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Comparison of the effect of FK506 and cyclosporin A on virus production in H9 cells chronically and newly infected by HIV-1

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Summary. The presence of FK506-binding protein-12 was demonstrated in virions of HIV-1, although its concentration was lower than that of cyclophilin A. The effect of two inhibitors of the peptidyl-prolyl *cis-trans* isomerases FK506 and cyclosporin A (CsA) was studied in H9 cells that were chronically infected by HIV-1. Both drugs inhibited virus production in the infected cells in a concentration-dependent manner, by decreasing the number of the producing cells. FK506 did not have an effect on Gag processing, based on the p24 antigen content of virions produced in the presence of this drug. Furthermore, FK506 treatment of uninfected H9 cells did not diminish their susceptibility toward HIV-1 infection, whereas CsA treatment decreased the degree of HIV-1 infection with an IC_{50} of 1–2 μg/ml. Also, pretreatment of the virus with CsA decreased its infectivity in HeLaCD4-LTR/β-gal cells; in contrast, at concentrations up to 10 μg/ml, FK506 did not have an effect. Our findings on the antiviral activity of FK506 and CsA suggest that FK506 is effective only in chronically infected cells, by selectively inhibiting the growth of HIV-1 infected cells, whereas CsA has a specific effect on virus replication.

Introduction

Member of two distinct classes of peptidyl-prolyl *cis-trans* isomerases, FK506-binding proteins (FKBPs) and cyclophilins, catalyze the isomerization of the amino acid-proline bond, and can accelerate the folding of proline-containing peptides both in vitro and in vivo [8]. Two well-studied members of these families are the 12-kDa FKBP-12 and the 18-kDa cyclophilin A (CyPA). Their isomerase

activity is inhibited by FK506 and cyclosporin A (CsA), respectively. Complexes of FKBP-12/FK506 as well as CypA/CsA disrupt T-cell signal transduction pathways by inhibiting calcineurin, a Ca^{2+}-dependent phosphoprotein phosphatase, leading to an immunosuppression that is capable of preventing tissue rejection in organ tansplantations [8].

We have been investigating the specificity of human immunodeficiency virus type 1 (HIV-1) proteinase by kinetic and modeling studies using oligopeptide substrates. Several cleavage sites in HIV-1 contain tyrosine (or phenylalanine) and proline at the site of cleavage [18]. Proline residues, especially those found after tyrosine and phenylalanine in the sequence, have a relatively high probability of forming the *cis* isomer rather than the *trans* ismer of the preceding peptide bond [14], and molecular modeling studies have indicated that the *trans* rather than the *cis* isomer provides a better fit into the substrate-binding pocket [24]. From these findings, we inferred that isomerization at proline in the retroviral cleavage sites may be involved in triggering the maturation events [24]. Subsequently, the conformational selectivity of HIV-1 proteinase (PR) toward the *trans* isomer of the cleaved peptide bond was demonstrated by nuclear magnetic resonance (NMR) and kinetic studies [15, 25]. The presence of CyPA has been demonstrated in HIV virions [7, 19, 23]. Interestingly, CyPA has a low specificity, whereas the specificity of FKBPs is much higher and one of their best substrate sequences contains-Phe-Pro- [10], characteristic of HIV-1 PR cleavage sites. However, the presence of FKBPs in virions has not been previously reported.

To elucidate the putative role of FKBP in the life cycle of HIV, we studied its incorporation into virions and used its inhibitor, FK506, to detect FKBP's effect on virus production in chronically and newly infected cells, as well as its effect on polyprotein processing in the virions.

Materials and methods

Cells, viruses, and immunosuppressive drugs

The human T-cell lymphoma cell line H9 (ATCC HTB 176) was obtained from American Type Culture Collection (Manassas, VA) and was cultured in RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS), 100 mg/ml streptomycin, 100 U/ml penicillin, and 2mM L-glutamine (all from Life Technologies, Inc., Gaithersburg, MD). H9 cells were infected with HIV-1_{IIIB}. HeLaCD4-LTR/β-gal cells were obtained from Michael Emerman and were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, containing 0.1 hygromycin B and 0.2 mg/ml G418. CsA and FK506 were obtained from Sandoz Pharmaceuticals (Basel, Switzerland) and Fujisawa (Deerfield, IL), respectively.

Detection of FKBP-12 in HIV-1 virions

HIV-1_{NL4-3} isolated from acutely infected H9 cells was treated and analyzed as previously described [19]. Briefly, equal amounts of virus (10 mg of total protein purified by sucrose density centrifugation) were incubated in a 20 mM Tris-HCl (pH 8.0) buffer containing 1 mM $CaCl_2$ for 18 h at 37 °C in the presence or absence of 2 mg/ml subtilisin (Boehringer Mannheim, Indianapolis, IN). The treated virions were then separated from the digestion mixture and protein fragments by sucrose density centrifugation as previously described [19]. After treatment, proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE),

transferred to a polyvinylidene difluoride membrane, and stained with antibodies as previously described [19]. The polyclonal anti-FKBP-12 antibody was a kind gift from Gregory Wiederrecht (Merck & Co., Inc., Rahway , NJ); the monoclonal antibody to the capsid antigen (anti-$p24^{CA}$) used was developed by the AIDS vaccine program, SAIC, NCI-FCRDC, Frederick, Maryland.

Effect of FK506 and CsA treatment on the growth of chronically infected cells

Choronically infected H9 cells (H9/HIV-1_{IIIB}) were washed twice with phosphate-buffered saline, pelleted, and resuspended at a concentration of 5×10^4 cells/ml in culture medium containing various concentrations of FK506 or CsA. After 3 days of incubation at 37 °C, virus-containing supernatants were clarified by centrifugatiion (1 100 **g** for 25 min at 4 °C). The supernatants were then passed through a 0.45-μm filter and virus production was quantitated by reverse transcription assay, performed as described by Popovic et al. [21]. Equal aliquots of cells were removed, stained with trypan blue, and counted.

Metabolic labeling, immunoprecipitation and electrophoresis of viral proteins

H9/HIV-1_{IIIB} cells (10^6) were pelleted at 180 **g** for 10 min at 4 °C, and resuspended in methionine-deficient RPMI 1640 containing 10% dialyzed FCS. The cells were incubated for 45 min, then labeled for 45 min in 2 ml of methionine-free RPMI 1640 with 10% dialyzed FCS containing 200 μCi [^{35}S]methionine (>800 Ci/mmol). The cells were washed, pelleted, and resuspended in growth medium containing 0–10 μg/ml FK506 and incubated for 72 h. Viral supernatants were collected, clarified at 1 100 **g** for 25 min and centrifuged at 104 000 **g** for 1.5 h through a 20% sucrose cushion to form a viral pellet. Viral lysates were prepared by the addition of 200 μl of cold lysis buffer [0.05 M Tris-HCl pH8.3), 0.15 M NaCl, 0.5% sodium deoxycholate, 1% NP-40, 0.1% SDS, 100 μg/ml phenylmethylsulphonyl fluoride, 1 μg/ml aprotinin, 0.02% sodium azide] to the viral pellet. After 1 h at 4 °C, an equal aliquot of each (50 000 cpm) was used for immunoprecipitation. The lysates were cleared with preimmune goat serum, then immunoprecipitated with goat anti-HIV-1 $p24^{CA}$ serum and protein A-Sepharose CL-4 overnight at 4 °C. The immunocomplexes were washed three times with lysis buffer and once with deionized water, then subjected to SDS-PAGE and autoradiography as described previously [5].

Effect of FK506 and CsA on HIV-1_{IIIB} infection of H9 cells

H9 cells (10^6) were incubated for 1 h at 37 °C, in the presence of FK506 or CsA. After a 1-h incubation with HIV-1_{IIIB} [0.01 mulitiplicity of infection (MOI)] with agitation, the cells were washed three times and resuspended in growth medium containing the same concentration of FK506 and CsA that was used in the pretreatment (final concentration: 5×10^4 cells/ml). As a control, H9 cells untreated with drugs prior to HIV-1 infection were handled in the same manner. Samples were removed on days 2, 5, 7, 9, and 12 for the reverse transcriptase (RT) assay, performed as described by Popovic et al. [21], and for HIV-1 $p24^{CA}$ antigen quantitation, performed by ELISA as described by Nagy et al. [16].

Effect of FK506 and CsA on HIV-1_{IIIB} infection of HeLa CD-4-LTRβ-gal cells

Cells (4×10^4 cells/well) were seeded in 24-well culture plates (Corning Costar Corp., Cambridge, MA) 18 h before infection with HIV-1_{IIIB}. The virus stock was treated with various concentrations of FK506 or CsA for 1 h at 37 °C, before infection. Then, a mixture of 10 μl of treated virus and 190 μl of culture medium containing 20 mg/ml-DEAE -dextran (Promega, Madison, WI) was added to the indicator cells (0.01 MOI). After a 2-h adsorption at 37 °C,

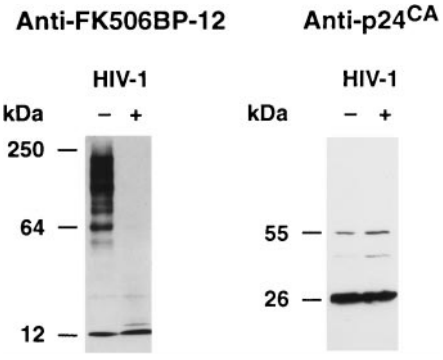

Fig. 1. Immunoblot analysis of HIV-1 virus preparations. HIV-1_{NL4-3} isolated from acutely infected H9 cells was incubated in the presence (+) or absence (−) of subtilisin and analyzed by SDS-PAGE and immunoblotting as described in Materials and methods. The antibodies used in immunoblotting are indicated above each blot and the molecular mass in kilodaltons (determined by the relative mobility compared with standards) is indicated on the left

fresh medium containing the drugs was added to each well and the cells were incubated for an additional 40–48 h. Cells were fixed and stained with X-gal as described [12]. Infected (blue) cells were scored by light microscopy.

Results

The presence of FKBP-12 inside the virion was revealed by immunoblot analysis (Fig. 1). Previous studies have shown that virion preparations also contain cellular proteins, mostly in the form of protein-laden microvesicles [20]. These contaminants can be removed from virions by subtilisin treatment followed by density gradient centrifugation [19]. The immunoblot of subtilisin-treated and mock-treated virions developed with the anti- FKBP-12 antibody showed very similar amounts of FKBP-12 in the samples (Fig.1). However, there was a significant amount of high-molecular-weight material that reacted with the antiserum in the mock digestioin but was not present in the treated sample, pesumably because this material was eliminated by the subtilisin treatment. The identity of these bands is unknown. Because FKBP-12 is a member of a large family of proteins, some higher molecular weight forms of FKBP might have been present in the virus preparation but not inside the virus itself. To demonstrate that the internal virion proteins are not lost using this procedure, we analyzed equal amounts of mock-treated and subtilisin-treated material for $p24^{CA}$ content. Our results demonstrated that the level of $p24^{CA}$ in the samples did not change after the protease digestion (Fig.1). Using the same methodology that we previously applied for the detection of CyPA [19], we confirmed the presence of FKBP-12 inside the virions by high-pressure liquid chromatography studies and protein sequencing of the proteins from subtilisin-treated HIV-1_{MN} (data not shown). Our data showed that FKBP-12 was present in approximately 1% of the molar amount of Gag, as compared with the amount of $p17^{MA}$ present. Assuming that there were 2500 Gag molecules per virion, we found an average of 25 FKBP-12 molecules in each virus, as compared with the estimated 250 molecules of CyPA [7, 19, 23].

To study the possible effect of FKBP-12 and CyPA on virus production of chronically infected cells, we treated H9/HIV-1_{IIIB} cells with increasing amounts of their inhibitors FK506 and CsA (Fig. 2A). Virus production, measured as RT

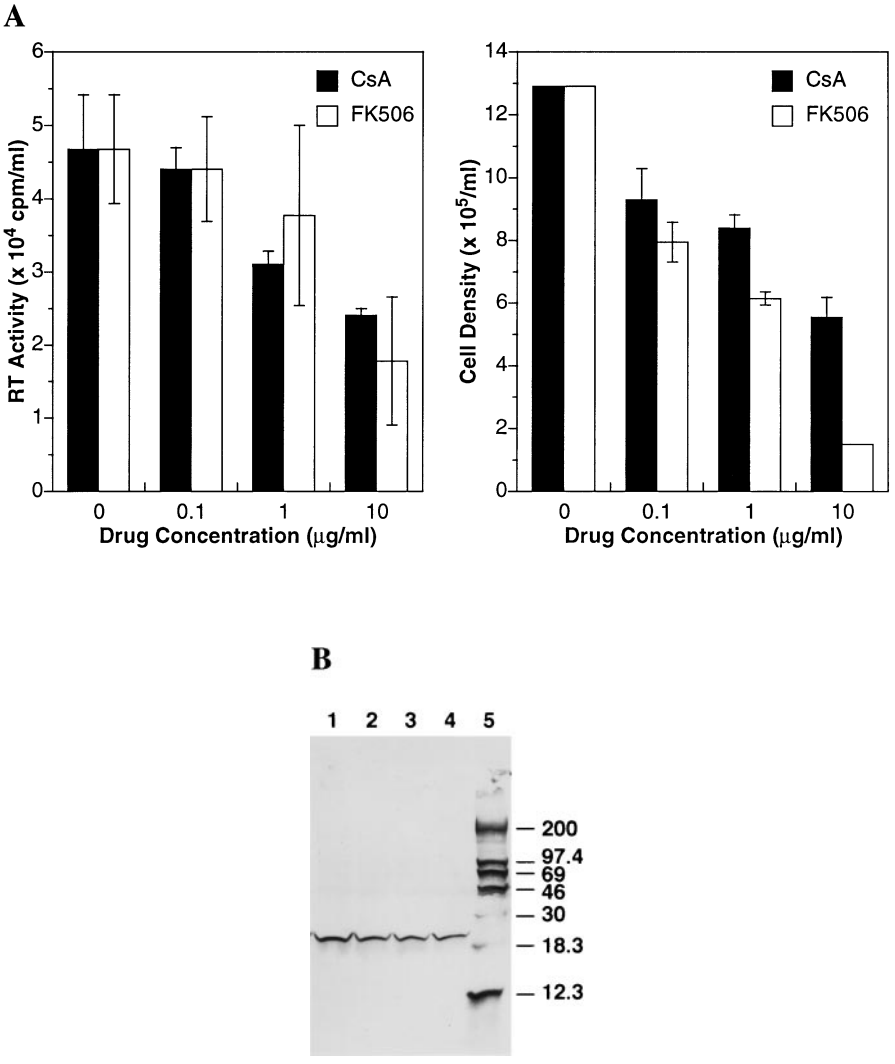

Fig. 2. Effect of FK506 and CsA on H9 cells chronically infected with HIV_{IIIB}. **A** H9/HIV-1_{IIIB} cells were resuspended in culture medium containing FK506 (open bars) and CsA (solid bars) at the indicated concentrations. After 3 days of incubation at 37 °C, the cell density was determined by staining with trypan blue, and virus production was quantitated by reverse transcriptase assays. The results shown are the average of two experiments. **B** H9/HIV-I_{IIIB} cells were labeled for 45 min with [^{35}S] methionine. The cells were washed, pelleted, resuspended in growth medium containing FK506, and incubated for 72 h. Viral supernatants were purified by centrifugation through a 20% sucrose cushion, then lysed; an equal aliquot of each sample (5000 cpm) was used for immunoprecipitation and autoradiography as described in Materials and methods. The FK506 concentrations used during the incubation are as follows: *1* 0 μg/ml; *2* 0.1 μg/ml; *3* 1 μg/ml; 4 10 μg/ml; *5* shows molecular weight markers

activity in cell culture supernatant, was decreased in a concentration dependent manner (Fig. 2A). Although FK506 and CsA did not have a measurable cytotoxic effect on noninfected cells at concentrations up to 10 μg/ml (data not shown), they substantially inhibited cell growth of chronically infected cells (Fig. 2A). To determine the effect of FK506 on Gag processing, [S^{35}] methionine-labeled viral lysate from H9/HIV-1_{IIIB} cells was immunoprecipitated with anti-$p24^{CA}$ antibody and analyzed by SDS-PAGE, similar to our previous studies of HIV-1 with CsA [4]. Although the amount of $p24^{CA}$ decreased in parallel with the level of cell growth inhibition, it did not result in an attendant appearance of unprocessed $Pr55^{gag}$, suggesting that the polyprotein processing in the lysates of FK-506-treated cells was uninhibited (Fig. 2B). These results are very similar to our findings on the effect of CsA virus production in H9/HIV-1_{IIIB} cells [5].

To compare the effect of FK506 and CsA on virus production in newly infected cells, we pretreated H9 cells with increasing concentrations of CsA and FK506, followed by infection with HIV-1. Infected cells were further cultured in the presence of these drugs, and virus production was measured as RT activity and $p24^{CA}$ antigen level in the cell supernatant up to 12 days. The drugs were used at concentrations up to 10 μg/ml, which were found to be nontoxic to the cells as ascertained by trypan blue exclusion (data not shown). Our results showed a dose-dependent reduction in both the RT activity and $p24^{CA}$ content of the supernatants of CsA treated cells, starting from day 5. As an example, results obtained 9 days after infection are shown in Fig. 3. Similar to the effect

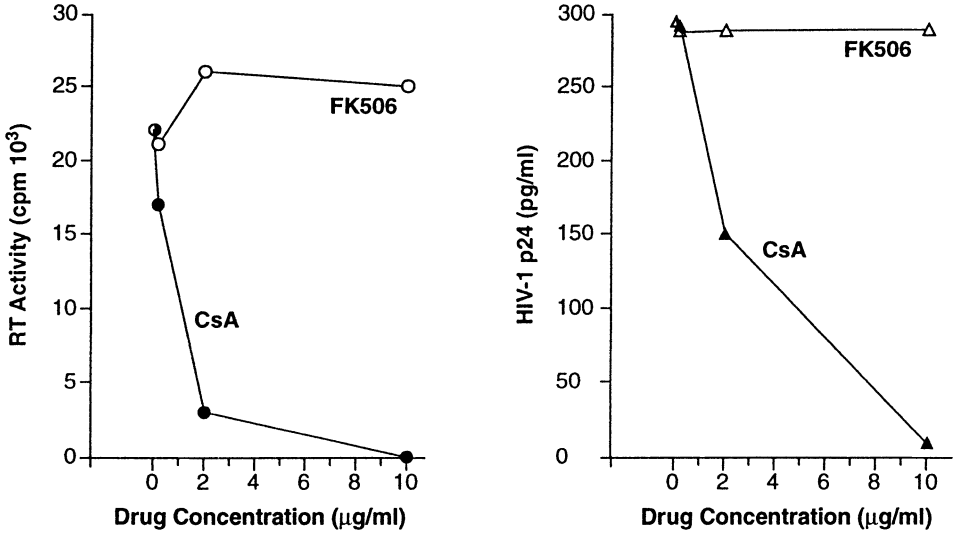

Fig. 3. Effect of FK506 and CsA on HIV-1_{IIIB} infection in H9 cells. H9 cells were incubated for 1 h at 37 °C, in the presence of the indicated concentrations of FK506 (open symbols) or CsA (solid symbols). After a 1-h incubation with HIV-1_{IIIB}, the cell were washed and resuspended in growth medium containing the same concentration of FK 506 or CsA that was used in the pretreatment (final concentration: 5×10^4 cells/ml). Control H9 cells without CsA or FK506 treatment prior to HIV-1 infection were handled in the same manner. Samples were removed on day 9 for the RT assay (left) and for HIV-1 p24 antigen quantitation (right). The mean value of three determinations is shown

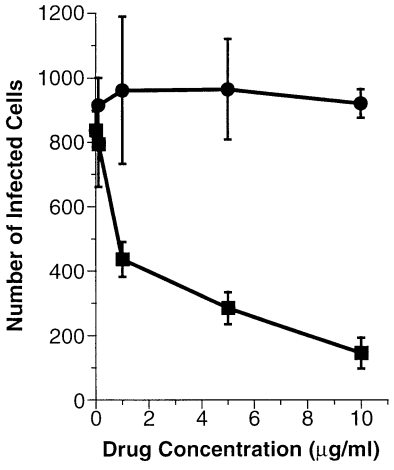

Fig. 4. Effect of FK506 and CsA on HIV-1_{IIIB} infection in HeLa CD4-LTR/β cells. The virus stock was treated with the indicated concentrations of FK506 (circles) or CsA (squares) before infection. A mixture of treated virus and culture medium containing DEAE-dextran was then added to the indicator cells. After a 2h adsorption at 37 °C, fresh medium containing these drugs was added to each well and the cells were incubated for 40–48h. Next, the cells were fixed and stained with X-gal, and infected (blue) cells were scored by light microscopy. The mean value of two determinations is shown

that was found in chronically infected cells [5], CsA had a dose-dependent effect on virus production (Fig. 3). FK506 did not show any inhibition at concentrations up to 10 μg/ml. In this experiment, which allowed for several rounds of infection, it was ineffective, although it was inhibitory in chronically infected cells (Fig. 2). Interestingly, newly infected cells grown in the presence of FK506 or CsA for 12 days showed no cell growth inhibition (data not shown), in contrast to our findings with chronically infected cells (Fig. 2A). These results suggest that long-term infection of the cells is required for the growth inhibition effect of immunosuppressive drugs.

The RT and $p24^{CA}$ assays were not sensitive enough to detect any difference in the virus production of newly infected H9 cells due to the presence of these inhibitory drugs in a single cycle of infection (after18 h). To study the effect of FK506 and CsA in the early phase of the viral life cycle, we used the multinuclear activation galactosidase infectivity (MAGI) assay [12]. As we reported previously [5], pretreatment of HIV-1 virions with CsA before infection of the HeLaCD4-LTR/β-gal cells decreased their infectivity in a dose-dependent manner, with an IC_{50} of 1 μg/ml (Fig. 4). These results are in good agreement with the findings that we obtained by infecting H9 cells (Fig. 3). No inhibition was observed using FK506, up to the tested 10 μg/ml concentration (Fig. 4). Furthermore, FK506 did not diminish the incorporation of the respective immunophilin (data not shown), in contrast to CsA [5].

Discussion

Capsid protein (CA) can bind CyPA, both in its processed form ($p24^{CA}$) and as a domain of the unprocessed Gag-polyprotein precursor [13]. Therefore, CyPA might be able to act in both the early and late phases of viral infection. In addition to its possible late-phase effect, our results with the MAGI assay support our findings of an early-phase effect and suggest that the binding of CsA to its target protein, CyPA, interferes with the interaction between the processed form of HIV-1 $p24^{CA}$ and cyclophilin. This interaction may be important during infection. However,

this interference effect may be true only for HIV-1 having the M phenotype, because the replication of isolates from the O phenotype was not inhibited by CsA despite the presence of cyclophillin in the virions [3]. Gag proteins from the other four lineages of primate immunodeficiency viruses and various oncoviruses do not package cyclophilin into virions, [3, 7, 13, 23], and their replication is not inhibited by CsA [1, 3, 13]. Interestingly, passage of HIV-1 in the presence of CsA or its nonimmunosuppressive analog in CsA -resistant and CsA-dependent mutant viruses that were still capable of binding CyPA [1].

The inability of FK506 to affect virus production of newly infected cells suggests that FKBP-12 does not play a substantial role as a chaperone in the early phase of the viral life cycle. This lack of effect may be due to the low concentration of FKBP-12 in the virions as compared with CyPA. Furthermore, cyclophilins A and B, but not FKBPs, can bind specifically to the HIV-1 Gag polyprotein at the N-terminal region of the $p24^{CA}$ protein [13].

Unlike CsA, FK506 did not inhibit the HIV-1-induced cytopathic effect in MT4 cells; however, both drugs prevented virus replication by blocking PHA stimulation of T4 lymphocytes [22]. Although FK506 was ineffective in cells that were newly infected with HIV-1 in an experiment that allowed several rounds of infection, FK506 did inhibit the growth of chronically infected cells and, as a consequence, the virus production (Fig. 2). Abnormalities of the signal transduction pathways have also been reported for lymphoid and myeloid cells chronically infected with HIV-1 [6, 9, 17]. Chronic HIV-1 infection of H9 cells substantially increases the intracellular Ca^{2+} concentration [9], which may cause increased activity of calcineurin, the target of the FKBP-12/FK506 and CyPA/CsA complexes. This increased calcineurin activity might explain the sensitivity of the chronically infected cells observed here. Therefore, although only CsA can act in the early phase of virus infection, both drugs can act on chronically infected cells via inhibition of T-cell activation by the CyPA/CsA and FKBP-12/FK506 complexes, as previously demonstrated in Molt-4 cells chronically infected with $HIV\text{-}1_{NDK}$ [11].

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References

1. Aberham C, Weber S, Phares W (1996) Spontaneous mutations in the human immunodeficiency virus type 1 *gag* gene that affect viral replication in the presence of cyclosporins. J Virol 70: 3 536–3 544
2. Billich A, Hammerschmid F, Peichl P, Wenger R, Zenke G, Quesniaux V, Rosenwirth B (1995) Mode of action of SDZ NIM 811, a nonimmunosuppressive cyclosporin A analog with activity against human immunodeficiency virus (HIV) type 1: interference with HIV protein-cyclophilin A interactions. J Virol 69: 2 451–2 461
3. Braaten D, Aberham C, Franke EK, Yin L, Phares W, Luban J (1996) Cyclosproine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. J Virol 70: 5 170–5 176
4. Braaten D, Franke EK, Luban J (1996) Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before initiation of reverse transcription. J Virol 70: 3 551–3 560
5. Briggs CJ, Tözsér J Oroszlan S (1996) Effect of cyclosporin A on the replication cycle of HIV-1 derived replication cycle of HIV-1 derived from H9 and Molt-4 producer cells. J Gen Virol 77: 2 963–2 967
6. DeLuca C, Roulston A, Koromilas A, Wainberg MA, Hiscott J (1996) Chronic human immunodeficiency virus type 1 infection of myeloid cells disrupts the autoregulatory control of the NF-κB/Rel pathway via enhanced IκBα degradation. J Virol 70: 5 183–5 193
7. Franke KE, Yuan HEH, Luban J (1994) Specific incorporation of cyclophilin A into HIV-1 virons. Nature 372: 359–362
8. Fruman DA, Burakoff SJ, Bierer BE (1994) Immunophilins in protein folding and immunosuppression. FASEB J 8: 391–400
9. Gupta S, Vayuvegula B (1987) Human immunodeficiency virus-associated changes in signal transduction. J Clin Immunol 7: 486–489
10. Harrison RK, Stein RL (1990) Substrate specificities of the peptidyl cis-trans isomerase activities of cyclophilin and FK-506 binding protein. Evidence for the existence of a family of distinct enzymes. Biochemistry 29: 3 813–3 816
11. Karpas A, Lowdell M, Jacobson SK, Hill F (1992) Inhibition of human immunodeficiency virus and growth of infected T cells by the immunosuppressive drugs cyclosporin A and FK506. Proc Natl Acad Sci USA 89: 8 351–8 355
12. Kimpton J, Emerman M (1992) Detection of replication-competent and pseudotyped human immunodeficiency virus with a sensitive cell line on the basis of activation of an integrated β galactosidase gene. J Virol 66: 2 232–2 239
13. Luban J, Bossolt KL, Franke EK, Kaplana GV, Goff SP (1993) Human immunodeficiency virus type 1 gag protein binds to cyclophilins A and B. Cell 73: 1 067–1 068
14. MacArthur WM, Thornton JM (1990) Influence of proline residues on protein conformation. J Mol Biol 218: 397–412
15. McCornack MA, Kakalis LT, Castera C, Handschumacher RE, Armitage IM (1997) HIV protease substrate conformation: modulation by cyclophilin A. FEBS Lett 414: 84–88
16. Nagy K, Young M, Baboonian C, Merson J, Whittle P, Oroszlan S (1994) Antiviral activity of human immunodeficiency virus type 1 protease inhibitors in a single cycle of infection: evidence for a role of protease in the early phase. J Virol 68: 757–765
17. Nye KE, Pinching AJ (1990) HIV infection of H9 lymphoblastoid cells chronically activates the inositol polyphosphate pathway. AIDS 4: 41–45
18. Oroszlan S, Tözsér J (1990) The retroviral proteinase. Semin Virol 1: 369–378

19. Ott DE, Coren LV, Johnson DG, Sowder RCII, Arthur LO, Henderson LE (1995) Analysis and localization of cyclophilin A found in the virions of human immunodeficiency virus type 1 MN strain. AIDS Res Hum Retroviruses 11: 1 003–1 006
20. Ott DE (1997) Cellular proteins in HIV-1 virions. Rev Med Virol 7: 167–180
21. Popovic MM, Sarngadharan G, Read E, Gallo RC (1984) Detection, isolation, and continous production of cytopathic retroviruses (HTLV- III) from patients with AIDS and pre- AIDS. Science 224: 497–500
22. Rosenwirth B, Billich A, Datema R, Donatsch P, Hammerschmid F, Harrison R, Hiestand P, Jaksche H, Mayer P, Peichl P, Quesniaux V, Schatz F, Schuurman H-J, Traber R, Wenger R, Wolff B, Zenke G, Zurini M (1994) Inhibition of human immunodeficiency virus type 1 replication by SDZ NIM 811, a nonimmunosuppressive cyclosporine analog. Antimicrob Agents Chemother 38: 1 763–1 772
23. Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Göttlinger HG (1994) Functional association of cyclophilin A with HIV virion. Nature 372: 363–365
24. Tözsér J, Weber IT, Gustchina A, Bláha I, Copeland TD, Louis JM, Oroszlan S (1992) Kinetic and modeling studies of S_3-S_3' subsites of HIV-1 proteinases. Biochemistry 31: 4 793–4 800
25. Vance Je, LeBlance DA, Wingfield P, London RE (1997) Conformational selectivity of HIV-1 protease cleavage of X-Pro peptide bonds and its implications. J Biol Chem 272: 15 603–15 606

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