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Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy

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Abstract

Effective immunosuppression is an essential pre-requisite for successful organ transplantation and improvements in outcome after transplantation have to a large extent been dependent on developments in immunosuppressive therapy. Here we provide an overview of the different immunosuppressive agents currently used in solid organ transplantation. A historical perspective on the development of immunosuppression for organ transplantation is followed by a review of the individual agents, with a focus on their mechanism of action and efficacy. Steroids, anti-proliferative agents (azathioprine and mycophenolate), calcineurin inhibitors (cyclosporine and tacrolimus) and TOR inhibitors (sirolimus and everolimus) are discussed along with both polyclonal and monoclonal antibody preparations. Many of the key clinical trials that underpin current clinical usage of these agents are described and side-effects of the different agents are highlighted. Finally, a number of newer agents still in various stages of clinical development are briefly considered.

Keywords: Immunosuppression; Solid organ transplant

1. Introduction

Organ transplantation is now the optimal treatment for many patients with end-stage organ failure and national and international transplant registries report 1-year graft survival rates of around 85% after kidney, liver and heart transplantation [1–3]. The current success of organ transplantation is in very large part attributable to advances in immunosuppressive therapy and very few allografts are now lost as a result of acute rejection. An increasing number of immunosuppressive agents are available and these target different steps of the immunological response to an allograft (Table 1 and Fig. 1). All cause non-specific immunosuppression and each has their own agent-specific side-effects (Table 2). This article provides an overview of the different immunosuppressive agents currently used in solid organ transplantation. A

Table 1
Immunosuppressive agents used in solid organ transplantation

Class of agent	Agent
Corticosteroid	Prednisolone Prednisone Methyl prednisolone
Anti-proliferative	Azathioprine Mycophenolate mofetil Mycophenolate sodium
Calcineurin inhibitor	Cyclosporine Tacrolimus
TOR inhibitor	Sirolimus Everolimus
Polyclonal anti-lymphocyte antibodies	ALG ATG ALS
Monoclonal antibodies	Muromonab-CD3 Basiliximab Daclizumab

brief historical perspective on the development of immunosuppression for organ transplantation is followed by a review of the individual agents, with a focus on their mechanism of action and efficacy. All currently available agents cause non-specific immunosuppression and hence increase the risk of infection and certain types of malignancy (skin cancer and post-transplant-lymphoproliferative disease) in the recipient. These general complications are not considered further here but specific side-effects of the different agents are highlighted. Key clinical trials that underpin current clinical use of the different immunosuppressive agents are described. There is an emphasis on kidney transplantation throughout since nearly all agents were first introduced into clinical transplantation on the basis of their ability to reduce acute rejection after kidney transplantation. A notable exception was tacrolimus which was first evaluated in liver transplantation.

2. Historical perspective

When Murray and co-workers performed the first successful kidney transplant in 1954 it was only possible because the donor and recipient were monozygotic twins. The immune system was slowly being characterised through the pioneering efforts of Owen and Medawar [4,5], but there were no effective immunosuppressive agents. Transplants between non-identical individuals suffered early acute rejection and graft failure, and while such pioneering procedures helped to refine the surgical technique they offered little life sustaining function to their recipients. Total body irradiation prior to transplantation was used both in America and France in the late fifties to ablate the recipient immune system and overcome rejection but apart from an occasional success, the results were invariably fatal [6–8].

The breakthrough in chemical immunosuppression for transplantation came with the observation that 6-

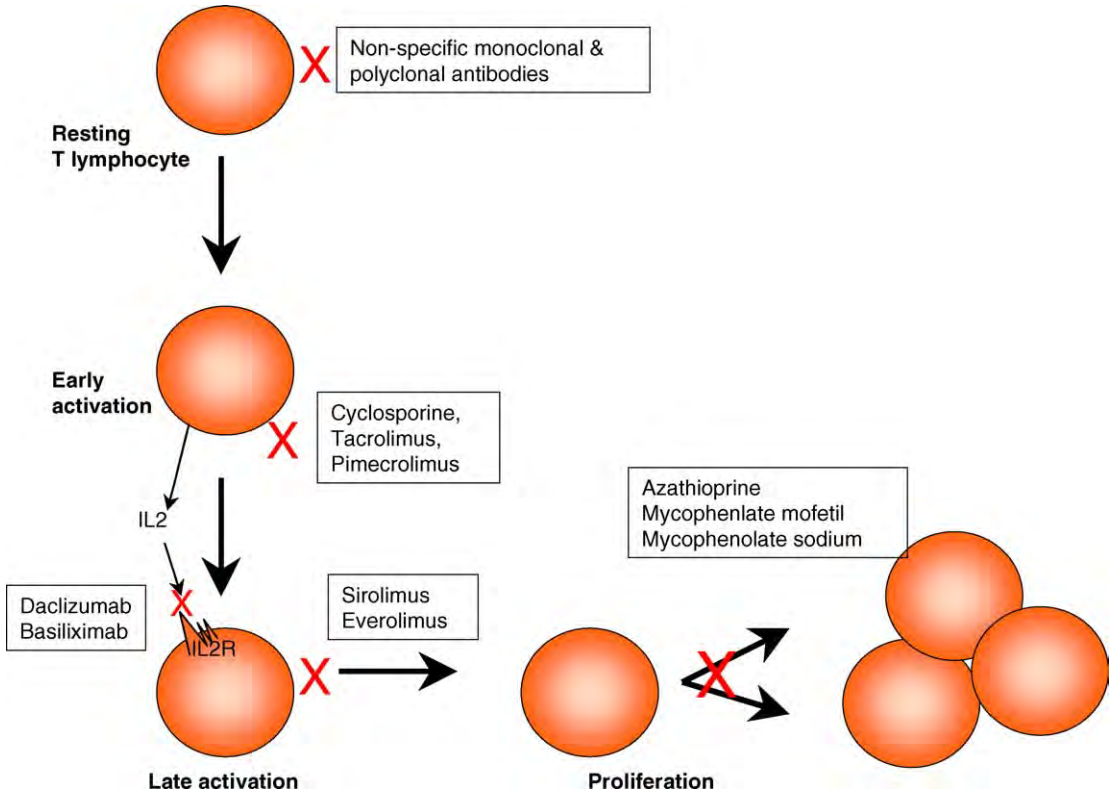

Fig. 1. Schematic sites of action of common immunosuppressants.

Table 2
Immunosuppressive drugs; potency and side effects

	Cyclosporine	Tacrolimus	Sirolimus	Azathioprine	Mycophenolate	Corticosteroids
Immunosuppressive potency	+++	+++±	++±	+	++	+
Nephrotoxicity	++	++	–	–	–	–
Neurotoxicity	+	++	–	–	–	–
Hirsutism/hypertrichosis	++	–	–	–	–	++
Skin rash	–	–	+	–	–	–
Diabetogenic	+	++	–	–	–	++
Diarrhoea	–	–	+	–	++	–
Hepatotoxicity	±	±	+	+	–	–
Marrow suppression	–	–	+	+	+	–

Key: –: equals no effect; +: mild (or low incidence) toxicity/potency; ++++: extreme toxicity or potency.

mercaptopurine (6-MP) could induce immunological unresponsiveness in rabbits to a foreign protein (human serum albumin), and later that it could prolong the survival of skin grafts in rabbits [9–11]. Following these observations Calne and co-workers demonstrated that 6-MP prolonged the survival of canine renal transplants, albeit with severe morbidity from the drug [12].

Around the same time Elion and Hitchings, working in the Burroughs Wellcome laboratories in New York, created a number of nucleotide analogues in the hope of finding novel chemotherapy agents for use in the treatment of leukaemia [13]. Calne obtained some of these from Elion and Hitchings and tested their ability to prolong kidney allograft survival in the dog in Murray's laboratory in Boston. One of the compounds, BW57-322 (azathioprine), stood out in terms of efficacy and tolerability [14,15]. Azathioprine was much less toxic than 6-MP and afforded better prolongation of allograft survival. It rapidly moved into clinical use and, while better than total or subtotal body irradiation, it was not potent enough to permit most recipients to keep their graft [16]. Although in France in the early fifties Rene Kuss had used corticosteroids to try to prolong kidney graft survival, it was only when corticosteroids were combined with azathioprine by Starzl in the early 1960s that effective chemical immunosuppression became a reality [17]. Nevertheless most kidney transplants suffered acute rejection, most of which was reversible with pulsed high dose steroids. Survival was improving, but even by the late 1970s kidney allograft survival barely exceeded 50% at 1 year.

Azathioprine and steroids remained the mainstay of immunosuppression for the next 25 years, as efforts were made to develop compounds that affected lymphocyte function. While interventions such as splenectomy, thymectomy and thoracic duct drainage were found to be unhelpful, anti-lymphocyte globulin (ALG), prepared from the serum of horses or rabbits innoculated with human lymphocytes, showed more promise. It proved a valuable adjunct to steroids and azathioprine, and was used both for the treatment of rejection and as part of the initial immunosuppressive regimen [18].

Prednisolone and azathioprine, with or without ALG, were powerful enough to permit successful renal transplantation but both heart and liver transplantation struggled for success with the level of immunosuppression available and in the face of exposure to such a large dose of steroids. It took the discovery of cyclosporine in 1976 for thoracic organ and liver transplantation to be truly successful. Cyclosporine was initially studied for its potential as an anti-fungal compound by the Sandoz laboratories in Basle, but when it was discovered to have potent anti-lymphocyte properties its development was temporarily halted. Borel, a scientist at Sandoz, showed that cycloporine permitted the survival of skin grafts in mice [19], and the next year it was shown to prolong the survival of kidney transplants in the dog [20]. Clinical trials began the following year in Cambridge, UK, and cyclosporine was shown to facilitate not only kidney transplantation, but also transplantation of the pancreas and liver [21], and later the heart and lungs.

Although initially used alone, cyclosporine proved more successful when combined with steroids and azathioprine as triple therapy [22–24]. The results of kidney transplants progressively improved such that by 2000 centres using cyclosporine based immunosuppression achieved graft survival between 85% and 95% at 1 year.

The 1970s were also notable for the development of monoclonal antibodies (mAbs) in Cambridge, UK [25]. Although the true potential of this discovery would not be fulfilled in the clinic for another two decades, one clinically important mAb was produced soon after the discovery. OKT3 (muromonab-CD3), a mouse anti-human CD3 mAb was used initially to treat acute rejection, and is still used occasionally for steroid-resistant acute rejection or as an induction agent [26].

Two agents with interesting results in rodent models were identified in the late 1980s, namely tacrolimus (FK506) and sirolimus (rapamycin). In the hitherto reliable canine renal transplant model both drugs caused a lethal vasculitis, and further clinical development was halted [27–29]. In spite of this, Starzl used tacrolimus in clinical liver transplantation and showed that it was not only potent, as predicted, but also devoid of the adverse effects seen in the dog [30]. Because of its perceived potency, tacrolimus was used initially as rescue therapy for patients with intractable rejection [31,32]. Further studies confirmed it to be of similar potency and with similar side effects to cyclosporine, albeit with slightly more neurological and diabetogenic effects in kidney and liver transplantation [33,34].

In the 1990s the pace of new drug development increased with the introduction of mycophenolate mofetil, sirolimus and everolimus, together with two anti-CD25 mAbs, daclizumab and basiliximab. Several other mAbs of different specificities are now in clinical trials, together with new drugs influencing lymphocyte trafficking (FTY720) and proliferation (FK778).

3. Corticosteroids

Corticosteroids are still widely considered an important component of most immunosuppressive regimens and are almost universally used as first-line treatment for acute allograft rejection. The two main corticosteroids used for the prevention of allograft rejection are prednisolone (used mainly in Europe) and prednisone (used mainly in the U.S.A.). The bioavailability of prednisone is 80% of that of prednisolone, though there is no apparent clinical difference between these two preparations in terms of efficacy or pharmacokinetics [35]. Prednisone is metabolised to the active component prednisolone in the liver. Both have predominantly glucocorticoid effects with minimal mineralocorticoid effects. First-line treatment of acute allograft rejection in most centres comprises high-dose intravenous steroids, usually methylprednisolone.

3.1. Chemical structure

The chemical structure of prednisolone and prednisone are illustrated in Fig. 2.

3.2. Mechanism of action

Corticosteroids have a variety of anti-inflammatory and immunomodulatory effects [36]. These include stabilisation of lysosomal membranes, suppression of prostaglandin synthesis, reduction of histamine and bradykinin release and lowering of capillary permeability. Corticosteroids cross into the cytoplasm and bind to glucocorticoid receptors, anchored in the cytoplasm by a complex of heat shock proteins. Binding permits release of heat shock proteins allowing the corticosteroid/glucocorticoid receptor complex to translocate to the cell nucleus where it influences gene transcription, including transcription of the nuclear activating factor family of genes. These genes are important in activating the transcription and production of several pro-inflammatory cytokines and the net result is a decrease in the inflammatory response through reduced production of cytokines, including IL-1, IL-2, IL-6, IFN-γ and TNF-α. Corticosteroids also impair monocyte/macrophage function and decrease the number of circulating $CD4^+$ T cells.

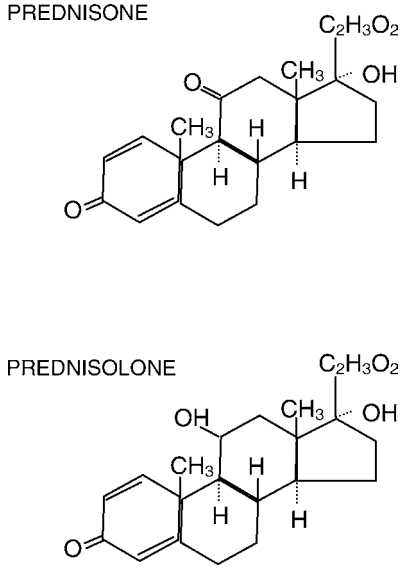

Fig. 2. Prednisolone and prednisone.

3.3. Side effects

The side effects of steroid treatment are numerous and well known. Metabolic effects include diabetogenesis due to altered carbohydrate metabolism, fat redistribution from the extremities leading to plethoric face and central obesity, and protein loss from skeletal muscle resulting in proximal weakness. Fluid retention is a consequence of mineralocorticoid activity, with hypokalaemia and hypertension. Long-term steroid therapy results in adrenal suppression and, eventually, adrenal atrophy. Psychosis, cataracts and glaucoma, peptic ulceration, abdominal wall striae and purpura, avascular necrosis of the femoral head and osteoporosis, and impaired wound healing are other common problems.

3.4. Clinical evidence

In the early 1960s steroids were given at high doses (2–4 mg/kg) after transplantation, inevitably with a high associated morbidity. A series of trials in the early 1980s compared high-dose to low-dose steroids in combination with azathioprine [37–39]. Patient survival, graft survival and incidence of acute rejection were similar irrespective of steroid dose, provided that azathioprine was given at a dose of greater than 2 mg/kg per day. There were significantly less steroid related complications in those patients receiving lower dose steroids, though patient mortality was similar in both groups. Nowadays doses of 20 mg/day are typical during the initial period and 5 mg/day is common in the maintenance phase. Most of the current literature on the role of steroids after organ transplantation focuses on the early withdrawal or reduction of steroids and on steroid avoidance.

3.4.1. Steroid withdrawal after renal transplantation

In general, studies examining early withdrawal (within the first 3 months post transplant) have shown a higher incidence of acute rejection. Studies of delayed steroid withdrawal show that cessation of steroid therapy is possible in most patients early in the maintenance phase, although there is still

a risk of late acute rejection and rise in the serum creatinine [40,41].

Several randomised controlled trials have examined steroid withdrawal beginning at 6–12 months following renal transplantation. In patients receiving cyclosporine and/or azathioprine/mycophenolate based immunosuppression, complete steroid withdrawal is successful in around 70–85% of patients [40–44]. There is a significant decrease in steroid related complications after steroid withdrawal, though mortality is not reduced. In two studies with a follow-up of over 3 years, both observed a continued decline in renal function in those patients who underwent steroid withdrawal [42,45]. In general, withdrawal of steroids after 3 months post-transplantation results in a small increase in the risk of acute graft rejection and fewer steroid related complications, though this does not appear to reduce cardiovascular mortality.

Steroid withdrawal at 3 months was examined in recipients taking cyclosporine and MMF and who had not suffered an episode of acute rejection [46]. A total of 266 patients were randomised to continue prednisone at a dose of 10–15 mg/day or to have prednisone withdrawn over 8 weeks. The cumulative incidence of acute rejection was 30.8% for the steroid withdrawal group compared to 9.8% in those patients continuing steroids. There was no difference in patient or graft survival at 1 year between the two groups, though the group in which steroids had been withdrawn had significantly lower serum cholesterol and required less anti-hypertensives. Serum creatinine levels tended to be higher after steroid withdrawal. Further randomised controlled studies have confirmed that the incidence of acute rejection is similar or higher in patients undergoing steroid withdrawal at 3 months post-transplantation, though benefits include improvement in hypertension, hypercholesterolaemia and bone density [47–50].

Withdrawal of steroids within the first week of transplantation is associated with a higher incidence and increased severity of rejection in patients on cyclosporine or tacrolimus based regimens [51–54], though the use of induction therapy with anti-CD25 mAb may reduce the high rate of acute rejection. The follow up in these studies is short (1 year) and longer term studies are required to determine effects on graft function as severe or recurrent rejection may predispose to chronic rejection and late graft loss.

3.4.2. Steroid withdrawal after liver transplantation

Steroid withdrawal can be safely performed following liver transplantation with little increased risk of acute rejection and with reduced hypertension, lower serum cholesterol, less obesity and a reduced incidence of post-transplantation diabetes [55–57]. As with kidney transplantation, very early steroid withdrawal after liver transplantation results in an increase in the incidence of rejection though without increased graft loss [55]. However, in recipients free from acute rejection at 3 months, early steroid withdrawal is safe and effective [58].

4. Azathioprine

4.1. Chemical structure

Fig. 3 illustrates the structures of both azathioprine and 6-mercaptopurine.

4.2. Mechanism of action

Azathioprine is metabolised to 6-mercaptopurine (6-MP) through reduction by glutathione, and then converted to 6-thiouric acid, 6-methyl-MP, and 6-thioguanine (6TG). These compounds are incorporated into replicating DNA and halt replication. They also block the de novo pathway of purine synthesis by formation of thio-inosinic acid. This latter effect confers specificity of action on lymphocytes which lack a salvage pathway for purine synthesis.

Recent evidence suggests that azathioprine also interferes with CD28 co-stimulation of alloreactive T lymphocytes. CD28 receptor signaling is mediated by phosphatases such as the GTPase Rac1. One of the metabolic products of azathioprine, 6TG, results in generation of 6-thioguanine triphosphate (6-thioGTP), which binds to the GTPase Rac1 in place of GTP. Blockade of Rac1 converts the co-stimulatory signal from CD28 into an apoptotic signal, thereby deleting activated lymphocytes. This recently described mechanism probably underlies the observations of Schwarz and Dameshek 44 years earlier that 6MP can induce tolerance to foreign proteins [10].

4.3. Pharmacokinetics and drug monitoring

The half-life of azathioprine and 6MP, its principle metabolite, is around 2 h but there is significant inter- and intra-patient variation in the pharmacokinetics [59]. In spite of this the drug is usually dosed according to body weight. Azathioprine is metabolised initially to 6MP, then by thiopurine *S*-methyltransferase (TPMT) to 6 me-MP and on to 6TG and the inactive 6-thiouric acid, as outlined above. A common polymorphism of TPMT exists causing low enzyme activity, resulting in acute azathioprine-induced myelosuppression [60]. This polymorphism is present in 10% of the popula-

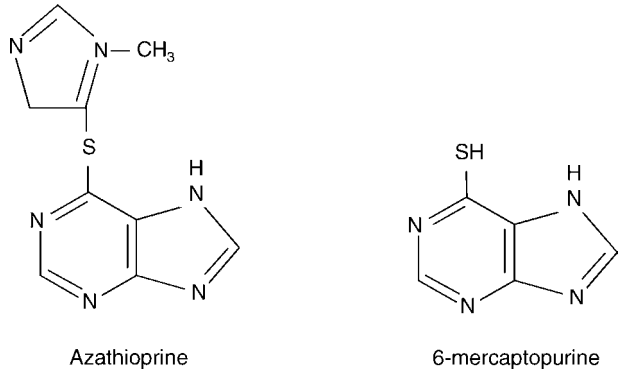

Fig. 3. Azathioprine and 6-mercaptopurine.

tion, of whom 0.5% are homozygous and at greatest risk of myelosuppression. Identification of the polymorphism, and consequent dose adjustment of azathioprine will overcome the risk of acute marrow failure, particularly where higher doses of azathioprine are used.

In spite of the safety benefits of monitoring for TPMT, no routine monitoring of the polymorphism, nor of azathioprine is undertaken in most transplant centres. Nevertheless there is evidence that monitoring could improve efficacy of azathioprine, as well as its safety. In one contemporary study, where azathioprine dose was controlled according to 6-thioguanine nucleotide concentration there was evidence of reduced incidence of acute rejection in renal transplantation [61].

4.4. Side effects

The principle side effect of azathioprine is dose-related bone marrow suppression but it may also cause occasional liver impairment and cholestatic jaundice; hepatic veno-occlusive disease has also been reported. In addition, a number of hypersensitivity reactions, usually manifesting as a rash, have been reported.

4.5. Clinical evidence

The evidence for the efficacy of azathioprine in transplantation comes from the pioneering studies in the 1960s [16]. Even after the advent of the calcineurin inhibitors (CNIs), azathioprine is still used in some centres as a component of triple therapy regimens and also as a long-term partner to prednisolone. The efficacy of azathioprine compared to placebo as a component of triple therapy (CNI, steroids and azathioprine) has not been subjected to extensive study by randomised trials. Several studies have examined the CNI-sparing efficacy of azathioprine when used in combination with prednisolone. In one such study, patients on triple therapy had their cyclosporine discontinued at 3 months, with improvement in renal function and uric acid concentrations [62]; in another patients receiving cyclosporine-based triple therapy had cyclosporine withdrawn at 12 months, again with improvement in renal function at the expense of a low incidence of acute rejection [63].

5. Mycophenolic acid

5.1. Chemical structure

Fig. 4 illustrates the chemical structure of the active drug, mycophenolic acid, and the two parent compounds, mycophenolate mofetil (CellCept) and mycophenolate sodium (Myfortic).

5.2. Mechanism of action

Mycophenolate mofetil (MMF) and mycophenolate sodium (MPS) are rapidly converted in the liver to mycophenolic acid which is the active compound. The target of mycophenolic acid is inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the de novo synthesis of guanosine nucleotides, themselves essential for DNA synthesis. Most cell types can generate guanosine nucleotides by two pathways, the IMPDH pathway and a salvage pathway. Lymphocytes do not possess such a salvage pathway, hence blockade of the IMPDH pathway results in relatively selective blockade of lymphocyte proliferation. There are two isoforms of the IMPDH enzyme, the type I isoform being found predominantly on resting cells and the type II isoform being induced and expressed on activated lymphocytes. Mycophenolic acid preferentially inhibits the type II isoform of IMPDH, expressed on the activated lymphocyte population [64].

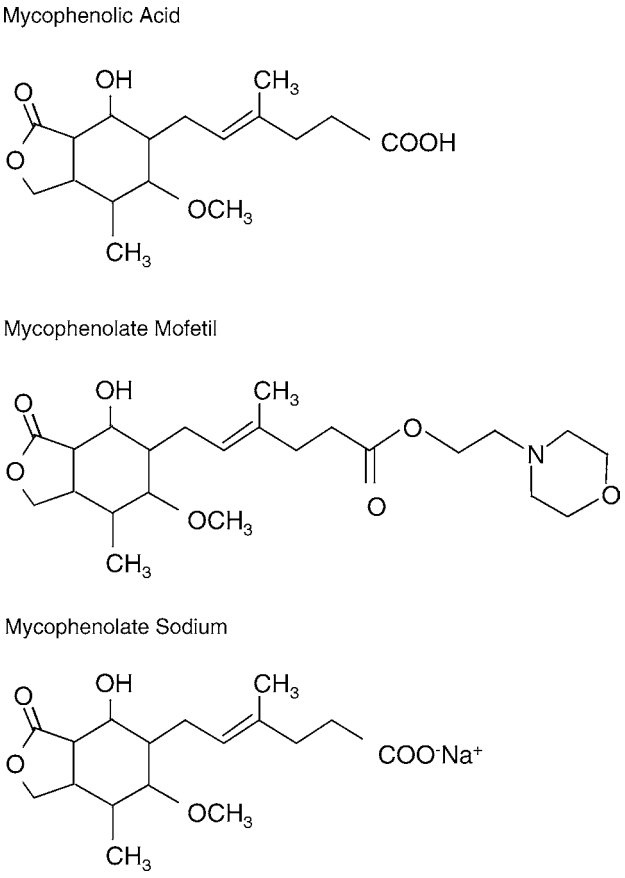

Fig. 4. Mycophenolate mofetil, mycophenolate sodium and the active compound mycophenolic acid.

5.3. Pharmacokinetics and drug monitoring

MMF and MPS are well absorbed and undergo immediate first-pass metabolism to the active form, mycophenolic acid. It has an apparent half-life of 18 h, and is metabolised by the liver to mycophenolic acid glucuronide (MPAG) which is eliminated primarily in the urine. MPAG is excreted via bile into the gut where it exhibits a significant entero-hepatic recirculation, with a concomitant second peak in plasma

mycophenolic acid concentration 6–12 h after dosing. This entero-hepatic circulation might contribute to its gastrointestinal intolerance. The AUC (area under concentration time curve) of mycophenolic acid is also increased by around 30% when cyclosporine is substituted with tacrolimus or sirolimus, with concomitant reduction in peak MPAG concentration. Mycophenolic acid exposure (AUC) immediately post renal, cardiac or liver transplant are around 30% lower than the AUC in the later post-transplant period (3–6 months) [65].

In the presence of severe renal impairment (GFR< 25 ml/min) the AUC of mycophenolic acid is increased. It is also increased as a consequence of MPAG competing for renal tubular secretion with anti-viral drugs such as ganciclovir and aciclovir. Monitoring of mycophenolic acid is not routinely undertaken, but there is a good argument in favour of monitoring in the light of the number of drugs and circumstances which can alter MPA levels [66,67].

5.4. Side effects

The drug-specific side effects of MMF and MPS are similar. The most common dose limiting adverse effect is diarrhoea, but other gastrointestinal side effects such as nausea, vomiting and abdominal pain are also common. Marrow suppression also occurs. In addition there is a suggestion from some of the clinical trials of an increased incidence of viral infections such as cytomegalovirus compared to placebo or azathioprine; candida and herpes simplex are also more common [68].

5.5. Clinical evidence

At the time of writing there is little published evidence regarding MPS and available evidence for clinical efficacy relates to studies with MMF.

5.5.1. MMF in renal transplantation

The efficacy of MMF was determined by three large double-blind randomised controlled multi-centre trials reported in the mid 1990s. Two of these compared MMF to azathioprine [69,70] and the third to placebo [71] in patients taking cyclosporine and steroids for immunosuppression. Two dose regimens of MMF were studied, 2 g/day and 3 g/day, and while immunosuppressive efficacy was slightly greater with 3 g/day the inevitable trade off was an increase in drug-related and immunosuppression-related side effects such that most centres now adopt a 2 g/day protocol. A combined analysis of the data from the three pivotal studies, amounting to 1493 patients, revealed that MMF significantly reduced the incidence of biopsy proven graft rejection in the first year post-transplant from 40.8% in patients taking placebo or azathioprine, to 19.8% in patients on 2 g/day MMF and 16.5% on 3 g/day MMF [72]. MMF was associated with less severe rejection, a greater proportion of steroid responsive rejection and a reduced requirement for antibody treatment of acute rejection. The incidence of graft loss at 1-year post transplant was 2.6% for patients taking MMF compared with 6.3% for patients taking azathioprine or placebo. Both the European and the U.S. studies showed a trend towards an improved graft survival at 3 and 4 years, respectively in patients taking MMF [44,73]. There was no significant difference in markers of chronic rejection (mean serum creatinine and proteinuria) between patients treated with MMF and control patients [70].

MMF may also have a role in the prevention of recurrent acute rejection. In one study, 221 patients receiving cyclosporine, azathioprine and steroids were randomised at the time of their first rejection episode to switch to MMF or to continue azathioprine [74]. At 6 months following randomisation, recurrent rejection occurred in 25% of patients on MMF compared with 58% of controls.

The ability of MMF to inhibit vascular smooth muscle cell proliferation in vitro led to hope that it might help to reduce chronic allograft rejection. Indirect evidence for such an effect has been provided by analysis of large databases of renal transplant recipients that suggest MMF reduces the known risk factors for chronic allograft nephropathy and improves long-term renal graft function [75,76]. Direct evidence has also recently been provided by a prospective randomised trial [77]. One-year protocol biopsies were examined in renal allograft recipients randomised to receive a cyclosporine-based immunosuppressive regimen combined with either MMF (37 recipients) or azathioprine (34 recipients). The incidence of chronic allograft nephropathy was 46% in the MMF group and 71% in the azathioprine group ($p = 0.03$). Although patient numbers were relatively small, the results suggested a beneficial effect of MMF on the incidence of chronic renal allograft nephropathy.

5.5.2. MMF in liver transplantation

Several studies have compared MMF with azathioprine in liver transplantation [78,79]. In one of the larger studies, 565 liver transplant recipients were randomised to receive either MMF or azathioprine in combination with cyclosporine and steroids [78]. The incidence of biopsy proven rejection was 40% in the azathioprine group compared to 31% in the MMF group, though this difference was not statistically significant. The incidence of acute rejection was lower in patients treated with MMF, but patient and graft survival rates were similar at 1 year. In addition to replacing azathioprine as part of a triple therapy regimen after liver transplantation, MMF may also have a role in permitting CNI dose reduction or withdrawal in recipients with renal dysfunction [80–82], although such a strategy is not completely without risk [81].

6. Calcineurin inhibitors (CNIs): cyclosporine, tacrolimus (FK506) and pimecrolimus (FK520, ascomycin)

Cyclosporine and tacrolimus are licensed for use in organ transplantation; pimecrolimus is currently licensed for use in the treatment of eczema and will not be discussed further.

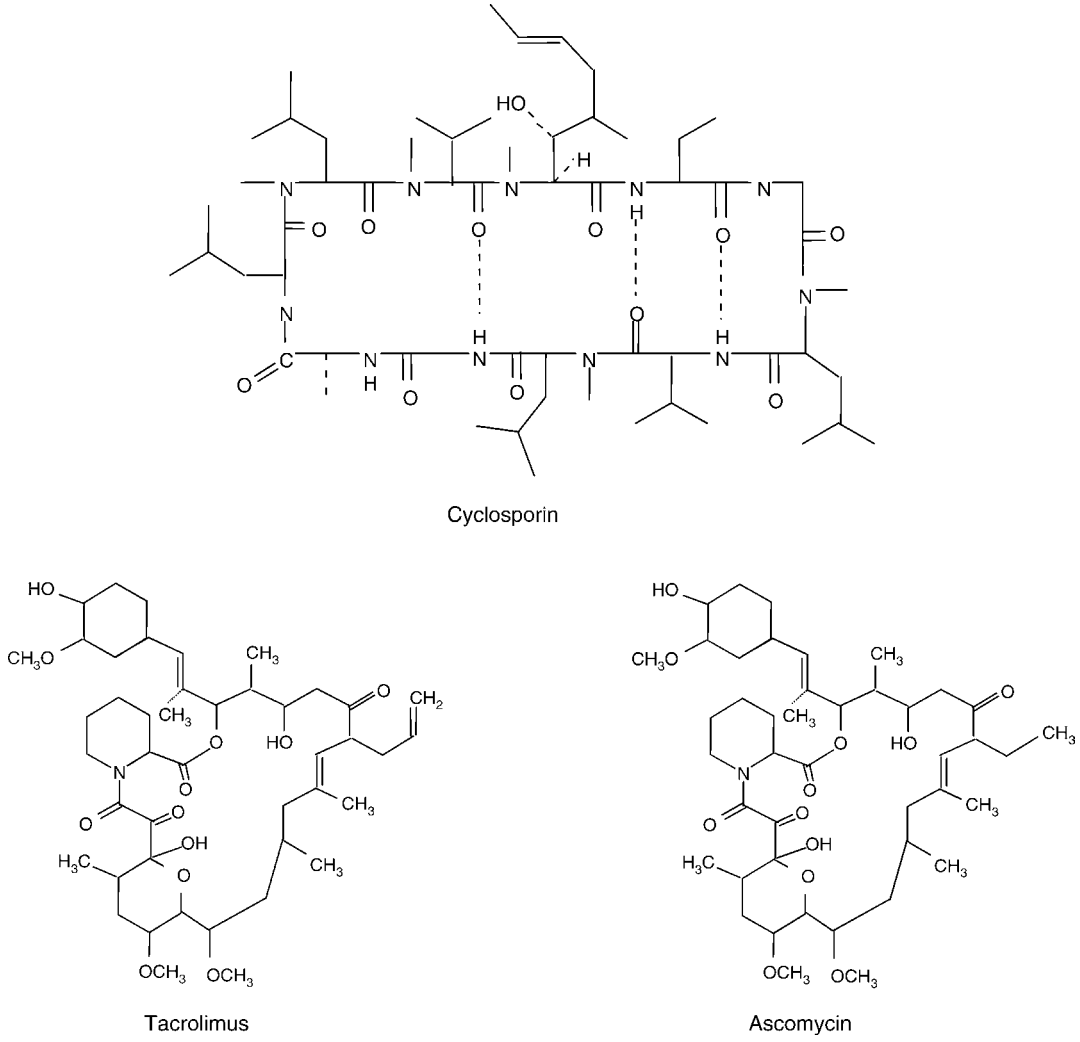

Fig. 5. Chemical structures of the calcineurin inhibitors.

6.1. Chemical structures

Fig. 5 shows the chemical formula of cyclosporine, tacrolimus and pimecrolimus.

6.2. Mechanism of action

The molecular mechanisms whereby CNIs inhibit T cell activation are well understood [83]. T cell receptor engagement with donor MHC/peptide normally triggers calcium-dependent intracellular signalling resulting in activation of the calcium/calmodulin-dependent phosphatase calcineurin. This leads to the de-phosphorylation of NF-AT allowing translocation into the nucleus where it enhances binding of transcription factors to genes encoding for pro-inflammatory cytokines such as IL-2, IL-3, IL-4, IFN-γ and TNF-α. After entering the cytoplasm, CNIs form complexes with their immunophilins. Cyclosporine binds to cyclophilin and tacrolimus and pimecrolimus bind to the 12 kDa FK506-binding protein (FKBP-12). The CNI-immunophilin complexes inhibit calcineurin activity, and hence prevent nuclear translocation of NF-AT and cytokine gene transcription. The net result is that CNIs block the production of cytokines such as IL-2 and inhibit T cell activation and proliferation.

6.3. Pharmacokinetics and drug monitoring

The immunosuppressive effect of the CNIs is related to the total drug exposure (AUC). For tacrolimus, the 12-h trough concentration (C_{min}) is a good surrogate of the AUC. This was not the case with the original cyclosporine formulation (Sandimmune), which was poorly absorbed from the GI tract, and exhibited marked inter- and intra-patient variability. To overcome this Novartis developed a microemulsion formulation (Neoral) which was much more reliably absorbed such that the C_{min} was a better surrogate of the AUC [84,85]. More recently it has been suggested that the 2-h concentration, C_2,

best reflects clinical efficacy [86]. CNIs are metabolised by the cytochrome P450 3A4 enzyme in the gut and liver, and drugs which interact with the CYP3A4 also affect the concentration of CNIs (and TOR inhibitors).

6.4. Side effects

CNIs are associated with a range of agent-specific side effects and these are summarised in Table 2. Many of the important side effects of CNIs are dose dependent and relate to the sites where calcineurin concentrations are highest, notably in the brain and the kidney [83]. Cyclosporine and tacrolimus share many dose-related side effects but there are also important differences. Both drugs are associated with nephrotoxicity and this is one of the most important side effects particularly after renal transplantation. It is due in part due to severe vasoconstriction of the afferent arteriole, with concomitant reduction in renal blood flow and glomerular filtration rate [87–89]; these changes are reversible with discontinuation of the CNI. In the longer-term CNIs cause chronic non-reversible changes that are characterised by interstitial fibrosis and obliterative arteriolar changes due to fibrous intimal thickening [90]. Although both cyclosporine and tacrolimus are nephrotoxic, recent studies suggest this may be less of a problem with tacrolimus [89,91,92]. Hypertension is a common consequence of CNI treatment, in part secondary to the renal effect [93] and again may be less common with tacrolimus.

The neurotoxicity of CNIs may manifest in many ways, is more common with tacrolimus than cyclosporine, and is exacerbated in the presence of low serum magnesium concentration [94]. Headache and tremor may occur, worse one to two hours following administration when the plasma concentration of the drugs are highest. Insomnia is also common. Agitation, convulsions, psychosis, hallucinations, encephalopathy and impaired consciousness are less common [95].

The metabolic effects of CNIs include diabetogenesis, which is two to four times more common with tacrolimus than cyclosporine, and may also reflect different sensitivity to the diabetogenic effects of corticosteroids [96,97]. Hyperkalaemia, hyperuricaemia and hyperlipidaemia are the other common metabolic side effects and the latter may occur less frequently with tacrolimus than cyclosporine. Gingival hyperplasia and hypertrichosis are drug specific side effects of cyclosporine while alopecia may accompany tacrolimus use [98].

6.5. Clinical evidence

6.5.1. Cyclosporine in renal transplantation

Cyclosporine was the mainstay of immunosuppression throughout the 1980s and early 1990s. During the early 1980s several large multi-centre trials showed a significant improvement in 1-year graft survival from around 50% with azathioprine to between 70% and 90% with cyclosporine [24,99–101] For the first time it was possible to achieve good graft survival in sensitised patients having second renal transplants [102]. The early studies even led to the use of cyclosporine monotherapy for renal transplantation in some centres [103]. More importantly, it allowed the development of other types of solid organ transplantation. In 1986 Opelz published data from a collaborative European database of renal transplants highlighting the nephrotoxicty of cyclosporine [104]. Such observations led clinicians towards using reduced doses of cyclosporine as part of a triple therapy regimen with prednisolone and azathioprine to minimise drug-specific side effects while optimising overall immunosuppression. Such triple therapy regimens resulted in 1-year graft survival rates of around 80% with around 40% of patients having no rejection episodes [105–107].

6.5.2. Cyclosporine induced nephrotoxicity

A number of trials have demonstrated improved renal function after cyclosporine withdrawal. In one such trial, 187 renal transplant recipients were randomised at 1-year post-transplant to continue with cyclosporine, MMF and steroids, or to have cyclosporine withdrawn gradually and continue steroids and MMF [108]. A statistically significant improvement in creatinine clearance and lower mean serum creatinine was observed in the cyclosporine withdrawal group. However, cyclosporine withdrawal was associated with a 10.6% incidence acute rejection over the study period compared with a 2.4% rejection rate in patients continuing cyclosporine. While it may be possible to withdraw cyclosporine completely in stable patients, many clinicians prefer dose reduction to complete withdrawal, to minimise nephrotoxicity but avoid precipitating acute rejection.

6.5.3. Tacrolimus in renal transplantation

The largest randomised controlled trial to examine the use of tacrolimus in renal transplantation entered 560 patients to receive either tacrolimus (287 patients) or cyclosporine microemulsion (273 patients) in combination with azathioprine and steroids [109]. At 6 months post-transplant, the incidence of biopsy-proven rejection was significantly lower in the tacrolimus group than the cyclosporine group (19.6% versus 37.3%, respectively); moreover when acute rejection did occur it was more likely to be steroid responsive in tacrolimus treated patients (9.4% versus 21.0%, respectively). There was no significant difference in patient or graft survival at 6 months post transplantation. An earlier randomised controlled trial of 412 patients also found a lower incidence of biopsy-proven acute rejection in patients receiving tacrolimus than the Sandimmune formulation of cyclosporine at 1 year follow up (30.7% versus 46.4%, respectively) with no significant difference in patient or graft survival [34]. A second randomised trial comparing tacrolimus with microemulsion cyclosporine showed that patients receiving tacrolimus experienced better kidney function than those receiving cyclosporine [110]. The main benefit

of tacrolimus administration was in patients with delayed graft function/acute tubular necrosis who experienced a 23% ($p = 0.06$) increase in allograft survival compared to those treated with cyclosporine.

These three large-scale randomised controlled trials of tacrolimus versus cyclosporine, with follow up from 6 to 24 months, show that patient and graft survival is comparable in patients receiving tacrolimus and cyclosporine, though with significantly less acute rejection in patients receiving tacrolimus [34,96,109,110]. There is no convincing evidence that the reduced rate and severity of acute rejection observed in patients treated with tacrolimus leads to improved long-term graft survival. A large European multi-centre study randomised 451 patients to receive either tacrolimus or cyclosporine in combination with azathioprine and steroids [111]. At 5 years, patient and graft survival rates were comparable in both groups. However, the incidence of chronic rejection was significantly lower in recipients treated with tacrolimus (6.6% versus 15.3%). Tacrolimus based immunosuppression may be beneficial in certain patient subgroups. The recently reported 3-year follow-up results from a randomised trial comparing tacrolimus plus MMF or azathioprine with cyclosporine plus MMF showed that graft survival in patients with delayed function was significantly better in the tacrolimus groups [112]. It has also been suggested that tacrolimus may be more effective than cyclosporine at preventing acute rejection in highly sensitised renal transplant recipients. Patients at high immunological risk were randomised to receive either tacrolimus ($n = 22$) or cyclosporine ($n = 11$) based triple therapy [113]. At 1 year the incidence of graft rejection was 31.8% in the tacrolimus group and 54.5% in the cyclosporine group. Graft survival was also improved in the tacrolimus group (86% versus 72%, respectively). Early conversion to tacrolimus may also be beneficial for renal allograft recipients who experience acute rejection while on cyclosporine treatment. The first prospective controlled study to examine this found that conversion to tacrolimus helped to resolve acute rejection and reduced the risk of recurrent acute rejection [114].

In summary, for the majority of adult patients, tacrolimus and cyclosporine both provide good immunosuppression and give equivalent graft and patient survival, though longer-term studies suggest that graft survival in patients receiving tacrolimus may be improved. Much of the early data on cyclosporine relates to the Sandimmune formulation and not the contemporary microemulsion formulation. Even with the microemulsion formulation consideration should be taken whether trough or C2 monitoring is used, since the latter is said to be more relevant by its advocates.

6.5.4. Calcineurin inhibitors in paediatric renal transplantation

Cyclosporins and tacrolimus also give equivalent patient and graft survival at 2 years post-transplant in paediatric recipients of kidney allografts though tacrolimus is associated with a lower incidence of acute rejection and improved graft function. A large multi-centre trial randomised 196 paediatric renal transplant patients to receive either cyclosporine ($n = 93$) or tacrolimus ($n = 103$) in combination with azathioprine and steroids [115]. The incidence of acute rejection was 36.9% in the tacrolimus treated group compared to 59.1% in the cyclosporine treated group ($p = 0.003$). Children treated with tacrolimus had a significantly higher GFR at 2 years and a lower incidence of graft loss through chronic rejection (9.7% versus 18.3%). A large retrospective study of 986 paediatric renal transplant recipients using data from the North America Paediatric Renal Transplant Cooperative Study database also showed that tacrolimus-treated patients have a significantly higher mean GFR at 2 years post transplant [116]. Steroid withdrawal may be more successful in children treated with tacrolimus and in one study long-term steroid withdrawal was achieved in 70% of paediatric renal transplant recipients on tacrolimus therapy [117]. Further studies are required to determine whether the improved graft function seen in children treated with tacrolimus translates into improved long-term graft survival.

6.5.5. Calcineurin inhibitors in liver transplantation

The efficacy of tacrolimus versus cyclosporine in liver transplantation has been examined in three large studies. A large European study randomised a total of 529 patients to receive tacrolimus and steroids ($n = 264$), or cyclosporine, azathioprine and steroids with or without ATG depending on the centre [118]. The incidence of acute, recurrent acute, and chronic rejection were all significantly lower in the group receiving tacrolimus at 3 years post-transplant, though patient and graft survival were similar. The United States FK506 Study Group published data at 5 years following randomisation of 529 patients to tacrolimus and steroids ($n = 263$) or cyclosporine and steroids, with ALG permitted in both arms [119]. The incidence of acute rejection was significantly lower in the tacrolimus treated group though there was little difference in the incidence of acute rejection beyond the first post-operative year. Patient and graft survival at 5 years were similar.

The UK and Republic of Ireland Liver Transplant Study Group conducted the third large trial comparing tacrolimus and cyclosporine in liver transplant recipients. A composite primary outcome measure was used comprising death, re-transplant or treatment failure for immunological reasons at 1-year post transplant [120]. Out of a total of 606 patients, the primary outcome was reached in 21% of patients in the tacrolimus group and 32% of patients in the cyclosporine group ($p = 0.001$). The authors concluded that tacrolimus therapy is the first choice calcineurin inhibitor for patients receiving their first liver graft. However, the results of the first multi-centre, randomised study to compare cyclosporine microemulsion with C2 monitoring versus tacrolimus in liver transplantation were recently published and the two agents were found to be equally efficacious with respect to acute rejection at 3 months and death or graft loss at 6 months

[121]. The incidence of adverse events was also similar with the exception of a significantly higher incidence of diabetes mellitus and diarrhoea in the tacrolimus group.

6.5.6. Calcineurin inhibitors in paediatric liver transplantation

Two large multi-centre randomised controlled trials have compared cyclosporine with tacrolimus in paediatric liver transplantation [122,123]. The U.S. multi-centre trial (50 patients) [122] and the European multi-centre trial (185 patients) [123] both found equivalent patient and graft survival for cyclosporine and tacrolimus treated children, with a lower incidence of acute rejection in those treated with tacrolimus at 12 months post-transplantation. The European study also showed that the incidence of corticosteroid-resistant rejection was significantly lower in the tacrolimus group (6% versus 29.6%). No prospective long term follow up data is available comparing tacrolimus with cyclosporine in paediatric liver transplantation, although one large retrospective study of 218 children compared outcome at 3–11 years post-transplantation [124]. Patients treated with tacrolimus had a significantly lower incidence of chronic rejection (1.4% versus 10.9%). At 1-year post transplant, 72.8% of cyclosporine-treated patients were still on corticosteroids compared to 42.3% of tacrolimus treated patients. The studies performed to date suggest that paediatric liver transplant recipients treated with tacrolimus show a lower incidence of acute and chronic rejection and have a reduced requirement for corticosteroids, although the trade-off may be a higher incidence of post-transplant lymphoproliferative disorder (PTLD) in tacrolimus treated patients. In the above retrospective study, the incidence of PTLD was 2.2% in cyclosporine-treated and 12.3% in tacrolimus treated patients [124]. However, no increase in PTLD was observed in the more recent European prospective multi-centre study [123]. This may be due to either a shorter duration of follow up or increased awareness of PTLD and early tacrolimus dose reduction in patients developing primary Epstein-Barr virus infection.

7. The TOR inhibitors sirolimus and everolimus

Sirolimus and everolimus belong to the group of immunosuppressive agents called mammalian target of rapamycin (mTOR) inhibitors.

7.1. Chemical structure

Fig. 6 illustrates the chemical structure of both drugs in this class. Both are macrocyclic lactones, with sirolimus being a naturally occurring fermentation product of the actinomycete *streptomyces hygroscopicus*, while everolimus represents a chemical modification of sirolimus to improve absorption.

7.2. Mechanism of action

Sirolimus (SRL) and everolimus (EVL) bind to the 12 kDa intracellular immunophilin FK506 binding protein (FKBP12) but, unlike tacrolimus, do not inhibit calcineurin activity. Instead the SRL/FKBP12 and EVL/FKBP12 complexes are highly specific inhibitors of mammalian target of rapamycin (mTOR) [125]. MTOR is a serine/threonine kinase involved in the phosphatidylinositol 3-kinase (P12K)/AKT (protein kinase B) signalling pathway. Inhibition of mTOR has a profound effect on the cell signalling pathway required for cell-cycle progression and cellular proliferation. The net effect is blockade of T cell activation by preventing progression of the cell cycle from the G1 to the S phase. In addition to their immunosuppressive effects, mTOR inhibitors inhibit fibroblast growth factors required for tissue repair, which can result in wound healing problems. The same effects on smooth muscle proliferation probably underlie the effects observed on the development of intimal proliferation following angioplasty.

7.3. Pharmacokinetics and drug monitoring

Sirolimus has a long half-life (around 60 h in patients on triple therapy). It is metabolised principally by the

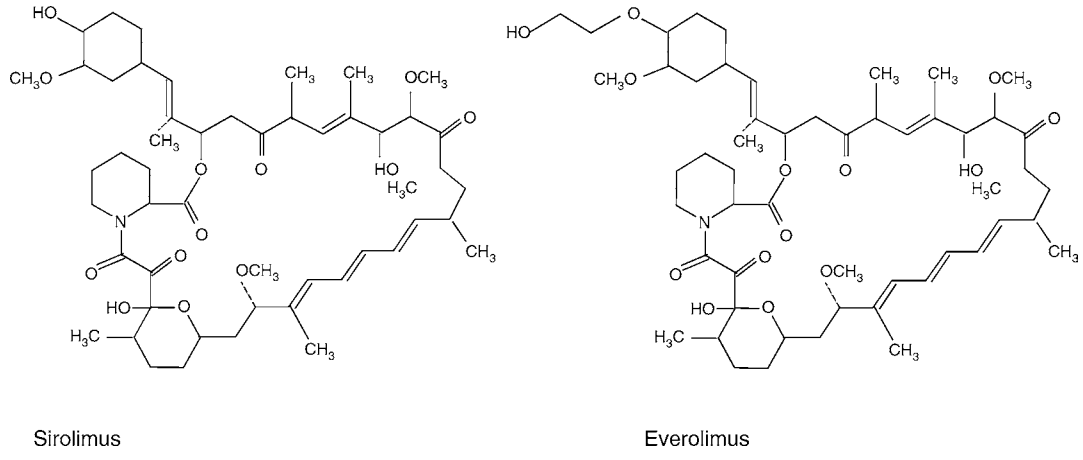

Fig. 6. Sirolimus and everolimus.

cytochrome P450 3A4 system, and therefore has the same interactions as the CNIs. Therapeutic drug monitoring is advisable, and trough concentrations of between 5 and 15 ng/ml are desirable in the maintenance phase in the absence of CNIs, with higher levels in the early post-transplant period. Everolimus is a more recent addition. The chemical modification that enhances its absorption also reduces its half-life (16–19 h) such that twice daily dosing is appropriate [126]. Monitoring of blood levels is also desirable. When given together with CNIs both sirolimus and everolimus have been reported to enhance nephrotoxicity.

7.4. Side effects

Everolimus and sirolimus have similar side effects, as might be expected from their similarity of structure and mode of action. The side effects are best categorised as metabolic, haematological, dermatological effects and effects related to growth factor inhibition [127,128]. Principal among the metabolic effects are increases in serum cholesterol and triglycerides. Other effects include reduction in uric acid, and elevation in liver function tests. Suppression of all three blood elements, leucocytes, erythrocytes and platelet count is common, with anaemia being particularly troublesome in the presence of renal impairment and responding to supplementary erythropoietin.

Skin rashes, particular acne, and mouth ulcers are more common in patients converted to mTOR inhibitors than those patients started de novo. The mouth ulcers often manifest like herpes simplex. Growth factor inhibition may well underlie the effects of sirolimus on the bone marrow. Inhibition of epithelial growth factor might contribute to the occurrence of mouth ulceration, while inhibition of fibroblast growth factor accounts for impaired wound healing [129]. A side effect that is assuming increased importance is the occurrence of interstitial pneumonitis, which appears to be dose related and which resolves on withdrawal of sirolimus [130]. In some instances granulomata have been seen on lung biopsy. Early reports of pneumocystis pneumonia in patients on sirolimus might have in fact been pneumonitis. Peripheral oedema, diarrhoea and lymphocoele formation post renal transplant are also well recognised complications [131].

7.5. Clinical evidence

7.5.1. Sirolimus in renal transplantation

Early randomised studies in the late 1990s confirmed the potency of sirolimus as an immunosuppressive agent in renal transplantation. When sirolimus was combined with cyclosporine and steroids it reduced the incidence of acute rejection in the first 6 months from 32% to 8.5% [132]. Early studies in Europe compared sirolimus head-to-head with cyclosporine in triple therapy regimens along with steroids and either azathioprine [133] or steroids and MMF [134]. Sirolimus was effective in both studies and neither graft survival or the incidence of acute rejection was significantly different between the sirolimus and cyclosporine treated groups, suggesting that the new agent was similar in efficacy and could be used as an alternative to CNIs as the mainstay of immunosuppressive regimens. These initial studies also showed that sirolimus had a distinct side effect profile and hypercholesterolaemia, hypertriglyceridaemia and leucopaenia were all seen significantly more frequently with sirolimus than with cyclosporine. Subsequently two pivotal licensing studies of sirolimus in renal transplantation were undertaken. The Rapamune Global Study Group reported a multi-centre randomised double-blinded clinical trial in which 576 patients were randomised to receive sirolimus 5 mg/day, sirolimus 2 mg/day or placebo in combination with cyclosporine and steroids [135]. The incidence of biopsy proven rejection during the first 6 months was 11%, 19% and 37%, respectively. There was no difference in patient or graft survival at 6 months. A similar three-arm study in the U.S. randomised patients to receive either 2 mg or 5 mg of sirolimus or azathioprine in combination with cyclosporine and steroids. The incidence of acute rejection was significantly lower in the sirolimus groups (2 mg: 18.7% and 5 mg: 16.8%, azathioprine 29.8%, $p < 0.05$); there was no difference in graft or patient survival [136]. One unexpected finding in both studies was an increase in serum creatinine in patients receiving a combination of cyclosporine and sirolimus. Sirolimus can also be combined with tacrolimus to provide effective immunosuppression after renal transplantation and in one recently reported randomised trial this combination was found to be more effective than sirolimus plus cyclosporine in terms of acute rejection at 1-year (4% versus 14%, $p = 0.03$) [137,138].

The most significant studies with sirolimus have involved its use to permit cyclospirine withdrawal. In a European study, 525 patients given cyclosporine, sirolimus and steroids were randomised at 3 months post-transplantation to continue this combination (215 patients) or to undergo cyclosporine withdrawal and remain on concentration-controlled sirolimus (215 patients). Patients suffering one or more episodes of moderate or severe rejection within the first 3 months were excluded from randomisation ($n = 95$). There were no significant differences in the incidence of biopsy proven acute rejection after randomisation (5.1% cyclosporine/sirolimus/steroids versus 9.8% sirolimus/steroids), patient survival (94.0% versus 95.3%) or graft survival (91.2% versus 93.5%) at 2 years. However serum creatinine, calculated creatinine clearance and systolic blood pressure were all significantly lower at 2 years in patients undergoing cyclosporine withdrawal [139,140]. A trend for improving calculated creatinine clearance was seen, an observation that continued out to the 5-year mark. Histological examination of biopsies from 57 patients in this study suggested that there was less evidence of chronic allograft nephropathy in the patients in whom cyclosporine had been withdrawn [141]. A similar multi-centre study in the U.S. of 246 patients confirmed that patients maintained on sirolimus without cyclosporine show better long-term graft function

[142]. These studies provided a foundation for a number of switch studies where CNIs have been withdrawn following transplantation, and other studies where CNIs have been avoided altogether [143,144]. Other innovative approaches include combining TOR inhibitors and CNIs with withdrawal of the CNI at 3–6 months [139].

7.5.2. Sirolimus in liver transplantation

Relatively few studies of sirolimus in liver transplantation have been reported to date. The first report illustrated the potency of the drug, and showed that sirolimus maintenance monotherapy was effective [145]. It also highlighted the wound healing problems, an observation repeated by subsequent users of the drug [146]. Two large studies of sirolimus as de novo therapy in combination with prednisolone were stopped early by Wyeth due to an excess of hepatic artery thrombosis in the sirolimus treated patients. The possible relationship between sirolimus and hepatic artery thrombosis has been disputed in subsequent studies. In one large retrospective analysis the incidence of thrombosis was lower in sirolimus treated patients than historic control patients on CNIs (5.3% versus 8.3%, $n = 170$ and 180, respectively), although the incidence of thrombosis was high in both groups. Thrombosis in patients on sirolimus in combination with low dose tacrolimus appears to be less, with only one case noted in 56 transplants [147]. Nevertheless concerns over arterial thrombosis led most investigators to focus not on de novo use of sirolimus but on conversion of patients to sirolimus for complications of CNI therapy, particularly nephrotoxicity. Conversion from CNIs to sirolimus appears to be associated with improvement in renal function [148–150], but no randomised studies have yet been published. If conversion is undertaken for declining renal function, it is probably best done early, since late conversion is unlikely to result in sustained restoration of worthwhile renal function [151].

7.5.3. Everolimus in renal transplantation

The clinical development of everolimus (SDZ RAD) in renal transplanation has followed similar lines to that of sirolimus, with it being assessed initially in combination with cyclosporine. In a dose ranging study using 1, 2 or 4 mg/day of everolimus in combination with steroids and microemulsion cyclosporine the incidence of biopsy proven rejection was lower in the higher dose groups [152]. A side effect profile similar to sirolimus was identified.

The results of two studies of 1.5 mg and 3 mg/day everolimus in combination with low dose cyclosporine were reported in one paper [153]. Cyclosporine monitoring was by the 2-h post dose concentration (C2), and one study included basiliximab induction therapy. In both studies 3 mg/day of everolimus produced low rates of acute rejection at 6 months (15.2% and 15.1%), although the addition of basiliximab was associated with a lower acute rejection in the 1.5 mg/day groups (25% versus 13.7%); there was no real difference in incidence in the 3 mg/day groups.

7.5.4. Everolimus in cardiac transplantation

Everolimus has also been trialled in cardiac transplantation and shown to be associated with a reduction in the incidence and severity of the coronary artery vasculopathy, as assessed by intravascular ultrasound [154]. This is the first direct evidence in humans that mTOR inhibitors may be able to prevent the vascular lesions that characterise chronic rejection in transplanted organs.

8. Polyclonal antibodies

8.1. Nature

Polyclonal antibodies (ATG, ALS and ALG)) prepared by inoculating rabbits or horses with human lymphocytes or thymocytes are still widely used in solid organ transplantation. The purified IgG fraction contains antibodies directed against many different cell-surface molecules expressed on T lymphocytes, B cells, NK cells and macrophages [155,156].

8.2. Mechanism of action

Administration of anti-lymphocyte polyclonal antibodies results in rapid and profound lymphopaenia in the majority of patients, probably due to complement mediated cell lysis and uptake by the reticulo-endothelial system of opsonised T cells [157]. In addition to depletion, polyclonal antibodies may cross-link the TCR, causing partial T cell activation and blockade of T cell proliferation [158].

8.3. Side effects

A so-called “first dose reaction” is seen in up to 80% of patients and may be caused by the presence of xenogeneic proteins or the initial activation of T cells following engagement of cell surface receptors and cytokine release [157,159,160]. The most common reaction is a febrile episode, which is seen much less frequently on subsequent infusions and is thought to result from pyrogen release due to the initial large lymphocytolysis. Other reactions include skin rash, pruritis, thrombocytopenia and rarely anaphylaxic shock. To minimise these reactions, a combination of steroids, antihistamines and paracetamol are given routinely 30–60 min before starting antibody therapy.

8.4. Clinical evidence

8.4.1. Polyclonal antibodies for the prevention and treatment of renal allograft rejection

Prior to the introduction of cyclosporine, ALS/ATG was often administered as induction therapy in combination with steroids and azathioprine. Early randomised controlled trials showed an improvement in allograft survival of nearly 20% at 4–36 months follow-up in addition to a reduction in the number of acute rejection episodes and less steroid usage

[161,162]. After the introduction of cyclosporine, some centres used antibody induction combined with cyclosporine, azathioprine and steroids (quadruple therapy). Although acute rejection was seen less frequently and graft survival was improved, there was a high incidence of PTLD [163–165]. Many centres abandoned the use of a prolonged course of antibody as induction therapy, though a short course or single administration of ALS at induction remains common practice in some centres, particularly for sensitized patients.

Anti-lymphocyte antibody preparations are still widely used to treat steroid resistant acute rejection episodes and are effective in 70–96% of patients [165–168]. Importantly the serum creatinine at 1 year in patients who respond to antibody therapy is similar to patients with steroid reversible rejection [167]. The efficacy of repeated treatment using antibody preparations from the same species may be reduced by the development of xeno-specific neutralising antibodies. For this reason, if a second course of antibody therapy is required, a preparation obtained from a different species should be chosen.

8.4.2. Use of polyclonal antibodies in liver transplant recipients

Several studies have examined the use of polyclonal antibody induction therapy as a component of quadruple immunosuppression for prophylaxis against acute rejection after liver transplantation [169–173]. In general, the addition of antibody induction protocols does not confer any further benefit in terms of reducing the incidence of acute rejection or improving patient of graft survival. Renewed interest has emerged recently in the use of antibody induction to permit steroid withdrawal or avoidance after liver transplantation. This is particularly advantageous in patients with hepatitis C because total steroid dose is related to early recurrent hepatitis C in the allograft [174]. Antibody induction therapy has been used successfully in several studies to allow complete steroid avoidance after liver transplantation with no increase in the incidence of acute rejection or clinically significant infections [175,176].

9. Monoclonal antibodies

9.1. Nature

In comparison to polyclonal antibody preparations, mAbs have a single well-defined specificity, are more standardised, and do not contain irrelevant proteins. The newer mAbs are all engineered to reduce the risk of stimulating a human anti-mouse antibody (HAMA) response that may limit the efficacy of mAb therapy and prohibit repeated courses. Humanised mAbs are engineered so that only the complementarity determining regions (CDR) on the mouse antibody are grafted onto the parent human IgG molecule whereas chimeric mAbs comprise the mouse variable region grafted onto the human IgG constant portion (Fc).

9.1.1. Nomenclature of monoclonal antibodies

The nomenclature of mAbs permits identification of their underlying construction. They follow the pattern

unique prefix–target–source–mab stem

The source of the antibody is denoted by the letters preceding the mab stem. For humanised antibodies the source identifier is “-zu-” and for chimeric antibodies it is “-xi-”. Other source identifiers include “-o-” for mouse and “-a-” for rat, such that the mouse CD3 monoclonal antibody OKT3 is called muromonab (murom-o-nab; conventionally should read muromomab but some modification out with convention does occur). When the antibody targets the immune system the target infix “-lim-” is incorporated into the word before source stem. For example, the CD25 mAbs daclizumab (dac-li-zu-mab) and basiliximab (basi-li-xi-mab) are examples of chimeric and humanised antibodies, respectively, with the same specificity on lymphocytes. Antibodies approved for the treatment of cancer have a “target” infix “-tu-”, hence alemtuzumab (alem-tu-zu-mab, Campath-1H, MabCampath) is a humanised version of Campath-1G, licensed for the treatment of certain lymphomas, and rituximab (ri-tu-xi-mab, MabThera, Rituxan) is a chimeric CD20 monoclonal licensed for use against B cell lymphomas.

9.1.2. Role of antibody isotype

Most mAbs are IgG, with different isotypes conferring different properties. This is well illustrated by the Campath 1 family of antibodies specific for CD52, an antigen expressed on T and B lymphocytes, as well as other circulating mononuclear cells [177]. Campath 1M is a rat IgM monoclonal antibody that lyses $CD52^+$ cells in the presence of complement, and in vivo produces a transient depletion of lymphocytes in the circulation. Campath-1G, a rat IgG_{2b} monoclonal, is also involved in antibody-dependent cell-mediated cytotoxicity in vitro and causes a sustained depletion of lymphocytic leukaemia cells from the circulation [177]. Campath-1H is a humanised IgG1 antibody that also causes sustained lymphocyte depletion.

9.1.3. Mechanism of action

mAbs bind to target epitopes on the cell surface following which they may trigger a variety of mechanisms. mAbs, such as alemtuzumab, fix complement and destroy the target cell, “depleting” it from the circulation. Other mAbs modulate the target molecule from the cell surface, either by antigen shedding or internalisation. Modulation, or physical blocking of the epitope may prevent ligation of cell surface molecules involved in signalling, such as the CD28 antigen.

9.2. Clinical evidence

9.2.1. Muromonab-CD3 (OKT3)

Muromonab-CD3 (OKT3, Orthoclone®) is a non-engineered mAb that targets the CD3 molecule on T cells.

The CD3 complex is essential for transducing intracellular signals that result from the engagement of the TCR with antigen. Within minutes of administration of OKT3, there is profound lymphodepletion by massive T cell lysis [178]. After three to 5 days, T lymphocytes are detectable in the circulation but they do not express CD3 and are, therefore, immunologically incompetent [179].

When OKT3 initially binds to CD3, there is T cell activation, cytolysis and massive cytokine release, often termed the "cytokine release syndrome" [180]. This is characterised by release of several cytokines, principally TNF-α, and sequestration of neutrophils in the lungs resulting in pulmonary oedema and acute respiratory distress [181,182], and more rarely intra-graft thrombosis and aseptic meningitis. To reduce these effects steroids and antihistamines are routinely given prior to administration of OKT3.

Clinically muromonab-CD3 has been used both for induction of immunosuppression and for the treatment of steroid resistant rejection, reversing rejection in around 75–86% of recipients [183–185].

The most frequent use of muromonab-CD3 in liver transplantation is for treatment of steroid resistant rejection where it results in a complete response in around 50% of patients and a partial response in most of the others [186,187]. Non-response to muromonab-CD3 is unusual but associated with a very poor prognosis. Overall, graft survival at 1 year following muromonab-CD3 administration is around 65–80% [186–188]. Use of muromonab-CD3 should be minimised in patients with hepatitis C as an increased disease recurrence is observed [189,190]. Muromonab-CD3 has also been used as induction therapy for liver transplantation. A large multi-centre trial randomised patients to receive either 14 days of muromonab-CD3 in combination with steroid and azathioprine commencing cyclosporine on day 11 post-transplant (46 patients) or to receive a standard triple therapy regimen of cyclosporine, azathioprine and steroids (50 patients) [191]. There was no significant difference in the incidence of acute rejection or in mean serum creatinine at 1 year, and at 4 years patient and graft survival were similar.

9.2.2. CD25 monoclonal antibodies

CD25 is the alpha chain of the IL-2 receptor (IL-2Rα), normally expressed only by activated T cells. Two CD25 mAbs have been approved for clinical use, basiliximab (Simulect) and daclizumab (Zenopax). Although they differ in the degree of humanisation and the recommended dosage regimen, there is little apparent difference in efficacy. Once given the antibodies remain in the circulation for several weeks, the duration depending on the other immunosuppressive therapy being taken. With prednisolone and cyclosporine, receptor saturating concentrations remain for around 7 weeks [192]; the addition of azathioprine or MMF extends the duration of action (50 and 59 days, respectively) [193]. Anti-CD25 mAbs do not trigger a strong antiglobulin (HAMA) response, although occasional anaphylactic reactions to re-challenge with the antibody are described [194]. Unlike muromonab-CD3, basiliximab and daclizumab do not produce a first-dose reaction, and have very few side effects directly attributable to the antibody.

The efficacy of basiliximab in renal transplantation was demonstrated in two pivotal randomised double-blind placebo controlled multi-centre trials. Basiliximab, in combination with prednisolone and cyclosporine, reduced the incidence of biopsy proven acute rejection at 6 months to 29.8%, compared with 44.0% in the control group receiving placebo ($p = 0.012$) [195]. In addition it also reduced the severity of acute rejection, with fewer episodes of steroid resistant rejection. There was no difference in the incidence of graft loss, infection or other adverse effects between groups at 12 months. Similar results were seen in a US study, again in combination with steroids and cyclosporine [196]. The incidence of acute rejection at 1 year was 35.3% in the basiliximab group as compared with 49.1% in the placebo group ($p = 0.009$). Patients who received basiliximab had a significantly lower incidence of renal dysfunction over the year. Basiliximab has also been used to allow the successful early withdrawal of steroids [197], and even steroid avoidance albeit with a high incidence of rejection [198].

The evidence for the efficacy of daclizumab is similar to that for basiliximab. In a randomised double-blind placebo-controlled study, addition of daclizumab to triple therapy with steroids, azathioprine and cyclosporine, led to a significant reduction in the incidence of rejection at 6 months (22% versus 35% in the control group, $p = 0.03$) [199]. No difference was seen between the groups in the incidence of infection or cancer. A similar reduction in the incidence of acute rejection was seen when daclizumab was combined with duel therapy comprising steroids and cyclosporine (47% in controls, 28% on daclizumab at 6 months) [200]. Graft function and patient survival were also better on daclizumab. In both studies of these studies, 3-year patient and graft survival were similar in the treatment and control groups, and there was no excess of adverse sequelae, in particular no excess of post-transplant lymphoproliferative disease (PTLD) [201]. Subset analysis also revealed that daclizumab reduced acute rejection in patients with delayed graft function [202]. Like basiliximab, daclizumab has also been used to facilitate steroid avoidance.

One of the drawbacks of daclizumab was the original dosage regimen of 1 mg/kg at transplant and then four additional doses at 2 week intervals, compared to basiliximab where dosing was 20 mg on day 0 and 4. Recent evidence suggests that adequate saturation of CD25 is achieved with just one or two doses of daclizumab [203].

In summary, basiliximab and daclizumab included in induction therapy reduce the incidence of acute rejection over the first year post renal transplant. These compounds have no place in treatment of acute rejection, since when IL-2 levels are raised cells are activated by the low affinity IL-2 receptor in the absence of the alpha chain.

9.2.3. Use of CD25 monoclonal antibodies in liver transplantation

Initial research in liver transplantation showed that therapeutic antibodies were often lost in the protein rich ascites or other post-surgical drainage, and while normal dose regimens were often adequate for patients with large fluid losses supplementary doses of antibody were beneficial [204,205]. CD25 mAbs have been used as induction agents, to permit low (or no) initial calcineurin inhibitor exposure [206], and to avoid steroid exposure in recipients with hepatitis C.

Induction therapy with basiliximab has been shown to reduce the incidence of acute rejection (35.1% versus 43.5%), the difference being largely confined to the HCV negative cohort [207]. The use of daclizumab to avoid exposure to steroids in patients with hepatitis C aims to reduce the rate and severity of graft infection with the virus. An early non-randomised study suggested that the opposite might be the case, with earlier and more severe HCV recurrence [208]. This observation has not been however been confirmed in other studies [209].

10. Immunosuppressive agents in late stages of testing

A large number of new immunosuppressive agents are in various stages of development and several of these have now entered clinical evaluation and some of those showing most promise are outlined below.

10.1. FTY720

FTY720 is a synthetic analogue of Myriocin, a product of the ascomycete *Isaria sinclarii.* In vivo FTY720 is phosphorylated to the active metabolite FTY720-P, and this molecule targets the cell receptors for the natural lipid sphingosine 1-phosphate (S1P) [210]. There are five S1P receptors on lymphoid tissue, and FTY720 is a strong agonist of S1P1, 3, 4, and 5. Increased expression of S1P1 and S1P4 on the surface of lymphocytes makes them more sensitive to the effects of homing chemokines such as CCR7. This results in homing of lymphocytes to lymphoid organs particularly the mesenteric lymph nodes, where they are sequestered leaving a much reduced population of circulating lymphocytes (Fig. 7).

FTY720 has a long half-life, estimated to be in the order of 8 days after multiple dosing [211]. In initial studies FTY720 was combined with cyclosporine and steroids and shown to be efficacious in terms of reducing acute rejection [212]. The principle toxicity of FTY720 is a "first-dose" negative chronotropic effect that persists over the first 48 h of treatment and is reversed with beta agonists and atropine. The mechanism behind this unusual toxicity is FTY720-P action on S1P1 and S1P3 receptors on sinoatrial cells reducing activity of adenylate cyclase and cAMP levels resulting in negative chronotropism.

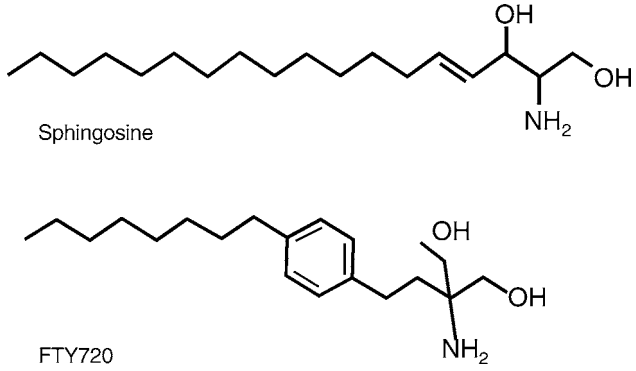

Fig. 7. Comparative structures of sphingosine and FTY720.

10.2. FK778

FK778 is a synthetic malononitrilamide derived from A77 1726, one of the active metabolites of leflunamide. Leflunamide has a long half-life and gastrointestinal side effects, and FK778 was engineered to overcome these problems. Early clinical studies combining the drug with tacrolimus suggest that the drug is well tolerated, but the optimal dosing regimen needs to be determined [213]. In preclinical models FK778 appears to prevent the development of graft vasculopathy, a hallmark of chronic rejection, making it a much awaited product [214,215].

10.3. LEA29Y

Cytotoxic T-lymphocyte antigen-4 (CTLA4 or CD152) is a cell surface molecule that is similar to CD28 and, like CD28 binds CD80 and CD86 (B7.1 and B7.2). Binding of CTLA4 inhibits T cell activation. A fusion protein of CTLA4 with the Fc domain of IgG has been created, CTLA4Ig, which has been shown to induce tolerance in rodent models [216]. LEA29Y (also known as BMS-224818) is derived from CTLA4Ig, and has been modified to have greater avidity of binding to CD80 and CD86 and a more sustained effect. It is given by intermittent injection. Initial studies suggest potency in renal transplantation and rheumatoid arthritis [217].

10.4. Alemtuzumab

Alemtuzumab is a humanised anti-CD-52 mAb that causes profound lymphocyte depletion. It is currently licensed for the treatment of chronic B cell lymphocytic leukaemia. Alemtuzumab was first used as induction therapy along with cyclosporine monotherapy in renal transplantation and was well tolerated and effective in the short-term [218,219]. A number of other studies have also reported using alemtuzumab induction therapy in renal transplantation as well as in liver and intestinal transplantation, again with satisfactory short-term results [220–223]. The efficacy and long-term safety of alemtuzumab has not yet, however, been determined in a randomised controlled trial.

11. Conclusions and future prospects

A range of different immunosuppressive agents, both pharmacological and biological, is now available for clinical use in solid organ transplantation. As new agents have emerged, their role in complementing or replacing existing agents in immunosuppressive regimens has become clearer and contemporary immunosuppressive regimens are highly effective at preventing graft loss from acute rejection. Indeed, the potency of the agents now available is such that the challenge is avoiding over-immunosuppression and the problems of infection and malignancy that accompany excessive non-specific immunosuppression. The increasing number of potential combinations of available agents has led to wide variations in immunosuppressive protocols between transplant centres. Further well-designed and properly powered clinical trials are needed to determine how best to combine the different agents now available. The current trend for tailoring immunosuppressive therapy to best suit the individual needs of the patients will undoubtedly continue and the application of pharmacogenetics may aid individualisation and refinement of immunosuppressive therapy. A priority is to select immunosuppressive regimens that mimimise unwanted agent-specific side effects, especially those that impact negatively on cardiovascular risk and renal function. It is important to avoid excessive levels of individual agents with serious side effects and to exercise particular caution when combining agents that share similar side-effects. For example although sirolimus and MMF act at different sites in the T cell activation pathway, they share the side effect of marrow suppression so it is no surprise that anaemia and leucopaenia are often dose limiting. Tacrolimus and corticosteroids both cause diabetes, so efforts are made to withdraw steroid therapy in patients on tacrolimus. There is currently a trend towards developing immunosuppressive strategies that minimise or even avoid the use of steroids and of CNIs for long-term maintenance immunosuppression therapy.

Graft loss from chronic rejection is a major problem after all types of solid organ transplantation and it remains to be seen whether the early promise of some of the newer agents, notably TOR inhibitors, in this regard will translate into real long-term benefit. Several newer agents including some with a completely novel mechanism of action (e.g. FTY720) are now undergoing early clinical trial and should prove a valuable addition to the immunosuppressive armamentarium. The long-term goal in transplantation has long been and remains to develop clinically applicable strategies for inducing specific immunological tolerance to an organ allograft so that there is little or no need for long-term exogenous immunosuppression. Although transplant tolerance is readily achievable in experimental animals it still poses a major challenge in the clinic. The pace of development in basic science underpinning immunological tolerance is such that it may become a reality sooner rather than later. However, until effective strategies for inducing tolerance after clinical transplantation are found improvements in transplant outcome will be dependent on further advances in immunosuppressive therapy.

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