REVIEW

Immunosuppression in Organ Transplantation

Takahiro Oka and Norio Yoshimura

The Second Department of Surgery, Kyoto Prefectural University of Medicine, 465 Hirokoji, Kawaramachi, Kamikyo-ku, Kyoto 602, Japan

Received February 27, 1996

ABSTRACT—The immunological barrier remains the major obstacle to the widespread use of transplantation as a replacement therapy for terminal organ failure. Since the first successful renal transplant, performed by Hume et al. (1952), there has been an elusive search for agents that can render the immune mechanism unresponsive to the specific alloantigen stimulus of the engrafted organ, while sparing non-specific host resistance. Immunosuppressive therapies in organ transplantation can be divided into the following four main classes: chemical (pharmaceutical), biological (immunological), physical (radiological) and surgical. Of these, chemical agents (drugs) have continued to play a principal role. The discovery of new immunosuppressive drugs such as corticosteroids, ciclosporin, azathioprine and FK506 have been epoch-making discoveries at each stage in the history of clinical organ transplantation. The recent immunosuppressants were designed to focus their action selectively on T and/or B cells by inhibiting cytokine synthesis (ciclosporin, FK506), cytokine action (rapamycin), or cell differentiation (15-deoxyspergualin) pathways, rather than to act on immune systems in a non-selective fashion. At the present time, however, there is no single panacea. To achieve the maximum preventive and therapeutic effects with the minimum toxicity, two or more immunosuppressive drugs are used appropriately in combination, taking the mechanisms of action of each into consideration.

Keywords: Immunosuppressive agent, Lymphokine, Organ transplantation, Toxicity

1. Introduction

The success of organ transplantation depends mainly on immunosuppressive therapy. The immunosuppressive methods can be divided into the following four classes: chemical (pharmaceutical), biological (immunological), physical (radiological) and surgical (Table 1). Of these, chemical agents (drugs) have continued to play a principal role in immunosuppression after transplantation. The rapid increase in number of kidney transplants began with combined use of azathioprine (Az) with corticosteroids in 1963, and the tremendous advances in results of heart and liver transplants were achieved by introduction of ciclosporin (CsA) in the early 1980s. However, the advantages and limitations of currently used immunosuppressive drugs are well-known. The immunosuppressive drugs have various degrees of toxic side effects on vital organs and tissue, and they can increase susceptibility to infections with microorganisms and the risk of malignancy, since they never suppress

transplantation immunity alone. At the present time, two or more immunosuppressive drugs are used in combination to achieve the maximum therapeutic effects with the minimum toxicity of each. The selection of immunosuppressive drugs used in combination depends on the mechanisms of action of each drug. The most ideal combination consists of drugs with different effects on the immune system and without overlapping toxicities. In recent years, many new immunosuppressive drugs have been discovered, developed and applied to clinical trials for their use in combination therapy, expecting them to exert complementary and synergistic effects.

Some drugs developed as anti-cancer agents, including Az, methotrexate (MTX) and mizoribine (Mz), were incidentally found to have immunosuppressive activity, whereas the immunosuppressive activity of other drugs such as CsA, tacrolimus (FK506), deoxyspergualin (DSG), mycophenolate mofetil (RS) and rapamycin (Rapa) were detected through screening of the fermentation products for anti-tumor or anti-microbial antibiotics

Table 1. Immunosuppressive methods in organ transplantation

1. Chemical (Pharmaceutical)
Corticosteroid
Azathioprine
Mizoribine
Ciclosporin
Tacrolimus (FK506)
15-Deoxyspergualin (Gusperimus)
Mycophenolate mofetil (RS-61443)
Rapamycin
2. Biological (Immunological)
Anti-lymphocyte globulin (ALG)
Anti-thymocyte globulin (ATG)
Monoclonal antibody against surface molecules of T cells
Blood transfusion
3. Physical (Radiological)
Irradiation (Local or total body)
Total lymphoid tissue irradiation
4. Surgical
Thymectomy
Splenectomy
Lymphapheresis
Plasmapheresis

Table 2. Classification of immunosuppressive drugs in clinical use

1. Corticosteroids
Prednisolone
Methylprednisolone
2. Cytotoxic agents (metabolic inhibitor)
a) Purine analogue
Azathioprine
Mizoribine
Mycophenolate mofetil (RS-61443)
b) Alkylating agent
Cyclophosphamide
c) Folic acid antagonist
Methotrexate
d) Pyrimidine synthesis inhibitor
Brequinar sodium
3. Antibiotics
Ciclosporin
Tacrolimus (FK506)
Rapamycin
15-Deoxyspergualin
4. Antibody
ATG
ALG
OKT-3

(Fig. 1, Table 2). These drugs can be classified according to their sites or phases of action for inhibition as follows:

1. Cytokine synthesis (G0 phase): CsA, FK506
2. Cytokine action (G1 phase): Rapa
3. DNA synthesis (S phase): Az, RS, Mz
4. Cell maturation (M phase): DSG

Thus, most immunosuppressive drugs block T cell function, but not all drugs suppress B cells directly. The immune response to alloantigen is initiated by recognition through binding of T cell receptors, CD4 and CD8 antigens to MHC class II and class I antigen, respectively, followed by intracellular signalling to activate T cells and then T cell proliferation and differentiation. The recognition of alloantigen can not be blocked by chemical agents but is blocked by monoclonal antibodies to surface molecules of T cells.

In this article, the proposed mechanisms of action, side effects and dosages in clinical use of the currently used immunosuppressive drugs including corticosteroids are reviewed.

2. Corticosteroids

Steroids bind receptors in the cytoplasm of target cells, and the complexes enter the nuclei and are involved in nucleic acid synthesis at an early stage of protein synthesis. Steroids are believed to affect the cAMP-prostaglan-

din system, rather than directly act on cAMP synthesis. Many leukocytes are affected by steroids, although their targets in cells remain unknown. Steroids not only reduce the number of monocytes and macrophages but also impair their functions, such as responses to lymphokines, phagocytosis and interleukin (IL)-1 secretion. In addition, steroids can inhibit killer T cell activity by blocking the production of IL-2 by helper T cells (1). Steroids show both preventive and therapeutic effects on allograft rejection, probably due to the above-mentioned effects on T cells, as well as potent antiinflammatory effects due to their inhibitory effects on cyclooxygenase activity, resulting in inhibition of prostaglandin synthesis. Although B cells are resistant to steroids, steroids seem to indirectly inhibit antibody production due to their effects on macrophages and helper T cells. Thus, steroids show a wider spectrum of effects than other immunosuppressive agents. However, their immunosuppressive effects are generally weak, and their efficacy decreases or is resistant in long-term treatment. The experience of transplant physicians plays a major role in their effective and safe use because their immunosuppressive and anti-inflammatory effects cause various side effects on gastrointestinal and metabolic systems, and there is no well-defined criteria regarding their optimal doses. In general, steroids are administered at a dose of 10–20 mg/kg as methylprednisolone

Fig. 1. Structures of immunosuppressive drugs.

on the day of transplantation. Doses are tapered thereafter from 1–2 mg/kg/day to 0.3 mg/kg/day in 3 months and then to 0.15–0.2 mg/kg/day (maintenance doses) in the subsequent 3 months. Upon onset of acute rejection, 500–1,000 mg/day of methylprednisolone is intravenously injected for 2 or 3 days (bolus therapy), and then the dose is gradually reduced, by 50% every 3 days.

3. Azathioprine (Az)

Az is obtained by substituting the position-6 hydrogen of 6-mercaptopurine with an imidazole ring. Its combined use with steroids was central to the immunosuppressive regimen following kidney transplantation for more than 15 years until 1980. Az affects the S-phase of the cell cycle and shows immunosuppressive effects by inhibiting the synthesis of nucleic acid and is effective for all T cell-mediated immune reactions. In large doses, it inhibits B cell functions and antibody production. These effects are useful for preventing rejection, but increase the risk of infections. The disadvantages of Az are bone marrow suppression resulting in leukocytopenia, thrombocytopenia, and anemia and hepatotoxicity, but severe leukocytopenia can be avoided by careful adjustment of the doses. In clinical use of this drug, it is critical to determine the optimal dose; i.e., the dose that results in the maximum immunosuppressive effects with minimum side effects. The optimal dose is generally determined based on blood leukocyte counts. According to our study on the relationship between the doses of Az and renal graft function in 104 living related renal transplant recipients, the optimal doses of Az were 2.45 mg/kg/day for the initial 60 days after transplantation and 2.43 mg/kg/day from 61 to 91 days after transplantation (2). The incidence of acute rejection in haploidentical donor kidney transplants with Az therapy was about 60%. Since the development of CsA, Az has become the drug of second choice. However, CsA has some side effects (mentioned in section

5.3.), such as nephrotoxicity and the induction of lymphoproliferative disease. At present, therefore, Az is often used in combination with CsA. Immunosuppressive effects of Az peak 1 to 2 hr after oral administration and decrease gradually thereafter. In various tissues, however, its immunosuppressive effects last for a long period of time. The final metabolites are excreted into urine. Since they have no immunosuppressive effects, immunosuppressive effects and toxicity of Az are not affected by renal function. In patients with impaired renal function due to rejection or renal tubular necrosis, however, daily administration of Az at 1 mg/kg/day or greater doses is reduced in most cases due to bone marrow suppression. Caution is required when administering Az in combination with allopurinol, because this enhances immunosuppressive effects and toxicity of Az.

4. Mizoribine (Mz)

Mz was developed in Japan as an alternative to Az in the late 1970's. Mz, an imidazole nucleotide, is an antibiotic with a molecular weight of 500 (3). It inhibits nucleic acid synthesis and cell proliferation by blocking the pathway from the xanthine-5'-nucleotide to the guanosine-5'-nucleotide in purine biosynthesis. It causes less hepatic dysfunction and bone marrow suppression than Az. Since its immunosuppressive effects are not as strong as those of Az, however, it has been mainly used in combination with other immunosuppressive agents. Co-administration of CsA with Mz is recommended by some authors who found synergistic effects. This approach is commonly used in patients with hepatic dysfunction caused by drugs or viral hepatitis. Due to its weak immunosuppressive effects, Mz must be administered at doses 2 to 3 times greater than Az in order to obtain comparable efficacy. The major side effects are gastrointestinal disorders and bone marrow suppression, but the latter is less severe than that caused by Az. Mizoribine 5-monophosphate is re-converted to mizoribine by 5'-

Table 3. Graft survival rate in kidney transplantation treated with CsA

Report	Ref. No.	CsA	Survival rate		
			CsA		non-CsA
European Multicentre Trial Group	(44)	CsA alone	CAD 1Y	72% (n=117)	52% (n=115)
			CAD 3Y	66% (n=117)	42% (n=115)
The Canadian Multicentre Transplant Study Group	(45)	CsA + Pred	CAD 3Y	69% (n=142)	58% (n=149)
Japanese CsA Study Group	(46)	CsA + Pred	LRD 1Y	91% (n=294)	77% (n=199)
			CAD 1Y	81% (n=159)	48% (n=84)
USA	(47, 48)	CsA + Pred	LRD 1Y	88% (n=407)	87% (n=3003)
			CAD 1Y	71% (n=2075)	63% (n=6318)

Pred: prednisolone, CAD: cadaveric donor, LRD: living related donor, Y: year.

nucleotidase before excretion into urine. For this reason, the drug is accumulated in the body in the presence of renal dysfunction, making dosage titration necessary in patients with impaired renal function.

5. Ciclosporin (CsA)

CsA is a metabolite generated by *Tolypocladium inflatum* (Gams), a kind of fungus, and is a cyclic peptide with a molecular weight of 1,202 composed of 11 amino acids. Since 5 amino acids are methylated, CsA is extremely hydrophobic. Borel et al. (4) reported in 1977 that it has immunosuppressive effects. CsA was first used clinically by Calne et al. (5) for kidney transplantation and Powles et al. (6) for bone marrow transplantation. This drug has attracted much attention because it has great immunosuppressive effects not only in kidney transplant patients (Table 3), but in recipients of other organs such as the heart and liver as well. CsA has entirely different mechanisms of action from conventional non-specific

immunosuppressive agents such as steroids, Az and cyclophosphamide. The action of CsA is very specific for lymphocytes and its effects are reversible.

5.1. Inhibition of immune responses

a) *Effects on mixed lymphocyte reaction (MLR) and cell-mediated lympholysis (CML):* MLR is an in vitro event that reflects host reactions to grafts. White et al. (7) reported that CsA completely inhibits human MLR at 100 ng/ml and that the presence of CsA from an initial stage is essential for this inhibitory effect. CsA was also found to dose-dependently inhibit the induction of killer T cell activity in CML following MLR.

b) *Inhibition of cytokine production (Fig. 2):* IL-2 is a lymphokine produced by activated helper T cells stimulated with IL-1 and is known to exert a wide spectrum of biological effects on immune responses. It affects a clonal expansion of activated T cells and natural killer cells (NK) and plays a central role in immune responses. Hess et al. (8) and Bunjes et al. (9) discovered, in human and mouse

Fig. 2. The role of immuno-regulatory molecules in transplantation immunity. APC: antigen presenting cell, MHC: major histocompatibility complex, Ag: antigen, MSF: macrophage stimulating factor, Th: helper T cell, Tc: cytotoxic T cell, mo: macrophage, B: B lymphocyte, IL: interleukin, Ig: immunoglobulin, IFN-γ: interferon-gamma, TNF: tumor necrosis factor.

MLR experiments, respectively, that production of IL-2 was decreased in the presence of CsA. In an *in vivo* study, we confirmed that the IL-2 production by peripheral lymphocytes was decreased markedly in renal transplant patients treated with CsA and was increased at the onset of acute rejection (10). We also found using complementary DNA for RNA of IL-2 that the amount of messenger RNA (mRNA) for IL-2 was decreased markedly in renal transplant recipients treated with CsA, indicating that CsA inhibited IL-2 production at a stage preceding mRNA transcription (11). Beside IL-2, CsA has been shown to inhibit the production of various immunomodulators, such as interferon- γ (IFN- γ), IL-6, macrophage chemotactic factor (MCF) and macrophage activating factor (MAF). Abbud-Filho et al. (12) reported, based on their findings obtained in the rat heart transplant model, that while the production of IL-1 and IL-2 was inhibited by CsA, IL-3 activity was enhanced, and suggested that IL-3 might be involved in the growth and differentiation of suppressor T cells.

c) Effects on suppressor T cells: Wang et al. (13) and Hess et al. (14) reported that specific and non-specific suppressor T cell activities were not affected by CsA at 1 $\mu\text{g/ml}$ or lower concentrations, although the activation of killer T cells was inhibited in MLR. DosReis et al. (15) showed that suppressor T cells were induced in the MLR of guinea pigs in the presence of CsA and that these suppressor T cells produced antigen non-specific suppressor factor (TsF). We confirmed in an *in vivo* study that suppressor T cells that inhibit the induction of killer T cells against alloantigens were present in mouse splenic cells treated with alloantigen and CsA and a humoral factor (TsF) mediated their activity (16). The fact that suppressor T cells are induced despite marked decreases of IL-2 production due to CsA administration suggests that their growth and differentiation do not entirely depend on IL-2 and that other mechanisms are involved in these processes. This hypothesis is supported by a study by Hess et al. (17), who found that the MLR supernatant containing CsA specifically enhanced suppressor T cell activity, antigen-specific OKT8⁺ suppressor T cell activity, despite the absence of IL-2, IL-1 (α and β) or IFN- γ activity, and the culture supernatant induced the proliferation of T cell clones stimulated by MLR.

d) Mechanisms of action on the molecular level: Merker et al. (18) discovered a substance of 16 kD that is bound to CsA in the cytoplasm, named it cyclophyllin and proposed a hypothesis that CsA exerts its immunosuppressive effects by binding cyclophyllin and blocking the influx of calcium necessary for lymphocyte activation into the cytoplasm. A recent study showed that CsA receptors are peptidylprolineisomerase (PPIase), and its enzymatic activity is lost when CsA is bound to

cyclophyllin or PPIase (19). In lymphocytes, calcineurin which is a protein phosphatase and requires calcium for its activation, has a key role in the calcium-dependent pathway to the IL-2 gene. The CsA-cyclophyllin complex binds calcineurin. Calcineurin involved in this supercomplex can no longer be activated by calcium, and thereby signal progression to the IL-2 gene is blocked (20).

5.2. Clinical use of CsA

CsA is administered either orally or intravenously. Intravenous administration is conducted over a period of at least 4 hr every 12 hr, while oral administration is carried out once or twice a day. When administered orally, about 40% of the dose is absorbed through the small intestine, while a part of it is transferred into the bile duct and absorbed from the small intestine. CsA is excreted mainly into bile and partly into urine. Blood concentrations peak 3 to 4 hr after oral administration and decrease rapidly thereafter. In general, concentrations in whole blood are measured by radioimmunoassay or fluorescence polarization immunoassay using monoclonal antibodies immediately before oral administration (trough level), and the dose is adjusted to 100–200 ng/ml in the case of renal transplantation. There are many reports of drug interactions with CsA. Hepatic metabolism of cytochrome P450-dependent mono-oxygenases is the primary means of CsA elimination. Therefore, co-administration of any drug with the ability to alter the activity of the mono-oxygenases has the potential to affect CsA blood levels. The inhibition of cytochrome P450 could cause decreased CsA metabolism, leading to high blood levels of CsA, whereas its induction could result in increased CsA metabolism resulting in low blood levels of CsA. Thus, the usual consequence of drug interactions with CsA is either a decrease in immunosuppression or the appearance of CsA toxicity. A list of drug interactions with CsA is given in Table 4. CsA itself is nephrotoxic, and its toxicity can be enhanced by co-administration with other potentially nephrotoxic drugs such as aminoglycoside an-

Table 4. Changes of CsA blood levels in relation to drug interaction

1. Increase due to inhibition of cytochrome P-450
ketoconazole, erythromycin, diltiazem, danazol,
androgenic hormones, cimetidine, verapamil,
oral contraceptives, josamycin, nicardipine*, ranitidine*
2. Decrease due to induction of cytochrome P-450
rifampicin (+ isoniazid), phenytoin, probucol,
phenobarbital, carbamazepine, sulphadimidine*,
trimethoprim-sulfamethoxazole*

*unknown mechanisms.

tibiotics, gentamicin, amphotericin B and trimethoprim. For the treatment of all transplant patients, CsA is commonly co-administered with several other drugs. It is very important to take drug interactions with CsA into consideration for prevention of post-transplant complications or drug-induced side effects.

5.3. Side effects and complications

The major side effect of CsA is nephrotoxicity. Impairment of renal function due to nephrotoxicity has a serious impact on the prognosis of renal grafts. It is important to differentiate nephrotoxicity from acute rejection at an early stage after transplantation and from chronic rejection at a later stage. In addition to clinical findings including changes in renal function test, amounts of urinary protein and fibrin-fibrinogen degradation products pro-

vide useful information for differentiating nephrotoxicity from acute rejection, but time-course changes in blood CsA concentrations, in particular the trough level, are critical for making this differentiation. Acute nephrotoxicity at an early stage after transplantation is closely correlated with blood trough levels of CsA and is reversible with dose reduction. Chronic nephrotoxicity and microangiopathy, which are irreversible and can lead to interstitial fibrosis, are thought to be induced by high initial doses of CsA or repetitive acute nephrotoxicity. Typical histological changes due to nephrotoxicity include vacuolation, calcification and lipofuscinosis of renal tubular epithelial cells. Recently, CsA with drastic reduction in initial dose is preferably combined with Az or Mz to prevent acute nephrotoxicity (Ref. 21, Fig. 3), especially in case of cadaveric kidney transplant with

Fig. 3. Immunosuppressive regimen in kidney transplantation. CsA: ciclosporin, Az: azathioprine, Pred.: prednisolone, MP: methylprednisolone, Tx.: transplantation.

ischemic damage. Although the precise pathogenesis of CsA nephrotoxicity remains unclear, tubular toxicity theory and vasoconstrictive theory in relation to activation of the renin-angiotensin system, inhibition of prostaglandin or stimulation of the sympathetic nervous system are postulated. Hepatotoxicity represented by slightly elevated serum bilirubin or transaminases levels is frequent during the early posttransplant period and is self-limited with dose reduction in most cases. The occurrence of hypertension and hyperkalemia may be related to CsA nephrotoxicity. We first proposed the toxic effect of CsA on the pancreas from our results that 32 (30.5%) of the 105 patients treated with CsA required insulin therapy, compared to only 38 (21.1%) of the 180 patients treated with AZ, and acute pancreatitis occurred in 4 of 105 patients treated with CsA.

The incidence of infections, in particular serious bacterial pneumonia and sepsis, has decreased since the introduction of CsA, while the incidence of CMV infection (22) and *Pneumocystis carinii* pneumonia (23) has been reported to be increased. To prevent these side effects or complications caused by CsA, careful monitoring of CsA blood levels are needed. However, the absorption, metabolism and excretion of CsA are variable within an individual, as well as among individuals, influenced by hepatic function, intestinal condition and/or diet intake. For more stable pharmacokinetics and better bioavailability of CsA, its new microemulsion formulation, Neoral[®] has been introduced and a multi-center clinical trial on this preparation is starting in Japan.

6. Tacrolimus (FK506)

FK506, a metabolite produced by *Streptomyces tsukubaensis*, microorganism belonging to the actinomycetes family, is a macrolide with a molecular weight of 822 (24). Although the molecular structure is quite different from CsA, which is a cyclic peptide, the mode of its immunosuppressive action is similar to that of CsA. FK506 also affects helper T cells and inhibits the differentiation of cytotoxic T cells by inhibiting the production of IL-2 and expression of IL-2 receptors. FK506 binds to another member of the cytoplasmic protein family (immunophilin), FK binding protein (FKBP), as CsA binds to cyclophilin. The FK506-FKBP complex binds calcineurin to form a super-complex in which calcineurin can not be activated by calcium. Thus, the signal transduction to the IL-2 gene and other cytokines is blocked. FK506 is 10 to 100 times more potent than CsA in inhibiting alloreactive T cell proliferation, B cell activation and the production of other cytokines such as IL-3, IL-4, IFN- γ and G-CSF, although it does not inhibit the secondary proliferation of activated T cells in response to IL-2. FK506 shows considerable variation in its pharmacokinetic profile among individuals. Since it is almost completely metabolized in the liver, hepatic dysfunction causes its accumulation in the body and elevated blood levels of FK506. In blood, FK506 is sequestered by erythrocytes and its plasma concentrations are influenced by the hematocrit, plasma protein levels and temperature (25). Therefore, levels of FK506 in whole blood must be measured instead of those in plasma or serum. In the early phase II clinical trials in Japan (25), FK506 was orally administered at a dose of 0.15 mg/kg every 12 hr

Fig. 4. Dosage of FK506 after kidney transplantation. TX: transplantation. Adapted from Refs. 25 and 27 for "early phase II" and "late phase II, phase III", respectively.

Table 5. One year survival rate in kidney transplantation treated with FK506

	Patient survival (%)		Graft survival (%)	
	FK506	control	FK506	control
LRD (n=51)	96.1	100.0	94.1	98.0
CAD (n=31)	100.0	96.8	86.9	77.4
Total (n=82)	97.6	98.8	91.3	90.2

LRD: living related donor, CAD: cadaveric donor. Kaplan-Meier method. Adapted from Ref. 27.

starting 2 days before transplantation, followed by continuous intravenous injection at 0.1 mg/kg/24 hr for 3 days from the day of transplantation and thereafter oral administration of 0.15 mg/kg twice a day (Fig. 4). Since the side effects of FK506 are related to blood concentrations as in the case of CsA, the doses have to be adjusted according to blood concentrations determined by ELISA. The target blood concentrations after transplantation are set at 20 ng/ml until 10 days, 15 ng/ml until 90 days and 10 ng/ml thereafter. Prednisolone is used as a concurrent drug. However, according to a report from Pittsburgh (26), the use of FK506 made it possible to completely withdraw steroids in 60% of kidney transplant patients. The steroid-sparing effects of FK506 may prove to be of particular value in children. Compared with CsA as a primary immunosuppressant, FK506 showed comparable or greater patient and graft survival rates in kidney and liver transplantation (Table 5). In a late-phase II clinical trial in Japan on kidney transplantation (27), a graft survival rate of 95.9% at 3 years after transplantation was achieved with a 36% incidence of acute rejection. In liver transplant patients treated with FK506, the graft survival rate at one year exceeded 80% (28). There was no significant difference in patient or graft survival rate between FK506 and CsA therapy, but the incidence of refractory rejection was significantly less in patients treated with FK506 (29). FK506 has been found to reverse advanced cellular rejection; in kidney transplantation, 76% of patients who experienced refractory rejection had remission when CsA was replaced with FK506 (30). The major side effects of FK506 are very similar to those of CsA, possibly because of the similarity of modes of action, and include nephrotoxicity, neurotoxicity, diabetogenicity, gastrointestinal symptoms and so on. FK506 has a direct glomeruloconstrictive effect that leads to reduced renal perfusion and glomerular flow. Associated with the nephrotoxicity, hyperkalaemia and hypertension can occur, but the former is more and the latter is less frequent in FK506 therapy, as compared with CsA. Neurological side effects can be categorized as major (seizures, aphasia,

psychosis, encephalopathy) and minor (tremors, headache, insomnia, dysesthesia) neurotoxicity with the incidence of about 10%. The neurotoxicity may occur because calcineurin and FKBP or cyclophillin are rich in the brain and central nervous system. Hyperlipidemia, which is common in CsA therapy, is rarely encountered in patients treated with FK506. In most cases, however, these side effects caused by FK506 resolve with dosage reduction or withdrawal. Careful monitoring of the blood concentration is essential for safe and effective use of FK506.

7. Gusperimus (15-deoxyspergualin, DSG)

DSG, a derivative of spergualin that is a metabolite produced by *Bacillus laterosporus*, is a compound with a molecular weight of 497 (31). It prevents rejection by inhibiting the growth and differentiation of cytotoxic T cells and B cells. Unlike CsA, DSG does not inhibit IL-2 or IFN- γ production by helper T cells and may affect monocyte/macrophage function predominantly, including inhibition of oxidative metabolism, lysosomal enzyme synthesis, IL-1 production and cell surface expression of MHC II antigens. The inhibitory effect of DSG on T cell activation is abolished by IFN- γ and partly by IL-2, suggesting that suppressive mechanism of DSG may be mediated by the inability of lymphocytes to produce IFN- γ (32). DSG also affects B cells directly and inhibits antibody production. For this reason, DSG is co-administered with other immunosuppressive agents in ABO-incompatible kidney transplantation. DSG has been shown to reverse ongoing rejection in patients and so can be used for rescue of acute rejection (33). DSG is administered at 3–5 mg/kg/day for 7 to 10 days by i.v. drip infusion. Major side effects include bone marrow suppression, and gastrointestinal discomfort. However, since the myelotoxic effects are cytostatic, bone marrow elements regenerate rapidly after the drug is withdrawn.

8. RS-61443 (mycophenolate mofetil, RS)

RS is a prodrug of mycophenolic acid (MPA) that is a fermentation product of several *Penicillium* products. GTP is essential for the synthesis of protein and nucleic acid and for various enzymatic reactions. Inosine 5'-monophosphate dehydrogenase (IMPDH) is a rate-limiting enzyme necessary for the biosynthesis of GTP. Therefore, an increase of IMPDH activity and GTP production is necessary for the proliferation of lymphocytes. RS, a morpholinoethyl ester of MPA, is an inhibitor of IMPDH, and selectively decreases intracellular GTP and inhibits purine biosynthesis, thereby inhibiting i) the differentiation and growth of T cells and ii) anti-

body production by B cells. It has been reported that RS not only prevents but reverses rejection of vascularized allografts in both small and large animals, and it has at least an additive effect without any increased toxicity when used in combination with CsA (34, 35). RS seems to be a promising alternative to Az for clinical use, because it has no toxic effect on the kidney, liver and bonemarrow and causes no increased susceptibility to infections at a dose of 40 mg/kg or more. In humans, RS combined with CsA and steroids decreased the incidence of rejection to 17% in comparison with 60% in the absence of RS and moreover could reverse ongoing rejection in the majority of recipients even when high-dose steroids and OKT3 were ineffective (36). A phase II study of RS for dose-finding (1,000 to 4,000 mg/day) in Japan has demonstrated that the optimal dose is 3,000 mg/day considering the incidence of rejection and side effects (37).

9. Rapamycin (Rapa)

Rapa, a compound with a molecular weight of 914 produced by the actinomyces *Streptomyces hygroscopicus*, was reported for the first time in 1975 as a compound with potent inhibitory activity against *Candida albicans* (38). The subsequent studies in rats showed that it prevents experimental allergic encephalomyelitis and adjuvant arthritis and inhibits the production of antibodies (39), suggesting that it might suppress transplant immunity. Rapa is a macrolide antibiotic like FK506 and has a chemical structure similar to FK506 but acts at a different site. Although Rapa also binds cytoplasmic immunophyllin, FKBP, it fails to inhibit calcineurin (20), because the Rapa-FKBP complex does not bind calcineurin and does not block its activity or G0 to G1 progression, resulting in preservation of signal transduction to the IL-2 gene. The failure to form a supercomplex in Rapa is reported to be caused by the difference of FKBP for the binding (40). Rapa inhibits the IL2-IL2 receptor interaction without affecting the production of IL-2 (41). T cell growth induced by IL-2 and IL-4 is only slightly inhibited by FK506 and CsA, but is effectively inhibited by Rapa (39). These findings suggest that unlike FK506 and CsA, Rapa affects T-cell activation by lymphokines at a late stage. While FK506 and CsA affect T-cell activation at an early stage and inhibit the production of lymphokines necessary for T-cell activation, Rapa exerts its immunosuppressive effects by inhibiting reactions to these lymphokines. Based on these modes of action, Rapa is expected to inhibit alloreactive cells which are resistant to FK506 or CsA. In fact, the mutually synergistic effect of low doses of Rapa and CsA has been reported in a rat cardiac allograft model (42). Although Rapa and FK506 are mutually antagonists with respect to T cell activation,

Morris et al. have reported that this combination acts synergistically to prolong mouse cardiac allograft survival (43). Rapa has been shown to have toxic side effects on the kidney, gastrointestinal system and central nervous system and to be diabetogenic in animal studies. The early phase of the clinical trial of Rapa in combination with CsA and steroids has just started in the United States to find the pharmacokinetic behavior and correlation between the trough levels and the occurrence of acute rejection or side effects.

10. Conclusion

Now a variety of prophylactic and therapeutic modalities are available for controlling of allograft rejection, but there is no single panacea. By proper combined use of these immunosuppressants acting at different sites, more specific suppression of transplantation immunity has been achieved without compromising the host's other immune responses. In reality, however, a rejection crisis can not completely be prevented or reversed, and a lowered quality of life or even death occurs due to immunosuppression. The final goal of clinical transplantation is to induce allotolerance, in which the graft survives without immunosuppression. Clinically, there are some allograft recipients having a functioning graft without any immunosuppressive drug, showing tolerance to alloantigens. In parallel with the development of new immunosuppressive drugs with more specificity and less toxicity, immunological procedures for induction of allotolerance remain to be established.

REFERENCES

- 1 Kaplan MP, Lysz K, Rosenberg SA and Rosenberg JC: Suppression of interleukin 2 production by methylprednisolone. *Transplant Proc* **15**, 407–412 (1983)
- 2 Nomura H: Immunosuppressive therapy in renal transplantation. *J Kyoto Pref Univ Med* **92**, 1067–1070 (1983)
- 3 Mizuno K, Tsujino M, Takeda M, Hayashi M, Atsumi K, Asano K and Matsuda T: Studies on bredinin. I. Isolation, characterization and biological properties. *J Antibiot (Tokyo)* **27**, 775–782 (1974)
- 4 Borel JF, Feurer C, Megnee C and Stabelin H: Effects of the new antilymphocytic peptide cyclosporin A in animals. *Immunology* **32**, 1017–1025 (1977)
- 5 Calne RY, White DJG, Thiru S, McMaster P, Graddock GN, Evans DB, Dunn DC, Pentlow BD and Rolles K: Cyclosporin A in patients receiving renal allograft from cadaver donors. *Lancet* **ii**, 1323–1327 (1978)
- 6 Powles RL, Clink H, Spence D, Mogenstern G, Watson JD, Selby PJ, Woods M, Barrett A, Jameson B, Sloane J, Lawler SD, Kay HEM, Lawson D, McElwain TJ and Alexander P: Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone marrow transplantation. *Lancet* **i**, 327–329 (1980)

- 7 White DJG, Plumb A and Calne RY: Mode of action of cyclosporine A, a new immunosuppressive agent. *Transplant Proc* **11**, 855–859 (1979)
- 8 Hess AD, Tutschka PJ and Santos GW: Effect of cyclosporin A on lymphocyte responses in vitro. II. Induction of specific alloantigen unresponsiveness mediated by a nylon wool adherent suppressor cell. *J Immunol* **126**, 961–968 (1981)
- 9 Bunjes D, Hardt C and Rollinghoff M: Cyclosporine A mediates immunosuppression of cytotoxic T-cell responses while impairing the release of IL-1 and IL-2. *Eur J Immunol* **11**, 656–661 (1981)
- 10 Yoshimura N and Kahan BD: Pharmacodynamic assessment of in vivo cyclosporine effect on interleukin-2 production by lymphocytes kidney transplant recipients. *Transplantation* **40**, 661–666 (1985)
- 11 Yoshimura N, Oka T, Kita M, Teraoka H and Hirai Y: Combination therapy of cyclosporine with steroid inhibits gamma-interferon and interleukin-1 gene expression at the level of mRNA synthesis in vivo. *J Clin Immunol* **6**, 322–328 (1989)
- 12 Abbud-Filho M, Kupiec-Weglinski JW, Araujo JL, Heideche CD, Tikney NL and Strom TB: Cyclosporine therapy of rat heart allograft recipients and release of interleukins (IL-1, IL-2, IL-3). A role for IL-3 in graft tolerance? *J Immunol* **133**, 2582–2586 (1984)
- 13 Wang BS, Heacock EH, Chang-Xue Z, Tilney NL, Strom TB and Mannick JA: Evidence for the presence of suppressor T cells in animals treated with cyclosporin A. *J Immunol* **128**, 1382–1388 (1982)
- 14 Hess AD and Tutschka PJ: Effect of CsA on human lymphocyte responses in vitro. I. CsA allows for the expression of alloantigen activated suppressor cells while preferentially inhibiting the induction of cytolytic effector lymphocytes in MLR. *J Immunol* **124**, 2601–2608 (1980)
- 15 Dos Reis GA and Shevach EM: Effect of cyclosporine A on T cell function in vitro. The mechanism of suppression of T cell proliferation depends on the nature of the T cell stimulus as well as the differentiation state of the responding T cell. *J Immunol* **129**, 2360–2367 (1982)
- 16 Yoshimura N, Matsui S, Hamashima T, Kita M and Oka T: The in vivo immunosuppressive action of suppressor cells from alloantigen cyclosporine treated mice and capacity of spleen cells to release interleukins (IL-1, IL-2, IL-3) and interferon. *Transplantation* **45**, 157–162 (1988)
- 17 Hess AD, Donnenberg A and Tutschka PJ: Effect of cyclosporine on human lymphocyte responses in vitro. V. Analysis of responding T-lymphocyte subpopulation in primary MLR with monoclonal antibodies. *J Immunol* **130**, 717–721 (1983)
- 18 Merker MM and Handschumacher RE: Uptake and nature of the intracellular binding of cyclosporine A in a murine thymoma cell line, BW5147. *J Immunol* **132**, 3064–3070 (1984)
- 19 Takahashi N, Hayano T and Suzuki M: Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* **337**, 473–475 (1989)
- 20 Liu J, Farmer JD, Lane WS, Freidman J, Weissman L and Schreiber SL: Calcineurin is a common target of cyclophilin-cyclosporin and FKBP-FK506 complex. *Cell* **86**, 807–815 (1991)
- 21 Oka T, Ohmori Y, Aikawa I, Yasumura T, Yoshimura N and Yoshida T: Early conversion from cyclosporine to combination therapy with azathioprine in living related kidney transplantation. *Transplant Proc* **21**, 1628–1630 (1989)
- 22 The Japanese CsA Study Group: The second report of a Japanese multi-center cooperative study for ciclosporin in renal transplantation. *Jpn J Transplant* **21**, 255–278 (1986)
- 23 Kahan BD: Cyclosporine. *N Engl J Med* **321**, 1725–1738 (1989)
- 24 Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaki M, Aoki H and Ochiai T: FK506, a novel immunosuppressant isolated from a streptomycetes. *J Antibiot (Tokyo)* **40**, 1256–1265 (1987)
- 25 Japanese FK506 Study Group: Japanese study of FK506 on kidney transplantation: Results of an early phase II study. *Transplant Proc* **23**, 3071–3074 (1991)
- 26 Shapiro R, Jordan M, Scantlebury V, Fung J, Jensen C, Tzakis A and McCauley J: FK 506 in clinical kidney transplantation. *Transplant Proc* **23**, 3065–3067 (1991)
- 27 Japanese FK506 Study Group: Japanese study of FK506 on kidney transplantation: Results of late phase II study. *Transplant Proc* **25**, 649–654 (1993)
- 28 Todo S, Fung JJ, Tzakis A, Demetris AJ, Jain A, Alessiani M, Takaya S, Day R, Gorden R and Starzl TE: One hundred ten consecutive primary orthotopic liver transplantation under FK506 in adults. *Transplant Proc* **23**, 1397–1402 (1991)
- 29 Fung JJ, Abu-Elmagd K, Jain A, Gordon R and Tzakis A: A randomized trial of primary liver transplantation under immunosuppression with FK506 versus cyclosporine. *Transplant Proc* **23**, 2977–2983 (1991)
- 30 Randhawa RS, Shapiro R, Jordan ML, Starzl TE and Demetris AJ: The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK506. Clinical significance and comparison with cyclosporine. *Am J Surg Pathol* **17**, 60–68 (1993)
- 31 Umezawa H, Ishizuka M and Takeuchi T: Suppression of tissue graft rejection by spargualin. *J Antibiot (Tokyo)* **38**, 283–284 (1985)
- 32 Nishimura K and Tokunaga T: Mechanisms of action of 15-deoxyspergualin. I. Suppressive effect on the induction of alloreactive secondary cytotoxic T lymphocytes in vivo and in vitro. *Immunology* **68**, 66–71 (1989)
- 33 Suzuki S and Amemiya H: 15-Deoxyspergualin – a novel immunosuppressant, experimental studies and clinical trial. *Transplant Immunol Lett* **7**, 17 (1990)
- 34 Morris RE, Hoyt EG, Murphy MP, Eugui EM and Allison AC: Mycophenolic acid morpholinoethyl ester (RS-61443) is a new immunosuppressive that prevents and halts heart allograft rejection by selective inhibition of T and B cell purine synthesis. *Transplant Proc* **22**, 1659–1662 (1990)
- 35 Platz KP, Sollinger HW, Hullett DA, Eckhoff DE, Eugui EM and Allison AC: RS-61443, a new immunosuppressive agent. *Transplantation* **51**, 27–31 (1990)
- 36 Sollinger HW, Eugui EM and Allison AC: RS-61443: Mechanisms of action, experimental and early clinical results. *Clin Transplant* **5**, 523–526 (1992)
- 37 RS-61443 Investigation Committee–Japan: Pilot study of mycophenolic mofetil (RS-61443) in the prevention of acute rejection following renal transplantation in Japanese patients. *Transplant Proc* **27**, 1421–1425 (1995)
- 38 Martel RR, Klicius J and Galet S: Inhibition of the immune response by rapamycin, a new antifungal antibiotics. *Can J Physiol Pharmacol* **55**, 48–51 (1975)
- 39 Dumont FJ, Staruch MJ, Koprak SL, Melino MR and Sigal

- NH: Distinct mechanism of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. *J Immunol* **144**, 251–258 (1990)
- 40 Kuo CJ, Chung J, Florentino DF, Flanagan WM, Blenis J and Crabtree GR: Rapamycin selectively inhibits IL-2 activation of P7056 kinase. *Nature* **358** 70–73 (1992)
- 41 Cramer DV, Chapman FA, Jaffee BD, Jones EA, Knoop M, Hreha-Elras G and Makawaka L: The effect of a new immunosuppressive drug, brequinar sodium, on heart, liver and kidney allograft rejection in the rat. *Transplantation* **53**, 303–308 (1992)
- 42 Kahan BD, Chang JY and Sehgal SN: Preclinical evaluation of a new potent immunosuppressive agent, rapamycin. *Transplantation* **52**, 185–191 (1991)
- 43 Morris RE, Meiser BM, Wu J, Shorthouse R and Wang J: Use of rapamycin for the suppression of alloimmune reaction in vivo: Schedule dependence, host-versus-graft and graft-versus-host reaction. *Transplant Proc* **23**, 521–524 (1991)
- 44 European Multicentre Trial Group: Cyclosporin in cadaveric renal transplantation: one-year follow-up of a multicentre trial. *Lancet* **2**, 986–989 (1983)
- 45 The Canadian Multicentre Transplant Study Group: A randomized clinical trial of cyclosporine in cadaveric renal transplantation. *N Engl J Med* **314**, 1219–1225 (1986)
- 46 Ochiai T, Toma H, Takagi H, Oka T, Ishibashi M, Kashiwabara H, Fukao K, Ota K, Hashimoto I, Sonoda T and Iwasaki Y: The second report of a Japanese multi-center cooperative study for cyclosporin in renal transplantation. *Jpn J Transplant* **23**, 255–278 (1986)
- 47 Spees EK, Krakauer H, Hodges JF and Milan R: The current experience with cyclosporine in the United States: Living related kidney transplant recipients. *Transplant Proc* **17**, 2657–2659 (1985)
- 48 Spees EK, Krakauer H, Hodges JF and Milan R: The current experience with cyclosporine in the United States: Cadaver kidney transplantation. *Transplant Proc* **17**, 2660–2662 (1985)