Immunosuppression and modulation in liver transplantation

Jens Encke1, Waldemar Uhl2, Wolfgang Stremmel1 and Peter Sauer1

1Department of Internal Medicine IV, University of Heidelberg, Bergheimerstrasse 58, 69115 Heidelberg, Germany and 2Department of Surgery, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany

Abstract
Recent advances in immunosuppressive drug regimens have changed the outcome after liver transplantation significantly in the last two decades. However, chronic rejection and long-term graft survival remains a major problem. Side effects like drug-induced nephrotoxicity, hypertension, osteoporosis, hyperlipidaemia and neuropathy of some immunosuppressive agents play an essential role in long-term allograft and patient survival. This review outlines the current treatment of short- and long-term immunosuppression in liver transplanted patients, it summarizes the treatment of acute and chronic rejection, describes the complications and side effects of immunosuppressive therapy and points out the current use of immunosuppressive therapy in living-related liver transplantation.

Keywords: antiproliferative agents; calcineurin inhibitors; corticosteroids; immunosuppression; liver transplantation; living-related liver transplantation

Introduction
Immunosuppressive drug regimens have evolved greatly and transformed solid-organ transplantation into a routine clinical procedure with impressive short-term results obtained in liver as well as kidney, heart, lung and pancreas transplantation. Despite early loss of organ allografts to acute rejection, the latter has almost been eliminated by the use of different combinations of immunosuppressive agents. However, chronic rejection and long-term graft survival remain a major problem. Late graft loss is caused predominantly by chronic rejection that is immunologically mediated but unresponsive as yet to immunosuppressive therapy. Moreover, side effects like drug-induced nephrotoxicity, hypertension and hyperlipidaemia due to some immunosuppressive agents may play an essential role in the pathogenesis of chronic allograft loss [1,2]. This review outlines the current treatment of short- and long-term immunosuppression in liver transplant patients, summarizes the treatment of acute and chronic rejection, describes the complications and side effects of immunosuppressive therapy and points out the current use of immunosuppressive therapy in living-related liver transplantation.

Acute cellular rejection in liver transplantation
Acute cellular rejection develops in ~75% of liver transplant recipients treated with cyclosporine- or tacrolimus-based immunosuppression [3,4]. Acute cellular rejection occurs 5–15 days after liver transplantation. With inadequate immunosuppression acute cellular rejection may also present later. An induction regimen with OKT3 for 10–14 days delays the onset of rejection by 1–2 weeks and reduces the incidence of acute cellular rejection to approximately one-third; however, it does not significantly improve 1 year patient or graft survival [5,6]. Acute cellular rejection is clinically suspected by elevations in serum aspartate aminotransferase and alkaline phosphatase, which typically precede clinical symptoms of jaundice and fever. Diagnosis should be confirmed by liver biopsy prior to initiating treatment for rejection. Histological features include endothelialitis, signs of cholangitis and mononuclear cell portal inflammation [7]. High-dose corticosteroids are the first line treatment for acute rejections; ~75% of episodes of acute cellular rejection resolve after a course of high-dose corticosteroids (3–5 days of 500–1000 mg of methylprednisolone), and a second course is effective in an additional 10% of cases [8,9]. Tacrolimus rescue therapy seems to be beneficial in some cases that have steroid refractory rejections. In
steroid-resistant rejection, OKT3 is another treatment of choice, however, new immunosuppressive agents such as sirolimus or mycophenolate mofetil (MMF) may successfully treat steroid-resistant rejection without severe side effects. These alternative therapies circumvent the increased susceptibility to infection and the association between OKT3 use and post-transplantation lymphoproliferative disease (PTLD). A small percentage of patients will be refractory to therapy for acute cellular rejection and progress to severe ductopenic rejection making liver retransplantation necessary.

**Maintenance immunosuppression in liver transplantation**

**Corticosteroids**

Besides the treatment of acute cellular reaction, corticosteroids continue to be part of the initial and maintenance immunosuppressive regimens after liver transplantation. However, recent studies have shown successful tapering of steroids in the first 6 months and further maintenance therapy can be obtained and is now used in most centres with calcineurin inhibitor (CnI) monotherapy or combined therapies without steroids and no increased risk of acute cellular reaction [1,10]. Corticosteroids inhibit the synthesis of cytokines through interaction with gene transcription. They also inhibit migration and phagocytosis of macrophages and increase membrane stability. Multiple side effects are associated with long-term steroid therapy and are mostly dose-dependent: diabetes, infections, Cushing syndrome, osteoporosis, hypertension, hyperlipidaemia, hirsutism, gastrointestinal symptoms such as stomach and duodenal ulcers and cataract. Cardiovascular complications with an increased morbidity and mortality are often due to long-term use of steroids in liver transplantation. Furthermore, severe complications from hepatitis B (HBV) and hepatitis C (HCV) after reinfection have been associated with increased doses of steroids [11]. Because of concerns related to steroid promotion of viral replication, tapering and discontinuation of steroid therapy in patients undergoing transplantation for viral-induced liver cirrhosis are strongly recommended.

**Calcineurin inhibitors**

Despite the introduction of new immunosuppressive agents such as sirolimus or MMF, CnIs remain the keystone of most immunosuppressive regimens in most liver transplant centres throughout the world. Both cyclosporine and tacrolimus bind to cytoplasmic receptors (cyclophylin and FK-binding protein 12, respectively). Through interaction with these receptors, calcineurin, a pivotal enzyme in T-cell receptor signalling and activation, is inactivated. Both substances inhibit calcineurin–calmodulin complex and therefore interleukin-2 production [12].

**Cyclosporine A (CsA).** Therapy with CsA is associated with a number of severe and significant side effects and include acute and chronic nephrotoxic effects, dyslipidaemia, hypertension, tremor, headache, hirsutism and gingival hyperplasia, whereas gastrointestinal symptoms, alopecia and diabetes are slightly more common with tacrolimus. Acute nephrotoxic effects of cyclosporine are reversible, unfortunately chronic effects are not [13]. To minimize cyclosporine-associated side effects, the dosage should be tailored in maintenance therapy and lower dosage regimens can be achieved by combination with other immunosuppressive agents, e.g. MMF. A reduction in both acute cellular rejection and toxicity has been described by measuring the blood concentration of CsA 2 h post-dose (C2); however the results of this approach allowing tapering CsA dose and reduction of side effects remain controversial [14].

**Tacrolimus.** Tacrolimus is a macrolide antibiotic with potent immunosuppressive properties. Similar to cyclosporine, tacrolimus interferes with T-cell activation by inhibiting the production of cytokines, including IL-2. It has ~100-fold greater in vitro and 10-fold greater in vivo potency than CsA in inhibiting T-cell responses. Large US and European multicentre trials demonstrated both a lower incidence and severity of rejection in maintenance therapy with tracolimus in both kidney and liver transplantation [15,16]. Recent results from a large British multicentre trial showed benefits for tacrolimus after 1 year for all major endpoints (graft and patient survival) compared to CsA [4]. Despite being a CnI with the same mechanisms of action, tacrolimus and CsA have different side effect profiles: although they have roughly the same nephrotoxicity, tacrolimus treated patients have a higher incidence of diabetes and neurotoxicity, but a lower incidence of hypertension, hyperlipidaemia, hirsutism and gingival hyperplasia.

**Antiproliferative agents**

**Azathioprine**

Antiproliferative agents prevent the expansion of alloactivated T- and B-cell clones. Azathioprine, a purine analogue inhibits DNA synthesis and has been used as an immunosuppressive agent since the 1960s. The dose-limiting toxicity is bone-marrow suppression with resulting significant leukopaenia. In a double-blind study in over 500 patients, MMF was superior to azathioprine in preventing acute rejection in the first 6 months post-transplantation. However, MMF and azathioprine were equivalent in preventing graft loss at 1 year, and the safety profiles between the
two immunosuppressive agents were similar. Many centres switched therefore to MMF in their standard immunosuppression protocol [17].

**Mycophenolate mofetil**

MMF inhibits the synthesis of purines necessary for lymphocyte activation. It was developed to replace azathioprine and has been used in kidney transplantation as a primary immunosuppressive agent, and for the treatment of refractory rejection. Several preliminary studies suggest that MMF used as a primary agent in combination with tacrolimus or cyclosporine in liver transplantation reduces the incidence of rejection and allows steroid withdrawal [18]. A large multicentre trial in liver transplantation demonstrated that the use of MMF reduces the incidence of acute cellular rejection [17,19]. MMF side effects are bone marrow suppression with leucopenia and anaemia, gastrointestinal symptoms, particularly nausea, abdominal cramps and diarrhoea. MMF therapy has also been associated with a slight increase of lymphoproliferative diseases and opportunistic infections.

**Rapamycin**

Despite having structural similarities to tacrolimus, rapamycin (sirolimus) blocks T-cell activation at a later signalling step. Sirolimus inhibits intracellular signalling distal to the interleukin-2 receptor and inhibits the progression of the T cell into the S phase of the cell cycle. Rapamycin also inhibits B-cell proliferation and growth factor-mediated proliferation of non-immune cells. Furthermore, it inhibits platelet-derived growth factor stimulation of smooth muscle cells, which may contribute to the efficacy of rapamycin in preventing and treating chronic rejection [20]. Some authors used rapamycin even as immunosuppressive monotherapy [21]. Furthermore, because rapamycin is thought to have antiproliferative activity against neoplastic cells, its use in patients who undergo transplantation for hepatocellular carcinoma has been advocated [22].

**Treatment of chronic rejection**

In liver transplant recipients, tacrolimus has shown efficacy as a primary immunosuppressive agent, and conversion from cyclosporine to tacrolimus has been used to treat refractory cellular rejection and early chronic rejection. Only small studies examined the effects of treatment of chronic cellular rejection in liver transplantation: in two studies, 56% of patients converted to tacrolimus for OKT3-refractory rejection and showed normalisation of liver enzymes after an average of 60 days, and a 1 year graft survival of 85% [23,24]. Patients who fail tacrolimus rescue therapy, or do not tolerate tacrolimus due to serious adverse effects such as neurotoxicity or nephrotoxicity, may be candidates for MMF rescue therapy. However, only few data are available on the use of MMF for refractory rejection in liver transplantation. MMF seems to be an effective alternative immunosuppressive in patients failing CsA-based conventional therapy, and may be of particular benefit in patients who do not tolerate CsA or tacrolimus. The long-term safety profile is similar to that of other immunosuppressives [25]. When the above described immunosuppressive agents as mono- or poly-immunosuppressive therapy fail to treat chronic rejection, some patients have to undergo re-transplantation.

**Immunosuppression in viral hepatitis**

Approximately 10–25% of HCV-infected recipients of liver allografts will develop cirrhosis within 5 years after transplantation. This accelerated course is in part caused by immunosuppression. Reducing immunosuppression is a balancing act between the attempt to control recurrence for HCV and the maintenance of adequate immunosuppression and prevention of acute and chronic cellular reaction. Several risk factors for aggressive recurrence and graft loss have been described, including the number of acute cellular reactions treated with prednisolone pulse therapy and the use of OKT3. Differences in maintenance immunosuppression by either CnI, cyclosporine or tacrolimus in hepatitis recurrence have not been observed and steroid withdrawal, although now commonly used in many transplant centres, has not shown any benefit. Despite the antiviral effects of MMF, no beneficial effect has been described in regimens using this medication. Effects of sirolimus or interleukin-2 receptor antibodies have not been adequately defined, but preliminary studies suggest a more severe disease activity with these immunosuppressive agents [11].

**Immunosuppression in living-donor liver transplantation (LDLT)**

In most transplant centres, immunosuppressive regimens for LDLT are essentially the same as in cadaveric liver transplantation. The largest series of LDLT reported from Chen et al. [26] and Lo [27] reported a lower incidence of acute rejections in LDLT (17–40%) compared to cadaveric series. However, to our knowledge the rate and severity of rejection in LDLT is controversial and larger studies are necessary to define the optimal immunosuppressive regimen after LDLT.

Conflict of interest statement. None declared.

**References**
