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T-Cell-Directed Immunointervention

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Chapter 6

FK 506: pharmacology and molecular action

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Historical perspective

During routine screening of the fermentation broths of soil fungi (*Streptomyces* spp.) for specific inhibitory effects on mouse mixed lymphocyte reactions (MLR), investigators of the Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, 1982–1983, identified a product of *Streptomyces tsukubaensis*, which exhibited powerful antilymphocytic and immunosuppressive activity (Goto *et al.*, 1987). The isolated product, designated FK 506, although classified as a macrolide antibiotic, exhibited no growth inhibitory effect on bacteria or yeast and showed only limited antifungal activity against *Aspergillus fumigatus* and *Fusarium oxysporum* (Kino *et al.*, 1987a). Early investigations revealed that FK 506 was very effective in suppressing immune responses both *in vivo* and *in vitro* and that the effective concentration was usually 10 to 100 times lower than that of cyclosporin A (CsA) (Goto *et al.*, 1987; Kino *et al.*, 1987a,b). An initial report on the capacity of FK 506 to prevent organ (heart) allograft rejection in rats was presented by T. Ochiai at the 11th International Congress of the Transplantation Society held in Helsinki in August, 1986 (Ochiai *et al.*, 1987). Since then, there has been rapid progress in elucidating the mode of action of FK 506 and in characterizing its immunosuppressive properties, including its effects on allograft survival in various animal models (reviewed by Thomson, 1989, 1990). This has led to the clinical evaluation of FK 506 in human organ (liver, kidney, heart, small bowel) transplantation in the University of Pittsburgh (Starzl *et al.*, 1989a,b, 1990; Todo *et al.*, 1990a,b; Armitage *et al.*, 1991; Shapiro *et al.*, 1991) and more recently, in 20 other centres in the United States, Europe and Japan. These centres are presently conducting prospective, randomized controlled trials of FK 506 in primary liver transplantation.

FK 506 shares many of the properties of CsA, a structurally unrelated fungal metabolite, but the former drug is considerably more potent. The two drugs share a very similar mode of action (Sawada *et al.*, 1987; Zeevi *et al.*, 1987; Tocci *et al.*, 1989) which inhibits the activation of CD4⁺T (helper) lymphocytes and the secretion of cytokines crucial to the induction and expression of immune reactivity. What follows is an overview of the pharmacology and molecular action of FK 506. The molecular action of FK 506 was recently reviewed by Schreiber (1991) and Schreiber and Crabtree (1992).

Physicochemical properties of FK 506

FK 506 is a white crystalline powder at room temperature and dissolves readily in non-polar solvents, such as methanol, ethanol and chloroform (Tanaka *et al.*, 1987) but it is insoluble in polar solvents. The molecular formula of FK 506, deduced by elemental analysis and mass spectrometry, is $C_{44}H_{69}NO_{12} \cdot H_2O$ (molecular weight 822) (Tanaka *et al.*, 1987). Infra-red spectral analysis has revealed the presence of hydroxy groups (3530/cm), carbonyl groups (1750, 1730, 1710/cm) and an amide group (1650/cm). Further detailed structural analysis by nuclear magnetic resonance has identified two ketones, one lactone, one hemiketal, three *O*-methyls and five *C*-methyls with the remainder being 12 methylenes and 13 methines. The structure of FK 506 is shown in Fig. 6.1. FK 506, a lactone with sugar substituents differs totally in structure from CsA, a cyclic peptide comprising 11 amino acids.

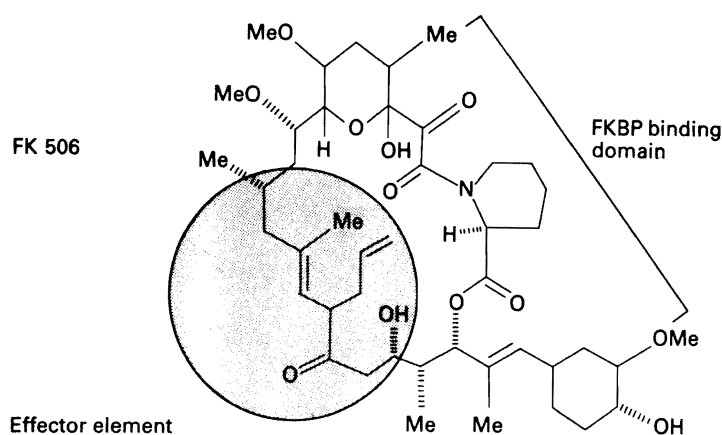


Fig. 6.1. The structure of FK 506 showing the immunophilin (FKBP) binding domain and the effector element. CsA is structurally dissimilar to FK 506.

FK 506 is relatively stable under normal laboratory conditions and has a melting point of 127–129°C. Maximal activity of FK 506 is retained after storage for 6 months at 40°C, 3 months at 82% relative humidity, 3 months under 500 Lux fluorescent light exposure and 24 months at room temperature (Tanaka *et al.*, 1987). Reports of its synthesis first appeared in 1989 (Harding *et al.*, 1989; Siekierka *et al.*, 1989).

Pharmacokinetics of FK 506 in animals and man

FK 506 is quantified in body fluids, following extraction of drug, by an enzyme-linked immunosorbant assay (ELISA), in which either a monoclonal or polyclonal antibody is employed. In blood, most of the FK 506 is bound to erythrocytes (mean trough blood:plasma ratio 10:1). The sensitivity limit is 20 pg/ml in plasma (Tamura *et al.*, 1987). A modified ELISA, using a solid phase extraction method and a mouse monoclonal anti-FK 506 antibody for quantitation of FK 506 in human plasma, has been described more recently (Cadoff *et al.*, 1990). The extent to which the antibody cross-reacts with FK 506 metabolites is unknown.

Monitoring of plasma FK 506 concentrations in the dog (Tamura *et al.*, 1987) has shown that, at immunosuppressive doses (1.0 mg/kg per os) the trough level lies between 0.08 and 0.4 ng/ml, while at non-immunosuppressive doses, a lower trough concentration is observed. This indicates that the effective, prophylactic plasma trough level is between 0.1 and 0.4 ng/ml (Todo *et al.*, 1988; Ochiai *et al.*, 1989). Coadministration of FK 506 with CsA can reduce the threshold effective FK 506 trough level (Ochiai *et al.*, 1989) and this may explain the synergy exhibited between these two drugs in experimental animals (Murase *et al.*, 1987; Todo *et al.*, 1987; Ochiai *et al.*, 1989) and reported earlier in *in vitro* models (Sawada *et al.*, 1987). In canine kidney recipients undergoing rejection, reduction in serum creatinine level and attenuation or disappearance of the cellular infiltrate during rejection was observed in FK 506-treated animals and was shown to correlate with an increase in FK 506 trough level (Ochiai *et al.*, 1989).

FK 506 is absorbed slowly after oral administration and distributed in various organs, including lung, spleen, heart and kidney (Venkataramanan *et al.*, 1990). Whilst absorption of CsA appears to be dependent on availability of bile in the gut, bile is unnecessary for FK 506 absorption (Jain *et al.*, 1990). The majority of FK 506 appears to be metabolized by *N*-demethylation and hydroxylation in the liver and is then excreted in bile, urine and faeces within 48 h of administration (Venkataramanan *et al.*, 1990, 1991). Activity of cytochrome P-450, the mixed function oxidase system of isoenzymes that metabolizes CsA, is downregulated by FK 506 both *in vivo* (Venkataramanan *et al.*, 1990) and *in vitro* (Burke *et al.*,

1990). This implies a possible mechanism whereby FK 506 affects the pharmacokinetics of CsA, which also depends on this degradation system.

Studies aimed at determining the optimal route of administration and dosage of FK 506 for clinical study were hampered by the severe toxic effects of the drug in dogs. Fortunately, the choice of method of drug delivery in the first clinical trial, i.e. 0.15 mg/kg intravenous (i.v.) over an hour soon after liver revascularization, followed by 0.075 mg/kg/12 h until the patient could eat, then oral doses of 0.15 mg/kg/12 h, was reasonably well tolerated (Todo *et al.*, 1990a). With i.v. therapy, continuous instead of 4-h infusions reduce the associated risks of transient renal and neurological dysfunctions, especially in high-risk patients (Abu-Elmagd *et al.*, 1991a,b). The i.v. doses used in man have required downward revision (Starzl *et al.*, 1991a; Abu-Elmagd *et al.*, 1991a,b). Peak plasma concentration is observed at the end of the infusion and then levels decline slowly over the next 24 h (Venkataramanan *et al.*, 1990, 1991). Plasma trough levels tend to be about 1 ng/ml, the effective immunosuppressive concentration *in vitro*. The half-life ranges from 3.5 to 40.5 h, with a mean of 8.7 h (Venkataramanan *et al.*, 1990). Drug absorption following oral administration is highly variable. The mean bioavailability is about 25% (range 6–57%). A peak plasma level of 0.4–3.7 ng/ml is reached after 1–4 h of an oral dose at 0.15 mg/kg (Venkataramanan *et al.*, 1990). The half-life of CsA is prolonged in patients receiving FK 506, from a normal 6–15 h to 26–74 h. This indicates that FK 506 may affect CsA metabolism, a phenomenon demonstrated recently *in vitro* (Burke *et al.*, 1990).

Experience gained from studies in CsA-treated liver transplant patients indicates that alterations in the absorption and metabolism of CsA occur with changing liver function (Grevel & Kahan, 1989). Since FK 506 and CsA share similar physical properties, the same issues might be expected to arise for FK 506. In five jaundiced patients with liver dysfunction, peak and trough FK 506 levels were higher than in patients with good hepatic function. Moreover, the half-life of FK 506 was increased and its clearance was reduced in patients with hepatic dysfunction (Jain *et al.*, 1990). As a result, the overall bioavailability of FK 506 was expected to increase because of the greatly reduced intrinsic clearance (Venkataramanan *et al.*, 1990, 1991).

The full impact of hepatic dysfunction on FK 506 pharmacokinetics was not appreciated until clinical trials were well established (Abu-Elmagd *et al.*, 1991a; Starzl *et al.*, 1991a). In liver transplant recipients, whose grafts do not function well initially and/or fail to recover quickly, the daily i.v. dose of 0.15 mg/kg quickly leads to enormously high trough plasma levels (> 100 ng/ml has been recorded), complete renal failure and neurotoxicity, which can progress to mutism, convulsions and coma. Prompt dose reduction is required and guidance for this is provided by rapid turn around time in the plasma assays. Dose control of the i.v. FK 506 is easier

if the drug is given by constant infusion instead of the 2-h bolus, which was originally used.

Even with well-functioning liver grafts, or in kidney and heart recipients whose hepatic function is normal, the University of Pittsburgh patients are now given a smaller i.v. dose of 0.075 or 0.10 mg/kg/day, instead of the 0.15 mg/kg originally employed. The larger doses cause unacceptable increases in plasma levels and can cause acute renal failure or neurotoxicity (Abu-Elmagd *et al.*, 1991a; Starzl *et al.*, 1991a). Failure to make these revisions constitutes an unnecessary risk. Plasma levels of 3–5 ng/ml can be accepted during the peri-operative period of constant drug infusion, if there is no evidence of toxicity, but otherwise the doses should be reduced to < 3 ng/ml.

A number of interactions of FK 506 with other drugs have been observed. Trough plasma concentrations of FK 506 are increased by co-administration of erythromycin, fluconazole and clotrimazole, whilst the effects of phenytoin, phenobarbital and acyclovir on FK 506 pharmacokinetics are currently being investigated.

Antilymphocytic activity of FK 506

The effects of FK 506 on T-cell responses, *in vitro* and *in vivo*, and the influence of the drug on gene expression and cytokine production are summarized in Table 6.1. Studies that are aimed at elucidating the immunosuppressive action of FK 506 have shown that the drug is very effective in suppressing both alloantigen- and T-cell mitogen-induced lymphocyte proliferation. The 50% inhibitory concentrations (IC_{50}) of FK 506 and CsA for human MLR are 0.21 nM and 20 nM respectively and for phytohaemagglutinin (PHA)-induced responses 8.6 nM and 750 nM respectively (Thomson, 1989; Yoshimura *et al.*, 1989a). Thus, compared with PHA-induced T-cell responses, those evoked by alloantigens are more FK 506-sensitive (Yoshimura *et al.*, 1989a; Woo *et al.*, 1990b; Zeevi *et al.*, 1990). In contrast, anti-CD28-induced responses are FK 506 insensitive (Kay & Benzie, 1990; Bierer *et al.*, 1991). Taken together, these observations reflect inherent differences between CD3- and CD28-activation pathways and their differential sensitivities to FK 506. Like CsA, FK 506 inhibits T-cell activation mediated not only by the T-cell receptor-CD3 complex, but also via another cell surface molecule, CD2 (Bierer *et al.*, 1991).

Delay in the addition of FK 506 to mitogen-stimulated cultures results in reduction of its antilymphocytic activity (Kay *et al.*, 1989), indicating a selective influence of FK 506 on early events in T-cell activation. The latter appear to include Ca^{2+} mobilization, protein kinase C (PKC) activation, cytokine gene transcription, cytokine secretion and cytokine-receptor expression, all of which occur within the first 2 h of T-

Table 6.1. Effects of FK 506 on T cells

Phenomenon	Effect	Reference
<i>Lymphocyte responses</i>		
<i>in vitro</i>		
MLR (human, mouse)	↓	Kino <i>et al.</i> , 1987b; Sawada <i>et al.</i> , 1987; Yoshimura <i>et al.</i> , 1989a
Con A, PHA-induced lymphocyte proliferation	↓	Sawada <i>et al.</i> , 1987; Yoshimura <i>et al.</i> , 1989a; Woo <i>et al.</i> , 1990b
Tc induction	↓	Sawada <i>et al.</i> , 1987; Yoshimura <i>et al.</i> , 1989a
Tc function	↓	Sawada <i>et al.</i> , 1987; Yoshimura <i>et al.</i> , 1989a
Ts induction	↓	Yoshimura <i>et al.</i> , 1989a
<i>Cell-mediated immunity</i>		
DTH (MBSA)	↓	Kino <i>et al.</i> , 1987a
Graft-versus-host disease	↓	Kino <i>et al.</i> , 1987a
Host-versus-graft popliteal lymph node assay	↓	Morris <i>et al.</i> , 1989
T-dependent humoral response:		
Alloantibody	↓	Propper <i>et al.</i> , 1990
Anti-SRBC Ab	↓	Woo <i>et al.</i> , 1990a
<i>Ex vivo</i> Con A-induced spleen cell proliferation	↓	Woo <i>et al.</i> , 1990c
<i>Lymphokine production</i>		
IL-1	?	
IL-2	↓	Kino <i>et al.</i> , 1987b; Sawada <i>et al.</i> , 1987
IL-3	↓	Kino <i>et al.</i> , 1987b
IL-4	?	
IL-5	?	
IL-6	NC	Yoshimura <i>et al.</i> , 1989b
INF γ	↓	Kino <i>et al.</i> , 1987b
GM-CSF	↓	Kino <i>et al.</i> , 1987b
<i>Lymphokine receptor expression</i>		
IL-2R	↓	Kino <i>et al.</i> , 1987b; Yoshimura <i>et al.</i> , 1989a,b; Woo <i>et al.</i> , 1990b

Table 6.1. *Continued*

Phenomenon	Effect	Reference
<i>MHC antigen expression</i>		
HLA-DR on activated T cells	↓	Woo <i>et al.</i> , 1990b
HLA-DR on monocytes	NC	Woo <i>et al.</i> , 1990b
<i>Gene expression</i>		
IL-1 α , β	NC	Tocci <i>et al.</i> , 1989
IL-2	↓	Tocci <i>et al.</i> , 1989
IL-3	↓	Tocci <i>et al.</i> , 1989
IL-4	↓	Tocci <i>et al.</i> , 1989
IL-10	NC	Wang <i>et al.</i> , 1991
GM-CSF	↓	Tocci <i>et al.</i> , 1989
TNF α	↓	Tocci <i>et al.</i> , 1989
IFN γ	↓	Tocci <i>et al.</i> , 1989
<i>c-myc</i>	↓	Tocci <i>et al.</i> , 1989
<i>c-fos</i>	NC	Tocci <i>et al.</i> , 1989
TfR	NC	Tocci <i>et al.</i> , 1989
IL-2R α -chain	NC	Tocci <i>et al.</i> , 1989
TNF β	NC	Tocci <i>et al.</i> , 1989
MHC class I HLA B-7	NC	Tocci <i>et al.</i> , 1989
FKBP	?	
TCR β -chain	↑	Kay <i>et al.</i> , 1989

Abbreviations: ↓, decrease; ↑, increase; NC, no change; ?, unclear; DTH, delayed-type hypersensitivity; TfR, transferrin receptor; FKBP, FK 506-binding protein; MLR, mixed lymphocyte reaction; SRBC, sheep red blood cells.

cell activation.

The generation of cytotoxic T lymphocytes (CTL) and suppressor T cells during human MLR is inhibited by FK 506 (Yoshimura *et al.*, 1989a), but the cytolytic activity of CTL against target cells during the effector phase is FK 506 resistant (Sawada *et al.*, 1987; Yoshimura *et al.*, 1989a). This indicates that FK 506 affects only the early induction phase of CTL development and has no effect on antigen recognition by CTL or on the cytolytic mechanism which results in target-cell destruction. Moreover, Zeevi *et al.* (1990) have reported that FK 506 does not affect human CTL differentiation or maturation from pre-effector to effector CTL. The recent finding that FK 506 has little or no effect on natural killer (NK) cell numbers or function or on antibody-dependent cytotoxicity (Markus *et al.*, 1991; Wasik *et al.*, 1991), suggests beneficial sparing of natural immunity against infectious agents.

Information concerning effects of FK 506 on processing and presentation of antigen by antigen-presenting cells (APC), is presently very limited.

Nevertheless, FK 506 concentrations which strongly inhibit antigen purified protein derivative (PPD)-induced human T-cell proliferation, have little effect on antigen processing and presentation by human blood monocytes (Woo *et al.*, 1990a). There is evidence, however, that FK 506 may impair alloantigen processing/presentation in human MLR (Thomas *et al.*, 1990). Cooper *et al.* (1991) observed that any effect of FK 506 on the presentation of microbial (*Listeria*) antigens by murine macrophages could be ascribed to drug carryover to the readout system (T-cell proliferation).

Experimental data concerning the inhibitory effect of FK 506 on mitogen- and alloantigen-induced T-cell responses in culture, indicate that FK 506, like CsA, exerts its primary influence on CD4⁺ (helper) T cells, with consequent effects on other cell types. Several studies have demonstrated that FK 506 inhibits the expression of the activation molecules interleukin (IL)-2R, MHC class II antigens and transferrin receptor (TfR), in a dose-dependent manner (Yoshimura *et al.*, 1989a; Woo *et al.*, 1990b). These effects, however, may be secondary to the potent inhibitory action of FK 506 on IL-2 production and to the reduction of its consequent, autocrine effect on the expression of IL-2R. Considered in conjunction with the reported failure of FK 506 to inhibit IL-2R mRNA expression (Tocci *et al.*, 1989), these observations suggest post-transcriptional inhibition of IL-2R expression by the drug.

Effects of FK 506 on early T-cell activation genes

FK 506 strongly inhibits expression of mRNA for early T-cell-activation genes, including those encoding IL-2, IL-3, IL-4, IFN γ , granulocyte-monocyte-colony-stimulating factor (GM-CSF) and *c-myc* (Tocci *et al.*, 1989; Dumont *et al.*, 1990b; Metcalfe & Richards, 1990). It is likely that the generation or transmission of a common activation signal for these genes is inhibited by FK 506, as prevention of gene expression by the drug is unaffected by the nature of the inducers used. On the other hand, recent studies have shown that FK 506 does not inhibit production of human IL-6 (Yoshimura *et al.*, 1989b) and may spare IL-10 (cytokine synthesis inhibitory factor) gene transcription by cloned murine helper T cells (T_{H2}) *in vitro*, whilst suppressing concomitant IL-4 mRNA production by these cells (Wang *et al.*, 1991). Thus, differential interference with cytokine gene expression may be an important mechanism whereby FK 506 and CsA inhibit immune-cell activation. Inhibition of gene expression by FK 506 is specific to early genes. No inhibition of constitutively expressed T-cell receptor (TCR)- β , MHC class I human leukocyte antigen (HLA)-B7, GPDH or late-phase genes, like IL-2R, TfR and tumour necrosis factor (TNF)- β (Kay *et al.*, 1989; Tocci *et al.*, 1989) is observed. Inhibition and superinduction, respectively, of *krox 20* and *krox 24* mRNA by FK 506 in

murine lymphocytes (Metcalf & Richards, 1990) may be of particular significance, since these genes encode proteins which are likely to regulate gene expression. In contrast to its influence on lymphokine gene expression and protein secretion, no inhibitory action of FK 506 on monokine gene expression (including IL-1 α or IL-1 β mRNA synthesis) or monokine production (TNF α and IL-6) after lipopolysaccharide (LPS) stimulation or human monocytes has been observed (Tocci *et al.*, 1989).

Experiments designed to ascertain the influence of FK 506 prior to gene transcription have shown that FK 506 does not affect Ca²⁺ mobilization (Bierer *et al.*, 1990), phosphatidylinositol turnover (Fujii *et al.*, 1989) or PKC activities (Gschwendt *et al.*, 1989) following binding of the antigen receptor. The target of FK 506 is thus probably a later event elicited by the T-cell antigen receptor (TCR) pathway and/or a separate activation pathway distinct from phosphoinositol breakdown.

Influence of FK 506 on gene transcription

The precise molecular events which occur in the cytosol during T-cell activation and lead eventually to gene activation within the nucleus are still unclear. Following cytoplasmic biochemical changes, one or more signals pass into the nucleus to influence the IL-2 gene, expression of which is regulated by a transcription initiation site (Durand *et al.*, 1987; Williams *et al.*, 1988). The nucleic acid sequence of the IL-2 enhancer (regulatory) region has been identified and several *cis*-acting transcriptional segments of this region that bind different nuclear factors have been investigated (Williams *et al.*, 1988; Muegge & Durum, 1990). Two sequences of this enhancer, - 285 to - 255 and - 93 to - 63 activate a linked promoter in response to signals generated from the antigen receptor (Durand *et al.*, 1987) (Fig. 6.2.). These two regions are bound by two distinct nuclear factors to protect them from DNase digestion (Durand *et al.*, 1987). One of these nuclear factors, NFIL-2A, which is present in both

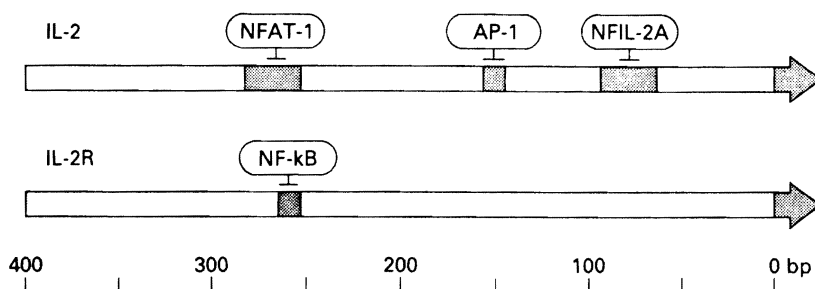


Fig. 6.2. IL-2 and IL-2R gene promoter sequences, showing the binding sites of nuclear regulatory proteins.

activated and non-activated T cells, binds to the regulatory site identified in the IL-2 enhancer between -93 and -63. This sequence is referred to as antigen receptor response element-1 (ARRE-1) (Fig. 6.2), since it can only be activated through the TCR/CD3 pathway and not by phorbol myristate acetate (PMA) which activates PKC. The IL-2 gene promoter is then activated by the sequence protected from DNase digestion, once an activation signal is elicited in the TCR. In contrast, the constitutive activity of the IL-2 promoter when the NFIL-2A binding is disrupted by mutation implies that NFIL-2A exerts a negative signal in resting T cells (Nabel *et al.*, 1988). Since NFIL-2A is present in both activated and non-activated cells, its presence and activity may not be influenced by immunosuppressive agents. A second antigen receptor response element (ARRE-2) present between -285 and -255 of the IL-2 enhancer is protected from DNase by the nuclear factor of activated T cells (NFAT) 1, which is only expressed in activated T cells (Shaw *et al.*, 1988). There is evidence that deletion of the NFAT binding site significantly impairs activity of the IL-2 enhancer (Durand *et al.*, 1987). Moreover, the increase in NFAT binding activity in nuclear extracts of activated T cells (Shaw *et al.*, 1988) and the appearance of NFAT about 10 min before the earliest detectable IL-2 mRNA implies that activation of the IL-2 gene is dependent on prior activation of NFAT. Cooperation between NFAT and NFIL-2A results in full enhancer activity, though each binds to a different sequence. Recent studies by Granelli-Piperno *et al.* (1990) have shown that the binding of NFAT to the transcriptional element is suppressed by FK 506, thereby inhibiting IL-2 gene activation and consequently, lymphokine production.

A third sequence, the AP-1 binding site, which is responsible for IL-2 transcription in response to PMA, is identified at position -240. AP-1 is the cellular homologue of the protein product of the *v-jun* oncogene (Ryder *et al.*, 1988). Binding of AP-1 to the AP-1 binding site makes a normally unresponsive promoter respond to agents that activate PKC. A functional AP-1 site in the IL-2 gene suggests a response to signals emanating from the TCR and to signals initiated by the activation of PKC. While the binding of AP-1 to its binding site has been shown to be markedly reduced by FK 506 in EL 4 lymphoma cells (Granelli-Piperno *et al.*, 1990), its transcriptional enhancing activity is not affected by FK 506 in human synovial fibroblasts.

Activation of the IL-2R gene is also essential for commitment of T lymphocytes to cell division and immunological functions. A binding site for the PMA-responsive transcriptional factor NF- κ B was localized between -255 and -268 of the IL-2R transcription initiation site. Granelli-Piperno *et al.* (1990) reported recently that FK 506 had only a slight inhibitory effect on NF- κ B binding, which is consistent with the minimal effect of FK 506 on IL-2R mRNA expression discussed earlier.

Role of FKBP in immunosuppression

A novel class of cytosolic proteins of 'immunophilins' has become the focus of attention with regard to the mechanism of action of FK 506 and CsA. Two families of immunophilins have been identified, namely the cyclophilins (predominant member cyclophilin A) and the FK 506-binding proteins (FKBPs) (predominant member FKBP 12) which are believed to play essential roles in the immunosuppressive actions of CsA and FK 506 respectively (Harding *et al.*, 1989; Siekierka *et al.*, 1989; Maki *et al.*, 1990). Although their physiological role is unknown, both proteins possess peptidyl-prolyl *cis-trans* isomerase (PPIase) activity, that catalyses the slow *cis-trans* isomerization of ala-pro bonds in oligopeptides and accelerates slow, rate-limiting steps in the folding of several proteins (Fischer *et al.*, 1989). The PPIase activity of cyclophilin and FKBP is strongly inhibited by their respective ligands (Fischer *et al.*, 1989; Siekierka *et al.*, 1989b), suggesting that PPIase activity may play a role in lymphocyte activation.

FK 506 binds with high affinity to FKBP (Harding *et al.*, 1989; Siekierka *et al.*, 1989), a heat-stable cytosolic component with a molecular weight (10–12 kD) lower than cyclophilin (17 737 D) (Siekierka *et al.*, 1989; Palaszynski *et al.*, 1991). No specific binding of FK 506 to purified calf thymus cyclophilin can be detected (Siekierka *et al.*, 1989) and anti-cyclophilin antibody does not cross-react with bovine or human FKBPs (Harding *et al.*, 1989). Cloning of FKBP (Standaert *et al.*, 1990; Tropschug *et al.*, 1990) has revealed that, despite their common enzymatic properties, FKBP and cyclophilin have dissimilar sequences.

The first 16 residues of human FKBP are identical to the corresponding bovine sequence (Harding *et al.*, 1989). cDNA of human lymphocyte FKBP has been synthesized and binds with mRNA species of 1.8 kb isolated from brain, lung, liver, placenta and leukocytes (Maki *et al.*, 1990; Standaert *et al.*, 1990), demonstrating the ubiquitous nature of this protein. The level of FKBP mRNA in (leukaemic) Jurkat T cells is, however, unaffected by cell activation stimulated through phorbol esters and ionomycin (Maki *et al.*, 1990). Inhibition of FKBP's PPIase activity is specific to the binding of FK 506 (or the structurally related macrolide rapamycin), while inhibition of cyclophilin's PPIase activity is specific to CsA (Siekierka *et al.*, 1989b; Dumont *et al.*, 1990a). 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 (Liu *et al.*, 1991). Both FK 506-FKBP and CsA-cyclophilin complexes have been shown to bind specifically to three polypeptides — calmodulin and the two subunits of calcineurin (a Ca^{2+} -activated, serine-threonine protein phosphatase). In each case, the interaction of the

immunophilin appears to be with calcineurin (Fig. 6.3). The drug-immunophilin complexes have been shown to block the Ca^{2+} -activated phosphatase activity of calcineurin (Liu *et al.*, 1991). Thus, calcineurin appears to be the target of the drug-immunophilin complexes.

A second, key observation in unravelling the molecular action of FK 506 is that the drug-immunophilin complexes block Ca^{2+} -dependent translocation of the pre-existing, cytoplasmic component of NFAT to the nucleus (Flanagan *et al.*, 1991). The nuclear component of NFAT is transcriptionally inactive in all cells other than activated T lymphocytes (see above) 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 NFAT which is required for its translocation to the nucleus (Fig. 6.4). In the absence of both nuclear and cytoplasmic components, binding of NFAT to DNA and transcriptional activation of the IL-2 gene is suppressed.

Conclusion

It is now clear that FK 506 and CsA are pro-drugs and that their pharmacological action, resulting in immunosuppression, is dependent on binding to the appropriate immunophilin (FKBP or cyclophilin, respectively). This results in modulation of the enzymic activity of the protein phosphatase calcineurin which, in turn, may impair translocation of the cytosolic component of the IL-2 gene transcription factor NFAT to the T-cell nucleus. Several key issues remain to be resolved, including the physiological role of calcineurin. Elucidation of these and related molecular events underlying T-cell activation will play an important role in the design of future clinical immunosuppressive agents.

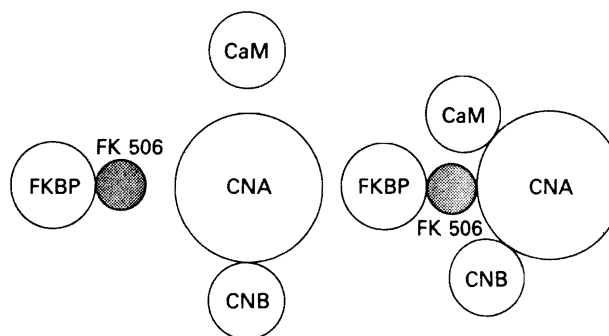


Fig. 6.3. Within the T lymphocyte, the FK 506-FKBP (FK 506 binding protein) complex binds with high affinity to calcineurin-calmodulin to form a pentameric complex which interferes with Ca^{2+} -dependent signalling pathways. Recent observations indicate that calcineurin (a protein phosphatase) is the target of the FK 506-FKBP complex. CaM, calmodulin; CNA, calcineurin A; CNB, calcineurin B.

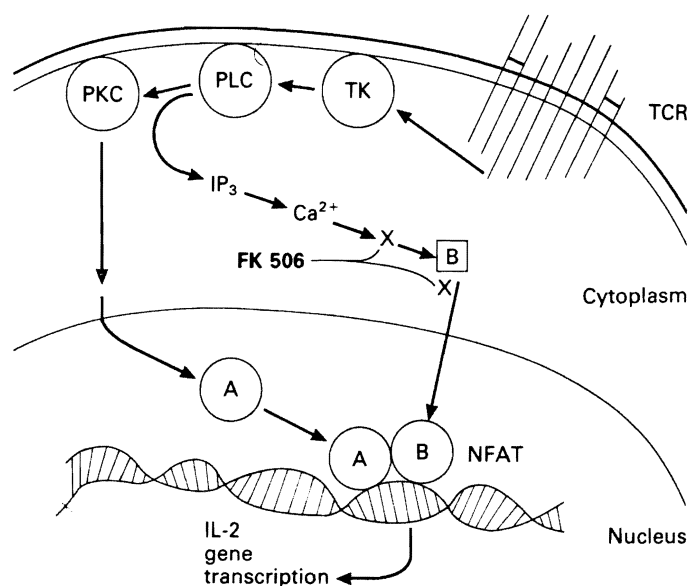


Fig. 6.4. Influence of FK 506 on signal transduction within T cells. FK 506 blocks translocation of the pre-existing cytoplasmic component of NFAT (B) to the nucleus by acting either on a Ca^{2+} signalling pathway or on translocation following the action of this pathway. Both components of NFAT are required for DNA binding and activation of the IL-2 gene. TCR, T-cell receptor; TK, tyrosine kinase; PLC, phospholipase C; PKC, protein kinase C; A, induced nuclear component of NFAT; B, existing cytoplasmic component of NFAT.

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