Immunosuppression

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Introduction

The aims of this chapter are to provide an overview of the processes involved in immunological rejection after liver transplantation, outline immunosuppression strategies post liver transplantation, review the pharmacology of immunosuppressive agents and provide an overview for treatment of liver graft rejection.

Immunological Rejection

Liver transplantation 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. Table 32.1 is a summary of patterns of rejection seen in liver transplant recipients.

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Acute cellular rejection (ACR) is graft dysfunction due to inflammation affecting interlobular bile ducts and vascular endothelia (Fig. 32.1) and, grading of severity is per the Banff Schema [1]. ACR does not usually affect long-term graft survival, except in hepatitis C infected recipients, 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%) [2]. Ongoing hepatitis C infection post liver transplantation is associated with approximately 10% risk of graft loss. Rejection episodes requiring bolus corticosteroid therapy however are associated with worse outcomes in patients with hepatitis C recurrence [3].

The incidence of acute cellular rejection was 60% in the 1990s [4]. Since 2000 it has improved to 15% due to the introduction of new therapeutic options and improved management of immunosuppression [5]. Most cases occur within 90 days of surgery and respond to high dose corticosteroids [6].

Chronic cellular rejection is mediated by both immunological and non-immunological factors. The greatest risk factor for chronic cellular rejection is frequency and severity of acute cellular rejection. Age of donor and quality of liver graft are non-immunological risk factors for chronic rejection.

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Table 32.1 Patterns of liver transplant rejection

Type	Timing	Incidence	Pathogenesis	Histology	Treatment	Outcome
Hyperacute	Minutes–hours	Rare	Pre-sensitization to donor antigen	Endothelial injury, fibrin deposition	Urgent retransplantation	
Acute cellular rejection	0–90 days	15–25%	Inflammation induced by T cell activation by antigen presenting cell	Portal inflammation, bile duct inflammation, venous endothelial inflammation. Fig. 32.1	High-dose corticosteroids ATG Alemtuzumab	No adverse effects on graft or patient outcomes, except in HCV, who have worse outcomes
Chronic cellular rejection	>90 days	<4%	Inadequate immunosuppression Donor age	Ductopaenia and obliterative vasculopathy affecting large and medium sized arteries and portal microcirculation Fig. 32.2 [7]	Augmentation of immunosuppression Re-transplantation in severe cases	Reduced graft survival
Acute antibody mediated rejection	0–90 days	2–6%, 10% in patients with idiopathic graft loss	Activation of complement pathway by donor specific antigens	Microvasculitis with diffuse portal and sinusoidal C4d staining Fig. 32.3	Multimodal including plasmapheresis and B-cell targeting agents	Limited data, can be associated with chronic rejection and graft failure
Chronic antibody mediated rejection	>90 days	Unknown	Yet to be defined	Yet to be defined	Unknown	Unknown

The steps involved in the development of acute cellular rejection are as follows (Fig. 32.2):

- *Allograft recognition*—Foreign antigens are presented to lymphocytes by antigen presenting cells (APC) (dendritic cells, macrophages, B lymphocytes) in lymphoid organs, e.g. spleen, regional lymph nodes. These are loaded onto the major histocompatibility complex (MHC) by the APC that are recognized by CD3 (as well as CD4/CD8). The T-cell receptor on CD3 interacts with the MHC of the APC, this is stabilized by CD4/CD8, resulting in "SIGNAL 1", a calcium-dependent pathway.
- *T-cell activation*—This is achieved by binding of co-stimulatory molecules (CD-28, 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 nuclear factor of activated T cells (NF-AT), nuclear factor kB (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 [8]. Inhibition of this pathway has been the predominant site of action in immunosuppression therapies utilizing calci-

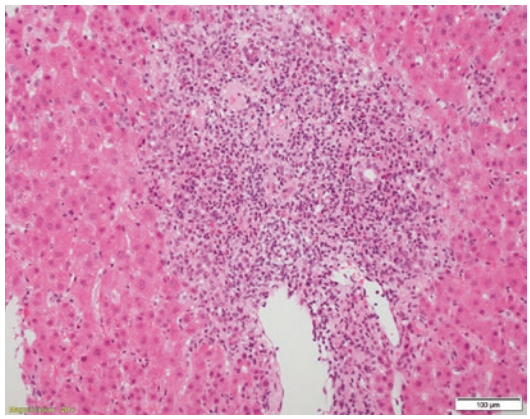

Fig. 32.1 H&E section shows a portal tract completely occupied by a florid mixed inflammatory cell infiltrate, including eosinophils, and involving bile ducts. Endotheliitis is present in the lower half of the field. H&E x 20. (Courtesy of Dr Alberto Quaglia, Institute of Liver Studies, King's College Hospital)

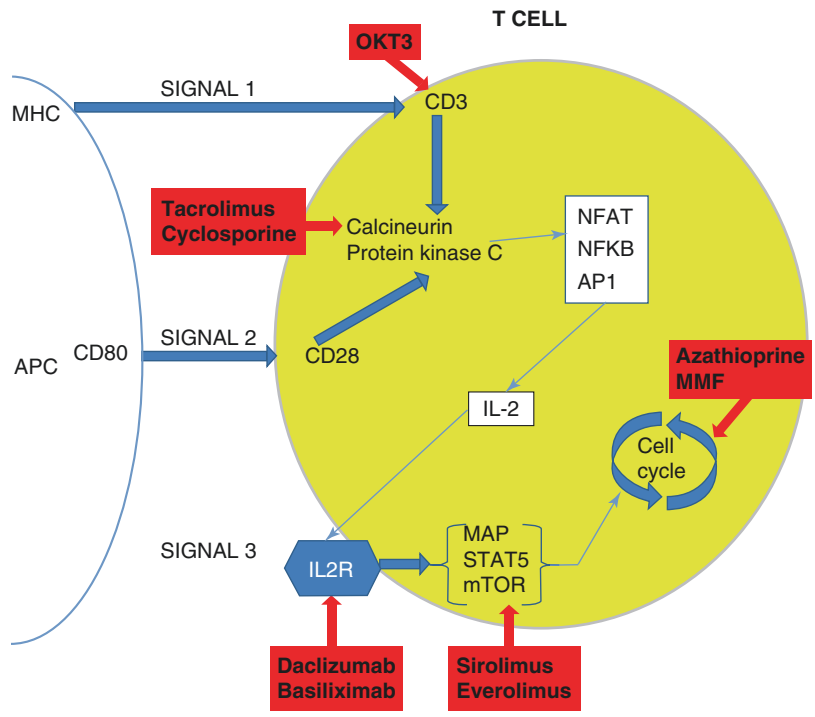

Fig. 32.2 Mechanism of allograft rejection and of immunosuppressive drugs. *APC* antigen presenting cell, *MHC* major histocompatibility complex, *IL2R* interleukin-20 receptor, *IL-2* interleukin 2, *NFAT* nuclear factor of activated T-cells, *NFKB* nuclear factor kappa-light-chain-enhancer of activated B cells, *AP1* activator protein 1, *mTOR* mammalian target of rapamycin, *MAP* mitogen-activated protein, *STAT5* signal transducer and activator of transcription 5, *OKT* orthoclone, *MMF* mycophenylate

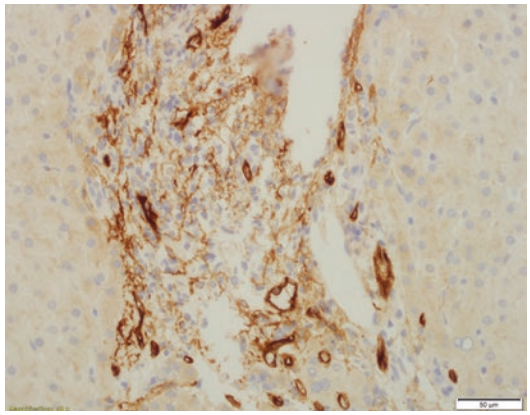

Fig. 32.3 Immunohistochemistry for C4d show stromal and vascular endothelial stain in a portal tract. (Courtesy of Dr Lara Neves-Souza, Institute of Liver Studies, King's College Hospital)

neurin inhibitors (CNIs) such as tacrolimus and cyclosporine.

- *Clonal expansion*—"SIGNAL3": auto/paracrine activation of T-cells. Receptor of the IL2 family activates JAK 1/3 in T-cells [9] which activates mammalian target of rapamycin (mTOR), STAT5 and Ras-Raf MAP kinase resulting in cell proliferation, DNA synthesis and cell division. Sirolimus and everolimus inhibit SIGNAL 3. Other molecules are produced that inhibit SIGNAL 2 (e.g. CD152) and decrease T-cell receptor signaling [10]. Azathioprine and mycophenolate mofetil inhibit purine and DNA synthesis.
- *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 release of TNF α/β perforin, granzymes. Corticosteroids and antilymphocyte antibody act via inhibition of this route.

Antibody mediated rejection (AMR) is due to de-novo or pre-existing anti-HLA donor-specific antibodies (DSA) and is seen in acute rejection with reported association with chronic rejection. The liver has been considered to be resistant to AMR due to large organ size and dual blood supply (arterial and portal) [11]. Anti-HLA class I DSA form complexes with soluble class I-MHC antigens released by the donor liver and are removed from circulation from liver Kupffer cells [11], providing further protection against AMR. Additionally, AMR can occur in the presence of acute cellular rejection. AMR is suspected in setting of unexplained graft dysfunction with thrombocytopenia, hypocomplementemia and microvasculitis with diffuse C4d staining on liver biopsy (Fig. 32.3) [12]. Whilst AMR is well recognized after renal and cardiac transplantation, its role in graft rejection post liver transplantation has only been recognized recently. The revised Banff criteria for AMR diagnosis requires histopathological pattern of injury consistent with AMR, positive serum DSA, diffuse microvascular C4d deposition and exclusion of other causes that may cause graft dysfunction [13].

Steps in the development of antibody mediated rejection [14] [15].

- Donor specific antigens can be present at time of transplantation or develop post-transplantation (*de novo* DSA). These antigens bind to HLA on graft endothelium. DSA can be directly measured in the serum.
- Classical complement pathway activation occurs when plasma C1q attaches to Fc segment on DSA
- A series of enzymatic reactions involving degradation of C2 and C4 follows. One of the byproducts of C4 degradation, C4d, is deposited on the allograft and can be detected through immunohistochemistry staining of liver biopsy specimen.
- Degradation products of C2 and C4 ultimately leads to formation of C3b which then activates C5 and allows formation of membrane attack complexes. The end result is endothelial damage and inflammation.

Immunosuppressive Agents

Most immunosuppressive regimens use a combination of drugs with different sites of action in the T-cell response pathway. This enables variable dos-

Table 32.2 Side effects of common immunosuppression medication

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	Hepatic artery thrombus, poor wound healing, hyperlipidemia, myelosuppression, pneumonitis, rash

age and treatment adjustment according to response and adverse effects. The current mainstay of treatment involves the use of calcineurin inhibitors (CNI) in combination with steroids. There is an increasing use of tailored protocols individualized to the patient and etiology to stratify risk of rejection and protect long-term graft function while minimizing adverse effects. See Table 32.2 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 post transplantation. It was derived from the fungus Tolyplocladium inflatum in 1972 and was evaluated for use as an immunosuppressive agent in 1976 [16]. Its use has now often been superceded by Tacrolimus (FK506) which is approximately 100 times more potent on a molar level [17]. Tacrolimus is a macrolide antibiotic similar to erythromycin that was derived from the fungus Streptomyces tsukubaensis in 1984 [18].

Method of Action

Cyclosporine binds to cyclophilin that causes inhibition of calcineurin, a calcium/calmodulin-dependent phosphatase. This prevents the dephosphorylation of activated T-cells that inhibits their nuclear entry and thus upregulation of proinflammatory cytokines including IL-2 (Signal 2 pathway) [19].

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. It is a corn oil based agent with a highly variable absorption and an average bioavailability of 10%. Absorption was dependent on the presence of bile salt availability and the use of T-tubes that 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. The half-life is 18 h and it is mainly excreted into bile [20].

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 [21]. 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 32.3).

Table 32.3 Drugs that increase and decrease CNI and sirolimus levels

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

Adverse Effects

Major long-term adverse effects are related to nephrotoxicity. CNIs cause a reduction in renal blood flow and GFR by vasoconstriction of the afferent renal arteriole [22]. 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 after transplant [23]. Both cumulative dose and duration of CNI exposure are related to the degree of renal injury. These changes are reversible in the short term however nearly 20% of liver transplant recipients go on to develop renal failure within 5 years [24]. 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 [25] 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 [26]. Tremor, headache and insomnia are the other adverse effects. Less common are convulsions, confusion, psychosis and reduced consciousness.

Metabolic effects: Diabetes, hyperlipidaemia, hyperkalaemia and metabolic acidosis are frequently observed. Gingival hyperplasia and hypertrichosis are specific to cyclosporine [27].

Clinical Use

Tacrolimus (Prograf™) has mostly superseded 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 [28–30].

In the immediate post-operative period tacrolimus can be administered orally or via an oro- or nasogastric tube if the patient 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 [31]. There is no clear consensus as to the optimal dosing regimen in transplantation. In the past levels as high as 10–20 ng/mL in the first post transplant month have been recommended. However, there is now increasing evidence for lower tacrolimus levels post liver transplantation. Trough concentration between 6 and 10 ng/mL in the first 4–6 weeks post transplantation with a reduction to 4–8 ng/mL long term has been recommended [32]. 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 [33].

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 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 32.2**.**

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. 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 [34]. 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 (MMF)

Antimetabolites were not initially used in liver transplantation. 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 (6-MP) 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 for the conversion of azathioprine to 6-MP. Polymorphisms of TPMT exist that cause decreased activity and allow toxic level of azathioprine to build up resulting in acute myelosuppression [35]. 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.

Use 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.

Mycophenolate Mofetil is derived from Penicillium and was first discovered in 1893 however its evaluation as an immunosuppressant was not until the 1990s [36]. 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 [37].

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 (MPAG) that 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 [38].

Adverse Effects

The most common dose related adverse effect is diarrhea. Other gastrointestinal adverse effects include nausea, vomiting and abdominal pain [39]. 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 cytomegaly virus (CMV), herpes simplex virus (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 [40]. MMF has replaced azathioprine as it is associated with a lower incidence of biopsy proven rejection when combined with CNI [41]. There is no role of MMF as monotherapy due to the high incidence of ACR, steroid resistant rejection and chronic rejection requiring re-transplantation [42].

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 [43].

mTOR Inhibitors: Sirolimus and Everolimus

The two mammalian target of rapamycin (mTOR) inhibitors licensed 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 [44]. 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 (Table 32.3) in the intestine and liver. Most of the metabolites are excreted in feces via a P-glycoprotein pump.

Adverse Effects

Hyperlipidaemia, thrombocytopenia, anemia and leucopenia are commonly seen. Less frequent adverse effects include aphthous ulceration, acne, arthralgia and intersititial pneumonitis (that resolves on withdrawal) [45]. Specifically in liver transplantation, an increased incidence of hepatic artery thrombosis and wound dehiscence in the first month post transplant has been reported [46].

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-tumor effect: patients transplanted with HCC have been found to have a prolonged survival with sirolimus compared to CNI [47] but further confirmatory studies are required.

Therapeutic Drug Monitoring

Sirolimus levels are measured 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 [48]. 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 anti-sera 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. Polyclonal antibodies are currently used as an induction agent, a steroid-sparing agent or as treatment of steroid-resistant rejection. Their action is on multiple T-cell antigens, B-cell antigens, HLA class 1 and 2, macrophages and NK cells causing lymphocyte depletion [49].

Adverse effects include fever, hypotension, headache, aseptic meningitis, ARDS, pulmonary

edema, graft thrombosis. Steroids, antihistamines and acetaminophen are given as pretreatment to counteract these adverse effects.

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 [50]. Various protocols are in use. Typically, 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 [51]. 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.

Approach to Immunosuppression Post Liver Transplantation

Each liver transplant center will have their own established protocols on immunosuppression regimes. Below is an overview of a broad approach according to timing post liver transplantation, special situations and treatment of graft rejection.

Induction

High dose intravenous steroids are administered during transplantation and continued for 2–3 days until the patient is able to tolerate oral intake. Induction immunosuppression post liver transplantation consists of intravenous methylprednisolone at doses of 500–1000 mg followed by a taper according to local practice. Once the patient is able to tolerate oral intake, methylprednisolone is ceased and prednisolone is commenced, usually at a dose of approximately 20 mg daily.

T-cell depleting (anti-thymocyte globulin (ATG), alemtuzumab) and non-depleting (basiliximab, daclizumab) antibodies can be used as an induction agent, either in conjunction or as an alternative to steroids. These strategies are not common and are used in select cases under the guidance of an experienced transplant physicians.

Maintenance

Maintenance immunosuppression with a calcineurin inhibitor (CNI) (tacrolimus or cyclosporine) is commenced within the first 24–48 h post-transplantation. In the absence of acute cellular rejection in the immediate post-transplant period, prednisolone is weaned and often ceased 3 months post transplantation. Patients are then frequently maintained on a CNI as sole immunosuppression agent. Tacrolimus is the preferred CNI over cyclosporine due to reduced incidence of graft loss, acute cellular rejection and steroid resistant rejection [52]. In the event of significant CNI toxicity (Table 32.2), a strategy involving reduction in the dose of CNI and commencement of an anti-metabolite agent (mycophenolate or azathioprine) or mTOR inhibitor (sirolimus or everolimus) is adopted.

Special Situations

Hepatitis C

In industrialized countries hepatitis C is now the single most common reason for liver transplantation. Re-infection of the graft is almost universal

[53] and occurs in the immediate post-transplant period. High dose steroid therapy for acute rejection causes an increase in viremia and more rapid progression of disease recurrence [54]. Ten to thirty percent develop cirrhosis at 5 years post transplantation [55]. Strategies used include early steroid withdrawal and the combination of induction therapy with IL-2 blockade [56]. Some in-vitro studies suggest that cyclosporine instead of tacrolimus has an inhibitory effect on replication but the concentrations used in these replication studies were greater than 1000 times of physiological concentration [57]. Furthermore cyclosporine is less diabetogenic than tacrolimus and diabetes is considered a risk factor for fibrosis progression post transplant for hepatitis C [58]. Treatment of hepatitis C either pre or post-transplant setting is now occurring with new oral-only direct acting agents (so called DAA agents). These regimes have the advantage of very high cure rates with few side effects. Some DAA regimes have minimum effect on immunosuppression levels and reinfection due to high levels of immunosuppression may be less concerning if hepatitis C is promptly treated.

Autoimmune Hepatitis

Incidence of recurrence of autoimmune hepatitis in the new graft is approximately 25% [59, 60]. A maintenance immunosuppression regime that includes prednisolone may reduce the risk of recurrence [61] and therefore prednisolone is often continued in addition to CNIs.

Renal Dysfunction

Renal dysfunction and acute kidney injury after liver transplantation is common and has important implications for subsequent patient morbidity and survival. Ten to sixty percent of liver transplant recipients develop postoperative acute kidney injury and 10–25% require postoperative renal replacement therapy [62]. The need for postoperative renal replacement is associated with a two to sixfold increased risk of 1-year mortality [63]. 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 [23]. 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 immunosuppression with IL-2 receptor blockers or ATG, and delayed or reduced dose start of CNI is commonly part of renal protective protocols. Some centers adopt the approach of converting from CNI to mTOR inhibitors in patient with acute kidney injury.

Hepatocellular Carcinoma

Liver transplantation is a curative management option for patients with hepatocellular carcinoma (HCC) and is an indicator for transplantation. Model for end stage liver disease score (MELD) of 15 is one of the minimum criteria required for listing for liver transplantation and exception points are allocated to those with HCC. Tumor recurrence post liver transplantation is approximately 10–20% and the type of immunosuppressive agent used is one of many factors which influences recurrence. High serum levels of CNI, prolonged steroid use, anti-lymphocytic antibody and ATG has been associated with increased risk of HCC recurrence. mTOR inhibitors, which have anti-neoplastic effects, may improve recurrence rates when compared with non-mTOR inhibitor regimes [64–66]. A randomized control study to address this very question is currently underway [67].

Graft Rejection

Once acute cellular rejection has been diagnosed treatment is instituted with high dose intravenous prednisolone (usually methylprednisolone at

500–1000 mg per day) and continued for 3 days. Patients are then switched to high oral steroids. Approximately 80% of ACR will respond to high dose prednisolone [68, 69]. The rest may require alternatives such as ATG or alemtuzumab. Following resolution of ACR a regime that involves adding either an anti-metabolite or mTOR inhibitor to CNI may be adopted.

Initial management of AMR is the same as that for ACR. Due to the paucity of evidence, presently there are no established protocols specifically for treatment of AMR [70]. Instead individual centers will have their own approach to management of AMR. The concepts underlying treatment of acute AMR are suppression of T-cell response, elimination of circulating antibodies, inhibition of residual antibodies and suppression or depletion of B-cells [71]. Limited studies have shown varying degrees of success with plasmaphoresis, IV immunoglobulins, rituximab and proteasome inhibitors [70]. Due to paucity of research there are presently no guidelines for management of chronic AMR.

In an era when organ shortage is amplified by increasing use of grafts for patients with significant co-morbidities or for retransplantations, marginal (so called) extended criteria grafts are frequently utilized. This necessitates individualized immunosuppression protocols. Whilst the main aim of immunosuppression is to prevent graft rejection, there is an emphasis now on minimizing immunosuppression-induced complications. It is now recognized that "less is more" with lower levels of immunosuppression as the long-term target. Bearing this in mind, there is increasing interest and research how to achieve complete withdrawal of immunosuppression in patients who have been stable for years after liver transplantation.

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