THE INFLUENCE OF TACROLIMUS ON EXPERIMENTAL AUTOIMMUNE DISEASE

Angus W. Thomson, Noriko Murase,
Michael A. Nalesnik and Thomas E. Starzl

INTRODUCTION

Four classes of immunosuppressive drugs have been used to treat human autoimmune diseases: corticosteroids, alkylating agents (cyclophosphamide and chlorambucil), antimetabolites (azathioprine and methotrexate) and more recently, cyclosporine A (CsA). Before the advent of CsA, which was approved by the US Food and Drug Administration (FDA) for the therapy of organ allograft rejection in 1983, the successful use of immunosuppressive drugs in autoimmune disease was restricted to a handful of disorders. These included systemic lupus erythematosus (SLE), rheumatoid arthritis, myasthenia gravis and nephrotic syndrome. The use of CsA, which selectively inhibits T-lymphocyte activation and cytokine production has resulted in a higher rate of improvement than previously observed in those autoimmune diseases with a presumed T cell pathogenesis. These diverse disorders include psoriasis, atopic dermatitis, uveitis, insulin-dependent (type I) diabetes mellitus, primary biliary cirrhosis, aplastic anemia and lichen planus. In contrast, autoimmune diseases that are believed to be mediated primarily by autoantibodies, such as the autoimmune cytopenias, myasthenia gravis, SLE, Graves' disease and the glomerulonephritides are relatively resistant to CsA. In addition to the improved rate of response seen with CsA, opportunistic infections (a potentially serious complication of cytotoxic drug administration) are very rare in CsA-treated autoimmune disease patients.

Despite the successes achieved with CsA, several important problems still exist with respect to the immunosuppressive therapy of autoimmune diseases. These include: (1) an insufficient response rate; (2) disease recurrence following drug withdrawal (failure to induce tolerance); and (3) the associated risks of drug toxicity or excessive immunosuppression (including infectious complications and malignancy). With respect to CsA, the most common side effects are nephrotoxicity (including arterial hypertension), cosmetic deformity with hirsutism and coarsening of the facies, hypercholesterolemia and an increased disposition to diabetes mellitus. Resolution of these difficulties has become the incentive for discovery of new immunosuppressive agents that act

specifically on components of the immune system that are involved in disease pathogenesis and not on "therapeutically irrelevant" cells. A wide range of new candidate drugs that act at different stages of lymphocyte activation/proliferation are currently under development (Table 10.3.1). Of these new agents, tacrolimus (formerly known as FK506), that has a similar mode of action to CsA but is considerably more potent, has recently been approved (April 1994) by the FDA for the therapy of liver allograft rejection. Its efficacy in the prevention of allograft rejection is well documented; more germane to the autoimmune disease field however, is the ability of tacrolimus to reverse established rejection—an immunopathologic process that shares cellular and molecular mechanisms with many autoimmune disorders. Preclinical evaluation of the efficacy and safety of tacrolimus in experimental autoimmune disorders is an important process in the evaluation of its therapeutic potential. This chapter briefly reviews the mode of action of tacrolimus and its influence on experimental autoimmunity.

MODE OF ACTION OF TACROLIMUS

The powerful anti-lymphocytic and immunosuppressive properties of tacrolimus ($C_{44}H_{69}NO_{12} \cdot H_2O$; mw 822), a fungally-derived macrocyclic lactone, were first documented in 1987.[1,2] Although totally distinct in structure from the fungal product and cyclic peptide CsA, tacrolimus shares many of the properties of the latter immunosuppressant. Both agents exhibit very similar molecular actions which result in the selective inhibition of CD4$^+$ T helper (TH) lymphocyte activation, cytokine-gene expression and consequently, lymphocyte proliferation. Like CsA, tacrolimus inhibits T cell activation mediated via the T cell receptor (TCR)-CD3 complex and the cell surface molecule CD2. It is very effective in suppressing lymphocyte proliferation in vitro at concentrations 100-fold lower than effective concentrations of CsA.[2-4] Tacrolimus inhibits the generation of cytotoxic T cells

in human mixed lymphocyte reactions but does not affect antigen recognition by cytotoxic T cells or the mechanism by which target cells are destroyed.[5] Tacrolimus does not appear to inhibit antigen processing or presentation by human monocytes at drug concentrations which strongly suppress T cell proliferation.[6] Tacrolimus does not affect Ca^{2+} mobilization, phosphatidylinositol turnover, or protein kinase C (PKC) activities. It 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, granulocyte macrophage-colony stimulating factor (GM-CSF) and c-myc.[4] On the other hand, recent studies have shown that tacrolimus may spare IL-10 (cytokine synthesis inhibitory factor) gene transcription by cloned murine T helper-2 (TH2) cells in vitro, whilst suppressing concomitant IL-4 mRNA production by these cells.[7] Thus, *differential* interference with T cell cytokine gene expression may be an important mechanism whereby tacrolimus inhibits lymphocyte activation and immune suppression is maintained.

Clues to the molecular actions of tacrolimus and CsA came from studies of their respective intracellular binding proteins—FK506 binding protein (FKBP) and cyclophilin, each of which is a *cis-trans* peptidyl prolyl isomerase.[8-10] Although binding of the drug by its receptor (or "immunophilin") inhibits isomerase activity, the immunosuppressive effects of tacrolimus and CsA result from the formation of complexes between the drug and its respective isomerase.[11] Both the complexes of tacrolimus (FK506)-FKBP and CsA-cyclophilin bind specifically to three polypeptides-calmodulin and the two subunits of calcineurin (a Ca^{2+}-calmodulin activated, serine-threonine protein phosphatase). In each case, the interaction of the immunophilin appears to be with calcineurin. The drug-immunophilin complexes but neither drug nor either immunophilin alone, have been shown to block the Ca^{2+}-activated phosphatase activity of calcineurin.[10] Calcineurin thus appears to be the molecular target of the drug-immunophilin complexes.

Table 10.3.1. New immunosuppressive drugs with potential for therapy of autoimmune disorders

Agent	Structure	Level of action
Deoxyspergualin	semisynthetic polyamine	macrophage function, cytotoxic T cells
FK506 (Tacrolimus)	carboxycyclic lactone	inhibits IL-2 production
Rapamycin	carboxycyclic lactone	inhibits IL-2 action
Leflunomide	isoxazole derivative	? B cell suppression
Mizoribine	imidazole nucleoside	inhibits DNA synthesis
Mycophenolate mofetil	mycophenolic acid derivative	inhibits DNA synthesis
Brequinar sodium	carboxylic acid derivative	inhibits DNA synthesis
SK&F 105685	azaspirane analog	? induction of suppressor cells

The second, important observation was that the drug-immunophilin complexes block Ca^{2+}-dependent translocation of the pre-existing, cytoplasmic component of the nuclear factor of activated T cells (NF-AT) to the nucleus.[12] The nuclear component of NF-AT is transcriptionally inactive in all cells other than activated T-lymphocytes, and is induced by signals from the TCR. Its appearance is not blocked by tacrolimus or CsA. It is now believed that tacrolimus and CsA block dephosphorylation of the cytoplasmic component of NF-AT which is required for its translocation to the nucleus. 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.

EFFECTS OF TACROLIMUS ON IMMUNE REACTIVITY

The very potent inhibitory effects of tacrolimus 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 tacrolimus in rodents, dogs and primates. These include models of organ allograft rejection, ranging from skin grafts to multi-visceral transplants (see recent reviews in refs. 13-17). Tacrolimus is also effective in preventing and reversing graft-versus-host disease after experimental bone marrow transplantation. In each instance, tacrolimus has been shown to be about 10-fold more potent than CsA.

TOXICITY OF TACROLIMUS IN EXPERIMENTAL ANIMALS

The toxic effects of tacrolimus in experimental animals (rodents, rabbits, dogs and primates) have been well documented.[18] Dogs are especially susceptible to tacrolimus toxicity and exhibit dose-related vasculitis and intususseptions. Several groups have shown that the toxic effects of tacrolimus in nonhuman primates are more pronounced when the drug is administered i.m. compared with orally, due presumably, to the greater bioavailability achieved using the former route. Tacrolimus does not exhibit mutagenic activity in either in vitro or in vivo tests. Fetotoxicity has been demonstrated in rats and teratogenic effects have been observed in rabbits.

An important issue is whether the nephrotoxic potential of tacrolimus and CsA (or their analogs/derivatives) correlates with the drugs' PPIase inhibitory activities. Recent data[19] indicate that immunosuppressive activity and not immunophilin binding or PPIase inhibitory activity determines the ability of CsA analogs to induce nephrotoxicity. It may thus be difficult to design new nonnephrotoxic drugs that retain the same potent immunosuppressive activity.

INFLUENCE OF TACROLIMUS IN AUTOIMMUNE DISEASE MODELS
(See Table 10.3.2 and refs. 20-43)

EXPERIMENTAL AUTOIMMUNE 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 cell mediated and a good model of autoimmune uveitis in humans. The influence of tacrolimus in EAU has been studied extensively in the Lewis rat by Mochizuki and his colleagues. Tacrolimus was found to be 10-30 times more potent than CsA in preventing induction of EAU when administered either from 0-5 days or from 7-12 days after S-antigen immunization. It appears that tacrolimus is effective in suppressing on-going immunopathological processes, even after the disease has been initiated.[20] As with CsA, the immunological unresponsiveness induced by a 2 week course of tacrolimus (days 0-14) was found to be specific to the S-antigen. Whilst splenic lymphocytes from animals treated with either drug showed markedly depressed responses to S-antigen in vitro, only tacrolimus was found to significantly depress serum antibody levels.[21] EAU induction, as well as immune responses to S-antigen were suppressed long after cessation of tacrolimus treatment.[22]

It has been reported that spleens of S-antigen immunized and tacrolimus-treated rats contain antigen-specific T suppressor (Ts) cells which, when transferred to naive 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.[23]

Immunohistochemical studies on lymphocytes infiltrating the ocular lesions in EAU have revealed that tacrolimus 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.[24] Further insight into the mode of action of tacrolimus 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). Tacrolimus also inhibits binding of CD4+ lymphocytes to RPE cells.[25]

In rhesus and cynomolgus monkeys, tacrolimus (0.5 mg/kg/day) administered i.m. for at least 2 weeks from 3 weeks after immunization with S-antigen prevented EAU. Antibody titers against S-antigen were reduced, whilst S-antigen-induced lymphocyte

Table 10.3.2. Experimental autoimmune diseases suppressed by tacrolimus (FK506)

Disease	Species	Tacrolimus Dose (mg/kg/day unless specified)	Reference
Arthritis (Type II collagen-induced)	Rat (Lewis)	0.32[a,b]	Inamura et al[27] (1988)
	Rat (Outbred)	2.5[c]	Arita et al[28] (1990)
	Mouse (DBA/1)	2.0	Takagishi et al[29] (1989)
Type I diabetes	NOD mouse	2.0 mg/kg/48 hr[a]	Miyagawa et al[31] (1990)
	Cyclophosphamide -treated NOD mouse	0.2, 1, 2	Carroll et al[32] (1991)
	BB rat	1.0[a]	Murase et al[30] (1990)
	BB rat	25 μg i.m.[d]	Nicoletti et al[33] (1991)
Uveoretinitis	Rat (Lewis)	1.0[a,e]	Kawashima et al[20] (1990)
	Rhesus & cynomolgus monkeys	0.5[f]	Fujino et al[26] (1990)
Thyroiditis	Rat (PVG)	2.0[g]	Tamura et al[34] (1992)
Lupus (SLE)	MRL-*lpr/lpr* mouse	2 mg[h]	Yamamoto et al[35] (1988)
	NZB/NZW F_1 mouse	2.5 mg/kg/48 hr[i]	Takabayashi et al[36] (1989)
Glomerulonephritis	Rat (Wistar)	0.3 mg[j]	Hara et al[37] (1990)
(Nephrotoxic antiserum nephritis)	Rat (Wistar)	0.64	Okuba et al[38] (1990)
Heymann nephritis	Rat (Wistar)	0.64[a]	Okuba et al[38] (1990)
	Rat (Lewis)	1.0[k]	Matsukawa et al[39] (1992)
Allergic encephalomyelitis	Rat (Lewis)	1.0[j]	Inamura et al[40] (1988)
Autoimmune myocarditis	Rat (Lewis)	0.1, 0.32, 1	Hanawa et al[41] (1992)
Experimental allergic contact dermatitis	Farm pig	0.04, 0.4% topical	Meingassner & Stutz[42] (1992)
Murine (coxsackie B3) myocarditis	Mice (C3H/He)	2.5	Hiraoka et al[43] (1992)

a Suppresses induction of disease
b Partially effective during efferent phase of response
c On day of immunization
d Administered daily from 27-120 days of age
e Effective only in induction phase
f Administered from 3 weeks after immunization
g Administered for 3 weeks following induction of disease
h Administered from 8 weeks of age
i From time of immunization
j Administered 5 days per week after immunization
k Administered from day 0-13 or 56-69

proliferation in vitro was unchanged or decreased during tacrolimus treatment.[26]

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[27] reported that administration of tacrolimus 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.[27] Studies by Arita et al[28] in Sprague-Dawley rats confirmed the efficacy of tacrolimus 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 tacrolimus was effective in suppressing existing disease and immune (humoral) responses to type II collagen. Moreover, pretreatment of rats with a single tacrolimus injection (10 mg/kg) (day -7 or -3) reduced disease severity and antibody production.

Similar results concerning the prophylactic effect of tacrolimus in collagen arthritis have been obtained in mice using doses of drug 25 times lower than effective doses of CsA.[29] As with CsA, tacrolimus 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 insulin-producing pancreatic β cells (Fig. 10.3.1(a, b)). Administration of tacrolimus (2 mg/kg/day) from 30 to 120 days of age prevented the destruction of insulin-producing cells (Fig. 10.3.1(c, d)) and the development of diabetes (Fig. 10.3.2) in 20/20 BB rats during the treatment period.[30] Blood glucose, renal and hepatic function tests remained normal, whilst histological examination confirmed the absence of insulitis. Glucose intolerance, which has been described in BB rats given CsA was not observed in this study.

In nonobese 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 tacrolimus (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 tacrolimus-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.[31]

In both the BB rat and NOD mouse studies, the preventive effect of tacrolimus 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 nonorgan-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[36] reported that tacrolimus (2.5 mg/kg, 3 times per week from 12 weeks of age) prolonged the lifespan of female NZB/W F₁ 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[36] reported similar effects of tacrolimus on survival, proteinuria and anti-dsDNA antibody levels in MRL/lpr female mice (given 2.5 mg/kg tacrolimus 3 times per week from 8 weeks old) to those observed in NZB/W F₁ 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 tacrolimus treatment protocol, Yamamoto et al[35] found that inhibition of disease activity in MRL/lpr mice was accompanied by significant reductions in serum anti-ssDNA and anti-dsDNA 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
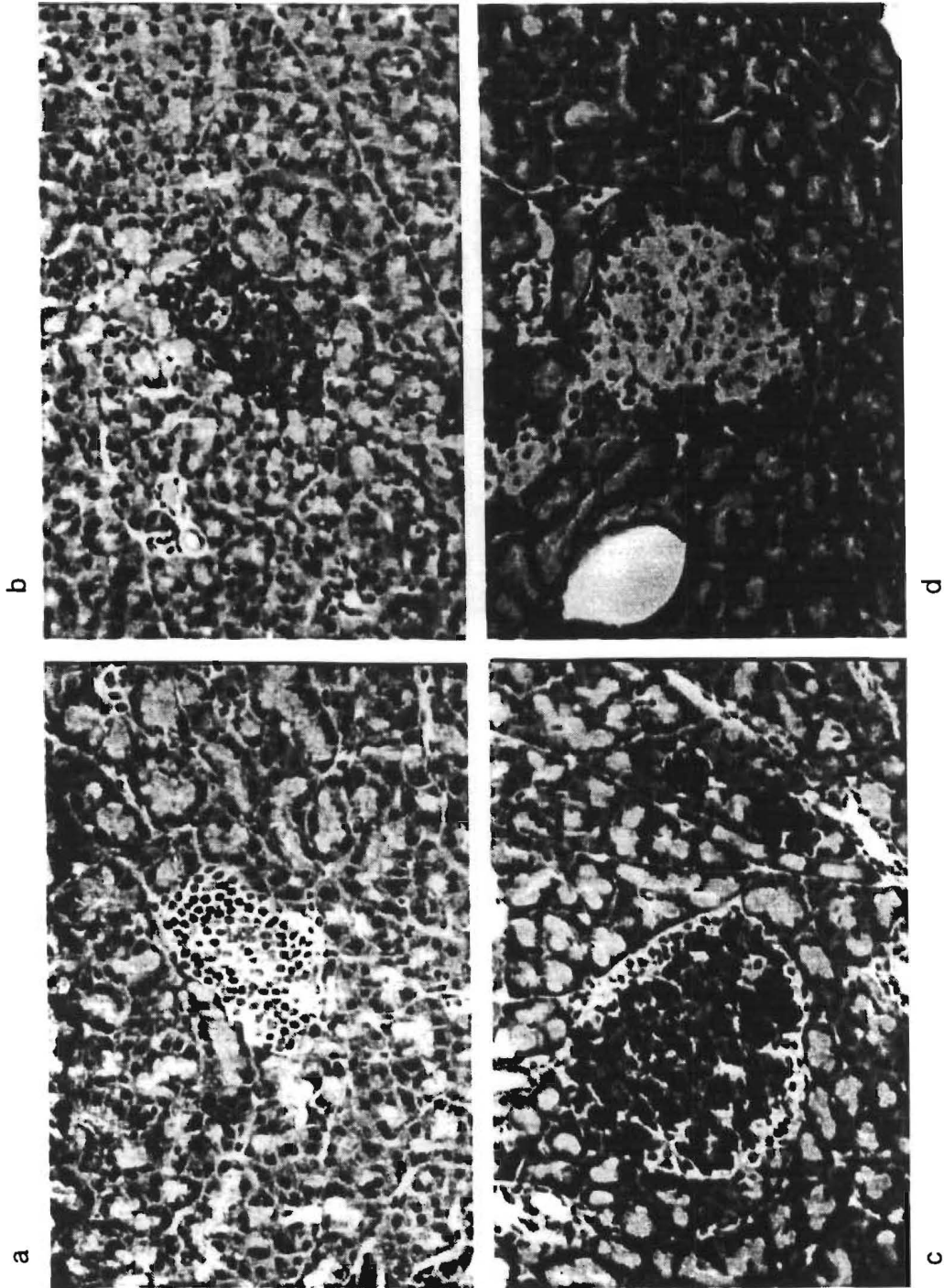
Fig. 10.3.1. Immunohistochemical staining of the pancreas of an untreated diabetic BB rat (80 days of age) showing (a) the absence of insulin-containing cells but (b) staining for glucagon. Continuous daily administration of tacrolimus (FK506) from 30 days of age up to 120 days preserved both (c) insulin and (d) glucagon-producing cells (120 days of age). (x 400.)
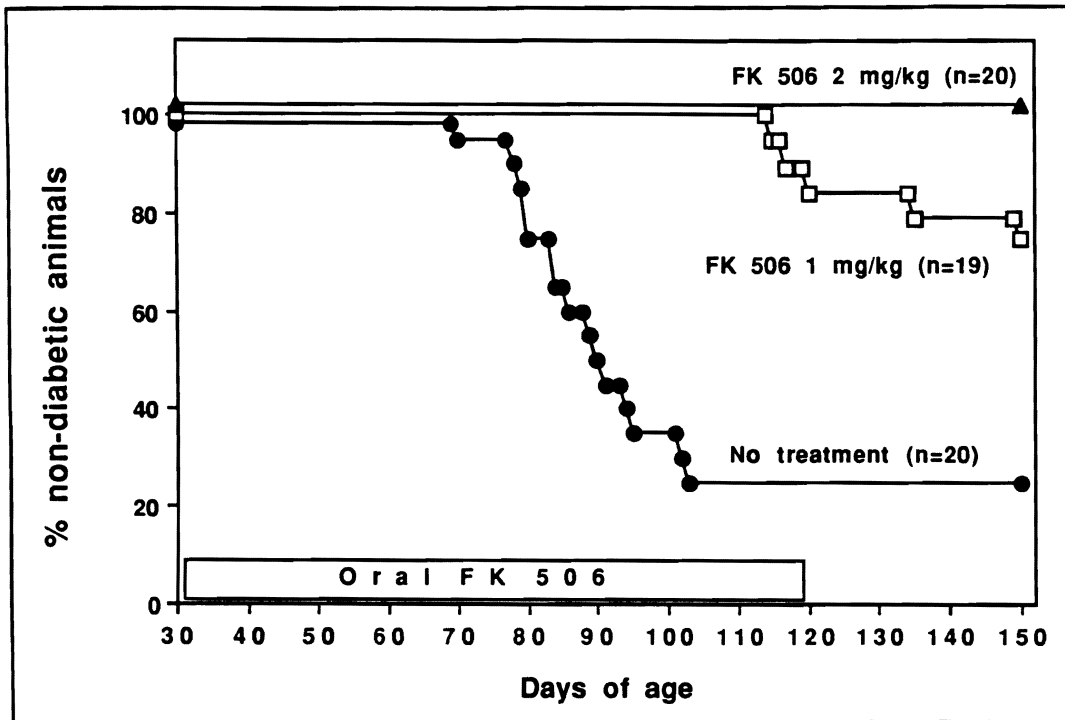
Fig. 10.3.2. Inhibition of development of diabetes in tacrolimus (FK506)-treated BB rats.

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[38] have shown that tacrolimus (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 tacrolimus 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 by tacrolimus 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, tacrolimus suppresses the autologous response to rabbit IgG and reduces substantially the glomerular injury.[37]

Experimental 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 autoantibodies. Administration of tacrolimus (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.[34]

Experimental 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. Tacrolimus (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.[40] It has been observed that the adoptive transfer of EAE can be suppressed by in vitro treatment of spleen cells with 1 nM tacrolimus, 100-fold lower than the effective concentration of CsA.[44]

COMBINED T AND B CELL DIRECTED THERAPY OF SPONTANEOUS AUTOIMMUNE DISEASE USING TACROLIMUS AND LOW DOSE CYCLOPHOSPHAMIDE

Autoimmune diseases in which autoantibodies and immune complexes appear to play a dominant role, such as SLE, various vasculitides and glomerulonephritis have not responded as favorably to CsA as those disorders that are thought to be predominantly T cell driven. The current management of the former disorders includes treatment with varying doses of corticosteroids and for certain organ system involvement, use of cyclophosphamide (CY).[45] Responses are frequently only partial however, and often associated with severe side effects, including life-threatening infections.

The search for improved strategies of immunosuppressive therapy includes the evaluation of combinations of drugs with complementary actions. In principle, two or more agents can be used together at reduced dosage to achieve additive or synergistic therapeutic effects while minimizing toxicity. Recently, it has been shown that combination of either of the T cell suppressants, CsA[46] or tacrolimus[47] with the alkylating agent CY can effectively control the strong antibody and cell-mediated responses evoked by concordant xenografts in experimental animals. Moreover, this approach has been found to prolong graft survival much longer than drug monotherapy. Therefore, it is possible that the combination of CsA or tacrolimus with CY may prove valuable in the treatment of autoimmune disorders in which both T cells and antibody-producing B cells are thought to play important pathogenic roles.

Mice homozygous for the *lpr* gene that encodes a defective allele of the apoptosis regulatory gene *Fas*[48] spontaneously develop an aggressive T cell dependent autoimmune disease that resembles human SLE.[49-51] MRL-*lpr/lpr* mice also develop high levels of autoantibodies, associated with glomerulonephritis and vasculitis, interstitial pneumonitis, arthritis (in some, but not all colonies) and premature death. It has been shown (see above) that high doses of tacrolimus can inhibit disease progression and prolong survival. The incidence of proteinuria and anti-DNA autoantibodies may however, remain high.[35,36] Since a combination of tacrolimus and CY has been shown to be effective in controlling both T cell and antibody-mediated immune responses in vivo, we have examined the effect of this drug combination on the development of autoimmune disease in MRL-*lpr* mice.

Groups of female MRL-*lpr* mice received either saline or tacrolimus (2 mg/kg i.p) thrice weekly, CY (20 mg/kg) once monthly, or both drugs from 8 weeks of age. Median survival for untreated and CY-treated mice was 26 weeks and for tacrolimus and tacrolimus + CY-treated groups was > 44 weeks.[52] Severity of skin lesions and lymph node hyperplasia was markedly reduced by the drug combination, whereas either drug alone was less effective (Table 10.3.3). Tacrolimus or CY alone delayed the onset of proteinuria, but by 24 weeks all of these animals were positive. In contrast, drug combination reduced the prevalence and severity of proteinuria throughout the 44 weeks of study (Fig. 10.3.3). Sequential monitoring of peripheral blood lymphocytes revealed that combination therapy but not monotherapy markedly reduced the proportion of atypical CD3+ B220+ and CD3+CD4-CD8- T cells. Serum levels of anti-dsDNA antibodies were reduced in all treatment groups. Analysis of tissue from saline-treated mice that died spontaneously showed as anticipated, evidence of extensive diffuse interstitial disease, diffuse proliferative glomerulonephritis with crescents, and vasculitis (Fig. 10.3.4(a)). Similarly, the CY-treated mice showed

Table 10.3.3. Influence of tacrolimus (FK506) and tacrolimus + cyclophosphamide (CY) on the clinical disease index (lymphoid hyperplasia, exudative dermatitis and ear necrosis) in female MRL-lpr mice

Treatment Age (weeks)	Saline	Tacrolimus	CY	Tacrolimus + CY
8	0 (10)	0 (10)	0 (6)	0 (10)
12	0 (10)	0 (10)	0 (6)	0 (10)
16	1.4 ± 0.9(9)	1.2 ± 0.4(10)	1.2 ± 0.4(4)	1.0 ± 0.5[a](10)
20	1.8 ± 0.6(7)	0.9 ± 0.4(10)	1.8 ± 0.9(4)	0.6 ± 0.6[b](10)
24	2.5 ± 0.6(5)	1.9 ± 0.8(10)	1.8 ± 0.9(4)	0.9 ± 0.9[b](10)
33	3.0(1)	2.8 ± 1.2(9)	3.5(1)	1.3 ± 1.2(10)
44	No survivors	2.6 ± 1.3(7)	2.5(1)	1.3 ± 1.3(7)

0-, no physical signs; clinical involvement scored 1-4 (most severe). Total score for each group was divided by the total number of mice alive (in parentheses) when determinations were made.
[a], p < 0.05; [b], p < 0.01 compared with saline-treated control.
Reproduced with permission from Woo J et al. Clin Exp Immunol 1995; 100:118-125.[52]

Proteinuria in MRL-*lpr/lpr* Mice

Albumin
Level mg/dL

16 Wk

	Untreated	FK506-Treated	CY-Treated	FK506+CY-Treated
> 2000				
300-2000	∞		∞	
100-300	∞	∞∞	○	∞∞
30-100	∞∞	∞		○
Trace	○	∞∞∞	∞∞	∞∞∞
Negative				∞

20 Wk

	Untreated	FK506-Treated	CY-Treated	FK506+CY-Treated
> 2000				
300-2000	∞		○	
100-300		∞		○
30-100	∞∞∞	∞∞∞	∞	∞∞
Trace	○	∞	○	∞∞∞
Negative				

24 Wk

	Untreated	FK506-Treated	CY-Treated	FK506+CY-Treated
> 2000	○			
300-2000	○	○	○	
100-300	∞	∞∞∞∞	○	
30-100	○	∞∞∞	○	∞∞∞∞
Trace				∞∞∞
Negative				○

33 Wk

	Untreated	FK506-Treated	CY-Treated	FK506+CY-Treated
> 2000				
300-2000		∞		∞∞
100-300		∞∞∞		○
30-100	○	∞∞	○	∞
Trace				∞∞
Negative				○

44 Wk

	Untreated	FK506-Treated	CY-Treated	FK506+CY-Treated
> 2000				
300-2000		○		
100-300	No Survivors	○		○
30-100		∞	○	∞
Trace		∞		∞∞
Negative				○

Untreated	FK506-Treated	Cy-Treated	FK506+Cy-Treated

Fig. 10.3.3. Effects of tacrolimus (FK506), CY or tacrolimus + CY therapy (started at 8 weeks of age) on the extent of proteinuria in individual female MRL-lpr mice from 16-44 weeks of age.
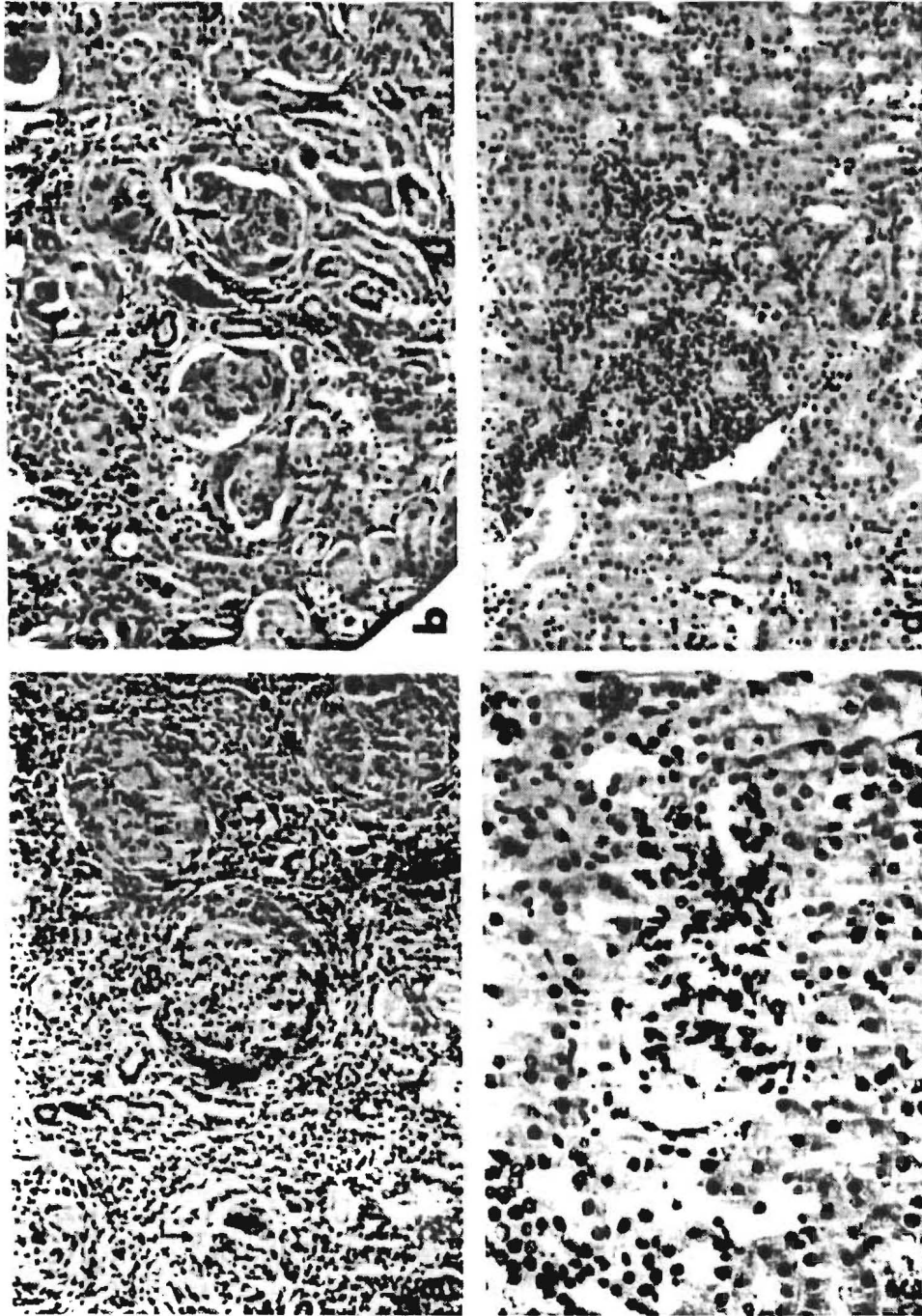
Fig. 10.3.4. Representative kidney histopathology of MRL-lpr mice from animals treated with (a) saline (29 weeks of age) that died spontaneously, showing extensive interstitial disease, and diffuse glomerulonephritis with crescents and tubular atrophy, (b) CY (44 weeks) showing marked interstitial and glomerular disease with tubular atrophy or (c) tacrolimus (FK506) (44 weeks) and (d) FK-506 + CY (44 weeks) both showing moderate local interstitial infiltrates and moderate, focal glomerulonephritis. Original magnifications: a,b,d, x200; c, x400. Reproduced with permission from Woo J et al. Clin Exp Immunol 1995; 100:118-125.[52]

severe interstitial and glomerular disease with vasculitis (Fig. 10.3.4(b)). In contrast, the kidneys of mice treated with tacrolimus or tacrolimus + Cy (Figs. 10.3.4 (c,d)), sacrificed at 44 weeks showed only moderate focal interstitial and glomerular changes. There was no significant difference in the severity of renal disease between the tacrolimus and tacrolimus + Cy groups. These data demonstrate the improved efficacy of combined T and B cell directed immunosuppression in murine lupus, associated with marked inhibition of atypical T cells within the affected lymphoid tissue. Since Cy is used currently in the treatment of SLE, the combined use of these powerful immunosuppressants for the more effective control of this debilitating disease is worthy of further evaluation.

REFERENCES

1. Kino T, Hatanaka H, Hashimoto M et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation and physiochemical and biological characteristics. J Antibiot 1987; 40:1249-55.
2. Kino T, Hatanaka H, Miyata S et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot 1987; 40:1256-65.
3. Zeevi A, Duquesnoy R, Eiras G et al. Immunosuppressive effect of FK-506 on in vitro lymphocyte alloactivation: synergism with cyclosporine A. Transplant Proc 1987; 19(Suppl. 6):40-4.
4. Tocci MJ, Matkovich DA, Collier KA et al. The immunosuppressant FK-506 selectively inhibits expression of early T cell activation genes. J Immunol 1989; 143:718-26.
5. Sawada S, Suzuki G, Kawase Y et al. Novel immunosuppressive agent, FK-506. In vitro effects on the cloned T cell activation. J Immunol 1987; 139: 1797-1803.
6. Woo J, Propper DJ, Thomson AW. Antigen presentation and HLA-DR expression by FK-506-treated human monocytes. Immunology 1990; 71:551-55.
7. Wang SC, Zeevi A, Tweardy DJ et al. FK-506, rapamycin and cyclosporine A: effects on IL-4 and IL-10 mRNA levels in a T helper cell line. Transplant Proc 1991; 23:2920-22.
8. Harding MW, Galat A, Uehling DE et al. A receptor for the immunosuppressant FK-506 is a cis-trans peptidyl-prolyl isomerase. Nature 1989; 341:758-60.
9. Siekierka JJ, Hung SHY, Poe M et al. A cytosolic binding protein for the immunosuppressant FK-506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 1989; 341:755-57.
10. Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 1991; 251:283-87.
11. Liu J, Farmer JD, Lane WS et al. Calcineurin is a common target of cyclophilin-cyclosporine A and

FKBP-FK-506 complexes. Cell 1991; 66:807-15.
12. Flanagan WM, Corthesy B, Bram RJ et al. Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporine A. Nature 1991; 352:803-7.
13. Thomson AW. FK-506. How much potential? Immunol Today 1989; 10:6-9.
14. Starzl TE, Thomson AW, Todo S, Fung JJ, eds. Proceedings of the first international congress on FK-506. Transplant Proc 1991; 23:2709-3380.
15. Starzl TE, Makowka L, Todo S, eds. FK-506: a potential breakthrough in imunosuppression. Transplant Proc 1987; 19:3-103.
16. Starzl TE, Todo S, Fung JJ, Groth C, eds. FK-506. A potential breakthrough in immunosuppression—clinical implications. Transplant Proc 1990; 22:5-113.
17. Thomson AW. FK-506: Profile of an important new immunosuppressant. Transplant Rev 1990; 4:1-13.
18. O'Hara K, Billington R, James RW et al. Toxicologic evaluation of FK-506. Transplant Proc 1990; 22:83-6.
19. Sigal NH, Dumont F, Durette P et al. Is cyclophilin involved in the nephrotoxic mechanism of action of cyclosporine A? J Exp Med 1991; 173:619-28.
20. 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: 565-72.
21. 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; 29:1265-71.
22. Mochizuki M, Kawashima H. Effects of FK-506, 15-deoxyspergualin and cyclosporine on experimental autoimmune uveoretinitis in the rat. Autoimmunity 1990; 8:37-41.
23. Kawashima H, Fujino Y, Mochizuki M. Antigen-specific suppressor cells induced by FK-506 in experimental autoimmune uveoretinitis in the rat. Invest Ophthal mol Vis Sci 1990; 31:31-8.
24. Ni M, Chan C-C, Nussenblatt RB et al. 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.
25. Liversidge J, Thomson AW, Forrester JV. FK-506 modulates accessary cell adhesion molecule expression and inhibits CD4* lymphocyte adhesion to retinal pigment epithelial cells in vitro; implications for therapy of uveoretinitis. Transplant Proc 1991; 23:3339-42.
26. Fujino Y, Mochizuki M, Raber J et al. FK-506 treatment of S-antigen induced uveitis in primates. Invest Ophthalmol Vis Sci 1990; 31:61.
27. Inamura N, Hashimoto M, Nakahara K et al. Immunosuppressive effect of FK-506 on collagen-induced arthritis in rats. Clin Immunol Immunopathol 1988; 46:82-90.
28. Arita C, Hotokebuchi T, Miyahara H et al. Inhibi-

tion by FK-506 of established lesions of collagen-induced arthritis in rats. Clin Exp Immunol 1990; 82:456-61.

29. Takagishi K, Yamamoto M, Nishimura A et al. Effects of FK-506 on collagen arthritis in mice. Transplant Proc 1989; 21:1053-55.

30. Murase N, Lieberman I, Nalesnik M et al. FK-506 prevents spontaneous diabetes in the BB rat. Lancet 1990; ii:373-4.

31. Miyagawa J, Yamamoto K, Hanafusa T et al. Preventive effect of a new immunosuppressant FK-506 on insulinitis and diabetes in non-obese diabetic mice. Diabetologia 1990; 33:503-5.

32. Carroll PB, Strasser S, Alejandro R. The effect of FK-506 on cyclophosphamide-induced diabetes in the NOD mouse model. Transplant Proc 1991; 23:3348-50.

33. Nicoletti F, Meroni PL, Barcellini W et al. FK-506 prevents diabetes in diabetes-prone BB/Wor rats. Int. J. Immunopharmac 1991; 13:1027-30.

33. Yamamoto K, Mori A, Nakahama T et al. Experimental treatment of autoimmune MRL-lpr/lpr mice with immunosuppressive compound FK-506. Immunology 1988; 69:222-7.

34. Tamura K, Woo J, Murase N et al. Suppression of autoimmune thyroid disease by FK-506: influence on thyroid-infiltrating cells, adhesion molecule expression and anti-thyroglobulin antibody production. Clin exp Immunol, 1993; 91:368-375.

35. Yamamoto K, Mori A, Nakahama T et al. Experimental treatment of autoimmune MRL-lpr/lpr mice with immunosuppressive compound FK-506. Immunology 1988; 69:222-7.

36. Takabayashi K, Koike T, Kurasawa K et al. Effect of FK-506, a novel immunosuppressive drug on murine systemic lupus erythematosus. Clin Immunol Immunopathol 1989; 51:110-7.

37. Hara S, Fukatsu A, Suzuki N et al. The effects of a new immunosuppressive agent, FK-506, on the glomerular injury in rats with accelerated nephrotoxic serum glomerulonephritis. Clin Immunol Immunopathol 1990; 57:351-62.

38. Okuba Y, Tsukada Y, Maezawa A et al. 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; 82:450-5.

39. Matsukawa W, Hara S, Yoshida F et al. Effects of a new immunosuppressive agent, FK-506, in rats with active Heymann nephritis. J Lab Clin Med 1992;

119:116-23.

40. Inamura N, Hashimoto M, Nakahara K et al. Immunosuppressive effect of FK-506 on experimental allergic encephalomyelitis in rats. Int J Immunopharmacol 1988; 10:991-5.

41. Hanawa H, Kodama M, Zhang S et al. An immunosuppressant compound, FK-506, prevents the progression of autoimmune myocarditis in rats. Clin Immunol Immunopathol 1992; 62:321-6.

42. Meingassner JG, Stutz A. Immunosuppressive macrolides of the type FK-506: a novel class of topical agents for treatment of skin diseases? J Invest Dermatol 1992; 98:851-5.

43. Hiraoka Y, Kishimoto C, Kurokawa M et al. The effects of FK 506, a novel and potent immunosuppressant, upon murine coxsackievirus B3 myocarditis. J Pharmacol Exp Therapeutics 1992; 260:1386-91.

44. 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; 23:3363-4.

45. Gladman DD. Indicators of disease activity, prognosis and treatment of systemic lupus erythematosus. Current Opinion in Rheumatology 1993; 5:587-95.

46. Hasan R, Van den Bogaerde JB, Wallwork J et al. Evidence that long-term survival of concordant xenografts is achieved by inhibition of antispecies antibody production. Transplantation 1992; 54:408-413.

47. Murase N, Starzl TE, Demetris AJ et al. Hamster-to-rat heart and liver xenotransplantation with FK-506 plus antiproliferative drugs. Transplantation 1993; 55:701-8.

48. Watanabe-Fukunaga R, Brannan CI, Copeland NG et al. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992; 356:314-7.

49. Andrews BS, Eisenberg RA, Theofilopoulos AN et al. Spontaneous murine-lupus like syndromes. Clinical and immunopathological manifestations in several strains. J Exp Med 1978; 148:1198-1215.

50. Murphy ED, Roths JB. New inbred strains. Mouse News Letter 1978; 58:51-4.

51. Theofilopoulos AN, Dixon FJ. 1981. Etiopathogenesis of murine SLE. Immunol Rev 1981; 55:179-216.

52. Woo J, Wright TM, Lemster B et al. Combined effects of FK 506 (tacrolimus) and cyclophosphamide on atypical B220⁺ T cells, cytokine gene expression and disease activity in MRL/MpJ-*lpr/lpr* mice. Clin Exp Immunol 1995; 100:118-125.