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Immunology

# Immunophilin ligands demonstrate common features of signal transduction leading to exocytosis or transcription

**(rat basophilic leukemia cells/cyclosporin A/FK506/rapamycin/cyclophilin)**

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**ABSTRACT** **Investigations of the actions and interactions of the immunophilin ligands FK506, cyclosporin A (CsA), rapamycin, and 506BD suggest that complexes of FK506 with an FK506-binding protein or of CsA with a cyclophilin (CsA-binding protein) inhibit the T-cell receptor-mediated signal transduction that results in the transcription of interleukin 2. Now we report an identical spectrum of activities of FK506, CsA, rapamycin, and 506BD on IgE receptor-mediated signal transduction that results in exocytosis of secretory granules from the rat basophilic leukemia cell line RBL-2H3, a mast cell model. Both FK506 and CsA inhibit receptor-mediated exocytosis (CsA $IC_{50}$ = 200 nM; FK506 $IC_{50}$ = 2 nM) without affecting early receptor-associated events (hydrolysis of phosphatidylinositol, synthesis and release of eicosanoids, uptake of $Ca^{2+}$). In contrast, rapamycin and 506BD, which share common structural elements with FK506, by themselves have no effect on IgE receptor-mediated exocytosis. Both compounds, however, prevent inhibition by FK506 but not by CsA. Affinity chromatography with FK506, CsA, and rapamycin matrices indicates that the same set of immunophilins present in RBL-2H3 cells have been found in Jurkat T cells and calf thymus; however, the relative amounts of these proteins differ in the two cell types. These results suggest the existence of a common step in cytoplasmic signaling in T cells and mast cells that may be part of a general signaling mechanism.**

Despite significant progress in our understanding of the events at the plasma membrane involved in receptor-mediated activation, little is known of the mechanisms of signal transduction through the cytoplasm. The immunosuppressive agents FK506, cyclosporin A (CsA), and rapamycin inhibit cytoplasmic signaling events in T cells (1–5), probably through the formation of complexes with cytoplasmic receptors termed immunophilins (1, 2, 4). The rat basophilic leukemia cell line RBL-2H3, a tumor analog of a mucosal mast cell that has been used to study the biochemical events of mast cell activation (6–8), was used to investigate the effects of FK506, rapamycin, and 506BD on IgE receptor-mediated exocytosis. These cells contain both CsA-sensitive (9) and FK506-sensitive (T.H. and R.J.H., unpublished results) rotamases. To date, every known immunophilin is a rotamase (i.e., a peptidyl-prolyl cis–trans isomerase).

Here we show that the same interactions of these immunosuppressive agents that take place in the human T-cell line Jurkat are operative in a rat mast cell line. IgE receptor-mediated exocytosis from these cells is inhibited by concentrations of CsA (10) and FK506 that are similar to those required to inhibit T-cell receptor (TCR)-mediated interleukin 2 transcription. Rapamycin [$K_d$ = 0.2 nM (2)] and 506BD [$K_d$ = 20 nM (1)], which, like FK506 [$K_d$ = 0.4 nM (2)], are high-affinity ligands for the FK506-binding protein (FKBP) (1, 2, 4, 11, 12), inhibit the actions of FK506, but not CsA, in both mast cell and T-cell lines. However, rapamycin and 506BD by themselves have no effect on the TCR-mediated pathway or IgE receptor-mediated pathway. A preliminary characterization of the immunophilins in this mast cell line by affinity chromatography indicates that all of the proteins observed from Jurkat cells are present in RBL-2H3 cells, although in different proportions.

These findings are consistent with the role of immunophilin–drug complexes as inhibitors of intracellular signaling and suggest that the mechanism for receptor-mediated activation in both cell types shares a common step that is susceptible to inhibition by FK506 and CsA. It is postulated that this step is part of a general mechanism for $Ca^{2+}$-dependent cytoplasmic signaling and that FK506, rapamycin, and CsA serve as a window for this as yet undefined event in signal transduction.

## MATERIALS AND METHODS

**Reagents.** CsA (Sandoz Pharmaceutical) was prepared as a 1 mM stock solution in ethanol. FK506 (Fujisawa Pharmaceutical, Osaka) and rapamycin (Wyeth–Ayerest) were prepared as 0.1 mM stock solutions in ethanol. Stock solutions were diluted directly into the experimental buffers. The final concentration of ethanol was <0.1%, which did not affect either the maximal response or the baseline value in any assay. Culture media were obtained from Biofluids (Rockville, MD). Other reagents were purchased from Sigma or Boehringer Mannheim.

**Buffers.** All experiments using oligomeric IgE (covalently crosslinked oligomers of IgE) were performed in Pipes buffer [25 mM $Na_2$Pipes, pH 7.1/100 mM NaCl/5 mM KCl/0.4 mM $MgCl_2$/1 mM $CaCl_2$/5.6 mM D-glucose/0.1% bovine serum albumin (BSA)]. All experiments measuring phosphatidylinositol (PI) hydrolysis were performed in the Pipes buffer described above, with the addition of 10 mM LiCl.

**Cells.** RBL-2H3 cells (hereafter called RBL cells) were maintained in 175-$cm^2$ tissue culture flasks (13). For serial passage and to prepare the cells for experiments, the cells were detached by incubation with trypsin/EDTA for 5–6 min at 37°C.

**Measurement of IgE Receptor-Mediated Release of Serotonin and Hydrolysis of PI.** Exocytosis was monitored by measuring the release of radioactive serotonin from labeled RBL cells, and IgE receptor-mediated hydrolysis of PI was measured exactly as previously described (10).

**Simultaneous Measurement of Arachidonic Acid Release and Serotonin Release.** Arachidonic acid release experiments were performed as described (17). RBL cells (4 × $10^5$ per ml

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Abbreviations: BSA, bovine serum albumin; CsA, cyclosporin A; FKBP, FK506-binding protein; PI, phosphatidylinositol; TCR, T-cell receptor.

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in medium) were labeled with both [$^{14}$C]arachidonic acid (Amersham, cat. no. CFA 711; final concentration, 0.1 $\mu$Ci/ml; 1 $\mu$Ci = 37 kBq) and [$^{3}$H]serotonin (DuPont, cat. no. NET 398; final concentration, 1 $\mu$Ci/ml), seeded in 24-well cluster plates (0.5 ml per well), and incubated for 18 hr in a humidified incubator with 5% $CO_2$/95% air. The Pipes buffer contained 1% delipidated BSA (Sigma, cat. no. B-7030) instead of 0.1% BSA. Cells were washed with this buffer, preincubated for 5 min with or without drug, and triggered with oligomeric IgE for 10 min. The reaction was stopped by removing the supernatants, and the radioactivity ($^{3}$H representing serotonin, and $^{14}$C representing arachidonic acid) was determined in both the cell lysates and corresponding supernatants. The percent arachidonate release and the percent serotonin release were calculated by dividing the radioactive counts in the supernatant by the sum of counts in the supernatant and the cell lysates.

**$^{45}Ca^{2+}$ Uptake Measurements.** $^{45}Ca^{2+}$ uptake experiments were performed essentially as described (10), with the following modifications. RBL cells were seeded in 24-well cluster plates ($2 \times 10^5$ cells per well) as described above. The cells were washed twice with Pipes buffer before the final assay buffer, containing CsA, FK506, or solvent alone, was added and the cells were preincubated for 5 min at 37°C. One minute before triggering, 10 $\mu$l of buffer containing $^{45}Ca^{2+}$ (600 mCi/ml) was added (DuPont, cat. no. NEZ 013). The cells were then triggered with oligomeric IgE, and the reaction was quenched by adding 2 ml of a 0°C quenching solution of Pipes buffer containing 10 mM $LaCl_3$ to each well and washing each well twice with the same solution. The cells were solubilized by washing each well three times with 250 $\mu$l of 1% (vol/vol) Triton X-100 in phosphate-buffered saline, and radioactivity in the lysates was measured by scintillation counting. Calcium uptake is expressed as pmol per $10^6$ cells. The solvent had no effect on $^{45}Ca^{2+}$ uptake.

**Detection of the FK506-, CSA-, and Rapamycin-Binding Proteins.** Three aliquots of RBL cells ($7.5 \times 10^7$ cells per aliquot) were lysed for 20 min at 0°C in 7.5 ml of lysis buffer [50 mM Tris·HCl, pH 7.5/1% Nonidet P-40/0.15 M NaCl/1 mM EDTA/5 mM 2-mercaptoethanol (added fresh)/1% BSA/2 mM phenylmethanesulfonyl fluoride (added fresh)/50 mM NaF]. The lysates were centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant from each aliquot was passed through a column packed with 50 $\mu$l of ethanolamine-capped Affi-Gel 10 (Bio-Rad) and then twice passed through a column packed with 50 $\mu$l of FK506-Affi-Gel (18) or CsA-acrylamide-based matrix. The FK506 column flowthrough was passed through a column packed with 50 $\mu$l rapamycin-Affi-Gel (≈5 mmol of drug per ml of matrix; ref. 18) at 4°C. The matrices were washed twice with 1 ml of lysis buffer, once with 1 ml of lysis buffer containing 0.65 M NaCl, and twice with 1 ml of 50 mM Tris·HCl, pH 7.5/0.1% Nonidet P-40/2 mM phenylmethanesulfonyl fluoride. Bound proteins were eluted by batch extraction of the matrices with their respective drug (0.3 mmol of drug in 25 ml of ethanol added to 250 ml 50 mM Tris·HCl, pH 7.5) for 2 hr at 4°C. The supernatant was removed, lyophilized, and resuspended in 50 $\mu$l of SDS loading buffer for SDS/14% PAGE followed by Coomassie blue staining. In a control experiment, the same washing and elution process was performed with the ethanolamine-capped Affi-Gel 10. Only BSA, which was used in the lysis buffer, was detected. The gel was electroblotted using a 10 mM Caps, pH 11/10% methanol tank buffer onto a ProBlott membrane (Applied Biosystems). The bands at 12 and 13 kDa from the FK506 matrix and those at 12, 13, and 27 kDa from the rapamycin matrix were excised for Edman degradation to determine their N-terminal sequences.

**Presentation of Data.** Assays were performed in duplicate or triplicate. Experiments were done at least three times.

## RESULTS

**Inhibition of IgE Receptor-Mediated Exocytosis by FK506.** Upon stimulation with oligomeric IgE, RBL cells release serotonin and other preformed mediators of immediate hypersensitivity as well as newly synthesized eicosanoids. As shown in Fig. 1, FK506 inhibits exocytosis in a dose-dependent manner. The $IC_{50}$ is similar to what has been reported for TCR-mediated activation of T cells (2, 5) and *in vivo* immunosuppression in patients who underwent organ transplantation (19); FK506 was ≈100-fold more potent than CsA in blocking exocytosis from RBL cells ($IC_{50}$ for FK506 and CsA is 2 nM and 200 nM, respectively).

**Rapamycin and 506BD Prevent the Inhibition of Exocytosis by FK506 But Not by CsA.** We examined the effects of rapamycin, a macrolide that is structurally related to FK506 and able to block the actions of FK506 in T cells (2, 3), on FK506-mediated inhibition of RBL cell activation. Indeed, rapamycin blocks the effects of FK506 on mast cells in a dose-dependent manner (Fig. 2) that reflects the relative affinity of these drugs to FKBP (see above). Equimolar concentrations (doses that are 50–100 times higher than the $IC_{50}$) of both drugs reverse approximately two-thirds of the inhibition by FK506; this 2:1 ratio is equivalent to the ratio of the dissociation constants of each drug with FKBP (see above). The inhibition by CsA is not affected by rapamycin (data not shown), and rapamycin alone has no effect on IgE receptor-mediated exocytosis (note data points in Fig. 2 for [FK506] = 0).

506BD was designed to preserve the common FKBP-binding domain of FK506 and rapamycin but to lack the putative biological effector elements of these agents (1, 4). In accord with the studies in T cells, where 506BD was shown to block the actions of FK506 but not of CsA, 506BD prevents the inhibition of exocytosis due to FK506 but not that due to CsA (Fig. 3). Thus, 506BD and rapamycin differentiate the effects of FK506 and CsA in both mast cells and T cells (1, 4). Interestingly, although rapamycin ($K_i$ = 0.2 nM) and 506BD ($K_i$ = 5 nM), like FK506 ($K_i$ = 0.5 nM), inhibit the rotamase activity of FKBP *in vitro* (1, 2), rapamycin and 506BD antagonize the effect of FK506 *in vivo* (Figs. 2 and 3). If the rotamase activity measured *in vitro* reflects its function *in vivo*, then the inhibition of this enzymatic activity is insufficient to block receptor-mediated activation of mast cells and T cells.

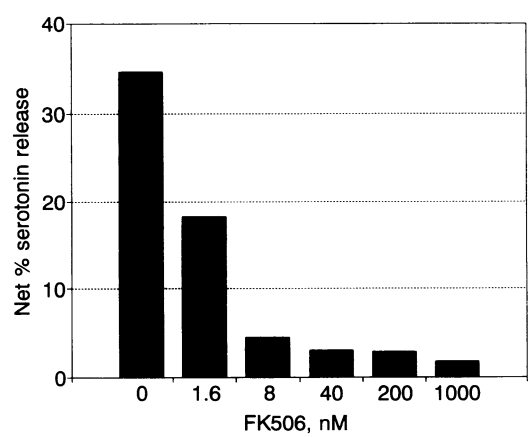


FIG. 1. FK506 inhibits IgE receptor-mediated exocytosis from RBL cells. Serotonin release was determined as described (10). RBL cells were preincubated with the indicated concentration of FK506 for 5 min at 37°C before addition of oligomeric IgE and incubation for 10 min. Data are presented as the average of triplicate determinations; the SD was always <1.5%. Exocytosis was assayed by measuring the release of radioactive serotonin as described in *Materials and Methods*.

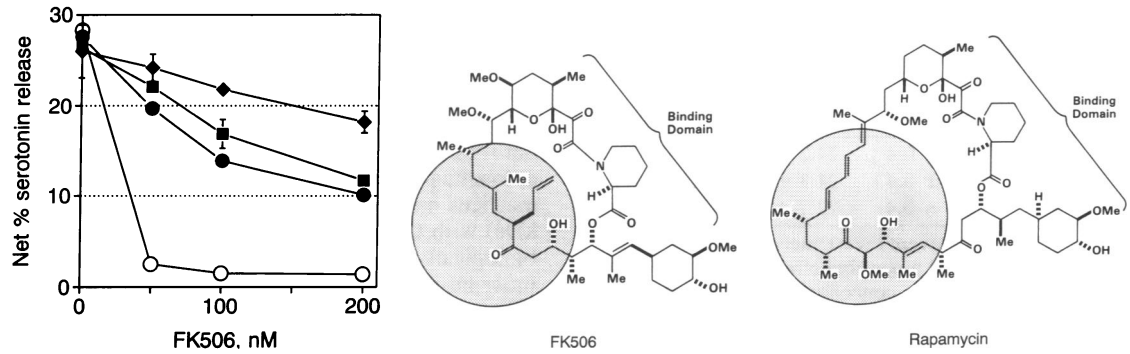


FIG. 2. Rapamycin prevents the inhibition of IgE receptor-mediated exocytosis from RBL cells by FK506. Cells were preincubated for 15 min with 0 nM (○), 50 nM (●), 100 nM (■), or 200 nM (◆) rapamycin before addition of the indicated concentration of FK506. Then the cells were incubated for an additional 5 min before addition of oligomeric IgE. The release of serotonin was measured after an additional 10-min incubation. Data are presented as the average of duplicate determinations; the SD was always <1.5%. Note that rapamycin alone did not inhibit exocytosis. The "binding domain" label refers to residues primarily responsible for binding to FKBP. The circled residues constitute effector domains of the two drugs (4).

**Effects of the Drugs on Early Membrane-Associated Events.** To determine the site of action of these drugs, the timing (at what stage) and location (in which compartment) of the inhibitory event in the signaling mechanism must be defined. In RBL cells, CsA has no effects on the early membrane-associated events of IgE receptor-mediated exocytosis, including aggregation of IgE receptors, hydrolysis of PI, uptake of $Ca^{2+}$ into the cell, or the increase in intracellular free $Ca^{2+}$ (10). In analogy to studies with CsA, concentrations of FK506 that result in maximum inhibition of exocytosis have no effect on the hydrolysis of PI, the uptake of $Ca^{2+}$, or the release of eicosanoids (Table 1). Hence, early membrane-associated events do not seem to be the site of action of these drugs. Exocytosis occurs within 10 min of receptor aggregation, making the involvement of nuclear processes unlikely. Therefore, these drugs probably inhibit cytoplasmic events.

**Identification of Cytoplasmic Immunophilins and Immunophilin-Associated Proteins in RBL Cells.** To identify potential receptors for FK506, CsA, and rapamycin, RBL cells were lysed with detergent and passed over either an FK506, a CsA, or a rapamycin affinity matrix (18), which was washed and then eluted with FK506, CsA, or rapamycin, respectively. SDS/PAGE with Coomassie blue staining shows proteins of 12, 13, and 60 kDa from the FK506 and rapamycin matrices (Fig. 4). An additional protein of 27 kDa is retained by the rapamycin matrix. The CsA matrix retains proteins of 18, 19, 20, 31, 43, and 80 kDa that are eluted specifically with CsA. N-terminal sequence analysis of the 12-, 13-, and 27-kDa proteins from the rapamycin matrix indicates that (*i*) the 12-kDa protein is identical to FKBP (12); (*ii*) the 13-kDa band has an N-terminal sequence that matches a sequence derived from FKBP-13 isolated from calf thymus (20), although the first three amino acids from the calf thymus sequence are missing; (*iii*) the 27-kDa band is blocked at the N-terminus, which is consistent with the corresponding protein isolated from calf thymus (W. S. Lane, A. Galat, R. F. Standaert, S.L.S., unpublished results).

## DISCUSSION

This paper reports the effects of the immunosuppressive agents FK506, CsA, and rapamycin on IgE receptor-mediated exocytosis from a rat mast cell line. The relative activities and interactions of these agents suggest that there is a common step in this signal-transduction pathway and a pathway emanating from the TCR in the T lymphocyte. FK506 and CsA inhibit these processes at nanomolar concentrations; rapamycin and 506BD reverse the inhibition by FK506 but have no effect on the actions of CsA; and rapamycin or 506BD alone have no effect on these processes. Furthermore, all three drug affinity matrices retained the same respective set of immunophilins from both cell types. These observations suggest that similar protein(s) may be involved in a step common to IgE receptor and TCR signaling pathways.

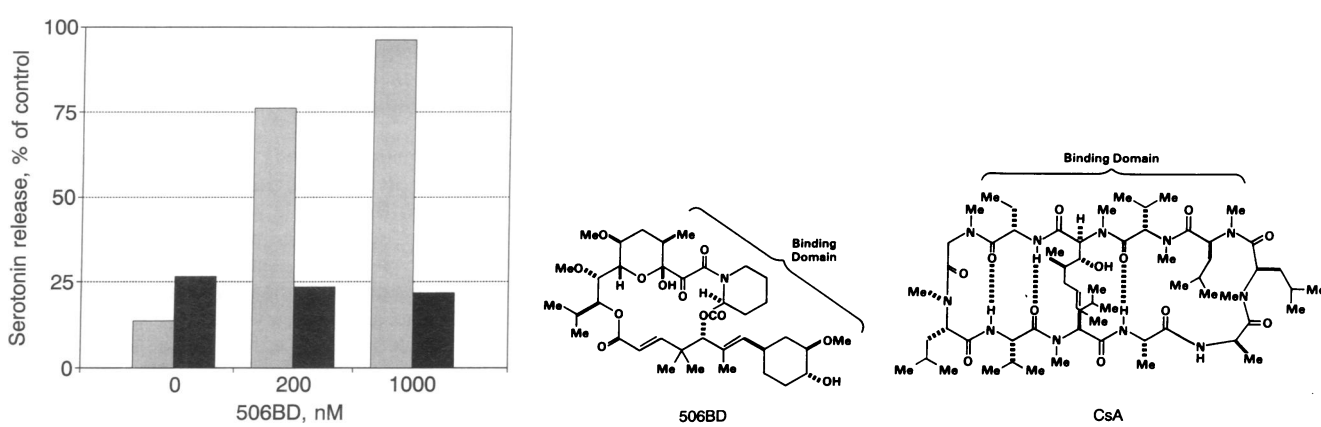


FIG. 3. 506BD prevents inhibition of IgE receptor-mediated mast cell activation by FK506 (gray bars) but not by CsA (black bars). RBL cells were preincubated for 15 min with the indicated concentration of 506BD before FK506 (3.2 nM) or CsA (330 nM) was added. After an additional 5-min incubation, the cells were triggered with oligomeric IgE and serotonin release was measured as described. Data are presented as the average of duplicate determinations; the SD was always <1.5%. The "binding domain" label refers to residues primarily responsible for binding to immunophilins (4).

Table 1. CsA and FK506 do not inhibit early receptor-mediated events

| Condition | % net release | | % net PI hydrolysis | Net $Ca^{2+}$ uptake* |
|---|---|---|---|---|
| | Serotonin | Eicosanoids | | |
| Buffer | 26.9 ± 0.2 | 12.3 ± 0.4 | 24.2 ± 0.6 | 1072 ± 68 |
| FK506 | 3.1 ± 0.1 | 12.3 ± 0.4 | 21.3 ± 0.6 | 1178 ± 71 |
| CsA | 2.4 ± 0.7 | 12.5 ± 0.4 | 22.7 ± 0.5 | 1310 ± 37 |

Four IgE receptor-mediated events were measured in RBL cells (see text for details). To rule out a possible effect of CsA or FK506 on early receptor-mediated events, concentrations of the inhibitors were used that were well above those required to inhibit exocytosis. In all assays, cells were preincubated for 5 min with or without the drug (330 nM CsA, 100 nM FK506). The cells were triggered by addition of an optimal dose of oligomeric IgE and incubated for 10 min as described (10). Assays were performed in triplicate, and each experiment was repeated at least three times. The concentration of the solvent for CsA and FK506 (ethanol) was <0.01% and did not affect these assays.
*Picomoles per $10^6$ cells.

There are other parallels between IgE receptor-mediated and TCR-mediated pathways. FK506 and CsA have no effect on early membrane-associated effects in either cell type. Both pathways involve a rise in intracellular $Ca^{2+}$ concentration. In addition, the $\zeta$ chain of the TCR exhibits 59% sequence identity with the $\gamma$ chain of the high-affinity IgE receptor on mast cells (21). We argue that the step inhibited by immunophilin ligands in these two cell types is part of a general $Ca^{2+}$-dependent signaling mechanism. Indeed, in addition to the findings described above, the actions of these immunosuppressive agents in both cell types show several similarities: (*i*) CsA inhibits TCR-mediated exocytosis in cytolytic T cells, without affecting TCR-mediated PI hydrolysis (22); (*ii*) CsA and FK506 inhibit IgE receptor-mediated activation of the nuclear factor AP-1 (T.H., J. Segars, and R.J.H., unpublished results), which is reminiscent of their effects in T cells (23, 24); (*iii*) CsA inhibits cytokine transcription in both mast cells and T cells (25–29).

We believe that a common mechanism of inhibition accounts for the common characteristics of the pathways in both cell types. As in the TCR-mediated pathway, it is an immunophilin–drug complex, and not inhibition of rotamase activity, that blocks IgE receptor-mediated exocytosis. Rapamycin and 506BD reverse the inhibition of FK506 by competitively binding to the immunophilin that confers the biological activity of FK506. Moreover, with the half-life of degranulation being 10 min, the kinetics of the mast cell process suggest that these drugs are inhibiting cytoplasmic events since there is insufficient time for nuclear processes to be involved.

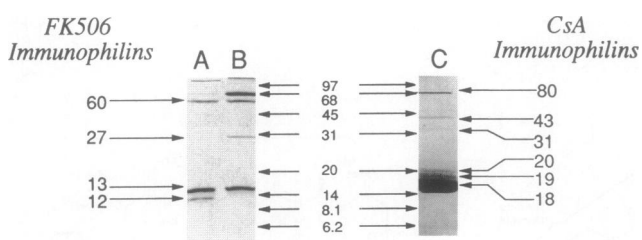


FIG. 4. Immunophilins and immunophilin-associated proteins from RBL cells as detected by SDS/PAGE of eluates from FK506 (lane A), rapamycin (lane B), and CsA (lane C) affinity columns. The apparent molecular masses (kDa) of the retained proteins from the FK506 and rapamycin matrices are indicated at left, and those from the CsA matrix at right. Positions of molecular mass markers are indicated in the center. The band at 68 kDa is BSA, a component of the lysis buffer. Note that since the FK506 column and rapamycin column were run sequentially, no FKBP-12 appears in the eluate from the rapamycin column, as it was completely removed by the FK506 matrix.

Several criteria defined by the results reported in this paper have to be met by the immunophilin that confers the biological activity of FK506. First, the immunophilin must bind both FK506 and rapamycin. Affinity chromatography of cytosolic protein from RBL cells with FK506 and rapamycin matrices has supplied several candidates (12, 13, 60, and 80 kDa) with this property. Second, the relative affinity of the biologically active immunophilin for FK506 and rapamycin must be in the ratio of 1:2. At concentrations where both drugs are in excess of the receptor inside the cell, the concentration of rapamycin needed to reverse the actions of FK506 by 50% is half the concentration of FK506. FKBP meets these criteria (see above) and thus may mediate the actions of FK506 in RBL cells. It is, however, essential to determine the dissociation constants of the other immunophilins.

Although the cytoplasmic proteins from RBL cells retained by the FK506 and rapamycin matrices have the same mobilities on SDS/PAGE as those isolated from Jurkat T cells (18), the relative proportions of FKBP (hereafter referred to as FKBP-12) and the 13-kDa protein (hereafter referred to as FKBP-13) are substantially different. In the T cell, FKBP-12 is the predominant binding protein for both FK506 and rapamycin; in RBL cells, FKBP-13 assumes this role. In addition to the quantitative differences in the relative amounts of FKBP-12 and FKBP-13 in Jurkat and RBL cells, the 80-kDa phosphoprotein from Jurkat cells that was retained on an FK506 affinity column (18) was not found in RBL cells. It remains to be determined whether this protein is absent from RBL cells or is present in concentrations below the level of sensitivity of the silver technique used to stain the gels. The recently cloned human FKBP-13 (20) has a signal sequence that can be cleaved by a microsomal signal peptidase and has a putative endoplasmic reticulum retention sequence (30). If FKBP-13 is localized to the endoplasmic reticulum, then it may play some role in protein trafficking, especially since the mast cell devotes considerable effort to synthesizing proteins that are incorporated into secretory granules, which constitute a major portion of the mast cell cytosol.

Cyclophilin (18 kDa) is the predominant protein retained on the CsA matrix. Purification of this protein from RBL cells shows that it has rotamase activity that is inhibited by CsA (9). The minor band at 43 kDa may correspond to the previously reported Jurkat cell phosphoprotein retained by a CsA matrix (31). The protein at 31 kDa may correspond to a protein from human spleen reported previously (12). The proteins at 20 and 19 kDa may correspond to the proteins from human spleen apparent in figure 2, lane 6, of ref. 12. If one of these bands is a cyclophilin-associated protein, it could lead to the identification of a physiological target for immunophilins.

These immunosuppressive drugs may be mimicking physiologically relevant molecules that serve to temporarily block signaling pathways in the cell. If so, the rotamase activity may be a relevant property of the immunophilins. In analogy to the GTPase activity of the $\alpha$ subunit of heterotrimeric guanine nucleotide-binding proteins (which turns off an activated G protein), the rotamase activity of the immunophilins may be responsible for causing the dissociation of these putative endogenous immunophilin ligands, thus relieving the inhibition of receptor-mediated signaling. In this scenario (Fig. 5), immunosuppressive drugs such as CsA, FK506, and rapamycin are not substrates for the rotamase, and therefore "lock" the immunophilin in an inhibitory configuration.

After the T cell, the mast cell is the second cell type where the actions of FK506 and rapamycin have been dissected at this level of detail; similar interactions between FK506 and

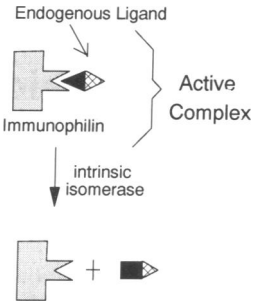


FIG. 5. Model to incorporate the rotamase activity of immunophilins into the mechanism of action of immunosuppressive drugs. In this model, immunophilins interact with endogenous ligands to form an active complex that blocks receptor-mediated cellular functions. The intrinsic isomerase activity of immunophilins is responsible for the dissociation of the complex. In analogy to the binding of guanosine 5'-[γ-thio]triphosphate to the α subunit of guanine nucleotide-binding proteins, the immunosuppressive drugs CsA, FK506, and rapamycin are not substrates for the isomerase; hence, the inhibitory complex remains active.

rapamycin have also been demonstrated in the B lymphocyte (32). How common is this signaling mechanism? Investigations of other $Ca^{2+}$-dependent signaling pathways with FK506, CsA, and rapamycin will illuminate the breadth of this signaling mechanism, which will have ramifications for the utility of these drugs as therapeutic agents. Indeed, the immunosuppression in patients resulting from the administration of CsA or FK506 may be due to inhibition of mast cell function in addition to inhibition of T-cell function.

Finally, the findings reported here suggest that the common step in the signaling pathways that the immunophilin ligands inhibit may be the intracellular transport of proteins. In the mast cell, FK506 and CsA inhibit the vectorial translocation of the contents of secretory granules across the plasma membrane, and in the T cell these agents may inhibit the translocation of nuclear factors across the nuclear envelope (4, 33). This inhibition could arise from direct interaction with the translocation apparatus or from blocking access of the target molecule to the translocation apparatus. Thus far, a strict association has been observed between $Ca^{2+}$ dependence and susceptibility to inhibition by FK506 or CsA. No functional or structural explanation for this correlation has been made. Studies of systems that use this common mechanism of signaling, defined by similar interactions of FK506, CsA, 506BD, and rapamycin, should illuminate the molecular details of these processes.

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1. Bierer, B. E., Somers, P. K., Wandless, T. J., Burakoff, S. J. & Schreiber, S. L. (1990) *Science* **250,** 556–559.
2. Bierer, B. E., Mattila, P. S., Standaert, R. F., Herzenberg, L. A., Burakoff, S. J., Crabtree, G. R. & Schreiber, S. L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9231–9235.
3. Dumont, F. J., Melino, M. R., Staruch, M., Koprak, S. L., Fischer, P. A. & Sigal, N. H. (1990) *J. Immunol.* **144,** 1418–1424.
4. Schreiber, S. L. (1991) *Science* **251,** 283–287.
5. Dumont, F. J., Staruch, M. J., Koprak, S. L., Melino, M. R. & Sigal, N. H. (1990) *J. Immunol.* **144,** 251–258.
6. Oliver, J. M., Seagrave, J., Stump, R. F., Pfeiffer, J. R. & Deanin, G. G. (1988) *Prog. Allergy* **42,** 185–245.
7. Metzger, H., Alcaraz, G., Hohman, R. J., Kinet, J. P., Pribluda, V. & Quarto, R. (1986) *Annu. Rev. Immunol.* **4,** 419–470.
8. Beaven, M. A. & Cunha-Melo, J. R. (1988) *Prog. Allergy* **42,** 1–62.
9. Hohman, R. J. & Hultsch, T. (1990) *New Biologist* **2,** 663–672.
10. Hultsch, T., Rodriguez, J. L., Kaliner, M. A. & Hohman, R. J. (1990) *J. Immunol.* **144,** 1000–1001.
11. Siekierka, J. J., Hung, S. H. Y., Poe, M., Lin, C. S. & Sigal, N. H. (1989) *Nature (London)* **341,** 755–757.
12. Harding, M. W., Galat, A., Uehling, D. E. & Schreiber, S. L. (1989) *Nature (London)* **341,** 758–760.
13. Barsumian, E. L., Isersky, C., Petrino, M. G. & Siraganian, R. P. (1981) *Eur. J. Immunol.* **11,** 317–323.
14. Maeyama, K., Hohman, R. J., Metzger, H. & Beaven, M. A. (1986) *J. Biol. Chem.* **261,** 2583–2592.
15. Beaven, M. A. & Cunha-Melo, J. R. (1988) *Prog. Allergy* **42,** 123–184.
16. Maeyama, K., Hohman, R. J., Ali, H., Cunha-Melo, J. R. & Beaven, M. A. (1988) *J. Immunol.* **140,** 3919–3927.
17. Collado-Escobar, D., Cunha-Melo, J. R. & Beaven, M. A. (1990) *J. Immunol.* **144,** 244–250.
18. Fretz, H., Albers, M. W., Galat, A., Standaert, R. F., Lane, W. S., Burakoff, S. J., Bierer, B. E. & Schreiber, S. L. (1991) *J. Am. Chem. Soc.* **113,** 1409–1411.
19. Starzl, T. E., Fung, J., Venkataramman, R., Todo, S., Demetris, A. J. & Jain, A. (1989) *Lancet* **ii,** 1000–1004.
20. Jin, Y.-J., Albers, M. W., Lane, W. S., Bierer, B. E., Schreiber, S. L. & Burakoff, S. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** in press.
21. Kinet, J.-P. (1989) *Cell* **57,** 351–354.
22. Trenn, G., Taffs, R., Hohman, R. J., Kincaid, R., Shevach, E. M. & Sitkovsky, M. (1989) *J. Immunol.* **142,** 3796–3802.
23. Emmel, E. A., Verweij, C. L., Durand, D. B., Higgins, K. M., Lacy, E. & Crabtree, G. R. (1989) *Science* **246,** 1617–1620.
24. Granelli-Piperno, A., Nolan, P., Inaba, K. & Steinman, R. M. (1990) *J. Exp. Med.* **172,** 1869–1872.
25. Kronke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldmann, T. A. & Greene, W. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5214–5218.
26. Elliott, J. F., Lin, Y., Mizel, S. B., Bleackley, R. C., Harnish, D. G. & Paetkau, V. (1984) *Science* **226,** 1439–1441.
27. Plaut, M., Pierce, J. H., Watson, C. J., Hanley-Hyde, J., Nordan, R. P. & Paul, W. E. (1989) *Nature (London)* **339,** 64–67.
28. Wodnar-Filipowicz, A., Heusser, C. H. & Moroni, C. (1989) *Nature (London)* **339,** 150–152.
29. Burd, P. R., Rogers, H. W., Gordon, J. R., Martin, C. A., Jayaraman, K. S., Wilson, S. D., Dvorak, A. M., Galli, S. J. & Dorf, M. E. (1989) *J. Exp. Med.* **170,** 245–257.
30. Varshavsky, A. (1991) *Cell* **64,** 13–15.
31. Foxwell, B. M. J., Hiestand, P. C., Wenger, R. & Ryffel, B. (1988) *Transplantation* **46,** 35S–40S.
32. Wicker, L. S., Boltz, R. C., Matt, V., Nichols, E. A., Peterson, L. B. & Sigal, N. H. (1990) *Eur. J. Immunol.* **20,** 2277–2283.
33. Hunt, T. (1989) *Cell* **59,** 949–951.