The development of a Rational Antiretroviral Therapy for Aids

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SUMMARY

Current information indicates that world-wide, through June 1992, 2.5 million people have died of AIDS, 160,000 in the United States alone. Especially alarming is the spread of the epidemic in some of the poorest countries of the Third world. Efforts made since 1983 to understand the molecular, cellular and clinical features of HIV-1 infection, the causative agent of AIDS in humans, have facilitated elaboration of strategies for therapeutic intervention. The in vitro activity of 3' - azido - 3' - deoxythymidine (AZT, zidovudine) against HIV-1, via the (viral) encoded reverse transcriptase (RT), established the enzyme as a major target for antiviral therapy, followed by the demonstration of the effectiveness of 2',3-dideoxynucleosides, in general, as potent inhibitors of HIV-1. Subsequent clinical trials led to the approval of zidovudine and then 2',3'-dideoxyinosine (ddI, didanosine) for treatment of AIDS. Recently, the combination of zidovudine and 2',3'-dideoxycytidine (ddC, zalcitabine), which exhibits different toxicity profiles, was granted limited approval for treatment of advanced HIV-1 infection.

A relatively wide spectrum of dideoxynucleoside analogues with modified sugar moitites, have shown impressive anti-HIV-1 activity. Among the more novel compounds are adenallene and cytallene in which the ribose ring is replaced by a rigid allene substituent. In addition, two structurally distinct, non-nucleoside inhibitors of HIV-RT, TIBO and HEPT, which share a common allosteric binding site to RT, have been identified as potent inhibitors of HIV-1.

Virally encoded aspartic protease (PR) comprises another unique aspect of HIV-1 biochemistry, which, in addition to RT, is indispensable for the generation of an infectious particle. The most effective inhibitors of HIV-1-PR reported thus far are pseudopeptide / peptidemimetic compounds containing transition state inserts in place of the residues occupying the P_1-P_1'-positions of the peptide. Analogs have been synthesized containing (among others) the hydroxyethylamine moiety which inhibit HIV-1-PR. These analogues exert a potent and highly specific antiretroviral activity in the nanomolar concentration range in multiple cell systems.

The trans-acting transcription activator, *tat*, appears to play a unique and pivotal role in the emergence of the virus from the latent state. Antagonists to this non-structural, regulatory protein would be distinctly different from antiviral agents directed to other functions of the virus. There are, at present, no known antagonists of *tat*; nonetheless, the present knowledge base appears sufficient to support the development of inhibitors of this regulatory protein.

It is likely that combinations of agents, targeting different sites in the HIV-1 replicative cycle, offer the hope of changing AIDS from a fatal to a manageable disease.

INTRODUCTION

Reports in 1981 of a new clinical entity, acquired immune deficiency syndrome (AIDS) (1-3), sparked an intensive research effort which led to the identification of a retrovirus of the *Lentiviridae* family as the etiological agent (4-6). The pathogen, which was originally referred to both as human T-lymphotropic virus (HTLV-III) (7) and lymphadenopathy associated virus (LAV) (8), is now designated human immunodeficiency virus (HIV-1). Two genetically distinct types, HIV-1 and HIV-2, have been characterized (9-11). The target of infection by the virus is the surface CD4 receptor leading to a severe depletion in $CD4^+$ T-Lymphocytes, and subverting, thereby, the body's defenses. Ultimately, the immune system collapses and the patient falls prey to opportunistic infections, neurological disorders, neoplastic disease which, at length, result in death.

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the United States alone. Especially alarming is the spread of the epidemic in some of the poorest countries of the Third world. The World Health Organization estimates that as many as 40 million people could become HIV-1 positive by the year 2000, up from some 10 million today. The lack of an effective vaccine or antiviral chemotherapy, coupled with the high cost of presently available treatment and a fatality rate of over 90% after four years from the time of clinical diagnosis, would be hard to contain and control not only in advanced nations of the industrialized world but even more so in the economically poor and underdeveloped countries that comprise the Third world.

HIV-1 Genome

HIV-1 is an enveloped, single stranded RNA virus, the genome of which exists as a dimer of identical RNA molecules. Structurally, the HIV-1 genome (Figure 1) encodes all of the proteins required for replication of this retrovirus; the four capsid proteins derived from the *gag* gene, three enzymes that are commonly found in retroviruses (protease, reverse transcriptase, and endonuclease) originating from the *pol* gene and the major exterior glycoprotein and transmembrane protein both derived from the *env* gene. The genome encodes, as well, the non-structural proteins *tat*, *rev* and *nef* and three additional proteins, *vif*, *vpu*, and *vpr*. Among the HIV-1 genes, *sor* (not shown) is a unique protein which, together with the envelope proteins, determines the infectivity of virus particles.

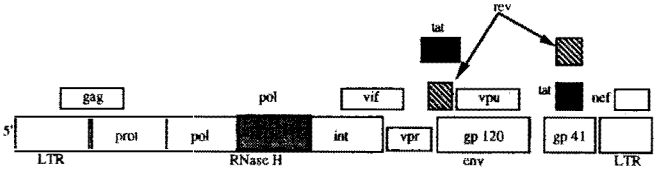


Figure 1. Structure of the HIV-1 genome

It is now clear that during replication, mutations are introduced into the genome that confer selective growth advantages to the virus. There are, as of today, at least five distinct subtypes of HIV-1, the principal form, each spreading rapidly. It is this proclivity to mutate that presents the greatest challenges to the development of treatments for AIDS. Indeed, medical science has never encountered a more devious and a more formidable adversary.

TARGETS OF ANTI-HIV CHEMOTHERAPY

1. Reverse Transcriptase

The elucidation of molecular events crucial to the life cycle of HIV-1, the most complex retrovirus encountered to date, has facilitated the elaboration of strategies (13) for therapeutic intervention. Among the major targets of antiviral therapy is reverse transcriptase (RT) which plays a central role in the retroviral life cycle, copying the genetic information in the genomic RNA into a double stranded DNA form.

In an elegant series of experiments, Mitsuya and Broder (14) showed that a broad family of 2',3'- dideoxynucleosides (Figure 2), which included 3'-azido-3'-deoxythymidine (AZT), and the 2', 3'-dideoxy derivatives of adenosine (ddA), guanosine (ddG), inosine (ddI,) and cytidine (ddC), all inhibited the infectivity and cytopathic analogues completely inhibited the replicated the infectivity and cytopathic effects of HIV toward cultured T Lymphoblasts. In vitro, these analogues completely inhibited the replication of HIV-1 and its capacity to destroy T cell cultures at concentrations that were 10-20 fold lower than those that impair the proliferation and survival of target cells.

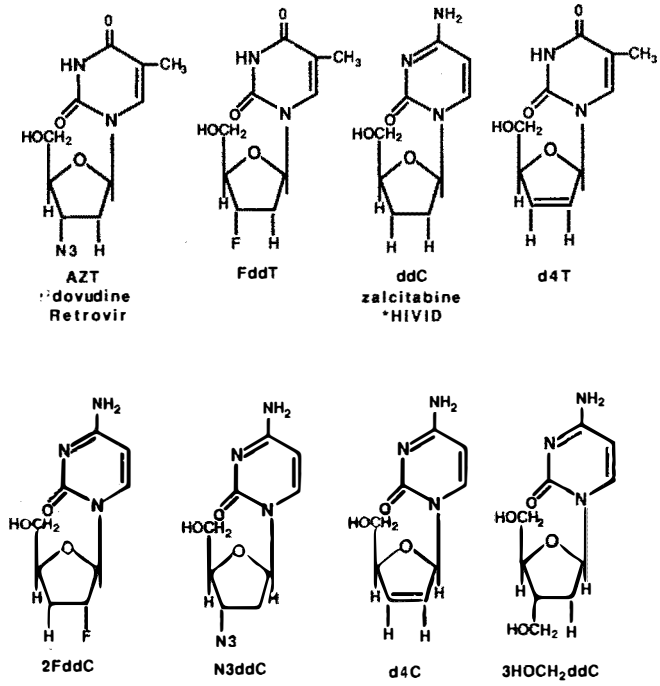


Figure 2. Structures of some HIV-1 active pyrimidine

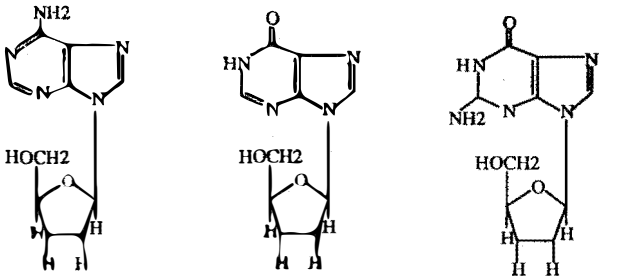
AZT, which appeared to be the most effective inhibitor of HIV-1 among the 2', 3'-dideoxy derivatives, is structurally related to the DNA constituent thymidine, but differs from the latter in having an azido (N_3) group in place of the hydroxyl group at the 3' position of the ribose ring (Figure 2). AZT enters the cell by passive diffusion (15) where it is phosporylated via (cellular) kinases to the corresponding 5'-triphospate derivative. The ability of the cell to phosphorylate AZT and the intracellular ratio of AZT triphosphate to thymidine 5'-triphosphate are the most important determinants of AZT's ability to inhibit HIV-1 (16, 17, 18). AZT triphosphate acts by competitively inhibiting the utilization of thymidine triphosphate by RT. In addition, AZT triphosphate terminates

DNA synthesis when incorporated into the proviral DNA chain because the 3'-azido group does not allow for the 5' to 3' phosphodiester linkage required for chain growth.

Synthesized nearly thirty-years ago as a potential cancer chemotherapeutic agent by Horwitz and coworkers (19), and shown in 1974 to inhibit a C-type murine retrovirus replication in vitro by Ostertag et al., (20), no medical application of AZT emerged. In this connection, it is important to point out that the triphosphate of AZT, and, in fact, all the 2', 3'-dideoxynucleoside triphosphates, were subsequently shown to be utilized by mammalian DNA polymerases β and γ (21). However, they are poor substrates for DNA polymerase-α which is the main enzyme responsible for both repair and replicative DNA synthesis in human lymphocytes. In part, these properties may, on the one hand, explain the selective anti-HIV-1 activity and, as well, the failure of the 2', 3'-dideoxynucleosides to manifest antitumor activities.

The clinical efficacy of AZT against AIDS was suggested in 1986 (22). The initial human study involving 19 patients with AIDS or advanced AIDS-related complex showed that oral AZT administration resulted in adequate serum levels and tolerable side effects. Moreover, the patients, on average, gained 2.2 Kg and showed a significant increase in their $CD4^+$ T cells. A subsequent placebo-controlled trial involving 282 patients (23), indicated that AZT improved survival and reduced the incidence of opportunistic infections in patients with AIDS or advanced AIDS-related complex. Of particular importance was the fact that only 1 of 145 patients treated with AZT (250 mg, every 4 hours; 1500mg/d) died compared with 19 of 137 placebo recipients. Thereupon, the study was terminated and the drug was offered to all participants. Approval of AZT (zidovudine, Burroughs Wellcome Co., trademark name, Retrovir) by the United States Food and Drug Administration (FDA) was granted in early 1987.

The purine 2', 3'-dideoxynucleosides, ddA and ddI (Figure 3), as indicated above, were observed to be effective in vitro inhibitors of the infectivity and cytopathic effect of HIV. It was found that ddA was subject to rapid conversion to ddI by the ubiquitous enzyme adenosine deaminase (24). Moreover, ddI exerts its antiretroviral activity by virtue of its ability to generate ddATP, and also that ddA can give rise to ddATP by either of two alternate routes, i.e., directly via 5'-phosporylation to ddAMP and indirectly via the intermediation of ddI (25). In vitro studies showed that ddI had a higher therapeutic index than other dideoxynucleoside analogues (26, 27) with minimal toxic effects on bone marrow progenitor cells (28). On the basis of these findings ddI was first tested in patients with symptomatic HIV-1 disease (29) and then employed in a phase I, dose-finding trial involving 34 patients with AIDS or AIDS-related complex (30). The data showed that ddI could safely



ddA ddI ddG

didanosine

*Videx

Figure 3. Structure of some purine 2',3'-dideoxynucleoside

be given to patients and documented a toxicity profile different from that of AZT. Approval of ddI (didanosine, Bristol-Meyers-Squibb Laboratories, trademark name, Videx) by the FDA for treatment of AIDS was granted in 1991.

Of the 2', 3'-dideoxynucleosides with in vitro activity against HIV-1, ddC, whose synthesis was first described by Horwitz and coworkers in 1967 (31), is about ten times more potent than AZT, (14). Activation of ddC in T cells occurs, as indicated above, by phosphorylation but by a pathway which is different in that it includes the anabolic formation of a ddC-diphosphate-choline derivative (32).

Several cytydine analogs (see Figure 2), including 2'-fluoro-2',3'-dideoxycytidine (FddC) (33), 3'-hydroxymethyl-2',3'-dideoxycytidine (HMddC) (34), and 2',3'-didehydro-2',3'-dideoxycytidine (D4C) (31) were prepared (see Figure 2) and evaluated (14) in comparison to ddC. Although all were found to be active against HIV-1, none of these analogues showed a potency comparable to ddC. Interestingly, 3'azido substitution in ddC, i.e., 3'-azido-2',3'-dideoxycytidine, results in almost total loss of in vitro antiretroviral activity (35).

In clinical trials ddC was shown to decrease serum HIV p24 antigen levels and possibly to raise circulating $CD4^+$ cell counts (36, 37). Both AZT and ddC produce dose limiting toxicities. AZT is associated with unacceptable levels of bone marrow suppression, and ddC can cause painful neuropathy. The different toxicity profiles of the two agents provided the rationale for testing them in alternating dosing combinations in an attempt to retain the antiviral activity of each against HIV while reducing the toxicities of both (38). The results of a phase I/II study of several different combinations of AZT and ddC showed the beneficial effects of the two-drug theraphy (39). In fact, the data from this study and a related Burroughs-Wellcome trial led the FDA in 1992 to approve ddC (zalcitabine, Hoffmann-La Roche, Inc. trademark, HIVID) in combination

with AZT for patients with advanced HIV-1 disease. It is important to note that there is, an increasing use of AZT as the core of combination therapy as is evident in multiple ongoing studies (40). These include, for example, a phase I/II study to evaluate the combination of AZT and ddI in asymptomatic patients.

3'-Deoxythymidine (ddT) did not prove to be particularly active against HIV on in vitro assay (14). By contrast, the thymidine analogue 3'-deoxy-2',3'-dehydrothymidine (D4T), first described by Horwitz et al. in 1966 (41), was shown to be comparable to AZT in potency against HIV in vitro (42). and 3'-deoxy-3'-fluorothymidine (FddT) (43) was at least ten-fold more potent (44). However, in mice bone marrow stem cell toxicity of FddT was observed to occur at a much lower dose than that with AZT which reduced the therapeutic advantage of FddT (44). By contrast, the dose-limiting toxicity of D4T in mice was hepatoxicity, which was observed at a higher dose level than bone marrow toxicity with AZT. In addition, D4T was substantially less toxic against human bone marrow stem cells, than the other two analogues.

It has been shown that AZT is metabolized in a rather inefficient manner to its corresponding triphosphate because the conversion of mono to diphosphate proceeds poorly (45). As a result there is an excessive accumulation of AZT monophosphate. The cellular pharmacology of D4T differs in a favorable manner from AZT in that the rate limiting step in D4T metabolism is the initial phosphorylation to the monophosphate (46). Thus, unlike AZT, excessive quantities of D4T metabolites do not accumulate in cells and possibly interfere, thereby, with normal cellular metabolism.

An additional advantage of the cellular metabolism of D4T is that the level of D4T triphosphate increases in proportion to the concentration of the nucleoside analogue incubated in the cells. This contrasts with AZT where the monophosphate increases in a dose-proportional manner, but the level of triphosphate remains relatively constant. Because the active form of a nucleoside drug is the triphosphate, the metabolic data predicted that D4T would be more likely to provide a clinical benefit proportional to the administered dose.

Upon completion of pharmacokinetic studies, which were carried out in the mouse, rat, dog and monkey (47) and in which the pharmacokinetic parameters were observed to be similar to those of AZT, clinical trials were initiated. It is expected that FDA approval of D4T for treatment of AIDS will be granted in 1993.

There are, in addition to the 2', 3'-dideoxynucleosides, several other agents in preclinical and clinical development as antiretroviral agents against AIDS, which will be mentioned only briefly because of limitations of space. These include the carbocyclic nucleosides (Figure 4), in which the furanose oxygen is replaced by a methylene group and, as a result are resistant to cleavage by phosphorylases and hydrolases. In particular, the carbocyclic 2', 3'-didehydro-2', 3'-dideoxyguanosine, designated carbovir, is active against HIV in vitro (48). The 5'-phosphate of this carbocyclic nucleoside analogue has a highly specific inhibitory effect on HIV-1-RT.

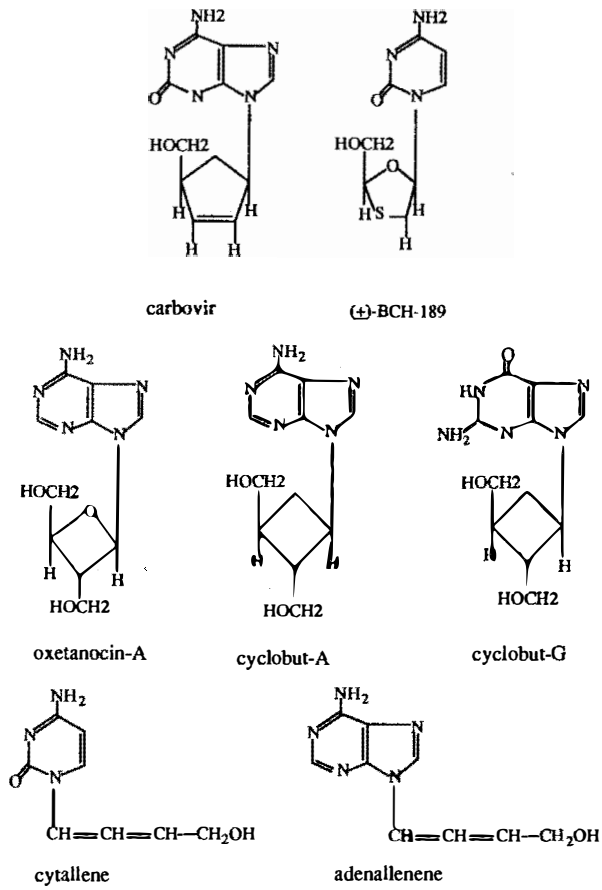


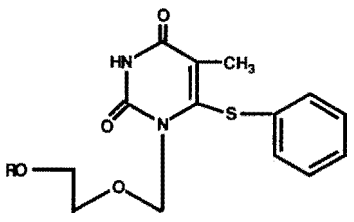
Figure 4. Structure of some 2',3'-dideoxynucleoside analogues

Racemic *cis*- 2', 3'-dideoxy-3'-thiacytidine [(±) - BCH - 189], which is characterized by the presence of the heteroatom sulfur at the 3'-position of the furanose ring, was reported to be active in vitro against HIV with a decreased toxicity as compared to AZT (49).

Oxetanocin-A, a member of a family of nucleoside analogues, which possess an oxetanosyl-N-glycoside in the sugar moiety, is active against HIV in vitro (50). In addition two carbocyclic analogues of the oxetanocins, designated cyclobut-A and cyclobut-G, are broad spectrum antiviral agents that block the infectivity and replication of HIV in vitro (51).

Zemlicka and conworkers have described a novel series of nucleoside analogues, named allenols, that are derived from nucleic acid bases (52). The derivatives (±)-adenallene and (±)-cytallene were found to inhibit the cytopathic effect and replication of HIV in vitro (53).

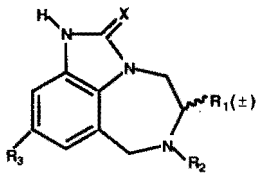
Among pyrimidine derivatives, 1-(2-hydroxyethoxy)methyl-6-phenylthiothymine (Figure 5, HEPT) and corresponding 2-thiothymine analogue (HEPT-S) are both potent and selective inhibitors of HIV-1 but not HIV-2. (54). These compounds are unique because they do not require phosphorylation in order to inhibit HIV-RT (55).



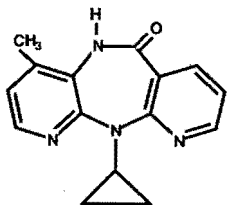
R = H, HEPT
R = alkyl

Figure 5. 1-[(2-hydroxyethoxy)methyl]-6-phenylthiothymine (HEPT) derivatives: inhibitors of HIV-1

Recently, two structurally distinct, non-nucleoside inhibitors of HIV-RT were described which share a common allosteric binding site in the enzyme. The inhibitors include analogues with variations of the five-membered urea ring of 4, 5, 6, 7-tetrahydro-5-methylimidazo [4,5,1-jk] [1,4]- benzodiazepine-2 (1H)-one (TIBO, Figure 6) and corresponding thione structures which block replication of HIV-1, but not HIV-2 (56). It appears that the TIBO analogues and the HEPT derivates share a similar mechanism of action (55). In addition, a series of dipyridodiazepinones have been identified as potent inhibitors of HIV-RT (57), which bind to a site on the enzyme distinct from the substrate binding sites (58). A compound from this series, 11-cyclopropyl-5, 11-dihydro-4-methyl-6H-dypyrido [3, 2-b:2', 3'-e] diazepin-6-one (nevirapine), is presently undergoing clinical evaluation.



TIBO derivatives
X = O, S



Nevirapine

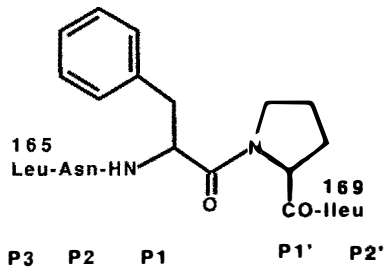
Figure 6. Benzodiazepin-2-(1H)-one (thione), TIBO and Dipyrido-diazepinone (Nevirapine) inhibitors of HIV-1

2. Protease

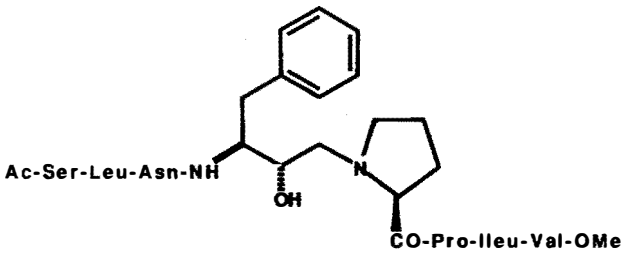
A virally encoded aspartic protease (PR) comprises another unique aspect of HIV-1 biochemistry, which, in addition to RT, is indispensable for the generation of an infectious particle. The PR processes the viral *gag* and *gag/pol* polyproteins in the final stages of viral maturation. It cleaves the p55 *gag* precursor into the four structural proteins, p17, p24, p8 and p7, that form the core of the virion. It also processes the p160 gag/pol precursor to liberate the four structural elements and the enzymes, protease itself, RT, and the endonuclease or integrase.

The three-dimensional structure of PR has been determined (59) both alone and in complexation with a variety of inhibitors. However, the details as to the mode, time and microenvironment of its action remain obscure. Nonetheless, it is clear that if the PR is catalytically defective or inhibited, viral maturation in HIV-infected cell culture is blocked and infection is arrested.

The most effective inhibitors of HIV-PR reported thus far are pseudopeptide / peptidemimetic compounds containing transition state inserts in place of the residues occupying the P1-P1'-positions [notation of Schechter and Berger (60)] of the peptide. These inserts include reduced peptide bond (methyleneamino, CH_2NH), hydroxyethylene [$CH(OH)CH_2$], hydroxy ethylamine [$CH(OH)CH_2N$] (see Figure 7) and



Substrate



Inhibitor

Figure 7. Structure of the physiological substrate and a hydroxyethylamine inhibitor (HEA) inhibitor of HIV protease

dihydroxyethylene [CH(OH)CH(OH)] moieties(61). Some of such peptide analogues can inhibit PR function in cultures of HIV-1 infected cells and, thus, can inhibit viral replication or attenuate the infectivity of the virions produced (62). Peptide analogs have been synthesized containing the hydroxyethylamine moiety which inhibit both HIV-1 and HIV-2 PRs with little effect on the structurally related aspartyl PRs (63). These analogues exert a potentand highly specific antiretroviral activity in the manomolar concentration range in multiple cell systems.

The generation of renin inhibitors as antihypertensive agents has resulted in much of the development of anti-HIV-PR inhibitors. In fact, knowledge of the peptomimetic inhibitors of renin, an enzyme closely related to HIV-1-PR has provided a guide in the design of templates and inserts for HIV-1-PR inhibitors (64). There have been sufficiently exciting data on several rationally designed, virus specific inhibitors to proceed to clinical trials in which the protease is the target.

3. TAT Protein

An important feature of the replicative cycle of HIV is its ability to establish a latent infection of $CD4^+$ T lymphocytes as a provirus integrated in the chromosome of the host cell (65), and possibly also as an unintegrated genome (66). The reactivation of the virus from this latent state and its entry into a destructive, replicati ve phase are important in the pathogenesis of HIV-induced disease.

The trans-acting transcription activator, *tat,* appears to play a unique and pivotal role in the emergence of the virus from the latent state. Antagonists to this non-structural, regulatory protein would be distinctly different from antiviral agents directed to other functions of the virus. Thus, there are, on the one hand, the nucleoside analogues that block initiation of infection in a cell freshly exposed to HIV by inhibition of the viral RT and, as suicide substrates, terminate DNA synthesis. On the other hand, inhibitors of viral protease and certain glycosidase inhibitors target events late in the replicative cycle, though they may also reduce the possibility of infection of fresh cells by blocking the formation of mature, infectious virions. However, neither the nucleoside analogues nor the inhibitors of viral protease would be expected to protect cells, that are already infected with the virus, from the cytopathicity associated with the late phase of HIV replication.

The cell culture assays which have been useful in the detection of, for example, inhibitors of RT should be suitable for screening antagonists of *tat.* The unique potential of a *tat* antagonist will be revealed, however, in its ability to block the reactivation of HIV from latently infected cells with an appropriate trigger, e.g., another virus such as cytomegalovirus, a cytokine or mitogen.

There are, at present, no known antagonists of *tat.* Nonetheless, the present knowledge base appears sufficient to support the development of inhibitors of this regulatory protein.

CONCLUSIONS

A major goal of a therapeutic strategy for treatment of HIV-1 infected individuals is the ability to achieve increased efficacy and/or reduced toxicity. This objective has led to the development of combination antiviral therapy for HIV infection. Thus, AZT and ddI (67), in addition to AZT in combination with ddC, as previously mentioned (39), have synergisitic activities against HIV. In this connection, it should be noted that there is ample precedent for the use of combination therapy for many bacterial and fungal infections, as a well as cancer chemotherapy. It is likely that combinations of agents, targeting different sites in the HIV-1 replicative cycle, may prevent or reduce the emergence of drug resistance and will afford, thereby, a prolonged and an effective antiretroviral therapy. Indeed, therapeutic intervention, utilizing drugs in combination, offers the hope of changing AIDS from a fatal to a manageable disease.

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