Immunosuppression

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The aims of this chapter are to provide an overview of the processes involved in immunological rejection after liver transplantation, explain the pharmacotherapy required to treat and prevent graft rejection and discuss alternative immunosuppressive strategies

Immune Rejection

The liver has a lower incidence of rejection compared to other organs and does not require HLA matching of donor and recipient prior to transplantation. However a substantial number of recipients still develop graft rejection. Early acute rejection usually does not affect long-term graft survival and has conversely been associated with increased patient and graft survival. One study found that patients who had at least one episode of acute rejection had improved 4-year patient (82.8% vs. 75.9%) and graft survival (76.5% vs. 71.7%) [1].

There are three main types of organ rejection:

- *Hyperacute rejection*. This is rare and occurs within minutes to hours of restoration of the hepatic circulation during transplantation. It is characterized by endothelial injury and fibrin deposition resulting in intravascular thrombosis. There is no lymphocytic infiltration or bile duct injury. It results from pre-sensitization to donor antigens.
- *Acute cellular rejection*. Characterized by portal inflammation, bile duct damage and endothelitis [2] (Fig. 30.1).
- *Chronic rejection*. Characterized by ductopenia and obliterative vasculopathy affecting large and medium-sized arteries and the portal microcirculation (Fig. 30.2). It has an incidence of less than 4% and requires augmentation of immunosuppression [3]. Severe cases can require re-transplantation and impact upon long-term graft survival.

The incidence of acute liver rejection was 60% in the 1990s [4] and decreased to 15% since 2000 [5] due to the introduction of new drugs and better management of immunosuppression. Most cases occur within 90 days of surgery and respond to high-dose corticosteroids [6]. In both acute and chronic rejection there is T-cell-mediated damage of donor-derived bile ducts and vascular endothelium.

Steps in the development of acute cellular rejection include (Fig. 30.3):

1. *Allograft recognition*—foreign antigens are presented to lymphocytes by antigen presenting cells (dendritic cells, macrophages, B lymphocytes) in lymphoid organs, e.g. spleen, regional nodes. These are loaded onto the major histocompatibility complex (MHC) by

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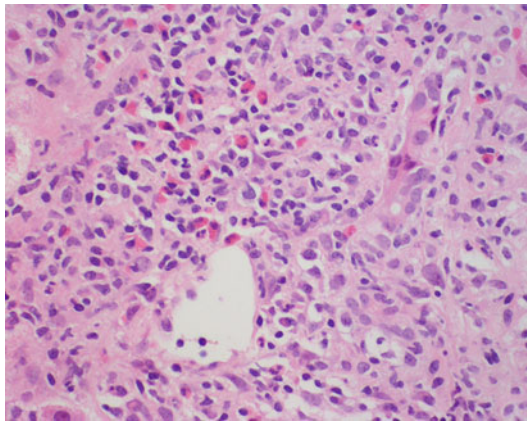

Fig. 30.1 Histological features of acute rejection. There is portal inflammation with cholangitis and endotheliitis

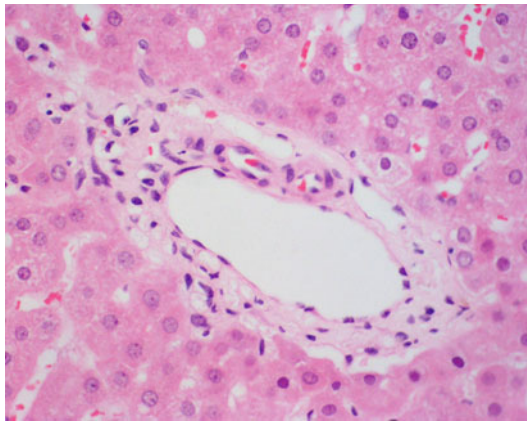

Fig. 30.2 Histological features of chronic rejection. Branches of portal vein and artery with bile ductopenia

the antigen presenting cell which are recognized by CD3 (and also CD4/CD8). The T-cell receptor on CD3 interacts with the MHC of the antigen presenting cell—this is stabilized by CD4/CD8 resulting in "SIGNAL 1" a calcium-dependent pathway.

2. *T-cell activation*—this is achieved by binding of co-stimulatory molecules (CD28, CD 40, PD1) on T-cells with ligands on the antigen presenting cell—"SIGNAL 2", a Ca^{2+}-independent process. Both signals are required for naïve T-cell activation and are mediated by calcineurin and Protein Kinase C activation of NF-AT, NF-KB and AP-1. These bind to gene promoters associated with T-cell activation and proliferation, i.e. promotes IL2 production which initiates G0 to G1 transition of the cell cycle [7]. Inhibition of this pathway has been the predominant site of action in immunosuppression therapies utilizing calcineurin inhibitors (CNIs) such as cyclosporin and tacrolimus.
3. *Clonal expansion*—"SIGNAL3": auto/paracrine activation of T-cells. Receptor of the IL2 family activate JAK 1/3 in T-cells [8]—which activates mammalian target of rapamycin (mTOR), STAT5, Ras-Raf MAP kinase [9] resulting in cell proliferation, DNA synthesis and cell division. Sirolimus and everolimus inhibit signal 3. Other molecules are produced which inhibit SIGNAL 2 (e.g. CD152) and decrease T-cell receptor signalling [10]. Azathioprine and mycophenolate mofetil (MMF) inhibit purine and DNA synthesis.
4. *Inflammation*—activated T-cells result in release of cytokines that recruit cytotoxic T-cells, B-cells, activated macrophages and adhesion molecules. Further activated T-cells are attracted by these leading to the release of TNF α/β perforin, granzymes. Corticosteroids and anti-lymphocyte antibody act via this route.

Immunosuppressive Agents

Immunosuppressive medication can be classified in several different ways: biologic vs. pharmacologic, induction therapy vs. maintenance therapy and by site or mechanism of action. Most regimens use a combination of drugs with different sites of action on the T-cell response pathway. This enables variable dosage and treatment adjustment according to response and adverse effects. The current mainstay of treatment involves the use of CNIs in combination with steroids. There is an increasing use of tailor-made protocols individualized to the patient and etiology to stratify risk of rejection and protect long-term graft function while minimizing adverse effects. For example, in cases with renal impairment, induction therapy with renal sparing agents are often given to enable a lower dose of nephrotoxic CNIs to be used in the early post-transplant

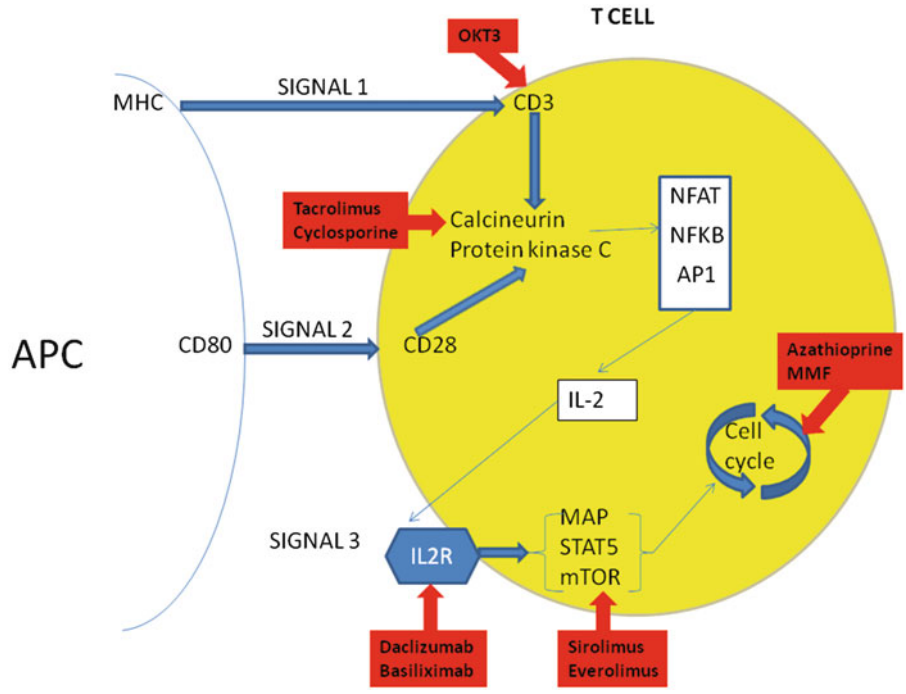

Fig. 30.3 Mechanisms of allograft rejection and of immunosuppressive drugs

phase. See Table 30.1 for an overview of currently used immunosuppressive agents and their adverse effects.

Calcineurin Inhibitors: Cyclosporine and Tacrolimus

Cyclosporine was the first CNI to be routinely used for post-transplantation. It was derived from the fungus *Tolypocladium inflatum* in 1972 and was evaluated for use as an immunosuppressive agent in 1976 [11]. Its use has now often been superceded by Tacrolimus (FK506) which is approximately 100 times more potent on a molar level [12]. Tacrolimus is a macrolide antibiotic similar to erythromycin that was derived from the fungus *Streptomyces tsukubaensis* in 1984 [13].

Method of Action

Cyclosporine binds to cyclophilin which causes inhibition of calcineurin, a calcium/

Table 30.1 Side effects of the most commonly used immunosuppressive drugs

Drug	Common adverse effects
Tacrolimus	Nephrotoxicity, neurotoxicity, hypertension, diabetes, metabolic acidosis
Cyclosporine	Nephrotoxicity, neurotoxicity, hypertension, diabetes, metabolic acidosis, hyperlipidemia, gingival hyperplasia, hypertrichosis
Corticosteroids	Hypertension, diabetes, osteoporosis, obesity, cataracts, poor wound healing
Mycophenolate mofetil	Myelosuppression, diarrhea, viral infections
Sirolimus	Poor wound healing, hyperlipidemia, myelosuppression, pneumonitis, rash

Table 30.2 Drugs that increase and decrease CNI and sirolimus levels

Increase levels	Decrease levels
Calcium antagonists	Anticonvulsants
Verapamil, nifedipine, diltiazem	Phenytoin, carbamazepine, phenobarbital
Antifungals	Antibiotics
Fluconazole, itraconazole, ketoconazole, voriconazole, clotrimazole	Rifampicin, rifabutin
Macrolides	St. John's wort
Azithromycin, erythromycin, clarithromycin	
Protease inhibitors	
E.g. ritonavir, darunavir, saquinavir	
Metoclopramide	
Amiodarone	

calmodulin-dependent phosphatase. This prevents the dephosphorylation of activated T-cells which inhibits their nuclear entry and thus upregulation of pro-inflammatory cytokines including IL-2 (Signal 2 pathway) [14].

Tacrolimus inhibits calcineurin by binding to FK-binding protein-12. This in turn binds to a separate site to cyclosporine/cyclophilin on calcineurin resulting in a similar inhibitory pathway for IL-2 production. These two drugs cannot be used simultaneously as they compete with other for immunosuppressive action.

Pharmacokinetics and Metabolism

The original formulation of cyclosporine was as Sandimmune, a corn oil-based agent with a highly variable absorption and only an average of 30% bioavailability. Absorption was dependent on the presence of bile salt availability. The use of T-tubes which interrupted enterohepatic circulation after transplantation necessitated intravenous administration. A microemulsion form, Neoral, was subsequently developed and adopted into regular practice. This formulation is less dependent on bile acids for absorption resulting in improved overall bioavailability. Distribution is concentration dependent and is predominantly in adipose, adrenal, hepatic, pancreatic and renal tissue. In blood it is primarily bound to lipoproteins in plasma. The half-life is 18 h and it is mainly excreted into bile [15].

Tacrolimus is well absorbed from the gastrointestinal tract with a bioavailability in liver transplant patients of approximately 22%. The rate of absorption is best under fasting conditions. It is 95% bound to erythrocytes, with 99% of the remaining 5% bound to plasma proteins. Less than 0.1% is unbound, and it is this fraction that exerts the pharmacological activity [16]. The half-life varies from 31 to 48 h.

CNIs are metabolized by the cytochrome P450 3A4 (CYP3A4) enzyme in the gastrointestinal epithelium (approximately 50%) and the liver where first pass hepatic metabolism accounts for a further 10%. The metabolites have minimal immunosuppressive effects. Drugs that interact with CYP3A4 will affect the concentration of CNIs (Table 30.2).

Adverse Effects

Major long-term adverse effects are related to the kidneys. CNIs cause a reduction in renal blood flow and GFR by vasoconstriction of the afferent renal arteriole [17]. Longitudinal studies of liver transplant patients with chronic renal insufficiency demonstrate that CNI toxicity is the most common clinical and histologic diagnosis in patients who progress to end stage renal failure [18]. Both cumulative dose and duration of CNI exposure are related to the degree of renal damage [19]. These changes are reversible in the short term. Nearly 20% of liver transplant recipients go on to develop renal failure within 5 years [20]. This is a major clinical issue in post-transplant care and the concern about renal toxicity has led to CNI sparing regimes in patients with pre-existing renal dysfunction.

Hypertension is commonly seen, often due to the renal changes [21] and amlodipine is the drug of choice used to treat CNI-induced hypertension. Neurotoxicity is potentiated by low magnesium levels and often improves with magnesium supplementation [22]. Tremor, headache and

insomnia are the other adverse effects. Less common are convulsions, confusion, psychosis and reduced consciousness.

Metabolic effects: Diabetes, hyperlipidemia, hyperkalemia and metabolic acidosis are frequently observed. Gingival hyperplasia and hypertrichosis are specific to cyclosporine [23].

Clinical Use

Tacrolimus (Prograf™) has mostly superceded cyclosporine as the first-line drug in liver transplantation. Several studies have demonstrated a lower incidence of acute cellular rejection with tacrolimus compared to cyclosporine with similar patient and graft survival, and tacrolimus is usually the first choice CNI in de novo transplants [24–26].

In the immediate post-operative period tacrolimus can be administered orally or via an oro- or nasogastric tube if the patients remains intubated, usually at a starting dose of 1–2 mg twice daily. It is given in combination with intravenous steroid. Levels are checked and the dose is adjusted accordingly.

Therapeutic Drug Monitoring

The immunosuppressive effects of CNIs are related to the total drug exposure that is represented by the area under the drug-concentration-time curve (AUC). Both drugs have a narrow therapeutic window. For tacrolimus, the 12-h trough concentration is a good estimation of the AUC: and blood samples taken 10–14 h after dosage are predictive of exposure [27]. There is no clear consensus as to the optimal dosing regimen in transplantation. One recommendation is to aim for trough concentrations of 10–20 ng/mL in the first post-transplant month provided good graft function and the absence of toxicity; 5–15 ng/mL in the next 2 months; and then 5–10 ng/mL [28]. Levels are adjusted according to renal function and the presence or absence of rejection.

A new once daily formulation of tacrolimus (Advagraf™) has recently been introduced. Once-daily dosing may improve compliance while allowing the same total daily dose and monitoring strategies [29]. Other generic formulations of tacrolimus will become available.

Corticosteroids

Corticosteroids are the most frequently used non-CNI drug immunosuppressants in liver transplantation and pulse dose methylprednisolone remains the first-line treatment for acute cellular rejection. Corticosteroids were initially used in high doses in the early era of transplantation and resulted in inevitable high morbidity. The current practice is based upon their use as induction therapy with early dose reduction over 6–8 weeks and possible withdrawal due to the myriad adverse effects.

Method of Action

Corticosteroids have a wide variety of immunomodulatory and anti-inflammatory actions. They bind to glucocorticoid receptors resulting in inhibition of gene transcription of pro-inflammatory cytokines including IL-2, IL-6, TNF-α and IFN-γ. These cytokines are required for the macrophage and lymphocyte response to allograft antigens. In addition, there is direct suppression of complement and antibody binding, stabilization of lysosomal enzymes, suppression of prostaglandin synthesis and reduction of histamine and bradykinin release.

Adverse Effects

These are well known and summarized in Table 30.1.

Clinical Use

Typical regimens use methylprednisolone 10–50 mg intravenously in the immediate post-operative period after a bolus of 500 mg

methylprednisolone in the operating room. Methylprednisolone is continued until enteral administration is possible and the dose is then converted to prednisolone 20 mg. The aim is to taper the dose gradually depending on the overall response to immunosuppression and etiology of the underlying liver disease. Withdrawal within 3–6 months in those with no evidence of rejection or autoimmune disease is often successful [30]. High-dose pulsed steroids are used to treat acute cellular rejection. Typically hydrocortisone 100 mg daily for 3 days or methylprednisolone 500 mg daily for 2 days is administered in conjunction with an increased dose of tacrolimus.

Antimetabolites: Azathioprine and Mycophenolate Mofetil

Antimetabolites were not initially used in liver transplantation. and they were used as part of strategies to reduce the frequency of CNI related renal failure and to treat refractory rejection.

Azathioprine is the pro-drug form of 6-mercaptopurine that is then converted to 6-thioguanine, 6-methyl-MP and 6-thiouric acid. These active compounds interfere with DNA replication. Thiopurine methyltransferase (TPMT) is the enzyme required in the conversion of azathioprine to 6-MP. Polymorphisms of TPMT exist which cause decreased activity and allow toxic level of azathioprine to build up resulting in acute myelosuppression [31]. It is therefore essential to check TPMT activity prior to commencing therapy. Further metabolism is via xanthine oxidase and therefore it must not be used with allopurinol, a xanthine oxidase inhibitor, as toxicity will be potentiated.

Usage in liver transplantation has been limited due to adverse effects including liver toxicity, cholestatic jaundice, hepatic veno-occlusive disease, hypersensitivity, pancreatitis and bone marrow suppression, particularly in patients with portal hypertension. It is currently used primarily as adjunctive therapy.

MMF is derived from Penicillium and was first discovered in 1893 [32]; however, its evaluation as an immunosuppressant was not until the 1990s [33]. Two forms are available: MMF (CellCept, Roche) and enteric coated mycophenolate sodium (Myfortic, Novartis).

Method of Action

The active compound is mycophenolate acid (MPA). MPA inhibits the action of inosine monophosphate dehydrogenase (IMDPH), the rate limiting enzyme in the synthesis of guanosine nucleotides which are essential for DNA synthesis. Most cell types have a second pathway for nucleotide synthesis; however, lymphocytes do not possess such activity. There are also two isoforms of the IMDPH enzyme. The second isoform is more prominent in lymphocytes, and has preferential selectivity for MMF [34].

Pharmacokinetics and Metabolism

MMF is well absorbed from the gastrointestinal tract and undergoes immediate hepatic first-pass metabolism to MPA. The half-life is approximately 18 h with bioavailability estimated at 90%. Food decreases MPA concentration so MMF should be administered at least 1 h before or 2 h after eating. MPA is 97% protein bound, with free MPA as the active fraction. MPA is further metabolized by the liver to mycophenolic acid glucuronide which has 93% urinary elimination. Liver disease impairs MPA conjugation, thus increasing its half-life. MPAG is also excreted into bile. Further hydrolysis back to MPA by gut organisms leads to enterohepatic recirculation of MPA and a second peak concentration 6–12 h post-ingestion [35].

Adverse Effects

The most common dose related adverse effect is diarrhea. Other gastrointestinal adverse effects include nausea, vomiting and abdominal pain [36]. Bone marrow suppression can also occur. If these adverse effect do not improve with dose reduction, MMF should be stopped. There is also an increased incidence of viral and fungal

infections including CMV, HSV and candida with the use of MMF. Its use is not recommended in pregnancy due to the increased risk of congenital malformation and spontaneous abortion.

Clinical Use

Predominant use is as a CNI-sparing agent as MMF is not nephrotoxic. It is more frequently used in patients requiring additional long-term immunosuppression, e.g. following documented previous rejection [37]. MMF has replaced azathioprine as it is associated with a lower incidence of biopsy proven rejection in combination with CNI [38]. There is no role of MMF as monotherapy due to the high incidence of ACR, steroid-resistant rejection and chronic rejection requiring re-transplantation [39].

Therapeutic Drug Monitoring

The data to support monitoring is of limited quality as drug levels and effects are affected by a variety of factors including serum protein levels, other immunosuppressive agents and renal function leading to significant inter-patient variability [40].

mTOR Inhibitors: Sirolimus and Everolimus

The two mTOR inhibitors licenced for use in transplantation are sirolimus and everolimus. Sirolimus was discovered in soil samples from Easter Island (Rapa Nui) in 1964 and initially developed as an anti-fungal [41]. It is structurally similar to tacrolimus and is a naturally occurring product of *Streptomyces hygroscopicus*. Everolimus is a chemically modified form of sirolimus to improve absorption.

Method of Action

Sirolimus and everolimus bind to the FK-binding protein-12 but do not inhibit calcineurin. Instead they inhibit mTOR that is required for mRNA translation necessary for cell cycle progression, (which is halted in the G1 phase), IL-2 production and cellular proliferation. T-cell activation occurs, but IL-2-induced proliferation does not occur.

Pharmacokinetics and Metabolism

Sirolimus is a highly lipophilic compound that is readily absorbed when in oily solution or microemulsion (bioavailability 14–18%). It has a half-life of 62 h and reaches steady state in 5–7 days. The long half-life necessitates regular drug monitoring. It is extensively bound to plasma proteins and metabolized by CYP3A4 (see Table 30.1) in the intestine and liver. Most of the metabolites are excreted in feces via a P-glycoprotein pump.

Adverse Effects

Hyperlipidemia, thrombocytopenia, anemia and leucopenia are commonly seen. Rarer adverse effects include aphthous ulceration, acne, arthralgia and interstitial pneumonitis (resolves on withdrawal) [42]. Specifically in liver transplantation, an increased incidence of hepatic artery thrombosis and wound dehiscence in the first month post-transplant has been reported [43].

Clinical Use

Studies of mTOR inhibitors as monotherapy have demonstrated the possibility of an increased risk of hepatic artery thrombosis and poor wound healing. There is also a higher incidence of rejection. Current practice is for introduction as combination therapy with tacrolimus in patients requiring broader immunosuppression or as a replacement monotherapy for patients intolerant of CNIs. In particular, early introduction of sirolimus may be most beneficial to prevent progression of renal complications of CNI.

Sirolimus has a potential anti-tumour effect: patients transplanted with HCC have been found to have a prolonged survival with sirolimus compared to CNI [44] but further confirmatory studies are required.

Therapeutic Drug Monitoring

Sirolimus levels are estimated by either immunoassay or chromatography. It is essential that the same method is consistently used. Trough levels <6 ng/mL are associated with an increased incidence of rejection; levels >15 ng/mL have an increased risk of hyperlipidemia and thrombocytopenia [45]. Trough levels obtained 5–7 days after dose adjustment are sufficient due to the long half-life of sirolimus.

Antibody-Based Therapies

These are generally utilized as induction of immunosuppression or as salvage for steroid refractory rejection.

Polyclonal Antibodies: Anti-thymocyte and Anti-lymphocyte Globulin

These agents are prepared by inoculation of rabbits with human lymphocytes or thymocytes. A purified gamma globulin fraction of antisera is used to prevent serum sickness. They were first used in the early era of transplantation with steroids and azathioprine prior to the introduction of CNI. Their action is on multiple T-cell antigens, B-cell antigens, HLA class 1 and 2, macrophages and NK cells causing lymphocyte depletion [46].

Adverse effects include fever, hypotension, headache, aseptic meningitis, ARDS, pulmonary edema and graft thrombosis. Steroids, antihistamines and acetaminophen are given as pretreatment to counteract these adverse effects. Polyclonal antibodies are currently used as an induction agent, a steroid-sparing agent or as the treatment of steroid-resistant rejection.

Monoclonal Antibodies

Anti IL-2 (*CD 25*) *receptor antibodies* such as daclizumab or basiliximab are used as induction therapy to prevent rejection, especially in cases with renal dysfunction peri-transplantation as they allow lower or later start of nephrotoxic CNI [47]. Various protocols are in use. Typically the anti IL-2 (CD 25) receptor antibodies are administered on the first post-operative day and then 4–7 days post-transplant and they remain in circulation for several weeks. There are few adverse effects and they are generally very well tolerated.

OKT3 (*muromonab-CD3*): binds to the CD3 receptor on mature T-cells, preventing signal 1 activation and depletion of lymphocytes by T-cell lysis and cytokine release [48]. Adverse effects are similar to ATG, but OKT3 is less well tolerated with a higher incidence of post-transplant lymphoproliferative disease (PTLD). Administration is by intravenous infusion and onset of action is within minutes, lasting 1 week. It is commonly used to treat steroid-resistant acute rejection and requires premedication antibodies with steroids, antihistamines and acetaminophen similar to polyclonal antibodies.

Campath (*Alemtuzumab*) is a humanized anti-CD52 monoclonal antibody that causes lymphocyte depletion from the circulation and peripheral nodes. Its role in immunosuppressive regimens is not yet identified, but it can be used as induction therapy to facilitate lower doses of CNI and in conjunction with sirolimus.

Special Situations

As individualized therapy becomes more common, immunosuppression for patients with hepatitis C infection and with renal failure are of particular relevance.

Hepatitis C: this is now the single most common reason for transplantation in industrialized countries. Re-infection of the graft is almost universal [49] and occurs in the immediate post-transplant period [50]. High-dose steroid therapy for acute rejection causes an increase in viremia

and more rapid progression of disease recurrence [51]. Strategies used include early steroid withdrawal and the combination of induction therapy with IL-2 blockade [52]. Some in vitro studies suggest that cyclosporine instead of tacrolimus has an inhibitory effect on replication [53] but the concentrations used in these replication studies were greater than 1,000 times of physiological concentration. Novel cyclophilin inhibitors (e.g. Debio 025) have demonstrated anti-HCV activity and are undergoing clinical trials as the treatment for HCV [54]. Therefore there may be a role for either cyclophilin inhibitors or cyclosporine in the post-transplant HCV. Furthermore cyclosporine is less diabetogenic than tacrolimus and diabetes is considered a risk factor for fibrosis progression post-transplant for HCV [55].

Renal failure: Renal dysfunction and acute kidney injury after liver transplantation is common and has important implications for subsequent patient morbidity and survival. Ten to 60% of LT recipients develop post-operative acute kidney injury and 10–25% require post-operative renal replacement therapy [56]. The need for post-operative renal replacement is associated with a two to six-fold increased risk of 1-year mortality [57]. Longitudinal studies of liver transplant patients with chronic renal insufficiency demonstrate that CNI toxicity is clinically and histologically the most common cause in patients who progress to end stage renal disease [58]. A number of strategies have been employed to minimize the dose of CNI in the immediate post-transplant period in patients at risk of developing renal injury, principally those with pre-existing renal dysfunction. Minimizing early acute CNI-induced renal injury will reduce the incidence of acute and chronic renal disease later after transplant. Induction of immunosuppression with IL-2 receptor blockers or ATG and delayed or reduced dose start of CNI is commonly part of renal-protective protocols. Some centre will also convert CNI to mTOR inhibitors in patient with acute kidney injury.

A wide range of different immunosuppressive agents are now available with varying degrees of potency and toxicity. Newer agents are in development that will enable more tailored regimens depending on the etiology of the underlying liver disease and to prevent renal toxicity. In an era of organ shortage that results in sicker patients with significant co-morbidities and the use of marginal, extended criteria grafts individualized immunosuppressive protocols are of increasing importance. The long-term aims are to develop agents and protocols that immunological tolerance and potentially immunosuppression withdrawal.

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