© 1991 Oxford University Press

The immunosuppressives FK 506 and cyclosporin A inhibit the generation of protein factors binding to the two purine boxes of the interleukin 2 enhancer

Thomas Brabletz, Ilona Pietrowski and Edgar Serfling*
Institut für Virologie und Immunbiologie der Universität Würzburg, Versbacher Strasse 7, D-87 Würzburg, FRG

Received October 1, 1990; Revised and Accepted December 4, 1990

ABSTRACT

Like Cyclosporin A (CsA), the macrolide FK 506 is a potent immunosuppressive that inhibits early steps of T cell activation, including the synthesis of Interleukin 2 (Il-2) and numerous other lymphokines. The block of Il-2 synthesis occurs at the transcriptional level. At concentrations that block T cell activation, FK 506 and CsA inhibit the proto-enhancer activity of Purine boxes of the Il-2 promoter and the generation of lymphocyte-specific factors binding to the Purine boxes. Under the same conditions, the DNA binding of other Il-2 enhancer factors remains unaffected by both compounds. These results support the view that FK 506 and CsA, which both inhibit the activity of peptidylprolyl cis/trans isomerases, suppress T cell activation by a similar, if not identical mechanism.

INTRODUCTION

The cyclic oligopeptide Cyclosporin A, a product of the fungus Tolypocladium inflatum, is widely used in transplantation medicine. Due to its selective inhibitory effect on the synthesis of lymphokines it suppresses adaptive immune responses after organ transplantation, thus preventing graft rejection (1). The inhibition of lymphokine synthesis occurs at the level of gene transcription (2, 3). CsA selectively interferes with the transcription of early phase T cell activation genes, such as the Il-2, Il-3, Il-4, gamma-interferon, GM-CSF and TNF-α genes, while the activity of many other genes remains unaffected by CsA (3, 4).

In studies of the effect of CsA on the expression of the murine and human Il-2 genes we and others showed that CsA interferes with the activity of and factor binding to the two so-called Purine-(Pu-) box motifs of the Il-2 enhancer (5, 6, 7). These motifs, which share the decanucleotide AAGAGGAAAA, also occur in a very similar form within the promoters and enhancers of numerous other CsA-sensitive lymphokine genes (5). When cloned in multiple copies the Pu-boxes exhibit strong inducible T cell-specific proto-enhancer activity. Upon induction, Pu-box polymers activate linked test genes in T lymphoma cells, but not in B cells or in any non-lymphoid cells tested so far (5, 8).

FK 506, a macrolide isolated from Streptomyces tsukubaensis, exhibits an immunosuppressive activity like CsA, though it is structurally unrelated to CsA. Since FK 506 is about 100 fold more potent in its immunosuppressive action than CsA, it will probably be used in place of CsA in transplantation medicine. Detailed experimental studies have shown that FK 506 and CsA inhibit the transcription of the same set of genes involved in the early activation of T cells, including the transcription of the Il-2 gene (3).

In this study, we show that FK 506 and CsA suppress the activity of the same set of proto-enhancer elements involved in the establishment of T cell-restricted Il-2 enhancer function. Both compounds inhibit the generation of protein factors that bind to the two Pu-box motifs of the Il-2 enhancer, whereas the occurrence of numerous other Il-2 enhancer factors is not affected by both drugs.

MATERIALS AND METHODS

Cells, DNA transfections and CAT assays

Murine El4 T lymphoma cells, 70 Z/3 pre-B cells and the B lymphoma cells lines A 20, X16C8.5 and P3X63-Ag8.653 were grown in RPMI medium supplemented with 5% fetal calf serum to a density of about 4×10^5 cells per ml. Twenty μg DNA of plasmid constructs (purified by two CsCl density gradient centrifugations) were transfected into about 3.2×10^7 cells in a final volume of 2.4 ml using the DEAE dextran method. After transfection the cells were incubated for 3 min in 15% dimethylsulfoxide and, 20 hr later, they were divided. One quarter was used as the uninduced control, one quarter was induced with the phorbolester 12-O-tetradecanoyl-phorbol-13-acetate (TPA; 10 ng/ml) and the plant lectin Concanavalin A (Con A; 2.5 μg/ml), one quarter was incubated with TPA/Con A and 1 ng/ml FK 506, and one quarter with TPA/Con A and 100 ng/ml CsA. After further incubation for 20 hr, the cells were sonicated and their CAT activities were

* To whom correspondence should be addressed

determined as described (5). Murine L cells and human tk⁻HeLa cells were grown in DMEM medium supplemented with 5% fetal calf serum. DNA was transfected into these cells using the calcium phosphate precipitation technique.

DNA cloning

The recombinant DNA work was carried out according to standard procedures (10). The construction of all except three of the recombinant plasmids used in this work has been described (5, 9). They were:

5xTCEp: Chemically synthesized oligonucleotides spanning the Il-2 enhancer nucleotides from −158 to −188 and containing Hind III ends (Fig. 1B) were cloned in five copies into pBLCAT2 (four copies in the direction of the CAT gene, the proximal copy in the opposite direction).

5xUPS: Five copies of the upstream promoter site (UPS) from −64 to −94 containing Hind III ends (Fig. 1B) were introduced into pBLCAT 2 in the direction opposite to the transcription of the CAT gene.

pIlCAT 2/1-N,3X: Three copies of the Il-2 enhancer segment from −156 to −250 containg Xba I ends were cloned into pBLCAT2 (the distal one in the direction of CAT gene transcription, the other two in the opposite direction).

Protein binding assays

The extraction of whole cell protein extracts of El4 lymphoma cells, their fractionation on heparin-agarose columns and the isolation of Pu-box factors by affinity chromatography on DNA columns have been described (5, 9). 'Quick' nuclear extracts were prepared according to the procedure of Schreiber et al. (12). In the electrophoretic mobility shift assays (EMSA), 4 μg protein of extracts was incubated with about 5000 cpm (equivalent to about 0.2 ng) of radioactively labelled probes and 4 μg poly dI·dC as unspecific competitor as described (5, 12, 13). After 30 min incubation at 0° the samples were fractionated in 5% native polyacrylamide gels at 200 V/15 cm at room temperature. In proteolytic clipping band shift assays (13) the pre-formed DNA/protein complexes were treated prior to fractionation on EMSA gels with 0.2−1 unit Staphylococcus aureus V8 protease or 0.2−1 unit trypsin for 10 min at 20°C.

RESULTS

FK 506 and CsA inhibit the proto-enhancer activity of the Pu-box and UPS motifs

To test the effect of FK 506 and CsA on the activity of single Il-2 enhancer motifs, four enhancer motifs were chemically synthesized and cloned in multiple copies in front of the tk promoter into the CAT vector pBLCAT2 (11). The resulting Il-2 CAT plasmids were transfected into El4 T lymphoma cells, in parallel with further CAT plasmids containing the entire Il-2 enhancer or Il-2 enhancer mutants. Their activity was determined upon induction of El4 cells by TPA and Con A, in the absence or presence of 1 ng/ml FK 506 or 100 ng/ml CsA.

The sequences of Il-2 enhancer motifs and their localisation within the Il-2 enhancer is shown in Fig. 1. The two Pu-boxes are bound by identical lymphocyte-specific transacting factors which appear to be newly generated when T lymphoma cells are induced (Ref. 5 and Fig. 2). The 4×Pu-b_d CAT construct is strongly expressed after TPA, TPA/ConA or TPA/ionomycin induction in murine El4 cells and in Jurkat cells, a human T cell line (5, 6 and Fig. 1C), while it is inactive in non-T cells (see below).

The two so-called T cell elements, TCEp and TCEd (9), are bound by the ubiquitous factors AP-3 and NF-κB in vitro, but only TCEd is able to act as a proto-enhancer in El4 cells (Fig. 1C and Refs. 9, 14 and 15). In all our transfection experiments, TCEp constructs carrying five copies of TCEp turned out to be inactive upon induction (Fig. 1C). The inactivity of TCEp is correlated with its inability to bind TCF-1, a NF-kB like factor, which is bound to TCEd in protein extracts from El4 cells and is essential for the activity of TCEd in these cells (14, 15).

The two putative TPA-responsive elements of the Il-2 enhancer, TREp and TREd, were found to be binding sites for purified factor AP-1 and bacterially synthesized v-Jun in vitro, albeit with different affinities. TREp DNA binds AP-1 several fold stronger than TREd DNA, and using crude protein extracts in DNase I footprint protections experiments, only the TREp appeared to be a genuine AP-1 binding site (9). In crude extracts from induced El4 cells the same factor binds to the TCEp and the TREcoll, a well-defined AP-1 binding site in the Collagenase promoter (16), while the TREd is bound by a different factor (Fig. 2E and unpubl. results). In the transfection experiments we used a 5×TREcoll construct which is TPA-inducible in lymphoid and non-lymphoid cells (see Fig. 1C and Ref. 16).

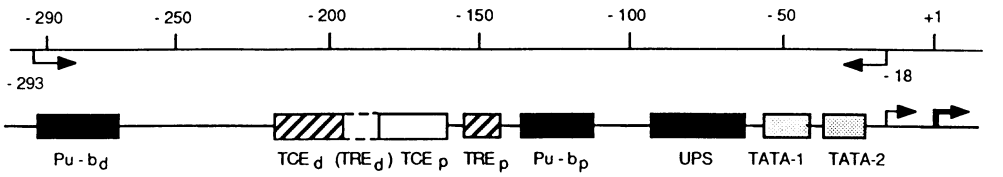
The upstream promoter sequences, UPS, harbour binding sites for numerous ubiquitous factors, including AP-1, AP-3 and Oct-1 (8, 9, 17, 18 and unpubl. results). The construct 5×UPS shows a strong activity in El4 cells (Fig. 1C) while it appeared to be inactive after transfection into non-T cells (not shown). This suggests that T cell-restricted and factors others than the ubiquitous factors AP-1, AP-3 and Oct-1, or factors in addition to the latter, are necessary to establish the activity of the UPS in T lymphoma cells.

The effect of FK 506 and CsA on the activity of proto-enhancer elements in TPA/ConA induced El4 cells is summarized in Fig. 1C. As we reported earlier for CsA (5), treatment of induced El4 cells with FK 506 strongly affected the activity of distal Pu-box, Pu-b_d. In the presence of FK 506 and CsA the expression of 4×Pu-b_d DNA decreased to less than 5% of the normal level. The activity of the TREcoll and TCEd plasmids, on the other hand, remained unaffected by CsA or FK 506. This suggests that the activity of AP-1, or of an AP-1 like factor, and TCF-1 is not influenced by either immunosuppressive. Apart from the Pu-b_d construct, the expression of the 5×UPS plasmid was also significantly inhibited by CsA and FK 506. The presence of drugs in the incubation medium led to a reduction of 5×UPS CAT activity to about 10% of normal activity (Fig. 1C). Thus, one (or several of the) factor(s) that takes part in the T cell-restricted activity of UPS sequences seems also to be a target for CsA and FK 506.

FK 506 and CsA prevent the inducible generation of factor complexes binding to the two Purine boxes

We recently reported that in protein extracts of TPA/Con A-induced El4 cells the two Purine boxes are recognized by three DNA binding factors, the occurrence of two of which appeared to be affected by CsA (Ref. 5, Fig. 2A and 3A). In these experiments, protein extracts of whole El4 cells were fractionated on heparin-agarose columns, and the major DNA binding fraction was used in the DNA-protein binding assays (5). The variations we observed when using these extract preparations prompted us to apply a very quick extraction procedure which allows the rapid

A. LOCALIZATION OF PROTEIN BINDING SITES WITHIN THE IL-2 ENHANCER:



B. PROTEIN BINDING MOTIFS:

Designation	Sequences
proximal Pu-box ($Pu\text{-}b_p$)	-145 -120 tcgaCAGAA GAGGAAAAAC AAAGGTAATG Cg
distal Pu-box ($Pu\text{-}b_d$)	-292 -264 tcgaCA AAGAGGAAAA TTTGTTTCAT ACAGAAG
proximal T cell element (TCEp)	-188 -158 agcTTCAGTCA GTGTATGGGG GTTTAAAGAA ATTa
distal T cell element (TCEd)	-215 -191 agcttGACCA AGAGGGATTT CACCTAAATCa
proximal TPA responsive element (TREp)	-157 -143 tcgaCCAGAGA GTCATCAG
distal TPA-responsive element (TREd)	-191 -178 tcgaC CATTCAGTCA GTG
upstream promotor site (UPS)	-96 -64 agcTTGAAA ATATGTGTAA TATGTAAAAC ATCGTGA
TPA responsive element from collagenase gene (TREcoll)	agcuGATGAGTCAGCCg

C. TRANSFECTION EXPERIMENTS:

Constructs	Induction Ratio (% of Acetylation)			No. of Experiments
	–	+CsA	+FK 506	
pII CAT 2/1+	13 (36)	1 (3)	0.6 (2)	2
4 x $Pu\text{-}b_d$	46 (22)	2 (0.9)	1 (0.4)	4
5 x UPS	119 (85)	16 (14)	10 (8)	3
5 x TCEd	37 (59)	50 (83)	37 (65)	3
5 x TREcoll	7 (9)	6 (7)	8 (10)	3
5 x TCEp	1 (0.2)	2 (0.2)	1 (0.2)	2
pII CAT 2/1-N3x	4 (2)	4 (2)	4 (2)	2

Figure 1. The effect of FK 506 and CsA on the inducible activity of the Il-2 enhancer in El4 T lymphoma cells. A. Scheme of the Il-2 enhancer. The position of the most prominent protein binding sites is indicated by boxes. The filled boxes indicate the FK 506- and CsA-sensitive proto-enhancer elements, the distal and proximal Purine-boxes, $Pu\text{-}b_d$ and $Pu\text{-}b_p$, and the upstream promoter sequences, UPS. The dashed boxes indicate the FK 506- and CsA-resistant proto-enhancer elements, namely the distal T cell element, TCEd, and the proximal TPA responsive element, TREp. The inactive proximal T cell-element, TCEp, is indicated by an open box; the two TATA boxes are indicated by stippled boxes. Although the TREd (in brackets) contains an AP-1 like binding sequence it did not bind any protein in DNase I footprint protection assays when crude protein extracts from El 4 cells were used (9). The activity of $Pu\text{-}b_p$ and TREp has been identified with deletion mutants (5,9), but so far not in proto-enhancer tests. B. Oligonucleotides used in the transfection experiments and DNA-protein binding studies. The nucleotides of recognition sites for restriction enzymes are written in lower case. C. FK 506 and CsA inhibit the inducible proto-enhancer activity of $Pu\text{-}b_d$ and UPS motifs. pILCAT 2/1+ contains the whole Il-2 enhancer sequences from position −7 to −293 (9). For the structure of other constructs and the transfection technique see Materials and methods and Refs. 5 and 9. The 'Induction Ratio' was calculated by dividing the percentage of chloramphenicol acetylation of induced cells (or of FK 506 and CsA treated cells) by the percentage of acetylation of uninduced cells. The '% of Acetylation' corresponds to the acetylated chloramphenicol determined for the induced cells.

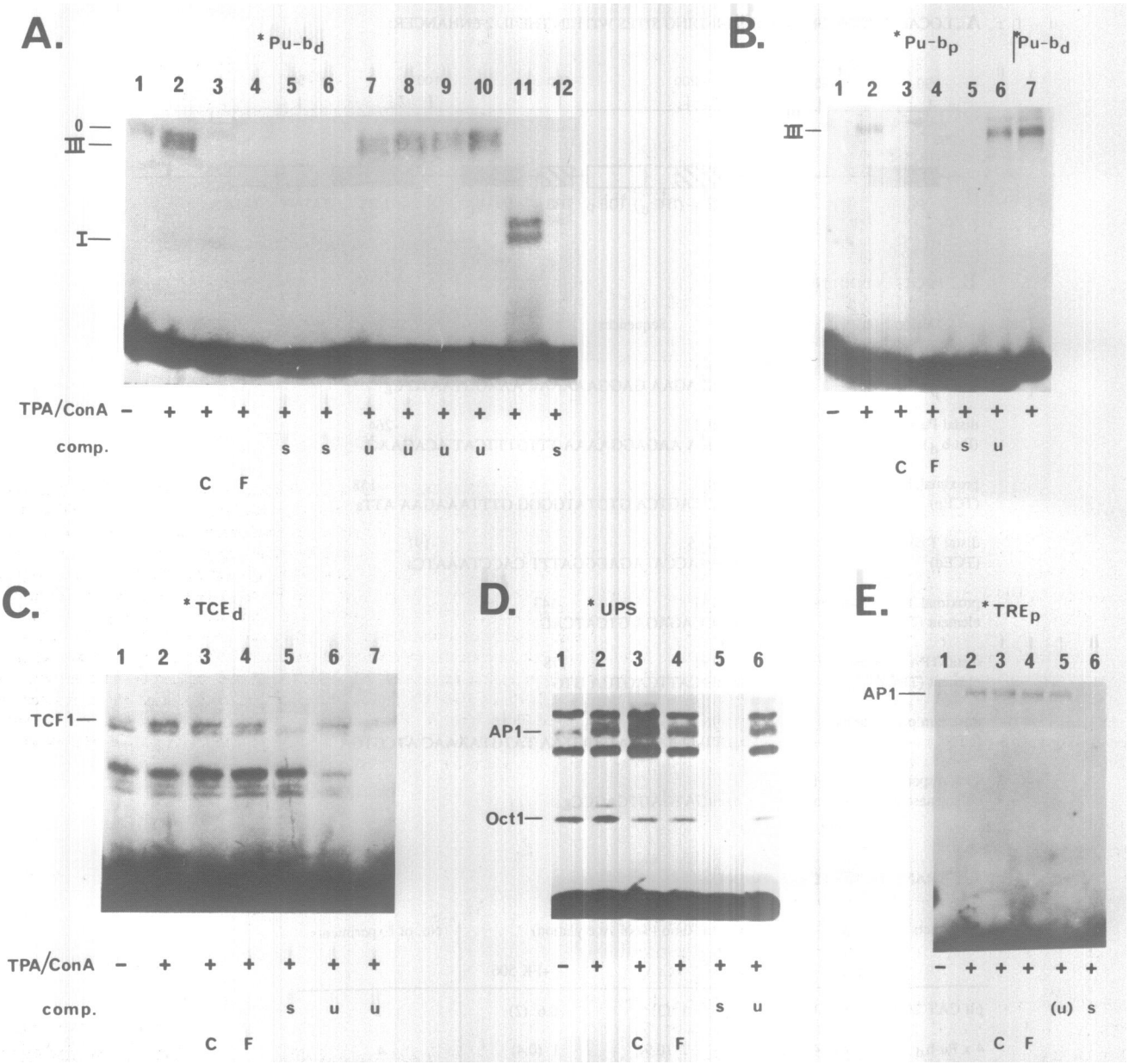


Figure 2. FK 506 and CsA suppress the generation of Pu-box factors, but not of factors binding to the TCEd, UPS and TREp motifs of the Il-2 enhancer. EMSA experiments. A. About 5000 cpm (equivalent to about 0.2 ng) of a Pu-b_d probe (Fig. 1B) was incubated with 4 μg protein of nuclear extracts from uninduced El4 cells (lane 1), from El4 cells induced with TPA/ConA (10 ng/2.5 μg per ml) for 4 h (lanes 2 and 5–10) or from induced cells treated with 100 ng/ml CsA (C; lane 3) or 1 ng/ml FK 506 (F; lane 4). For competition, 5 ng (lane 5) or 50 ng (6) unlabelled Pu-b_d DNA, 5 ng (7) or 50 ng TCEd (8) and 5 ng (9) or 50 ng UPS DNA (10) was added to the incubation reactions. In lanes 11 and 12, 4 μg protein from the 0.4 M KCl peak of a heparin/agarose column eluate of whole cell extract from induced El4 cells was used in the incubation mixture. In lane 12, 50 ng Pu-bd TCEd DNA was added for competition. s,u, specific and unspecific competition. Factor complexes 0, I and III are indicated. B. A Pu-b_p probe (Fig. 1B) was incubated with 4 μg protein of nuclear extracts from uninduced El4 cells (lane 1), induced El4 cells (lanes 2, 5 and 6) or from induced cells treated with CsA (lane 3) or FK 506 (lane 4). In lanes 5 and 6, 50 ng unlabelled Pu-b_p or TCEd DNA was added, respectively. In lane 7, a Pu-b_d probe was incubated with nuclear protein from induced El4 cells for comparison. C. A TCEd probe (Fig. 1B) was incubated with nuclear extracts from uninduced El4 cells (lane 1), induced El4 cells (lanes 2 and 5–7) or from induced cells treated with CsA (3) or FK 506 (4). In lanes 5–7, 50 ng unlabelled TCEd (5), Pu-b_d (6) or UPS DNA (7) was added for competition. The position of factor TCF-1 (Refs. 14 and 15) is indicated. D. A UPS probe (Fig. 1B) was incubated with nuclear extracts from uninduced El4 cells (lane 1), induced El4 cells (lanes 2, 5 and 6) or from induced cells treated with CsA (3) or FK 506 (4). In lanes 5 and 6, 50 ng unlabelled UPS (5) or Pu-bd DNA (6) was added for competition. The putative complexes of UPS DNA with factors AP-1 and Oct-1 are indicated. They were identified by co-electrophoresis of complexes generated by incubating a UPS probe with enriched AP-1 from El4 cells (Ref. 9) and Oct-1 from HeLa cells (a kind gift from N. Dathan and I. Mattaj). E. A TREp probe (Fig. 1B) was incubated with nuclear extracts from uninduced El4 cells (lane 1), induced El4 cells (lanes 2, 5 and 6) or from induced cells treated with CsA (3) or FK 506 (4). In lanes 5 and 6, 50 ng TREd or TREp DNA was added for competition. s, specific competition; (u), 'unspecific' competition with TREd DNA.

and gentle isolation of Pu-box (and other DNA binding) factors from El4 cell nuclei (12). Using such nuclear extracts in electrophoretic mobility shift assays (EMSA), the Pu-box factor III appeared to be the most prominent factor binding to both Pu-boxes. As shown in Fig. 2A and B, the generation of factor complex III depends on the induction of El4 cells, and its formation is inhibited by 1 ng/ml FK 506 or 100 ng/ml CsA. In the nuclear extracts of uninduced EL4 cells another, less

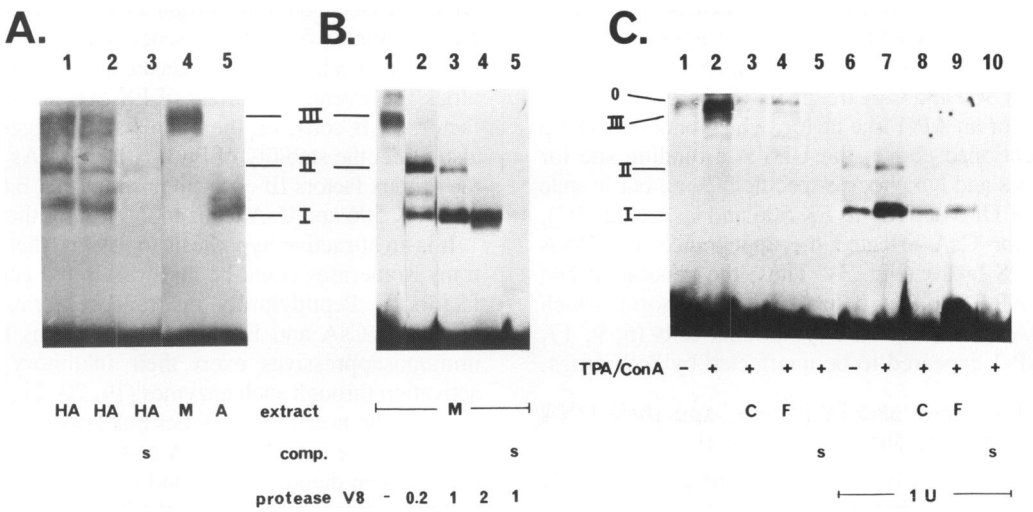


Figure 3. Demonstration by means of proteolytic clipping that Pu-box factor complexes I and II are part of the larger factor complexes III and 0. A. The pattern of Pu-box factors differed according to the mode of their extraction. A Pu-b_d probe was incubated with 4 μg protein from two eluate fractions of the 0.4 M KCl peak of a heparin/agarose column on which protein from a whole cell extract of induced El4 cells (lanes 1 and 2) was fractionated; with a crude nuclear 'mini' extract of induced El4 cells prepared according to Schreiber et al. (12) (lane 4), or with purified Pu-box factors isolated by affinity column with Pu-b_d oligonucleotides (5). In lane 3, 50 ng unlabelled Pu-b_d was added to the incubation mixture of lane 1 for competition. s, specific competition; HA, extract fractionated on a heparin-agarose column; M, nuclear 'mini' extract; A, Pu-box factors enriched by affinity chromatography. The position of the Pu-box factor complexes is indicated. B. Proteolytic clipping band shift assay of Pu-box factors. About 20,000 cpm (equivalent to about 0.8 ng) Pu-b_d probe was incubated with 20 μg protein of nuclear extracts of induced El4 cells (designated M). After incubation for 30 min, 0.2 units (lane 2) or 1 unit (lanes 3 and 5) or two units (lane 4) of V8 protease was added, and the samples were digested for 10 min at room temperature followed by electrophoresis on an EMSA gel. In lanes 5, 50 ng Pu-b_d DNA was added for specific (s) competition. C. Proteolytic clipping band shift assay with Pu-box factors in nuclear extracts of induced El4 cells and induced cells treated with CsA or FK 506. A Pu-b_d probe was incubated with nuclear extracts of uninduced El4 cells (lanes 1 and 6), induced El 4 cells (lanes 2,5,7 and 10) or induced cells treated with CsA (lanes 3 and 8; designated C) or FK 506 (lanes 4 and 9; designated F). In lanes 6–10, after the incubation, one unit V8 protease was added, and the samples were digested for 10 min followed by electrophoresis on an EMSA gel. Note the similar protease digestion patterns of protein-DNA complexes from induced and CsA/FK 506 treated cells. In lanes 5 and 10, 50 ng Pu-b_d DNA was added for specific (s) competition.

prominent complex, complex 0, was observed which is also found in extracts of FK 506 and CsA treated cells (Figs. 2A and 3C).

The differences in the factor patterns between different extract preparations (Fig. 3A) prompted us to investigate whether the large Pu-box factor complex III could be converted to the smaller complexes I and II by protease digestion. In proteolytic clipping band shift assays (13) using Pu-box probes and nuclear El4 extracts or El4 extracts fractionated on heparin-agarose columns, we treated the generated protein-DNA complexes with V8 protease or trypsin. Untreated and protease-treated incubations were then fractionated on EMSA gels. The autoradiographs of these gels showed that V8 digestion converted complex III to complexes with the electrophoretic mobilities of complexes I and II (Fig. 3B), whereas trypsin treatment resulted in the appearance of complex I-like factor complexes (not shown). These results suggest that proteolytic degradation led to the occurrence of factors I and II in our former extract preparations and favour factor(s) III to be the native Pu-box factor(s).

Mild V8 protease treatment of complex 0 in extracts of uninduced or induced El4 cells treated with FK 506 or CsA also resulted in the appearance of factor complexes I and II (Fig. 3C). This leads to the hypothesis that complex 0 corresponds to an (inactive) precursor of complex III, and that FK 506 and CsA inhibit the conversion of complex 0 to complex III (see Discussion).

FK 506 and CsA do not interfere with the generation and DNA binding of other Il-2 enhancer factors

In the EMSA assays with the TCEd, TREp and UPS probes no differences were observed between the nuclear extracts of induced control cells and induced cells treated with FK 506 or CsA (Fig.

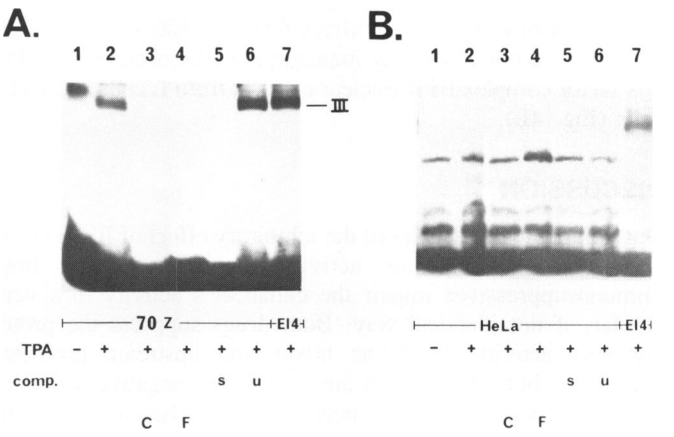


Figure 4. Detection of Pu-box factor complex III in 70 Z/3 pre-B cells. EMSA experiments. A. Analysis of protein extracts from 70 Z/3 pre-B cells. About 5000 cpm (equivalent to about 0.2ng) of a Pu-b_d probe was incubated with 4 μg protein from nuclear extracts of uninduced 70 Z/3 pre-B cells (lane 1), 70 Z cells induced with 25 ng TPA for 4 hr (lanes 2, 5 and 6), and induced 70 Z cells treated with CsA (lane 3) or FK 506 (lane 4). In lane 7, nuclear extract of induced El4 cells was incubated with the Pu-b_d probe for comparison. For specific (s) and unspecific competition (u), 50 ng Pu-b_d (lane 5) or TCEd DNA (lane 6) was added. Note the appearance of factor complex III in the extract of uninduced B cells, and the disappearance of complex III in CsA- and FK 506-treated cells. B. Analysis of protein extracts from tk^-HeLa cells. The Pu-b_d probe was incubated with nuclear extract of uninduced tk^-HeLa cells (lane 1), HeLa cells induced with 50 ng/ml TPA for 4 hr (lanes 2, 5 and 6), and induced HeLa cells treated with CsA (lane 3) or FK 506 (lane 4). Fifty ng Pu-b_d (lane 5) or TCEd DNA (lane 6) were added for specific (s) and unspecific competition (u). Lane 7: nuclear extract of induced El 4 cells.

2C, D and E). Among the TCEd-binding factors, one factor (probably TCF-1; Refs. 14 and 15) appeared upon induction, but in contrast to the Pu-box factors its occurrence turned out to be resistant to FK 506 and CsA treatment (Fig. 2C). The same was true for AP-1, or an AP-1 like factor, which bound to TREp (Fig. 2E). As mentioned above, the UPS is a binding site for numerous ubiquitous and lymphocyte-specific factors, but in spite of the sensitivity of UPS activity to FK 506 and CsA (Fig. 1C), neither FK 506 nor CsA affected the appearance and DNA binding of any UPS factor (Fig. 3). Thus, the generation and DNA-binding of AP-3 and Oct-1 (or of similar factors), which bind to UPS DNA in extracts of T lymphoma cells (6, 9, 17, 18), as well as AP-1 appeared to be unaffected by both drugs.

The Pu-box factors occur also in B cells, and their DNA binding is affected by FK 506 and CsA in these cells

The Pu-box construct 4×Pu-b_d exhibited strong activity in El4 T lymphoma cells (Fig. 1C), but showed only a very weak basic, uninducible and FK506+CsA-resistant expression in non-T cells. This was found after transfecting 4×Pu-b_d DNA into the murine pre-B cell line 70 Z/3 and murine B cell lymphoma lines A20 and X16C8.5, as well as into murine L cells and human tk^-HeLa cells (unpubl. results). The lack of any protection of Pu-boxes using column-fractionated extracts of 70 Z cells in DNase I footprint protection experiments and complex III led us to conclude that Pu-box factors III correspond to T cell-specific factors (5). Similar results have been published by Shaw et al. (8, see also 18). However, using 'quick' nuclear extracts in EMSA experiments, we detected Pu-box factor complex III in all B cell lines tested (i.e. in 70 Z/3 pre-B cells and the B cell lymphoma lines A20, X16C8.3 and P3X63-Ag8.653)(Fig. 4A and unpubl. results). In contrast to their absence in uninduced El4 cells, factor complex III is present in uninduced B cells, and their DNA binding is again affected by FK 506 and CsA (Fig. 4A). We did not obtain any indication for the occurence of Pu-box factor complex III in nuclear extracts from L cells and HeLa cells (Fig. 4B).

DISCUSSION

Our comparative analyses of the inhibitory effect of FK 506 and CsA on Il-2 enhancer activity demonstrate that both immunosuppressives impair the enhancer's activity in a very similar, if not identical way. Both drugs suppress the proto-enhancer activity of Purine boxes and upstream promoter sequences, but do not exert any significant negative influence on the activity of other enhancer elements. The concentrations at which FK 506 and CsA exert their inhibitory effect on the Il-2 enhancer correspond to those at which they block T cell activation and function. Thus, it is very likely that FK 506 and CsA suppress T cell activation by inhibiting the function of transacting factors that bind to the enhancer of the Il-2 gene and the promoters and enhancers of other lymphokine genes.

One target for FK 506 and CsA are the Pu-box factors. These factors (also named NFAT-1: Refs. 6, 8 and 18) seem to be newly generated when T lymphoma cells are induced, since inhibition of RNA and protein synthesis block their appearance (5, 8). These findings led us to conclude that CsA might inhibit the synthesis of these factors, including their putative processing and/or transport from the cytoplasm to the nucleus (5). The detection of factors (complex 0) larger than the (native) factors III (Figs. 2A and 3C) in nuclear extracts of uninduced cells suggests that upon induction a factor precursor (complex 0) is transformed into an active DNA-binding complex (complex III). The presence of factor complex 0 and the absence of complex III in FK 506 and CsA treated cells seems to indicate that both immunosuppressives block this event. The effect of FK 506 and CsA on the Pu-box factors in B cells, on the other hand, suggests that both drugs also affect the stability of Pu-box factors. As shown in Fig. 4A, the Pu-box factors III occur in unstimulated B cells, and treatment with FK 506 and CsA leads to the loss of their binding activity.

It is an attractive hypothesis to assume that peptidylprolyl cis-trans isomerases could be involved in the generation of Pu-box factors III. Peptidylprolyl cis-trans isomerases are the common target for CsA and FK 506, and it seems likely that the two immunosuppressives exert their inhibitory effect on T cell activation through such enzymes (19, 20, 21, 22, 23). However, it should be noted that a direct link between nuclear events and the isomerase activity of CsA and FK 506 binding proteins has not yet been demonstrated, and it remains to be shown directly that these cytosolic proteins actually play a role in the generation of Pu-box factors. It is also conceivable that other enzymes, such as proteases, are involved in the putative processing of Pu-box factors, and that FK 506 and CsA could inhibit their activity.

The occurrence of Pu-box factors III in B cells, in which the Pu-boxes are inactive (as is the whole Il-2 gene), suggests that more factors than detected in our DNA-protein binding assays are involved in the control of Pu-box activity in T cells. The same seems to be true for the UPS sequences, since although the proto-enhancer activity of the UPS motif is inhibited by FK 506 and CsA, no changes in the UPS factor patterns were detected after treatment with FK 506 or CsA (Fig. 2D). Such factors could also be a target for FK 506 and CsA.

ACKNOWLEDGEMENTS

The authors are very much indebted to J.F. Borrell (Sandoz,Basel) for a gift of Cyclosporin A, G. Fischer (Halle/Saale) for FK 506 and N. Dathan and I. Mattaj (Heidelberg) for Oct-1 from HeLa cells. For critical reading of the manuscript we wish to thank K. Erb, W.K. Joklik and A. Schimpl. This work was supported by grants from the Deutsche Forschungsgemeinschaft, SFB 165, and the Bundesgesundheitsamt, research program AIDS, grant no. II-087-89.

REFERENCES

1. Kahan,B.D. (1989) New England J.Medic. 321, 1725–1738.
2. Krönke,M., Leonard,W.J., Depper,J.M., Arya,S.K., Wong-Staal,F., Gallo,R.C., Waldmann,T.A. and Greene,W.C. (1984) Proc. Natl. Acad. Sci. USA 81, 5214–5218.
3. Tocci,M.J., Matkovich,D.A., Collier,K.A., Kwok,P., Dumont,F., Lin,S., Degudicibus,S., Siekierka,J.J., Chin,J. and Hutchinson, N.I. (1989) J. Immunol. 143, 718–726.
4. Zipfel,P.F., Irving,S.G., Kelly,K. and Siebenlist,U. (1989) Mol.Cell Biol. 9, 1041–1048.
5. Randak,C., Brabletz,T., Hergenröther,M., Sobotta,I. and Serfling,E. (1990) EMBO J. 9, 2529–2536.
6. Emmel,E.A., Verweij,C.L., Durand,D.B., Higgins,K.M., Lacy,E. and Crabtree,G.R. (1989) Science 226, 1439–1441.
7. Schmidt,A., Hennighausen,L. and Siebenlist,U. (1990) J. Virol. 64, 4037–4041.
8. Shaw,J.P., Utz,P.J., Durand,D.B., Toole,J.J., Emmel,E.A. and Crabtree,G.R. (1988) Science 241, 202–205.
9. Serfling,E., Barthelmäs,R., Pfeuffer,I., Schenk,B., Zarius,S., Swoboda,R., Mercurio,F. and Karin,M. (1989) EMBO J. 8, 465–473.
10. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning. A Laboratory Manual. 2.Edition. Cold Spring Harbor Laboratory Press. Cold Spring Harbor,N.Y.
11. Luckow,B. and Schütz,G. (1987) Nucleic Acids Res. 15, 5490.

12. Schreiber,E., Matthias,P., Müller,M.M. and Schaffner,W. (1989) Nucleic Acids Res. 17, 6419.
13. Schreiber,E., Matthias,P., Müller,M.M., and Schaffner,W.(1988) EMBO J. 7, 4221–4229.
14. Radler-Pohl,A., Pfeuffer,I., Karin,M. and Serfling,E. (1990), The new biologist 2, 566–573.
15. Briegel,K., Hentsch,B., Stricker,K., Falk,W. and Serfling,E. (1990) submitted for publication.
16. Angel,P., Imagawa,M., Chiu,R., Stein, P., Imbra,R.J., Rahmsdorf,H.J., Jonat,C., Herrlich,P. and Karin,M. (1987) Cell 49, 729–739.
17. Shibuya,H. and Taniguchi,T. (1989) Nucleic Acids Res. 17, 9173–9184.
18. Ullman,K.S., Northrop, J.P., Verweij,C.L. and Crabtree,G.R. (1990) Annu.Rev.Immunol. 8, 421–452.
19. Fischer,G., Wittmann-Liebold,B., Lang,K., Kiefhaber,T. and Schmid,F.X. (1989) Nature 337, 476–478.
20. Takahashi,N., Hayano,T. and Suzuki,M. (1989) Nature 337, 47–475.
21. Tropschug,M., Wachter,E., Mayer,S., Schönbrunner,E.R. and Schmid,F.X. (1990) Nature 346, 674–677.
22. Standaert,R.F., Galat,A., Verdine,G.L. and Schreiber,S.L. (1990) Nature 346, 671–674.
23. Maki,N., Sekiguchi,F., Nishimaki,J., Miwa,K., Hayano,T., Takahashi,N. and Suzuki,M. (1990) Proc.Natl.Acad.Sci. USA 87, 5440–5443.