Immunosuppressive therapy in liver transplantation

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1. Immunosuppressive drugs

1.1. Mechanisms of allograft rejection and targets of immunosuppressive therapies

Recipient CD4 T helper cells recognise foreign antigens derived from the allograft, encoded predominantly by the major histocompatibility complex (signal 1). A second (costimulatory) signal must be provided by cognate ligands on the antigen-presenting cell. The best characterised costimulatory signal is provided by the ligation of CD28 on the surface of the CD4 T cell with a member of the B7 family of molecules (B71 and B72, also named CD80 and CD86) on the antigen-presenting cell. CD40-CD40 ligand (CD154) interaction provides another costimulatory signal. When both signals 1 and 2 are provided, the T cell secretes optimum concentrations of interleukin (IL)-2, that induces T cell proliferation and clonal expansion (Fig. 1). If the second signal is absent, the CD4 T cell becomes unresponsive to further exposure to the antigen. This dependence on a second signal has formed the basis for the development of agents able to induce allograft tolerance.

Allo-activated CD4 T cells subsequently interact with effector cells of the rejection response via direct cell–cell contact and cytokine secretion. Through an increase in the activation and function of B cells, cytotoxic CD8 T cells and monocyte macrophages, allo-activated CD4 T cells promote allo-antibody production, target cell lysis, and delayed type hypersensitivity response, respectively. These effector mechanisms ultimately result in graft destruction (Fig. 1).

1.2. Classification of immunosuppressive drugs

Immunosuppressive drugs can be classified into five groups: corticosteroids, calcineurin inhibitors (cyclosporine and tacrolimus), inhibitors of purine biosynthesis [azathioprine and mycophenolate mofetil (MMF)], inhibitors of mammalian target of rapamycin (mTOR) [sirolimus (rapamycin) and everolimus], and monoclonal or polyclonal antibodies [OKT3, antithymocyte globulin, antilymphocyte globulin and anti-IL2 (anti-CD25) receptor antibodies] (Table 1). Corticosteroids are prescribed during the early post-transplantation period, but are now commonly withdrawn after 3 months. Calcineurin inhibitors currently remain the keystone of most immunosuppressive regimens following organ transplantation. Azathioprine and MMF are used to enhance immunosuppression or to allow dose reductions in either cyclosporine, tacrolimus, or corticosteroids. mTOR inhibitors may be used with the same aim. Antibodies and high-dose corticosteroids are used for induction and to treat acute cellular rejection.

1.3. Corticosteroids

Corticosteroids (prednisone and prednisolone or methylprednisolone) are non-specific anti-inflammatory and immunosuppressive agents. They depress leukocyte and macrophage function, cytotoxic T cell activity, cytokine (IL2, IL3, IL4, IL6, interferon-γ) release, prostaglandin and leukotriene release, eosinophil and mast cell function, and MHC and adhesion molecule expression. At high concentrations, glucocorticosteroids induce lymphopenia (caused by redistribution, associated with some lympholysis).

Corticosteroids have numerous side effects. Hypertension, dyslipidaemia and glucose intolerance are effects shared by both corticosteroids and calcineurin inhibitors. Osteopenia, growth retardation in children, digestive and psychiatric disorders are more specific to steroids. The results of most studies showed that steroid withdrawal at 3 months could be achieved in approximately 85% of liver
graft recipients without a significant increase in acute rejection [1]. Chronic rejection was not increased, and patient and graft survival were not adversely affected. Steroid withdrawal was associated with reduced levels and better control of hypertension, reduced total cholesterol levels, and reduced values and improved control of diabetes. Even after very early steroid withdrawal (<3 months), acute rejection rates were similar to or less than those reported under steroid-containing regimens [1]. However, the potential deleterious effects of steroid withdrawal on long-term graft function remain unknown.

The introduction of newer agents may enable the safer withdrawal of steroids.

1.4. Calcineurin inhibitors

Both cyclosporine and tacrolimus bind to cytoplasmic receptors, and the resulting complexes inactivate calcineurine, a key enzyme in T cell signalling. Calcineurin inhibition prevents the transcription of target genes, such as IL2 gene, thereby inhibiting T cell IL2 production. The introduction of cyclosporine dramatically improved graft and patient survival. However, calcineurin inhibitors induce several major side effects, which include acute and chronic nephrotoxic effects, hypertension, dyslipidaemia, and tumour promotion [2].

1.4.1. Cyclosporine

The original oil-based oral formulation of cyclosporine (Sandimmun®) was characterised by poor bioavailability and high intra- and interpatient pharmacokinetic variability. A novel microemulsion formulation (Neoral®) was therefore developed [2]. Studies have shown an improvement in pharmacokinetic parameters, attributable mainly to improved absorption in patients with poor absorption, with no clinically significant differences in terms of tolerability or drug interaction profiles. When compared with the original formulation, there has been a trend towards a reduced incidence of acute rejection with the microemulsion formulation.

1.4.2. Therapeutic drug monitoring (TDM) of cyclosporine

Variable degrees of absorption and a narrow therapeutic index have resulted in the need to determine cyclosporine

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**Table 1**

<table>
<thead>
<tr>
<th>Class of immunosuppressors</th>
<th>Main cell target(s)</th>
<th>Main molecular target(s)</th>
<th>Main side effect(s)</th>
<th>Use</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>All leukocytes, antigen-presenting cells</td>
<td>Nuclear receptors (blockade of the production of cytokines and of mediators of inflammation)</td>
<td>Diabetes, osteopenia, hyperlipidaemia, hypertension</td>
<td>P,T</td>
<td>3–12 months</td>
</tr>
<tr>
<td>Anticalcineurin drugs</td>
<td>T lymphocytes</td>
<td>Calcineurin</td>
<td>Nephrotoxicity, hypertension, diabetes, neurotoxicity</td>
<td>P, T (tacrolimus)</td>
<td>Definitive</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>T,B lymphocytes, endothelial cells</td>
<td>Enzymes of purine synthesis TOR</td>
<td>Bone marrow toxicity, GI disorders (MMF) Hyperlipidaemia, thrombopenia, hepatic artery thrombosis</td>
<td>P</td>
<td>3 months-definitive</td>
</tr>
<tr>
<td>mTOR inhibitors</td>
<td>T,B lymphocytes, endothelial cells</td>
<td>TOR</td>
<td>Bone marrow toxicity, GI disorders (MMF) Hyperlipidaemia, thrombopenia, hepatic artery thrombosis</td>
<td>P</td>
<td>Definitive</td>
</tr>
<tr>
<td>Polyclonal antilymphocyte antibodies</td>
<td>T lymphocytes</td>
<td>Membranous molecules</td>
<td>Cytokine release syndrome</td>
<td>P,T</td>
<td>Days</td>
</tr>
<tr>
<td>Anti-CD3 mAb</td>
<td>T lymphocytes</td>
<td>CD3</td>
<td>Cytokine release syndrome</td>
<td>P,T</td>
<td>Days</td>
</tr>
<tr>
<td>Anti-CD25 mAb</td>
<td>Activated T lymphocytes</td>
<td>CD25 (alpha chain of IL2-R)</td>
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P, prophylaxis; T, treatment; mTOR, mammalian target or rapamycin; mAb, monoclonal antibodies.
blood concentrations. However, the trough whole blood concentration (C0) remains an imperfect measure of total exposure to cyclosporine [3]. Monitoring of the area under the concentration–time curve (AUC) has failed to gain widespread acceptance because of practical difficulties. Another option is the cyclosporine blood concentration at 2-h postdose (C2); cyclosporine absorption during the first 4 h postdose (AUC0–4) represents the period of the greatest variability in blood concentration profiles between patients, and AUC0–4 is highly predictive of acute rejection [2]. Studies in liver transplant recipients have demonstrated that C2 is the single time point that correlates most closely with AUC0–4. C2 monitoring identifies whether patients are high or low absorbers of cyclosporine.

C2 monitoring reduces the incidence and severity of acute rejection when compared with C0 monitoring following liver transplantation [4]. The initial C2 target of 1000 ng/ml should be achieved by days 3–5 post-transplant for maximum clinical benefit [4]. Single-centre experience has shown that when the C2 target is achieved during the early post-transplant period, the acute rejection rate may be as low as <10% [4]. C2 monitoring can also improve the renal function and reduce the incidence and severity of hypertension in maintenance patients by identifying those who are receiving excessive cyclosporine doses. Indeed, up to 40% of maintenance liver patients have C2 levels exceeding predefined targets [4].

1.4.3. Tacrolimus

Tacrolimus (FK506) was developed as an alternative agent to cyclosporine [5]. After liver transplantation, its greater water solubility and lesser dependence on bile salt absorption results in improved bioavailability over cyclosporine. Large US and European multicentre trials in liver transplantation showed that tacrolimus-based immunosuppression was associated with a lower incidence of acute rejection than standard cyclosporine-based therapy [6,7]. In addition, patient survival was higher in the tacrolimus group after 3 years of follow-up. Tacrolimus was associated with a lower incidence of hypertension and hyperlipidaemia. In contrast, neurological and diabetogenic adverse effects were increased under tacrolimus [7]. During these initial studies, tacrolimus was used at higher doses, the initial doses being given intravenously, which may have led to excessively high blood levels. More recent studies have shown that tacrolimus can be given orally (or via the intragastric route) as from the immediate post-operative period, and intravenous tacrolimus is no longer employed.

Continuing trials are now comparing tacrolimus with cyclosporine microemulsion after liver transplantation. In a trial including all liver transplant centres in the UK and the Republic of Ireland [8], the primary outcome combining death, retransplantation or treatment failure for immunological reasons was attained in 21% in the tacrolimus group vs. 32% of the patients allocated to microemulsified cyclosporine (P = 0.001); death occurred in 17% vs. 24%, retransplantations in 4% vs. 10%, and treatment failure in 2% vs. 4%. Renal dysfunction and the need for antihypertensive therapy were much the same in both groups. Tacrolimus was more diabetogenic (relative risk = 2, P = 0.0006). The findings of this study are at odds with those of another European (continental) trial [9], which found no significant differences in the incidence and severity of rejection, steroid-resistant rejection or chronic rejection between the two groups at 1 year [10]. In both trials, about 600 recipients were randomised, and the study designs were similar. One explanation for these conflicting results may be that in the UK/Republic of Ireland trial, target drug concentrations were generally achieved for tacrolimus but not for cyclosporine. A second may have been the lower rate of patient and graft survival at 1 year in this trial (80%) than in the continental trial (more than 90%), suggesting that some additional factor has increased patient and graft loss in the former: causes of death unrelated to immunosuppressive therapy were indeed more numerous in the cyclosporine than in the tacrolimus group (42 vs. 19); graft failure linked to hepatic artery thrombosis or graft infarction was also more frequent in the cyclosporine group (22/31 vs. 6/11 in the tacrolimus group), although these complications were probably unrelated to immunosuppressive therapy. The long-term results of the two trials will require careful analysis.

Tacrolimus is commonly used as rescue therapy for refractory acute rejection in patients on cyclosporine-based therapy. Studies have reported a 75% rescue rate with tacrolimus with a mean 5-year follow-up period [11,12]. This ability to reverse refractory acute rejection is less evident with cyclosporine. The efficacy of tacrolimus in improving the outcome of patients with chronic rejection was evaluated prospectively in a multicentre study [13]. Patient and graft survivals at 2 years reached 81.2% and 48.5%, respectively. Two prognostic factors were identified: Patients with a total bilirubin ≤10 mg/dl and converted ≥90 days after transplantation exhibited better graft and patient survival. These data suggest that conversion to tacrolimus should be proposed at an early stage of chronic rejection, prior to the onset of extensive biliary duct loss.

1.4.4. Therapeutic Drug Monitoring (TDM) of tacrolimus

As yet, trough whole blood concentration monitoring is still the usual method employed to monitor tacrolimus therapy. The use of other timed samples and AUC monitoring has been investigated but, unlike cyclosporine, they have not been adopted in clinical practice [14]. This may, in part, be due to the strong correlation observed between trough concentration and AUC values [15].

1.5. Antiproliferative agents

Antiproliferative agents prevent the expansion of allo-activated T cell and B cell clones. Azathioprine, a purine
analogue that inhibits DNA synthesis, has been used as an immunosuppressive agent since the 1960s. In many centres, mycophenolate mofetil (MMF) is now replacing azathioprine in standard immunosuppression protocols.

1.5.1. Azathioprine

Azathioprine is metabolised by the liver to the active drug 6-mercaptopurine, which inhibits adenosine and guanosine monophosphate production, and thus inhibits DNA and RNA synthesis in rapidly proliferating cells. Bone marrow suppression and hepatotoxicity can be serious and limiting adverse effects of azathioprine. Azathioprine is currently used in combination with cyclosporine and corticosteroids, mainly to achieve corticosteroid or cyclosporine sparing. Recent studies have examined the use of tacrolimus in combination with azathioprine. When full-dose tacrolimus and identical corticosteroid regimens were used, azathioprine provided no additional benefit [16]. The monitoring of azathioprine is not ensured in clinical practice.

1.5.2. Mycophenolate mofetil (MMF)

MMF works by a mechanism similar to that of azathioprine but in a more selective manner. Mycophenolic acid (MPA), the active metabolite of MMF, inhibits the synthesis of de novo guanine nucleotide production, and B and T lymphocytes are deeply vulnerable to this effect. Other cell types have a salvage pathway that allows nucleotide synthesis to continue. When compared with azathioprine, MMF appears to have fewer myelotoxic and hepatotoxic adverse effects [17]. Since both B and T cells are inhibited, MMF may be effective against both acute and chronic rejection. Gastrointestinal side effects of MMF occur in around 25% of patients, and may be dose-limiting. They include nausea, vomiting, diarrhoea and abdominal pain [18]. Haematological disorders (thrombocytopenia, anaemia, leukopenia) are currently reported and an increased risk of cytomegalovirus infection has been observed.

1.5.3. MMF versus azathioprine

In the setting of primary immunosuppression, MMF presents certain advantages over azathioprine with respect to safety and efficacy. During a randomised trial, 565 liver transplant recipients were assigned to treatment with MMF, 3 g daily orally, or azathioprine, 1–2 mg/kg per day, in combination with cyclosporine and corticosteroids. The incidence of acute rejection or graft loss was 47.7% in azathioprine patients vs. 38.5% in MMF patients (P < 0.03). The incidence of rejection was 40.0% vs. 31.0% (P < 0.06) and that of steroid-resistant rejection 8.2% vs. 3.8% (P < 0.02) [19]. MMF and azathioprine, in combination with equivalent doses of lymphocyte antibodies, cyclosporine microemulsion and corticosteroids, were also compared during a controlled trial [20]. After a median follow-up period of 10 months, MMF-treated patients experienced fewer episodes of acute rejection (21.4% vs. 44.8%, P = 0.06), thrombocytopenia (21.4% vs. 48.3%, P < 0.05) and leukopenia (7.1% vs. 20%, P = 0.14).

1.5.4. MMF as steroid-sparing agent

In a major trial using MMF and an identical corticosteroid taper with the randomisation of patients to microemulsion cyclosporine or tacrolimus [21], the 6-month patient and graft survival rates were 98% and 94%, respectively. There was no difference in the number of patients with rejection episodes (22% vs. 17%, P = 0.61) or infection. Steroid-free immunosuppression with tacrolimus and MMF has also been assessed by a study during which acute rejection occurred in 26.2% of patients, and was associated with subtherapeutic tacrolimus blood levels [22]. Acute renal failure was seen in 33% of patients, and was related to high tacrolimus blood levels. Close drug monitoring was therefore advised by the authors. A randomised analysis has shown that steroid-free immunosuppression with tacrolimus and MMF was at least as effective as the standard protocol with tacrolimus and steroids [23]; the incidence of acute rejection was 26% in the tacrolimus/MMF group vs. 46% in the tacrolimus/prednisolone group.

1.5.5. MMF as a calcineurin inhibitor-sparing agent

The addition of MMF to full dose tacrolimus and corticosteroids does not appear to be beneficial. In a randomised trial comparing tacrolimus and corticosteroids vs. full dose tacrolimus, 2 g/day MMF and corticosteroids after liver transplantation [24], there was no significant difference in patient or graft survival up to 3 years, or in the incidence of rejection (38.9% vs. 45.2% at 1 year). However, approximately half of the patients withdrew from the MMF group because of side effects. The combination of tacrolimus with MMF may permit the safe reduction of calcineurin inhibitor dosage. A retrospective study included patients who had received oral MMF 2 g daily, tacrolimus and steroids, compared with historical controls who received tacrolimus and the same steroid taper [25]. The rate of acute rejection was significantly lower in the MMF group, although blood tacrolimus levels were lower in that group. When reduced dosages of cyclosporine and tacrolimus were used in combination with MMF [26], the 1-year acute rejection rates were 75% for the cyclosporine group versus 45% for the tacrolimus group (P < 0.05). Part of the reason for the difference may be that exposure to the active metabolite, MPA, was higher when MMF was used in combination with tacrolimus. This pharmacokinetic difference could be circumvented by simply increasing the MMF dosages when it is used with cyclosporine.

1.5.6. Secondary reduction of calcineurin inhibitors

MMF has been used secondarily to reduce or suppress the calcineurin inhibitor, in patients experiencing side effects with this agent. Following liver transplantation,
replacement of the calcineurin inhibitor by MMF leads to an improvement in chronic renal dysfunction in most cases. Side effects of MMF or the occurrence of rejection may be limiting in some patients. In a prospective series of liver graft recipients with renal dysfunction, calcineurin inhibitor was replaced by MMF at a final dose of 1.5–3 g/day [27]. Six months after study entry, renal function had improved in 17 of the 22 patients included. An improvement was observed less frequently (11/15) in patients with creatinine elevation ≥12 months than in patients with creatinine elevation ≤6 months (6/6). A randomised trial of MMF monotherapy in liver transplant patients who developed renal failure was stopped when three of five patients on monotherapy developed organ rejection requiring a second transplantation [28]. During a second randomised study, the replacement of calcineurin inhibitor by MMF, achieved in a more stepwise manner, was compared with calcineurin inhibitor continuation in 28 liver graft recipients with renal dysfunction [29]. By the end of the study, mean serum creatinine levels had fallen by 44.4 μmol/l in study patients (vs. 3.1 μmol/l in controls). Three reversible episodes of acute graft rejection occurred in the study patients, vs. none in the control group. During a prospective study, liver graft recipients with renal failure were administered 2 g daily MMF either as monotherapy (group I), or in combination with low-dose calcineurin inhibitor, without (group II) or with (group III) previous refractory rejection [30]. In group I, only 6% experienced cellular rejection, and serum creatinine normalized in five out of eight patients. In group II, none of the 18 patients experienced rejection, and serum creatinine levels normalized in 6/10. In group III, 45% of patients experienced further rejection.

1.5.7. Therapeutic Drug Monitoring (TDM) of MMF

When given orally, MMF undergoes rapid and complete absorption. It is hydrolysed to the active metabolite, MPA, and no MMF is measurable in plasma. Low plasma MPA AUC is a significant risk factor for developing rejection after renal transplantation [31]. Maintaining the MPA trough plasma concentration between 2.5 and 4 mg/l may also have a beneficial effect on the rejection rate. TDM should be used to establish that adequate MPA concentrations are achieved soon after surgery and it could be useful in cases of adverse reaction to MMF [32].

1.5.8. Enteric-coated mycophenolate sodium (EC-MPS)

An enteric-coated formulation of mycophenolate sodium (EC-MPS) has been developed with the aim of improving the gastrointestinal disorders associated with MMF. During early studies, 720 mg EC-MPS were shown to result in MPA exposure equivalent to 1000 mg MMF. Two pivotal studies in kidney transplantation designed to prove the therapeutic equivalence of the two drugs (combined with cyclosporine microemulsion and corticosteroids) showed that the failure of efficacy (acute rejection, graft loss, death or loss to follow-up) and incidence of acute rejection and tolerance (notably gastrointestinal adverse events) at 6 months were comparable between the two drugs [28]. However, MPA exposure associated with 2 × 720 mg EC-MPS was higher than with 2 × 1000 mg MMF (increase: 30%).

1.6. mTOR inhibitors

Sirolimus (rapamycin) and its derivative everolimus (RAD) constitute a new class of compounds designated as the mTOR inhibitors, which exhibit potent antifungal, antiproliferative, and immunosuppressive effects. mTOR inhibitors inhibit intracellular signalling distal to the IL2 receptor and subsequent progression of the T cell into the S phase of the cell cycle. These activities are complementary to those of calcineurin inhibitors [33]. Sirolimus has been approved by most Western countries for renal transplantation but not yet for liver transplantation. Large international randomised trials using sirolimus and everolimus in combination with cyclosporine and steroids after renal transplantation have demonstrated that these agents are safe, and enable excellent patient and graft survival [33]. In addition, experimental data suggest that sirolimus may prevent the development of chronic rejection [34]. This finding may be related to an antiproliferative effect of the drug. Everolimus also inhibits the growth of human Epstein-Barr virus-transformed B lymphocytes [35].

Reversible leukopenia, thrombocytopenia, and dose-dependent hyperlipidaemia have been the principal toxicities associated with sirolimus and everolimus [33]. Triglyceride and cholesterol elevations are responsive to dietary modification and lipid-lowering drugs. Interstitial pneumopathies have been found in patients receiving sirolimus [36]. Sirolimus is considered to exhibit no nephrotoxic or neurotoxic effects. However, significant elevations in serum creatinine were identified among patients receiving sirolimus and everolimus in combination with full therapeutic doses of cyclosporine. Nephrotoxicity, presumably linked to pharmacokinetic and pharmacodynamic interactions with calcineurin inhibitors, appeared to be well controlled when lower doses of cyclosporine are given. Recent results have suggested that a low (around 10%) incidence of acute rejection may be observed using sirolimus or everolimus with low doses of cyclosporine or tacrolimus after renal transplantation [33]. This is particularly true when sirolimus trough levels are maintained within the 10–20 ng/ml range, even when very low blood concentrations of calcineurin inhibitor are obtained [33].

Immunosuppression using sirolimus without calcineurin inhibitors seems possible in de novo renal recipients [37] as well as among patients converted from calcineurin inhibitor to sirolimus-based therapy [33]. This possibility has not been proven after liver transplantation.

1.6.1. Sirolimus and liver transplantation

During a pilot study, sirolimus was combined with microemulsion cyclosporine with or without
corticosteroids, or was used alone after liver transplantation [38]. Rejection was more common on monotherapy than under double therapy, and absent under triple therapy. Liver transplantation may be performed successfully with a minimal use of corticosteroids by using sirolimus; in patients undergoing liver transplantation with either tacrolimus or microemulsion cyclosporine and sirolimus (6 mg/day for 1 day, followed by 2 mg/day), with a 3-day tapered dose of corticosteroids [39], patient survival was 92%, and graft survival reached 89%; 30% of patients experienced rejection, compared with 70% in historical controls (P < 0.01).

The combination of sirolimus (target trough levels 4–11 ng/ml) and low-dose tacrolimus (target trough levels: 3–7 ng/ml, days 1–90; 3–5 ng/ml thereafter) plus corticosteroids was compared with conventional tacrolimus (trough levels 7–15 ng/ml, days 1–90; 5–10 ng/ml thereafter) plus corticosteroids in a randomised study including 222 patients [40]. At 12 months, patient survival (86.4% vs. 94.6%, P = 0.04) and graft survival (80.0% vs. 91.1%, P = 0.02) were lower in the sirolimus group. The rates of acute rejection were comparable between the two arms. There was a trend towards better renal function in the sirolimus arm. Rates of wound infection (18.2% vs. 8%, P = 0.03) and sepsis (12.7% and 6.3%, P = NS) were higher in the sirolimus group. The rate of hepatic artery thrombosis was numerically higher at 12 months in the sirolimus arm (5.4% vs. 0.9%, P = 0.06), with five out of six of these cases resulting in graft loss; all cases occurred within 1 month of transplantation; however, most of these patients had other mitigating factors that increased the risk of thrombosis. The occurrence of an unusual frequency of hepatic artery thrombosis has stopped trials involving sirolimus in de novo liver transplant recipients, but it remains to be determined if this finding is really linked to the use of sirolimus.

Much of the experience gained using sirolimus in liver transplantation has arisen from studies where the drug was introduced because of calcineurin inhibitor toxicity [41]. Fourteen patients with renal failure or neurotoxicity were administered sirolimus, combined with MMF and corticosteroids [42]. Once neurological or renal dysfunction had been resolved, tacrolimus therapy was initiated. Six patients experienced steroid-sensitive acute rejection. Serum creatinine levels improved at 3 months. All three patients with neurological indications also improved. Sirolimus and MMF were compared retrospectively in another study as agents capable of reducing the calcineurin inhibitor dosage in long-term liver transplant recipients with renal failure [43]. Patients received sirolimus 4 mg daily (N = 27) to maintain levels of 8–10 ng/ml or MMF 2 g daily (N = 34), and then the tacrolimus dose was reduced in anticipation of eliminating tacrolimus. The reduction in serum creatinine levels was higher in the MMF than in the sirolimus group at 3 months (P = 0.001). In the MMF group, treatment with tacrolimus was stopped in 35.3% of patients, vs. 14.8% in the sirolimus group. The rejection rates were 29.6% vs. 5.8% (P = 0.03) and the drop-out rates were 37% and 5.8%, in the sirolimus and MMF groups, respectively.

Sirolimus has been shown to have potential benefits as a rescue treatment in refractory rejection [44].

1.6.2. Therapeutic Drug Monitoring (TDM) of sirolimus

The half-life of sirolimus is 62 h in stable kidney recipients treated with cyclosporine. The correlation between trough concentration levels (C0) and drug exposure is excellent (r² = 0.80–0.95) in patients treated with cyclosporine or tacrolimus [45]. Cyclosporine increases the bioavailability of sirolimus by 240% when administered simultaneously, but by only 80% when administered 4 h apart. A 4-h interval between intakes of the two drugs is thus recommended. In patients receiving tacrolimus and sirolimus, neither the pharmacokinetic profiles of sirolimus nor those of tacrolimus were altered by simultaneous administration [45]. When sirolimus is used in combination with cyclosporine or tacrolimus, predose concentrations are generally targeted within the range 5–15 ng/ml [45].

1.6.3. Everolimus

Everolimus has been studied in large, randomised, multicentre studies designed to evaluate its equivalence of efficacy with MMF after renal transplantation [46]. These ongoing studies have shown that everolimus (1.5 or 3 mg/day) and MMF 2 g/day produce similar low rates of acute rejection in recipients receiving triple immunosuppressive therapy with conventionally dosed cyclosporine microemulsion and corticosteroids [46]. Low rates of acute rejection, excellent rates of patient and graft survival, a lower incidence of CMV infections, improved cholesterol, triglyceride and creatinine profiles, and better renal function have been demonstrated with everolimus and lower doses of cyclosporine microemulsion in renal transplant recipients. In an ongoing randomised study in renal transplantation using everolimus 3 mg/day and either full-dose or reduced-dose cyclosporine microemulsion (plus basiliximab and corticosteroids), acute rejection rates were lower in patients receiving the reduced-dose regimen [46] (7.0% vs. 16.7%). Furthermore, renal function was significantly improved by the reduced-dose cyclosporine regimen. Studies of everolimus after liver transplantation are currently being performed.

1.6.4. Therapeutic Drug Monitoring (TDM) of everolimus

The bioavailability of everolimus is higher than that of sirolimus, and its half-life is shorter (around 30 h). The correlation between AUC and dose is excellent, and the pharmacokinetics of everolimus are less affected by cyclosporine than sirolimus. It is still not clear whether an interval of time between intakes of the two drugs is necessary [47]. The lower therapeutic concentration limit is
around 3 ng/ml when everolimus is used with cyclosporine. The upper limit is probably more than 15 ng/ml.

1.7. Antilymphocyte antibodies

1.7.1. Polyclonal antibodies

Antilymphocyte antibodies were amongst the first immunosuppressive agents to be used in clinical liver transplantation. Antilymphocyte globulins were derived from horse or rabbit serum following inoculation with human lymphocytes. Although such preparations were potent immunosuppressives, severe allergic reactions and serum sickness were common complications. In addition, the variable effectiveness of these preparations was responsible for unpredictable effects. Today, purer forms of antilymphocyte and antithymocyte globulins are available, thus inciting renewed interest in antithymocyte globulin (ATG).

The complete elimination of corticosteroids ab initio through induction with rabbit ATG was assessed in a randomised trial [48]. Seventy-one adult patients were randomised to the administration of ATG (1.5 mg/kg on days 0 and 1), or that of corticosteroids. Maintenance immunosuppression consisted of tacrolimus and MMF. The overall survival rate was 91% and the graft survival rate 89%, in both groups. The rejection rate was 20.5%, but in all cases with was treated successfully by increasing the tacrolimus dosage in patients receiving ATG. This rate reached 32% in corticosteroid patients, 64% of whom required additional corticosteroids for treatment ($P = 0.01$ for rejection requiring corticosteroid therapy). The incidence and severity of infectious complications were similar in both groups.

ATG has been monitored in renal transplant patients using the effect of ATG on CD3+ lymphocytes. The dose of ATG administered was titrated to maintain the absolute CD3+ lymphocyte count at <50 cells/μl of blood.

1.7.2. Monoclonal antibodies

One approach to increasing the specificity of induction immunosuppression is the development of monoclonal antibodies with highly specific targets.

1.7.2.1. Muromonab CD3

Muromonab CD3 is a murine preparation of monoclonal antibodies directed against CD3, which is found on the cell surface of all T lymphocytes [49]. The administration of muromonab CD3 is associated with a variety of adverse effects, known as the cytokine release syndrome. Symptoms include fever, diarrhea, nausea and vomiting, severe headaches, myalgia and shortness of breath. These adverse effects are generally most severe with the first dose and can be reduced by the administration of corticosteroid premedication.

Three randomised trials have evaluated the administration of muromonab CD3 during the first 14 days in order to delay the start of cyclosporine therapy after liver transplantation [49]. The control group treatment in all three studies consisted of cyclosporine, corticosteroids and azathioprine. In the muromonab CD3 group, patients received a similar regimen, but cyclosporine was delayed until day 11. No advantage was demonstrated in terms of graft or patient survival. Acute rejection was significantly less and the renal function improved during the first 2 weeks in the muromonab CD3 group, but at 6 months, the incidence of acute rejection and impaired renal function equalised in the two groups. Concern over an increased risk of lymphoproliferative disease, and the availability of more specific immunosuppressive drugs have led to a marked decrease in the use of muromonab CD3 in liver transplantation, both as induction therapy and in the treatment of steroid-resistant acute rejection.

OKT3 therapy can be monitored by measuring the OKT3 concentration, antiOKT3 antibody concentration and the number of CD3+ cells by flow cytometry [49]. TDM for OKT3 is not in widespread use.

1.7.2.2. Antireceptor antibodies

AntiIL2 receptor (anti-chain alpha, or anti-CD25) antibodies are being used increasingly as induction immunosuppression therapy after liver transplantation. Antibody binding to the IL2 receptor competes with IL2 and inhibits lymphocyte activation and clonal expansion. To circumvent the formation of anti-murine antibodies, chimeric and humanised forms of anti-CD25 antibodies were developed. Their advantages include prolonged action and more potent effector activity as a result of the presence of human Fc. Two such antibodies are now available. Basiliximab is a chimeric antibody with a half-life of approximately 6.5 days in renal transplant patients [50]. Daclizumab is a humanised antibody with a half-life of approximately 11 days [51]. The half-life was shown to be decreased in liver recipients when compared with renal transplant recipients [52]. Loss of ascites was weakly correlated with monoclonal antibody clearance, and adjustments should only be considered for patients with massive ascites fluid drainage [52]. These antibodies are well tolerated [53].

1.7.2.2.1. Daclizumab

Low dose daclizumab (1 mg/kg after reperfusion and 0.5 mg/kg on day 4 post-transplant), with cyclosporine and corticosteroids, was administered during a pilot study of 28 liver transplant patients [53]. Only one patient underwent steroid-sensitive acute rejection. The rate of opportunistic infections did not differ from that seen with conventional immunosuppressive regimens.

1.7.2.2.2. Daclizumab to delay the use of calcineurin inhibitors

Daclizumab without calcineurin inhibitor is ineffective in liver transplant recipients, but can be used safely to reduce or delay the introduction of calcineurin inhibitors: in a pilot study [54], seven patients received daclizumab combined with corticosteroids and MMF; the study was halted after all seven patients developed acute rejection. In this study, daclizumab concentrations fell to subtherapeutic levels (<5 mg/l) by postoperative day 4–6.
In the same paper, the remaining 25 patients received daclizumab, MMF, and corticosteroids, with the introduction of a calcineurin inhibitor on average 7 days postoperatively. The rate of rejection was 36%, similar to that seen in historical controls who received a calcineurin inhibitor, MMF and prednisone.

In one study, three different approaches to induction were evaluated in patients with preoperative renal failure [55]: a single dose of daclizumab with delayed introduction of tacrolimus, muromonab CD3 for the first 2 weeks, or low-dose tacrolimus immediately postoperatively. The incidence of acute rejection was similar between the groups. Renal function improved less quickly in the tacrolimus group than in the two other groups. In the daclizumab group, 2-year patient and graft survival rates were significantly better when compared with the low-dose tacrolimus group. In a prospective trial comparing daclizumab with the delayed introduction of tacrolimus, with tacrolimus immediately postoperatively (both groups receiving MMF and corticosteroids), the incidence of acute rejection was lower in the daclizumab group than the control group (26 vs. 49%), and serum creatinine levels were lower in the daclizumab group at 7 days [56].

Since daclizumab concentrations may fall rapidly after liver transplantation, a double dose regimen has been assessed in liver graft recipients. This double dose regimen produced an effective blockade of IL-2R alpha for at least 14 days [51]. A non-randomised study looked at a double dose schedule in patients with a risk of renal dysfunction [57], who were compared with those with normal renal function, receiving conventional immunosuppression. While the daclizumab group exhibited markedly elevated preoperative creatinine levels, these values had equalised in the two groups by 1 week post-transplant. The rejection rate was lower in the induction group at 6 months (18 vs. 40%, \( P = 0.02 \)).

The avoidance of corticosteroids was assessed in a randomised trial [58] comparing tacrolimus plus MMF and corticosteroids, with daclizumab plus tacrolimus, MMF and corticosteroids for 2 days only. Patient and graft survival rates at 18 months were 93% and 100%, respectively. More rejections were diagnosed in patients without corticosteroids (25% vs. 6.7%) \( (P = 0.03) \).

1.7.2.2.3. Basiliximab. A pilot study assessed basiliximab in combination with cyclosporine, azathioprine and corticosteroids after liver transplantation [59]. At 6 months, the acute rejection rate was 24%; no episodes of severe rejection were observed. Both patient and graft survival were excellent. The efficacy and safety of basiliximab were recently assessed during a large controlled trial [60], where patients were randomised to basiliximab or placebo, plus cyclosporine and corticosteroids. The incidence of acute rejection was reduced in patients receiving basiliximab, particularly in the hepatitis C virus-negative subgroup (33.1 vs. 47.6%, \( P = 0.034 \)). In a retrospective analysis [61], four groups were compared: cyclosporine and corticosteroids (group A); basiliximab plus cyclosporine and corticosteroids (group B); tacrolimus and corticosteroids (group C) and tacrolimus, basiliximab, and corticosteroids (group D). The acute rejection rates were 78%, 50%, 20%, and 9%, respectively, in the four groups. No patients in groups C or D experienced any corticosteroid-resistant rejection.

Basiliximab was assessed prospectively as rescue therapy in a setting of corticosteroid-resistant rejection in seven paediatric recipients, associated with conversion to tacrolimus and the addition of MMF [62]. All five children were rejection-free after a median follow-up period of 22 months. Chronic rejection developed in the other two children.

1.8. Future immunosuppressive drugs

1.8.1. Leflunomide and FK 778

Leflunomide, a member of the malonitrilamide family, reversibly blocks dihydro-orotate dehydrogenase, an enzyme required for de novo pyrimidine synthesis in lymphocytes and other cells, and inhibits selected tyrosine kinases. Leflunomide exhibits synergistic interaction with calcineurin inhibitors, prevents acute rejection and delays the progression of chronic rejection in animal models, and can halt the replication of herpes viruses, specifically CMV and HSV. Its active metabolite, A77,1726, seems to be responsible for the drug’s many activities. Because of a broad inter-patient range of half-lives for the active metabolite, the monitoring of serum levels seems to be an important part of its evaluation. Leflunomide is currently being used in rheumatoid arthritis. The recommended dose in this population, 20 mg daily, produces a serum level of the active metabolite of approximately 25–45 ng/ml, which is associated with few side effects. A retrospective review of the use of leflunomide was performed in 53 liver and kidney transplant recipients [63]. Cyclosporine or tacrolimus immunosuppression could be completely stopped in seven patients, without any evidence of acute rejection. In this series, no patients with serum levels of A77, 1726 above 50 ng/ml showed any evidence of acute rejection despite the reduction or discontinuation of calcineurin inhibitors. The primary toxicities were anaemia in renal transplant patients and an elevation of liver enzymes in liver transplant patients. Dose-limiting side effects occurred in fewer than 15% of patients when drug serum levels were lower than 80 ng/ml. Conversion of kidney graft recipients from azathioprine or MMF (+ cyclosporine and corticosteroids) to leflunomide reversed the progression of chronic renal allograft dysfunction with minimal toxicity in a pilot, crossover trial [64].

FK778, a synthetic malonitrilamide derived from A77 1726, was synthesized to reduce the extended half-life of leflunomide, while maintaining similar therapeutic efficacy. FK778 may be used in combination with current standard drugs in organ transplantation. In a canine kidney transplantation model, FK778 at a dose of 4 mg/kg
prolonged median survival, displaying a synergistic effect with calcineurin inhibitors [65].

1.8.2. FTY720

FTY720, a synthetic small molecule with functional homology to sphingosine-1 phosphate, is a novel immunomodulator with an unique mechanism of action, i.e. chemokine-dependent lymphocyte sequestration into secondary lymphoid organs and FTY720-induced apoptosis, associated with profound lymphocyte depletion in the blood [66]. FTY720 proved to be an effective immunosuppressant in preventing acute kidney allograft rejection in cynomolgus monkeys, displaying a synergistic effect with cyclosporine and/or everolimus [67].

In stable renal transplant patients, single oral doses of FTY720 ranging from 0.25 to 3.5 mg were well tolerated and caused reversible selective lymphopenia within 6 h, the nadir being 42% of baseline. The lymphocyte count returned to baseline within 72 h in all dosing cohorts except the highest. Transient but asymptomatic bradycardia was the most common adverse event. The long half-life (89–157 h) suggests long dosing intervals [68].

1.8.3. Biological agents

1.8.3.1. Cytokines. IL-10 production is low during allograft rejection. Thus, recombinant human IL-10 therapy in association with cyclosporine or FK506 might be proposed after liver transplantation [69].

1.8.3.2. Antagonists of chemokine receptors. Antagonists to several chemokine receptors, including CCR1, CXCR3 and CCR5, have been shown to be effective in experimental transplantation and are likely to be considered for clinical development [70].

1.8.3.3. Blockade of T cell costimulation. The blockade of T cell costimulation soon after transplantation may render recipient CD4 T cells unresponsive to donor antigen. CD28/B7 interactions can be blocked by CTLA4Ig, a molecule formed by fusing CTLA4 to the constant region of a human IgG1. CTLA4Ig binds B71 and B72 with stronger affinity than CD28 and acts as a potent competitive inhibitor of CD28-mediated T cell activation. Humanized anti-B7.1/B7.2 and a second-generation CTLA4Ig are currently under investigation (Vincenti). Much research has also focused on the effects of interrupting the CD40-CD154 costimulatory pathway. CD40/CD154 interactions are critical in the activation of both CD4 T cells and B cells, as well as proinflammatory effects on endothelial cells and macrophages. The blockade of CD40/CD154 interactions may thus be beneficial in preventing chronic rejection. Recent data suggest that a combination of anti-CD154 and anti-CD80 plus anti-CD86 monoclonal antibodies may be synergistic in non-human primate renal transplantation [71]. However, anti-CD154 antibodies have been associated with vascular thromboses.

1.8.3.4. Blockade of T cell adhesion and accessory molecules. Antibodies directed against intercellular adhesion molecule-1 (ICAM-1) inhibit CD4 T cell activation and extend allograft survival in animal models [70]. A monoclonal anti-ICAM-1 antibody was shown to reduce the incidence of acute rejection after kidney transplantation. Antibodies directed against leukocyte function antigen-1 (LFA1), a ligand for ICAM1, may ensure similar prophylaxis against acute rejection to antilymphocyte globulin when given during the early post-transplant period. A humanized anti-CD11a (anti-LFA1) is currently under investigation [70]. LFA3TIP, a human fusion protein, has been developed to block CD2/LFA3 interactions and has been shown to extend cardiac allograft survival in a primate model. In a small randomised trial, an antihuman CD2 monoclonal antibody reduced the incidence of acute rejection and delayed graft function after renal transplantation [70].

Monoclonal antibodies directed against the CD4 molecule inhibit T cell activation. During preliminary studies, monoclonal antibodies to CD4 were well tolerated, but did not reduce the incidence of acute rejection. CD45 is a protein tyrosine phosphatase present on all leukocytes and is a critical regulator of T cell activation. Monoclonal antibodies directed against the CD45RB isoform extend renal graft survival in mice.

1.8.4. Inhibition of the efferent limb of the rejection process

Most of the drugs currently in use inhibit the afferent limb of the immune response. Only monoclonal or polyclonal antibodies act partly at the efferent phase, by killing activated effector cells. Inhibition of the efferent limb has not so far been explored. Inhibition of apoptosis or of the production of reactive oxygen-derived species, and cytoprotective drugs (which are mainly devoid of significant side-effects) could be used as adjuvant therapies in immunosuppressive regimens.

2. Immunosuppressive regimens in specific situations

2.1. Current immunosuppressive regimens

At present, immunosuppression is initiated at high levels when there is the greatest risk of graft rejection. Several immunosuppressive agents are used in combination in order to minimise the side effects of any single drug whilst maintaining adequate overall immunosuppression by targeting multiple steps in T cell activation (Fig. 2). Standard combinations include corticosteroids with a calcineurin inhibitor (cyclosporine or tacrolimus) and an antiproliferative agent.
Whereas early acute rejection episodes have a negative impact on the long term survival of a renal allograft, early episodes of acute rejection have not been shown to have a similar effect on a liver allograft [72]. Rather, the principal concern after liver transplantation lies in the numerous side effects associated with immunosuppression, which may reduce the patient survival [73]. Indeed, 30–40% of transplant recipients are predicted to develop neoplasia within 30 years. Renal failure may affect 5% of liver transplant recipients at 5 years and more than 10% at 10 years. Diabetes, predating liver transplantation or occurring de novo, is observed in 10–30% of patients. Hypertension and hyperlipidaemia may also require specific therapies. One means of preventing such complications is to reduce the dosage or even discontinue corticosteroids and calcineurin inhibitors, which are responsible for most of these side effects. A second approach involves the development of drugs, such as monoclonal antibodies, that are more specific to the prevention of organ rejection. Since single episodes of acute rejection do not give rise to significant long-term impairment of liver graft function [72], a third approach is deliberately to use low-dose initial immunosuppressive regimens, with the possibility of converting any patients who develop early episodes of acute rejection to a heavier immunosuppressive regimen, while leaving at least one-half of the recipients on lifelong, light immunosuppression.

A more specific problem in liver transplantation is the frequent risk of recurrence of the initial disease, which may be facilitated by the immunosuppressive regimen. Thus the next step will probably be to adjust the immunosuppressive regimen to the recipient. ‘A la carte’ immunosuppression could help to reduce the side effects of currently available drugs, while at the same time reducing the severity of disease recurrence.

2.2. Hepatitis C

The histological progression of chronic hepatitis C is more rapid after liver transplantation and leads to the development of cirrhosis in 15–20% of HCV-positive recipients [74]. Acute rejection and immunosuppression account to a major extent for this accelerated progression. Graft failure and death are more common in patients with hepatitis C who undergo acute cellular rejection, and the risk is directly related to the number of rejection episodes, steroid-resistant rejection and the use of steroid pulses and OKT3 [75,76]. However, increasing maintenance immunosuppression in order to reduce the incidence of acute rejection favours viral replication and facilitates viral-mediated graft injury [77].

Among the drugs currently used in immunosuppressive regimens, corticosteroids clearly worsen recurrence: the histological recurrence of hepatitis C and patient survival correlate with the cumulative corticosteroid dose [75,78]. Steroid withdrawal may thus be beneficial in patients with chronic hepatitis C, but few data are available. When corticosteroids were withdrawn 3 months after transplantation [79], there were no differences in the percentages of patients with recurrent HCV, progression to fibrosis stage 3 or 4 or death from endstage liver disease. In contrast, very early corticosteroid withdrawal (14 days post-transplantation) or steroid avoidance may be associated with lower HCV RNA levels, a reduced rate of HCV recurrence and a slower progression of liver fibrosis [80]. Long-term follow-up is required to determine the beneficial effects of this strategy.

Few studies have examined the relationship between azathioprine and a recurrence of hepatitis C. In a retrospective review [81], histological findings of recurrent hepatitis C was seen in 43 of 65 patients, with progression (fibrosis, grade 2 by last follow-up) in 19 patients; those who received azathioprine-containing immunosuppressive regimens experienced less recurrence (6 of 17 vs. 37 of 48 patients; P = 0.005) and progression (1 of 17 vs. 18 of 48 patients; P = 0.014) than those without azathioprine. MMF may act as an antiviral agent because of its ribavirin-like effects. A controlled trial compared MMF with azathioprine combined with cyclosporine and corticosteroids [19]. The rates of acute rejection were 31% in MMF-treated patients and 40% in azathioprine-treated patients. In HCV-positive patients, the rates of recurrent hepatitis at 6 months were 18.5% and 29.1%, respectively. In a retrospective review, HCV-positive patients receiving cyclosporine or tacrolimus with corticosteroids were compared with patients receiving the same regimen plus MMF at a low (<1.5 g/j) or high (≥1.5 g/j) dosage [82]. The rate of extensive fibrosis (METAVIR F3 or F4) was lower in the group receiving
a high MMF dosage than in the other two groups (33%, 22%, and 7%, respectively, *P* = NS). The viral load was also lower in the group with a high MMF dosage than in the two others (*P* = 0.007). Conversely, no benefits of MMF could be observed in a randomised trial comparing tacrolimus and prednisone (dual) versus tacrolimus, prednisone, and MMF (triple) therapy in HCV-positive patients [83]. Actuarial patient survival at 4 years (72.6% in dual vs. 73.8% in triple groups), actuarial graft survival at 4 years (65.6% vs. 65.4%), recurrent HCV (46.4% vs. 46.0%), mean hepatitis activity index scores, and mean fibrosis scores were similar in both groups.

There appears to be no consistent difference between cyclosporine and tacrolimus in terms of their effects on hepatitis C [78,81]. The effects of sirolimus have not as yet been adequately defined.

HCV recurrence was examined in HCV-positive transplant recipients receiving OKT3 for steroid-resistant rejection, and well-matched HCV-positive recipients administered at least one steroid pulse, but no OKT3 [84]. The rates of recurrent HCV infection were higher (84% vs. 52%), and histological severity was greater, in OKT3-treated patients.

Early experience with basiliximab has indicated an increased risk of rejection in HCV-positive transplant recipients [59]. Similar results were found in a large controlled trial [60]: the reduction in rejection episodes was concentrated in the HCV-negative cohort (14.5% relative to tacrolimus and a steroid taper [85]. Compared with a well-matched HCV-positive population without daclizumab, recurrent hepatitis progressed more rapidly in the daclizumab group and the HCV viral load was significantly higher at both 4 months and 1 year.

### 2.3. Cancer

Patients transplanted for neoplastic disease (mainly hepatocellular carcinoma) may benefit from reduced immunosuppression and from immunosuppressive drugs without effects on tumour promotion. Patients at a higher risk of de novo cancer may also benefit from the same regimens. In particular, patients transplanted for alcoholic cirrhosis experience a high incidence of postoperative oopharyngeal squamous cell carcinoma (16.7% vs. 0% in patients receiving a transplant for non-alcoholic cirrhosis, *P* = 0.001). This could be due to an additional effect of post-transplantation immunosuppression in patients exposed to alcohol and tobacco prior to transplant [86].

The role of anti-calcineurin drugs in cancer promotion has been well documented. It was shown in kidney transplant recipients [87] that the risk was dose-dependent.

In a series of patients transplanted for hepatocellular carcinoma, the principal factor influencing outcome and tumour recurrence appeared to be the cumulative dosage of cyclosporine administered during the first postoperative year rather than the staging of the cancer [88].

In contrast, other drugs do not seem to increase the risk of cancer: azathioprine has been used for years in autoimmune diseases without significantly increasing the neoplastic risk [89]; it can reasonably be extrapolated that MMF does not involve an increased risk of tumour promotion. The risk associated with sirolimus and everolimus remains unknown, but in vitro data suggest that these drugs may tend to have anti-proliferative properties [90]. However, convincing clinical data to support the anti-tumour effects of mTOR inhibitors in transplant patients are still lacking.

Monoclonal and polyclonal anti-lymphocyte antibodies may increase the risk of tumour promotion. In a retrospective study, the use of anti-lymphocyte antibodies (*P* = 0.007, relative risk [RR] = 4.2), age older than 50 years and liver transplantation for hepatitis C virus cirrhosis or alcoholic cirrhosis were independently associated with the onset of lymphoproliferative disease under multivariate analysis [91]. In contrast, anti-IL2R antibodies do not seem to increase the risk of lymphoproliferative diseases, after several years of follow-up [53].

#### 2.4. HIV

Human immunodeficiency virus (HIV)-positive patients treated with highly active antiretroviral therapy can expect a significant prolongation of life. HIV-positive patients experiencing complications of liver failure—mainly associated with HCV infection—are at a greater risk of dying from their end-stage liver disease rather than from their HIV. Several transplant centres no longer consider HIV infection as a contraindication to liver transplantation. The liver toxicity of antiretroviral therapy and HCV recurrence are the two main complications seen in HIV/HCV co-infected patients. In addition, protease inhibitors markedly inhibit cytochrome P450 3A isoenzymes, and thus the metabolism of calcineurin inhibitors. Cautious monitoring of drug interactions is therefore mandatory [92,93]. MMF, which exhibits significant anti-HIV activity may thus be useful in these patients [94].

#### 2.5. Patients at a low or high risk of rejection

A reduced risk of rejection (in parallel with an increasing risk of infection) has been demonstrated in elderly liver transplant recipients [95]. Following liver transplantation, the incidence of rejection may also be a function of the primary disease: patients transplanted for alcoholic liver disease or hepatitis B virus-related disease experience a reduced incidence of rejection (and a higher risk of infection). The link between alcohol and the immune response is poorly understood. The reduced risk of rejection...
patients without autoimmune disease. In addition, acute rejection occurred in 38% of patients in only 68% of a series of patients with autoimmune disease and/or more frequent episodes of the original disease and/or more frequent episodes of acute rejection may occur. Steroid withdrawal was possible in only 68% of a series of patients with autoimmune hepatitis; withdrawal was easier to achieve in patients without enterocolitis than in patients suffering from that condition; acute rejection occurred in 38% of patients during the weaning process, a higher figure than that seen in patients without autoimmune disease. In addition, steroid-resistant acute rejection seems to be more frequent in patients transplanted for autoimmune diseases or having received pre-transplant corticosteroid therapy.

In the future, cytokine gene polymorphisms may assist the selection of those patients requiring higher or lower immunosuppressive regimens. Preliminary data suggest that polymorphisms associated with low TNF-alpha and high IL-10 production may help to detect liver graft recipients in whom immunosuppression could be reduced or even stopped (Tambur, Mazariegos). A low level of IL-1Ra production is associated with steroid resistance of acute rejection and is due to a constitutional defect. The early identification of such patients may qualify them for stronger anti-rejection therapy.

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References


