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Efficacy of Constant Infusion of A-77003, an Inhibitor of the Human Immunodeficiency Virus Type 1 (HIV-1) Protease, in Limiting Acute HIV-1 Infection In Vitro

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A-77003, a human immunodeficiency virus type 1 (HIV-1) protease inhibitor, is effective for both acute and chronic infection in vitro and was evaluated clinically by continuous intravenous infusion administration. The minimum effective dose (the concentration required to completely inhibit viral replication) was determined in vitro in a population of uninfected (99%) and HIV-infected (1%) cells exposed to A-77003 by continuous infusion in hollow-fiber bioreactors. The production of infectious HIV and release of p24 antigen from infected cells were completely inhibited in cultures exposed to A-77003 at or above a concentration of 0.5 μM. Measurement of unintegrated HIV-1 DNA synthesis and flow cytometric analysis for cells expressing HIV p24 antigen demonstrated that the spread of HIV to uninfected cells was also blocked at 0.5 μM A-77003. Dose deescalation to 0.25 μM or removal of A-77003 resulted in the limited spread of the virus throughout the culture, the resumption of viral DNA synthesis, and release of p24. HIV produced after exposure to 0.5 μM A-77003 was noninfectious for a period of 72 h after the removal of the drug. Addition of 1 mg of α_1-acid glycoprotein per ml to this in vitro system completely ablated the anti-HIV effect of 0.5 μM A-77003. These data suggest that determination of the minimum effective dose under conditions which simulate human pharmacodynamic patterns may be useful in determining the initial dose and schedule for clinical trials. However, other factors, such as serum protein binding, may influence the selection of a therapeutic regimen.

Human immunodeficiency virus (HIV) encodes an aspartic proteinase which, as a homodimer, posttranslationally and proteolytically cleaves the viral *gag* and *pol* gene precursors to the mature viral proteins (16, 24). Site-specific mutagenesis of the HIV protease or inhibition of the enzyme with specific inhibitors results in the production of noninfectious virions which have the morphological features of immature particles (4, 7, 14, 17). The critical role of protease in virus maturation has established the HIV protease as an important target for therapeutic intervention during the late (postintegration) phase of HIV replication. HIV protease was both cloned and chemically synthesized, and the resultant structural information (18–20) led to the development of substrate analogs with exquisite substrate specificity (6, 12, 25). Earlier studies had indicated that A-77003, a symmetric inhibitor of the HIV protease, had antiviral activity at submicromolar levels against a spectrum of HIV strains and an excellent therapeutic index in primary and transformed human cell lines (11, 15).

Interest in whether the in vitro activity of A-77003 would also result in clinical efficacy led to preclinical and clinical studies of this compound. We have developed an in vitro test system which is based upon hollow-fiber (HF) technology and has the following attributes: (i) it is able to grow and maintain cells at near tissue densities, (ii) it provides access for serial evaluation of drug exposure, metabolism, and efficacy, and (iii) it permits the exposure of a defined cell population to a concentration-time profile of antiretroviral agents identical to that achievable in humans. In this study, we have utilized this HF pharmacodynamic model system to determine a minimum effective dose for A-77003 in vitro, given the continuous infusion mode of administration.

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MATERIALS AND METHODS

Cell culture. Human T-lymphoblastoid cell lines obtained from the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) were cultured as described previously (1b). HF units (Cellco Inc., Germantown, Md.) were initiated by the addition of a 1:100 mixture of HIV_{IIIB}-infected ($CEM\text{-}H9_{IIIB}$) and uninfected CEM cells (3.5×10^7 cells per unit) to the extracapillary space (ECS). The percentage of infected cells was determined by flow cytometry of methanol-fixed, permabilized cells with fluorescein isothiocyanate-conjugated anti-p24 (15). In continuous infusion experiments, the drug-containing medium was recirculated and replenished daily.

Drugs and compounds. A-77003 was provided by Abbott Laboratories (Abbott Park, Ill.). The concentrations of A-77003 used in HF studies were monitored by bioassay. Human α_1-acid glycoprotein (α_1-AGP; G-9885; purity, 99%) was purchased from Sigma Chemical Company (St. Louis, Mo.).

HIV antigen assay. HIV *gag* p24 protein in cell-free culture supernatants was measured by the Coulter p24 enzyme-linked immunosorbent assay (ELISA) (Coulter Immunology, Hialeah, Fla.) as described previously (1b).

DNA PCR. High- and low-molecular-weight DNA was isolated (8), and a PCR with the SK38 and SK39 primers (21) was performed as described previously (1b).

HIV infectivity assays. The titers of the infectious HIV were determined with CD4-transfected HeLa cell line 1022 (3). The residual drug was reduced by dilution (or by addition of α_1-AGP at 1 mg/ml) to a concentration which did not inhibit HIV. The cytotoxicity of HIV type 1 (HIV-1) in the presence and absence of A-77003 was correlated to the formation of formazan in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described (15).

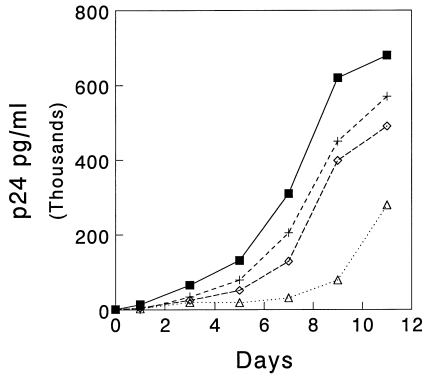

FIG. 1. The effect of continuous exposure of HIV-infected cells to A-77003 at 0.5-, 1-, and 2-fold the EC_{50}. Four HF bioreactors were set with 10^7 CEM cells and 10^5 CEM-$H9_{IIIB}$ cells (infected-to-uninfected ratio, 1:100). The four bioreactors were set in parallel, and the cells were continuously exposed to medium with A-77003 concentrations 0.5-, 1-, and 2-fold the EC_{50} (0.063 [+], 0.125 [◇], and 0.25 [△] μM A-77003) and to medium without the drug (■). Samples were taken from the ECS at the indicated time points, and p24 in the cell-free culture medium was measured as described previously.

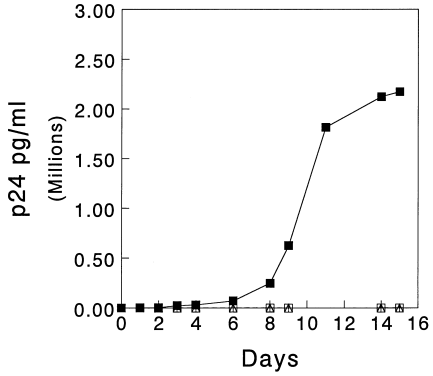

FIG. 2. The effect of continuous exposure of HIV-infected cells to A-77003 at concentrations 4-, 8-, and 16-fold the EC_{50}. Four bioreactors were set with 10^7 CEM cells and 10^5 CEM-$H9_{IIIB}$ cells (infected-to-uninfected ratio, 1:100). The A-77003-treated bioreactors and untreated unit were set in parallel, and the cells were continuously exposed to medium with (0.5 [+], 1 [□], and 2 [△] μM A-77003) and without (■) the drug. Samples were taken from the ECS at the indicated time points, and p24 in the cell-free culture medium was measured as described previously.

RESULTS

Concentration response analysis. We measured the release of p24 from a 1:100 mixture of infected and uninfected CEM cells treated with 0 to 25 μM A-77003 in microwell plates. The 50% effective concentration (EC_{50}) was calculated to be 0.124 μM, which is similar to data presented earlier (2, 15) on the EC_{50} for treatment of acute infection of fresh human peripheral blood lymphocytes and several cell lines (e.g., MT-2). The EC_{50}s of A-77003 for several of the HIV-1 strains that we tested, i.e., MN and several clinical isolates, including the zidovudine-resistant strain G910-11, were similar (data not shown).

HF pharmacodynamics. Both animal and human pharmacokinetic data (oral bioavailability for humans, <2%) indicated that A-77003 would be best administered by continuous infusion (1). Therefore, we determined the minimal effective dose by exposure of 1:100 mixtures of infected and uninfected CEM cells to various doses of A-77003 in a simulated constant infusion as described previously (1b). The continuous infusion regimen was simulated by addition of A-77003 at the indicated concentration to the medium circulating in the central reservoir. Figure 1 shows the effect of continuous exposure of HIV-infected cells to 0.063, 0.125, and 0.25 μM A-77003 (0.5-, 1-, and 2-fold the EC_{50}, respectively) on p24 levels in the cell-free culture medium in the ECS. Exposure to the drug was maintained for a total of 11 days. While each of the treated cultures showed some suppression of p24 release, HIV was actively replicating in the culture, as judged by the slow progressive increase in p24 levels in the cell-free medium removed from the ECS of the bioreactor. Further analysis of the cells in the ECS of the treated bioreactors by flow cytometry indicated that HIV was able to spread to uninfected cells. At day 11, untreated cultures had approximately 63% infected cells, while those treated with 0.063, 0.125, and 0.25 μM A-77003 had 60, 58, and 28% p24-positive cells, respectively. The viability of the cells in each of the HF bioreactors was greater than 85% as determined by trypan blue dye exclusion, and cells were actively growing, as measured by increased glucose utilization and lactate production.

In order to determine a drug concentration which effectively limited HIV infection and spread of the virus, 1:100 HIV-infected-to-uninfected CEM cell mixtures were exposed to 0, 0.5, 1, and 2 μM A-77003. Assay of the cell-free medium by a p24 antigen ELISA indicated that while p24 rapidly increased in the untreated cells, little to no p24 was released from cells treated with concentrations 4-, 8-, and 16-fold the EC_{50} of A-77003 (Fig. 2). High copy numbers of unintegrated DNA are usually associated with active HIV-1 DNA synthesis and acute infections (1b, 5, 13, 22). Linear and circular forms of unintegrated HIV proviral DNA are not pelleted by the Hirt DNA extraction, while integrated proviral DNA is associated with the bulk of chromosomal DNA in the pellet. Analysis of unintegrated HIV-1 DNA by PCR amplification of DNA in the Hirt supernatants indicated that HIV-1 was actively replicating in untreated cells and cells treated with two times the EC_{50} but not in cells treated with four times the EC_{50} (Fig. 3). Thus, A-77003, at concentrations of 0.5 μM and above, was able to prevent the de novo replication of HIV in HF bioreactors. The findings of flow cytometric analysis were consistent with these

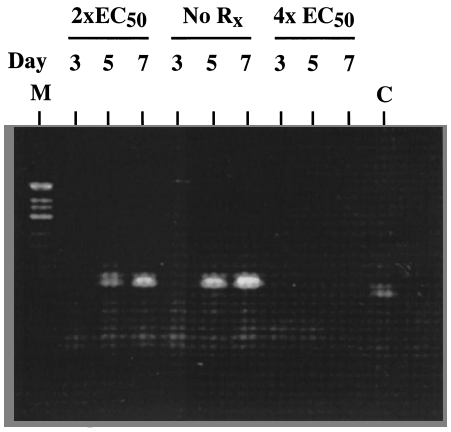

FIG. 3. Analysis of unintegrated HIV-1 DNA by PCR. DNA was prepared on days 3, 5, and 7 of treatment by the Hirt extraction method described in Materials and Methods. HIV-1 DNA in the Hirt supernatant fraction was amplified by PCR with the *gag* gene-specific primers SK38 and SK39 as described previously. Lanes M and C contain DNA markers and an HIV-1 control DNA (100 copies), respectively. Amplification of HIV-1 DNA results in a 115-bp fragment. A-77003 was used at two times the EC_{50} (2× EC_{50}), four times the EC_{50} (4× EC_{50}), and not at all (No R_x).

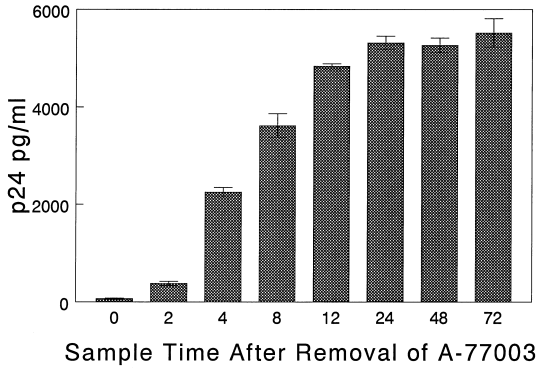

FIG. 4. Removal of A-77003 from treated cultures. An HF bioreactor set with a 1:100 mixture of HIV-1_{IIIB}-infected and uninfected CEM cells was treated with 0.5 μM A-77003 for 5 days. At time 0 minus 2 h, the cells were removed from the ECS, washed twice with drug-containing medium, and returned to the bioreactor. The cells were removed from the ECS at time 0 and washed twice with medium without the drug. Concurrently, the medium in the reservoir and central circulating lines was replaced with drug-free medium. The cells were returned to the bioreactor and incubated for 2-h periods at the indicated time points. After the 2-h incubation, cell-free medium was obtained from the ECS (sample times are in hours) and HIV p24 was measured as described in Materials and Methods. Error bars indicate standard deviations.

findings and suggest that the spread of HIV was completely inhibited. The number of p24-positive CEM cells after exposure of the cells to 0.5 μM A-77003 was identical to that of a 1:100 infected-to-uninfected cell mixture at baseline. In contrast, cells grown in the parallel untreated bioreactor were greater than 75% positive for HIV p24 (data not shown).

While these studies were not specifically designed to isolate HIV resistant to A-77003, we analyzed the EC_{50} of the virus harvested from the culture prior to treatment and at day 11 (one and two times the EC_{50}) and at day 15 (four times the EC_{50}). No significant difference in the sensitivities of the virus exposed to A-77003 at optimal and suboptimal doses was measured (data not shown).

Reversal of the antiviral effect. The use of HF bioreactor cultures permits the rapid change of drug concentration by removal of the drug from the central reservoir. In a wash-out experiment, a culture inhibited with 0.5 μM A-77003 for 5 days was washed free of the drug and both the infectivity and p24 production were measured at 2-h intervals after the removal of the drug (Fig. 4). Viral p24 released into the medium rose after 2 to 4 h after drug removal, and the yield progressively increased over the next 24 h (Fig. 4). In contrast, infectious HIV was not isolated from the cultures for 72 h after drug removal (data not shown).

α_1-AGP specifically binds to and inhibits the antiviral activity of A-77003 in cells acutely and chronically infected with HIV (2, 4a). The EC_{50}s for A-77003 were increased approximately 5.6-fold upon the addition of 1 mg of α_1-AGP per ml to cultures initiated at infected-to-uninfected cell ratios of 1:10 and 1:100 (Fig. 5). The point estimate of the EC_{50} with α_1-AGP has a 95% confidence bound which does not overlap the 95% confidence bound of the point estimate of the EC_{50} determined in the absence of α_1-AGP, which indicates that the effect of α_1-AGP is statistically significantly different. The 95% confidence bounds for the determinations at infected cell ratios of 1:10 and 1:100 overlap, and there was no statistically significant effect of increased virus load. Since our in vitro HF model was designed to expose cells to the drug under physiological conditions, we tested whether α_1-AGP added to an already inhibited (5 days of exposure at 0.5 μM A-77003) HF culture could bind and reduce the activity of A-77003. α_1-AGP (1 mg/ml) added directly to the ECS reversed the antiviral effect of A-77003 (0.5 μM). Increased accumulation of p24 (24,994 ± 76 pg/ml) was measured in the cell-free tissue culture medium from the ECS 24 h after the α_1-AGP was added. By comparison, a parallel HF culture with continued drug exposure had much less accumulation (586 ± 68 pg/ml).

DISCUSSION

We have used an HF pharmacodynamic model to determine the efficacy of A-77003 for HIV infection when it is administered as a constant infusion. A mixture of infected and uninfected cells was used to mimic the in vivo situation, in which virus is transmitted to uninfected cells by contact with infected cells capable of spreading HIV. We have previously shown that this system could predict the minimum effective dose of an antiviral agent found by dose-ranging clinical studies (1b). In this study, we have shown that concentrations of A-77003 at or above 0.5 μM were required to completely prevent the spread of HIV in the culture. The ability of A-77003 to prevent de novo HIV infection was shown by flow cytometric analysis of cells from the bioreactors. This analysis indicated that mixtures of infected and uninfected cells exposed to the drug showed a marked reduction in the number of cells positive for intracellular viral p24 after 7 to 14 days of cocultivation. The virus load (the percentage of infected cells used to initiate the bioreactor) did not seem to markedly affect the efficacy of A-77003 in vitro (Fig. 5) (unpublished data). We have previously shown that

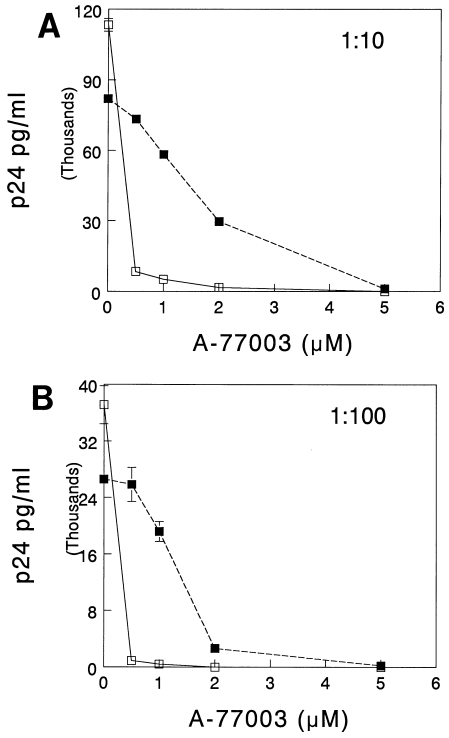

FIG. 5. Effect of α_1-AGP on the anti-HIV activity of A-77003 at different viral loads. Cocultures of CEM-$H9_{IIIB}$ and CEM cells (1:10 [A] and 1:100 [B] infected-to-uninfected cell ratios) were exposed to the indicated concentrations of A-77003 in the presence (■) and absence (□) of 1 mg of α_1-AGP per ml. At day 10, the supernatants from the cells were removed and tested for the amount of p24 as described in Materials and Methods. The graphs show p24 produced by treated cultures initiated at 1:10 (A) and 1:100 (B) infected-to-uninfected cell ratios.

A-77003 was able to block the release of p24 and infectious virus from chronically infected cells (15). Similar results were obtained upon measurement of HIV p24 antigen or infectious virus in cell-free supernatant sequentially removed from the ECS at various time points. HF bioreactors exposed to effective concentrations of A-77003 had essentially baseline levels of HIV p24, while untreated controls had progressively increasing amounts of p24.

Reedijk et al. (23) studied A-77003 in patients treated by continuous intravenous infusion. Steady-state concentrations in plasma at the EC_{50} showed no evidence for antiviral activity. These clinical data agree with data from our HF pharmacodynamic studies which suggested that A-77003 would not be effective when administered as a continuous infusion at the EC_{50} (Fig. 1). Furthermore, we ablated the anti-HIV activity of A-77003 in an experiment in which we added α_1-AGP (1 mg/ml) to an HF culture in which HIV replication was completely inhibited by continuous exposure to 0.5 μM A-77003. In view of the potential for avid plasma protein binding of A-77003, these data predict that A-77003 would not be clinically efficacious at steady-state concentrations in plasma that were fourfold the EC_{50} in vivo.

Since the HF model system makes it possible to sequentially monitor the high-density cell population, we examined the cells at multiple time points after they were exposed to A-77003 at several dose levels. Both this and earlier studies with 2′,3′-didehydro-3′-deoxythymidine (D4T) (1b) had indicated that measurement of HIV proviral DNA in Hirt DNA supernatants was useful in monitoring viral replication and integration in vitro. HIV DNA was not detectable in Hirt DNA supernatants prepared from cells exposed to efficacious concentrations of A-77003 (at or above 0.5 μM). Synthesis of unintegrated DNA preceded the release of p24 or infectious HIV into the media of infected cell mixtures treated with suboptimal amounts of A-77003, suggesting that unintegrated DNA may be used to rapidly distinguish effective from non-efficacious doses or schedules of the drug being tested.

While the population of virus released from cell cultures which were actively synthesizing HIV DNA contained virus capable of replicating while exposed to 0.25 μM A-77003, there was no evidence for selection of virus mutants with altered sensitivity to the protease inhibitor as described previously (10). While virus resistant to A-77003 was not selected under the culture conditions and profiles of drug exposure utilized, the large cell numbers achievable in HF bioreactors should result in increased numbers of rounds of viral replication which could favor the selection of resistant virus populations. The cultures described herein were monitored for only a limited time period, 11 to 15 days. We are presently involved in studies to determine whether HIV resistant to protease inhibitors would emerge upon culture for extended periods with the periodic addition of uninfected CEM cells.

The HF model is also useful for performing reversal experiments in which the drug is rapidly removed from the system. In earlier experiments, p24 production and the spread of HIV rapidly (<24 h) followed the removal of D4T, a nucleoside analog, from the cellular milieu (1b). In marked contrast, HIV p24 release rapidly increased when A-77003 was removed while the appearance of infectious virus was delayed (>72 h), suggesting that A-77003 treatment has a potent postantiviral effect. A recent study by Kageyama et al. also indicated that the particles produced in the presence of A-77003 had markedly reduced infectivity and that this was essentially irreversible (9). It remains to be seen if the postantiviral effect, which results in the release of noninfectious virus, will permit extensions of the time required between dosing intervals for A-77003 and other protease inhibitors. Noninfectious particles released from infected cells treated with these inhibitors may help to elicit cell-mediated or humoral immune responses. However, cells treated with protease inhibitors express normal levels of gp120/160 and still can fuse with uninfected cells, leading to syncytium formation (1a), which may contribute to the continued loss of $CD4^+$ cells.

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REFERENCES

1. **Abbott Laboratories.** Data on file.
1a. **Bilello, J.** Unpublished data.
1b. **Bilello, J. A., G. Bauer, M. N. Dudley, G. A. Cole, and G. L. Drusano.** 1994. Effect of 2′,3′-didehydro-3′-deoxythymidine in an in vitro hollow-fiber pharmacodynamic model system correlates with results of dose-ranging clinical studies. Antimicrob. Agents Chemother. **38:**1386–1391.
2. **Bilello, J. A., P. A. Bilello, M. Prichard, T. Robins, and G. L. Drusano.** 1995. Reduction of the in vitro activity of A-77003, an inhibitor of the human immunodeficiency virus protease, by human α_1-acid glycoprotein. J. Infect. Dis. **171:**546–551.
3. **Chesebro, B., and K. Wehrly.** 1988. Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. J. Virol. **62:**3779–3788.
4. **Debouck, C., G. J. Gorniak, J. E. Strickler, T. D. Meek, B. W. Metcalf, and M. Rosenberg.** 1987. Human immunodeficiency virus protease expressed in *Escherichia coli* exhibits autoprocessing and specific maturation of the gag precursor. Proc. Natl. Acad. Sci. USA **84:**8903–8906.
4a. **Dennissen, J., and R. Granneman (Abbott Laboratories).** Personal communication.
5. **Dickover, R. E., R. M. Donovan, E. Goldstein, S. H. Cohen, V. Bolton, R. G. Huth, G. Liu, and J. R. Carson.** 1992. Decreases in unintegrated HIV DNA are associated with antiretroviral therapy in AIDS patients. J. Acquired Immune Defic. Syndr. **5:**31–36.
6. **Erickson, J., D. J. Neidhart, J. VanDrie, D. J. Kempf, X. C. Wang, D. W. Norbeck, J. J. Plattner, J. W. Rittenhouse, M. Turon, N. Wideburg, W. E. Kohlbrenner, R. Simmer, R. Helfrich, D. A. Paul, and M. Knigge.** 1990. Design, activity and 2.8Å crystal structure of a C_2 symmetric inhibitor complexed to HIV-1 protease. Science **249:**527–533.
7. **Gottlinger, H. G., J. G. Sodroski, and W. A. Haseltine.** 1989. Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **86:**5781–5785.
8. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse embryo cell cultures. J. Mol. Biol. **26:**365–369.
9. **Kageyama, S., D. T. Hoekzema, Y. Murakawa, T. Shirsaka, D. J. Kempf, D. W. Norbeck, J. Erickson, and H. Mitsuya.** 1994. A C_2 symmetry-based HIV protease inhibitor, A-77003, irreversibly inhibits infectivity of HIV-1 *in vitro*. AIDS Res. Hum. Retroviruses **10:**735–743.
10. **Kaplan, A. H., S. F. Michael, R. S. Wehbie, M. F. Knigge, D. A. Paul, L. Everitt, D. J. Kempf, D. W. Norbeck, J. W. Erickson, and R. Swanstrom.** 1994. Selection of multiple human immunodeficiency virus type 1 variants that encode viral proteases with decreased sensitivity to an inhibitor of the viral protease. Proc. Natl. Acad. Sci. USA **91:**5597–5601.
11. **Kempf, D. J., K. C. Marsh, D. A. Paul, M. F. Knigge, D. W. Norbeck, W. E. Kohlbrenner, L. Codacovi, S. Vasavanonda, P. Bryant, X. C. Wang, N. E. Wideburg, J. J. Clement, J. J. Plattner, and J. Erickson.** 1991. Antiviral and pharmacokinetic properties of C_2 symmetric inhibitors of the human immunodeficiency virus type 1 protease. Antimicrob. Agents Chemother. **35:**2209–2214.
12. **Kempf, D. J., D. W. Norbeck, L. Codacovi, S. C. Wang, W. C. Kohlbrenner, N. E. Wideburg, D. A. Paul, M. F. Knigge, S. Vasavanonda, A. Kraig-Kennard, A. Saldivar, A. Rosenbrook, Jr., J. J. Clement, J. J. Plattner, and J. Erickson.** 1990. Structure-based C_2 symmetric inhibitors of HIV protease. J. Med. Chem. **33:**2687–2689.
13. **Kim, S. Y., R. Byrn, J. Groopman, and D. Baltimore.** 1989. Temporal aspects of DNA and RNA synthesis during human immunodeficiency virus infection: evidence for differential gene expression. J. Virol. **63:**3708–3713.
14. **Kohl, N. E., E. A. Emini, W. A. Schleif, L. J. Davis, J. C. Heimbach, R. A. Dixon, E. M. Scolnick, and I. S. Sigal.** 1988. Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA **85:**4686–4690.
15. **Kort, J. J., J. A. Bilello, G. Bauer, and G. L. Drusano.** 1993. Preclinical evaluation of antiviral activity and toxicity of Abbott A77003, an inhibitor of

the human immunodeficiency virus type 1 protease. Antimicrob. Agents Chemother. **37:**115–119.
16. **Krausslich, H. G., and E. Wimmer.** 1988. Viral proteinases. Annu. Rev. Biochem. **57:**701–754.
17. **Loeb, D. D., C. A. I. Hutchison, M. H. Edgell, W. G. Farmerie, and R. Swanstrom.** 1989. Mutational analysis of human immunodeficiency virus type 1 protease suggests functional homology with aspartic proteinases. J. Virol. **63:**111–121.
18. **Louis, J. M., E. M. Wondrak, T. D. Copeland, C. A. D. Smith, P. T. Mora, and S. Oroszlan.** 1989. Chemical synthesis and expression of the HIV-1 protease gene in E. coli. Biochem. Biophys. Res. Commun. **159:**87–94.
19. **Miller, M., B. K. Sathyanarayana, M. V. Toth, G. R. Marshall, L. Clawson, L. Selk, J. Schneider, S. B. H. Kent, and A. Wlodawer.** 1989. Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 Å resolution. Science **246:**1149–1152.
20. **Navia, M. A., P. M. D. Fitzgerald, B. M. McKeever, C.-T. Leu, J. C. Heimbach, W. K. Herber, I. S. Sigal, P. L. Darke, and J. P. Springer.** 1989. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. Nature (London) **337:**615–620.
21. **Ou, C.-Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **238:**295–297.
22. **Pauza, C. D., J. E. Gallindo, and D. D. Richman.** 1990. Reinfection results in the accumulation of unintegrated DNA in cytopathic and persistent human immunodeficiency virus type 1 infection of CEM cells. J. Exp. Med. **172:**1035–1042.
23. **Reedijk, M., C. A. B. Boucher, T. van Bommel, D. D. Ho, T. B. Tzeng, D. Sereni, P. Veyssier, S. Jurriaans, R. G. Granneman, A. Hsu, J. M. Leonard, and S. A. Danner.** 1995. Safety, pharmacokinetics, and antiviral activity of A77003, a C_2 symmetry-based human immunodeficiency virus protease inhibitor. Antimicrob. Agents Chemother. **39:**1559–1564.
24. **Robins, T., and J. Plattner.** 1993. HIV protease inhibitors: their anti-HIV activity and potential role in treatment. J. Acquired Immune Defic. Syndr. **6:**162–170.
25. **Wlodawer, A., and J. W. Erickson.** 1993. Structure-based inhibitors of the HIV-1 protease. Annu. Rev. Biochem. **62:**543–585.