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Tacrolimus

A Review of its Pharmacology, and Therapeutic Potential in Hepatic and Renal Transplantation

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Synopsis

Tacrolimus (FK 506) is a macrolide immunosuppressant which possesses similar but more potent immunosuppressant properties compared with cyclosporin, inhibiting cell-mediated and humoral immune responses. Like cyclosporin, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. This has caused difficulty in defining the optimum dosage regimen and has highlighted the usefulness of therapeutic drug monitoring. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomised investigations of tacrolimus versus cyclosporin, particularly in renal transplantation. Despite these drawbacks, tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids in adult and paediatric recipients following liver or kidney transplantation. Indeed, graft salvage rates in patients experiencing rejection or drug-related toxicity were ≥50%, although data in renal transplantation are limited. Compared with cyclosporin as a primary immunosuppressant, tacrolimus showed comparable or greater patient/graft survival rates in liver allograft recipients (where cost savings associated with reduced hospitalisation costs were evident in one study), and comparable patient/graft survival in patients following kidney transplantation. Worthy of note was the efficacy of tacrolimus as a primary immunosuppressant in patients who received en bloc *kidney allografts. The incidence of rejection was largely reduced following rescue therapy with tacrolimus and was generally lower (notably for refractory rejection) than that observed for cyclosporin, at least in liver allograft recipients. This was reflected in less need for adjunct immunotherapy including antilymphocyte preparations for the treatment of rejection episodes. The potential for reduction or withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with cyclosporin, and this may be enhanced by the reduced incidence of infectious complications and of hypertension and hypercholesterolaemia reported by some investigators. In other respects, however, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporin.*

Against this background, preliminary data indicate that tacrolimus provides a valuable therapeutic alternative to retransplantation in patients experiencing liver or kidney graft rejection or drug-related toxicity. Pending confirmation of initial randomised studies and preliminary results from large randomised investigations, tacrolimus may well be considered as an alternative primary immunosuppressant to cyclosporin in hepatic (particularly) and renal transplantation. Furthermore, the steroid-sparing effects of tacrolimus, although of benefit to all patient groups, may prove to be of particular worth in children and in en bloc *kidney recipients. In these patients tacrolimus may well emerge as the drug of choice.*

Clearly, further experience in the clinical setting will help clarify the role of tacrolimus in transplantation surgery. Nevertheless, this new immunosuppressant has already demonstrated its usefulness as an addition to the limited immunotherapeutic options available to date.

Pharmacodynamic Properties

The macrolide immunosuppressant, tacrolimus, displays similar, but more potent, immunosuppressive properties to cyclosporin, inhibiting cell-mediated and humoral immune responses. Through its interaction with a specific cytoplasmic immunophilin, tacrolimus inhibits calcium-dependent signal transduction pathways in T cells, thereby preventing transcription of a discrete

set of lymphokine genes. *In vitro*, tacrolimus is 10 to 100 times more potent than cyclosporin in inhibiting allogen- and mitogen-induced stimulation of T cell proliferation and the production of interleukin (IL)-2 and other growth-promoting cytokines (IL-3, IL-4, interferon-γ, tumour necrosis factor-α and granulocyte-macrophage colony-stimulating factor), the mixed lymphocyte response, generation of cytotoxic T cells, and B cell activation. Tacrolimus does not, however, inhibit the secondary proliferation of activated T cells in response to IL-2. *In vivo*, tacrolimus is approximately 10 times more potent than cyclosporin in suppressing T cell-dependent antibody production, graft-versus-host reactivity and delayed type hypersensitivity. Tacrolimus interferes with a variety of exocytosis-related events in cells of haematopoietic lineage (basophils, neutrophils and mast cells), but at concentrations which suppress T cell proliferation, tacrolimus does not modify mononuclear phagocyte function. Tacrolimus displays hepatotrophic properties, stimulating hepatic regeneration following hepatectomy, and confers hepatoprotective and renoprotective effects against ischaemia/reperfusion injury. Tacrolimus has a direct glomeruloconstrictive effect and on subacute administration reduces renal perfusion and glomerular flow. Tacrolimus-induced nephrotoxicity has variously been attributed to altered prostaglandin metabolism, lipid peroxidation of the plasma membrane, and enhanced endothelin secretion. In animal models of organ transplantation, tacrolimus has been shown to prolong survival of hepatic, renal, cardiac, small intestine, pancreatic and skin allografts, and to reverse cardiac and renal allograft rejection.

Pharmacokinetic Properties

Enzyme-linked immunoabsorbant assay (ELISA) employing a monoclonal anti-tacrolimus antibody has been used to quantify tacrolimus (and its immunoreactive metabolites) in biological fluids. Absorption of tacrolimus following oral administration is highly variable, as reflected in a peak plasma concentration (C_{max}) of 0.4 to 5.6 μg/L after a single oral dosage of 0.15 mg/kg. C_{max} values following intravenous infusion of tacrolimus 0.15 mg/kg over 2 hours ranged from 10 to 24 μg/L. Tacrolimus is highly lipophilic and undergoes extensive tissue distribution. In blood, tacrolimus is sequestered by erythrocytes, with the result that plasma drug concentrations are approximately 10 to 30 times lower than whole blood concentrations. Tacrolimus is metabolised extensively in the liver, primarily by demethylation and hydroxylation, with less than 1% of the parent compound being excreted unaltered in the bile and urine. Hepatic dysfunction is associated with elevated plasma concentrations of tacrolimus, prolongation of the plasma elimination half-life, and reduced plasma clearance.

Clinical Efficacy

The vast majority of investigations which evaluated the efficacy of tacrolimus as a rescue or primary immunosuppressive therapy in liver or kidney allograft recipients were presented at symposia and were only briefly reported or subsequently published to document symposia proceedings. The results of these studies should therefore be interpreted with caution as they have not been subjected to extensive peer review. Furthermore, there has only been one large study which evaluated the efficacy of tacrolimus as a rescue therapy in renal allograft recipients and there is a paucity of randomised investigations of tacrolimus versus cyclosporin as a primary immunosuppressant, particularly in renal transplantation.

In paediatric/adult patients experiencing liver allograft rejection or adverse effects associated with conventional immunosuppressive therapy who were subsequently treated with tacrolimus plus corticosteroids, patient survival was $\geq$85% and graft survival exceeded 70% at initial follow-up ($\geq$2 months). These results were maintained in the longer term ($\approx$9 to 18 months) in one study, although actuarial patient and graft survival rates were 70 or 72% and 50 or 70%, respectively, after 12 months in 2 others. Rescue therapy with tacrolimus was associated with histological and biochemical improvements in allograft function in a large proportion of patients, and this reflected in improvements in performance status when assessed in one study. Histological assessment of graft function revealed that prognosis was notably superior in patients with acute versus chronic rejection in one investigation; however, in another more detailed study, outcome was dependent on preconversion liver and kidney function, and on a history of retransplantation.

When administered as a primary immunosuppressant in combination with corticosteroids to

patients who received a primary liver transplant, therapy with tacrolimus in the medium term (6 to 18 months) was associated with patient survival rates generally ≥84% (although 63% was obtained in one study), and graft survival rates approaching or exceeding 80%. There were no significant differences in patient or graft survival rates for tacrolimus versus cyclosporin in the 2 largest randomised investigations (total patient numbers of 520 and 545), although both these parameters were numerically greater for tacrolimus in one of these studies (+4 to +5%). In the majority of the other comparative investigations evaluated, graft and patient survival rates were statistically and/or numerically greater with tacrolimus than with cyclosporin (+8 to +17% and +15 to +20%, respectively). Retransplantation rates were either similar or lower with tacrolimus compared with cyclosporin. Furthermore, in one small randomised investigation, 73% of cyclosporin-treated patients were switched to therapy with tacrolimus, and rejection accounted for the switch in approximately 75% of these recipients. Comparison of hospital costs of liver transplantation revealed that primary immunosuppression with cyclosporin was nearly twice as expensive as that with tacrolimus.

Although only assessed in one large investigation, tacrolimus has demonstrated efficacy as a rescue therapy when administered in combination with corticosteroids to kidney allograft recipients experiencing rejection despite optimum standard immunotherapy. Patient survival exceeded 90%, and 70% of patients were successfully converted after a mean follow-up of 10.6 months. Serum creatinine levels and the overall need for dialysis declined.

In adult or paediatric kidney allograft recipients, administration of tacrolimus as a primary immunosuppressant, plus corticosteroids, resulted in patient survival of ≥90% and graft survival of ≥70%, during follow-up periods of 1 to 21 months. In patients at lower risk of treatment failure, however, graft survival up to 1 year was noticeably greater (≈80%). Patient and graft survival rates did not differ significantly between patients receiving tacrolimus or cyclosporin, or those treated with tacrolimus in the presence or absence of azathioprine. Worthy of note was the efficacy of tacrolimus in recipients of paediatric *en bloc* allografts, in whom urological complications did not manifest, even in grafts from the smallest of donors.

The incidence of rejection with tacrolimus was largely reduced following rescue therapy, and was generally lower than that noted for cyclosporin when administered as a primary immunosuppressant therapy in liver allograft recipients. Notably, in the latter setting, the overall and refractory rejection incidence was significantly less in patients treated with tacrolimus versus cyclosporin in 2 large randomised investigations. Not surprisingly, the lower incidence of rejection noted for patients treated with tacrolimus generally reflected less need for adjunct immunotherapy (azathioprine, high dose steroids and antilymphocyte preparations) for the treatment of rejection episodes. Tacrolimus was also associated with a steroid-sparing effect, which resulted in lower maintenance steroid requirements when compared with cyclosporin. Indeed, steroid withdrawal was often possible for patients (notably children) treated with tacrolimus. The incidence of hypertension was also lower with tacrolimus in some (but not all) investigations when compared with cyclosporin, and tacrolimus was not associated with hypercholesterolaemia.

Tolerability

The variety of tacrolimus dosage regimens employed in clinical investigations has complicated interpretation of the tolerability profile of this drug. Indeed, its adverse effects have been pivotal in the temporal refinement of tacrolimus dosage regimens. Generally, toxicity is reduced by lowering the tacrolimus dosage, although such measures may not improve some reactions (such as the development of dysarthrias) which may be idiosyncratic or require multiple factors to emerge.

The clinical presentation and morphology of tacrolimus nephrotoxicity are identical to those of cyclosporin, and the incidence of this adverse effect is broadly similar for patients treated with either drug. The incidence of nephrotoxicity necessitating withdrawal from therapy was similar for tacrolimus and cyclosporin in a large randomised study when the former drug was administered at initial intravenous dosages ≤0.06 mg/kg, but was greater when these initial dosages were exceeded. In another large study of similar design haemodialysis requirements were similar for tacrolimus and cyclosporin recipients. Acute nephrotoxicity (characterised by increased serum creatinine levels within 1 month of treatment), has occurred in most tacrolimus recipients and

necessitated haemodialysis in up to about 25% of patients who received a liver allograft. Chronic nephrotoxicity (after 1 month of treatment) has been reported to occur in about 30 to 50% of patients. Nephrotoxicity may respond to a reduction in tacrolimus dosage, although reports differ as to the degree of correlation between plasma tacrolimus concentrations and renal function variables. In one study nephrotoxicity, assessed by histopathological changes in biopsy specimens, was recorded with trough whole blood tacrolimus concentrations within the currently accepted optimum range (≈15 to 20 μg/L). Mild hyperkalaemia, associated with low or low-normal renin and aldosterone levels commonly occurs with tacrolimus therapy, but usually responds to treatment with potassium-binding resins, potassium-restricted diets and/or fludrocortisone. Cyclosporin and tacrolimus appear to induce a similar incidence of nephrotoxicity, and although similar changes in serum creatinine levels occur with either drug following transplantation, adverse effects on glomerular filtration rate appear to occur less severely with tacrolimus in the long-term. Histopathological examination of renal allografts from patients experiencing rejection revealed some of the features associated with cyclosporin toxicity in patients treated with tacrolimus; however, the effect of prolonged tacrolimus administration on renal structural integrity with prolonged requires further study.

Infections have been reported to occur in ≤50% of patients treated with tacrolimus, and were severe in 38% of the population studied. In clinical trials, tacrolimus was often associated with a numerical and/or statistical lower incidence of overall, severe, bacterial, viral or fungal infections when compared with cyclosporin. Post-transplant lymphoproliferative disorders apparently occur with a similar incidence among patients treated with either tacrolimus or cyclosporin (<2%).

Neurological adverse effects associated with post-transplant tacrolimus immunosuppression most commonly occur with intravenous administration and can be categorised as major (e.g. akinetic mutism, expressive aphasia, seizures, confusion requiring investigation, psychosis, encephalopathy, persistent coma) or minor (e.g. tremors, headache, sleep disturbances, nightmares, dysesthesias, photophobia) neurotoxicity. Major neurotoxicity has been reported in less than 10%, and minor neurotoxicity in about 20% of patients. In most cases tacrolimus-induced neurological effects resolve with dosage reduction or withdrawal from therapy. The incidence of neurotoxicity necessitating withdrawal from therapy was similar for tacrolimus and cyclosporin when the former drug was administered at initial intravenous dosages ≤0.06 mg/kg in a large randomised study, but was greater when these initial dosages were exceeded.

Association of post-transplant hyperglycaemia with tacrolimus is confounded by the influence of perioperative events in the short term. With chronic administration (≥3 months), hyperglycaemia requiring insulin therapy appears to vary temporally; up to 20% of patients require insulin at 6 months but as few as 5.5% require it after 1 year. The incidence of new-onset diabetes among paediatric allograft recipients treated with tacrolimus appears to be low (<2%). Insulin-dependent new-onset diabetes does not appear to occur more frequently with tacrolimus than with other immunosuppressive regimens, but the diabetogenic effect of this drug is likely to be as significant. Indeed, the incidence of hyperglycaemia was greater with tacrolimus when compared with cyclosporin in one large randomised investigation, although the converse was seen among children treated with either of these drugs in another.

Although hypertension is a common finding in transplant recipients, treatment with tacrolimus allows withdrawal of antihypertensive therapy from a significant portion of patients. Furthermore, cessation of antihypertensive therapy is generally more common with tacrolimus than with cyclosporin. Gingival hyperplasia and hirsutism, which are complications of therapy with cyclosporin, do not appear to be of significance among patients treated with tacrolimus. Furthermore, hypercholesterolaemia is apparently not a complication of tacrolimus therapy.

In common with cyclosporin, tacrolimus has been associated with rare instances of acute haemolytic anaemia; 8 of 1400 patients treated with the drug developed this phenomenon at one centre in the US.

Dosage and Administration

In hepatic or renal transplantation tacrolimus has usually been administered intravenously commencing after revascularisation of the graft and continuing until oral administration was feasible. However, the optimum dosage regimen for tacrolimus is continuing to be refined. Most

recent experience in large randomised studies indicate that initial continuous intravenous dosages of ≤0.1 mg/kg/day or 0.035 to 0.075 mg/kg/day should be employed, with subsequent initial oral dosages of 0.1 to 0.2 mg/kg/day. Preoperative administration of tacrolimus has been rare, although it has been given orally at 0.15 mg/kg (single dose) or intravenously at 0.15 mg/kg/12h for 2 days before renal transplantation. In the clinical setting dosage adjustments are made based on liver and kidney function, rejection status and trough tacrolimus concentrations in plasma or whole blood. Dosage requirements generally decline temporally, but long term therapy is necessary. Maintenance tacrolimus dosages expressed as a function of bodyweight are greater in children than in adults. A computer programme is available to select the optimum tacrolimus dosage based on patient characteristics.

Tacrolimus should not be administered with cyclosporin due to potential nephrotoxicity. In patients who simultaneously receive drugs which are metabolised by the cytochrome P450 IIIa enzyme system tacrolimus should be administered with caution.

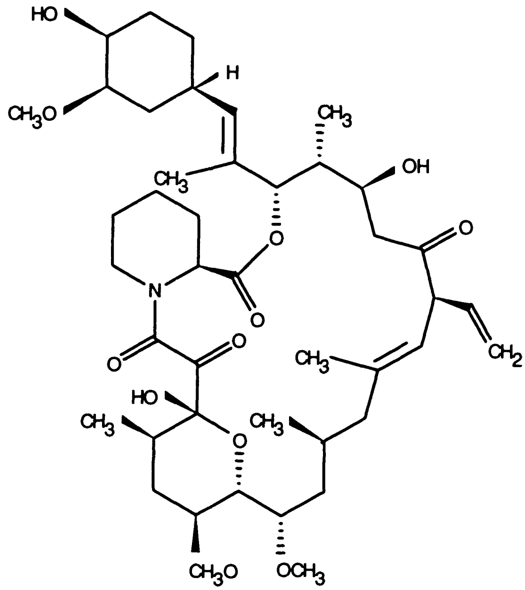
Tacrolimus (FK 506), a hydrophobic macrocyclic lactone (fig. 1), was first isolated in 1985 from the fermentation broth of *Streptomyces tsukubaensis*, a soil microorganism found in the Tsukuba region of northern Japan (Kino et al. 1987a; Tanaka et al. 1987). Although structurally unrelated to cyclosporin, tacrolimus shows a similar spectrum of immunosuppressive effects to this agent at the cellular and molecular level. Initial studies indicated that tacrolimus was a powerful immunosuppressant, displaying approximately 100-fold greater *in vitro* potency than cyclosporin in inhibiting T cell activation. Subsequent *in vivo* studies have shown tacrolimus to be effective both in suppressing spontaneous and experimental autoimmune disease, and in preventing allograft and xenograft rejection in animal models of organ transplantation. Although tacrolimus has been used clinically in the treatment of rejection following transplantation with a variety of organs and also as an immunosuppressive agent in the therapy of autoimmune disorders, the focus of this review is its use in hepatic and renal transplantation.

1. Pharmacodynamic Properties

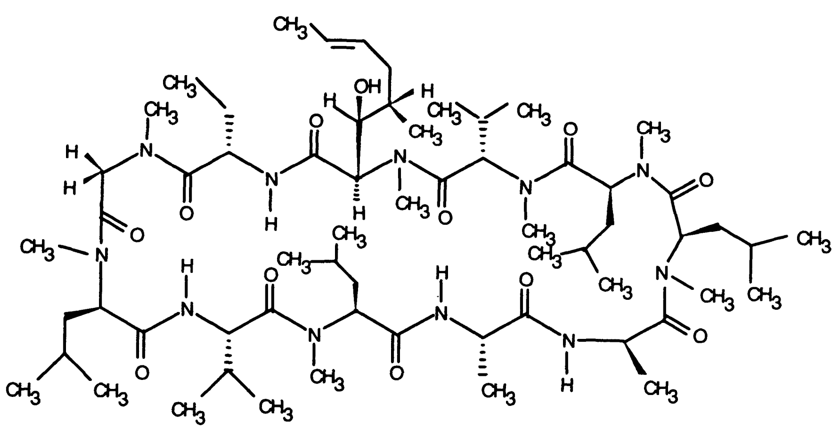
1.1 Intracellular Mechanism of Action

In common with cyclosporin and rapamycin, tacrolimus binds with high affinity to a family of cytoplasmic immunosuppressant binding proteins (immunophilins). FK 506 binding protein-12 (FKBP12), the predominant tacrolimus-binding immunophilin, is a peptidyl-prolyl *cis-trans* isomerase (rotamase) [Harding et al. 1989; Siekierka et al. 1989a,b] which catalyses proline peptide bond isomerisation, a rate limiting step in protein folding (Rothman 1989; Tropschug et al. 1990). Although tacrolimus is a potent inhibitor of rotamase activity [inhibition constant K_i = 0.2 to 1.7 nmol/L (Bierer et al. 1990a; Harrison & Stein 1990)], it is unlikely that this effect is directly implicated in the drug's immunosuppressive action (Bierer et al. 1990c; Sigal et al. 1990). Instead, the immunophilin-drug complex is proposed as the biologically active moiety which interacts with intracellular molecules involved in signal transduction (Bierer et al. 1990a,c; DeFranco 1991; Schreiber 1991). The tacrolimus-FKBP12 complex specifically and competitively binds to and inhibits calcineurin (Fruman et al. 1992; Lane et al. 1993; Liu et al. 1991, 1992), a calmodulin- and calcium-dependent serine/threonine phosphatase (Stewart et al. 1982).

Calcineurin is believed to be the common molecular target mediating the immunosuppressive actions of both tacrolimus and cyclosporin (Schreiber & Crabtree 1992). Support for this hypothesis comes from recent evidence that calcineurin is a tacrolimus-sensitive component of the T cell receptor-mediated signal transduction pathway (Clipstone & Crabtree 1992; O'Keefe et al. 1992), and the demonstration of a close correlation between the inhibitory effects of immunophilin-bound tacrolimus and cyclosporin analogues on calcineurin phosphatase activity and their suppression of



Tacrolimus



Cyclosporin

Fig. 1. Structural formulae of the immunosuppressive agents tacrolimus and cyclosporin.

interleukin (IL)-2 gene transcription (Liu et al. 1992).

Inhibition of calcineurin phosphatase activity by the tacrolimus-FKBP12 complex may impair the generation and/or activation of nuclear transcription factors required for lymphokine gene expression. Tacrolimus blocks IL-2 gene transcription mediated by the interaction of specific nuclear transcription factors with their respective DNA binding sites on the IL-2 gene enhancer/promoter (Banerji et al. 1991; Baumann et al. 1991; Brabletz et al. 1991; Chang et al. 1991; Granelli-Piperno et

al. 1990; Henderson et al. 1991; Mattila et al. 1990; Su & Semerjian 1991). Among these, the T cell-specific cytosolic nuclear factor of activated T cells (NF-AT) is of particular interest, as it appears to be responsible for the restricted expression of IL-2 by antigen-stimulated T cells. Tacrolimus blocks the calcium-dependent nuclear translocation of the cytosolic subunit of NF-AT in human T cells (DeFranco 1991; Flanagan et al. 1991; McCaffrey et al. 1993), a process apparently dependent on calcineurin-mediated dephosphorylation (DeFranco 1991).

Both cyclosporin and tacrolimus appear to block transcription of a discrete set of early-phase T cell activation genes, including the *c-myc* proto-oncogene and those encoding IL-2, IL-3, IL-4, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), but they do not affect the transcription of late-phase genes such as the tumour necrosis factor-β (TNF-β), IL-2 receptor, and transferrin receptor genes (Dumont et al. 1990a,b; Hanke et al. 1992; Mattila et al. 1990; Metcalfe & Richards 1990; Sakuma et al. 1988; Staruch et al. 1989; Tocci et al. 1989). Tacrolimus additionally induces transcription of *krox-24*, a putative transcriptional activator (Dumont & Altmeyer 1990; Metcalfe & Richards 1990), and the transforming growth factor-β (TGF-β) gene (Rao et al. 1991). Tacrolimus can also selectively enhance IL-2 and GM-CSF mRNA degradation in T cells, indicating that it modulates IL-2 and GM-CSF gene expression at both the transcriptional and post-transcriptional level (Hanke et al. 1992). Expression of mRNA for the IL-2 receptor (Sakuma et al. 1988) and mRNAs for IL-1α or IL-1β in lipopolysaccharide (LPS)-stimulated human monocytes (Tocci et al. 1989) is, however, unaffected by tacrolimus. Mitogen-stimulated DNA replication in T cells is minimally affected by tacrolimus (Kimball et al. 1990), thereby suggesting that the drug's antiproliferative effect is mediated by a mechanism(s) independent of DNA replication activation.

The nephrotoxic and immunosuppressive actions of tacrolimus appear to be mechanistically related, with inhibition of calcineurin phosphatase activity representing a possible common feature (Dumont et al. 1992). Both tacrolimus and cyclosporin selectively inhibit expression of the renal phosphoenolpyruvate carboxykinase gene (Morris et al. 1991), thereby suggesting that inhibition of specific transcription factors in the proximal tubule may be critical in the pathogenesis of nephrotoxicity.

1.2 Immunomodulating Properties of Tacrolimus

T cell activation in response to antigen-specific T cell receptor recognition is associated with integrin-mediated cell adhesion, increased phosphatidylinositol turnover, generation of the intracellular second messengers inositol triphosphate and diacylglycerol (resulting, respectively, in calcium release from the endoplasmic reticulum and activation of protein kinase C), and phosphorylation of cytosolic proteins (Crabtree 1989; Krensky et al. 1990; Nisbet-Brown et al. 1985) [fig. 2]. Once initiated, these biochemical processes lead to the coordinated expression of gene products, most notably IL-2, crucial for lymphocyte growth and proliferation.

In common with cyclosporin, tacrolimus does not inhibit T cell adhesion (Eiras et al. 1991), the generation of early second messengers (Bierer et al. 1990b; Fidelus & Laughter 1986; Fujii et al. 1989) or the increase in intracellular calcium (Bierer et al. 1990b; Jordan et al. 1991) subsequent to T cell receptor recognition; however, the drug does appear to block more distal components of the T cell activation pathway that link these early membrane-associated events and gene expression (Sigal & Dumont 1992; Sigal et al. 1990). Furthermore, tacrolimus appears to be selective for a subset of calcium-associated signal transduction pathways (Bierer et al. 1990a; Dumont et al. 1990b; Mattila et al. 1990), and these may predominate in the T cell receptor-triggered cascade leading to lymphokine production, thereby accounting for the drug's preferential effect on the immune system (Chang et al. 1991).

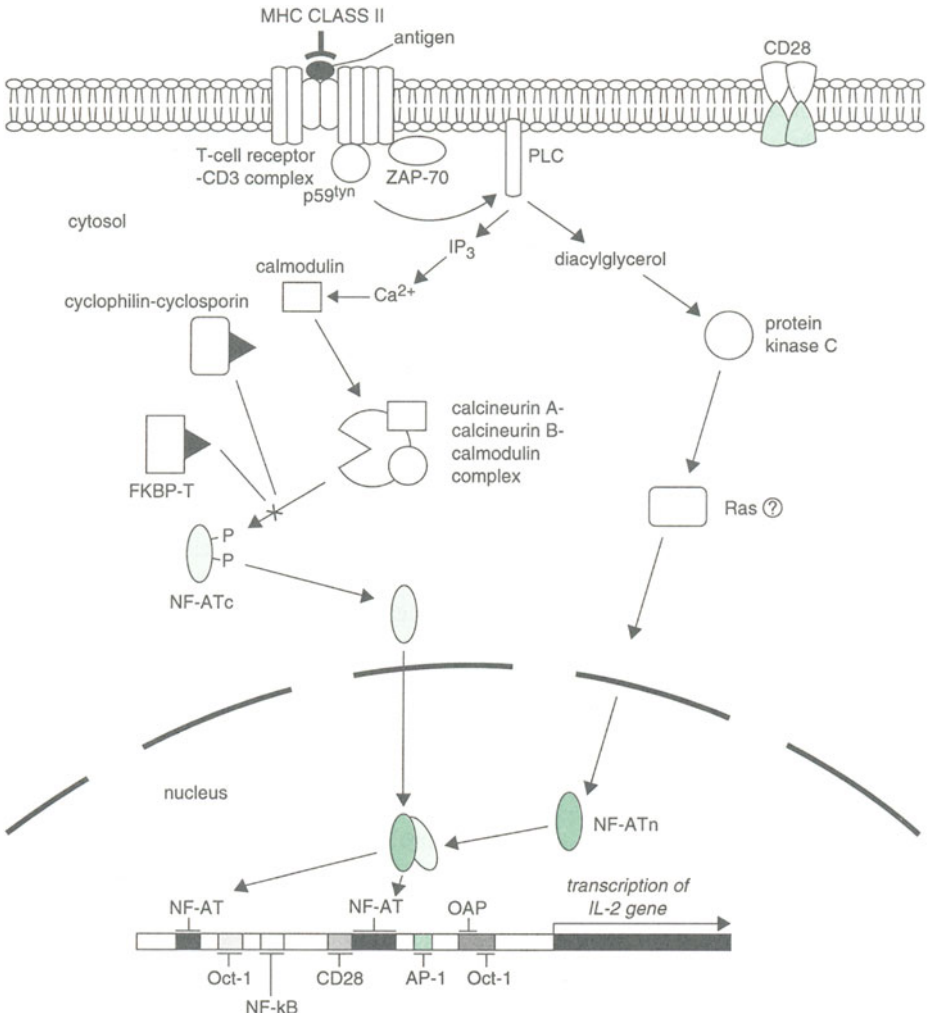


Fig. 2. T cell receptor-mediated signal transduction pathways leading to interleukin-2 (IL-2) transcription. Antigen-stimulated T cell activation via the T cell receptor leads to phospholipase C (PLC)-mediated generation of diacylglycerol and inositol triphosphate (IP_3), activation of protein kinase C, an increase in cytosolic calcium levels, formation of an activated calmodulin-calcineurin complex, and formation of a competent transcription activator, the nuclear factor of activated T cells (NF-AT). Tacrolimus (T) blocks nuclear translocation of the cytoplasmic subunit ($NF\text{-}AT_c$) of the NF-AT transcription factor. Both the nuclear ($NF\text{-}AT_n$) and cytoplasmic ($NF\text{-}AT_c$) subunits of the NF-AT transcription factor are required for DNA binding and gene transcription (after Liu 1993). *Other Abbreviations:* AP-1, CD28, OAP, Oct-1, NF-AT and NF-kB = Factors involved in the transcription of the IL-2 gene FKBP = FK 506 binding protein.

1.2.1 Effects on T Cells

In the thymus, developing T cells acquire the antigenic and functional characteristics of mature T cells as they migrate from the cortex to the medulla, before being returned to the circulation. During this process of thymic maturation, immature thymocytes bearing self-reactive T cell receptors are deleted (clonal elimination). Tacrolimus appears to inhibit thymocyte differentiation (Pugh-Humphreys et al. 1990; Thomson & Pugh-Humphreys

1991) and the expression of major histocompatability complex (MHC) class II antigen on thymic epithelial cells (Takai et al. 1990). Tacrolimus additionally damages thymic epithelial cells (Pugh-Humphreys et al. 1990; Stephen et al. 1989; Thomson & Pugh-Humphreys 1991) and prevents programmed cell death (apoptosis) of antigen- and mitogen-stimulated T cell hybridomas (Bierer et al. 1991; Staruch et al. 1991), suggesting a potentially deleterious effect on clonal elimination (Jenkinson et al. 1989). Thymocyte mobilisation appears to be unaffected by tacrolimus (Takai et al. 1990).

Tacrolimus potently inhibits the proliferative response of murine and human T cells to specific antigens, allogeneic lymphocytes and mitogenic lectins *in vitro* (Bierer et al. 1991; Dumont et al. 1990b; Kay et al. 1989a; Kino et al. 1987a,b; Sawada et al. 1987). Both antigen-specific proliferation of cloned helper and cytotoxic T cells (Borg & Kumagai 1991; Sawada et al. 1987) and the secondary proliferation of alloreactive T cells generated from mixed lymphocyte reactions (regarded as an *in vitro* correlate of allograft rejection) [Borg & Kumagai 1991; Henderson et al. 1991; Kino et al. 1987b; Lane et al. 1993; Thomas et al. 1990; Yoshimura et al. 1989a] and allograft biopsies (Zeevi et al. 1987) are sensitive to tacrolimus. Tacrolimus is approximately 100 times more potent than cyclosporin in inhibiting T cell proliferative responses, including mixed lymphocyte reactivity and cytotoxic T cell generation, displaying an IC_{50} (concentration required for 50% inhibition of response) of $\approx$ 0.1 nmol/L (Kay et al. 1989b; Kino et al. 1987a,b; Sawada et al. 1987; Woo et al. 1988). However, tacrolimus has no effect, at micromolar concentrations, on lymphokine (IL-2 and IL-4)-dependent T cell proliferation (Bierer et al. 1990b; Dumont et al. 1990a,b; Eiras et al. 1991; Kay et al. 1989a; Kino et al. 1987b; Sawada et al. 1987; Woo et al. 1988; Yoshimura et al. 1989a; Zeevi et al. 1987,1990a). The inhibitory effect of tacrolimus on T cell proliferation *in vitro* is partially reversed by addition of recombinant IL-2 (Dumont et al. 1990b; Lin et al. 1991), suggesting that, with the exception of IL-2, those gene products and pathways distal to IL-2 remain intact in cells treated with tacrolimus.

The ability of tacrolimus to inhibit lymphocyte proliferation and lymphokine production is governed by the manner of cellular activation. *In vitro* studies have indicated that tacrolimus inhibits calcium-dependent T cell activation triggered via the T cell receptor-CD3 complex (Bierer et al. 1991; Jiang et al. 1991; Johansson & Möller 1990; Kay & Benzie 1989; Lin et al. 1991), the cell surface CD2 receptor (Bierer et al. 1991; Kay & Benzie 1989), and the combination of protein kinase C activation and calcium influx (Dumont et al. 1990a,b; Kay et al. 1989b), but has no effect on calcium-independent T cell activation, such as that triggered via the CD28 surface molecule or protein kinase C activation alone (Bierer et al. 1991; Chang et al. 1991; Kay & Benzie 1989; Lin et al. 1991).

Tacrolimus blocks calcium-dependent T cell division between the resting phase (G0) and activation phase (G1) of the cell cycle, and suppresses the transcription of early phase cytokine genes (Chang et al. 1991; Morris et al. 1991). Consequently, an antiproliferative effect is only observed when the drug is added within the first few hours of T cell stimulation (Dumont et al. 1990b; Henderson et al. 1991; Kay et al. 1989b, 1990; Metcalfe & Richards 1990; Tocci et al. 1989).

Tacrolimus produces a selective inhibition of cytokine expression by antigen- and mitogen-stimulated T cells, suppressing IL-2, IL-3, IL-4, IL-5, IFN-γ, TNF-α, and GM-CSF production (Andersson et al. 1992; Kino et al. 1987b,c; Sawada et al. 1987; Tocci et al. 1989), IL-1-stimulated IFN-γ production (Dumont & Altmeyer 1990), as well as transferrin and IL-2 receptor expression on the T cell surface (Dumont et al. 1990a,b; Kino et al. 1987c; Lin et al. 1990; Sawada et al. 1987; Staruch et al. 1989; Tocci et al. 1989; Yoshimura et al. 1989a,b; Woo et al. 1990c), while leaving IL-6 (B cell stimulating factor 2) [Yoshimura et al. 1989b] and IL-10 (cytokine synthesis inhibitory factor) [Wang et al. 1991] production unaffected. These effects are achieved at concentrations (IC_{50} values of $\approx$ 0.1 nmol/L) which have no effect on murine

bone marrow colony formation *in vitro* (Kino et al. 1987b).

Tacrolimus suppresses mixed lymphocyte reactions and the generation of cytotoxic lymphocytes against allogeneic targets, indicating that one of the drug's actions on cellular immune responses is directed against T cells proliferating in response to an alloantigenic stimulus (Beck & Akiyama 1989). *In vitro* cellular cytotoxicity mediated by natural killer (NK) and killer (K) cells and antibody-dependent cell-mediated cytotoxicity is unaffected by tacrolimus (Beck & Akiyama 1989; Markus et al. 1991; Wasik et al. 1991). *In vivo*, tacrolimus is approximately 10 times more potent than cyclosporin in suppressing graft-versus-host reactivity and delayed type hypersensitivity in mice (Kino et al. 1987a).

1.2.2 Effects on B Cells

The proliferative response of murine and human B cells to anti-Ig antibody, mitogen or ionomycin plus the protein kinase C activator phorbol myristate acetate (PMA) is sensitive to tacrolimus at concentrations which inhibit T cell responses (Lagodzinski et al. 1991; Morikawa et al. 1992; Walliser et al. 1989; Wasik et al. 1990; Wicker et al. 1990), whereas lipopolysaccharide (LPS)-induced B cell proliferation is unaffected by the drug (Walliser et al. 1989; Wicker et al. 1990). *In vitro*, tacrolimus inhibits IgM and IgG production by mitogen-stimulated human B lymphocytes, but does not inhibit IL-6 production (Yoshimura et al. 1989b) or IL-6-induced IgM and IgG production (Stevens et al. 1991; Yoshimura et al. 1989b). *In vivo*, tacrolimus suppresses T cell-dependent IgM production by murine and rat splenic plasma cells (Kino et al. 1987a; Lagodzinski et al. 1991; Tsuji et al. 1990; Woo et al. 1988, 1990b). Although these effects may be attributed in part to inhibition of lymphokine production by activated T cells (Suzuki et al. 1990; Woo et al. 1988), tacrolimus also has a direct inhibitory effect on calcium-dependent B cell activation. Thus, tacrolimus blocks induction of TNF-α gene transcription by anti-Ig antibody in human B cells (Goldfeld et al. 1992). Tacrolimus additionally appears to inhibit human B cell proliferation in response to certain calcium-independent stimuli, including the protein kinase C activator PMA and IL-2 (Morikawa et al. 1992).

In contrast to the situation with the T cell, tacrolimus blocks B cell division in the late activation phase (G1) of the cell cycle, with the result that substantial inhibition of B cell proliferation is evident when the drug is added as late as 24 hours after stimulation with anti-IgG (Wicker et al. 1990). Moreover, tacrolimus causes cell death upon activation of murine B cells (Wicker et al. 1990), whereas in T cells it inhibits apoptosis (section 1.2.1).

1.2.3 Effects on Nonlymphoid Cells

At concentrations 10- to 100-fold higher than those required to inhibit T cell proliferation, tacrolimus partially suppresses IL-1α release from LPS-activated human monocytes and alveolar macrophages (Keicho et al. 1991), and inhibits TNF-α production by antigen- and mitogen-stimulated monocytes (Andersson et al. 1992).

Through its ability to block T cell lymphokine secretion, tacrolimus might be expected to affect *in vivo* antigen presentation by inhibiting lymphokine-mediated MHC expression. However, it appears unlikely that direct inhibition of antigen processing contributes significantly to the drug's immunosuppressive action (Sigal & Dumont 1992). Moreover, at concentrations which markedly depress T cell proliferation, tacrolimus does not appreciably modify mononuclear phagocyte function (Woo et al. 1990a).

Tacrolimus has been shown to interfere with a variety of exocytosis-related events in cells of haematopoietic lineage, including IgE receptor-mediated histamine and serotonin release from a rat mast cell line (Hultsch et al. 1991), human skin mast cells (De Paulis et al. 1992) and human basophils (De Paulis et al. 1991a, 1992), and calcium ionophore-induced degranulation of human basophils (De Paulis et al. 1992) and neutrophils (Forrest et al. 1991). The anti-inflammatory activity of tacrolimus is reflected in its inhibition of prostaglandin D_2 synthesis in anti-IgE-stimulated human skin mast cells (De Paulis et al. 1992) and leuko-

triene C_4 release from human basophils and alveolar mast cells (De Paulis et al. 1991a,b). *In vivo*, tacrolimus attenuates antigen-induced lung eosinophilia in the guinea pig (Morris et al. 1992), platelet activating factor- and leucotriene B_4- induced leucocyte adhesion and emigration in postcapillary venules (Asako et al. 1992), and ischaemia/reperfusion-induced neutrophil infiltration in the cat small intestinal mucosa (Kubes et al. 1991). However, calcium-mediated platelet activation and aggregation is unaffected by tacrolimus (≤ 100 nmol/L) [Johnson et al. 1990; Pelekanou et al. 1991], indicating that sensitivity to tacrolimus is not a universal characteristic of cells of haemopoietic lineage.

Tacrolimus has a direct stimulatory effect on human haematopoietic progenitor cells, enhancing growth of blood-derived (but not bone marrow-derived) colony-forming units/granulocyte-macrophage (CFU-GM) and burst-forming units/erythroid (BFU-E) [Hirao et al. 1993]. Haematopoietic recovery after murine bone marrow depletion is unaltered by tacrolimus (Boggs et al. 1991).

1.3 Hepatic Effects of Tacrolimus

On subacute administration to rats, tacrolimus (0.4 or 0.8 mg/kg/day orally for 5 to 6 weeks) had no significant deleterious effect on hepatic excretory function (Farghali et al. 1991b). Tacrolimus pretreatment attenuated hepatic injury produced by the hepatotoxin D-galactosamine in the rat (Farghali et al. 1991a) and ameliorated the hepatic injury associated with ischaemia/reperfusion in the rat and dog (Dhar et al. 1992; Kawano et al. 1991; Sakr et al. 1991; Wakabayashi et al. 1992). The drug's hepatoprotective effect against ischaemia/reperfusion injury, exemplified by a reduction in hepatic necrosis and neutrophil infiltration, restoration of hepatic ATP content and a reduction in hepatic peroxidation and serum alanine aminotransferase and lactic dehydrogenase levels (Dhar et al. 1992; Kawano et al. 1991; Sakr et al. 1991; Wakabayashi et al. 1992), has been attributed to inhibition of hepatic TNF and IL-6 production (Sakr et al. 1993).

Tacrolimus stimulates hepatic regeneration following acute chemical injury (Fagiuoli et al. 1992b) or partial hepatectomy (Francavilla et al. 1989; Il Kim et al. 1992; Okamura et al. 1992) in the rat. This latter effect is organ-specific in that the drug does not modify the proliferative response of the kidney following unilateral partial nephrectomy or that of the remnant intestine following intestinal resection (Francavilla et al. 1990). In addition, the stimulatory effect of portacaval shunt on hepatocyte replication in the dog is markedly enhanced by intrahepatic infusion of tacrolimus (Starzl et al. 1991). This hepatotrophic effect of tacrolimus may underlie the drug's observed stimulatory effect on experimental liver tumour growth in the rat (Shinozuka et al. 1991).

The regenerative response to tacrolimus does not appear to be mediated through a direct hepatocellular effect: tacrolimus (≤400 μg/L) was devoid of mitogenic activity on cultured rat hepatocytes (Francavilla et al. 1990), and at higher concentrations (≤1.8 mg/L) blocked the proliferative response of cultured human hepatocytes to epidermal growth factor and transforming growth factor-α (Blanc et al. 1991). The hepatotrophic effect of tacrolimus can be blocked by IL-1α and IL-2 (Okamura et al. 1992), suggesting an immunological mechanism of action, possibly through modulation of liver cell growth regulatory factors (Shinozuka et al. 1991). However, the regenerative response does not appear to be mediated through altered lymphocyte or NK cell function (Francavilla et al. 1991), and modification of hepatic growth control mechanisms through nonimmunological pathways is equally feasible (Francavilla et al. 1991; Schreiber 1991; Starzl et al. 1991).

1.4 Renal Effects of Tacrolimus

On acute administration, tacrolimus (≤20 mg/kg intravenously) has no deleterious effect on renal function in the rat (Benigni et al. 1992; Perico et al. 1991), and at serum concentrations (≤1.0 mg/L) well in excess of those achieved therapeutically in humans (≤50 μg/L), the drug shows minimal effect on perfused rat kidney function (Sumpio &

Phan 1991). In contrast, a therapeutic concentration of cyclosporin (0.5 mg/L) causes immediate and irreversible nephrotoxicity in this model (Sumpio 1988). Nevertheless, tacrolimus (1 to 10 nmol/L; 0.8 to 8.2 μg/L) has a direct glomeruloconstrictive effect *in vitro* (Lieberman et al. 1991), and on subacute administration reduces glomerular flow rate (Kumano et al. 1991) and renal cortical blood flow (Ueda et al. 1991) and increases renal vascular resistance (Kumano et al. 1991) in the mouse and rat. Similarly, in patients undergoing orthotopic liver transplant, reductions in effective renal plasma flow and glomerular flow rate are frequently observed after initiation of tacrolimus therapy (McCauley et al. 1991c).

As with the liver, tacrolimus pretreatment reduces TNF production and acute renal injury associated with renal ischaemia and reperfusion in the rat (Sakr et al. 1992b; Van Thiel et al. 1992). At supratherapeutic concentrations (0.1 to 10 mg/L) tacrolimus exerts a direct cytotoxic effect on cultured porcine tubular epithelial LLC-PK_1 cells (Moutabarrik et al. 1992) and inhibits porcine (McCauley et al. 1991a) and human (Blaehr et al. 1993) proximal tubular cell proliferation. Toxicological studies in the rodent, dog, and baboon have indicated that, on subacute administration, tacrolimus produces renal vascular changes, including arteriolar vascular lesions (Ueda et al. 1991; Yamada et al. 1991), focal medial arteriolar necrosis (Kumano et al. 1991; Ochiai et al. 1989b; Todo et al. 1988), renal vasculitis (Collier et al. 1987), interstitial inflammation (Ohara et al. 1990) and epithelial damage to the proximal tubule (Ueda et al. 1991; Yamada et al. 1991).

The nephrotoxic action of tacrolimus has variously been attributed to altered prostaglandin metabolism and lipid peroxidation of the cell membrane (Yamada et al. 1992a,b), and to enhanced endothelin secretion (Moutabarrik et al. 1992). In the rat, juxtaglomerular hyperplasia and tubular damage were accompanied by enhanced urinary thromboxane B_2 excretion, indicative of increased production of the vasoconstrictor thromboxane A_2 in the renal parenchyma (Benigni et al. 1988), decreased urinary excretion of 6-keto-$PGF_{1\alpha}$, and an increase in renal malondialdehyde levels, the latter effect suggesting enhanced lipid peroxidation of the cell membrane (Yamada et al. 1992a,b). Tacrolimus-induced arteriolar vasoconstriction may reduce glomerular blood flow, resulting in compensatory juxtaglomerular hyperplasia and tubular damage (Yamada et al. 1992b); direct stimulation of intrarenal renin production by tacrolimus may also contribute to this effect (Yamada et al. 1992a). Endogenous endothelins, potent renal vasoconstrictors, are believed to play a pivotal role in acute cyclosporin nephrotoxicity (Kon et al. 1990), and may also contribute to the pathogenesis of tacrolimus-induced glomerular dysfunction. At non-cytolytic concentrations (0.01 to 1.0 μmol/L) tacrolimus has a stimulatory effect on endothelin-1 secretion by cultured porcine renal tubular LLC-PK_1 cells, and on repeated administration elevates serum endothelin levels in the rat (Moutabarrik et al. 1992).

1.5 Effects on Animal Models of Allograft Transplantation

In general, short term post-transplant administration of tacrolimus is effective in preventing allograft rejection in various experimental animal models at doses 10 to 100 times lower than those of cyclosporin (Inamura et al. 1988; Morris et al. 1990; Ochiai et al. 1987b, 1989a,b; Sato et al. 1991; Todo et al. 1988, 1989). Thus, in the rat and dog, tacrolimus dosages of 0.1 to 0.3 mg/kg/day intramuscularly or 1.0 to 1.5 mg/kg/day orally are sufficient to suppress hepatic, renal, cardiac, and skin allograft rejection (Inamura et al. 1988; Ochiai et al. 1987a,b,d; Sato et al. 1991; Todo et al. 1987b).

Animal studies indicate that prolonged allograft survival is achieved only with postoperative administration of tacrolimus. Tacrolimus was shown to prolong cardiac, lung, renal and hepatic allograft survival in the rat and dog even when started as late as day 4 or 5 postoperatively (Katayama et al. 1991; Murase et al. 1987, 1990a; Ochiai et al. 1987d), whereas administration to the donor or recipient prior to transplantation failed to prolong allograft survival (Fabrega et al. 1991;

Ochiai et al. 1987d). Similarly, the beneficial effect of pretransplant donor-specific antigen presentation (donor spleen cell injection or blood transfusion) on pancreatic islet (Fukuzaki et al. 1991), small intestine (Fukuzawa et al. 1991), and cardiac (Fabrega et al. 1991) allograft survival was enhanced by acute administration of tacrolimus over the following 3 to 5 days. The possibility of inducing immunological tolerance with a short course of high dose tacrolimus has been reported for rodent skin (Wada et al. 1991), cardiac (Murase et al. 1987, 1990a; Ochiai et al. 1987c), pancreatic (Fukuzaki et al. 1991; Gotoh et al. 1992), hepatic (Murase et al. 1990a,b), small bowel (Langrehr et al. 1991; Lee et al. 1990), and lung (Katayama et al. 1991) allotransplants, as well as renal and hepatic transplants in larger animals (Gotoh et al. 1991; Monden et al. 1990; Ochiai et al.1989a; Todo et al. 1989; Ueda et al. 1990).

1.5.1 Hepatic Transplantation

Tacrolimus prolonged orthotopic liver allograft survival in the rat (Inagaki et al. 1989; Isai et al. 1990; Murase et al. 1990a; Tsuchimoto et al. 1989), dog (Todo et al. 1987b; Yokota et al. 1989) and monkey (Gotoh et al. 1991; Monden et al. 1990). Although indefinite (>100 days) liver allograft acceptance has been described following either short or long term tacrolimus therapy (Gotoh et al. 1991; Murase et al. 1990a), this does not appear to represent classical tolerance, as skin grafts from the same donor animals were acutely rejected (Isai et al. 1990). Nevertheless, immunological tolerance to hepatic allografts was obtained in the monkey after withdrawal of long term tacrolimus therapy (Gotoh et al. 1991). Tacrolimus prolonged orthotopic liver survival in the hamster-to-rat xenograft model (Valdivia et al. 1991).

1.5.2 Renal Transplantation

Tacrolimus can prolong renal allograft survival in the rat (Ochiai et al. 1987a,b), dog (Calne et al. 1987; Griffin et al. 1992; Ochiai et al. 1987a,b; Todo et al. 1987a, 1988; Watanabe et al. 1989) and baboon (Calne et al. 1987; Todo et al. 1988, 1989). Intramuscular tacrolimus 0.16 mg/kg/day prolonged median allograft survival time from 16 days to 176 days in the dog, while at an oral dosage of 1.0 mg/kg/day graft survival exceeded 130 days (Ochiai et al. 1987a,b). A delayed 3-day course of tacrolimus, initiated on the fourth day post-transplantation, was effective in prolonging renal allograft survival in the baboon, and rejection episodes were reversed with a second 3-day course of tacrolimus (Todo et al. 1989).

1.5.3 Other Organ Transplantation

Among the nonhuman primates, tacrolimus prolongs cardiac allograft survival in the baboon (Hildebrandt et al. 1991) and monkey (Flavin et al. 1991). In contrast to cyclosporin, tacrolimus has also been reported to inhibit ongoing acute cardiac allograft rejection in the rat (Ochiai et al. 1987c) and baboon (Hildebrandt et al. 1991), but is ineffective in the dog (DeValeria et al. 1991). Furthermore, tacrolimus inhibited the development of transplant arteriosclerosis in rats receiving cardiac allografts (Wu et al. 1991a).

Prolongation of pancreatic allograft function and survival by tacrolimus has been described in the rodent (Fukuzaki et al. 1991; Gotoh et al. 1992; Ohtsuka et al. 1991; Tze et al 1992; Yabuuchi et al. 1991; Yamashita et al. 1991; Yasunami et al. 1989, 1990), dog (Imai et al. 1991; Kenmochi et al. 1988; Morimoto et al. 1990; Sato et al. 1989) and monkey (Ericzon et al. 1992).

Tacrolimus can prolong graft and recipient survival after small intestine allotransplantation in the rat (Hatazawa et al. 1992; Hoffman et al. 1990; Iga et al. 1990; Langrehr et al. 1991; Lee et al. 1990; Takeda et al. 1991; Utsunomiya et al. 1992) and dog (Yoshimi et al. 1991). Moreover, tacrolimus is more effective than cyclosporin in preventing acute rejection of small intestine allografts in the rat (Hoffman et al. 1990) and can reverse ongoing allograft rejection (Graeb et al. 1992).

Tacrolimus can prolong the survival of allogeneic skin grafts in the mouse (Wada et al. 1991) and rat (Inamura et al. 1988; Ochiai et al. 1987d; Sakamoto et al. 1989), although, in contrast to cardiac allografts, long term survival of skin allo-

grafts appears to require maintenance therapy (Inamura et al. 1988).

Corneal graft rejection is suppressed by subconjunctival tacrolimus in the rabbit (Kobayashi et al. 1989), and vascularised limb graft survival is prolonged by intramuscular tacrolimus in the rat (Arai et al.1989; Kuroki et al. 1989). Tacrolimus induces long term survival of lung allografts in the dog (Hasegawa et al. 1992; Hirai et al. 1993) and rat, where it is capable of reversing ongoing rejection (Katayama et al. 1991).

2. *Pharmacokinetic Properties*

In the majority of pharmacokinetic studies, an enzyme-linked immunoabsorbant assay (ELISA) employing a monoclonal anti-tacrolimus antibody has been used for quantification of tacrolimus in biological fluids (Cadoff et al. 1990; Kobayashi et al. 1991; Tamura et al. 1987; Warty et al. 1991b). A semiautomated immunoassay system is also available (Grenier et al. 1991). Warty et al. (1993) suggested possible cross-reactivity of the ELISA system with non-immunosuppressive metabolites might necessitate additional assay development. However, Kobayashi et al. (1991) demonstrated good correlation between mixed lymphocyte reaction immunosuppressive activity and immunocross-reactivities of tacrolimus and its metabolites to monoclonal antibody. Thus, current ELISA results may reflect the immunosuppressive state of the patient. In this respect, radioreceptor assay may provide more clinically meaningful results than conventional immunoassay (Murthy et al. 1992). While high performance liquid chromatography (HPLC)-based methods specific for tacrolimus and its metabolites are available, these are less sensitive than immunoassay (Takada et al. 1990; Venkataramanan et al. 1987). An ELISA method combined with HPLC, allowing quantification of unchanged tacrolimus and possible metabolite(s), has been described (Friob et al. 1991). An *in vitro* bioassay based on inhibition of the primed T cell response (Zeevi et al. 1990b) can be useful in cases of severe hepatic dysfunction, where accumulation of ELISA-active but pharmacologically inactive drug metabolites may inflate ELISA values (Abu-Elmagd et al. 1991a; Nossal 1991; Zeevi et al. 1991).

Tacrolimus is poorly and erratically absorbed after oral administration, with peak plasma concentrations (C_{max}) of 0.4 to 5.6 μg/L occurring at 0.5 to 8 hours (t_{max}) after a single oral dose of 0.15 mg/kg (Japanese FK 506 Study Group 1991; Venkataramanan et al. 1990). C_{max} values following intravenous infusion of tacrolimus 0.15 mg/kg over 2 hours ranged from 10 to 24 μg/L (Venkataramanan et al. 1990). Continuous intravenous infusion of tacrolimus 0.1 mg/kg/day to patients undergoing hepatic transplant resulted in steady-state plasma concentrations ranging from 4.5 to 14.4 μg/L (Jain et al. 1993). Trough plasma tacrolimus concentrations are reported to correlate poorly with dose (Cadoff et al. 1990; Venkataramanan et al. 1991). Nevertheless, there appears to be close correlation between the area under the plasma tacrolimus concentration-time curve and trough plasma tacrolimus concentrations in whole blood and plasma (Lee et al. 1993). The absolute oral bioavailability of tacrolimus ranged from 5 to 67% (mean 27%) in transplant recipients with various degrees of hepatic function (Venkataramanan et al. 1991). The presence of food may decrease the absorption of tacrolimus in liver transplant recipients (Mekki et al. 1992).

Tacrolimus is highly lipophilic and undergoes extensive tissue distribution, as reflected in a large volume of distribution (1300L) [Venkataramanan et al. 1990]. Animal studies indicate that drug concentrations in the lung, spleen, heart, kidney and pancreas exceed those in plasma (Venkataramanan et al. 1990). In the human vascular compartment, erythrocytes sequester tacrolimus, binding 75 to 80% of the drug, with the result that whole blood concentrations are approximately 10 to 30 times higher than plasma concentrations (Japanese FK 506 Study Group 1991; Jusko & D'Ambrosio 1991; Warty et al. 1991a). The distribution of tacrolimus between erythrocytes and plasma is concentration-, haematocrit- and temperature- dependent: plasma concentrations at 37°C are approximately twice those at 24°C (Beysens et al. 1991; Japanese FK 506 Study Group 1991; Machida et al. 1991; Ven-

kataramanan et al. 1990). Consequently, whole blood and plasma concentrations of tacrolimus are nonlinearly related (Jusko & D'Ambrosio 1991).

In the plasma, tacrolimus is highly bound (88%) to plasma proteins (Habucky et al. 1992), predominantly the nonlipoprotein fraction containing albumin and α-1 acid glycoprotein (Habucky et al. 1992; Warty et al. 1991a).

Plasma clearance of tacrolimus following intravenous administration ranged from 87 to 269 (mean 143) L/hour, while the plasma elimination half-life was 5.5 to 16.6 (mean 8.7) hours (Venkataramanan et al. 1990). Tacrolimus is virtually completely metabolised by the liver, with less than 1% of the parent drug being excreted unchanged in the bile, urine and faeces during the initial 48-hour period following oral or intravenous administration (Venkataramanan et al. 1990). Hepatic metabolism of tacrolimus in humans is catalysed primarily by cytochrome P450 subtypes Ia and IIIa (Sattler et al. 1992; Shah et al. 1991; Stiff et al. 1992; Vincent et al. 1992), with demethylation and hydroxylation representing the main metabolic pathways (Christians et al. 1991a,c). Of 9 metabolites of tacrolimus identified during its incubation with human hepatic microsomes, two exhibited significant immunosuppressive activity *in vitro* (Christians et al. 1991c). In liver transplant recipients, demethylated and didemethylated metabolites of tacrolimus predominated in the blood and urine, while hydroxylated metabolites were prominent in the bile (Christians et al. 1991a).

In view of its extensive hepatic metabolism, the pharmacokinetics of tacrolimus would not be expected to be appreciably modified in patients with renal impairment. In contrast, hepatic dysfunction is associated with increased plasma concentrations, prolonged half-life and reduced clearance of tacrolimus (Abu-Elmagd et al. 1991a; Jain et al. 1990, 1993). Plasma tacrolimus concentrations correlate directly with serum bilirubin levels, suggesting that hepatic dysfunction interferes with tacrolimus metabolism (Abu-Elmagd et al. 1991a).

3. Clinical Efficacy in Hepatic and Renal Transplantation

The end-point of any clinical trial of an immunosuppressant should include an assessment of patient and graft survival, and also rejection status. Other factors which may influence this outcome or confound its interpretation should ideally be controlled. This highlights an inherent problem in the design of such investigations, since for most patients there is a void between theoretical optimisation of outcome (e.g. in terms of histocompatibility), and that which is possible in the clinical setting. Elements which affect the success of transplantation may be divided into 3 categories relative to the timing of surgery;

- The preoperative period (e.g. donor/patient selection, graft patency)
- The intraoperative period (e.g. surgical technique)
- The postoperative period (e.g. patient management).

In the absence of absolute or relative contraindications, potential candidates for liver or kidney transplantation are children or adults who have severe, irreversible liver or kidney disease for whom alternative medical or surgical treatments have been exhausted or are restrictive (e.g dialysis). In patients undergoing liver transplantation, at least, the prognosis is dependent on the primary disease, the age of the patient and on the severity of the patient's condition (Gordon et al. 1991). The greatest impact on patient selection, however, appears to have occurred with an increased understanding of immunological mechanisms. This has led to recognition of the importance of histocompatibility between donor and recipient. Indeed, early recognition of the relevance of ABO compatibility followed by human leucocyte antigen (HLA) matching, and the avoidance of allograft transplantation into individuals with preformed antibodies have been major contributions to the success of transplantation surgery (Diethelm 1992). However, with improvements in operative techniques and the use of more effective immunosuppressant agents such as cyclosporin, the effects of HLA mismatching on graft

survival, although still considerable, have been diminished.

The importance of tissue typing is established in intrafamilial transplantation, but there has been controversy regarding its value in recipients of organs from cadaveric donors (Starzl & Fung 1990). Improved graft survival, however, has been reported for recipients of unrelated cadaveric liver allografts matched for HLA-A and -B antigens (Yagihashi et al. 1992), and for recipients of unrelated cadaveric kidney allografts matched for HLA-A, -B and -DR antigens (Opelz et al. 1991). Indeed, relatively small gains in terms of superior early success rates with a good HLA match (even at centres with good overall success rates) eventually translate into substantial long term benefits, at least with renal transplantation (Opelz et al. 1991). Similarly, lymphocytotoxic crossmatch testing among recipients of hepatic allografts was considered to be of little value based on early experience (Gordon et al. 1986), although the presence of preformed antibodies had long been associated with reduced graft survival among recipients of renal allografts (Terasaki et al. 1965). More recently, however, the adverse effect of preformed cytotoxic antibodies on hepatic graft survival has been demonstrated, emphasising the importance of testing for compatibility of this variable (Nakamura et al. 1991; Takaya et al. 1991, 1992). Despite all of the foregoing observations and the availability of networks for organ sharing, full utilisation of the HLA and lymphocytotoxic matching effect is often not possible, given the mosaic of HLA antigens expressed, the usual urgency of transplantation and the shortage of suitable organs. Clinical judgement is therefore exercised and compromise is the rule rather than the exception.

With regard to donor selection, most healthy kidneys or livers suitable for transplantation are obtained from cadavers, although some are obtained from living donors. Indeed, in Japan the use of organs from donors who are brain-dead is not widely accepted, neither are the criteria for the confirmation of death. Cadaveric organ transplantation in this country is therefore less common than in many other countries.

Major improvements in organ preservation, particularly that of the liver, were made with the introduction of the University of Wisconsin Solution in 1987. This solution has extended renal preservation to 50 hours with pulsatile perfusion, and to 35 to 40 hours with cold storage; liver preservation is now possible for 24 hours (Diethelm 1992), although preservation for >18 hours appears to carry a greater risk of graft failure (Morel et al. 1991).

The clinical efficacy of tacrolimus has been principally evaluated in established 'transplantation centres' in the US, Japan and Europe; notably, the greatest clinical experience with tacrolimus has been documented by workers at the University of Pittsburgh, Pennsylvania in the US. Concentration of expertise in these tertiary care facilities has allowed optimisation of outcome given the present constraints associated with transplantation surgery. Most clinical trials conducted in these centres (and therefore in the US) have been prospective in design with rapid publication of results followed by periodic updates. Thus, patients reported in early studies have also often been included in later published reports of larger cohorts followed over extended periods of time. This review has attempted to differentiate between such studies and has concentrated on the most recent reports detailing the most extensive clinical experience available.

The results of clinical studies with tacrolimus should be closely scrutinised. Indeed, the vast majority of investigations evaluated were presented at symposia and were either only available in abstract form or were subsequently published to document symposia proceedings. Since these studies have therefore not been subjected to extensive peer review, they should be interpreted with caution. Furthermore, there has only been one large study which evaluated the efficacy of tacrolimus as a rescue therapy in renal allograft recipients, and there is a paucity of randomised investigations of tacrolimus versus cyclosporin as a primary immunosuppressant, particularly in renal transplantation. In addition, since the majority of rejection episodes (≈90%) are known to occur within 3 months of transplantation (Jain et al. 1991b), circumspection

should be used when interpreting rejection incidence in those studies that detailed a minimum follow-up period of less than 3 months. Only those investigations which used the University of Wisconsin Solution for organ preservation were included in this analysis.

In the clinical investigations evaluated, tacrolimus was usually commenced after revascularisation of the graft, and was most often administered intravenously until oral administration was feasible. Furthermore, intravenous and oral administration often overlapped, usually by 1 to 2 days. Dosage regimens have varied, principally reflecting a trend to reduce the magnitude of the initial post-transplantation intravenous dosage and prolong the daily period of its administration in an effort to avoid potential adverse effects. The most commonly used regimen involved intravenous administration of tacrolimus 0.1 to 0.15 mg/kg/day until oral therapy with a dosage of 0.3 mg/kg/day was feasible. It should be noted however, that the optimum dosage regimen for tacrolimus is continuing to be refined and these dosages may be superseded (see section 6). Indeed, most recent experience in large randomised studies indicate that initial intravenous dosages of $\leq$0.1 mg/kg/day (Klintmalm 1993) or 0.035 to 0.075 mg/kg day (data on file, Fujisawa GmbH, Germany) should be employed, with subsequent oral dosages of 0.1 to 0.2 mg/kg/day (data on file, Fujisawa GmbH, Germany). Dosage adjustments in the studies evaluated were usually made based on liver and kidney function, rejection status and trough plasma or whole blood tacrolimus concentrations. However, in some studies circulating tacrolimus concentrations were either only monitored or not assessed. Adjunct maintenance steroid therapy was administered in all investigations, and rejection usually treated with increased dosages of the primary immunosuppressant, high dosages of steroids and/or antilymphocyte preparations.

3.1 Hepatic Transplantation

3.1.1 Rescue Therapy in Hepatic Allograft Recipients

The efficacy of tacrolimus in the rescue of liver allografts has been principally assessed in 3 large noncomparative studies and in 2 smaller studies of similar design in children (table I). Most investigations recruited recipients of cadaveric-donor organs; however, one investigation conducted in children examined recipients of allografts from living-related donors (Uemoto et al. 1993). Patients experiencing rejection of their first or subsequent graft and/or drug-related adverse effects while receiving cyclosporin-based therapy were evaluated.

Following conversion of treatment to tacrolimus, steroid therapy was tapered or discontinued (Demetris et al. 1992; Tzakis et al. 1991a; Uemoto et al. 1993), azathioprine was stopped (Demetris et al. 1992), and requirements for antihypertensive therapy reduced (Tzakis et al. 1991a). After a minimum follow-up of 2 months in studies which evaluated recipients of cadaveric organs, patient survival was $\geq$85% and graft survival exceeded 70% (Fung et al. 1991c; Tzakis et al. 1991a, US Multicenter FK 506 Study Group 1993a,b,c). Demetris et al. (1992) showed that these results were maintained in the longer term ($\approx$9 to 18 months); however, the US Multicenter FK 506 Study Group (1993a,b,c) reported a decline in actuarial patient and graft survival rates to 72 and 50%, respectively, after 12 months. In children with liver allografts donated by living relatives, 12-month patient and graft survival rates were both 70%.

In the largest clinical trial which evaluated tacrolimus as a rescue therapy, 70% of 246 patients previously received muromonab-CD3 and were thus considered to have failed conventional immunosuppressive therapy (Fung et al. 1991c). Following conversion of therapy, improvement in biochemical and histological indices of graft function was evident for patients with acute, and notably, chronic rejection. Indeed, over 70% of patients with chronic rejection showed a biochemical and histological response; improvements in liver function tests were also noted for a sub-group with end-stage liver disease. Among those patients with prior steroid toxicity, conversion to therapy with tacrolimus afforded steroid-withdrawal. Hepatitis appeared a factor for poor prognosis as 50% of biopsy specimens showed worsening of the histological diagnosis at follow-up.

Demetris and colleagues (1992) stratified

Table I. Summary of clinical studies of tacrolimus (T) in hepatic allograft recipients

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
Rescue Therapy								
Demetris et al. (1992)	96 (1-74, mean 42)	p, nc	T 0.15-0.3/d IV then 0.15 bid PO	Previous: C-based including S. mAb used in 7 patients	≈9 to 18mo	89	70	75 primary and 21 subsequent transplants. Dosage adjustments made without regard to circulating tacrolimus concentrations. S dosages ↓ or stopped. Aza stopped
Fung et al. (1991c)	246 (1-66, mean 42)	p, nc	T 0.075-0.15 bid over 4h IV then 0.15 bid PO	Previous: C-based including S +/− Aza. mAb used in 70% of patients Mtnce: S (dosages NA)	≥2mo (median 240d)	87	79	203 primary and 43 subsequent transplants
Tzakis et al. (1991a)	26 (mean 8.7)[a]	p, nc	T 0.15/d IV then 0.15 bid PO	Previous: C-based Mtnce: S (PDN) dosages initially 10-20 mg/d Rejection: ↑ T, S or mAb	≥3mo (mean 150d)	88	73	S dosages ↓ or stopped
Uemoto et al. (1993)	22 (0.7-15)[a]	p, nc	T 0.075 bid over 4h IV or 0.03 bid then 0.15 bid PO	Previous: C-based Mtnce: S (MPDN) dosages initially 10 mg/d then 2 mg/d ↓ 0.5 mg/d Rejection: ↑ T or S	1-16mo	70 (12mo)	70 (12mo)	All primary transplants from living-related donors. S dosages stopped in all survivors
US Multicenter FK 506 Study Group (1993a,b,c)	125 (0-70, mean 35)	p, nc, mc	T 0.075/12h IV then 0.15 bid PO	Previous: C-based including S, mAb (AR) &/ or Aza (CR)	≈6-19mo	85 (3mo) ≈70 (12mo)[b]	>70 (3mo) ≈50 (12mo)[b]	98 primary and 24 subsequent transplants.[c] Target T C_{min} 0.2-5 μg/L, but subsequently 0.2-2 μg/L based on clinical experience in some centres
Primary Immunosuppression[d]								
Noncomparative Studies								
Jain et al. (1991a)	125 (0.3-69)	p	T 0.15 d1 IV then 0.075 bid IV then 0.15 bid PO	Mtnce: S (MPDN) high dosages (1g bolus then 200	≈6-12mo (mean 242d)	92	87	S requirements limited

Table I. Contd

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
				mg/d ↓ to 20 mg/d initially; n = 63; dosages ↓ for children) or low dosages (10-20 mg/d initially; n = 62). Rejection: included ↑ S and/or mAb; (Aza used for a few patients)				
Tzakis et al. (1991a)	30 (mean 4.2)[a]	p	T 0.15/d IV then 0.15 bid PO	Mtnce: S (PDN) dosages 10-20 mg/d initially. Rejection: ↑ T, S or mAb	⩾3mo (mean 150d)	90	90	S dosages ↓ or stopped
Comparative Studies								
Esquivel (1993)	30 (⩽12)[a]	p, r, pl, mc	T mean 0.39-0.49 PO 1-6mo	Mtnce: S. C-based TT (10 sites), DT (1 site), QT (1 site) Rejection: included ↑ S &/or mAb	1-6mo	92 (3mo),[b] 92 (6mo)[b]	85 (3mo),[b] 85 (6mo)[b]	Paediatric data from Klintmalm et al. (1993)/ McDiarmid et al. (1993a). Incidence of rejection at 6mo[b]: T 42%, C 72%. Mab usage at 6mo[b]: T 16%, C 29%
	20 (⩽12)[a]		C (NA)			89 (3mo),[b] 89 (6mo)[b]	77 (3mo),[b] 77 (6mo)[b]	
Fung et al. (1991a)	41 (med 42)	p, r, pl	T 0.1/d IV then 0.15 bid PO	Mtnce: S (MPDN or PDN) dosages 1g bolus, then 20 mg/d ↓ initially to 10 mg/d then 5 mg/d Rejection: ↑ T, C, S or mAb or switch from C to T	≈8-15mo (med 12mo)	100 (3mo), 95 (6mo), 93 (12mo)	95* (3mo), 93* (6mo), 90* (12mo)	Target T C_{min} 1-5 μg/L. Rejection free status at 1mo: T 61%, C 18%.† 73% of C patients were switched to T, 22 with rejection; 1 T patient required RTX AHT requirements at 3mo: T 27%, C 53%*
	40 (med 42)		C 4/d IV d1 then 8 bid PO			90 (3mo), 85 (6mo), 81 (12mo)	83 (3mo), 78 (6mo), 70 (12mo)	S requirements T < C

Continued over

Table I. Contd

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
European FK506 Study Group (1993)	270	p, r, pl, mc	T (NA)		6mo	84	81	Rejection: T 39%, C 50%*. Withdrawal due to refractory rejection: T 0.7%, C 7.6%‡. Usage of high dose S: T 15.1 mg/d, C 17.1 mg/d‡
	275		C(NA)		6mo	79	77	
Klintmalm (1993)/ McDiarmid et al. (1993a)	259	p, r, pl, mc	T 0.075 bid over 4h or ≤0.05 bid IV before PO dosage (NA)	Mtnce: S. C-based TT (10 sites), DT (1 site), QT (1 site) Rejection: included ↑ S &/or mAb	1-12mo	91 (3mo),[b] 90 (6mo),[b] 86 (12mo)[b]	86 (3mo),[b] 86 (6mo),[b] 79 (12mo)[b]	Rejection 1-12mo[b]: T 54-66%, C 65-76%** Refractory rejection: T 1.9%, C 10%† mAb usage 1-12mo[b]: T 15-19%, C 28-33%.†
	261		C (NA) but either DT, TT or QT		1-12mo	92 (3mo),[b] 90 (6mo),[b] 87 (12mo)[b]	86 (3mo),[b] 85 (6mo),[b] 80 (12mo)[b]	IV S usage to 6mo: T 3.8 g/patient, C 6.1 g/patient†
Takaya et al. (1991)	409 (18-68)	retro, hc	T (NA)	Mtnce: S (low dosages for T). Rejection: included ↑ S &/or mAb	≥6mo (med 13mo)	86 (1mo), 63 (6mo)		Graft PNF: T 4.1%, C 7.4%* Rejection: T 0.5%, C 2.9%* RTX at ≤6mo of primary grafts: T 8.6%, C 15.4%**
	631 (18-74)		C (NA)		≥6mo (med 13mo)	69 (1mo), 55 (6mo)		
Todo et al. (1991)	110 (18-70, mean 46)	retro, hc	T 0.075/12h IV then 0.15 bid PO		≈6-12mo	92 (12mo)[b]	87 (overall)	T data (adults only) from Jain et al. (1991a) T patients had ↑ UNOS scores
	325 (18-73, mean 46)		C (NA)			<80 (12mo)**	NA but comparable with T	Usage of high dose S, mAb and Aza, and rejection episodes were lower for T** Mtnce S dosages

Table I. Contd

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
Todo et al. (1991) Contd								T < C (S stopped for 45% of T patients ≤6mo) Hypertension incidence low for T, ≤22% up to 6mo
Tzakis et al. (1991b)	59 (0.2-17, med 2.7)[a]	p, hc	T 0.15/d IV then 0.15 bid PO	Mtnce: S dosages: T initially 10-20 mg/d (PDN), C 1g bolus (HCN or PDN) then 100 or 200 mg/d (PDN) ↓ to 20 mg/d initially	≤18mo	92 (3mo),[b] 90 (6mo),[b] 90 (12mo),[b] 90 (18mo)[b]	85 (3mo),[b] 83 (6mo),[b] 83 (12mo),[b] 83 (18mo)[b]	Plasma T C_{min} monitored only. Rejection incidence similar but more manageable for T. S requirements: T 17% (3mo), 12% (mo), <10% (9mo) C, >95% (throughout)*.
	50 (0.3-15, med 1.8)[a]		C 3 bid over 4h IV then 17.5/d PO			80 (3mo),[b] 78 (6mo),[b] 78 (12mo),[b] 76 (18mo)[b]	70 (3mo),[b] 68 (6mo),[b] 68 (12mo),[b] 66 (18mo)[b]	Aza requirements: T 0%, C 55% AHT requirements: T, 17% (d1) 8% (15mo); C, 65% (d1), 45% (15mo)*

a Children.
b Actuarial survival.
c Data only detailed for 122 of 125 patients.
d Patients undergoing a primary liver transplant.

Abbreviations and symbols: AHT = antihypertensive therapy; AR = acute rejection; Aza = azathioprine; bid = twice daily; C = cyclosporin; C_{min} = minimum plasma concentration; CR = chronic rejection; d = day; DT = double therapy; h = hours; hc = historic control; HCN = hydrocortisone; IV = intravenous; mAb = muromonab-CD3 or other antilymphocyte preparations; med = median; mo = months; mc = multicentre; MPDN = methylprednisolone; Mtnce = maintenance; NA = data not available; nc = noncomparative; PDN = prednisone or prednisolone; PNF = primary nonfunction; p = prospective; pl = parallel; PO = oral; QT = quadruple therapy; r = randomised; retro = retrospective; RTX = retransplantation; S = corticosteroids; TT = triple therapy; UNOS = united network for organ sharing; significant difference between treatments * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$; ‡ $p < 0.0001$; ↑ = increased; ↓ = decreased.

patients according to the cause of graft dysfunction which ultimately led to conversion of therapy and principally evaluated histological and biochemical indices of graft function. The histological outcome of the switch is detailed in figure 3. Patients who had experienced acute rejection (mild to severe) during optimal standard cyclosporin therapy and adjuvant immunosuppression (steroids and/or muromonab-CD3) responded most favourably: 14 of 18 grafts survived (78%), with histological and biochemical improvement noted for 10 and slight biochemical improvement for the remaining 4. Of the 22 patients with chronic rejection, 13 (59%) showed histological improvement, with 10 also showing improved biochemical indices; 6 of the 9 patients with no histological response showed biochemical improvement. Further analysis of this subgroup revealed that the switch to tacrolimus was more beneficial among those with early-stage versus late-stage chronic rejection. Patients in whom it was difficult to differentiate between chronic rejection and chronic persistent or low-grade chronic active hepatitis were mostly unaffected by conversion to tacrolimus. Furthermore, corroborating the

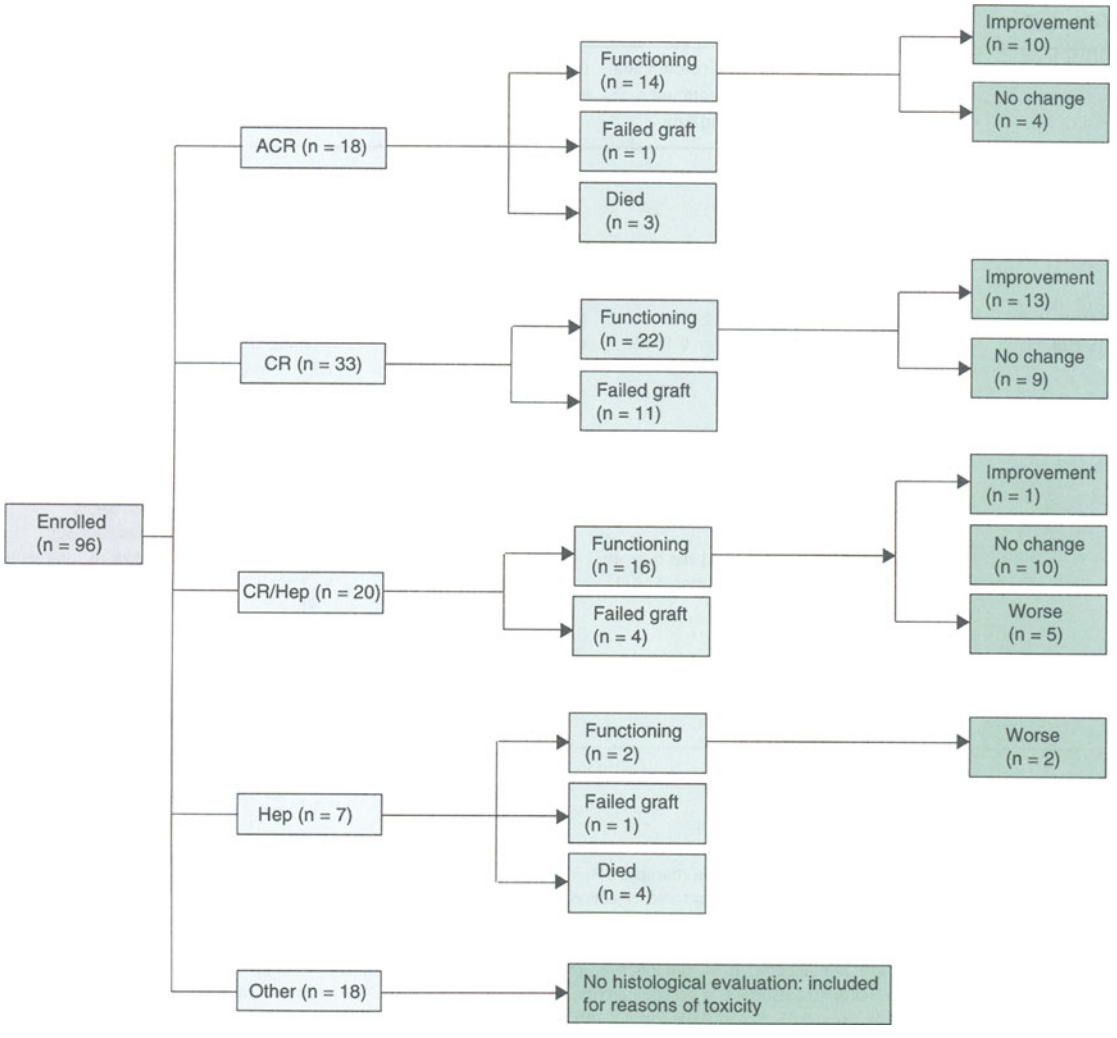


Fig. 3. Results of histological evaluation of liver biopsies from 96 patients switched from therapy with cyclosporin to tacrolimus (follow-up ≈9-18 months) [after Demetris et al. 1992]. *Abbreviations:* ACR = acute cellular rejection; CR = chronic rejection; CR/Hep = chronic rejection and chronic persistent or low grade chronic active hepatitis; Hep = active hepatitis.

results of Fung et al. (1991c), active hepatitis was a poor prognostic indicator as patients with this condition either experienced a decline in graft function, graft rejection or they died from liver failure.

The US Multicenter FK 506 Liver Study Group (1993a,b,c) reported that for patients who survived and remained on tacrolimus with a functioning liver transplant at 3 months, there was a clinically significant improvement in biochemical indices of liver function. Overall, the mean Karnofsky score, used to evaluate performance status, increased from 58 at baseline (n = 117) to a plateau of 85 after 6 months of therapy (n = ≤50). Clinical response was rated according to an arbitrary scale of 1 (complete recovery) to 5 (progressive debilitating disease); a complete/partial response or an improvement in this variable was noted for 50 of 92 patients (54%)

after 1 month, and for 39 of 58 patients (67%) after 6 months. Stratification according to histological rejection status revealed 61 patients with only acute rejection and 32 patients with only chronic rejection that were successfully converted to tacrolimus therapy (US Multicenter FK 506 Study Group 1993b). Recurrent rejection episodes were markedly reduced during therapy with tacrolimus compared with the preconversion period. Furthermore, 35 of 61 patients (57%) with acute rejection and 7 of 32 patients (22%) with chronic rejection became rejection-free. Despite this difference, and in contrast to the results of Demetris et al. (1992), the type of rejection (acute *vs* chronic) at entry was not independently correlated with outcome, although allowance was made for impairment of liver function. Whereas acute rejection episodes occurring during tacrolimus therapy were easily controlled and did not lead to graft loss, 16 of 61 patients with acute rejection at entry developed chronic rejection. Thus, although acute and chronic rejection are thought to be mechanistically distinct, these data suggest that histologically, chronic rejection may not infrequently follow the successful treatment of severe refractory acute rejection. Multivariate analysis of prognostic factors revealed a correlation between treatment failure or death and the following preconversion elements among patients with:

- total serum bilirubin levels of ≥22 versus 3.4 μmol/L; the relative risk was 4.9 for treatment failure and 5.0 for death ($p < 0.01$)
- AST levels of ≥300 versus 80 IU/L; the relative risk was 2.7 for treatment failure ($p < 0.01$)
- >1 prior liver transplantation versus 1; the relative risk was 4.0 for treatment failure ($p < 0.01$; 4 months postconverision)
- serum creatinine levels of ≥133 versus 53 μmol/L; the relative risk was 1.9 for death ($p < 0.05$) [US Multicenter FK 506 Liver Study Group 1993a].

Overall improvements in liver function tests were also observed in the paediatric study conducted by Tzakis et al. (1991a), and of 7 children who required retransplantation, 5 commenced primary therapy with tacrolimus after reoperation. Among paediatric patients who received a liver allograft from a living-related donor, hepatic dysfunction indicative of allograft rejection was only recorded for 2 of 22 patients, and was successfully treated with high dose steroids or increased dosages of tacrolimus (Uemoto et al. 1993).

In a brief analysis of 22 paediatric recipients of a cadaveric liver transplant experiencing rejection or complications associated with cyclosporin-based therapy and subsequently converted to tacrolimus (dosage not stipulated), patient and graft survival were 91% after a mean follow-up period of 7.3mo (Esquivel et al. 1993).

Eight of 10 children with refractory rejection survived with histological or biochemical reversal of rejection.

The efficacy of tacrolimus as a rescue therapy in patients experiencing liver allograft rejection, nephrotoxicity or malabsorption with cyclosporin-based therapy has also been demonstrated in a small study (n = 37) conducted in Europe (Winkler et al. 1993b).

3.1.2 Primary Therapy in Hepatic Allograft Recipients

The efficacy of tacrolimus as a primary immunosuppressive therapy has been assessed in a series of noncomparative and comparative studies which recruited adults or children undergoing their first liver transplant (table I). The investigations in which tacrolimus and cyclosporin were compared were either randomised or employed historical control data for the comparator drug. However, there are limitations to the interpretation of these studies. Indeed, of the 4 randomised investigations [one of which evaluated a paediatric subset of patients recruited to the study conducted by Klintmalm (1993)/McDiarmid et al. (1993a)], 3 have been reported only in summary form. Furthermore, those studies which utilised historical control data should be interpreted with caution when considered in isolation; given the vast experience and commitment of the transplantation centres involved in these investigations to enhanced success, it would not be unreasonable to assume an improvement over historic controls even if no new immunosuppressive therapy had been available.

Nevertheless, in 2 of the 3 investigations evaluated which employed historical data, patients were matched for age and primary diagnosis. United Network for Organ Sharing (UNOS) scores were also matched or reported, and in the investigation conducted by Todo et al. (1991), UNOS classification showed that tacrolimus recipients had a greater urgency rating. This had the effect of subjecting tacrolimus to a more stringent evaluation.

In the medium-term (6 to 18 months), therapy with tacrolimus was associated with patient survival rates generally ≥84%, and graft survival rates approaching or exceeding 80% (table I). In one retrospective investigation, however, patient survival declined to 63% after 6 months (Takaya et al. 1991). There were no significant differences in patient and graft survival rates for tacrolimus versus cyclosporin in the 2 largest randomised clinical trials (European FK506 Study Group 1993; Klintmalm 1993/McDiarmid et al. 1993a). However, patient and graft survival rates were numerically greater for tacrolimus in one of these investigations after 6 months (+4 to 5% $p \leq 0.2$; European FK506 Study Group 1993). Although this finding might be considered of doubtful significance when examined alone, statistically and/or numerically greater patient survival (+8 to +17%) and graft survival (+15 to +20%) rates were recorded in the majority of other studies evaluated; graft survival was similar with both drugs in only one of these investigations, although in this investigation tacrolimus recipients were disadvantaged by greater UNOS scores (Todo et al. 1991).

Reflecting the incidence of graft survival in the 2 largest clinical trials, retransplantation rates for tacrolimus were either similar to those for cyclosporin in the investigation reported by Klintmalm (1993) [6.6 *vs* 5.4% $p > 0.05$] or lower in that reported by the European FK506 Study Group (1993) [6.4 *vs* 10.3%; data on file, Fujisawa GmbH, Germany]; however, statistical results were not reported for the latter comparison. Takaya et al. (1991) also reported lower retransplantation rates of primary allografts during the first 6 months of therapy with tacrolimus compared with cyclosporin (8.6 *vs* 15.4%; $p < 0.005$). This finding reflected the higher incidence of primary nonfunction (although cold ischaemia times were similar) and rejection noted for cyclosporin recipients. Indeed, the incidence of rejection was lower for patients treated with tacrolimus in the majority of comparative studies (table I). Notably, the incidence of overall and refractory rejection was significantly lower for tacrolimus versus cyclosporin in the 2 largest randomised clinical trials (European FK506 Study Group 1993; Klintmalm 1993/McDiarmid et al. 1993a). Furthermore, in a smaller randomised study Fung et al. (1991a) reported that the cumulative percentage of patients who remained rejection-free was far greater in the tacrolimus group after 1 month (61 *vs* 18%; $p < 0.001$), and during follow-up (fig. 4). Only one investigation detailed a similar rejection incidence for patients treated with either drug, and in this evaluation rejection episodes were less severe when occurring with tacrolimus and were therefore more manageable (Tzakis et al. 1991b).

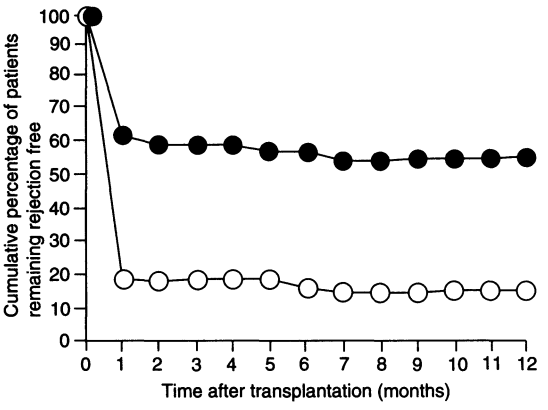


Fig. 4. Cumulative rejection-free status among 81 patients treated with tacrolimus (●) or cyclosporin (○) following primary liver transplantation in a randomised prospective investigation (after Fung et al. 1991a).

Not surprisingly, the lower incidence of rejection noted for patients treated with tacrolimus versus cyclosporin also resulted in a lower requirement for azathioprine (Todo et al. 1991; Tzakis et al. 1991b), high dose steroids (European FK506 Study Group; Fung et al. 1991a; Klintmalm 1993/

McDiarmid et al. 1993a; Todo et al. 1991) and antilymphocyte preparations (Esquivel 1993; Klintmalm 1993/McDiarmid et al. 1993a; Todo et al. 1991). In the randomised investigation conducted by Fung et al. (1991a), 29 of 40 (73%) patients treated with cyclosporin were converted to therapy with tacrolimus, 22 of whom made the switch due to rejection. In contrast, only one patient treated initially with tacrolimus experienced rejection that required retransplantation and therapy with muromonab-CD3; this patient also had evidence of persistent preservation injury. The incidence of rejection among patients treated with tacrolimus was greater when adjunct steroid dosages were low (Jain et al. 1991a,b). However, this finding was not of critical clinical importance as rejection episodes were generally mild and readily controlled with adjunct immunosuppression.

In both noncomparative investigations, primary immunosuppression with tacrolimus was associated with a steroid sparing-effect (table I). Reflecting this facet, patients treated with tacrolimus required less maintenance steroid therapy than those treated with cyclosporin in the comparative investigations that reported steroid usage (Fung et al. 1991a; Todo et al. 1991; Tzakis et al. 1991b). Notably, Tzakis et al. (1991b) reported a decline in the percentage of children requiring steroid therapy to 17% after 3 months and to <10% after 9 months, markedly contrasting with the almost universal requirement for concomitant steroid therapy among children treated with cyclosporin. Withdrawal of steroid therapy was possible for 45% of patients treated with tacrolimus in the investigation conducted by Todo et al. (1991).

The incidence of hypertension was lower for adults or children treated with tacrolimus versus children treated with cyclosporin in one of the largest randomised studies (40 *vs* 75%; Esquivel 1993), but was similar for both groups of patients in the other (European FK506 Study Group 1993). Nevertheless, other investigators have reported hypertension (as assessed by the need for antihypertensive therapy) to be significantly lower for tacrolimus when compared with cyclosporin (Fung et al. 1991a; Tzakis et al. 1991b). Diltiazem was not considered as an antihypertensive drug for cyclosporin recipients, because it is frequently given to increase plasma cyclosporin concentrations (Tzakis et al. 1991b). Hypertension was reported by Todo et al. (1991) for 22% of patients treated with tacrolimus over the first 6 months of therapy, and all almost exclusively responded to monotherapy.

In an effort to determine the effect of the type of immunosuppression on primary liver transplant expenditure, a cost comparison study based on hospital costs was conducted in 42 patients treated with either tacrolimus or cyclosporin matched for age, gender, primary liver disease and UNOS score (Staschak et al. 1990). Transplantation using cyclosporin was nearly twice as expensive as that using tacrolimus ($US244 863 *vs* $US134 169; $p < 0.004$). This difference emerged despite inflated costs associated with tacrolimus resulting from additional compulsory hepatic biopsies and nuclear medicine scans. The differences in costs reflected differences in the duration of hospitalisation, and presumably the differences in the immediate postoperative care of these 2 patient groups.

3.2 Renal Transplantation

3.2.1 Rescue Therapy in Renal Allograft Recipients

The efficacy of tacrolimus as a rescue therapy for patients experiencing renal allograft rejection despite standard optimum immunosuppression has been assessed in only one large study to date (table II; Jordan et al. 1993). Among the 54 patients enrolled, 37 (69%) had previously received at least one course of an antilymphocyte preparation, and were thus considered resistant to conventional immunosuppressive therapy. Conversion of therapy was deemed successful for 38 recipients (70%), and patient survival exceeded 90% after a mean follow-up period of 10.6 months. Analysis by subgroup revealed successful conversion for 26 of 34 patients (76%) with acute rejection, 9 of 13 patients (69%) with a vascular component of rejection, and 3 of 7 patients (43%) with acute rejection and primary graft nonfunction. The use of azathioprine as a

Table II. Summary of clinical studies of tacrolimus (T) in kidney allograft recipients

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
Rescue Therapy								
Jordan et al. (1993)	54 (8-58, mean 34)	p, nc	T 0.025-1.0/d IV or 0.15 bid PO	Previous: C-based with S, Aza &/or mAb. mAb used in 69% of patients	mean 10.6 ± 7.3mo	91	70[a]	43 primary and 11 subsequent transplants including 19 from a living donor. Target T C_{min} (P) 1-2 μg/L. SCr in rescued patients: PrC 292 μmol/L, PoC 212 μmol/L*. Dialysis requirements: PrC 28%, PoC 13%. Conversion success: greater with PrC C/S/Aza than PrC C/S** S requirements: PrC 21%, PoC 9%. S withdrawn in 6 patients
Primary Immunosuppression								
Darras et al. (1991)	39	p, nc	T 0.03-0.24 bid PO[b]	Mtnce: S	6-21 (mean 11) mo	92	74	*En bloc* allografts from paediatric donors aged <4 years. 36 primary and 3 subsequent transplants. Rejection: 54%, reversed for 71%. S withdrawal: 45% of patients. No urological complications
Japanese FK 506 Study Group (1993a)	70 (≥16)	p, nc, mc	T 0.15/12h IV x2d preop then 0.1/d x 3d IV then 0.15 bid PO	Mtnce: S dosages 250 mg/d (MPDN) then 50 mg/d (PDN) ↓ 10 mg/d. Rejection: ↑ T, S, Aza, mizoribine or switch to C	3mo	96	94	Primary transplants, 39 from living related donors and 31 from cadavers. Target T C_{min} (WB) 15-20 μg/L. Rejection: 36%, reversed for 96%

Table II. Contd

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
Jensen et al. (1991)	16 (3-16, mean 10)	p, nc	T 0.1/d IV then 0.15 bid PO	Mtnce: S dosages 1g/d (MPDN) then (PDN) ↓ 20 mg/d initially. Rejection: High dose S or mAb	1-15 (mean 8.5) mo	100	94	12 primary and 4 subsequent transplants including 4 from living related donors. Rejection: 38%, reversed for all. S withdrawal: 47%. S use minimised (≤5 mg/d) 33%
Shapiro et al. (1991a)	65 (mean 40 ±13.5)	p, nc	T 0.075 or 0.15 over 2-4h IV then 0.075/12h IV then 0.15 bid PO	Mtnce: S high dosages [1 g/d (MPDN) then (PDN) ↓ to 20 mg/d n = 25] or low dosages [20 mg/d (PDN) n = 40]. Rejection: ↑ T, S or mAb	≈3-11mo	98	79	43 primary and 23 subsequent transplants[c] including 65 from cadavers. S withdrawal: 60% S use minimised (2.5-5 mg/d): 15%. AHT withdrawal: 40%. Graft loss mainly due to rejection (57%), although 75% of these patients were CCT^{+ve}
Shapiro et al. (1991b)	234 (mean 41 ±14.7)	p, nr, pl	T 0.075 bid over 4h IV, or 0.1-0.15/d IV then 0.15 bid PO		>2 to 27 (med 13) mo	90 (12mo)[d]	74 (12mo)[d]	Incidence of living donors lower, RTX, use of en bloc kidneys and sensitisation higher for T patients. Biochemical indices of renal function were similar for both groups. Rejection: T 59%, C 57%. mAb usage T 19%, C 21%.
	191 (mean 38 ±15.0)		C (NA but not sequential therapy)			94 (12mo)[d]	77 (12mo)[d]	

Continued over

Table II. Contd

Reference	Number of patients (age in years)	Study design	Principal initial treatment (mg/kg)	Other immunotherapy	Duration of follow-up	Survival (%)		Comments
						patient	graft	
								S withdrawal: T 44%, C 0%. S minimised (2.5-5 mg/d): T 24%, C 22%. AHT withdrawal: T 43%, C 25%. Chol: T 4.8 mmol/L, C 6.1 mmol/L†
	28 (mean 37 ±11.6)	p, r, pl	As above		As above	96 (12mo)[d]	82 (12mo)[d]	9 of 29 C patients converted to T. Biochemical indices of graft function were similar in both groups. S withdrawal: T 56%, C 0% S minimised (2.5-5 mg/d): T 11%, C 6%. AHT withdrawal: T 44%, C 22%. Chol: T 4.7 mmol/L, C 6.1 mmol/L**
	29 (mean 39 ±9.9)					89 (12mo)[d]	79 (12mo)[d]	
Shapiro et al. (1993)	63 (19-66, mean 42)	p, r, pl	T 0.15/d PO preop then 0.1/d IV then 0.15 bid PO	Mtnce: S dosages 1g (MPDN) then 200 mg/d ↓ 20 mg/d initially Rejection: High dose S, mAb	mean 5.5 ±2.5mo	100 (6mo)[d]	92 (6mo)[d]	85 primary and 40 subsequent transplants including 17 from a living donor. Incidence of rejection: T 51%, T + Aza 40% ($p > 0.05$).
	62 (18-78, mean 44)		As above + Aza 3/d PO preop then postop			98 (6mo)[d]	85 (6mo)[d]	S withdrawal: 23%. AHT withdrawn: 29%

a Patients showing an improvement in biochemical/histological indices of graft function, and/or freedom from dialysis if previously required.
b Maintenance dosage at last follow-up.
c One patient received a subsequent transplant during the study.
d Actuarial survival.

Abbreviations and symbols: AHT = antihypertensive therapy; Aza = azathioprine; bid = twice daily; BUN = blood urea nitrogen; C = cyclosporin; CCT = cytotoxic antibody crossmatch test; Chol = cholesterol; C_{min} = minimum concentration in plasma (P) or whole blood (WB); d = day; IV = intravenous; mAb = muromonab-CD3 or other antilymphocyte preparations; mc = multicentre; mo = months; MPDN = methylprednisolone; Mtnce = maintenance; NA = data not available; nc = noncomparative; nr = nonrandomised; p = prospective; PDN = prednisone or prednisolone; pl = parallel; PO = oral; PoC = postconversion; postop = postoperatively; PRA = panel reactive antibody; PrC = preconversion; preop = preoperatively; r = randomised; RTX = retransplantation; S = corticosteroids; SCr = serum creatinine concentration; significant difference between treatments * $p < 0.05$, ** $p < 0.01$, † $p < 0.0001$; ↑ = increased; ↓ = decreased.

component of the preconversion immunosuppressive regimen was an indicator of good prognosis. Serum creatinine levels in rescued patients and the overall need for dialysis notably reduced following conversion, demonstrating that the need for dialysis was not a precluding factor for change. Steroid tapering was possible for the majority of patients successfully converted, and no patient required treatment for recurrent rejection.

3.2.2 Primary Therapy in Renal Allograft Recipients

The efficacy of tacrolimus as a primary immunosuppressant therapy following kidney transplantation has been evaluated in a series of noncomparative studies, and compared with that of cyclosporin in one investigation (table II). The design of this latter study conducted by Shapiro et al. (1991b) requires some comment. Indeed, in the overall evaluation, patients were not randomly assigned to therapy, although those treated with tacrolimus were at a higher risk of treatment failure; notably in the tacrolimus cohort the incidence of retransplantation was higher (32 *vs* 19%; $p < 0.005$), and the use of grafts from living donors lower (5 *vs* 14%; $p < 0.02$). A marginally greater incidence of en bloc kidney usage and sensitisation further contributed to the possibility of failure. Together, these differences had the effect of subjecting tacrolimus to a more stringent evaluation. In addition, within the study population evaluated, a small group ($n = 57$) of patients were entered into a randomised investigation which compared tacrolimus with cyclosporin. These patients were all considered to possess good prognosis at baseline. One other randomised study compared the efficacy of tacrolimus in combination with, and in the absence of azathioprine (Shapiro et al. 1993; table II). However, only preliminary data have been published for this investigation.

One to 27 months after transplantation, primary immunosuppression with tacrolimus was associated with patient and graft survival rates of ≥90% and ≥70%, respectively (table II). However, in patients who appeared to be at lower risk of treatment failure, graft survival up to 1 year was noticeably greater (≈80%) [Shapiro et al. 1991a,b]. Graft and patient survival did not differ significantly between patients receiving tacrolimus or cyclosporin, or those treated with tacrolimus in the presence or absence of azathioprine. Worthy of note was the efficacy of tacrolimus in patients who received an en bloc kidney allograft from paediatric donors (Darras et al. 1991). Indeed, the use of kidney allografts from paediatric donors has been controversial because of high rates of graft loss due to technical and immunological complications (Wengerten et al. 1986). Nevertheless, in the study evaluated there were no urological complications, even when en bloc kidneys were transplanted from the smallest of donors. This may have been attributable to rapid tapering of the steroid dosages (see below), thus avoiding delayed healing and the possibility of technical complications.

The incidence of rejection with tacrolimus varied from 36 to 59%, and was similar to that noted for cyclosporin when these drugs were compared in the nonrandomised investigation (table II). Nevertheless, 9 of 29 patients treated with cyclosporin in the randomised investigation were switched to therapy with tacrolimus, presumably due to rejection or drug-related toxicity (Shapiro et al. 1991b). Furthermore, in a study of the histopathological profile of renal allograft rejection in 19 tacrolimus-treated patients and 26 contemporaneous recipients of cyclosporin who experienced a biopsy-confirmed rejection episode within 3 months of transplantation, there was a lower incidence of findings associated with more severe rejection in the tacrolimus group, although the difference did not achieve statistical significance with the small group size employed (Demetris et al. 1991). Rejection episodes were reversed for 71 to 100% of tacrolimus-treated patients with adjuvant therapy (Darras et al. 1991; Japanese FK 506 Study Group 1993a; Jensen et al. 1991). Nevertheless, Shapiro et al. (1991a) reported rejection to be the major cause of graft loss (57% of patients), although in 75% of the patients affected a positive cytotoxic crossmatch test result was obtained. The incidence of muromonab-CD3 usage was similar among recipients of tacrolimus or cyclosporin, al-

though tacrolimus recipients were at a higher risk of treatment failure (Shapiro et al. 1991b). The incidence of rejection was numerically, but not statistically lower, when azathioprine was added to the tacrolimus regimen (51 *vs* 40%; Shapiro et al. 1993).

In all studies evaluated, steroid usage with tacrolimus was tapered and/or stopped (table II). Indeed, withdrawal of steroid therapy was reported to vary in incidence from 23 to 60%. Furthermore, in the randomised investigation which compared tacrolimus and cyclosporin, although it was possible to minimise steroid therapy (2.5 to 5 mg/day) for a similar proportion of patients treated with either of these drugs, steroids were withdrawn for 56% of tacrolimus recipients but remained a component of the immunosuppressive regimen of all cyclosporin recipients (Shapiro et al. 1991b). Addition of azathioprine to tacrolimus-based therapy did not significantly alter steroid usage (Shapiro et al. 1993).

There was also a limited need for antihypertensive therapy among recipients of tacrolimus (table II). Withdrawal of such medication was possible for ≥40% of patients treated with tacrolimus in the majority of studies which reported this facet, although 29% in one. This compared favourably with the incidence recorded for those receiving cyclosporin (22 to 25%). Mean serum cholesterol levels were also significantly greater in patients treated with cyclosporin.

4. Tolerability

Interpretation of the tolerability profile of tacrolimus is complicated by the variety of dosage regimens employed in clinical investigations. Indeed, the adverse effects of tacrolimus have been pivotal in the temporal refinement of dosage regimens for this drug. Generally, toxicity due to the drug decreases with lowering of its dosage, although some reactions (such as the development of dysarthrias) may be idiosyncratic or require multiple factors to emerge, and therefore may not respond to such measures (Fung et al. 1991b).

The principal adverse effects of tacrolimus are nephrotoxicity, infectious and malignant complications, neurotoxicity and diabetogenic effects, all of which have also been associated with conventional immunosuppressive therapies, including cyclosporin (reviewed by Fagiuoli et al. 1992a; Faulds et al. 1993; Fung et al. 1991b; Li et al. 1990). The tolerability profile of tacrolimus appears to be similar in adult and paediatric transplant recipients (Tzakis et al. 1991a, 1993).

4.1 Nephrotoxicity

Like cyclosporin, tacrolimus demonstrates significant nephrotoxicity. Indeed, the clinical presentation and morphology of tacrolimus-induced nephrotoxic changes are identical to those of cyclosporin (McCauley 1993). Furthermore, clinical studies indicate that the incidence of nephrotoxicity with these 2 drugs is approximately equivalent (McCauley 1993).

Nephrotoxicity which necessitated withdrawal from therapy occurred at the same frequency (2%), for patients treated with tacrolimus (n = 182) or cyclosporin (n = 222) in a large randomised investigation when the former drug was administered at initial intravenous dosages ≤0.06 mg/kg (Klintmalm 1993). However, this finding was more common among 47 tacrolimus recipients treated with initial dosages exceeding 0.06 mg/kg (17 *vs* 2%). In another large randomised trial which compared these 2 drugs, dialysis requirements were similar among both groups (European FK506 Study Group 1993).

In an effort to define the nephrotoxic liability of tacrolimus, McCauley et al. (1991d) studied postoperative renal function in primary liver and thoracic organ recipients. Patients received intravenous tacrolimus 0.075 to 0.15 mg/kg, infused over 4 hours in the operating room and repeated at 0.075 mg/kg every 12 hours until oral intake was possible. Oral tacrolimus often overlapped for 1 day with intravenous therapy and was administered at 0.15 mg/kg every 12 hours. In the majority of cases, corticosteroids were also used as a component of immunosuppressant therapy. Virtually all patients exhibited increased serum creatinine levels peri-

operatively, which generally resolved by the end of the first postoperative month with downward adjustment of tacrolimus dosage. Mean serum creatinine levels after 279 postoperative days were 124 and 168 μmol/L among liver and thoracic organ transplant recipients, respectively. Dialysis was required for the first time postoperatively by 9 of 120 patients (7.5%) who received a liver transplant, and 1 of 25 patients (4%) who underwent a heart transplant, although the latter patient had cardiac arrest and massage while being transferred to the operating room. Fagiuoli et al. (1992a) also concluded that tacrolimus-induced nephrotoxicity necessitating haemodialysis generally does not occur in thoracic organ transplant recipients.

A similar, early pattern of nephrotoxicity was observed with tacrolimus in a small prospective randomised trial comparing tacrolimus with standard sequential therapy (Minnesota antilymphoblast globulin followed by cyclosporin) in patients receiving a liver transplant (Stock et al. 1993). Nephrotoxic effects of tacrolimus occurred during the first month following transplantation but subsequently stabilised; standard sequential therapy induced less initial nephrotoxicity but renal function deteriorated between 1 and 6 months postoperatively (table III).

Fung et al. (1991b) reported that 134 of 370 liver transplant recipients (36.2%) exhibited renal dysfunction (serum creatinine >177 μmol/L) during the first month of tacrolimus therapy (acute nephrotoxicity), although almost one-half (64) of these patients had one or more contributing factors such as co-administration of nephrotoxic antibiotics. A total of 81 patients (23.2%) required haemodialysis following transplantation. Chronic nephrotoxicity (after one month of treatment) was observed in 115 patients (31.1%).

Mild nephrotoxicity, responding to dose reduction, occurred during the first postoperative year in 10 of 20 liver transplant recipients (50%) randomised to receive tacrolimus plus corticosteroids, and in 9 of 17 patients (53%) receiving a combined regimen of cyclosporin, corticosteroids plus azathioprine in a nonblinded study (Porayko et al. 1993). Elevation of serum creatinine levels from baseline was similar in both treatment groups, but glomerular filtration rate (GFR) after 12 months of treatment was significantly lower in tacrolimus than in cyclosporin recipients (42 *vs* 64 ml/min/1.73m^2 body surface area). Plasma tacrolimus concentrations correlated poorly with renal function parameters (serum creatinine levels and GFR), as demonstrated in a previous study (McCauley et al. 1990), although Winkler et al. (1991) showed an association between high plasma tacrolimus concentrations and elevated serum creatinine levels. In patients with renal impairment, as assessed by histopathological changes in renal biopsy specimens, tacrolimus trough whole blood concentrations were 18.7 μg/L (Japanese FK 506 Study Group 1993b). These findings indicate that renal toxicity may occur with tacrolimus even if such concentra-

Table III. Renal function among patients randomised to receive tacrolimus or standard sequential therapy (Minnesota antilymphoblast globulin and cyclosporin) following liver transplantation (after Stock et al. 1993)

Immunosuppressive agents	Number of patients	Time post-transplant (months)		
		baseline	1	6
	Glomerular filtration rate (ml/min)			
Tacrolimus	7	55.5	38.6	36.2
Sequential standard therapy	12	75.2	71.8	54.0
	Serum creatinine level (μmol/L)			
Tacrolimus	14	83.1	106.1	110.5
Sequential standard therapy	21	78.7	86.6	106.9

tions are maintained at currently accepted optimum levels (≈15 to 20 μg/L).

Mild hyperkalaemia, associated with low or low-normal renin and aldosterone levels, commonly occurs during tacrolimus therapy, but usually responds to treatment with potassium-binding resins, potassium-restricted diets and/or fludrocortisone (Fagiuoli et al. 1992a; Fung et al. 1991b; Li et al. 1990; McCauley et al. 1990). The hyperkalaemia may be due to an effect of tacrolimus on either mineralocorticoid secretion or altered mineralocorticoid activity at the renal tubules (Fagiuoli et al. 1992a). Another possible mechanism thought to be related to tacrolimus nephrotoxicity is that involving changes in renal cortical haemodynamics leading to reduced GFR and subsequent tubular dysfunction (Fagiuoli et al. 1992a; Sumpio & Phan 1991).

Fries et al. (1991) reported 2 cases of significant myocyte vacuolisation in the arteriolar media at renal biopsy following tacrolimus-associated renal dysfunction in liver transplant patients. Histopathological changes in renal allograft biopsies from patients receiving tacrolimus were similar to those observed in needle biopsies obtained from patients receiving cyclosporin (Randhawa et al. 1993). Early markers of immunosuppressant toxicity were tubular vacuolation and myocyte vacuolation, while long-term administration of tacrolimus was associated with striped interstitial fibrosis and arteriolar hyalinosis, as previously seen with cyclosporin. The Japanese FK 506 Study Group (1993b) categorised early tacrolimus nephropathy of renal allografts as tubular (vacuolation and calcification), vascular (arterioles and glomerulus) and diffuse interstitial fibrosis, although further studies are required to confirm whether vascular lesions and diffuse interstitial fibroses are morphological evidence of tacrolimus nephropathy.

Patients previously treated with cyclosporin predictably exhibit deterioration in renal function with tacrolimus rescue therapy (McCauley et al. 1990; 1991b), and transplant recipients who developed hyperuricaemia and acute gouty arthritis while receiving cyclosporin may also be at risk for marked hyperuricaemia during rescue treatment with tacrolimus (Williams & Lewis 1992).

4.2 Infections and Lymphoproliferative Disorders

Infectious complications remain the leading cause of mortality and morbidity following liver transplantation. Early experience with tacrolimus indicated a lower incidence of severe infection among 20 patients receiving the drug than among a matched group of patients receiving cyclosporin (20 *vs* 55%; $p < 0.05$) as primary immunosuppression after a maximum follow-up period of 62 days (Alessiani et al. 1990). This early experience with tacrolimus was subsequently extended in a non-comparative study of 110 liver allograft recipients treated with this drug and followed over 5 to 12 months (Alessiani et al. 1991). Serious infections developed in 38% of patients receiving tacrolimus, and 50% acquired some type of infection. The majority of cases were bacterial (64 episodes); severe infections included pneumonia, peritonitis and cholangitis. Cytomegalovirus (CMV) was the most frequently reported viral infection (27 episodes), with the liver, gastrointestinal tract and lungs being predominant sites of infection. Fungal infections were less common (13 episodes) but included serious candidiasis and aspergillosis infections. In a retrospective analysis of 2180 liver transplant recipients, the incidence of aspergillosis was significantly lower among patients receiving tacrolimus than cyclosporin (0.2 *vs* 2.4%; $p < 0.01$) [Torre-Cisneros et al. 1991].

In a large randomised investigation which compared tacrolimus with cyclosporin as a primary immunosuppressant therapy in liver allograft recipients ($n = 545$), the overall incidence of infection was lower for tacrolimus-recipients (30 *vs* 40% $p < 0.05$; European FK506 Study Group 1993). Furthermore, although the incidence of infection was similar for both these drugs in another large randomised study which recruited patients with similar characteristics ($n = 520$; McDiarmid et al. 1993a), the incidence of sepsis among children recruited to this latter investigation was reported to

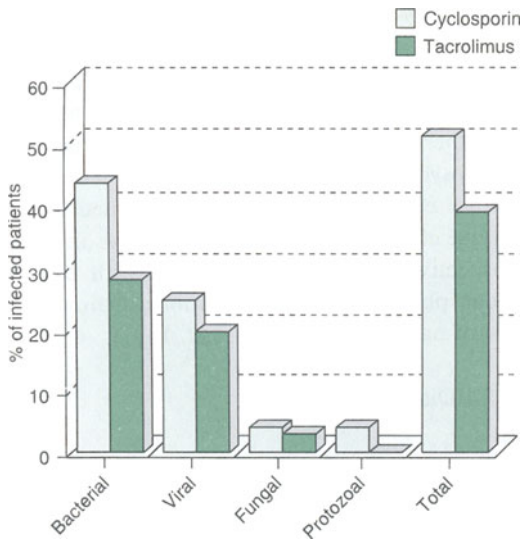


Fig. 5. Percentage of patients with infectious complications following liver transplantation (median follow-up 341 days) in a prospective randomised study comparing tacrolimus (n = 57) versus cyclosporin (n = 53) as primary immunosuppressive therapy (after Kusne et al. 1992).

be lower in the tacrolimus group (17 *vs* 35%; Esquivel 1993).

Infectious complications were also compared in a prospective trial in which 110 liver transplant recipients were randomised to receive tacrolimus (0.1 mg/kg/day intravenously then 0.15 mg/kg twice daily orally) or cyclosporin (4 mg/kg/day intravenously then 4 mg/kg twice daily orally) as primary immunosuppression (Kusne et al. 1992). The frequency of various infection categories was consistently lower with tacrolimus than cyclosporin, but these differences were not statistically significant (fig. 5). Overall, 38.6% of 57 tacrolimus recipients had at least one infection compared with 50.9% of 53 cyclosporin recipients. Tacrolimus was associated with a significantly lower incidence of bacteraemia (7 *vs* 26.4%; $p = 0.013$) which may have been due to a decreased requirement for corticosteroids and muromonab-CD3 therapy among this group of patients. Again, CMV was the most frequently reported viral infection, occurring in 17.5% of tacrolimus recipients and 20.8% of cyclosporin recipients. Kusne et al. (1991) also reported an infection rate of 33% among kidney transplant recipients treated with tacrolimus compared with a rate of 61% for a group of 64 historical control patients who received cyclosporin. In this study, all infections occurred within 4 months of transplantation, and those patients undergoing secondary retransplantation had a significantly higher number of infectious episodes than primary transplantation patients (0.68 *vs* 0.27 per patient; $p < 0.05$).

Patients receiving tacrolimus as primary immunosuppressive therapy had a significantly lower rate of CMV enteritis than cyclosporin recipients during the first month following liver transplantation (0 *vs* 11.5%; $p < 0.05$) in a randomised trial of 140 patients (Sakr et al. 1992a). The overall incidence of enteric CMV infection (after >6-month follow-up) was also lower with tacrolimus (20 *vs* 27.7%), but the difference was not statistically significant. In a separate study, approximately 1% of patients receiving tacrolimus following solid organ transplantation developed CMV retinitis (Paul et al. 1991).

The number of infectious episodes in children up to 90 days following liver transplantation was lower in tacrolimus recipients than matched historical controls who received cyclosporin (1.2 to 1.4 *vs* 1.8 to 2.0 episodes/patient $p < 0.05$: Green et al. 1991; Tzakis et al. 1991b). However, these results may have been influenced by reductions in the use of central venous catheterisation which preceded the onset of the trials.

Post-transplant lymphoproliferative disorders (PTLD) occur in approximately 1.7% of solid organ transplant patients receiving cyclosporin (Nalesnik et al. 1988). Tacrolimus appears to have a similar incidence of PTLD, ranging from 0.7 to 1.6% in this patient population (Nalesnik et al. 1991, 1992; Reyes et al. 1991). There is a strong association between the development of PTLD and infection with Epstein-Barr virus (EBV); Reyes et al. (1991) found all 15 tacrolimus patients who developed PTLD had serological evidence of EBV (Reyes et al. 1991). It has been reported that tacrolimus may have mutagenic potential at suprathrapeutic concentrations in human lymphocytes *in*

vitro (Yu et al. 1993). However, no mutagenic activity was recorded for this compound in a battery of *in vitro* and *in vivo* tests (Hirai et al. 1992).

4.3 Neurotoxicity

Neurological adverse effects associated with post-transplant tacrolimus immunosuppression most commonly develop during the intravenous phase of drug administration, and can be categorised as major (e.g. akinetic mutism, expressive aphasia, seizures, confusion requiring investigation, psychosis, encephalopathy, persistent coma) or minor (e.g. tremors, headache, sleep disturbances, nightmares, dysesthesias, photophobia) neurotoxicity (Eidelman et al. 1991; Fung et al. 1991b). Of 370 consecutive liver transplant recipients receiving tacrolimus postoperatively, 8.4% exhibited major neurotoxicity associated with the immunosuppressant (Fung et al. 1991b). In another large group of 294 solid organ transplant recipients treated with tacrolimus, 5.4% of patients developed major neurological toxicities with a mean onset of 12.8 days after commencing therapy, and minor neurological adverse effects occurred in approximately 20% of the population studied (Eidelman et al. 1991).

In most instances, tacrolimus-induced neurotoxicity is reversible following temporary discontinuation of tacrolimus therapy or dosage reduction (Eidelman et al. 1991; Reyes et al. 1990), although some reactions such as dysarthrias may not always respond to decreased doses of tacrolimus (Fung et al. 1991b). The incidence of neurotoxicity necessitating withdrawal from therapy was similar (3 *vs* 2%) for patients treated with tacrolimus (n = 182) or cyclosporin (n = 222) when the former drug was administered at initial intravenous dosages $\leq$0.06 mg/kg in a large randomised study, but was greater when these initial dosages were exceeded (9 *vs* 2%) [Klintmalm 1993]. Qualitatively, the neuropathological changes observed with tacrolimus appear to be similar to those associated with cyclosporin immunosuppression (Freise et al. 1991; Lopez et al. 1991). In a small randomised study of 24 liver transplant recipients, no difference was demonstrated between tacrolimus and cyclosporin treatment groups during the first postoperative week with respect to cognitive and psychiatric symptoms; plasma concentrations of either drug correlated with clinician-rated neuropsychiatric symptom checklist scores (DiMartini et al. 1991). Similarly, major neurological adverse effects observed with tacrolimus in a large prospective study were associated with elevated trough plasma drug concentrations in more than 50% of patients (Eidelman et al. 1991).

4.4 Diabetogenic Effects

Glucose metabolic disorders were reported more frequently for patients treated with tacrolimus versus cyclosporin in a large randomised investigation (17 *vs* 10% $p < 0.05$; European FK506 Study Group 1993). However, the incidence of hyperglycaemia was similar among children randomised to therapy with either of these drugs in a similar investigation (Esquivel 1993). Furthermore, the incidence of new-onset insulin-dependent diabetes in tacrolimus- versus cyclosporin-treated patients was comparable following liver transplantation in a smaller randomised study (Fung et al. 1991a). Three months following transplantation 17% of tacrolimus recipients and 17.5% of cyclosporin recipients required insulin therapy ($p > 0.05$). Scantlebury et al. (1991) also evaluated this finding among kidney allograft recipients randomised to therapy with either of these 2 drugs. Diabetes mellitus developed in 4 of 20 patients (20%) treated with tacrolimus and in 1 of 14 patients (7%) treated with cyclosporin. This difference was not statistically significant given the small group size, and the finding was reversible for 2 tacrolimus recipients and the one recipient of cyclosporin.

The association of insulin requirements with tacrolimus appears to be confounded by perioperative events. Indeed, 151 of 370 patients (35.5%) who received a liver allograft and tacrolimus-based immunosuppression required insulin therapy for hyperglycaemia within a median of 2 days, suggesting that perioperative events were more likely to lead to insulin requirements than the use of tac-

rolimus. Corroborating this supposition, recovery was noted in 106 of these patients and only 12.1% of patients required long term insulin treatment (median follow-up 13.5 months). In a smaller but more extensive analysis, the long term insulin requirement among 46 adult patients who were treated with tacrolimus after liver transplantation was assessed over a mean follow-up period of approximately 1 year (Tabasco-Minguillan et al. 1993). *De novo* insulin was needed for 15% of patients at 3 months and in 20% of them at 6 months post-transplant, but this declined to 5.5% after 12 months. Insulin-dependent diabetes mellitus did not develop in any patient beyond the 18-month evaluation point. Furthermore, insulin dependence did not affect graft or patient survival over the period studied, and did not correlate with tacrolimus dosage.

The incidence of new-onset diabetes mellitus in allograft recipients treated with tacrolimus appears to be lower among the paediatric than the adult population. Indeed, of 206 children who received a solid organ transplant and subsequent treatment with tacrolimus, 3 (1.5%) developed insulin-dependent diabetes, and all were receiving tacrolimus as rescue therapy (Carroll et al. 1991).

Glycaemia and insulin requirements changed only minimally in patients following a switch to tacrolimus therapy in 76 adults or children experiencing toxicity due to previous administration of cyclosporin and/or steroids; only one patient who was insulin-free before the switch required insulin afterwards (Mieles et al. 1991a). This analysis was complicated by the increased immunosuppression (especially with prednisone) given prior to rescue therapy in an effort to overcome graft dysfunction, and presumably contributed to the increases in blood glucose and serum creatinine which only reversed after weeks or months.

4.5 Other Adverse Effects

Hypertension is often observed in allograft recipients. In those who were previously normotensive this may be due to excessive water volume, intrinsic renal damage or increased vasomotor tone (Fung et al. 1991b). Use of corticosteroids may also lead to retention of excess water. Furthermore, patients with liver failure manifest a low peripheral resistance prior to transplantation which may mask hypertension. The incidence of newly diagnosed hypertension among 370 patients treated with tacrolimus who received a liver allograft was 42.4%; however, withdrawal from antihypertensive medication was possible for 38 of these 157 patients as their fluid status normalised. Similarly, although most of the 65 patients treated with tacrolimus who received a renal allograft required antihypertensive medication at entry to a noncomparative study, after a follow-up period of $\approx$3 to 11 months, 40% of those with functioning kidneys no longer required this medication, and 46% only required monotherapy (Shapiro et al. 1991a). Among 309 transplant recipients the incidence of withdrawal from antihypertensive therapy was greater among recipients of tacrolimus (43% of 177) than cyclosporin (25% of 132) [Shapiro et al. 1991b]. Similar findings were recorded for a subset of these patients with good prognosis at entry entered into a randomised trial comparing these 2 drugs (Shapiro et al. 1991b). Corroborating these findings, hypertension was recorded less frequently for children treated with tacrolimus than those treated with cyclosporin in a more recent randomised investigation (Esquivel 1993). However, in one large randomised investigation the incidence of hypertension was similar for recipients of either drug (European FK506 Study Group 1993).

Gingival hyperplasia and hirsutism, which commonly occur with cyclosporin therapy, do not appear to be a significant complication associated with tacrolimus (Fung et al. 1991b). Furthermore, tacrolimus-associated hypercholesterolaemia has not been observed, and may be of importance with long-term administration (Fung et al. 1991b).

Other adverse events associated with tacrolimus in decreasing frequency (incidence not given) were insomnia, tremors, headache, tingling sensations, muscle aching, itching, fatigue, visual sensitivity to light, and gastrointestinal symptoms (Fung et al. 1991b). In randomised investigations tremor has been reported to occur more frequently with tac-

rolimus than with cyclosporin (Esquivel 1993; European FK506 Study Group 1993).

In common with cyclosporin, tacrolimus has been associated with instances of acute haemolytic anaemia. This finding was recorded for 8 of 1400 patients (0.6%) who received tacrolimus at the University of Pittsburgh in the US (Abu-Elmagd et al. 1991b). Haemolysis appeared to be triggered by different aetiological factors. However, *in vitro* studies and the high affinity of tacrolimus for the red blood cell membrane implicate the potential role of this drug in inducing and/or promoting red blood cell destruction in patients with acquired anti-erythrocyte antibodies (Abu-Elmagd et al. 1991b).

5. Drug Interactions

Results of *in vitro* studies suggest that tacrolimus is a potent inhibitor of cytochrome P450-dependent drug metabolism in human liver microsomes and hepatocytes, with a degree of specificity for P450 IIIa (Ali Shah et al. 1991; Burke et al. 1990; Christians et al. 1993; Pichard et al. 1991). This enzyme system is responsible for oxidation of calcium channel blockers, corticosteroids, cyclosporin, macrolide antibiotics and other drugs.

Since tacrolimus and cyclosporin are both substrates of hepatic cytochrome P450 IIa, each drug can act as a competitive inhibitor of the other's metabolism *in vitro* (Omar et al. 1991). Cyclosporin metabolism by human liver microsomes was inhibited by 86 and 88% by tacrolimus 100 μmol/L in two different human liver samples (Burke et al. 1990). Studies in dogs suggest tacrolimus may also inhibit cytochrome P450 IIIc in the intestinal tract, resulting in increased absorption of cyclosporin (Wu et al. 1991b). Indeed, plasma cyclosporin concentrations in humans were increased on coadministration with tacrolimus (Starzl et al. 1989a), suggesting that intestinal metabolism of cyclosporin is inhibited by tacrolimus. However, in liver transplant patients, short term treatment with tacrolimus does not appear to alter the pharmacokinetics of cyclosporin (Jain et al. 1991b). Despite these conflicting observations, combined use of tacrolimus and cyclosporin results in synergistic immunosuppression (Zeevi et al. 1987), and nephrotoxicity (McCauley et al. 1991d) in humans.

In vitro metabolism of tacrolimus by human hepatic microsomes was inhibited by bromocriptine, corticosterone, ethinylestradiol, methylprednisolone, erythromycin, josamycin, troleandomycin, ketoconazole, miconazole, nifedipine, verapamil, omeprazole, ergotamine and midazolam (Christians et al. 1993). However, the *in vitro* metabolism of tacrolimus did not appear to be affected by cyclosporin (Christians et al. 1991b).

Elimination of tacrolimus was impaired in a liver transplant recipient when clotrimazole troches 10mg four times daily were given in conjunction with tacrolimus 6 mg/day orally; the trough plasma tacrolimus concentration increased from 3.5 to 9 μg/L over an 8-day period (Mieles et al. 1991b). The area under the tacrolimus plasma concentration-time curve (AUC) doubled after addition of clotrimazole therapy, but the elimination half-life increased only slightly from 10.5 to 11.8 hours. The authors speculated that clotrimazole may compete with tacrolimus for binding sites of the cytochrome P450 enzyme system in the intestine, resulting in decreased metabolism of tacrolimus by intestinal mucosa and increased absorption of the immunosuppressant. Increased plasma tacrolimus concentrations (measured by ELISA or bioassay) have been associated with coadministration of cytochrome P450 inhibitors such as ketoconazole, clotrimazole, diltiazem, fluconazole, and erythromycin (Rui et al. 1992; Venkataramanan et al. 1991). Coadministration of steroids increases plasma tacrolimus concentrations in humans, as measured by ELISA, without causing a proportionate rise in bioassay-based concentrations, suggesting an inhibitory effect of steroids on the biliary excretion of tacrolimus (Zeevi et al. 1990b).

6. Dosage and Administration

Despite over 10 years of experience with cyclosporin, treatment regimens with this drug vary considerably between individual transplantation centres and are continually being modified. Experience with tacrolimus to date has demonstrated a

similar evolution process, particularly with regard to dosage reduction.

Following hepatic or renal transplantation, tacrolimus is usually administered by intravenous infusion commencing after revascularisation of the graft and continuing until oral therapy with the drug is feasible. In early clinical trials tacrolimus 0.075 mg/kg as a 2- to 4-hour infusion twice daily was initially used. However, to avoid the potential adverse effects related to high plasma concentrations, the same daily dosage was subsequently given over 24 hours. With additional clinical experience, an intravenous infusion of 0.1 mg/kg/24h has been employed most extensively.

The switch to oral therapy with tacrolimus was usually made within 4 days of transplantation, and an oral dosage of 0.15 mg/kg twice daily was then most frequently employed in clinical trials. Despite these observations, it should be noted that the optimum dosage regimen for tacrolimus is continuing to be refined and these dosages may be superseded. Indeed, most recent experience in large randomised studies indicate that initial intravenous dosages of $\leq$0.1 mg/kg/day (Klintmalm 1993) or 0.035 to 0.075 mg/kg day (data on file, Fujisawa GmbH, Germany) should be employed, with subsequent initial oral dosages of 0.1 to 0.2 mg/kg/day (data on file, Fujisawa GmbH, Germany). In patients with early liver dysfunction, however, an initial intravenous tacrolimus dosage of 0.025 mg/kg/day reduced the incidence of adverse effects but not the incidence of rejection (Winkler et al. 1993a). Dosage adjustments are made based on the clinical status of the patient, the functional status of the liver and kidney, and trough tacrolimus concentrations in plasma (target range $\approx$0.5 to 2 μg/L) or whole blood ($\approx$15 to <20 μg/L). In the majority of centres measurements of tacrolimus in whole blood is the method of choice. A computer programme is available to select the optimum tacrolimus dosage based on multiple patient parameters (McMichael et al. 1991).

Tacrolimus requirements generally decline with time, although long term therapy is necessary to prevent rejection. Children require higher maintenance tacrolimus dosages than adults. Indeed, comparison of 16 paediatric and 33 adult patients followed for 1 year after liver transplantation revealed daily tacrolimus dosages were 2.7- to 4.4-fold greater among children when expressed as a function of bodyweight (McDiarmid et al. 1993b). Preoperative administration of tacrolimus has been rare, although based on prior experience, it has been given intravenously at 0.15 mg/kg/12h for 2 days prior to renal transplantation in Japan or as a single oral dosage of 0.15 mg/kg in this setting in the US.

Available evidence suggest that tacrolimus should not be co-administered with cyclosporin due to potential nephrotoxicity. Furthermore, there have been reports of patients treated previously with cyclosporin exhibiting a deterioration in renal function with tacrolimus rescue therapy. Tacrolimus should be administered with caution to patients receiving drugs which are known to undergo metabolism via the cytochrome P450 IIIa enzyme system.

7. Place of Tacrolimus in Hepatic and Renal Transplantation

The success of organ transplantation has improved significantly over the past few decades, reflecting refinement of many facets of this area of surgery including patient selection, surgical technique, organ preservation and postoperative management. However, the greatest impact on transplantation success has occurred with the introduction of cyclosporin. Indeed, whereas only one in three patients undergoing liver transplantation during the pre-cyclosporin era were alive one year after transplantation, survival has more than doubled since then (Starzl et al. 1989b). It is not surprising therefore that virtually all post-transplant immunosuppressive regimens include cyclosporin, generally in association with either azathioprine and/or prednisolone. For the treatment of rejection crises, steroids given in high dosages have remained the most common modality. However, antilymphocyte preparations are used in the treatment of steroid-resistant rejection episodes, and for induction immunotherapy where cyclosporin is introduced sequentially.

Despite the impressive advances in transplantation made during the cyclosporin epoch, there still remains considerable scope for further improvement. Rejection (particularly chronic rejection), infection and drug toxicity are the three main problems that continue to hamper success. Indeed, rejection and infection are the leading causes of morbidity and mortality in patients who receive a liver transplant (Cosimi 1991). This observation reflects the inability of current immunosuppressive regimens to selectively block the allograft response while leaving other host defences intact (Simmons & Wang 1991). In addition, each immunosuppressive drug presents serious tolerability problems which are mainly drug-specific. Furthermore, it is often difficult to differentiate between chronic rejection and cyclosporin-nephrotoxicity in recipients of kidney allografts. Against this background it is not surprising that the search for more effective and less toxic immunosuppressive drugs continues.

Tacrolimus is a new immunosuppressant that displays similar but more potent immunosuppresant properties than cyclosporin. Its pharmacokinetic profile, however, like that of cyclosporin, shows considerable interindividual variation. This has presented clinicians with a perplexing problem with regard to dosage selection and is reflected in the variety of dosage regimens used in clinical trials. The optimum intravenous induction dosage regimen for tacrolimus continues to be refined, and although some centres appear to have established the subsequent initial oral maintenance dosage, it continues to be modified by others. To overcome the complexity of dosage selection, a computer programme has been developed to predict the optimum dosage for tacrolimus tailored to multiple patient parameters. Dosage adjustments based on tacrolimus concentrations in whole blood or plasma have been aided by the improved availability of assays for this drug. Indeed, monitoring of plasma or whole blood tacrolimus concentrations is recommended where dosage adjustment is necessary (particularly in patients with hepatic insufficiency in whom concentrations may become rapidly elevated causing toxicity) or when co-administering drugs which may affect the disposition of tacrolimus.

The clinical efficacy of tacrolimus has been assessed in studies conducted in the US, Japan and Europe, but mostly by workers at the University of Pittsburgh, Pennsylvania in the US. The majority of these investigations have not been published in their entirety, or have only been published to document symposia proceedings. Consequently, most have not been subjected to extensive peer review and should therefore be interpreted with caution. Furthermore, there has only been one large study which evaluated the efficacy of tacrolimus in the rescue of patients with failing kidney allografts, and there is a paucity of published randomised investigations of tacrolimus versus cyclosporin as a primary immunosuppressant therapy, particularly in renal transplantation. Despite these drawbacks associated with the data evaluated, tacrolimus demonstrated notable efficacy as a rescue or primary immunosuppressive therapy in adult or paediatric recipients of liver or renal allografts.

Available clinical evidence suggests that like cyclosporin, tacrolimus is more effective in suppressing acute rather than chronic rejection episodes. Nevertheless, as a rescue therapy in combination with corticosteroids, tacrolimus improves the biochemical and histological indices of graft function which results in prolonged graft survival for a significant proportion of patients (≥50%) experiencing rejection or toxicity associated with cyclosporin-based therapy. As a primary immunosuppressant when combined with corticosteroids in patients undergoing liver transplantation, patient and graft survival rates did not differ significantly for tacrolimus versus cyclosporin in the 2 largest randomised clinical trials conducted to date, although in one, both rates were marginally greater for tacrolimus. In other investigations conducted in this setting, compared with cyclosporin, tacrolimus demonstrated statistically and/or numerically superior graft survival (+8 to +17%) and patient survival (+15 to +20%) rates. This may reflect, at least in part, the marked hepatotrophic effect of the drug. Graft and patient survival were

essentially similar for patients treated with tacrolimus or cyclosporin following renal transplantation. However, tacrolimus was of particular worth in en bloc kidney transplantation, an area traditionally hampered by complications associated with slow healing.

The incidence of rejection was largely reduced following rescue therapy with tacrolimus and was generally lower (notably for refractory rejection) than that noted for cyclosporin, as a primary immunosuppressant, at least in liver allograft recipients. This was reflected in less need for adjunct therapy for the treatment of rejection episodes. Tacrolimus was also associated with a steroid-sparing effect. Indeed, reduction or withdrawal of maintenance steroid therapy is a common feature of tacrolimus therapy. In terms of health economics, reduced hospitalisation costs are evident with tacrolimus usage when compared with cyclosporin as a primary immunosuppressant in the field of liver transplantation.

Definition of the adverse effects of tacrolimus has been hampered by the variety of dosage regimens used in clinical trials. Indeed, the adverse effects of tacrolimus have been pivotal in the temporal refinement of dosage regimens. Generally, most adverse effects improve with dosage reduction, although some which are idiosyncratic or require multiple factors to emerge may not respond to such measures. Qualitatively, the adverse effects of tacrolimus are similar to those seen with cyclosporin; nephrotoxicity, infectious and malignant complications, neurotoxicity and diabetogenic effects are the most troublesome. Quantitatively, some adverse effects may occur less frequently with tacrolimus than with cyclosporin. Notably, the incidence of infectious complications appear to be less with tacrolimus, and a lower frequency of hypertension and hypercholesterolaemia reported by some investigators may be an additional benefit. Whether these differences reflect the lower steroid requirements associated with tacrolimus is open to question. In addition, tacrolimus also provides immunosuppressant therapy without the disfiguring complications of hirsutism and gum hyperplasia which are common features of therapy with cyclosporin. This is of particular importance in children and may aid long term patient compliance.

The place of tacrolimus in the therapy of patients undergoing hepatic or renal transplantation is still emerging. Nevertheless, preliminary clinical data (although limited for kidney transplant recipients) show this new immunosuppressant clearly provides a valuable therapeutic alternative for patients experiencing rejection or drug-related toxicity with other immunosuppressive regimens in whom retransplantation (and dialysis in kidney transplantation) is often the only alternative. Pending confirmation of initial randomised studies, and preliminary results from larger randomised investigations, primary immunosuppression with tacrolimus may well be considered an option to cyclosporin, particularly in patients undergoing liver transplantation. Furthermore, the steroid-sparing effects of tacrolimus (although of potential benefit to all patients) may be of notable benefit in special patient groups where the adverse effects of these drugs are particularly worrisome. Recipients of en bloc kidney allografts will probably benefit through avoidance of complications associated with slow healing, but a greater beneficial impact will most likely be seen for children in whom growth retardation is a problem. With additional clinical experience, tacrolimus may well emerge as the drug of choice in both these settings.

In conclusion, although further clinical experience will help define the role of tacrolimus in renal and hepatic transplantation, this drug already provides a worthwhile addition to the limited range of immunosuppressants available to the transplant surgeon.

References

Abu-Elmagd K, Fung J, Alessiani M, Jain A, Venkataramanan R, et al. The effect of graft function on FK506 plasma levels, dosages, and renal function, with particular reference to the liver. Transplantation 52: 71-77, 1991a

Abu-Elmagd KM, Bronsther O, Kobayashi M, Yagihashi A, Iwaki Y, et al. Acute hemolytic anaemia in liver and bone marrow transplant patients under FK 506 therapy. Transplantation Proceedings 23: 3190-3192, 1991b

Alessiani M, Kusne S, Martin FM, Fung JJ, Jain A, et al. Infections with FK 506 immunosuppression: preliminary results with

primary therapy. Transplantation Proceedings 22 (Suppl. 1): 44-46, 1990

Alessiani M, Kusne S, Martin M, Jain A, Abu-Elmagd K, et al. Infections in adult liver transplant patients under FK 506 immunosuppression. Transplantation Proceedings 23: 1501-1503, 1991

Ali Shah I, Whiting PH, Omar G, Thomson AW, Burke MD. Effects of FK 506 on human hepatic microsomal cytochrome P450-dependent drug metabolism in vitro. Transplantation Proceedings 23: 2783-2785, 1991

Andersson J, Nagy S, Groth CG, Andersson U. Effects of FK506 and cyclosporin A on cytokine production studied in vitro at a single-cell level. Immunology 75: 136-142, 1992

Arai K, Hotokebuchi T, Miyahara H, Arita C, Mohtai M, et al. Limb allografts in rats immunosuppressed with FK506. I. Reversal of rejection and indefinite survival. Transplantation 48: 782-786, 1989

Asako H, Kubes P, Baethge BA, Wolf RE, Granger DN. Reduction of leucocyte adherence and emigration by cyclosporine and L683,590 (FK506) in postcapillary venules. Transplantation 54: 686-690, 1992

Banerji SS, Parsons JN, Tocci MJ. The immunosuppressant FK-506 specifically inhibits mitogen-induced activation of the interleukin-2 promoter and the isolated enhancer elements NFIL-2A and NF-AT1. Molecular and Cellular Biology 11: 4074-4087, 1991

Baumann G, Geisse S, Sullivan M. Cyclosporin A and FK-506 both affect DNA binding of regulatory nuclear proteins to the human interleukin-2 promoter. New Biology 3: 270-278, 1991

Beck Y, Akiyama N. Effect of FK-506 and cyclosporine on human lymphocyte responses in vitro. Transplantation Proceedings 21: 3464-3467, 1989

Benigni A, Morigi M, Perico N, Zoja C, Amuchastegui CS, et al. The acute effect of FK506 and cyclosporine on endothelial cell function and renal vascular resistance. Transplantation 54: 775-780, 1992

Benigni A, Chiabrando C, Piccinelli A, Perico N, Gavinelli M, et al. Increased urinary excretion of thromboxane B_2 and 2,3-dinor-TxB_2 in cyclosporine A nephrotoxicity. Kidney International 34: 164-174, 1988

Beysens AJ, Wijnen RMH, Beuman GH, Van der Heyden J, Kootstra G, et al. FK 506: monitoring in plasma or in whole blood? Transplantation Proceedings 23: 2745-2747, 1991

Bierer BE, Mattila PS, Standaert RF, Herzenberg LA, Burakoff SJ, et al. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. Proceedings of the National Academy of Sciences of the United States of America 87: 9231-9235, 1990a

Bierer B, Schreiber SL, Burakoff SJ. The effect of the immunosuppressant FK-506 on alternate pathways of T-cell activation. European Journal of Immunology 21: 439-445, 1991

Bierer B, Schreiber SL, Burakoff SJ. Mechanisms of immunosuppression by FK506. Preservation of T-cell transmembrane signal transduction. Transplantation 49: 1168-1170, 1990b

Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL. Probing immunosuppressant action with a nonnatural immunophilin ligand. Science 250: 556-559, 1990c

Blaehr H, Andersen CB, Ladefoged J. Acute effects of FK506 and cyclosporine A on cultured human proximal tubular cells. European Journal of Pharmacology 228: 283-288, 1993

Blanc P, Etienne H, Daujat M, Fabre I, Pichard L, et al. Antiproliferative effect of FK 506 and cyclosporine on adult human hepatocytes in culture. Transplantation Proceedings 23: 2821-2824, 1991

Boggs S, Patrene K, Vecchini F, Markus P, Cooper M, et al. FK 506 has no short-term effects on endogenous or exogenous myeloid reconstitution in irradiated mice. Transplantation Proceedings 23: 3236-3237, 1991

Borg AJ, Kumagai M. FK 506 and xenogeneic human anti-porcine cellular reactivity. Transplantation Proceedings 23: 2972-2973, 1991

Brabletz T, Pietrowski I, Serfling E. The immunosuppressives FK-506 and cyclosporin-A inhibit the generation of protein factors binding to the 2 purine boxes of the interleukin-2 enhancer. Nucleic Acids Research 19: 61-67, 1991

Burke MD, Omar G, Thomson AW, Whiting PH. Inhibition of the metabolism of cyclosporine by human liver microsomes by FK506. Transplantation 50: 901-902, 1990

Cadoff EM, Venkataramanan R, Krajack A, Jain AS, Fung JJ, et al. Assay of FK 506 in plasma. Transplantation Proceedings 22 (Suppl. 1): 50-52, 1990

Calne R, Collier DStJ, Thiru S. Observations about FK-506 in primates. Transplantation Proceedings 19 (Suppl. 6): 63, 1987

Carroll PB, Rilo H, Reyes J, Alejandro R, Zeng Y, et al. FK-506-associated diabetes mellitus in the pediatric transplant population is a rare complication. Transplantation Proceedings: 3171-3172, 1991

Chang JY, Sehgal SN, Bansbach CC. FK506 and rapamycin: novel pharmacological probes of the immune response. Trends in Pharmacological Sciences 12: 218-223, 1991

Christians U, Guengerich FP, Schmidt G, Sewing KF. In vitro metabolism of FK506, cytochrome P450 and drug interactions. Abstract. Therapeutic Drug Monitoring 15: 145, 1993

Christians U, Braun F, Kosian N, Schmidt M, Schiebel HM, et al. High performance liquid chromatography/mass spectrometry of FK 506 and its metabolites in blood, bile and urine of liver grafted patients. Transplantation Proceedings 23: 2741-2744, 1991a

Christians U, Braun F, Sattler M, Almeidal VMF, Linck A, et al. Interactions of FK 506 and cyclosporine metabolism. Transplantation Proceedings 23: 2794-2796, 1991b

Christians U, Kruse C, Kownatzki R, Schiebel HM, Schwinzer R, et al. Measurement of FK 506 by HPLC and isolation and characterization of its metabolites. Transplantation Proceedings 23: 940-941, 1991c

Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 357: 695-697, 1992

Collier DStJ, Calne R, Thiru S, Friend PJ, Lim S, et al. FK-506 in experimental renal allografts. Transplantation Proceedings 19: 3975-3977, 1987

Cosimi AB. Update on liver transplantation. Transplantation Proceedings 23: 2083-2090, 1991

Crabtree GR. Contingent genetic regulatory events in T lymphocyte activation. Science 246: 1617-1620, 1989

Darras FS, Jordan ML, Shapiro R, Sundberg R, Scantlebury VP, et al. Transplantation of pediatric en bloc kidneys under FK 506 immunosuppression. Transplantation Proceedings 23: 3089-3090, 1991

DeFranco AL. Immunosuppressants at work. Nature 352: 754-755, 1991

Demetris AJ, Fung JJ, Todo S, McCauley J, Jain A, et al. Conversion of liver allograft recipients from cyclosporine to FK506 immunosuppressive therapy – a clinicopathologic study in 96 patients. Transplantation 53: 1056-1062, 1992

Demetris AJ, Banner B, Fung J, Shapiro R, Jordan M, et al. Histopathology of human renal allograft rejection under FK 506: a comparison with cyclosporine. Transplantation Proceedings 23: 944-946, 1991

De Paulis A, Cirillo R, Ciccarelli A, de Crescenzo G, Oriente A, et al. Characterization of the anti-inflammatory effect of FK-506 on human mast cells. Journal of Immunology 147: 4278-4285, 1991b

De Paulis A, Cirillo R, Ciccarelli A, Condorelli M, Marone G. FK-506, a potent novel inhibitor of the release of proinflammatory mediators from human $F_{\epsilon}\epsilon RI+$ cells. Journal of Immunology 146: 2374-2381, 1991a

De Paulis A, Stellato C, Cirillo R, Ciccarelli A, Oriente A, et al. Anti-inflammatory effect of FK-506 on human skin mast cells. Journal of Investigative Dermatology 99: 723-728, 1992

DeValeria PA, Gillinov AM, Chacko VP, Handa N, Bando K, et al. FK 506 fails to reverse moderate cardiac allograft rejection in a canine heterotopic model. Transplantation Proceedings 23: 3288-3290, 1991

Dhar DK, Nagasue N, Kimoto T, Uchida M, Takemoto Y, et al. The salutary effect of FK506 in ischemia-reperfusion injury of the canine liver. Transplantation 54: 583-588, 1992

Diethelm AG. A critical appraisal of renal transplantation. Transplantation Proceedings 24: 2387-2390, 1992

DiMartini A, Pajer K, Trzepacz P, Fung J, Starzl T, et al. Psychiatric morbidity in liver transplant patients. Transplantation Proceedings 23: 3179-3180, 1991

Dumont FJ, Altmeyer A. Differential modulation of IL-1-induced IFN-γ production by the immunosuppressive macrolides rapamycin (RAP) and FK-506. Lymphokine Research 9: 574, 1990

Dumont FJ, Melino MR, Staruch MJ, Koprak SL, Fischer PA, et al. The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T cells. Journal of Immunology 144: 1418-1424, 1990a

Dumont FJ, Staruch MJ, Koprak SL, Melino MR, Sigal NH. Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. Journal of Immunology 144: 251-258, 1990b

Dumont FJ, Staruch MJ, Koprak SL, Siekierka J, Kindt V, et al. The immunosuppressive and toxic actions of FK-506 are mechanistically related. Abstract. FASEB Journal 6: A1692, 1992

Eidelman BH, Abu-Elmagd K, Wilson J, Fung JJ, Alessiani M, et al. Neurologic complications of FK 506. Transplantation Proceedings 23: 3175-3178, 1991

Eiras G, Shimizu Y, van Seventer GA, Duquesnoy RJ, Zeevi A. Effects of FK 506 and cyclosporine on T-cell activation: integrin-mediated adhesion of T cells, proliferation, and maturation of cytotoxic T cells. Transplantation Proceedings 23: 936-939, 1991

Ericzon BG, Wijnen RMH, Tiebosch A, Kubota K, Kootstra G, et al. The effect of FK506 treatment on pancreaticoduodenal allotransplantation in the primate. Transplantation 53: 1184-1189, 1992

Esquivel C. FK506 therapy after pediatric liver transplantation: comparison with cyclosporin (CyA)-treated pediatric patients and adult FK506-treated patients. Abstract. Presented at The American Society of Transplant Physicians 12th Annual Meeting, Houston, Texas, May, 1993

Esquivel CO, Egawa H, Cox K, Concepcion W, Lawrence L, et al. Experiences with FK506 conversion therapy in pediatric liver transplantation (OLT). Abstract. Presented at The American Society of Transplant Physicians 12th Annual Meeting, Houston, Texas, May, 1993

European FK506 Study Group. A European multicentre randomized study to compare the efficacy and safety of FK506 with that of cyclosporine in patients undergoing primary liver transplantation. Abstract. Presented at The American Society of Transplant Surgeons 19th Annual Scientific Meeting, Houston, Texas, May, 1993

Fabrega AJ, Blanchard JM, Braucher E, Dumble L, Rivas P, et al. FK 506 enhances the beneficial effects of donor-specific blood transfusion on allograft survival in rats. Transplantation Proceedings 23: 3312-3313, 1991

Fagiuoli S, Gasbarrini A, Nikolaidis N, Farghali H, Wang Z, et al. Does FK 506 pretreatment promote healing following acute chemical injury in the rat? Abstract. Gastroenterology 102: A804, 1992a

Fagiuoli S, Gasbarrini A, Azzarone A, Francavilla A, Van Thiel DH. FK 506: a new immunosuppressive agent for organ transplantation. Pharmacology, mechanism of action and clinical applications. Italian Journal of Gastroenterology 24: 355-360, 1992b

Farghali H, Sakr M, Gasbarrini A, Venkataramanan R, Gavaler J, et al. Effect of FK 506 chronic administration on bromosulphthalein hepatic excretion in rats. Transplantation Proceedings 23: 2802-2804, 1991b

Farghali H, Gasbarrini A, Borle AB, Nalesnik MA, Francavilla A, et al. FK 506 modulates D-galactosamine-induced hepatitis in rats. Transplantation Proceedings 23: 2809-2811, 1991a

Faulds D, Goa KL, Benfield P. Cyclosporin: A review of its phrmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. Drugs 45: 953-1040, 1993

Fidelus RK, Laughter AH. Protein kinase activation and the immunosuppressant cyclosporine. Transplantation 41: 187-192, 1986

Flanagan WF, Corthesy B, Bram RJ, Crabtree GR. Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. Nature 352: 803-807, 1991

Flavin T, Ivens K, Wang J, Gutierrez J, Hoyt EG, et al. Initial experience with FK 506 as an immunosuppressant for nonhuman primate recipients of cardiac allografts. Transplantation Proceedings 23: 531-532, 1991

Forrest MJ, Jewell ME, Koo GC, Sigal NH. FK-506 and cyclosporin: selective inhibition of calcium ionophore induced polymorphonuclear leukocyte degranulation. Biochemical Pharmacology 42: 1221-1228, 1991

Francavilla A, Barone M, Todo S, Zeng Q, Porter KA, et al. Augmentation of rat liver regeneration by FK 506 compared with cyclosporine. Lancet 2: 1248-1249, 1989

Francavilla A, Starzl TE, Barone M, Zeng QH, Porter KA, et al. Studies on mechanisms of augmentation of liver regeneration by cyclosporine and FK 506. Hepatology 14: 140-143, 1991

Francavilla A, Barone M, Starzl TE, Zeevi A, Scotti C, et al. FK 506 as a growth control factor. Transplantation Proceedings 23 (Suppl. 1): 90-92, 1990

Freise CE, Rowley H, Lake J, Hebert M, Ascher NL, et al. Similar clinical presentation of neurotoxicity following FK 506 and cyclosporine in a liver transplant recipient. Transplantation Proceedings 23: 3173-3174, 1991

Fries D, Rucay P, Samuel D, Hiesse C, Meduri G, et al. Development of renal dysfunction and renal histological lesions in two patients treated with FK 506 for acute rejection following liver transplantation. Transplantation Proceedings 23: 3099-3100, 1991

Friob MC, Hassoun A, Latinne D, Lhoest G, Otte JB, et al. A combined HPLC-ELISA evaluation of FK-506 in transplant patients. Transplantation Proceedings 23: 2750-2752, 1991

Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proceedings of the National Academy of Sciences of the United States of America 89: 3686-3690, 1992

Fujii Y, Fujii S, Kaneko T. Effect of a novel immunosuppressive agent, FK506, on mitogen-induced inositol phospholipid degradation in rat thymocytes. Transplantation 47: 1081-1083, 1989

Fukuzaki T, Gotoh M, Monden M, Dono K, Wada H, et al. Induction of tolerance to islet allografts with preoperative donor spleen cell injection and a short course of FK 506 treatment. Transplantation Proceedings 23: 3221-3222, 1991

Fukuzawa M, Santiago S, Nakata S, Shirakura R, Okada A. Effect of donor-specific transfusion and FK 506 on small intestine allotransplantation. Transplantation Proceedings 23: 3252-3254, 1991

Fung J, Abu-Elmagd K, Jain A, Gordon R, Tzakis A, et al. A randomised trial of primary liver transplantation under immunosuppression with FK506 vs cyclosporine. Transplantation Proceedings 23: 2977-2983, 1991a

Fung JJ, Alessiani M, Abu-Elmagd K, Todo S, Shapiro R, et al.

Adverse effects associated with the use of FK 506. Transplantation Proceedings 23: 3105-3108, 1991b

Fung JJ, Todo S, Tzakis A, Demetris A, Jain A, et al. Conversion of liver allograft recipients from cyclosporine to FK 506-based immunosuppression: benefits and pitfalls. Transplantation Proceedings 23: 14-21, 1991c

Goldfeld AE, Flemington EK, Boussiotis VA, Theodos CM, Titus RG, et al. Transcription of the tumor necrosis factor α gene is rapidly induced by anti-immunoglobulin and blocked by cyclosporin A and FK506 in human B cells. Proceedings of the National Academy of Sciences of the United States of America 89: 12198-12201, 1992

Gordon RD, Fung J, Markus B, Fox I, Iwatsuki S, et al. The antibody crossmatch in liver transplantation. Surgery 100: 705-715, 1986

Gordon RD, Todo S, Tzakis AG, Fung JJ, Stieber A, et al. Liver transplantation under cyclosporins: a decade of experience. Transplantation Proceedings 23: 1393-1396, 1991

Gotoh M, Fukuzaki T, Dono K, Wada H, Kanai T, et al. FK-506 and islet transplantation: a potential protocol for inducing tolerance. Diabetes, Nutrition and Metabolism 5 (Suppl. 1): 125-128, 1992

Gotoh M, Monden M, Kanai T, Umeshita K, Wang K, et al. Tolerance induction by liver grafting and FK 506 treatment in nonhuman primates. Transplantation Proceedings 23: 3265-3268, 1991

Graeb C, Stangl MJ, Mebert H, Fischer T, Hammer C. Cytoimmunologic monitoring of small bowel allograft recipients. Transplantation Proceedings 24: 1153-1154, 1992

Granelli-Piperno A, Nolan P, Inaba K, Steinman RM. The effect of immunosuppressive agents on the induction of nuclear factors that bind to sites on the interleukin 2 promoter. Journal of Experimental Medicine 172: 1869-1872, 1990

Green M, Tzakis A, Reyes J, Nour B, Todo S, et al. Infectious complications of pediatric liver transplantation under FK 506. Transplantation Proceedings 23: 3038-3039, 1991

Grenier FC, Luczkiw J, Bergmann M, Lunetta S, Morrison M, et al. A whole blood FK 506 assay for the IMx® analyzer. Transplantation Proceedings 23: 2748-2749, 1991

Griffin AD, Hawthorne WJ, Inutsuka S, Chapman JR, Allen RDM. FK 506 in canine renal transplantation. Transplantation Proceedings 24: 2305-2306, 1992

Habucky K, Flowers J, Warty VJ, Venkataramanan R, Fung JJ, et al. Blood protein binding (BPB) of FK-506 (F) in various species. Abstract. Pharmaceutical Research 9 (Suppl.): S-334, 1992

Hanke JH, Nichols LN, Coon ME. FK506 and rapamycin selectively enhance degradation of IL-2 and GM-CSF mRNA. Lymphokine and Cytokine Research 11: 221-231, 1992

Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK506 is a *cis-trans* peptidyl-prolyl isomerase. Nature 341: 758-760, 1989

Harrison RK, Stein RL. Substrate specificities of the peptidyl prolyl cis-trans isomerase activities of cyclophilin and FK-506 binding protein: evidence for the existence of a family of distinct enzymes. Biochemistry 29: 3813-3816, 1990

Hasegawa S, Yokomise H, Hirai T, Fukuse T, Muro K, et al. Combination use of suboptimal dose of FK 506 and cyclosporine in canine lung transplantation. Journal of Thoracic and Cardiovascular Surgery 104: 1340-1348, 1992

Hatazawa C, Yamaguchi M, Kato T, Koyama K. Effect of FK 506 on bowel transplantation in rats. Transplantation Proceedings 24: 1177, 1992

Henderson DJ, Naya I, Bundick RV, Smith GM, Schmidt JA. Comparison of the effects of FK-506, cyclosporin A and rapamycin on IL-2 production. Immunology 73: 316-321, 1991

Hildebrandt A, Meiser B, Human P, Reichenspurner H, Rose A, et al. FK 506: short- and long-term treatment after cardiac transplantation in nonhuman primates. Transplantation Proceedings 23: 509-510, 1991

Hirai O, Miyamae Y, Izumi H, Miyamoto A, Takashima M, et al. Mutagenicity test of tacrolimus (FK506). The Clinical Report 26: 989-1001, 1992

Hirai T, Waddell TK, Puskas JD, Wada H, Hitomi S, et al. Prolonged lung allograft survival with a short course of FK 506. Journal of Thoracic and Cardiovascular Surgery 105: 1-8, 1993

Hirao A, Kawano Y, Takaue Y. Effects of immunosuppressants, FK506, deoxyspergualin, and cyclosporine A on immature human hematopoiesis. Blood 81: 1179-1183, 1993

Hoffman AL, Makowka L, Banner B, Cai X, Cramer DV, et al. The use of FK-506 for small intestine allotransplantation. Inhibition of acute rejection and prevention of fatal graft-versus-host disease. Transplantation 49: 483-490, 1990

Hultsch T, Albers MW, Schreiber SL, Hohman RJ. Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription. Proceedings of the National Academy of Sciences of the United States of America 88: 6229-6233, 1991

Iga C, Okajima K, Takeda Y, Tezuka K. Prolonged survival of small intestinal allograft in the rat with cyclosporine A, FK 506, and 15-deoxyspergualin. Transplantation Proceedings 22: 1658, 1990

Il Kim Y, Kawano K, Iwao Y, Ono M, Goto S, et al. Stimulation of liver regeneration by pretreatment with azathioprine as well as cyclosporine and FK506. Transplantation 53: 949-951, 1992

Imai K, Sato K, Nakayama Y, Takishima T, Osakabe T, et al. Histopathological study of canine pancreaticoduodenal allotranspantation with FK506, cyclosporine, and triple regimen immunosuppression. Transplantation Proceedings 23: 1589-1592, 1991

Inagaki K, Fukuda Y, Sumimoto K, Matsuno K, Ito H, et al. Effects of FK506 and 15-deoxyspergualin in rat orthotopic liver transplantation. Transplantation Proceedings 21: 1069-1071, 1989

Inamura N, Nakahara K, Kino T, Goto T, Aoki H, et al. Prolongation of skin allograft survival in rats by a novel immunosuppressive agent, FK-506. Transplantation 45: 206-209, 1988

Isai H, Painter DM, Sheil AGR. FK 506 and orthotopic liver transplantation in the rat. Transplantation Proceedings 22: 2125-2126, 1990

Jain AB, Abu-Elmagd K, Abdallah H, Warty V, Fung J, et al. Pharmacokinetics of FK 506 in liver transplant recipients after continuous intravenous infusion. Journal of Clinical Pharmacology 33: 606-611, 1993

Jain AB, Todo S, Fung JJ, Venkataramanan R, Day R, et al. Correlation of rejection episodes with FK 506 dosage, FK 506 level, and steroids following primary orthotopic liver transplantation. Transplantation Proceedings 23: 3023-3025, 1991a

Jain AB, Venkataramanan R, Cadoff E, Fung JJ, Todo S, et al. Effect of hepatic dysfunction and T tube clamping on FK 506 pharmacokinetics and trough concentrations. Transplantation Proceedings 22 (Suppl. 1): 57-59, 1990

Jain AB, Venkataramanan R, Fung J, Burckart G, Emeigh J, et al. Pharmacokinetics of cyclosporine and nephrotoxicity in orthotopic liver transplant patients rescued with FK 506. Transplantation Proceedings 23: 2777-2779, 1991b

Japanese FK 506 Study Group. Japanese study of FK 506 on kidney transplantation: the benefit of monitoring the whole blood FK 506 concentration. Transplantation Proceedings 23: 3085-3088, 1991

Japanese FK 506 Study Group. Japanese study of FK 506 on kidney transplantation: results of an late phase II study. Transplantation Proceedings 25: 649-654, 1993a

Japanese FK 506 Study Group. Morphological characteristics of renal allografts showing renal dysfunction under FK 506 therapy: is graft biopsy available to reveal the morphological

findings corresponding with FK 506 nephropathy? Transplantation Proceedings 25: 624-627, 1993b

Jenkinson EJ, Kingston R, Smith CA, Williams GT, Owen JJT. Antigen-induced apoptosis in developing T cells: a mechanism for negative selection of the T cell receptor repertoire. European Journal of Immunology 19: 2175-2177, 1989

Jensen CWB, Jordan ML, Schneck FX, Shapiro R, Tzakis A, et al. Pediatric renal transplantation under FK 506 immunosuppression. Transplantation Proceedings 23: 3075-3077, 1991

Jiang H, Suguo H, Takahara S, Takano Y, Li D, et al. Combined immunosuppressive effect of FK 506 and other immunosuppressive agents on PHA- and CD3-stimulated human lymphocyte proliferation in vitro. Transplantation Proceedings 23: 2933-2936, 1991

Johansson A, Möller E. Evidence that the immunosuppressive effects of FK506 and cyclosporine are identical. Transplantation 50: 1001-1007, 1990

Johnson PC, Douglas R, Fung J, Janosky J, Sheppeck R, et al. Cyclosporin A and FK-506: effects on platelet function. Abstract. Clinical Research 38: 548A, 1990

Jordan ML, Hignet S, Wright J, Hoffman RA, Starzl TE. Inhibition of T-cell function by FK 506 and cyclosporine is not accompanied by alterations in intracellular calcium. Transplantation Proceedings 23: 2950-2952, 1991

Jordan ML, Shapiro R, Vivas CA, Scantlebury VP, Darras FS, et al. FK 506 salvage of renal allografts with ongoing rejection failing cyclosporine immunosuppression. Transplantation Proceedings 25: 638-640, 1993

Jusko WJ, D'Ambrosio RD. Monitoring FK 506 concentrations in plasma and whole blood. Transplantation Proceedings 23: 2732-2735, 1991

Katayama Y, Takao M, Onoda K, Hiraiwa T, Yada I, et al. Immunosuppressive effects of FK 506 and 15-deoxyspergualin in rat lung transplantation. Transplantation Proceedings 23: 349-353, 1991

Kawano K, Il Kim Y, Goto S, Ono M, Kobayashi M. A protective effect of FK506 in ischaemically injured rat livers. Transplantation 52: 143-145, 1991

Kay JE, Benzie CR. T lymphocyte activation through the CD28 pathway is insensitive to inhibition by the immunosuppressive drug FK-506. Immunology Letters 23: 155-159, 1989

Kay JE, Benzie CR, Goodier MR, Wick CJ, Doe SAE. Inhibition of T-lymphocyte activation by the immunosuppressive drug FK-506. Immunology 67: 473-477, 1989a

Kay JE, Doe SE, Benzie CR. The mechanism of action of the immunosuppressive drug FK-506. Cellular Immunology 124: 175-181, 1989b

Kay JE, Moore AL, Doe SEA, Benzie CR, Schönbrunner R, et al. The mechanism of action of FK 506. Transplantation Proceedings 22 (Suppl. 1): 96-99, 1990

Keicho N, Sawada S, Kitamura K, Yotsumoto H, Takaku F. Effects of an immunosuppressant, FK506, on interleukin 1α production by human macrophages and a macrophage-like cell line, U937. Cellular Immunology 132: 285-294, 1991

Kenmochi T, Asano T, Enomoto K, Goto T, Nakagori T, et al. The effect of FK506 on segmental pancreas allografts in mongrel dogs. Transplantation Proceedings 20 (Suppl. 1): 223-225, 1988

Kimball PM, Kerman RH, Kahan BD. Sensitivity of intracellular signals responsible for cell cycle progression to cyclosporine. Transplantation 49: 186-191, 1990

Kino T, Hatanaka H, Hashimoto H, Nishiyama N, Goto T, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physicochemical and biological characteristics. Journal of Antibiotics 40: 1249-1255, 1987a

Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK 506 in vitro. Journal of Antibiotics 40: 1256-1265, 1987b

Kino T, Inamura N, Sakai F, Nakahara K, Goto T, et al. Effect of FK-506 on human mixed lymphocyte reaction in vitro. Transplantation Proceedings 19 (Suppl. 6): 36-39, 1987c

Klintmalm GBG. US multicenter prospective randomized trial comparing FK506 to cyclosporine after liver transplantation: primary outcome analysis. Abstract. Presented at The American Society of Transplant Surgeons 19th Annual Scientific Meeting, Houston, Texas, May, 1993

Kobayashi C, Kanai A, Nakajima A, Okumura K. Suppression of corneal graft rejection in rabbits by a new immunosuppressive agent, FK-506. Transplantation Proceedings 21: 3156-3158, 1989

Kobayashi M, Tamura K, Katayama N, Nakamura K, Nagase K, et al. FK 506 assay past and present- characteristics of FK 506 ELISA. Transplantation Proceedings 23: 2725-2729, 1991

Kon V, Suguira M, Inagami T, Harvie BR, Ichikawa I, et al. Role of endothelin in cyclosporine-induced glomerular dysfunction. Kidney International 37: 1487-1491, 1990

Krensky AM, Weiss A, Crabtree G, Davis MM, Parham P. Lymphocyte-T antigen interaction in transplant rejection. New England Journal of Medicine 322: 510-517, 1990

Kubes P, Hunter J, Granger DN. Effects of cyclosporin A and FK506 on ischemia/reperfusion-induced neutrophil infiltration in the cat. Digestive Diseases and Sciences 36: 1469-1472, 1991

Kumano K, Wang G, Endo T, Kuwao S. FK506 induced nephrotoxicity in rats. Transplantation Proceedings 23: 512-515, 1991

Kuroki H, Ikuta Y, Akiyama M. Experimental studies of vascularized allogeneic limb transplantation in the rat using a new immunosuppressive agent, FK-506: morphological and immunological analysis. Transplantation Proceedings 21: 3187-3190, 1989

Kusne S, Fung J, Alessiani M, Martin M, Torre-Cisneros J, et al. Infections during a randomized trial comparing cyclosporine to FK 506 immunosuppression in liver transplantation. Transplantation Proceedings 24: 429-430, 1992

Kusne S, Martin M, Shapiro R, Jordan M, Fung J, et al. Early infections in kidney transplant recipients under FK 506. Transplantation Proceedings 23: 956-957, 1991

Lagodzinski Z, Górski A, Stepien-Sopniewska B, Wasik M. Effect of FK 506 on B-cell responses. Transplantation Proceedings 23: 942-943, 1991

Lane BC, Miller LN, Kawai M, Or YS, Wiedeman P, et al. Evaluation of calcineurin's role in the immunosuppressant activity of FK 506, related macrolactams, and cyclosporine. Transplantation Proceedings 25: 644-646, 1993

Langrehr JM, Hoffman RA, Müller AR, Lee KKW, Demetris AJ, et al. Indefinite small bowel allograft survival after limited immunosuppressive therapy is not due to specific hyporesponsiveness. Transplantation Proceedings 23: 3248-3250, 1991

Lee C, Jusko W, Shaefer M, Klintmalm G, Hebert M, et al. Pharmacokinetics of tacrolimus (FK506) in liver transplant patients. Abstract. Clinical Pharmacology and Therapeutics 53: 181, 1992

Lee KKW, Stangl MJ, Todo S, Langrehr JM, Starzl TE, et al. Successful orthotopic small bowel transplantation with short-term FK 506 immunosuppressive therapy. Transplantation Proceedings 22 (Suppl. 1): 78-79, 1990

Li PKT, Nicholls MG, Lai KN. The complications of newer transplant antirejection drugs: treatment with cyclosporin A, OKT3, and FK506. Adverse Drug Reactions and Acute Poisoning Reviews 9: 123-155, 1990

Lieberman KV, Lin WG, Reisman L. FK 506 is a direct glomeruloconstrictor, as determined by electrical resistance pulse sizing (ERPS). Transplantation Proceedings 23: 3119-3120, 1991

Lin CS, Boltz RC, Siekierka JJ, Sigal NH. FK-506 and cyclosporin A inhibit highly similar signal transduction pathways

in human T lymphocytes. Cellular Immunology 133: 269-284, 1991

Liu J. FK 506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunology Today 14: 290-295, 1993

Liu J, Albers MW, Wandless TJ, Luan S, Alberg DG, et al. Inhibition of T cell signalling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Biochemistry 31: 3896-3901, 1992

Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, et al. Calcineurin is a common target of cyclophilin-cyclosporin and FKBP-FK506 complexes. Cell 66: 807-815, 1991

Lopez OL, Martinez AJ, Torre-Cisneros J. Neuropathologic findings in liver transplantation: a comparative study of cyclosporine and FK 506. Transplantation Proceedings 23: 3181-3182, 1991

Machida M, Takahara S, Ishibashi M, Hayashi M, Sekihara T, et al. Effect of temperature and hematocrit on plasma concentration of FK 506. Transplantation Proceedings 23: 2753-2754, 1991

Markus PM, van den Brink MRM, Luchs BA, Fung JJ, Starzl TE, et al. Effects of in vivo treatment with FK506 on natural killer cells in rats. Transplantation 51: 913-915, 1991

Mattila PS, Ullman KS, Fiering S, Emmel EA, McCutcheon M, et al. The actions of cyclosporin A and FK506 suggest a novel step in the activation of T lymphocytes. EMBO Journal 9: 4425-4433, 1990

McCaffrey PG, Perrino BA, Soderling TR, Rao A. NF-ATp, a lymphocyte-T DNA-binding protein that is a target for calcineurin and immunosuppressive drugs. Journal of Biological Chemistry 268: 3747-3752, 1993

McCauley J. The nephrotoxicity of FK506 as compared with cyclosporine. Current Opinion in Nephrology and Hypertension 2: 662-669, 1993

McCauley J, Farkus Z, Prasad SJ, Plummer HA, Murray SA. Cyclosporine and FK 506 induced inhibition of renal epithelial cell proliferation. Transplantation Proceedings 23: 2829-2830, 1991a

McCauley J, Fung JJ, Brown H, Deballi P, Jain A, et al. Renal function after conversion from cyclosporine to FK 506 in liver transplant patients. Transplantation Proceedings 23: 3148-3149, 1991b

McCauley J, Fung JJ, Jain A, Todo S, Starzl TE. The effects of FK506 on renal function after liver transplantation. Transplant Proceedings 22 (Suppl. 1): 17-20, 1990

McCauley J, Fung JJ, Todo S, Jain A, Deballi P, et al. Changes in renal function after liver transplantation under FK 506. Transplantation Proceedings 23: 3143-3145, 1991c

McCauley J, Takaya S, Fung J, Tzakis A, Abu-Elmagd K, et al. The question of FK 506 nephrotoxicity after liver transplantation. Transplantation Proceedings 23: 1444-1447, 1991d

McDiarmid S and the US Multi-Center FK506 Liver Study Group. A multi-center prospective randomized trial comparing FK506 to cyclosporine (CyA) after liver transplantation: comparison of efficacy based on incidence, severity and treatment of rejection. Abstract. Presented at The American Society of Transplant Physicians 12th Annual Meeting, Houston, Texas, May, 1993a

McDiarmid SV, Colonna JO, Shaked A, Vargas J, Ament ME, et al. Differences in oral FK506 dosae requirements between adult and pediatric liver transplant patients. Transplantation 55: 1328-1322, 1993b

McMichael J, Irish W, McCauley J, Shapiro R, Gordon R, et al. Evaluation of a novel ,intelligent, dosing system for optimizing FK 506 therapy. Transplantation Proceedings 23: 2780-2782, 1991

Mekki O, Lee C, Carrier S, Klintmalm G, Shaefer M, et al. The effect of food on oral bioavailability of tacrolimus (FK506) in liver transplant patients. Abstract. Clinical Pharmacology and Therapeutics 53: 229, 1992

Metcalfe SM, Richards FM. Cyclosporine, FK506, and rapamycin. Some effects on early activation events in serum-free, mitogen-stimulated mouse spleen cells. Transplantation 49: 798-802, 1990

Mieles L, Gordon RD, Mintz D, Toussaint RM, Imventarza O, et al. Glycemia and insulin need following FK 506 rescue therapy in liver transplant recipients. Transplantation Proceedings 23: 949-953, 1991a

Mieles L, Venkataramanan R, Yokoyama I, Warty VJ, Starzl TE. Interaction between FK506 and clotrimazole in a liver transplant recipient. Transplantation 52: 1086-1087, 1991b

Monden M, Gotoh M, Kanai T, Valdivia LA, Umeshita K, et al. A potent immunosuppressive effect of FK 506 in orthotopic liver transplantation in primates. Transplantation Proceedings 22 (Suppl. 1): 66-71, 1990

Morel P, Rilo HLR, Tzakis AG, Todo S, Gordon RD, et al. Liver retransplantation in adults: overall results and determinant factors affecting the outcome. Transplantation Proceedings 23: 3029-3031, 1991

Morikawa K, Oseko F, Morikawa S. The distinct effects of FK506 on the activation, proliferation, and differentiation of human B lymphocytes. Transplantation 54: 1025-1030, 1992

Morimoto T, Yamada F, Kobayashi T, Miyaishi S, Tokumaru T, et al. Pancreaticoduodenal allotransplantation with FK 506 in the dog. Transplantation Proceedings 22: 717-719, 1990

Morris AA, Jackson DM, Eady RP. Protective effects of cyclophosphamide, cyclosporin A and FK506 against antigen-induced lung eosinophilia in guinea-pigs. Clinical and Experimental Immunology 89: 347-350, 1992

Morris RE, Wu J, Shorthouse R. Comparative immunopharmacologic effects of FK 506 and CyA in in vivo models of organ transplantation. Transplantation Proceedings 22 (Suppl. 1): 110-112, 1990

Morris SM, Kepka-Lenhart D, Curthoys NP, McGill RL, Marcus RJ, et al. Disruption of renal function and gene expression by FK 506 and cyclosporine. Transplantation Proceedings 23: 3116-3118, 1991

Moutabarrik A, Ishibashi M, Fukunaga M, Kameoka H, Kawaguchi N, et al. FK506-induced kidney tubular cell injury. Transplantation 54: 1041-1047, 1992

Murase N, Kim DG, Todo S, Cramer DV, Fung JJ, et al. Suppression of allograft rejection with FK506. I. Prolonged cardiac and liver survival in rats following short-course therapy. Transplantation 50: 186-189, 1990a

Murase N, Kim DG, Todo S, Cramer DV, Fung JJ, et al. FK506 suppression of heart and liver allograft rejection. II. The induction of graft acceptance in rats. Transplantation 50: 739-744, 1990b

Murase N, Todo S, Lee PH, Lai HS, Chapman F, et al. Heterotopic heart transplantation in the rat receiving FK-506 alone or with cyclosporine. Transplantation Proceedings 19 (Suppl. 6): 71-75, 1987

Murthy JN, Chen Y, Warty VS, Venkataramanan R, Donnelly JG, et al. Radioreceptor assay for quantifying FK-506 immunosuppressant in whole blood. Clinical Chemistry 38: 1307-1310, 1992

Nakamura K, Yagihashi A, Iwaki Y, Takaya S, Hartman GG, et al. The lymphocytotoxic crossmatch in liver transplantation: a clinicopathologic analysis. Transplantation Proceedings 23: 3021-3022, 1991

Nalesnik MA, Demetris AJ, Fung JJ, Starzl TE. Lymphoproliferative disorders arising under immunosuppression with FK 506: initial observations in a large transplant population. Transplantation Proceedings 23: 1108-1110, 1991

Nalesnik MA, Jaffe R, Starzl TE, Demetris AJ, Porter K, et al. The pathology of posttransplant lymphoproliferative disorders occurring in the setting of cyclosporine A-prednisone im-

munosuppression. American Journal of Pathology 133: 173-192, 1988
Nalesnik M, Locker J, Jaffe R, Reyes J, Cooper M, et al. Experience with posttransplant lymphoproliferative disorders in solid organ transplant recipients. Clinical Transplantation 6: 249-253, 1992
Nisbet-Brown E, Cheung RK, Lee JW, Gelfand EW. Antigen-dependent increase in cytosolic free calcium in specific human T-lymphocyte clones. Nature 316: 545-547, 1985
Nossal GJV. Summary of the first international FK 506 congress: perspectives and prospects. Transplantation Proceedings 23: 3371-3375, 1991
Ochiai T, Gunji Y, Sakamoto K, Suzuki T, Isegawa N, et al. Optimal serum trough levels of FK506 in renal allotransplantation of the beagle dog. Transplantation 48: 189-193, 1989a
Ochiai T, Nagata M, Nakajima K, Sakamoto K, Asano T, et al. Prolongation of canine renal allograft survival by treatment with FK-506. Transplantation Proceedings 19 (Suppl. 6): 53-56, 1987a
Ochiai T, Nagata M, Nakajima K, Suzuki T, Sakamoto K, et al. Studies on the effects of FK-506 on renal allografting in the beagle dog. Transplantation 44: 729-733, 1987b
Ochiai T, Nakajima K, Nagata M, Hori S, Asano T, et al. Studies on the induction and maintenance of long-term graft acceptance by treatment with FK-506 in heterotropic cardiac allotransplantation in rats. Transplantation 44: 734-738, 1987c
Ochiai T, Sakamoto K, Gunji Y, Hamaguchi K, Isegawa N, et al. Effects of combination treatment with FK 506 and cyclosporine on survival time and vascular changes in renal-allograft-recipient dogs. Transplantation 48: 193-197, 1989b
Ochiai T, Sakamoto K, Nagata M, Nakajima K, Goto T, et al. Studies on FK506 in experimental organ transplantation. Transplantation Proceedings 20 (Suppl. 1): 209-214, 1987d
Ohtsuka S, Hayashi S, Sato E, Tanaka Y, Hachisuka T, et al. The effect of short-term FK 506 therapy on pancreas transplantation in rats. Transplantation Proceedings 23: 3217-3218, 1991
Okamura N, Tsukada K, Sakaguchi T, Ohtake M, Yoshida K, et al. Enhanced liver regeneration by FK 506 can be blocked by interleukin-1α and interleukin-2. Transplantation Proceedings 24: 413-415, 1992
O'Keefe SJ, Tamura J, Kincaid RL, Tocci MJ, O'Neill EA. FK506- and CSA- sensitive activation of the interleukin-2 promoter by calcineurin. Nature 357: 692-694, 1992
Omar G, Shah A, Thomson AW, Whiting PH, Burke MD. FK 506 inhibition of cyclosporine metabolism by human liver microsomes. Transplantation Proceedings 23: 934-935, 1991
Opelz G, Schwarz V, Engelmann A, Back D, Wilk M, et al. Long-term impact of HLA matching on kidney graft survival in cyclosporine-treated recipients. Transplantation Proceedings 23; 373-375, 1991
Paul AA, Leeper HF, Friberg TR. CMV retinitis and the use of FK 506. Transplantation Proceedings 23: 3042-3043, 1991
Pelekanou V, Trezise AE, Moore AL, Kay JE. FK 506 and rapamycin do not affect platelet aggregation or mitochondrial function. Transplantation Proceedings 23: 3200-3201, 1991
Perico N, Amuchastegui CS, Zoja C, Benigni A, Morigi M, et al. FK 506 does not affect the glomerular filtration rate and renal plasma flow in the rat. Transplantation Proceedings 23: 3123-3125, 1991
Pichard L, Fabre I, Domergue J, Joyeux H, Maurel P. Effect of FK 506 on human hepatic cytochromes P-450: interaction with CyA. Transplantation Proceedings 23: 2791-2793, 1991
Porayko MK, Textor SC, Krom RAF, Hay JE, Gores GJ, et al. Nephrotoxicity of FK 506 and cyclosporine when used as primary immunosuppression in liver transplant recipients. Transplantation Proceedings 25: 665-668, 1993
Pugh-Humphreys RGP, Ross CSK, Thomson AW. Influence of FK 506 on the thymus. An immunophenotypic and structural analysis. Immunology 70: 398-404, 1990
Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The histopathological changes associated with allograft rejection and drug toxicity in renal transplant recipients maintained on FK506. American Journal of Surgical Pathology 17: 60-68, 1993
Rao P, Sun H, Snyder J, Fung JJ, Starzl TE. Effect of FK 506 on FK-binding protein and transforming growth factor beta gene expression. Transplantation Proceedings 23: 2873-2874, 1991
Reyes J, Gayowski T, Fung J, Todo S, Alessiani M, Starzl TE. Expressive dysphasia possibly related to FK506 in two liver transplant recipients. Transplantation 50: 1043-1045, 1990
Reyes J, Tzakis A, Green M, Nour B, Nalesnik M, et al. Post-transplant lymphoproliferative disorders occurring under primary FK 506 immunosuppression. Transplantation Proceedings 23: 3044-3046, 1991
Rothman JA. Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells. Cell 59: 591-601, 1989
Rui X, Flowers J, Warty VS, Venkataramanan R. Drug interactions with FK 506. Abstract. Pharmaceutical Research 9 (Suppl.): S314, 1992
Sakamoto K, Nakajima K, Ochiai T, Asano T, Isono K. Analysis of long-term acceptance of xenografts in rats induced by short-term administration of FK506. Transplantation Proceedings 21: 527-529, 1989
Sakr M, Hassanein T, Gavaler J, Abu-Elmagd K, Fung J, et al. Cytomegalovirus infection of the upper gastrointestinal tract following liver transplantation - incidence, location, and severity in cyclosporin- and FK506-treated patients. Transplantation 53: 786-791, 1992a
Sakr M, McClain C, Gavaler J, Zetti G, Starzl TE, et al. FK-506 pretreatment is associated with reduced levels of tumor necrosis factor and interleukin 6 following hepatic ischemia/reperfusion. Journal of Hepatology 17: 301-307, 1993
Sakr M, Zetti G, Hassanein T, Farghali H, Nalesnik M, et al. FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. Hepatology 13: 947-951, 1991
Sakr M, Zetti G, McClain C, Gavaler J, Nalesnik M, et al. The protective effect of FK506 pretreatment against renal ischemia/reperfusion injury in rats. Transplantation 53: 987-991, 1992b
Sakuma S, Kohsaka H, Kiyotaki C, Fujitsu T, Senoh H, et al. Mechanism of the inhibition of T cell activation by immunosuppressant FK506. Abstract. International Journal of Immunopharmacology 10 (Suppl. 1): 108, 1988
Sato K, Yamagishi K, Nakayama Y, Yokota K, Uchida H, et al. Pancreaticoduodenal allotransplantation with cyclosporine and FK506. Transplantation Proceedings 21: 1074-1075, 1989
Sato K, Yokota K, Matsui T, Imai K, Takishima T, et al. Comparative study of FK506, cyclosporine, and triple regimen immunosuppression with FK 506, cyclosporine and mizoribine. Transplantation Proceedings 23: 3296-3299, 1991
Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metabolism and Disposition 20: 753-761, 1992
Sawada S, Suzuki G, Kawase Y, Takaku F. Novel immunosuppressive agent, FK 506. In vitro effects on the cloned T cell activation. Journal of Immunology 139: 1797-1803, 1987
Scantlebury V, Shapiro R, Fung J, Tzakis A, McCauley J, et al. New onset diabetes in FK-506 vs cyclosporine-treated kidney transplant recipients. Transplantation Proceedings 23: 3169-3170, 1991
Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 251: 283-287, 1991
Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. Immunology Today 13: 136-142, 1992
Shah IA, Whiting PH, Omar G, Thomson AW, Burke MD. Ef-

fects of FK 506 on human hepatic microsomal cytochrome P-450-dependent drug metabolism in vitro. Transplantation Proceedings 23: 2783-2785, 1991

Shapiro R, Jordan M, Fung J, McCauley J, Johnston J, et al. Kidney transplantation under FK 506 immunosuppression. Transplantation Proceedings 23: 920-923, 1991a

Shapiro R, Jordan M, Scantlebury V, Fung J, Jensen C, et al. FK 506 in kidney transplantation. Transplantation Proceedings 23: 3065-3067, 1991b

Shapiro R, Jordan M, Scantlebury V, Fung J, Jensen C, et al. Randomized trial of FK 506/prednisone vs FK 506/azathioprine/prednisone after renal transplantation: a preliminary report. Transplantation Proceedings 25: 669-672, 1993

Shinozuka H, Warty VS, Masuhara M, Murase N, Iwatsuki S. Effect of FK 506 on experimental liver carcinogenesis. Transplantation Proceedings 23: 3197-3199, 1991

Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH. A cytosolic binding protein for the immunosuppressant FK-506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 341: 755-757, 1989a

Siekierka JJ, Staruch MJ, Hung SHY, Sigal NH. FK-506, a potent novel immunosuppressive agent, binds to a cytosolic protein which is distinct from the cyclosporin A-binding protein, cyclophilin. Journal of Immunology 143: 1580-1583, 1989b

Sigal NH, Dumont FJ. Cyclosporin A, FK-506, and rapamycin: pharmacologic probes of lymphocyte signal transduction. Annual Review of Immunology 10: 519-560, 1992

Sigal NS, Siekierka JJ, Dumont F. Observations on the mechanism of action of FK 506. Biochemical Pharmacology 40: 2201-2208, 1990

Simmons RL, Wang SC. New horizons in immunosuppression. Transplantation Proceedings 23: 2152-2156, 1991

Staruch MJ, Melino S, Koprak PA, Fischer PA, Norton G, et al. FK 506 and rapamycin inhibit murine T cell activation through different mechanisms. Abstract. FASEB Journal 3: A813, 1989

Staruch MJ, Sigal NH, Dumont FJ. Differential effects of the immunosuppressive macrolides FK-506 and rapamycin on activation-induced T cell apoptosis. International Journal of Immunopharmacology 13: 677-685, 1991

Starzl TE, Fung JJ. Transplantation. Journal of the American Medical Association 263: 2686-2687, 1990

Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung JJ, et al. Hepatotrophic effects of FK 506 in dogs. Transplantation 51: 67-70, 1991

Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramanan R, et al. FK 506 for liver, kidney, and pancreas transplantation. Lancet 2: 1000-1004, 1989a

Starzl TE, Todo S, Tzakis AG, Gordon RD, Makowka L, et al. Liver transplantation: An unfinished product. Transplantation Proceedings 21: 2197-2200, 1989b

Staschak S, Wagner S, Block D, Van Thiel DH, Jain A, et al. A cost comparison of liver transplantation with FK 506 or CyA as the primary immunosuppressive agent. Transplantation Proceedings 22 (Suppl. 1): 47-49, 1990

Stephen ME, Woo J, Hassan NU, Whiting PH, Thomson AW. Immunosuppressive activity, lymphocyte subset analysis and acute toxicity of FK 506 in the rat: a comparative and combination study with cyclosporine. Transplantation 47: 60-65, 1989

Stevens C, Lempert N, Freed BM. The effects of immunosuppressive agents on in vitro production of human immunoglobulins. Transplantation 51: 1240-1244, 1991

Stewart AA, Ingebritsen TS, Manalan A, Klee CB, Cohen P. Discovery of a Ca^{2+}- and calmodulin-dependent protein phosphatase. FEBS Letters 137: 671-674, 1982

Stiff DD, Venkataramanan R, Tata TNV. Metabolism of FK 506 in differentially induced rat liver microsomes. Research Communications in Chemical Pathology and Pharmacology 78: 121-124, 1992

Stock PG, Ascher NL, Osorio RW, Tomlanovich S, Lake JR, et al. Standard sequential immunosuppression with Minnesota antilymphoblast globulin and cyclosporine vs FK 506: a comparison of early nephrotoxicity. Transplantation Proceedings 25: 675-676, 1993

Su MSS, Semerjian A. Activation of transcription factor NFκB in Jurkat cells is inhibited selectively by FK506 in a signal-dependent manner. Transplantation Proceedings 23: 2912-2915, 1991

Sumpio BE. Cyclosporine toxicity in the isolated perfused rat kidney. Transplantation Proceedings 20 (Suppl.3): 714-716, 1988

Sumpio BE, Phan S. Nephrotoxic potential of FK 506. Transplantation Proceedings 23: 2789-2790, 1991

Suzuki N, Sakane T, Tsunematsu T. Effects of a novel immunosuppressive agent, FK506, on human B-cell activation. Clinical and Experimental Immunology 79: 240-245, 1990

Tabasco-Minguillan J, Mieles L, Caroll P, Gavaler J, Van Thiel DH, et al. Long-term insulin requirement after liver transplantation with FK 506 in american veterans. Transplantation Proceedings 25: 677-678, 1993

Takada K, Oh-hashi M, Yoshikawa H, Muranishi S, Nishiyama M, et al. Determination of a novel potent immunosuppressant (FK-506) in rat serum and lymph by high-performance liquid chromatography with chemiluminescence detection. Journal of Chromatography 530: 212-218, 1990

Takai K, Jojima K, Sakatoku J, Fukumoto T. Effects of FK506 on rat thymus: time-course analysis by immunoperoxidase technique and flow cytofluorometry. Clinical and Experimental Immunology 82: 445-449, 1990

Takaya S, Bronsther O, Iwaki Y, Nakamura K, Abu-Elmagd K, et al. The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors. Transplantation 53: 400-406, 1992

Takaya S, Bronsther O, Todo S, Abu-Elmagd K, Jain A, et al. Retransplantation of liver: a comparison of FK 506 and cyclosprine-treated patients. Transplantation Proceedings 23: 3026-3028, 1991

Takeda Y, Okajima K, Iga C, Date K, Ryo T, et al. Effect of FK 506 on survival of rat small bowel allografts. Transplantation Proceedings 23: 3251, 1991

Tamura K, Kobayashi M, Hashimoto K, Kojima K, Nagase K, et al. A highly sensitive method to assay FK-506 levels in plasma. Transplantation Proceedings 19 (Suppl. 6): 23-29, 1987

Tanaka H, Kuroda A, Marusawa H, Hashimoto M, Hatanaka H, et al. Physicochemical properties of FK-506, a novel immunosuppressant isolated from *Streptomyces tsukubaensis*. Transplantation Proceedings 19 (Suppl. 6): 11-16, 1987

Terasaki PI, Marchioro TL, Stazl TE. Histocompatability testing. Washington DC, National Academy of Sciences, p. 83. National Research Council, 1965

Thomas J, Matthews C, Carroll R, Loreth R, Thomas F. The immunosuppressive action of FK506. In vitro induction of allogeneic unresponsiveness in human CTL precursors. Transplantation 49: 390-396, 1990

Thomson AW, Pugh-Humphreys RGP. Antilymphocytic activity of FK-506 and its influence on the thymic microenvironment. In Kendall & Ritter (Eds) Thymus Update 4, pp. 93-127, 1991

Tocci MJ, Matkovich DA, Collier KA, Kwok P, Dumont F, et al. The immunosuppressant FK 506 selectively inhibits expression of early T cell activation genes. Journal of Immunology 143: 718-726, 1989

Todo S, Demetris A, Ueda Y, Imventarza O, Cadoff E, et al. Renal transplantation in baboons under FK-506. Surgery 106: 444-451, 1989

Todo S, Demetris AJ, Ueda Y, Imventarza O, Okuda K, et al. Canine kidney transplantation with FK-506 alone or in combination with cyclosporine and steroids. Transplantation Proceedings 19 (Suppl. 6): 57-61, 1987a

Todo S, Fung JJ, Tzakis A, Demetris AJ, Jain A, et al. One hundred

ten consecutive primary orthotopic liver transplants under FK 506 in adults. Transplantation Proceedings 23: 1397-1402, 1991

Todo S, Podesta L, ChapChap P, Kahn D, Pan CE, et al. Orthotopic liver transplantation in dogs receiving FK-506. Transplantation Proceedings 19 (Suppl. 6): 64-67, 1987b

Todo S, Ueda Y, Demetris JA, Imventarza O, Nalesnik M, et al. Immunosuppression of canine, monkey and baboon allografts by FK506: with special reference to synergism with other drugs and to tolerance induction. Surgery 104: 239-249, 1988

Torre-Cisneros J, Mañez R, Kusne S, Alessiani M, Martin M, et al. The spectrum of aspergillosis in liver transplant patients: comparison of FK 506 and cyclosporine immunosuppression. Transplantation Proceedings 23: 3040-3041, 1991

Tropschug M, Wachter E, Mayer S, Schonbrunner ER, Schmidt FX. Isolation and sequence of an FK 506-binding protein from *N. crassa* which catalyses protein folding. Nature 346: 674-677, 1990

Tsuji RF, Yamamoto M, Nakamura A, Kataoka T, Magae J, et al. Selective immunosuppression of prodigiosin 25-C and FK506 in the murine immune system. Journal of Antibiotics 43: 1293-1301, 1990

Tzakis AG, Fung JJ, Todo S, Reyes J, Green M, et al. Use of FK 506 in pediatric patients. Transplantation Proceedings 23: 924-927, 1991a

Tzakis AG, Reyes J, Todo S, Green M, Ohya T, et al. FK 506 versus cyclosporine in pediatric liver transplantation. Transplantation Proceedings 23: 3010-3015, 1991b

Tzakis AG, Reyes J, Todo S, Nour B, Shapiro R, et al. Two-year experience with FK 506 in pediatric patients. Transplantation Proceedings 25: 619-621, 1993

Tze WJ, Tai J, Cheung SSC, Ricordi C, Murase N, et al. FK 506-an effective immunosuppressant for achieving long-term functional islet allograft survival in diabetic rats. Transplantation Proceedings 24: 1034-1036, 1992

Ueda D, Tajima A, Ohtawara Y, Ishikawa A, Nakano M, et al. Influence of FK 506 on renal blood flow. Transplantation Proceedings 23: 3121-3122, 1991

Ueda Y, Todo S, Eiras G, Furukawa H, Imventarza O, et al. Induction of graft acceptance after dog kidney or liver transplantation. Transplantation Proceedings 22 (Suppl. 1): 80-82, 1990

Uemoto S, Tanaka K, Honda K, Tokunada Y, Sano K, et al. Experience with FK506 in living-related liver transplantation. Transplantation 55: 288-292, 1993

US Multicenter FK 506 Study Group (Investigators: Klintmalm GBG, Goldstein R, Gonwa T, Wiesner RH, Krom RAF, et al.). Prognostic factors for successful conversion from cyclosporine to FK 506-based immunosuppressive therapy for refractory rejection after liver transplantation. Transplantation Proceedings 25: 641-643, 1993a

US Multicenter FK 506 Study Group (Investigators: Klintmalm GBG, Goldstein R, Gonwa T, Wiesner RH, Krom RAF, et al.). Use of FK 506 for the prevention of recurrent allograft rejection after successful conversion from cyclosporine for refractory rejection. Transplantation Proceedings 25: 635-637, 1993b

US Multicenter FK 506 Study Group (Investigators: Klintmalm GBG, Goldstein R, Gonwa T, Wiesner RH, Krom RAF, et al.). Use of prograf (FK 506) as rescue therapy for refractory rejection after liver transplantation. Transplantation Proceedings 25: 679-688, 1993c

Utsunomiya H, Tanaka K, Uemoto S, Kato H, Nishizawa T, et al. Effect of FK 506 on orthotopic small bowel transplantation in rats. Transplantation Proceedings 24: 1191, 1992

Valdivia LA, Fung JJ, Demetris AJ, Starzl TE. Differential survival of hamster-to-rat liver and cardiac xenografts under FK 506 immunosuppression. Transplantation Proceedings 23: 3269-3271, 1991

Van Thiel DH, Sakr M, Zetti G, McClain C. FK 506 reduces the injury experienced following renal ischemia and repefusion. Renal Failure 14: 285-288, 1992

Venkataramanan R, Jain A, Cadoff E, Warty V, Iwasaki K, et al. Pharmacokinetics of FK 506: preclinical and clinical studies. Transplantation Proceedings 22 (Suppl. 1): 52-56, 1990

Venkataramanan R, Jain A, Warty VJ, Abu-Elmagd K, Alessiani M, et al. Pharmacokinetics of FK 506 in transplant patients. Transplantation Proceedings 23: 2736-2740, 1991

Venkataramanan R, Warty VS, Zemaitis MA, Sanghvi AT, Burckart GJ, et al. Biopharmaceutical aspects of FK-506. Transplantation Proceedings 19 (Suppl. 6): 30-35, 1987

Vincent SH, Karanam BV, Painter SK, Chiu SHL. In vitro metabolism of FK-506 in rat, rabbit, and human liver microsomes: identification of a major metabolite and of cytochrome P450 3A as the major enzymes responsible for its metabolism. Archives of Biochemistry and Biophysics 294: 454-460, 1992

Wada H, Monden M, Gotoh M, Nakano H, Kita Y, et al. An attempt to induce tolerance to skin grafts in congenic mice with FK 506. Transplantation Proceedings 23: 3280-3281, 1991

Wakabayashi H, Karasawa Y, Maeba T, Tanaka S. Effect of FK 506 and cyclosporine A on hepatic energy status in the art after warm ischemia, as monitored by ^{31}P nuclear magnetic resonance spectroscopy in vivo. Transplantation Proceedings 24: 1993-1995, 1992

Walliser P, Benzie CR, Kay JE. Inhibition of murine B-lymphocyte proliferation by the novel immunosuppressive drug FK-506. Immunology 68: 434-435, 1989.

Wang SC, Zeevi A, Jordan ML, Simmons RL, Tweardy DJ. FK 506, rapamycin, and cyclosporine: effects on IL-4 and IL-10 mRNA levels in a T-helper 2 cell line. Transplantation Proceedings 23: 2920-2922, 1991

Warty VS, Venkataramanan R, Zendehrouh P, McKaveney T, Chao J, et al. Distribution of FK 506 in plasma lipoproteins in transplant patients. Transplantation Proceedings 23: 954-955, 1991a

Warty VS, Venkataramanan R, Zendehrouh P, Mehta S, McKaveney T, et al. Practical aspects of FK 506 analysis (Pittsburgh experience). Transplantation Proceedings 23: 2730-2731, 1991b

Warty VS, Zuckerman S, Venkataramanan R, Lever J, Fung J, et al. FK 506 measurement: comparison of different analytical methods. Therapeutic Drug Monitoring 15: 204-208, 1993

Wasik M, Górski A, Stepien-Sopniewska B, Lagodzinski Z. Effect of FK-506 versus cyclosporine on human natural and antibody-dependent cytotoxicity reactions in vitro. Transplantation 51: 268-270, 1991

Wasik M, Stepien-Sopniewska B, Lagodzinski Z, Górski A. Effects of FK-506 and cyclosporine on human T and B lymphoproliferative responses. Immunopharmacology 20: 57-61, 1990

Wengerten K, Matas AJ, Tellis VA, Quinn T, Soberman R, et al. Transplantation of paediatric donor kidneys to adult patients. Annals of Surgery 204: 172-175, 1986

Wicker LS, Boltz RC, Matt V, Nichols EA, Peterson LB, et al. Suppression of B-cell activation by cyclosporin-A, FK506 and rapamycin. European Journal of Immunology 20: 2277-2283, 1990

Williams ME, Lewis D. Hyperuricemic acute renal failure associated with FK506 nephrotoxicity. Clinical Transplantation 6: 194-195, 1992

Winkler M, Jost U, Ringe B, Gubernatis G, Wonigeit K, et al. Association of elevated FK 506 plasma levels with nephrotoxicity in liver-grafted patients. Transplantation Proceedings 23: 3153-3155, 1991

Winkler M, Ringe B, Jost U, Gubernatis G, Pichlmayr R. Plasma level-guided low-dose FK506 therapy in patients with early liver dysfunction after liver transplantation. Transplantation Proceedings 25: 2688-2690, 1993a

Winkler M, Ringe B, Jost U, Melter M, Rodeck B, et al. Con-

version from cyclosporin to FK506 after liver transplantation. Transplantation Proceedings 6: in press, 1993b

Woo J, Propper DJ, Thomson AW. Antigen presentation and HLA-DR expression by FK-506-treated human monocytes. Immunology 71: 551-555, 1990a

Woo J, Ross CS, Milton JI, Thomson AW. Flow cytometric analysis of lymphocyte populations and activation antigen expression in blood and lymphoid tissues of FK 506-treated rats. Transplantation Proceedings 22: 1642-1644, 1990b

Woo J, Sewell HF, Thomson AW. The influence of FK-506 and low-concentration cyclosporin on human lymphocyte activation, antigen expression and blastogenesis: a flow cytometric analysis. Scandinavian Journal of Immunology 31: 297-304, 1990c

Woo J, Stephen M, Thomson AW. Spleen lymphocyte populations and expression of activation markers in rats treated with the potent new immunosuppressive agent FK-506. Immunology 65: 153-155, 1988

Wu GD, Cramer DV, Chapman FA, Cajulis E, Wang HK, et al. FK 506 inhibits the development of transplant arteriosclerosis. Transplantation Proceedings 23: 3272-3274, 1991a

Wu YM, Venkataramanan R, Suzuki M, Zhu Y, Abdallah H, et al. Interaction between FK 506 and cyclosporine in dogs. Transplantation Proceedings 23: 2797-2799, 1991b

Yabuuchi H, Nakajima Y, Segawa M, Kanehiro H, Murao Y, et al. Prominent prolongation of islet xenograft survival in combination therapy with FK 506 and 15-deoxyspergualin. Transplantation Proceedings 23: 859-861, 1991

Yagihashi A, Kobayashi M, Noguchi K, Konno A, et al. HLA matching in liver transplantation. Transplantation Proceedings 24: 2432-2433, 1992

Yamada K, Sugisaki Y, Akimoto M, Yamanaka N. Short-term FK 506-induced morphological changes in rat kidneys. Transplantation Proceedings 23: 3130-3132, 1991

Yamada K, Sugisaki Y, Akimoto M, Yamanaka N. FK 506-induced juxtaglomerular apparatus hyperplasia and tubular damage in rat kidney- morphologic and biologic analysis. Transplantation Proceedings 24: 1396-1398, 1992a

Yamada K, Sugisaki Y, Suzuki S, Akimoto M, Amemiya H, et al. New morphological changes induced by FK506 in a short period in the rat kidney and the effect of superoxide dismutase and OKY-046 on them: the relationship of FK506 nephrotoxicity to lipid peroxidation and change in production of thromboxane A_2 in the kidney. Transplantation International 5 (Suppl.1): S564-S567, 1992b

Yamashita T, Maeda Y, Ishikawa T, Ohira M, Nakagawa H, et al. Prolongation of pancreaticoduodenal allograft survival in rats by treatment with FK 506. Transplantation Proceedings 23: 3219-3220, 1991

Yasunami Y, Ryu S, Kamei T. FK506 as the sole immunosuppressive agent for prolongation of islet allograft survival in the rat. Transplantation 49: 682-686, 1990

Yasunami Y, Ryu S, Kamei T, Konomi K. Effects of a novel immunosuppressive agent, FK-506, on islet allograft survival in the rat. Transplantation Proceedings 21: 2720-2721, 1989

Yokota K, Takishima T, Sato K, Osakabe T, Nakayama Y, et al. Comparative studies of FK506 and cyclosporine in canine orthotopic hepatic allograft survival. Transplantation Proceedings 21: 1066-1068, 1989

Yoshimi F, Nakamura K, Zhu Y, Suzuki M, Funakoshi Y, et al. Canine total orthotopic small bowel transplantation under FK 506. Transplantation Proceedings 23: 3240-3242, 1991

Yoshimura N, Matsui S, Hamashima T, Oka T. Effect of a new immunosuppressive agent, FK506, on human lymphocyte responses in vitro. I. Inhibition of expression of alloantigen-activated suppressor cells, as well as induction of alloreactivity. Transplantation 47: 351-356, 1989a

Yoshimura N, Matsui S, Hamashima T, Oka T. Effect of a new immunosuppressive agent, FK506, on human lymphocyte responses in vitro. II. Inhibition of the production of IL-2 and γ-IFN, but not B cell-stimulating factor 2. Transplantation 47: 356-359, 1989b

Yu Y, Yuzawa K, Otsuka M, Fukao K. Mutagenicity of the new immunosuppressive drugs, FK 506, and spergualin. Transplantation Proceedings 25: 2116-2118, 1993

Zeevi A, Duquesnoy R, Eiras G, Rabinowich H, Todo S, et al. Immunosuppressive effect of FK-506 on in vitro lymphocyte alloactivation: synergism with cyclosporine A. Transplantation Proceedings 19 (Suppl. 6): 40-44, 1987

Zeevi A, Eiras G, Bach FH, Fung JJ, Todo S, et al. Functional differentiation of human cytotoxic T lymphocytes in the presence of FK 506 and CyA. Transplantation Proceedings 22 (Suppl. 1): 106-109, 1990a

Zeevi A, Eiras G, Burckart G, Jain A, Kragack A, et al. Bioassay of plasma specimens from liver transplant patients on FK 506 immunosuppression. Transplantation Proceedings 22 (Suppl. 1): 60-63, 1990b

Zeevi A, Eiras G, Kaufman C, Alessiani M, Demetris AJ, et al. Correlation between bioassayed plasma levels of FK 506 and lymphocyte growth from liver transplant biopsies with histological evidence of rejection. Transplantation Proceedings 23: 1406-1408, 1991

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