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Clinical and mechanistic differences between FK506 (tacrolimus) and cyclosporin A

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Introduction

Cyclosporin A (CsA) and FK506 (tacrolimus) are potent immunosuppressive drugs widely used in reducing the incidence and severity of allograft rejection after organ transplantation. Owing to their capacity to antagonize calcineurin activity and hence calcium-dependent T-cell activation [1], both drugs were thought to have identical cellular and molecular effects, despite differences in their structure [2]. This notion was challenged by both clinical and experimental evidence clearly showing that CsA and FK506 immunosuppressive effects are not identical, and that FK506 affects CsA-sensitive and CsA-insensitive T-cell activation pathways. Here we discuss recent findings, including our own, which highlight differences in efficacy and mechanisms between CsA and FK506.

Overview of T-cell activation

T-cell activation is a highly co-ordinated process which requires binding of the T-cell receptor (TcR)-CD3 complex to antigen:MHC class II molecules expressed on the surface of antigen-presenting cells [3], and the provision of cell-bound and secreted co-stimulatory molecules which, while not imparting antigenic specificity, significantly augment T-cell activation. As a result, several signal transduction pathways become operational, leading to induction of cytokine gene expression and stimulation of cellular activation (Figure 1).

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Whereas initial events of T-cell activation are calcium-dependent, later events, in particular those associated with interaction of cytokines with their high-affinity receptors, are less dependent on calcium [4]. Interruption of any of the events of T-cell activation by the immunosuppressive drugs CsA, FK506, glucocorticoids, and rapamycin (Sirolimus) results in downstream inhibition of cytokine expression and T-cell proliferation [2,5].

Pharmacology of FK506 and CsA

Although structurally not related, CsA and FK506 belong to the family of immunophilin-binding drugs, each drug binding to its respective cytosolic immunophilin: CsA to cyclophilin and FK506 to FKBP respectively [6]. The drug-immunophilin complex in turn binds to the calcium/calmodulin-dependent phosphatase, calcineurin [7,8], leading to inhibition of the activation and nuclear translocation of nuclear factor of activated T cells (NF-AT), and hence IL-2 gene transcription [1]. Collectively, this suggested that (i) the calcineurin–NF-AT pathway is required for proper transcription of IL-2 and other cytokine genes, and (ii) that inhibition of NF-AT-supported transcription may be required for suppression of early proximal (and calcium-dependent) events of T-cell activation. More recently, inhibition of calcineurin activation by CsA and FK506 reportedly antagonizes the interaction of another transcription factor, cAMP response element-binding protein (CREB) with its putative DNA binding site, CRE, which in turn inhibited cAMP-directed transcriptional events [9]. Rapamycin, structurally related to FK506, binds to the same cytosolic

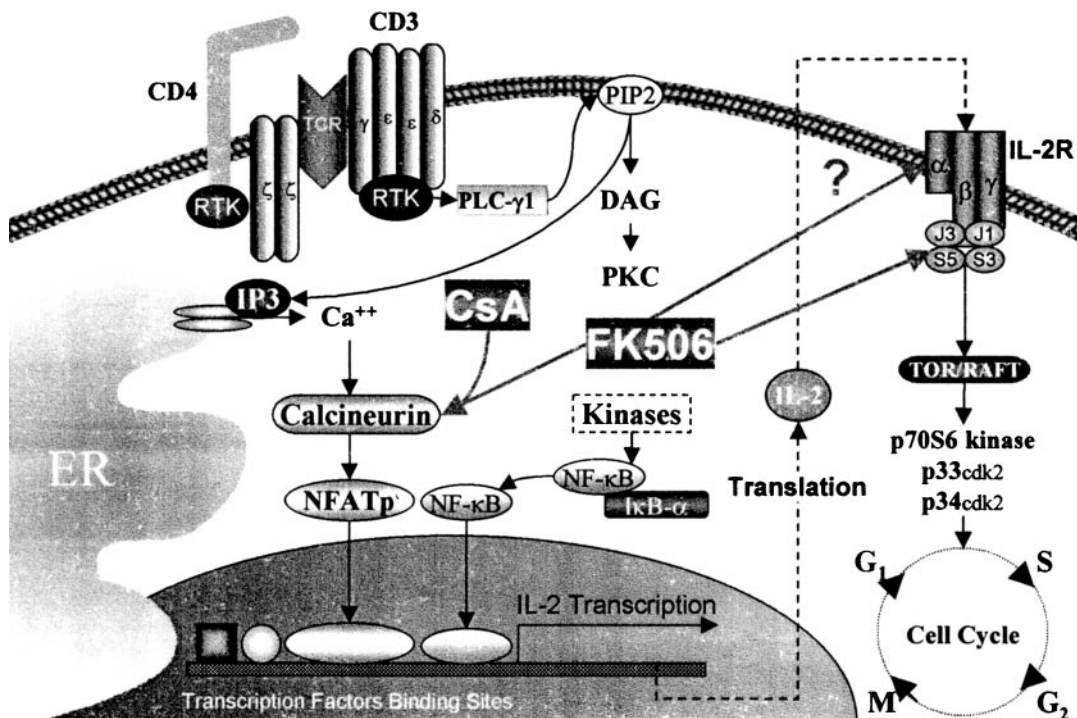


Fig. 1. Site of action of cyclosporin A and FK506. Antigen-specific T-cell activation pathways, including activation of calcineurin and expression of cytokines and their high-affinity receptors. CsA and FK506 antagonize calcineurin activation, leading to inhibition of NF-AT-supported IL-2 gene expression. FK506, but not CsA, acts more distally by attenuating the response to cytokine stimulation, possibly by altering the expression of their high-affinity receptors and/or through antagonizing a key step in cytokine receptor signalling.

receptor (FKBP) [5,6], but acts by inhibiting cytokine-mediated signalling through antagonism of cyclin-dependent kinases, without any appreciable effect on cytokine production [2,5].

CsA vs FK506 immunosuppressive effects

Like CsA but unlike rapamycin, FK506 inhibits cytokine production principally by antagonizing calcineurin [2], hence by attenuating activation and nuclear translocation of NF-AT. It had therefore been anticipated that the immunosuppressive effects of CsA and FK506 are almost identical, and mediated *via* antagonism of calcineurin activity. Recent experimental results [10; Almawi *et al.*, unpublished findings], and clinical observations [11,12], clearly showed, however, that the effects of FK506 and CsA are not identical, and that FK506-induced immunosuppression is not exclusively due to interruption of the NF-AT pathway [13]. The drug apparently acts on other pathways of T-cell activation as well, including blockade of cytokine receptor expression and cytokine effects on target cells [14].

Differences between FK506 and CsA immunosuppressive effects were highlighted by the finding that FK506, but not CsA, inhibits (i) IL-2 induced IL-5 production by human CD4⁺ T cells [14], and (ii) T-cell proliferation stimulated by IL-2 and IL-7 [Almawi *et al.*, unpublished findings]. CsA, while sharing with FK506 the capacity to inhibit cytokine pro-

duction [5], had negligible (if any) effect on cytokine-stimulated T-cell activation. FK506 was also reported to inhibit the expression of the high-affinity IL-7 receptor, in contrast to CsA which was ineffective in this respect. In addition, FK506 was more effective than CsA in decreasing the frequency of IL-2-producing T cells in stable renal allograft recipients. This observation implies that some of the effects of FK506 are mediated by CsA-insensitive pathways [15]. Furthermore, CsA, but not FK506, inhibited the expression of nitric oxide synthase [10], thus causing hypertension and nephrotoxicity, well-known sequelae of the long-term use of CsA [16].

Some reports claimed that FK506 did not act post-transcriptionally. For example, it was reported that FK506 failed to affect cytokine-stimulated responses [17,18]. It should be noted, however, that in these experiments the effects of FK506 were assessed in cytokine-dependent cell lines, including IL-2-dependent CTLL-2 [17,18], and IL-4-dependent D10.G4.1 cells [18]. The possibility exists that such discrepant results are explained by differences in cytokine dependency for growth and activation between primary T-cell cultures and established T-cell lines. In primary non-immortalized cells, FK506, similar to CsA, inhibited the expression of T-cell cytokines. In contrast to CsA, it acted post-transcriptionally and inhibited the response to cytokines, possibly through modulation of the expression of their high-affinity receptors and/or through attenuation of signalling through cytokine receptors. The exact target of FK506 post-transcriptional events remains to be determined.

Conclusion

The proven superiority of FK506 over CsA in attenuating rejection episodes in patients with renal [16,19], heart-lung [12], and liver [20] allografts is well documented. Although a major component of the immunosuppressive effects of CsA and FK506 is accounted for by the antagonism to the activity of calcineurin [2,5], it is apparent that FK506 inhibits steps distal to calcineurin activation in the T-cell activation cascade. This is evidenced by the demonstrated capacity of FK506 monotherapy to reverse steroid-resistant allograft rejection episodes. This is a very important difference between FK506 and CsA, the latter of which is ineffective in the treatment of allograft rejection [11]. While the exact cellular and molecular targets of FK506 remain to be investigated, the use of FK506 in combating refractory allograft rejection episodes [11,12,19] appears rational in view of the above findings.

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