

Inhibition of T-Cell Function by FK 506 and Cyclosporine Is Not Accompanied by Alterations in Intracellular Calcium

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FK 506 is a novel immunosuppressant that is more potent than cyclosporine (CyA) and has been used with encouraging results in preliminary clinical trials of organ transplantation.¹⁻⁴ FK 506 is a macrolide whereas CyA is a cyclic peptide. Despite their structural differences and the fact that FK 506 and CyA bind to different cytosolic peptidyl-prolyl isomerases (PPIases), both agents have shown remarkably similar qualitative effects on in vitro T-cell function.⁵⁻⁸ Both FK 506 and CyA inhibit interleukin-2 (IL-2), IL-3, IL-4, tumor necrosis factor alpha (TNF- α), and interferon gamma (INF- γ) synthesis as well as the mRNA levels of these cytokines during T-cell activation.⁵⁻⁹ Although these mechanisms may involve calcium-dependent pathways, it is unclear whether FK 506 and CyA actually alter signal transduction via changes in intracellular calcium ($[Ca^{2+}]_i$). Since FK 506 and CyA possess distinct cytosolic binding sites, it is conceivable that intracellular signaling via second messengers may be differentially affected by these two agents. Such differences could have important consequences in their mechanism of immunosuppression. The purpose of this study was to determine whether $[Ca^{2+}]_i$ is involved in the suppression of T-cell function by FK 506 and CyA.

MATERIALS AND METHODS

Animals

Female C57BL/6 (B6, H-2^b) and DBA/2J (DBA, H-2^d) mice were purchased from the Jackson Laboratory, Bar Harbor, Me. Mice were housed in plastic cages, provided with Purina Rodent Chow and tap water ad libitum, and used at 10 to 16 weeks of age.

Cells

Allosensitized T cells were derived from a day 7-10 B6 anti-DBA mixed leukocyte culture (MLC) as previously described.¹⁰

Ca²⁺_i Determinations

After washing three times, 2×10^7 MLC cells were loaded with the Ca²⁺-sensitive fluorescent indicator Indo-1 (2 μ mol/L) for 30 minutes and both baseline and stimulated (2 μ g/mL concanavalin A [Con A]) $[Ca^{2+}]_i$ was measured in a Shimadzu RF5000 fluorescence spectrophotometer, as previously described.¹¹ FK 506, CyA, or the appropriate vehicle control was added to the cell suspensions with or without Con A.

Assessment of Proliferation

In order to correlate potential changes $[Ca^{2+}]_i$ with concomitant alterations in T-cell function, proliferation (³HTdR uptake) in response to Con A (1 μ g/mL) was also determined. Briefly, MLC cells (20,000 cells/well) were incubated in a final volume of 0.2 ml

Dulbecco's Modified Eagle's Medium (DMEM) in triplicate flat-bottomed microtiter wells for 24 hours and pulsed with 2 μ Ci ³HTdR/well for the final 6.5 hours of the incubation period. Plates were then harvested onto glass fiber filter paper and the ³HTdR determined by liquid scintillation spectrophotometry, as previously described.¹⁰

Data Analysis

The data shown represent the mean \pm SEM of results from three experiments.

RESULTS

As shown in Fig 1, both FK 506 and CyA inhibited MLC cell proliferation to Con A in a dose-dependent manner. FK 506 was approximately 100-fold more potent than CyA in this regard. In further experiments, FK 506 and CyA also inhibited MLC cell proliferation to monoclonal antibody (MAb) to CD3 (data not shown). In contrast, neither FK 506 nor CyA had any effect on either baseline or Con A-stimulated increases in $[Ca^{2+}]_i$, even at the highest doses tested (Fig 2). This held true when the drugs were added just before Con A as well as simultaneously or following Con A. Cell viability was unaffected by 24 hour exposure of MLC cells to the highest concentrations of FK 506 (10.0 ng/mL) or CyA (1,000.0 ng/mL) tested as determined by trypan blue exclusion.

DISCUSSION

We and others have previously shown that T-cell activation by mitogen is accompanied by an elevation in $[Ca^{2+}]_i$.¹¹⁻¹⁴ This is true both for unsensitized and allosensitized T cells.¹¹ In previous work, we have observed that increases in basal $[Ca^{2+}]_i$ occur prior to maximal allosensitized T-cell proliferation in culture after antigen exposure but that even higher $[Ca^{2+}]_i$ levels are detected well after the completion of DNA synthesis.¹¹ This raises the question of whether $[Ca^{2+}]_i$ may be a marker of the allosensitized state and whether elevated $[Ca^{2+}]_i$ is in fact required for optimal T-cell function. It is tempting to speculate that $[Ca^{2+}]_i$ may play an important role in maintaining or

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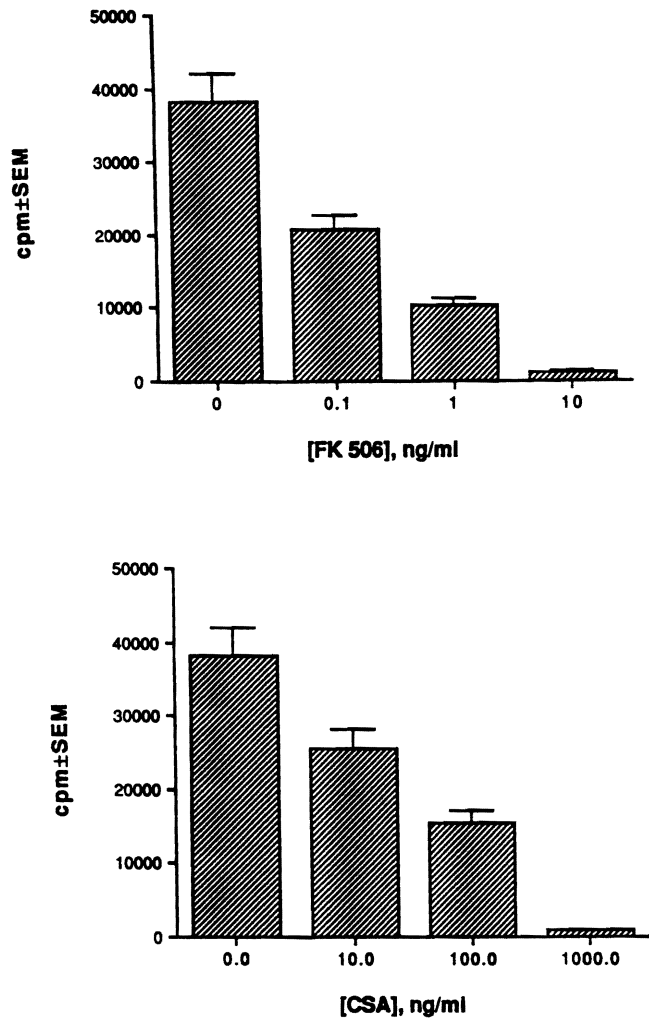


Fig 1. FK 506 and CyA inhibit Con A-induced T-cell proliferation. Twenty-thousand cells derived from a day 7-9 MLC were cultured in the presence or absence of various concentrations of the indicated drug. The $^3\text{HTdR}$ uptake was determined after 24 hours. Data represent the mean \pm SEM of results from three experiments.

altering T-cell function within the allograft. It therefore became of interest to us to determine whether the immunosuppressive agents FK 506 and CyA, in addition to their inhibitory effects on T-cell proliferation and lymphokine synthesis, were capable of altering T-cell $[\text{Ca}^{2+}]_i$. At the concentrations of FK 506 and CyA that inhibit Con A-induced proliferation of allosensitized MLC cells, we failed to observe any inhibition of the rise in $[\text{Ca}^{2+}]_i$ induced by triggering the TcR-CD3 complex with Con A. In subsequent experiments, we have also found that the rise in $[\text{Ca}^{2+}]_i$ is preserved even following a 2-hour preincubation with either FK 506 or CyA.

Thus, even though it has been shown that FK 506 and CyA inhibit certain T-cell activation steps that are calcium-

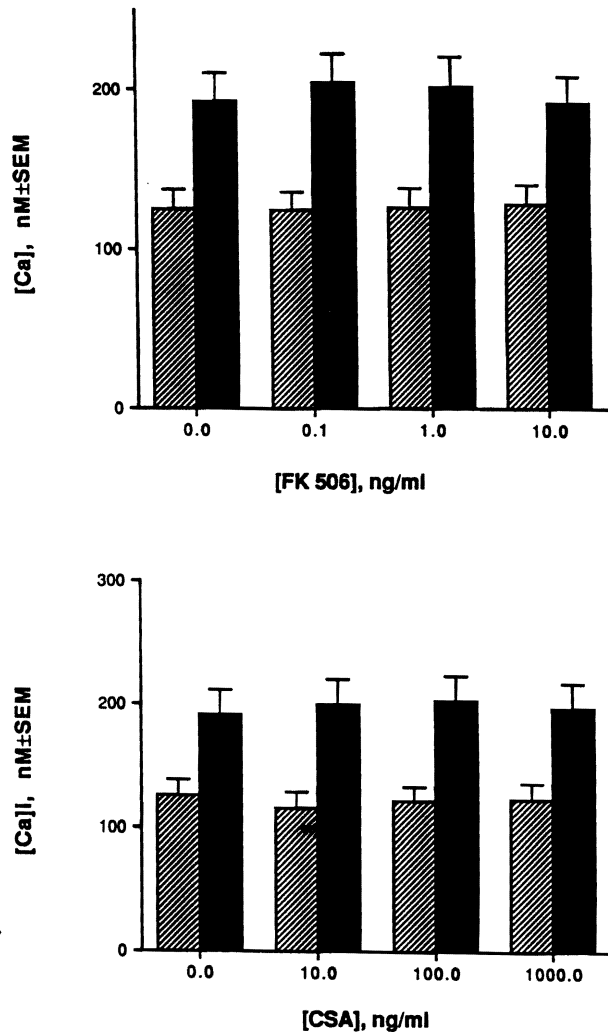


Fig 2. Effects of FK 506 and CyA on T-cell calcium signaling. 2×10^6 day 7-9 MLC cells were loaded with indo-1 ($2 \mu\text{mol/L}$) for 30 minutes and basal (▨) and Con A-stimulated (■) $[\text{Ca}^{2+}]_i$ determined by fluorescence spectrophotometry. Data represent the mean \pm SEM of results from three experiments.

dependent,^{6,15-19} these agents in and of themselves do not appear to alter calcium fluxes in allosensitized murine T cells. Similar observations have been made in human tumor cell lines that are not allosensitized.¹⁸ It would appear that FK 506 and CyA inhibit T-cell function subsequent to the rise in $[\text{Ca}^{2+}]_i$ that accompanies T-cell activation via the TcR-CD3 complex. Another novel immunosuppressive agent, rapamycin, appears to affect even later events during T-cell activation.^{6,20} We have not yet tested the effects of rapamycin on allosensitized T-cell calcium signaling. However, the observation that the calcium signal is preserved in the presence of FK 506 and CyA suggests that potential synergism of these drugs with agents inhibiting $[\text{Ca}^{2+}]_i$ may exist.

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