The Mechanism of Action of FK-506 and Cyclosporin A

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INTRODUCTION

The T lymphocyte plays a fundamental role in the process of organ transplant rejection as well as in the generation of autoimmune disease. Upon presentation with antigen by the antigen presenting cell (APC), the T cell undergoes a differentiation process that results in lymphokine secretion and its own proliferation.[1] The secreted lymphokines are responsible for a number of subsequent events, including conversion of B cells into antibody-secreting plasma cells, chemotaxis of macrophages and natural killer cells, and conversion of precytotoxic T lymphocytes (pre-CTLs) into CTLs, all of which contribute to the eventual destruction of the targeted tissue. In order to prevent tissue rejection, it is clear then why the prevention of T cell activation is an important pharmacological target.

Cyclosporin A (CsA) was introduced in 1983 as a therapeutic agent for renal allograft rejection and revolutionized the field of clinical transplantation due to the improved organ transplant survival rate.[2] Since that time, its use has expanded to cardiac, liver, heart-lung, and multiple organ transplants as well as to the treatment of certain autoimmune diseases such as psoriasis and insulin-dependent type I diabetes.[3,4] Use of CsA is limited by its side-effect profile, particularly its chronic nephrotoxicity, and so safer immunosuppressive agents have been sought. FK-506, a compound 10- to 100-fold more potent than CsA, began clinical testing in 1989 for the prevention of liver transplant rejection.[5,6] Surprisingly, both FK-506 and CsA have almost identical toxicities when used at equivalent therapeutic doses.

FK-506 AND CsA INHIBIT EARLY EVENTS IN T CELL ACTIVATION

FK-506 and CsA block early events in T lymphocyte activation. Both compounds exert their immunosuppressive effects downstream from the very early membrane-associated events, such as phosphoinositide turnover, that occur upon contact between the antigen presenting cell and the T cell receptor-CD3 complex.[7–9] These early membrane-associated events result in a rise in the levels of intracellular calcium and activation of the protein kinase C family, neither of which is affected by CsA or FK-506. Both drugs act proximally to transcription of a set of early lymphokine genes, IL-2, IL-3, IL-4, TNF-α, GM-CSF, and IFN-γ, which are expressed within two to four hours following the initiation of T cell activation.[10] Administration of either drug after expression of these early lymphokine mRNAs has no effect upon the T

cell.[9] The inhibitory effects of both drugs are IL-2 reversible because addition of IL-2 to drug-treated cells rescues the cells from their antiproliferative effects. In addition to their effects upon T cells, FK-506 and CsA block mast cell, neutrophil, basophil, and cytotoxic T lymphocyte degranulation—events that do not require active transcription—at a potency comparable to that observed for inhibition of T cell activation.[11,12] A stringent requirement for drug sensitivity is that the T cell activation or exocytosis pathway utilized by the cell involve an increase in intracellular calcium levels.[13] Pathways not involving a rise in intracellular calcium concentration, such as T cell activation via the CD28 pathway or C5a-induced neutrophil degranulation, are not sensitive to the effects of either FK-506 or CsA.[14–16] These results demonstrate that FK-506 and CsA do not inhibit specific transcriptional or exocytosis events. Rather, the two drugs block a calcium-dependent signal transduction event common to certain calcium-dependent pathways.

To understand the biochemical mechanism of action of FK-506 and CsA, two directions of research have been fruitful—forward from the identification of the intracellular receptors for these drugs, and backward from the inhibition of expression of IL-2.

THE MAJOR INTRACELLULAR RECEPTORS FOR FK-506 AND CsA

Based upon the clear similarities in the mechanisms of action of FK-506 and CsA, it was not surprising when it was found that the intracellular receptors for these chemically dissimilar drugs share at least four interesting properties. First, both of the major receptors are small cytosolic proteins. The molecular weight of the major receptor for FK-506 is 12 kilodaltons (kDa) and is termed FKBP12.[17,18] The major receptor for CsA is a 17-kDa protein and is named CypA.[19] Second, CypA and FKBP12 are among the most abundant proteins within the cell, comprising between 0.2% and 0.4% of the total cytosolic protein. Third, both receptors appear to be ubiquitous and are extraordinarily well conserved throughout phylogeny.[20,21] Not only have they been found in nearly every cell type and tissue examined, but the amino acid sequences within each receptor family are highly similar in organisms ranging from yeast to man.

The fourth, and perhaps the most interesting, characteristic shared by the two receptors is that they share an enzymatic activity. Both proteins catalyze the *cis*-to-*trans* isomerization of peptidyl-prolyl bonds and are termed peptidyl-prolyl-isomerases (PPIases).[17,22,23] CypA has little preference for the amino acid preceding the proline residue, while the substrate preferences for FKBP12 can vary over three orders of magnitude with Leu-Pro substrates being preferred.[24] CsA and FK-506 are potent inhibitors of their cognate receptors' PPIase activity. Initially, it seemed possible that inhibition of PPIase activity would be relevant to the immunosuppressive activity of the drugs. However, FK-506 and CsA analogues were soon discovered that were potent PPIase inhibitors but that had no inhibitory effect upon IL-2 transcription. One such compound is rapamycin, which is chemically related to FK-506, but which antagonizes the ability of FK-506 to block IL-2 transcription.[25,26] Rapamycin is at least equipotent to FK-506 in its ability to inhibit the PPIase activity of FKBP12. Another compound is the MeAla[6] derivative of CsA, which has no immunosuppressive activity but which is 70% as active as CsA at inhibiting the PPIase activity of CypA.[27] These observations demonstrate that inhibition of PPIase activity is not sufficient to block T cell activation.

The great abundance of FKBP12 and CypA within cells as well as their high degree of conservation throughout phylogeny suggest that these proteins would have

some fundamental and critical role in cellular physiology. Their PPIase activity led to the suggestion that these proteins might be "foldases," required for proper protein folding within the cell. However, disruption of the genes encoding CypA and FKBP12 in *Saccharomyces cerevisiae* demonstrated that, in yeast, neither gene is essential for viability.[28,29] The precise roles for the CypA and FKBP12 proteins within the cell are presently an area of active investigation. FKBP12 is specifically associated with the ryanodine receptor, a calcium channel in the sarcoplasmic reticulum of smooth muscle cells involved in excitation-contraction coupling.[30] The role of FKBP12 in mediating calcium flux through the channel is unknown at present. It is possible that the PPIase activity of FKBP12 functions to help open and close the channel.

The absence of any correlation between inhibition of PPIase activity and immunosuppression showed that loss of function of FKBP12 or CypA was not the underlying mechanism of immunosuppression mediated by FK-506 or CsA, respectively. Further evidence against the loss-of-function model came from cell-loading experiments which indicated that only a fraction of the intracellular pool of FKBP12 or CypA is bound by either of the drugs at immunosuppressive doses. Alternatively, a gain-of-function model suggested that FK-506 and CsA were not active by themselves but were, in fact, prodrugs that became active upon binding to their cognate receptors. This model would help to explain the observations listed above. Lending further support to the gain-of-function model were the observations that CsA and FK-506 undergo radical changes when bound by their receptors and exist in conformations not observed when they are free in solution. The resulting drug-receptor complex is a novel chemical entity that might then be the active immunosuppressive drug.

CALCINEURIN IS A SIGNALING COMPONENT OF IL-2 EXPRESSION

The hunt for targets of the FKBP/FK-506 and the Cyp/CsA drug-receptor complexes led to the isolation of the calcium- and calmodulin-dependent serine-threonine phosphatase calcineurin (CaN), on FKBP12/FK-506 and CypA/CsA affinity columns.[31,32] Calcineurin is a heterotrimer of a 59-kDa catalytic subunit (CaNα), a 19-kDa regulatory subunit (CaNβ), and the 15-kDa calmodulin (CaM).[33] A strong correlation exists between the immunosuppressive activity of the FK-506 or CsA analogues and the ability of the receptor-analogue complex to bind calcineurin *in vitro* and to inhibit its phosphatase activity against an artificial substrate *in vitro*.[25] For example, rapamycin, which does not inhibit IL-2 synthesis, does not bind calcineurin in the presence of FKBP. Likewise, the nonimmunosuppressive CsA analogue MeAla^6-CsA binds to cyclophilin with an affinity similar to that of CsA, but the Cyp/MeAla^6-CsA complex does not inhibit CaN phosphatase activity. Careful characterization of the requirements for FKBP/FK-506/CaN complex formation using a high-performance liquid chromatography (HPLC) gel filtration assay demonstrated an absolute requirement for the presence of calmodulin.[34] A requirement for the presence of the CaNβ regulatory subunit was demonstrated in experiments measuring CypA/CsA-mediated inhibition of calcineurin phosphatase activity.[35]

The calcineurin catalytic subunit consists of a catalytic domain in the N-terminal half of the protein, followed by two allosteric control regions in the C-terminal third of the protein, a calmodulin-binding domain and an autoinhibitory domain. It has been proposed that, *in vivo,* calmodulin releases the autoinhibitory domain from the catalytic site of calcineurin, allowing the FKBP/FK-506 complex to bind. This hypothesis would be consistent with the observation that a 43-kDa proteolytic

fragment of CaN, which lacks the calmodulin and autoinhibitory domains, is constitutively active and calcium and calmodulin independent.[36,37] The proteolytic subunit is bound by drug-receptor complexes even in the absence of calmodulin. The PPIase activity of FKBP12 has no role in calcineurin complex formation. This was demonstrated using a PPIase-deficient FKBP12 mutant, F37Y, which has FK-506 binding activity but only 0.01% of the wild-type PPIase activity.[34] Titration of wild-type and F37Y proteins into incubation mixtures that contained FK-506 and calcineurin demonstrated that both proteins have equivalent IC_{50}s for inhibiting phosphatase activity.

Some of the first experiments confirming calcineurin as a relevant *in vivo* target for FK-506 and CsA were performed by measuring calcineurin activity in extracts prepared from T cells cultured in the presence of the drugs. By treatment of the extracts with okadaic acid, a potent inhibitor of protein phosphatases 1 and 2A, it was shown that calcineurin activity decreased in a dose-dependent manner when the cells had been incubated with FK-506 or CsA.[38] IL-2 production by the drug-treated cells correlated with the decrease in calcineurin phosphatase activity. Rapamycin had no effect upon calcineurin activity and reversed the effects of FK-506, evidence that it is an FK-506/receptor complex that is the actual inhibitory molecule. That FKBP12 is the receptor relevant to FK-506's effects is suggested by experiments performed in mast cell lines deficient in FKBP12. Treatment of these FKBP12-deficient cells with FK-506 failed to inhibit calcineurin phosphatase activity or lymphokine secretion.[39]

Validation of calcineurin as a physiological component of the TCR signal transduction pathway was confirmed in transfection experiments using a construct, named ΔCaM-AI, encoding the 43-kDa calcium- and calmodulin-independent form of CaN described above and Jurkat cells, a human T-lymphoma cell line.[40] Jurkat cells can be activated to produce IL-2 in response to the mitogens ionomycin and PMA which provide the stimuli needed to increase the intracellular calcium levels and activate the protein kinase C family, respectively. The Jurkat cells were cotransfected with a reporter gene that measured IL-2 promoter activity. In cells transfected with wild-type calcineurin, there was no IL-2 promoter activity when the cells were stimulated with either PMA or ionomycin alone. In cells transfected with ΔCaM-AI, there was no activity of the IL-2 promoter in response to ionomycin alone. However, treatment of the ΔCaM-AI transfected cells with PMA alone bypassed the calcium requirement and resulted in a >150-fold induction of promoter activity that was sensitive to FK-506 and CsA. Thus, ΔCaM-AI acted in synergy with PMA to render the cells independent of calcium signaling. This provided the first *in vivo* demonstration that calcineurin is a component of calcium-dependent transcriptional activation of the IL-2 gene via the TCR signal transduction pathway.

Further corroboration of calcineurin as the drug-sensitive target came from drug titration experiments with CsA and FK-506.[40] Transfection of Jurkat cells containing an IL-2 reporter construct with either wild-type calcineurin or ΔCaM-AI increased the IC_{50} of FK-506 and CsA between four- and sixfold. Rapamycin reversed the inhibitory effects of FK-506. These results confirmed that calcineurin is the FK-506- and CsA-sensitive component of the TCR signal transduction pathway.

THE HUMAN IL-2 PROMOTER

As a mediator of the calcium signaling emanating from the T cell receptor, calcineurin probably dephosphorylates one or more substrates that are either directly or indirectly involved in transcribing the IL-2 gene, as well as other early

lymphokine genes. One strategy aimed at identifying the substrates for calcineurin and, hence, identifying novel therapeutic targets for immunosuppression has been to start with the IL-2 promoter and work backward. The human IL-2 promoter has been extensively characterized, and the region required for maximal IL-2 expression extends approximately 300 base pairs (bp) upstream from the transcription initiation site.[41] Deletion and mutational analysis has identified five enhancer sites that have been labeled NFIL-2A through NFIL-2E. These enhancers contain binding sites for several transcription factors. Some of these transcription factors such as octamer/octamer associated protein (Oct/OAP), NF-κB, and AP-1 are relatively well characterized proteins that are required for transcribing genes in other cell types. Other enhancer elements on the IL-2 promoter bind factors that appear to be unique to T cells such as nuclear factor of activated T cells (NF-AT) and a CD28-responsive factor.

To understand the role that each enhancer element plays in the FK-506 and CsA sensitivity of the IL-2 promoter as well as to understand which enhancer elements are responding to signals from calcineurin, each of the enhancer elements has been multimerized, placed upstream of a basal promoter element (containing the IL-2 TATA box), fused to a chloramphenicol acetyl transferase (CAT) reporter gene, and the constructs transfected into Jurkat cells.[42] The IL-2A (Oct/OAP), IL-2E (NF-AT), and NF-κB multimerized elements yield maximal levels of CAT expression when the cells are costimulated with ionomycin and PMA. In all three cases, that activity is inhibited by FK-506 and CsA, indicating the involvement of calcineurin. Further evidence of calcineurin's role in the transcription driven by these factors is that when the cells are cotransfected with ΔCaM-AI, the requirement for ionomycin treatment is bypassed.[42] Interestingly, ionomycin/PMA cotreatment of ΔCaM-AI transfected cells results in hyperactivation of transcription driven by the IL-2A and IL-2E elements, suggesting that additional calcium-dependent signaling pathways may be required for maximal induction through these elements. The ΔCaM-AI/PMA-induced transcriptional activity exhibited by these reporter constructs is, in all cases, inhibited by FK-506 and CsA.

In contrast to the observations made with the IL-2A, IL-2E, and NF-κB elements, transcription driven by multimers of the AP-1 element is maximal when the cells are treated with PMA alone.[42] Ionomycin/PMA cotreatment actually results in less CAT expression driven by the AP-1 elements. Pretreatment of PMA/ionomycin-stimulated cells with FK-506 results in more AP-1-dependent CAT expression. This result suggests that inhibition of calcineurin appears to enhance transcription through the AP-1 element.

The transcriptional results, taken together, make it clear that calcineurin activates the IL-2 promoter via at least three different transcription factors (NF-AT, NF-κB, and Oct/OAP) and that multiple calcium-sensitive signals regulate IL-2 promoter activity. One model for calcineurin's action in the T cell is that it dephosphorylates one or more transcription factors in the cytoplasm that can then be translocated to the nucleus to activate the IL-2 promoter. Precedent for this model is found in *Saccharomyces cerevisiae* where the transcription factor SWI5 is dephosphorylated in the cytoplasm and is then translocated to the nucleus.[43] It has been proposed that, in the T cell, calcineurin dephosphorylates a constitutive cytosolic component of the NF-AT complex ($NF\text{-}AT_C$) which is then translocated to the nucleus, combines with a nuclear NF-AT component of the complex ($NF\text{-}AT_N$), and the resulting $NF\text{-}AT_C/NF\text{-}AT_N$ heterodimer binds to its regulatory site on the IL-2 promoter contributing to IL-2 expression.[44] Experimental support for this hypothesis has recently been reported.[45] The FK-506 sensitivity of the NF-κB site is particularly intriguing as it participates in the induction of numerous promoters in a variety of

cell types and might therefore be associated with some of the toxic effects of FK-506 and CsA. In addition to its effects on these transcription factors, calcineurin almost certainly dephosphorylates other, as yet unidentified, substrates in the T cell and in other cells that are not involved in IL-2 expression. For instance, inhibition of FK-506- and CsA-sensitive exocytosis pathways does not require new transcription. Therefore, the substrates for calcineurin in granulocytic cells are almost certainly different from those in T cells. Other calcineurin substrates relevant to the neuro- and nephrotoxic side effects of FK-506 and CsA will be discussed in a later section.

THE HUMAN FKBP, CYCLOPHILIN, AND CALCINEURIN GENE FAMILIES

The binding activity for FK-506 was first identified in the cytosol of Jurkat cells.[46] Later, a 12-kDa binding activity, FKBP12, was purified to homogeneity by two groups.[17,18] In addition to FKBP12, three more human FKBPs have been identified and are named, according to their mass, FKBP13, FKBP25, and FKBP52.[46–53] We have cloned, expressed in *Escherichia coli,* purified, and characterized each of these

TABLE 1. Characterization of Human FK-506 Binding Proteins[a]

FKBP	Binds FK-506	PPIase Activity Inhibited by FK-506	Drug-FKBP Inhibits Calcineurin
12	+	+	+
13	+	+	–
25	+	+	–
52	+	+	–

[a]Known human FK-506 binding proteins are listed in order of increasing molecular weight in kilodaltons. Binding of FK-506, inhibition of PPIase activity by FK-506, and inhibition of bovine calcineurin phosphatase activity by the FKBP/FK-506 complex are indicated by pluses and minuses.

proteins. Northern analysis has demonstrated that all three are expressed at the message level in Jurkat cells. All of them bind FK-506, and all have PPIase activity that is antagonized by FK-506 and rapamycin. The FKBP12/FK-506 complex is, by far, the most potent at inhibition of bovine calcineurin phosphatase (TABLE 1). At our present level of understanding, FKBP12 is probably the relevant FKBP mediating the immunosuppressive effects of FK-506. However, a formal demonstration of this awaits gene knockout experiments in mice.

The fact that all of the FKBPs are PPIases is the only clue that we have concerning their *in vivo* function. Given their PPIase activity, it is not surprising that two of the FKBPs, FKBP12 and FKBP52, have been found associated with other proteins within the cell. The association of FKBP12 with the ryanodine receptor has already been discussed. Cloning of the cDNA encoding rabbit FKBP52 revealed that it contains three FKBP homologous regions, the most conserved of which is contained N-terminally and which is probably most responsible for its FK-506 binding activity.[54] FKBP52 binds to hsp90 alone as well as when hsp90 is complexed with untransformed steroid receptors.[55] The amino acid sequence of FKBP52 contains consensus elements indicating binding to calmodulin, and FKBP52 has been purified using calmodulin affinity columns.[56] FKBP52's relevance to steroid receptor function as well as FK-506's effects upon steroid function is, as yet, undetermined.

Binding of FKBP13 or FKBP25 to other proteins has not yet been reported, although the two proteins have been characterized to some extent. FKBP13 contains a signal sequence and an endoplasmic reticulum retention sequence.[47] The processed protein is associated with heavy membrane fractions. Interestingly, while FKBP12 is the most abundant FKBP in most cells and tissues, FKBP13 is more abundant than FKBP12 in rat basophilic leukemia cells.[12] However, FKBP13 has not been implicated in the drug sensitivity of serotonin release in these cells. FKBP25 is homologous to FKBP12 in the C-terminal half of the protein.[49,52] The N-terminal half of the protein is unrelated to any protein in the current databases. FKBP25 binds rapamycin with much greater avidity than it binds FK-506. Cloning and sequencing of the FKBP25 cDNA as well as sequencing of the protein have revealed that it has five nuclear localization sequences in the N-terminal half of the protein and one in the C-terminal, FKBP12 homologous, half. Nuclear localization of FKBP25 has recently been confirmed.[57] The *in vivo* function of FKBP25 or whether it mediates the effects of rapamycin on IL-2 dependent T cell proliferation is not known.

Like the FKBPs, the cyclophilins comprise an ever-expanding gene family. Southern analysis of human genomic DNA suggests the presence of at least 20 related genes or pseudogenes.[58] Thus far, in addition to the 165 amino acid major human cytosolic cyclophilin, CypA, three other human cyclophilins have been purified or their cDNAs cloned. CypB, also called Cyp2 or S-cyclophilin, is a 208 amino acid protein having a 32 amino acid hydrophobic leader sequence, an endoplasmic reticulum retention sequence, and has been detected in human milk.[59–61] Cyp3 is a 207 amino acid protein having a 42 amino acid hydrophobic leader sequence.[59] Subcellularly, CypB and Cyp3 are associated with membrane fractions. NK-TR (natural killer-tumor recognition molecule) is a 1403 amino acid protein with a cyclophilin like domain at its *N*-terminus.[62] Like FKBP12, CypA appears to have a ubiquitous tissue and cell distribution. By northern analysis, CypB and Cyp3 appear to have a more restricted tissue distribution relative to CypA, although all three have message expressed in T lymphocytes. However, the message levels of CypB and Cyp3 are 10 to 100 fold lower, respectively, than the message level of CypA in a number of tissues and cell types. CypA, CypB, and Cyp3 all have PPIase activity that is inhibitable by CsA. CypA and CypB have been examined for their ability, in the presence of CsA, to inhibit CaN phosphatase activity. Both CypA/CsA and CypB/CsA drug complexes inhibit CaN phosphatase activity with the CypB/CsA complex being the more potent inhibitor.[35] NK-TR is found only on the surface of natural killer cells. As yet, there have been no reports measuring NK-TR's affinity for CsA or whether it has PPIase activity. In addition to these human cyclophilins, a 40-kDa cyclophilin-related protein that will almost certainly have a human counterpart has been purified from bovine brain and characterized.[63] Cyp40 appears to be ubiquitously expressed, binds CsA, and has PPIase activity that is inhibitable by CsA.

Based upon isoelectric focusing experiments, six isoforms of the catalytic subunit of calcineurin can be discerned in extracts prepared from brain.[64,65] To date, the accumulated data indicate that there are at least three genes encoding the catalytic subunit of mammalian calcineurin.[66–73] These genes, encoding the type 1, type 2, and type 3 isoforms, can undergo alternative splicing events to give additional variants (type 1δ, type 2δ, and type 3δ) in which 10 amino acids in the resulting protein are deleted between the calmodulin binding and autoinhibitory domains. All of the calcineurin catalytic subunits are extremely well conserved. Message for the type 1, type 2, and type 3 variants has been detected in T lymphocytes.[74] Activation of the IL-2 promoter by PMA and either the type 1, type 2, and type 3 isotypes is inhibited by FK-506 suggesting that, in contrast to the FKBPs, at our present level of understanding, there is little to distinguish the calcineurins from one another.[75]

TOXICITY

Although FK-506 appears to have some benefits over CsA, both drugs have significant toxic side effects. Both compounds show acute neurological toxicity, which improves over time, and both drugs exhibit chronic nephrotoxicity. The similar toxicity profiles indicate that toxicity is mechanistically linked to the similar biochemical effects exerted by the two drugs. Support for mechanism-based toxicity came from an analysis of CsA analogues demonstrating that more immunosuppressive compounds had greater nephrotoxic potential.[27] Further support for mechanism-linked toxicity came from an analysis of the effects of L-685,818, an FK-506 antagonist. L-685,818 and FK-506 bind with equal affinity to FKBP12. However, the FKBP12/L-685,818 complex is a much poorer inhibitor of CaN phosphatase activity and, in the cell, it reverses the effects of FK-506. Unlike FK-506, L-685,818 exhibits no toxicity and can, in fact, antagonize the toxic effects of FK-506.[25] These data indicate that binding of FK-506 or CsA by their cognate receptors is not responsible for toxicity but that inhibition of calcineurin by the drug-receptor complex is. As discussed earlier, inhibition of calcineurin-mediated dephosphorylation of substrates in cells other than T cells may be deleterious to the organism. These toxic side effects may be the consequence of perturbations in transcriptional regulation, inhibition of other cellular signaling pathways in which CaN plays a role, or both. Potential mediators of toxicity include calcineurin-regulated Ca^{2+} channels in the brain and the membrane Na^+/K^+ pump in renal tubule cells, which is also regulated by calcineurin and which is sensitive to FK-506 and CsA.[76–78] Long-term disruption of sodium and potassium homeostasis in tubule cells might be responsible for the problems in renal clearance that occur during CsA and FK-506 therapy. While the FKBPs and cyclophilins may not be relevant to toxicity from a mechanistic perspective, it is possible that these receptors act as potential drug sinks in cells and in tissues and that the ability of a particular analogue to penetrate a particular organ may be related to the FKBP or cyclophilin concentration within the cells of that organ.

CONCLUSION

The efforts to understand the mechanistic basis for the immunosuppressive effects of FK-506 and CsA have led to a partial dissection of the signal transduction pathway between the T cell receptor and expression of IL-2. Calcineurin has been identified as a component of the pathway that is activated in response to increases in intracellular calcium. However, the calcineurin substrates relevant to activation of IL-2 synthesis remain to be identified. Identification of the calcineurin substrates and an understanding of the FK-506 and CsA receptors may yield safer and more beneficial immunosuppressive drugs.

SUMMARY

FK-506 and cyclosporin A (CsA) are potent immunosuppressive agents used clinically to prevent tissue rejection. Interest in the development of more effective immunosuppressive drugs has led to an intense effort toward understanding their biochemical mechanism of action with the result that these compounds have now become powerful tools used in deciphering the signal transduction events in T lymphocyte activation. Although chemically unrelated, FK-506 and CsA exert nearly

identical biological effects in cells by inhibiting the same subset of early calcium-associated events involved in lymphokine expression, apoptosis, and degranulation. FK-506 binds to a family of intracellular receptors termed the FK-506 binding proteins (FKBPs). CsA binds to another family of intracellular receptors, the cyclophilins (Cyps), distinct from the FKBPs. The similarities between the mechanisms of action of CsA and FK-506 converge upon the calcium- and calmodulin-dependent serine-threonine protein phosphatase calcineurin (CaN). Both the FKBP/FK-506 complex and the Cyp/CsA complex can bind to calcineurin, thereby inhibiting its phosphatase activity. Calcineurin, a component of the signal transduction pathway resulting in IL-2 expression, catalyzes critical dephosphorylation events required for early lymphokine gene transcription.

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