82-84).

Monoclonal antibodies: Monoclonal antibodies used as induction agents consist of OKT-3, daclizumab, basiliximab, and alemtuzumab. The target of OKT-3 is the CD3 complex on T-lymphocytes, resulting in their rapid depletion. Daclizumab and basiliximab are monoclonal antibodies that antagonize the IL-2 receptor. These agents prevent rejection by blocking IL-2-mediated T-cell activation, not by depleting T cells. Both antibodies are specific for the alpha chain of the IL-2 receptor, which is only expressed on activated T-cells. This type of selective T-cell receptor targeting is associated with fewer adverse effects in comparison to anti-thymocyte globulins, or other general immunosuppressive drugs (5).

Muromonab-CD3: Muromonab-CD3 is a monoclonal antibody directed against a T-cell surface molecule. Muromonab-CD3 targets the CD3 molecule on T cells and causes depletion of lymphocytes by massive T-cell lysis and cytokine

release (85, 86). This profound cytokine release can lead to pulmonary edema and acute respiratory distress and rarely, intragraft thrombosis and aseptic meningitis (87, 88). Several days after OKT3 administration, T lymphocytes no longer express CD3, and are considered to be immunologically incompetent (89). The current use of OKT3 in OLT is primarily for steroid-resistant acute rejection and has a success rate of complete recovery in 50% of patients (90, 91). OKT3 use should be limited in the hepatitis-C–virus population as several studies have confirmed exacerbation of disease recurrence increasing mortality (92, 93).

The use of OKT3 has been reported as a risk factor for the development of posttransplant lymphoproliferative disorder. One study showed that the introduction of OKT3 into clinical practice increased the incidence of posttransplant lymphoproliferative disorder from 1.3% to 11.4% (94). More-recent studies using lower cumulative doses of OKT3 suggest there is no increase in the incidence of posttransplant lymphoproliferative disorder after OKT3 (95).

Alemtuzumab: Alemtuzumab is a humanized anti-CD52 antibody, which targets lymphocytes, monocytes, macrophages, natural killer cells and thymocytes, but spares plasma cells and memory lymphocytes. Its use results in a profound and long-lasting depletion of leukocytes, which offers the potential for greater efficacy in high immunologic risk liver transplant recipients (5). The use of alemtuzumab in liver transplant has been found to be associated with increased viral loads in hepatitis-C–infected recipients, and also greater risk of *cytomegalovirus* disease (96, 97).

Conclusion

The evolution of immunosuppression agents has resulted in many patients enjoying long and productive lives posttransplant; however, simply using the same regimen for all liver patients is not likely to yield the best results. Ultimately, we should find an optimal balance of efficacy with adverse effects for each individual patient.

Selection of immunosuppressive agents is not straightforward or universally applicable. The goal of therapy is to preserve graft function without creating an environment for increased infections, recurrent viral infections, and recurrent or de novo malignancies. The ultimate goal remains finding the balance between preserving graft function and optimizing immunosuppression while minimizing toxicities.

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