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Review

FK 506 AND AUTOIMMUNE DISEASE: PERSPECTIVE AND PROSPECTS

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INTRODUCTION: THE NEED FOR BETTER IMMUNOSUPPRESSANTS

Four classes of drugs have been used as immunosuppressive agents in human autoimmune diseases—corticosteroids, alkylating agents (cyclophosphamide and chlorambucil), antimetabolites (azathioprine and methotrexate) and more recently, cyclosporin A (CsA). Before the introduction of CsA within the last decade, the successful use of immunosuppressive drugs in autoimmune disease was restricted to systemic lupus erythematosus (SLE), rheumatoid arthritis, myasthenia gravis and nephrotic syndrome. CsA, which acts selectively to inhibit T cell activation and cytokine production has changed the situation. Now, using CsA, significant improvement in a higher proportion of patients than previously observed can be achieved in autoimmune diseases with a presumed T cell pathogenesis. These include psoriasis, uveitis, insulin-dependent (type 1) diabetes mellitus, primary biliary cirrhosis, aplastic anaemia, lichen planus and severe allergic asthma. Autoimmune disorders mediated by autoantibodies however, such as the autoimmune cytopenias, myasthenia gravis, SLE, Graves' disease and glomerulonephritides are relatively resistant to CsA. In addition to the improved rate of response seen with CsA, opportunistic infections are very rare in CsA-treated autoimmune disease patients. Despite the successes achieved with CsA, however, several problems continue to exist. These are (1) an insufficient response rate, (2) disease recurrence following drug withdrawal and (3) the associated risks of drug toxicity or excessive immunosuppression. The most common side effects are infections, lymphoproliferative disease, nephrotoxicity including arterial hypertension, cosmetic deformity with hirsutism and coarsening of the facies, hypercholesterolemia and an increased disposition to diabetes mellitus. These difficulties have driven the search for new agents that act with precision on those components of the immune system involved in disease pathogenesis but not on therapeutically irrelevant cells. Amongst the most promising new candidate drugs is FK 506. Already the advent of FK 506 and its use as an investigational tool has had a major impact on our understanding of molecular mechanisms underlying T cell activation and at a clinical level, on the practice of organ transplantation. Its efficacy in the prevention of allograft rejection is well documented; more germane to the autoimmune field is its ability to reverse established rejection which is thought to follow the same mechanisms as many autoimmune diseases. In this context, the rejecting organ graft (or bone marrow) may be thought of as a direct analogue of many autoimmune disorders.

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FK 506 IN PERSPECTIVE

The powerful immunosuppressive properties of FK 506, a fungally-derived macrolide antibiotic, were first documented in 1987[1,2]. Although totally distinct in structure from the fungal product and cyclic peptide CsA, FK 506 shares many of the properties of the latter drug. The two agents exhibit very similar molecular actions which inhibit T helper lymphocyte activation, cytokine production and lymphocyte proliferation. FK 506, however, is a considerably more potent immunosuppressant than CsA[1–3] and significantly, has a superior ability to reverse acute and early chronic liver allograft rejection in man[4]. A closely related structural analogue of FK 506, rapamycin (RAPA), is also an extremely potent inhibitor

of T cell responses, although its mode of action is quite distinct from that of FK 506 and CsA and affects a later stage in the T cell activation-proliferation pathway (reviewed by Kahan *et al.*[5]). Because of a better therapeutic margin (before the advent of limiting side effects), FK 506 appears to have a greater steroid sparing effect than CsA in the management of human organ transplant rejection and thus may be of special benefit to children. Infectious complications are no greater and may be reduced as a consequence. The principal potential side effects of FK 506 (nephro- and neurotoxicity and diabetogenicity) are similar to those of CsA[6]. FK 506, however, does not cause such severe elevations of blood pressure, gum hyperplasia or hirsutism, which are commonly encountered side effects of highdose CsA. Single centre studies[7–9] reporting improved results with FK 506 in human liver, heart and kidney transplantation compared with historical, CsA-treated controls, have been followed by multicentre, prospective randomized controlled studies of FK 506 in primary liver transplantation trials currently in progress in Europe, the United States and Japan. These centres have already confirmed the ability of FK 506 to reverse liver allograft rejection in up to 50% of patients unresponsive to other therapies. Reversal of heart and kidney rejection has also been documented[10]. It is established that FK 506 is compatible with steroids, azathioprine, and antilymphoid agents (such as OKT3), although the increased immunologic nonspecificity of "cocktail" therapy increases the risk of infections and neoplastic complications as is the case with CsA. In Japan and in Pittsburgh, clinical studies of the efficacy and safety of FK 506 in kidney and bone marrow transplantation are in progress. Armed with experience and encouraging results under all of these circumstances, preliminary studies of the efficacy and safety of FK 506 in human autoimmune diseases have begun in the United States and Japan.

This short review will outline the properties of FK 506, its molecular action and anti-lymphocytic activity and its pharmacokinetic and toxic properties in animals and man. The effects of FK 506 in experimental and clinical organ transplantation have been reviewed extensively elsewhere[10–14].

The influence of FK 506 in experimental autoimmune disorders will be reviewed and the limited clinical experience with FK 506 in human autoimmune disease will be examined.

PHYSICOCHEMICAL PROPERTIES OF FK 506

FK 506 was discovered by the Fujisawa Pharmaceutical Co., Osaka, Japan, during routine screening of the fermentation broths of *Streptomyces* spp. for specific, inhibitory effects on mouse mixed lymphocyte reactions (MLR)[1,2,15]. Although classified according to its structure (Figure 1) and function as a macrolide antibiotic, FK 506 exhibits only limited antifungal activity. It is insoluble in water, but dissolves readily in non-polar solvents, such as ethanol. The molecular formula of FK 506, deduced by elemental analysis and mass spectrometry, is $C_{44}H_{69}NO_{12}\cdot H_2O$ (mw 822 da). It is relatively stable under normal laboratory conditions. Reports of its synthesis appeared in 1989[16,17].

MODE OF ACTION AT THE CELLULAR AND MOLECULAR LEVELS

There is substantial evidence based on *in vitro* cell culture studies that, like CsA, FK 506 inhibits selectively, $CD4^+$ T lymphocyte activation and cytokine production, with consequent inhibitory effects on other T and non-T cell components of the immune system. Like CsA, FK 506 inhibits T cell activation mediated by the T cell antigen receptor (TCR)-CD3 complex and also via the cell surface molecule CD2. It is very effective in suppressing alloantigen or T cell mitogen-induced lymphocyte proliferation at concentrations 100-fold lower than effective concentrations of CsA[2,18,19]. The drug also inhibits the generation of cytotoxic and suppressor T cells in human MLR but does not affect antigen recognition by cytotoxic T cells or the mechanism by which target cells are destroyed[20]. FK 506 does not appear to inhibit antigen processing or presentation by human monocytes at drug concentrations which strongly suppress T cell proliferation[21]. There is also evidence that FK 506 does not affect directly human natural killer (NK) cell activity or antibody dependent cytotoxicity. Interestingly, however, both FK 506 and CsA inhibit pro-inflammatory mediator release from human basophils[22]. Thus, the ability of FK 506 to inhibit mediator release from basophils and mast cells may contribute to some of its therapeutic effects in graft rejection and autoimmune disease.

In T lymphocytes, FK 506 disrupts an unknown step in the transmission of signals from the TCR to genes that coordinate the immune response. Considerable effort has been expended by several laboratories to identify the precise target of FK 506 in the T cell activation pathway. Experiments designed to ascertain the influence of FK 506 on very early events prior to gene transcription following binding of the antigen receptor have shown that the drug does not affect Ca^{++} mobilization, phosphatidylinositol turnover or protein kinase C (PKC) activities. FK 506 does, however, strongly and specifically inhibit expression of early T cell activation genes encoding interleukin-2 (IL-2)—the main growth factor for T cells, IL-3, IL-4, IFN-γ, GM-CSF and *c-myc*[18]. On the other hand, recent studies have shown that FK 506 may spare IL-10 (cytokine synthesis inhibitory factor=CSIF) gene

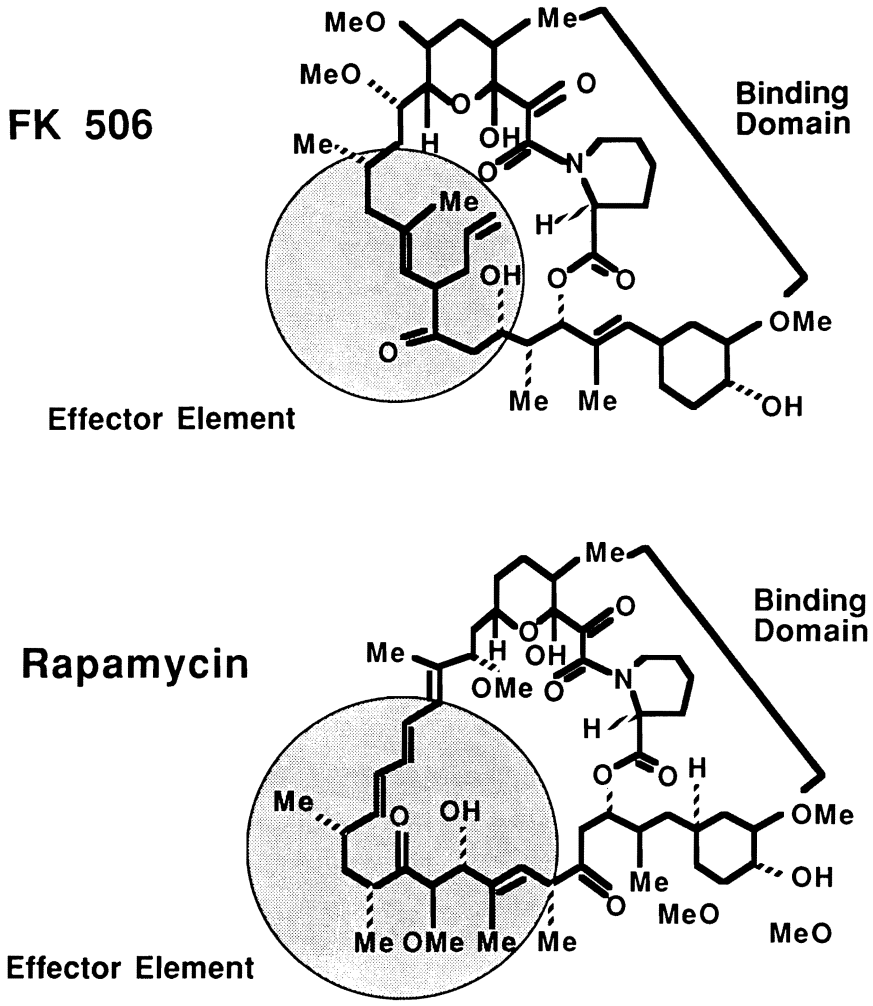

Figure 1 Structures of the immunosuppressive macrolides FK 506 and Rapamycin showing the FKBP binding domain and the effector element of each molecule.

transcription by cloned murine T helper-2 (TH2) cells *in vitro*, whilst suppressing concomitant IL-4 mRNA production by these cells[23]. Thus, *differential* interference with T cell cytokine gene expression may be an important mechanism whereby FK 506 inhibits immune cell activation and immune suppression is maintained.

Clues to the molecular action of FK 506 and CsA come from studies of their intracellular receptors, FK 506 binding protein (FKBP) and cyclophilin, respectively, each of which is a *cis-trans* peptidyl prolyl isomerase(PPIase)[16,17,24]. Although binding of the drug by its receptor (or "immunophilin") inhibits isomerase activity, recent results indicate that the immunosuppressive effects of FK 506 and CsA result from the formation of complexes between the drug and its respective isomerase[25]. Both the complex of FK 506 and FKBP and the complex of CsA and cyclophilin have been shown to bind specifically to three polypeptides—calmodulin and the two subunits of calcineurin (a Ca^{++}-activated, serine-threonine protein phosphatase). In each case, the interaction of the immunophilin appears to be with calcineurin (Figure 2). The drug-immunophilin complexes have been shown to block the Ca^{++}-activated phosphatase activity of calcineurin[24]. Thus, calcineurin appears to be the target of the drug-immunophilin complexes.

A second, important observation in unravelling the molecular action of FK 506 is that the drug-immunophilin complexes block Ca^{++}-dependent translocation of the pre-existing, cytoplasmic component of NF-AT (nuclear factor of activated T cells) to the nucleus[26]. The nuclear component of NF-AT is transcriptionally inactive in all cells other than activated T lympho-

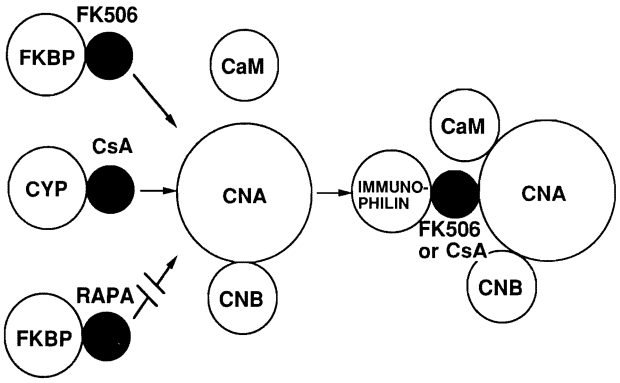

Figure 2 Within the T lymphocyte, FK 506-FKBP or CsA-cyclophilin complexes bind with high affinity to calcineurin-calmodulin to form a pentameric complex which interferes with Ca^{++}-dependent signalling pathways. Complexes of RAPA-FKBP do not bind to calcineurin. Recent observations[25] indicate that calcineurin (a protein phosphatase) is the target of the FK 506-FKBP and CsA-cyclophilin complexes. CaM=calmodulin; CNA=calcineurin A; CNB=calcineurin B; CYP=cyclophilin; FKBP=FK 506 binding protein.

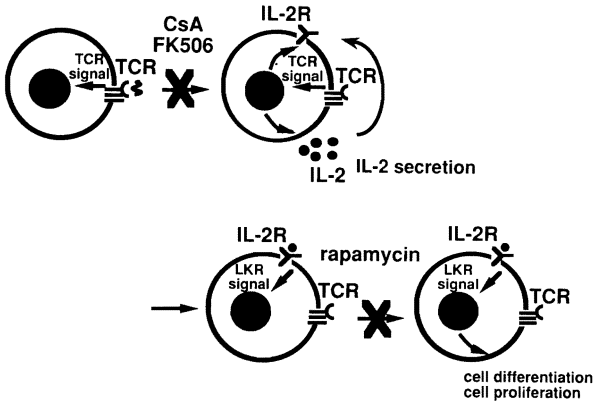

Figure 4 Differential effects of the immunosuppressive macrolides FK 506 and RAPA on T-cell activation and proliferation. FK 506 (and CsA) inhibit pre-transcriptional events in $CD4^+$ T cell activation, resulting in suppression of production of IL-2 and other cytokines. In contrast, RAPA does not impair IL-2 production, IL-2R expression or ligand binding to IL-2R but blocks the response of T-cells to IL-2 and other cytokines (IL-4, IL-6). TCR=T cell receptor; LKR=lymphokine receptor.

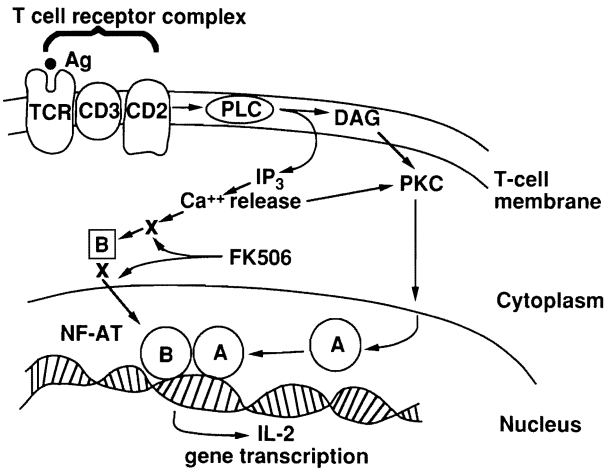

Figure 3 Influence of FK 506 on signal transduction within T cells. The crucial event in T-cell activation stimulated by antigen plus IL-1 would appear to be activation of the phospholipase which splits phosphatidyl inositol diphosphate into the reactive products diacylglycerol and inositol triphosphate. The increased intracellular calcium concentration activates a number of different enzyme systems which lead to new synthesis of RNA, protein and IL-2. FK 506 blocks translocation of the pre-existing cytoplasmic component (B) of the nuclear protein NF-AT at the nucleus[26] by acting either on a Ca^{++} signalling pathway or on translocation following the action of this pathway (arrows). Both components of NF-AT are required for DNA binding and activation of the IL-2 gene. TCR=T cell receptor; DAG=diacylglycerol; PLC=phospholipase C, PKC=protein kinase C; A=induced nuclear component of NF-AT; B=existing cytoplasmic component of NF-AT.

cytes and is induced by signals from the TCR. Its appearance is not blocked by FK 506 or CsA. Current thinking is that FK 506 and CsA block dephosphorylation of the cytoplasmic component of NF-AT which is required for its translocation to the nucleus (Figure 3). In the absence of both nuclear and cytoplasmic components, binding of NF-AT to DNA and transcriptional activation of the IL-2 gene is suppressed. The molecular action of the immunosuppressive macrolide RAPA, which inhibits Ca^{++}-independent signalling and which binds to the same cytosolic receptor (FKBP) as FK 506 is quite distinct from that of the latter drug (see Figures 2 and 4).

FK 506 MONITORING AND PHARMACOKINETICS

FK 506 may be quantified in body fluids, following extraction of the drug, by enzyme-linked immunosorbant assay (ELISA), using either a monoclonal or polyclonal antibody. The sensitivity limit for the assay described originally by Tamura *et al.*[27] is 20 pg/ml FK 506 in plasma. A modified ELISA, in which a solid phase extraction method and a mouse monoclonal anti-FK 506 antibody are used for quantitation of FK 506 in human plasma has recently been described[28]. The extent to which the antibody cross-reacts with FK 506 metabolites is unknown and thus plasma levels as determined at present denote native molecule plus metabolites.

In whole blood, FK 506 is highly bound to erythrocytes, with a mean blood:plasma trough concentration ratio of 10:1. FK 506 is slowly absorbed after oral administration and distributed in various organs, including lung, spleen, heart and kidney[29]. Unlike CsA, the absorption of FK 506 does not appear to be

dependent on the availability of bile in the gut. It seems that the majority of FK 506 is metabolized by N-demethylation and hydroxylation in the liver and is then excreted in bile, urine and faeces within 48 hr of administration[29,30]. In patients with hepatic dysfunction, the half-life of FK 506 is increased and its clearance reduced, increasing drug bioavailability and thus the risk of associated toxicity.

With intravenous administration, peak FK 506 plasma concentration in transplant patients is observed at the end of infusion. Levels decline slowly over the next 24 hr[29,30]. Plasma trough levels tend to be about 1 ng/ml, but the effective immunosuppressive concentration *in vitro* may be much lower than this. The half-life of FK 506 ranges from 3.5 to 40.5 hr, with a mean of 8.7 hr. Drug absorption following oral administration is highly variable with mean bioavailability of about 25% (range 6–57%). Peak plasma level of 0.4 to 3.7 ng/ml is reached after 1 to 4 hr of an oral dose at 0.15 mg/kg.

A number of interactions of FK 506 with other drugs has been observed in studies at the University of Pittsburgh. Trough plasma concentrations of FK 506 in man are increased by co-administration of erythromycin, fluconazole and clotrimazole, whilst the effects of phenytoin, phenobarbital and acyclovir on FK 506 pharmacokinetics are currently under study.

TOXICITY

The toxic effects of FK 506 in experimental animals (rodents, rabbits, dogs and primates) have been well documented[31]. Dogs are susceptible to FK 506 toxicity and exhibit dose-related vasculitis and intususseptions. Studies by several groups have shown that the toxic effects of FK 506 in non-human primates are more pronounced when the drug is administered i.m. compared with orally, due presumably, to be greater bioavailability achieved using the former route. FK 506 does not exhibit mutagenic activity in either *in vitro* or *in vivo* tests. Fetotoxicity has been demonstrated in rats and in rabbits, teratogenic effects have been observed.

In humans, adverse reactions requiring treatment or dose reduction are impairment of renal function (attributable in part to reduced renal blood flow), alterations in glucose metabolism (15% incidence of new onset diabetes in transplant patients) and neurotoxicity[6]. The reported overall incidence of post-transplant lymphoproliferative disorders in all (936) first organ transplant recipients receiving FK 506 as primary immunosuppressive therapy at the University of Pittsburgh Medical Centre is 1.6%[32]. Although a 20% incidence of CMV infections has been observed in FK 506-treated transplant patients[6], no patient treated with FK 506 for non-transplant indications has developed CMV infection. These side effects are similar to those associated with CsA administration. FK 506 use, however, has a lower incidence of hypertension than CsA, and gum hyperplasia and hirsutism are not seen.

An intriguing issue is whether the nephrotoxic potential of FK 506 and CsA correlates with the drugs' PPIase inhibitory activities. Recent data[33] indicate that immunosuppressive activity and not immunophilin binding or PPIase inhibitory activity determines the ability of CsA analogues to induce nephrotoxicity. It may thus be difficult to design new nonnephrotoxic drugs that retain the same potent immunosuppressive activity.

EFFECTS OF FK 506 ON IMMUNE REACTIVITY

The very potent inhibitory effects of FK 506 on humoral and cell-mediated immune responses were first reported by Kino *et al.* in 1987[1], using mice as experimental models. Subsequent reports have confirmed the powerful immunosuppressive properties of FK 506 in rodents, dogs and primates. These include models of organ allograft rejection, ranging from skin grafts to multi-visceral transplants (see recent reviews[3,10–13]). FK 506 is also effective in preventing and reversing graft-versus-host disease after experimental bone marrow transplantation. In each instance, FK 506 has been shown to be about 10-fold more potent than CsA.

There is little doubt that FK 506-induced immunosuppression results from the inhibition of $CD4^+$ T helper cell activities. Enumeration of activated, $CD4^+$ cells in immunized animals has revealed significant reductions following FK 506 treatment[34]. At the same time, FK 506 may selectively inhibit the development of mature, $CD4^+$ T cells within the thymus and the numbers of these cells reaching peripheral lymphoid tissue[35,36].

INFLUENCE OF FK 506 IN AUTOIMMUNE DISEASE MODELS

Uveitis

Experimental autoimmune uveitis (EAU) is an organ-specific autoimmune disease of the eye that can be induced by immunization with retinal antigens, i.e. retinal soluble antigen (S-antigen) or interphotoreceptor retinoid-binding protein (IRBP). EAU is believed to be T lymphocyte mediated and a good model of autoimmune uveitis in humans. The influence of FK 506 in EAU has been studied extensively in the Lewis rat by Mochizuki and his colleagues. FK 506 was found to be 10–30 times more potent than CsA in pre-

venting induction of EAU when administered either from 0–5 days or from 7–12 days after S-antigen immunization. It appears that FK 506 is effective in suppressing on-going immunopathological processes, even after the disease has been initiated[37]. As with CsA, the immunological unresponsiveness induced by a 2-week course of FK 506 (days 0–14) was found to be specific to the S-antigen. Whilst splenic lymphocytes from animals treated with either drug showed markedly depressed *in vitro* responses to S-antigen, only FK 506 was found to significantly depress serum antibody levels[38]. EAU induction, as well as immune responses to S-antigen were suppressed long after cessation of FK 506 treatment[39].

It has been reported that spleens of S-antigen immunized and FK 506-treated rats contain antigen-specific T suppressor cells which, when transferred to live recipients can inhibit the induction of EAU. Moreover, Ts cells from the same donors suppress antigen-specific proliferative responses of S-antigen primed cells without influencing responses of IRBP-sensitized cells[40].

Immunohistochemical studies on lymphocytes infiltrating the ocular lesions in EAU have revealed that FK 506 reduces the absolute number of T cells, potentiates the recruitment of $CD8^+$ (Tc/s) cells, and inhibits both IL-2R expression on T cells and expression of MHC class II antigens on ocular resident cells[41]. Further insight into the mode of action of FK 506 in uveitis comes from the observation that the drug reduces intercellular adhesion molecule (ICAM-1) expression on both $CD4^+$ lymphocytes and retinal pigment epithelial (RPE) cells (candidate antigen presenting cells). FK 506 also inhibits binding of $CD4^+$ lymphocytes to RPE cells[42].

In rhesus and cynomolgus monkeys, FK 506 (0.5 mg/kg/day) administered i.m. for at least 2 weeks from 3 weeks after immunization with S-antigen prevented EAU. Antibody titres against S-antigen were reduced, whilst S-antigen-induced lymphocyte proliferation *in vitro* was unchanged or decreased during FK 506 treatment[43].

To date, there are no published data concerning effects of local (topical or intravitreal) FK 506 administration in experimental uveitis.

Arthritis

Collagen arthritis can be induced readily in many rat strains by immunization with homologous or heterologous native type II collagen emulsified in complete Freund's adjuvant (CFA).

The disease is characterized by the development of cellular and humoral responses to type II collagen and can be transferred by sensitized spleen and lymph node cells and by antibodies to type II collagen. Inamura *et al.*[44] reported that administration of FK 506 to Lewis rats for 12 days following immunization suppressed arthritis and inhibited both antibody and delayed-type hypersensitivity (DTH) skin tests to type II collagen. Failure of the animals to respond to re-immunization on day 50, but to develop experimental allergic encephalomyelitis in response to myelin basic protein, suggested the development of long-lasting, antigen-specific unresponsiveness[44]. Studies by Arita *et al.*[45] in Sprague–Dawley rats confirmed the efficacy of FK 506 in preventing collagen-induced arthritis; a single injection of 10 mg/kg at the time of immunization suppressed arthritis completely and almost abolished IgG antibody and DTH responses to type II collagen. They further showed that if withheld until day 12 or 15 after immunization, the same single high dose of FK 506 was effective in suppressing existing disease and immune (humoral) responses to type II collagen. Moreover, pretreatment of rats with a single FK 506 injection (10 mg/kg) (day −7 or −3) reduced disease severity and antibody production.

Similar results concerning the prophylactic effect of FK 506 in collagen arthritis have been obtained in mice using doses of drug 25 times lower than effective doses of CsA[46]. As with CsA, FK 506 was ineffective in treatment of established lesions in the mouse.

Insulin-dependent diabetes

Spontaneously diabetic BB rats are considered an excellent model for type I, insulin-dependent diabetes mellitus (IDDM). The disease shows genetic predisposition, abrupt onset of insulin-dependent ketosis-prone diabetes (60–120 days of age) associated with lymphocytic insulitis and virtually complete destruction of pancreatic β cells. Administration of FK 506 (2 mg/kg/day) from 30 to 120 days of age prevented the development of diabetes in 20/20 BB rats during the treatment period. Blood glucose and renal and hepatic function tests remained normal whilst histological examination confirmed the absence of insulitis[47]. Glucose intolerance, which has been described in BB rats given CsA was not observed in this study.

In non-obese diabetic (NOD) mice, IDDM develops spontaneously in about 80% of female mice between weeks 12 and 26. The disease is thought to have a $CD4^+$ T cell dependent autoimmune pathogenesis. Administration of FK 506 (2 mg/kg/48 hr) to female NOD mice from weeks 5–20 inhibited both the insulitis and the occurrence of diabetes (cumulative incidence up to 40 weeks: 86% in control mice and 23% in FK 506-treated animals). These effects were accompanied by significant reductions in splenic $CD4^+$ and $CD8^+$ T cells compared with untreated controls, suggesting that the suppression of disease activity may be linked to inhibition of cell-mediated autoimmune reactivity[48].

In both the BB rat and NOD mouse studies, the pre-

ventive effect of FK 506 in diabetes often outlasted the duration of treatment by many weeks or in some animals permanently.

Spontaneous autoimmune lupus disease

The New Zealand black/white (NZB/W) hybrid mouse spontaneously develops non organ-specific, autoimmune immune complex type disease that resembles systemic lupus erythematosus in man. Nephritis and proteinuria develop within 2–3 months of age, leading to chronic renal failure and 50% mortality by 8–9 months. Takabayashi *et al.*[49] reported that FK 506 (2.5 mg/kg, 3 times per week from 12 weeks of age) prolonged the lifespan of female NZB/W F_1 mice and significantly reduced proteinuria. There were, however, no differences in anti-dsDNA antibody levels or IgG subclass distribution between drug-treated animals and controls.

The MRL/lpr mouse spontaneously develops glomerulonephritis, marked lymphoid hyperplasia, arteritis and chronic polyarthritis, with 50% mortality at about 5 months. Takabayashi *et al.*[49] reported similar effects of FK 506 on survival, proteinuria and anti-dsDNA antibody levels in MRL/lpr female mice (given 2.5 mg/kg FK 506 3 times per week from 8 weeks old) to those observed in NZB/W F_1 hybrids. Compared with untreated MRL/lpr controls, minimal glomerulonephritis with only mild proliferation of endothelial and mesangial cells was noted, whilst immunoglobulin and C3 deposits were restricted mainly to the mesangia. Using a similar FK 506 treatment protocol, Yamamoto *et al.*[50] found that inhibition of disease activity in MRL/lpr mice was accompanied by significant reductions in serum anti-ssDNA and anti-ds DNA activities.

Experimental autoimmune glomerulonephritis

There are several ways of inducing immune-mediated nephritis in experimental animals. Heymann's nephritis is a membranous type of chronic glomerulonephritis with glomerular subepithelial immune deposits induced by autologous antibody against renal tubular brush border (TBB) antigens. It is induced in rats by footpad immunization with TBB antigen in FCA. Another type of glomerulonephritis (nephrotoxic antiserum nephritis or Masugi nephritis) is induced by the intravenous injection of (rabbit) antibody to rat glomerular basement membrane (GBM). Okuba *et al.*[51] have shown that FK 506 (0.64 mg/kg) given for 14 days from the time of TBB immunization completely suppresses development of Heymann's nephritis. They have also found that FK 506 prevents the autologous phase (i.e. production of antibody against rabbit gamma globulin fixed on the GBM) of nephrotoxic antiserum nephritis. In both models, the rats remained tolerant to the immunizing antigens but not to other antigens for at least 14 weeks after drug withdrawal. The induction of FK 506 of long lasting, antigen-specific unresponsiveness is similar to the effects of the drug in EAU and collagen-induced arthritis (see above). The underlying mechanism(s) has not been elucidated but possible explanations include clonal deletion or the induction of anti-idiotypic antibody or antigen-specific suppressor cells.

Glomerulonephritis can also be induced by preimmunization of rats with normal rabbit IgG and the subsequent i.v. injection of rabbit anti-GBM antibody (accelerated nephrotoxic serum glomerulonephritis). In this model, FK 506 suppresses the autologous response to rabbit IgG and reduces substantially, the glomerular injury[52].

Autoimmune thyroiditis

Experimental autoimmune thyroiditis can be induced in female PVG/c rats by early thymectomy followed by a course of sublethal total body irradiation (TBI). This results in progressive, lymphocytic infiltration of the thyroid, follicular obliteration and production of anti-thyroglobulin. Administration of FK 506 (2 mg/kg/day) from week 8 after the last dose of TBI significantly decreases the disease severity and is associated with reversal of the splenic $CD4^+$: $CD8^+$ T cell ratio (G. Carrieri, N. Murase, J. Woo and A.W. Thomson, unpublished observations).

Allergic encephalomyelitis

Experimental allergic encephalomyelitis (EAE) is induced in susceptible rat strains by a single injection of myelin basic protein (MBP) in FCA. An acute paralytic disease develops within 10–14 days after immunization with features similar to human acute inflammatory central nervous system disease. FK 506 (1 mg/kg 5 days per week for 2 weeks from the time of immunization) prevented development of EAE for at least 50 days and completely suppressed both antibody production and cell-mediated immunity to MBP. The effectiveness of the drug, however, was considerably reduced when treatment was delayed until 7 days after immunization[53]. Recently, it has been observed that the adoptive transfer of EAE can be suppressed by *in vitro* treatment of spleen cells with 1 nM FK 506, 100-fold lower than the effective concentration of CsA[54].

FK 506 AND HUMAN AUTOIMMUNE DISEASE

Dermatological disorders

Experience with FK 506 in human autoimmune dis-

orders is very limited. Nevertheless, both the efficacy and therapeutic potential of the drug have been demonstrated in several diseases. The capacity of FK 506 to clear psoriatic skin lesions was first observed in organ transplant recipients, whilst a group of seven patients (median follow up time 7 months) with severe, recalcitrant, chronic plaque psoriasis unresponsive to conventional therapy showed a marked reduction (93%) in psoriasis area and severity index within 4 weeks of commencement of oral FK 506 treatment (starting dose 0.3 mg/kg/day)[55,56]. Psoriatic arthritis present in 4 patients also improved markedly within this period. Examination of lesional biopsies revealed that disease remission was accompanied by reduction in dermal and epidermal T cells[57]. Elevations in serum creatinine were observed after 1–6 months but responded quickly to dose reduction. FK 506 did not induce severe hypertension in any of the patients and blood glucose, liver enzymes, bilirubin, cholesterol and uric acid were maintained within normal limits. Clinical side effects were minor and included nausea, mild tremors, headaches and insomnia not necessitating drug withdrawal. FK 506 monotherapy has also been used effectively in four patients with pyoderma gangrenosum[58]. Each subject had been previously treated unsuccessfully with conventional immunosuppressive therapy. Complete healing of the skin, as well as remission of the associated non-dermatological lesions, including perianal fistulae (due to Crohn's disease) in one patient was achieved within 6 weeks with no serious side effects.

Uveitis

Mochizuki and his colleagues have reported 13 cases of refractory uveitis (mean follow up 6 weeks), including 12 patients with Behçet's disease, in which beneficial effects of FK 506 (0.05–0.2 mg/kg/day) i.e. improved visual acuity and decreased uveitis were achieved in the majority of patients, without serious side effects[59].

Nephrotic syndrome

McCauley *et al.*[60] reported successful treatment of 2 patients with steroid-resistant nephrotic syndrome with FK 506. More recently, the same group[61] has reported marked and sustained reductions in proteinuria in 6 out of 7 patients with primary glomerulonephritis resistant to all other forms of therapy. Creatinine clearance decreased in all patients after starting FK 506 but improved upon dose reduction. Disease recurrence was prompt in two patients who stopped taking FK 506 temporarily, but their nephrotic syndrome was controlled rapidly following reinstatement of FK 506 therapy. Patients with transplant-related nephrotic syndrome without chronic rejection were

Table 1 Experimental autoimmune diseases suppressed by FK 506.

Disorder	*Species*	*FK 506 dose mg/kg/day unless specified*	*Reference*
Arthritis (Type II collagen-induced	Rat (Lewis)	0.32[a,b]	Inamura *et al.*[41] (1988)
	Rat (outbred)	2.5[c]	Arita *et al.*[42] (1990)
	Mouse (DBA/1)	2.0	Takagishi *et al.*[43] (1989)
Type I diabetes	NOD mouse	2.0 mg/kg/48 hr[a]	Miyagawa *et al.*[45] (1990)
	BB rat	1.0[a]	Murase *et al.*[44] (1990)
Uveoretinitis	Rat (Lewis)	1.0[a,d]	Kawashima *et al.*[35] (1990)
	Rhesus & cynomolgus monkeys	0.5[e]	Fujino *et al.*[40] (1990)
Thyroiditis	Rat (PVG)	2.0[f]	Carrieri *et al.* (1991) unpublished
Lupus (SLE)	MRL-lpr/lpr	2 mg[g]	Yamamoto *et al.*[47] (1990)
	NZB/NZW F_1 mouse	2.5 mg/kg/48 hr[g]	Takabayashi *et al.*[46] (1989)
Glomerulonephritis	Rat (Wistar)	0.3 mg[h]	Hara *et al.*[49] (1990)
(Nephrotoxic antiserum nephritis)	Rat (Wistar)	0.64	Okuba *et al.*[48] (1990)
Heymann's nephritis	Rat (Wistar)	0.64[a]	Okuba *et al.*[48] (1990)
Allergic encephalomyelitis	Rat (Lewis)	1.0[i]	Inamura *et al.*[50] (1988)

[a]Suppresses induction of disease.
[b]Partially effective during efferent phase of response.
[c]On day of immunization.
[d]Effective only in induction phase.
[e]Administered from 3 weeks after immunization.
[f]Administered for 3 weeks following induction of disease.
[g]Administered from 8 weeks of age.
[h]From time of immunization.
[i]Administered 5 days per week after immunization.

Table 2 FK 506 in human autoimmune disease.

FDA-Approved Protocols University of Pittsburgh
Renal disease
Nephrotic syndrome
Liver disease
Primary biliary cirrhosis
Connective tissue disorder
Systemic sclerosis
Skin diseases
Psoriasis
Pyoderma gangrenosum
Recent onset type I diabetes
Ophthalmic disorders
Uveitis
Scleritis
Cicatricial pemphigoid
Inflammatory bowel disease
Crohn's disease
Crohn's colitis
Ulcerative colitis

Other autoimmune disease protocols, FDA approval of which is pending, include chronic active hepatitis, rheumatoid arthritis, systemic lupus erythematosus, polymyositis-polydermatomyositis, sprue (celiac disease), multiple sclerosis, Behçet's syndrome, systemic vasculitidies.

less benefited from FK 506, the majority failing to respond but with notable exceptions.

The efficacy and safety of FK 506 in these and a variety of other human autoimmune disorders, including recent onset type 1 diabetes and inflammatory bowel diseases are presently being evaluated at the University of Pittsburgh Medical Centre.

CONCLUSIONS

Although it is too early to draw firm conclusions about the value of FK 506 in human autoimmune disease from the limited clinical investigations which have been undertaken to date, preliminary observations are encouraging. There is no doubt that FK 506 is at least as efficacious an immunosuppressant as CsA. Its capacity (unlike CsA) to reverse allograft rejection suggests that it may be more broadly effective than CsA in autoimmune disease and may be useful in patients unresponsive to high dose steroids and/or other forms of immunosuppressive therapy. Further studies must include dose range and long term evaluation of FK 506 therapy. Because FK 506 augments hepatic repair and regeneration, it may prove especially valuable in patients with autoimmune liver disease. Although FK 506 and CsA share several potential side effects, including nephrotoxicity, FK 506 is less apt to induce hypertension and does not cause gingival hyperplasia or hirsutism at all. Amongst the beneficiaries of FK 506 therapy have been a high proportion of paediatric heart transplant recipients[62] in whom steroids have never been used and children with nephrotic syndrome, in which prior high dose steroids have been discontinued[60]. The inclusion of FK 506 on the limited list of immunosuppressive drugs available for human autoimmune disease therapy appears justifiable.

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References

1. Kino T, Hatanaka H, Hashimoto M, Nishiyama M, Goto T, Okuhara M, Kohsaka M, Aoki H, Imanaka H. FK 506, a novel immunosuppressant isolated from Streptomyces. I. Fermentation, isolation and physicochemical and biological characteristics. *J Antibiot* 1987; **40:** 1249–1255
2. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, Goto T, Okuhara M, Kohsaka M, Aoki H, Ochiai T. FK 506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK 506 in vitro. *J Antibiot* **40:** 1256–1265
3. Thomson AW. FK 506. How much potential? *Immunol Today* 1989; **10:** 6–9
4. Starzl TE, Fung J, Venkataramanan R, Todo S, Demetris AJ, Jain A. FK 506 for liver, kidney and pancreas transplantation. *Lancet* 1989; **ii:** 1000-1004
5. Kahan BD, Chang JY, Seghal SN. Preclinical evaluation of a new potent immunosuppressive agent rapamycin. *Transplantation* 1991; **52:** 185–191
6. Fung JJ, Alessiani M, Abu-Elmagd K, Todo S, Shapiro R, Tzakis A, Van Thiel D, Armitage J, Jain A, McCauley J, Selby R, Starzl TE. Adverse effects associated with the use of FK 506. *Transplant Proc* 1991; **23:** 3105–3108
7. Todo S, Fung JJ, Demetris AJ, Jain A, Venkataramanan R, Starzl TE. Early trials with FK 506 as primary treatment in liver transplantation. *Transplantation Proc* 1990; **22:** 13–16
8. Shapiro R, Jordan M, Fung J, McCauley J, Johnston J, Iwaki Y, Tzakis A, Hakala T, Todo S, Starzl TE. Kidney transplantation under FK 506 immunosuppression. *Transplant Proc* 1991; **23:** 920–923
9. Armitage JM, Kormos RL, Fung J, Lavee J, Fricker FJ, Griffith BP. Preliminary experience with FK 506 in thoracic transplantation. *Transplantation* 1991; **52:** 164–167
10. Starzl TE, Thomson AW, Todo S, Fung JJ (eds.). Proceedings of the first international congress on FK 506. *Transplant Proc* 1991; **23:** 2709–3380
11. Starzl TE, Makowka L, Todo S (eds.) FK 506: a potential breakthrough in immunosuppression. *Transplant Proc* 1987; **19:** 3–103
12. Starzl TE, Todo S, Fung JJ, Groth C. (eds.) FK 506. A potential breakthrough in immunosuppression—clinical implications. *Transplant Proc* 1990; **22:** 5–113
13. Thomson AW. FK 506: Profile of an important new immunosuppressant. *Transplant Rev* 1990; **4:** 1–13
14. Macleod AM, Thomson AW. FK 506: an immunosuppressant for the 1990's? *Lancet* 1991; **337:** 25–27
15. Goto T, Kino T, Hatanaka H, Nishiyama M, Okuhara M, Kohsaka M, Aoki H, Imamaka H. Discovery of FK 506, a novel

immunosuppressant isolated from Streptomyces tsukubaensis. *Transplant Proc* 1987; **19:** Suppl 6, 4–8
16. Harding MW, Galat A, Uehling DE, Schreiber SL. A receptor for the immunosuppressant FK 506 is a *cis-trans* peptidyl-prolyl isomerase. *Nature* 1989; **341:** 758–760
17. Siekierka JJ, Hung SHY, Poe M, Lin SC, Sigal NH. A cytosolic binding protein for the immunosuppressant FK 506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 1989; **341:** 755–757
18. Tocci MJ, Matkovich DA, Collier KA, Kwok P, Dumont F, Lin S, Degudicibus S, Siekierka JJ, Chin J, Hutchinson NI. The immunosuppressant FK 506 selectively inhibits expression of early T cell activation genes. *J Immunol* 1989; **143:** 718–726
19. Zeevi A, Duquesnoy R, Eiras G, Rabinowich H, Todo S, Makowka L, Starzl TE. Immunosuppressive effect of FK 506 on in vitro lymphocyte alloactivation: synergism with cyclosporine A. *Transplant Proc* 1987; **19** (Suppl. 6): 40–44
20. Sawada S, Suzuki G, Kawase Y, Takaku F. Novel immunosuppressive agent, FK 506. In vitro effects on the cloned T cell activation. *J Immunol* 1987; **139:** 1797–1803
21. Woo J, Propper DJ, Thomson AW. Antigen presentation and HLA-DR expression by FK 506-treated human monocytes. *Immunology* 1990; **71:** 551–555
22. DePaulis A, Cirillo R, Ciccarelli A, Condorelli M, Marone G. FK 506, a potent novel inhibitor of the release of proinflammatory mediators from human FcRI$^+$ cells. *J Immunol* 1991; **146:** 2374–2381
23. Wang SC, Zeevi A, Tweardy DJ, Jordan ML, Simmons RL. FK 506, rapamycin and cyclosporine A: effects on IL-4 and IL-10 mRNA levels in a T helper cell line. *Transplant Proc* 1991; **23:** 2920–2922
24. Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* 1991; **251:** 283–287
25. Liu J, Farmer JD, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK 506 complexes. *Cell* 1991; **66:** 807–815
26. Flanagan WM, Corthesy B, Bram RJ, Crabtree GR. Nuclear association of a T-cell transcription factor blocked by FK 506 and cyclosporin A. *Nature* 1991; **352:** 803–807
27. Tamura K, Kobayashi M, Hashimoto K, Kojima K, Nagase K, Iwasaki K, Kaizu T, Tanaka H, Niwa M. A highly sensitive method to assay FK 506 in plasma. *Transplant Proc* 1987; **19:** Suppl 6, 23–29
28. Cadoff EM, Venkataramanan R, Krajack A, Jain AS, Fung JJ, Todo S, Starzl TE. Assay of FK 506 in plasma. *Transplant Proc* 1990; **22:** 50–51
29. Venkataramanan R, Jain A, Cadoff E, Warty V, Iwasaki K, Nagase K, Krajack A, Imventarza O, Todo S, Fung JJ, Starzl TE. Pharmacokinetics of FK 506: Preclinical and clinical studies. *Transplant Proc* 1990; **22:** 52–56
30. Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Furukawa H, Imventarza O, Fung JJ, Todo S, Starzl TE. Pharmacokinetics of FK 506 following oral administration. A comparison of FK 506 and cyclosporine. *Transplant Proc* 1991; **23:** 931–933
31. O'Hara K, Billington R, James RW, Dean GA, Nishiyama M, Noguchi H. Toxicologic evaluation of FK 506. *Transplant Proc* 1990; **22:** 83–86
32. Reyes J, Tzakis A, Green M, Nour B, Nalesnik M, Van Thiel D, Martin M, Breinig MK, Fung JJ, Cooper M, Starzl TE. Post-transplant lymphoproliferative disorders occurring under primary FK 506 immunosuppression. *Transplant Proc* 1991; **23:** 3044–3046
33. Sigal NH, Dumont F, Durette P, Siekierka JJ, Peterson L, Rich DH, Dunlap BE, Staruch MJ, Melino MR, Koprak SL, Williams D, Witzel B, Pisano JM. Is cyclophilin involved in the nephrotoxic mechanism of action of cyclosporin A? *J exp Med* 1991; **173:** 619–628
34. Woo J, Ross CS, Milton JI, Thomson AW. Immunosuppressive activity of FK 506 in rats: flow cytometric analysis of lymphocyte populations in blood, spleen and thymus during treatment and following drug withdrawal. *Clin Exp Immunol* 1990; **79:** 115–122
35. Pugh-Humphreys RGP, Ross CSK, Thomson AW. The influence of FK 506 on the thymus: an immunophenotypic and structural analysis. *Immunoloy* 1990; **70:** 398–404
36. Woo J, Ildstad ST, Hronakes ML, Thomson AW. Kinetics of early T cell repopulation in the mouse following syngeneic bone marrow transplantation: FK 506 causes a maturational defect of CD4$^+$ CD8$^-$ T cells. *Transplant Proc* 1991; **23:** 2323–2924
37. Kawashima H, Mochizuki M. Effects of a new immunosuppressive agent FK 506, on the efferent limb of the immune responses. *Exp Eye Res* 1990; **51:** 565–572
38. Kawashima H, Fujino Y, Mochizuki M. Effects of a new immunosuppressive agent, FK 506, on experimental autoimmune uveoretinitis in rats. *Invest Ophthalmol Vis Sci* 1988; **29:** 1265–1271
39. Mochizuki M, Kawashima H. Effects of FK 506, 15-deoxyspergualin and cyclosporine on experimental autoimmune uveoretinitis in the rat. *Autoimmunity* 1990; **8:** 37–41
40. Kawashima H, Fujino Y, Mochizuki M. Antigen-specific suppressor cells induced by FK 506 in experimental autoimmune uveoretinitis in the rat. *Invest Ophthalmol Vis Sci* 1990; **31:** 31–38
41. Ni M, Chan C-C, Nussenblatt RB, Mochizuki M. FR900506 (FK 506) and 15-deoxyspergualine (15-DSG) modulate the kinetics of infiltrating cells in eyes with experimental autoimmune uveoretinitis. *Autoimmunity* 1990; **8:** 43–51
42. Liversidge J, Thomson AW, Forrester JV. FK 506 modulates accessory cell adhesion molecule expression and inhibits CD4$^+$ lymphocyte adhesion to retinal pigment epithelial cells *in vitro*; implications for therapy of uveoretinitis. *Transplant Proc* 1991; **23:** 3339–3342
43. Fujino Y, Mochizuki M, Raber J, Kotake S, Gery I, Nussenblatt RB. FK 506 treatment of S-antigen induced uveitis in primates. *Invest Ophthalmol Vis Sci* 1990; **31:** 61
44. Inamura N, Hashimoto M, Nakahara K, Aoki H, Yamaguchi I, Kohsaka M. Immunosuppressive effect of FK 506 on collagen-induced arthritis in rats. *Clin Immunol Immunopathol* 1988; **46:** 82–90
45. Arita C, Hotokebuchi T, Miyahara H, Arai K, Sugioka Y, Takagishi K, Kaibara N, Inhibition by FK 506 of established lesions of collagen-induced arthritis in rats. *Clin Exp Immunol* 1990; **82:** 456–461
46. Takagishi K, Yamamoto M, Nishimura A, Yamasaki G, Kanazawa N, Hotokebuchi T, Kaibara N. Effects of FK 506 on collagen arthritis in mice. *Transplant Proc* 1989; **21:** 1053–1055
47. Murase N, Lieberman I, Nalesnik M, Mintz D, Todo S, Drash AL, Starzl TE. FK 506 prevents spontaneous diabetes in the BB rat. *Lancet* 1990; **ii:** 373–374
48. Miyagawa J, Yamamoto K, Hanafusa T, Itoh N, Nakagawa C, Otsuka A, Katsura H, Yamagata K, Miyazaki A, Kono N, Tarui S. Preventive effect of a new immunosuppressant FK 506 on insulinitis and diabetes in non-obese diabetic mice. *Diabetologia* 1990; **33:** 503–505
49. Takabayashi K, Koike T, Kurasawa K, Matsumura R, Sato T, Tomioka H, Ito I, Yoshiki T, Yoshida S. Effects of FK 506, a novel immunosuppressive drug on murine systemic lupus erythematosus. *Clin Immunol Immunopathol* 1989; **51:** 110–117
50. Yamamoto K, Mori A, Nakahama T, Ito M, Okudaira H, Miyamoto T. Experimental treatment of autoimmune MRL-lpr/lpr mice with immunosuppressive compound FK 506. *Immunology* 1988; **69:** 222–227
51. Okuba Y, Tsukada Y, Maezawa A, Ono K, Yano S, Naruse T. FK 506, a novel immunosuppressive agent, induces antigen-specific immunotolerance in active Heymann's nephritis and in the autologous phase of Masugi nephritis. *Clin Exp Immunol* 1990; **82:** 450–455

52. Hara S, Fukatsu A, Suzuki N, Sakamoto N, Matsuo S. The effects of a new immunosuppressive agent, FK 506, on the glomerular injury in rats with accelerated nephrotoxic serum glomerulonephritis. *Clin Immunol Immunopathol* 1990; **57:** 351–362
53. Inamura N, Hashimoto M, Nakahara K, Nakajima Y, Nishio M, Aoki H, Yamaguchi I, Kohsaka M. Immunosuppressive effect of FK 506 on experimental allergic encephalomyelitis in rats. *Int J Immunopharmacol* 1988; **10:** 991–995
54. Bolton C. The ability of myelin basic protein-sensitized leukocytes to adoptively transfer experimental allergic encephalomyelitis following coculture with FK 506, cyclosporine, or prednisone. *Transplant Proc* 1991; **23:** 3363–3364
55. Jegasothy B, Ackerman CD, Todo S, Fung J, Starzl TE. FK 506—a new therapeutic agent for severe, recalcitrant psoriasis. *Arch Dermatol* In press
56. Abu-Elmagd K, Van Thiel DH, Jegasothy BV, Ackerman CD, Todo S, Fung JJ, Thomson AW, Starzl TE. FK 506–a new therapeutic agent for severe, recalcitrant psoriasis. *Transplant Proc* 1991; **23:** 3322–3324
57. Thomson AW, Nalesnik M, Abu-Elmagd K, Starzl TE. Influence of FK 506 on T lymphocytes, Langerhans cells, and the expression of cytokine receptors and adhesion molecules in psoriatic skin lesions: a preliminary study. *Transplant Proc* 1991; **23:** 3330–3331
58. Abu-Elmagd K, Jegasothy BV, Ackerman CD, Thomson AW, Rilo H, Nikolaidis N, Van Thiel D, Fung JJ, Todo S, Starzl TE. Efficacy of FK 506 in the treatment of pyoderma gangrenosum. *Transplant Proc* 1991; **23:** 3328–3329
59. Mochizuki M, Masuda K, Sakane T, Inaba G, Ito K, Kogure M, Sugino N, Usui M, Mizushima Y, Ohno S, Miyanaga Y, Hayasaka S, Ohizumi K. A multicentre clinical open trial of FK 506 in refractory uveitis, including Behçet's disease. *Transplant Proc* 1991; **23:** 3343–3346
60. McCauley J, Tzakis AG, Fung JJ, Todo S, Starzl TE. FK 506 in steroid-resistant focal sclerosing glomerulonephritis of childhood. *Lancet* 1990; **i:** 674
61. McCauley J, Shapiro R, Scantlebury V, Gilboa N, Jordan M, Jensen C, Naik A, Tzakis A, Ellis D, Starzl TE. FK 506 in the management of transplant-related nephrotic syndrome and steroid-resistant nephrotic syndrome. *Transplant Proc* 1991; **23:** 3354–3356
62. Armitage JM, Fricker FJ, Del Nido P, Cipriani L, Starzl TE. The clinical trial of FK 506 as primary and rescue immunosuppression in pediatric cardiac transplantation. *Transplant Proc* 1991; **23:** 3058–3060