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Present Status and Future Prospects for HIV Therapies

Margaret I. Johnston* and Daniel F. Hoth

Since the discovery of human immunodeficiency virus (HIV) in 1983, significant progress has been made toward the discovery, development, and licensing of anti-HIV drugs. In vitro screens against whole virus are now being complemented by screens against specific viral targets, resulting in the development of clinical candidates acting at several critical stages of the viral life cycle. Despite these advances, clinical therapy remains largely palliative. In addition, it has recently been recognized that HIV resistance to most drugs may pose even greater obstacles. Moreover, emerging data on immunopathogenesis raise the possibility that even if virus was eliminated from an infected individual, the patient's immune system might not be capable of restoration to normal function. In the face of such obstacles, deeper insights into the pathogenic mechanisms of disease, aggressive exploitation of those mechanisms for therapeutic gain, and continued commitment of both public and private sectors to support and collaborate in this research are needed.

Introduction

In 1983, when HIV was discovered, the only antiviral agents licensed in the United States were amantadine, vidarabine, and acyclovir (*1*). Research was initially slow because only a limited number of facilities were willing to handle HIV, a new, lethal infectious agent. Fortunately, a significant body of information on the genomic structure and replication cycle of retroviruses had accumulated over the previous two decades (*2*) (Fig. 1).

Nucleoside analogs were a logical first place to search for anti-HIV agents because reverse transcriptase (RT) catalyzes a reaction not known to occur in humans and because several companies had libraries of nucleoside analogs synthesized in the search for anticancer or antiviral agents. In 1984, 3′-azidothymidine (AZT) was identified as active, first against murine retroviruses and then against HIV in cell culture (*3*). Clinical testing began in 1985. The phase II trial that conclusively showed a survival advantage for individuals with advanced disease taking AZT versus placebo was completed in September 1986, only 3 years after identification of HIV. The speed at which AZT was discovered, moved through clinical trials, and approved was unprecedented. Recognition that AZT did not completely suppress disease and had associated toxicities served as a stimulus for expanded research to identify additional agents.

The first inhibitors of HIV replication were discovered as a result of cell culture–based screening efforts, and such efforts continue to be valuable in identifying new agents that act at any step in the viral replication cycle. Recombinant DNA technology made possible the eventual cloning

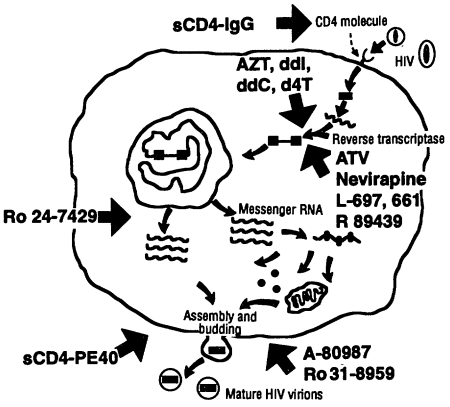

Fig. 1. Life cycle of HIV, showing the steps at which several anti-HIV agents act. Abbreviations are explained in the text.

M. I. Johnston is the associate director, Basic Research and Development Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD 20892. D. F. Hoth is the director, Division of AIDS, NIAID, NIH, Bethesda, MD 20892.

*To whom correspondence should be addressed.

of several key HIV proteins, which in turn stimulated development of mechanism-based screens that could accommodate large numbers of agents rapidly, inexpensively, and safely. Only recently have products identified by mechanism-based screens and confirmed in cell culture assays advanced to clinical trial. Structure-based primary screening activities have also been described, and although novel inhibitors have been identified, none has yet demonstrated sufficient activity against HIV in cell culture assays at nontoxic concentrations to warrant further development.

Typically, a clinical candidate is selected because of demonstrated potency in vitro at concentrations (usually micromolar or lower) that are anticipated to be maintained in the bloodstream or intracellularly for several hours and which are significantly lower than concentrations toxic to cells or animals. Several agents have shown potent activity in cell culture assays but have not been considered for further development because pharmacologically active blood levels could not be maintained or tolerated (*4–6*). Because both $CD4^+$ T cells and monocytes-macrophages are infected in the body, agents that show activity in both cell types are favored. In addition, because cells in the central nervous system (CNS) can be infected with HIV, the ability to penetrate the blood-brain barrier is considered. Finally, because almost all are virustatic and must be taken over a prolonged period, orally administered agents are preferable.

This article will focus on therapies for HIV infection and immune restoration. Although opportunistic infections (OIs) arising late in the course of disease secondary to severe immunosuppression are the principal cause of morbidity, discussion of this diverse group of diseases is beyond the scope of this article. Furthermore, unless interventions that result in fundamental improvements in immune function or reduction in viral replication are developed, effective treatment of one OI will only permit emergence of yet another.

Current State-of-the-Art Treatment

Although it is difficult to prove, treatment of HIV has likely resulted in prolonging the life of HIV-infected individuals and improving their quality of life. There are currently three antiretrovirals approved for use in HIV disease: 3′-azidothymidine (AZT, zidovudine, Retrovir), 2′,3′-dideoxyinosine (ddI, didanosine, Videx), and 2′,3′-dideoxycytidine (DDC, zalcitabine, HIVID). Numerous others are in clinical trial.

Approval of AZT was based on a placebo-controlled trial in individuals with advanced acquired immunodeficiency syndrome (AIDS) (<200 $CD4^+$ cells per cubic millimeter); the trial demonstrated a significant difference in mortality in the drug and placebo groups (*7*). Subsequently, AZT administered earlier in disease (<500 $CD4^+$ cells per cubic millimeter) was shown to delay the onset of AIDS-associated OIs (*8*). Furthermore, a recent preliminary report of a European trial of AZT versus placebo in asymptomatic HIV-infected persons suggests that the duration of AZT benefit may be limited, because there was no difference in survival at 3 years (*9*). Additional trials of AZT in asymptomatic HIV-infected persons are in progress.

Although ddI, another potent inhibitor of RT, produced a moderate rise in CD4 cell number, it was not shown to be superior to AZT as initial therapy (*10, 11*). Dideoxyinosine is approved for use in individuals who have been on AZT for 4 months or longer or who are hematologically intolerant to AZT. The combination of AZT and ddI has shown promising results in phase I evaluation (*12*). DDC, on a molar basis, is the most potent of the three approved drugs in cell culture assays (*13, 14*) and has been approved for use with AZT in adults with <300 $CD4^+$ cells per cubic millimeter who have experienced significant clinical or immunologic deterioration (*15*). Additional efficacy trials of AZT, ddI, and DDC are in progress.

Antiretroviral therapy is routinely used in the United States to treat individuals with moderate to advanced disease (for example, <500 $CD4^+$ cells per cubic millimeter). Experimental monotherapies are typically evaluated for their ability to delay progression to disease; determining the clinical impact of new therapies on survival has been problematic. Although the first randomized trial with AZT was placebo-controlled and demonstrated a survival advantage, subsequent trials have used active controls, and survival gains over AZT have not been shown. However, it is possible that the therapies evaluated to date have an equivalent effect on survival, so that such trial designs may not be capable of detecting mortality impact. Even drug-induced delay of disease progression is a problematic end point because trials among patients with early disease may require years before sufficient clinical end points accumulate. This has led to the search for laboratory tests, such as measurement of $CD4^+$ cells, or quantitative measurement by polymerase chain reaction (PCR) of circulating virus that could be used as surrogates and that could yield evidence of a drug effect more quickly and with greater sensitivity. Development of reliable assays and validation of these markers remain a challenge (*16*).

Since late 1989 there has been a reduction in the quarterly incidence of AIDS, that is, in the transition from the asymptomatic to the symptomatic state, as compared with the predicted AIDS rates (*17*). This result cannot be fully explained by a reduced rate of infection. The change in rate of progression to disease is probably associated with the widespread introduction of AZT into the population (as well as with prophylaxis for *Pneumocystis carinii* pneumonia) and is confined to those populations with more ready access to adequate health care.

Why are licensed RT inhibitors not entirely successful? The failure of existing therapies to completely block clinical progression remains one of most important questions facing therapeutic researchers. AZT and ddI, at clinically used doses, reduce circulating viral burden by only about one-half to one-tenth of initial values (*18, 19*). Although an RT inhibitor would not be anticipated to decrease the production of virus from cells already infected with HIV, effective concentrations should protect new cells from becoming infected. However, individuals do progress to disease while on antiretrovirals. Further, changes in the genomic sequences of HIV circulating in patients suggest that active infection of new cells and the error-prone process of reverse transcription occurs.

One possible explanation for the failure of existing therapies to halt progression is a failure to maintain adequate drug levels at the site of viral replication over extended periods. Current anti-HIV therapies, once begun, require frequent administration of drug owing to the relatively short half-lives of these drugs and are usually continued throughout life. Hence, drug compliance remains a major issue, and drug failure may be due to the inability to maintain drug concentrations at adequate levels. In particular, drug concentration in infected tissues and especially in intracellular compartments remains poorly studied. For example, for certain nucleosides [ddI, DDC, and the (−) enantiomer of 2′-deoxy-3′-thiacytidine (3TC, Lamivadine) (*20*)] the ratio of active nucleoside triphosphate to natural substrate is higher in resting than activated cells, whereas for others [AZT and didehydrothymidine (d4T, Stavudine)] the opposite is found (*21*). The impact of cell activation on the intracellular levels of other anti-HIV agents remains unknown.

Another possible explanation for drug failure is the emergence of drug resistance. Resistant virus can be isolated in cell culture and from individuals after about 6 months to 1 year of AZT treatment; resistance appears to become more frequent in later stage patients (*22*). Resistance may arise more quickly in patients with more advanced disease because they have a higher and more genetically diverse viral burden and are thus more likely to have preexisting resistant species that become selected in the

presence of drug. However, it has not been proven that the emergence of drug-resistant phenotypes, as measured in cell culture assays, is associated with clinical deterioration, although correlations have been suggested (*23, 24*).

Resistance has been described for each of the widely used antiretroviral nucleosides. The molecular basis for resistance to AZT inhibition is associated with mutations at at least four key loci of the HIV RT, with multiple mutations resulting in the highest degree of resistance (*25*). In addition, certain loci are more critical than others in maintaining drug susceptibility (*26*). Mutations associated with resistance to ddI and DDC have also been found in clinical isolates (*27*). There are now troublesome reports of possible transmission of AZT-resistant phenotypes (*23, 28*). Determining the extent and significance of such transmission is a public health priority.

Mutations associated with resistance, although seemingly counter to successful therapy, could have a beneficial outcome. Certain combinations of mutations linked with drug-resistant phenotypes might yield nonfunctional RT and noninfectious HIV (*29*). Four mutations associated with resistance to three different drugs (AZT, ddI, and a nonnucleoside RT inhibitor) were engineered into HIV and shown to result in replication-incompetent virus. Exposure of chronically infected cells to the above drug combination, and passage of the infected cells for several weeks, first in the presence of the drug combination and then in its absence, resulted in a sterile culture. However, it has not yet been demonstrated that exposure of HIV to drug combinations in cell culture resulted in selection of genotypic mutations associated with multidrug-resistant, replication-incompetent phenotype. In addition, other combinations of AZT, ddI, and nonnucleoside RT inhibitor (NNRTI)–induced mutations were consistent with HIV replication (*30*). Furthermore, this was not the first report of a drug-induced sterile culture, which could be the result of complete inhibition of HIV replication coupled with death or diluting out of infected cells (*31, 32*). Regardless, the combination of AZT, ddI, and nonnucleoside inhibitor was highly potent. A definitive clinical trial of this combination is in progress.

Other approaches attempting to overcome or prevent selection for drug resistance include, first, the use of drug-resistant HIV, particularly resistant clinical isolates, in screens for new drugs. Second, early treatment before significant numbers of mutants are generated may be worthwhile if resistance is the result of selection of preexisting mutants and given current data suggesting that initial infection is usually established by a single genotype. Two studies are under way to evaluate AZT treatment initiated within weeks of infection. Third, the benefit of switching patients onto a different antiretroviral therapy when evidence of a resistant genotype first appears is currently being evaluated.

Therapies in Development

Other RT inhibitors. The successful results obtained with AZT stimulated researchers to find additional RT inhibitors, many of which are now undergoing clinical evaluation. Use of d4T has resulted in increased $CD4^+$ cell counts and a decline in p24 antigenemia in previous trials and is currently being evaluated versus AZT use in phase II and phase III clinical trials in HIV-infected individuals with at least 6 months of prior AZT therapy (*33*). Toxicities associated with higher doses of d4T have included peripheral neuropathy and hepatitis. Another nucleoside, 3TC, is currently in phase I and phase II clinical trial; preliminary reports cite a transient increase in $CD4^+$ cell counts and decline in serum p24 levels, although no clear dose-response relation has been observed (*34*). No significant toxicity has been noted at the doses evaluated. On the basis of cell culture data, 3′-fluoro-thymidine (FLT) is among the most potent of the 3′-halo-dideoxypyridine analogs reported to date (*35, 36*). Preliminary clinical results suggested some activity in vivo (*37*). Further development of FLT has recently been discontinued, suggesting that significant antiviral activity was not observed at plasma concentrations that were well tolerated in a recent controlled phase II study. 9-(2-Phosphonomethoxyethyl)-adenine (PMEA), an acyclic nucleotide, recently entered phase I clinical trial (*38*). PMEA was more effective than AZT in blocking simian immunodeficiency virus (SIV) infection of macaques under optimal conditions of drug administration before infection (*39*). Clinical development of PMEA should be undertaken with caution in view of the narrow therapeutic range observed in cultured cells and in studies with mice demonstrating significant side effects, including fetal resorption and death of pregnant female mice (*40*). Other nucleosides are at earlier stages of development.

The development of a nonnucleoside inhibitor of HIV RT and replication was first reported in 1990; subsequently, other classes of RT inhibitors were reported, including tetrahydro-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one (TIBO, R82913) (*41, 42*), 11-cyclopropyl-7-methyl-dipyrido-[2,3-b:3′3′-f]1,4-diazepin-6H-5-one (BI-RG-587, nevirapine) (*43*), pyridones (L-697,661 and L-696,229) (*44*), and bis(heteroaryl)piperazines (BHAPs, U-87201E, Atevirdine Mesylate, ATV) (*45*). Two additional agents in this class, R-89439 [an α-anilino-phenylacetamide (α-APA) derivative (*46*)] and a second generation BHAP U-90,152 (*47*), recently entered clinical trial.

Although structurally distinct, NNRTIs have several features in common. All are extremely potent in cell culture assays and inhibit HIV replication at nanomolar concentrations (*41–49*). Unlike AZT, ddI, or DDC, the NNRTIs do not require conversion to active drug once within the cell. These agents typically have therapeutic indices defined in cultured cells in excess of 1000. They are highly specific noncompetitive inhibitors of HIV and do not inhibit other retroviruses, including the closely related SIV and HIV-2. Most appear to bind to a site on RT near Tyr^{181} and distinct from the substrate binding site (*48–51*). Decreased sensitivity of HIV to these agents develops rapidly, both in cultured cells and in vivo (*31, 47, 50–53*). HIV resistance to one NNRTI is usually cross-resistant to the other classes of NNRTIs, with the possible exception of the BHAPs class of RT inhibitors (*31, 47, 50, 51*).

In phase I trials, NNRTIs produce a rapid but transient decline in serum p24 levels (*54*). Virus with drug-resistant phenotype can be isolated within weeks after the onset of treatment. The favorable oral bioavailability of the NNRTIs and the lack of significant toxicities of these agents have stimulated a search for more potent NNRTIs that might overcome resistance. To date, NNRTIs appear to be additive or synergistic with nucleosides (*55*), and several combinations are currently being, or will soon be, evaluated in clinical trials in the hope that highly resistant isolates will not emerge. In addition to agents that block HIV replication at the RT stage, therapeutics that act at other stages are being sought. These will be discussed in an order that reflects the relative priorities currently being accorded to their development.

Protease inhibitors. HIV protease cleaves polyprotein precursors into mature structural proteins and enzymes during particle assembly and maturation (*56*). Although genetically engineered virus containing mutated protease that lacks enzymatic activity replicates, the virions that are produced are noninfectious in cultured cells (*57*). Protease inhibitors are an attractive target for therapeutic intervention because they act at a postintegration step of HIV replication. Recent cell culture data suggesting that cell-associated virus is more infectious than free virus magnify the need to evaluate agents that block the spread of HIV from infected cells (*58*). Whereas RT inhibitors are only effective in blocking HIV replication when added to cultured cells before HIV infection, protease inhibitors can also

inhibit HIV production from chronically infected cells (59, 60). Additional factors combine to make the HIV protease an attractive target for therapeutic intervention, including its unique cleavage specificity relative to human aspartic proteases, availability of cloned and chemically synthesized protease, detailed structure information, and the availability of rapid protease assays (61).

Peptide-based substrate analogs were the first inhibitors of protease reported to be active in vitro (59, 60, 62) and were the first to enter clinical trial. Development of peptide-based inhibitors has presented several challenges. In general, attempts to synthesize many of these inhibitors have required multistep, low-yield efforts. Modifications were needed to protect the peptide from degradation, while maintaining bioavailability, solubility, and activity. The first protease inhibitor to enter clinical trial, Ro 31-8959 [reported by Roberts (59, 60)], was an orally administered hydroxyethylamine mimetic of the transition state and had fairly low solubility and low bioavailability (62). Finally, development of protease-resistant HIV in cell culture has been reported, although the level of resistance has been low (10- to 30-fold) and cross-resistance is usually not complete (63). Thus, resistance to protease drugs may not prove to be as problematic as NNRTI resistance, which can be 1000-fold (31, 47, 50–53).

Recent studies by Kempf (59, 60) on the C-2 symmetric protease inhibitor A-77003 [originally designed to fit the C-2 symmetric protease active site (64)] led to identification of a second generation inhibitor, A-80987, with improved oral bioavailability and serum half-life in animals (65). A-80987 is currently in phase I trials in Europe. Other protease inhibitors, including several that will probably be orally bioavailable, are at earlier stages of development and will probably soon enter clinical trial. Protease inhibitors tested are additive or synergistic with AZT, ddI, or DDC, which suggests that combinations of therapies directed to different drug targets will prove to be useful (66).

Tat inhibitors. Tat, a regulatory protein required for HIV replication in cultured cells, is a positive transactivator that stimulates transcription (67) and that may have other activities (68). An anti-Tat agent capable of blocking HIV replication in both acutely and chronically HIV-infected cells, 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepin-2(H)-one (Ro 5-3335), was first reported in 1991 (69). A less toxic clinical candidate, Ro 24-7429, entered clinical trial in 1992. A multisite trial is under way to study safety and to determine the impact on viral load and $CD4^+$ cell count. Although Tat-resistant HIV has not been observed even after 2 years of virus passage in cell culture under conditions similar to those used to generate resistance to other anti-HIV therapies (70), examination of clinical isolates will be needed. The clinical usefulness of anti-Tat agents in combination with anti-RT agents should be investigated.

Blocking of viral entry. HIV entry begins with highly specific binding of the HIV gp120 envelope protein with a CD4 molecule on the surface of most susceptible cells. In addition, binding of gp120 on the surface of an infected cell with CD4 on the surface of an uninfected cell is involved in syncytia formation and cell-to-cell spread of HIV. A recombinant soluble form of the CD4 receptor (sCD4) or the chimeric CD4–immunoglobulin G (IgG), designed to extend the serum half-life of sCD4, effectively blocked HIV infection and syncytia formation in cultured cells at levels that were attainable clinically (4, 71). However, in initial clinical studies viral markers were not affected (5). Primary isolate virions were shown to have a significantly decreased ability to bind sCD4 and were less sensitive to neutralization by sCD4 in vitro as compared with cell-cultured adapted isolates (72). Higher doses of sCD4 may be tolerated without toxicity, but it may not be practical to pursue such studies.

Another agent designed to exploit the interaction of CD4 and gp120 is CD4-PE40, a fusion protein between CD4 and two domains of the *Pseudomonas aeruginosa* exotoxin A (32, 73). CD4-PE40 binds to infected cells through interaction with gp120 expressed on the cell surface. One toxin domain facilitates entry of the lethal second domain into the cell, resulting in death of infected cells in culture. Phase I trials demonstrated dose-limiting hepatotoxicity. Because CD4-PE40 and AZT synergize in vitro, clinical studies are in progress to determine whether any combination of dose and schedule has a beneficial effect without unacceptable toxicity. This strategy represents one of the few virucidal approaches to the treatment of HIV. However, toxicity may eventually prove to limit the clinical usefulness of this approach.

Other HIV targets. HIV RT has three distinct enzymatic functions: (i) the polymerase domain, which catalyzes the transfer of nucleotides onto the growing DNA chain, is the primary target for existing nucleoside and nonnucleoside inhibitors of HIV RT; (ii) a ribonuclease (RNase) H domain cleaves genomic RNA after first strand synthesis to allow synthesis of the second strand DNA; and (iii) a double-stranded RNA-dependent RNase cleaves in the primer-template pair at specific sites (74). RNase H is required for HIV replication (75). Only a few agents have been reported to target RNase H (76, 77). 3′-Azidothymidine monophosphate, which accumulates to millimolar levels in cells treated with AZT, inhibits RNase at millimolar levels; RNase inhibitors may be a secondary mechanism by which AZT inhibits RT activity (76, 78).

Two other HIV-encoded proteins are being examined as possible targets for therapeutic intervention. Integrase, an enzyme characteristic of retroviral infection, is essential to replication (79, 80). A rapid microtiter assay for the joining activity catalyzed by HIV integrase has been described (81), although no inhibitors of integrase have been reported to date. Rev permits the export of unspliced HIV mRNAs from the nucleus and is also required for HIV replication (82). Recently, a high-throughput screen based on Rev-dependent expression of gp160 in *Drosophila* cells has been established (83).

A cellular myristoylCoA:N-myristoyltransferase (NMT) catalyzes transfer of myristate from myristoylCoA to the HIV-encoded proteins Nef and Gag in a process required for HIV replication. Heteroatom-containing analogs of myristic acid, such as 4-oxatetradecanoic acid, serve as substrates for NMT (84, 85). Addition of these analogs to HIV Gag results in alteration of protein hydrophobicity and localization of Gag in the cytosol rather than the plasma membrane. Inhibition of HIV replication in cultured cells can be achieved with these analogs at concentrations that are not detectably toxic to uninfected cells (85, 86). It remains to be seen whether addition of myristic acid analog will be sufficiently restricted to viral rather than cellular proteins to provide an acceptable therapeutic window. Finally, a number of natural products whose mechanism of action may or may not be known are at various stages of development (87).

Nucleic acid–based therapies. Nucleic acid–based therapeutics, many of which target virally encoded nucleic acids, offer unique opportunities for intervention, but remain wholly unproven. Nucleic acid–based therapies being evaluated in cell culture include antisense oligonucleotides (88, 89), catalytic RNAs or ribozymes (90, 91), RNA analogs or decoys (92), and genes that encode proteins, such as CD4 or transdominant peptides, that have direct antiviral activity (93).

Antisense oligonucleotides directed against sites proximal to and including the translation initiation codon, splice sites, and single-strand loops were reported to be successful in blocking HIV replication in cultured cells at micromolar levels (94, 95). The use of catalytic RNAs or ribozymes that recognize and cleave specific viral sequences in trans has been proposed as an

approach to decrease the amount of oligonucleotide needed to target a specific intracellular RNA (*91*). Cultured cells stably transfected to express a ribozyme gene targeted against the HIV *gag* mRNA were shown to be partially resistant to HIV infection; cleavage of *gag* mRNA in the predicted location was demonstrated (*96*). In theory, catalytic RNAs can inactivate many target RNA molecules within the same cell, although turnover of catalytic RNA in intact cells has not yet been demonstrated. Oligonucleotides, such as TAR decoys, polyTAR, and RRE decoys, that inhibit the function of viral proteins are also under investigation (*92*).

Exogenous delivery of oligonucleotides has been plagued by problems of nonspecific inhibition and toxicity, inefficient cellular uptake, and instability in plasma. Stability issues have been addressed to some extent through chemical modifications of the oligonucleotide or, in the case of ribozymes, through DNA-RNA chimeras. Liposome or lipofectin encapsulation or lipophilic modification increases the efficiency of uptake of nucleic acids into cultured cells (*97*). A critical step in exogenous delivery of nucleic acid therapies will be to assure that the delivered material escapes the lysosomal degradation pathway after internalization into the cell. Despite the difficulty of obtaining sufficient quantities of nucleic acid to administer systematically, clinical trials are likely to occur within the next year as several antisense and ribozyme approaches are developed further.

Recent advances in the application of gene therapy to several diseases have stimulated interest in the therapeutic potential of nucleic acids expressed endogenously by cells. Although several approaches have succeeded in producing anti-HIV activity in cultured cells, the difficulty in obtaining sufficient expression of the desired gene in a sufficient number of cells in vivo has remained an obstacle. Currently, transfection with retroviral vectors results in expression of the desired gene in only a small percentage of cells. Other methods of delivery have been proposed, including a retroviral vector that makes use of the highly efficient HIV long terminal repeat (LTR) to control gene expression (*98*). Although HIV vectors have the advantage of infecting the same target cell as HIV, there may be serious drawbacks associated with their use. Approaches are needed to ensure that these vectors are devoid of pathogenic capability and to eliminate the risk of recombination or mutation. Adeno-associated virus vectors that can infect diverse cell types with higher efficiency have also been described but are not yet available for clinic use (*99*). Another approach is the use of adenovirus capsids that bind the gene to be delivered through an antibody-polylysine complex attached to the capsid (*100*). Capsids may be readily formulated with the nucleic acid to be delivered and may deliver large amounts of nucleic acid into the cell, although gene expression is transient and the efficiency of T cell transduction is low.

Immune reconstitution. Approaches to block HIV replication are complemented by approaches to manipulate the immune system. The use of candidate HIV vaccines to increase existing immune responses or stimulate new ones in HIV-infected individuals is reviewed elsewhere (*101*). Another immunization-based approach is the ex vivo retrovirally mediated introduction of the *env* gene into autologous fibroblasts (*102*), which would then be given back to the patient to stimulate anti-*env* immune responses. Studies of cytotoxic T lymphocytes (CTLs) generated by immunization of mice with syngeneic cells expressing HIV (IIIB) envelope demonstrated that these CTLs recognize common determinants on diverse HIV strains, including several clinical isolates (*103*). Direct administration of the *env*-expressing vector may provide a more feasible long-term approach and should be accorded a high priority once safety concerns have been addressed. Delivery of naked DNA in the form of circular plasmid DNA engineered to express HIV proteins is also being pursued, with promising results (*104*). This approach offers several advantages, including low cost and the ease of preparation of DNA, and could revolutionize immunization strategies. However, the clinical benefit of any of these approaches in HIV disease remains uncertain.

Certain cytokines, such as tumor necrosis factor (TNF) and interleukin-6 (IL-6), may have a direct up-regulatory effect on HIV synthesis and should be considered as potential targets for intervention (*105*). Reported inhibitors of TNF action, pentoxifylline (Trental) and BRL 61063, are currently being evaluated (*106*). Certain thiol-based agents, such as *N*-acetylcysteine (NAC) and 2-oxothiazolidine-4-carboxylate (OTC, Procysteine), have been reported to prevent activation of HIV in latently infected cells, presumably as a result of the ability to raise the intracellular levels of glutathione, which is required for a variety of immune functions (*107*). More recently, a new class of anti-HIV agents, 1,2-dithiole-3-thiones, exemplified by oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione], not only elevated levels of glutathione but also appeared to irreversibly inhibit HIV RT (*108*).

Interferon-α (IFN-α) blocks HIV replication in vitro probably by interfering with the assembly or release (or both) of mature virions (*109*). Clinical trials addressing the in vivo effects of IFN-α, both alone and in combination with other agents, in patients at all stages of HIV-1 infection have suggested that patients at earlier stages may benefit from treatment with this agent (*110*). Controlled clinical trials of IFN-α alone and in combination with antiretrovirals are under way.

$CD8^+$ major histocompatibility complex (MHC) class I–restricted CTLs kill HIV-infected cells in culture and may also block HIV replication by release of a soluble factor (*111*). Expansion and reinfusion of HIV-specific autologous $CD8^+$ T cells from HIV-infected individuals (*112*) is undergoing initial clinical evaluation. Given the difficulty and expense in expanding cells ex vivo, this approach is unlikely to have widespread use in the near future. This early trial, however, may provide valuable information on the role of $CD8^+$ cells in controlling viral replication and may stimulate research on alternative sources of $CD8^+$ cells, including allogeneic or xenogeneic cells or universally accepted CTLs.

Several approaches to broadly reconstitute immune competence are being evaluated. IL-2 induced a transient but significant increase in the number of $CD4^+$ cells (*113*), and low-dose polyethylene glycol–modified IL-2 (PEG–IL-2) increased killer cell activity and enhanced proliferative responses in infected individuals (*114*). Additional trials of PEG–IL-2 in combination with AZT or ddI are under way. Thymic humoral factor (THF) and thymopentin (TP5) are two hormone-based therapies that have entered clinical trial (*115*). THF is reported to augment cell-mediated immunity, whereas thymopentin is reported to enhance T cell function by increasing lymphokine production.

If procedures for purging HIV from infected cells could be developed, it may be worthwhile to pursue ex vivo expansion and reinfusion of $CD4^+$ cells. Finally, a long-range goal to achieve complete immune function would be the administration of multipotent progenitor cells genetically engineered to resist HIV infection. Significant advances in gene transduction and expression in human progenitor cells, and information on the ability of engineered progenitors to differentiate in HIV-infected individuals, will be needed prior to attaining such a lofty goal. In the interim, evaluation of syngeneic bone marrow transplantation and adoptive transfer of peripheral blood lymphocytes in combination with antiretroviral regimens continues (*116*).

The Future of HIV Therapeutics

Three inhibitors of RT have been licensed, and other inhibitors of RT, Tat, and protease are currently in clinical trial. In addition, several combinations of anti-RT

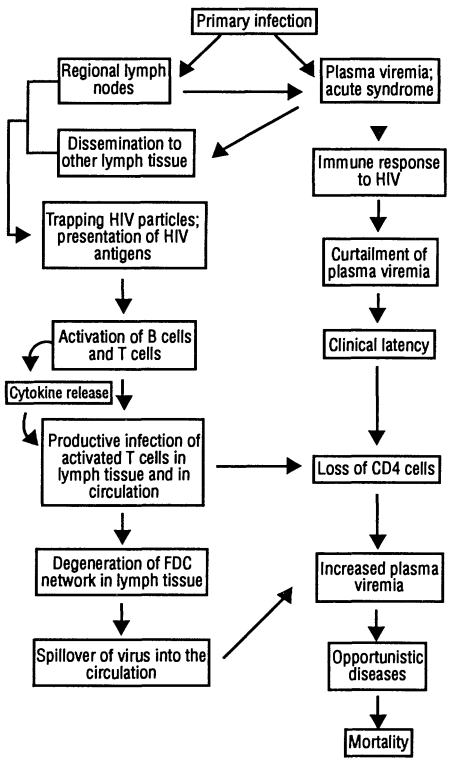

Fig. 2. Progression of HIV infection from acute infection to morbidity and mortality. The right side depicts information obtained by monitoring of the blood from infected individuals. The left side summarizes information on the role of lymphoid organs in disease progression. FDC, follicular dendritic cells. [Adapted from (*123*)]

agents are undergoing clinical evaluation, both in simultaneous and alternating regimens. The use of combinations of agents that act at pre- and post-integration events is an attractive theoretical approach. However, there is currently no clinical information proving that mechanistically diverse combinations are more efficacious than single agents or combinations of agents that act at the same step in the viral life cycle. The orderly evaluation of drug combinations offers significant preclinical and clinical challenges. Indeed, it is expected that the use of combinations either to overcome or avoid resistance, to provide a synergistic antiviral effect, or to manage drug-related toxicities will improve the management of HIV disease in the next few years.

It is likely that the speed of discovery of new drugs will be accelerated by newer technologies, such as screens based on combinatorial libraries of peptides and oligonucleotides, permitting the rapid screening of millions of compounds and equally rapid optimization of candidate drugs (*88, 117*).

Although most current antiretroviral approaches are based on an understanding of the life cycle of HIV in cell culture, much less is understood about the interactions of HIV with the host and the mechanisms by which HIV causes disease. As new information from in vivo studies emerges, new therapeutic concepts will likely follow. For example, wild-type SIV containing *nef* and SIV with a *nef* deletion were indistinguishable in their growth kinetics in cultured cells (*118*). Yet, animals infected with SIV deleted in *nef* became infected but did not develop disease, whereas the wild-type virus caused disease and death (*118*).

Information on the earliest stages of disease may also yield valuable clues to new therapeutic strategies. During acute infection, HIV replication may be amplified because a very high percentage of cells are activated, probably by various cytokines that act by autocrine or paracrine routes (Fig. 2) (*105, 118a*). Methods to downregulate this process may prove particularly beneficial. Further, deciphering the specificity of cells or antibodies that clear the early burst of infectious virus from the circulation will facilitate the design of therapeutic immunization strategies. Finally, emerging data suggest that viral burden in early infection is relatively low, and the number of genomic variants appears to be small. Aggressive therapy with antiretrovirals, with or without immune-targeted therapies, might impact long-term outcome. In addition, one of the highest priorities of current research is determining the stage at which antiretroviral therapy should begin, particularly in view of data indicating that viral replication occurs at all stages of disease (*18, 119*).

Recent reports have suggested that the transition from apparent clinical latency to a stage of more rapid decline is associated with a change in the phenotype of the predominant virus in the body, specifically from nonsyncytium-inducing (NSI) to syncytium-inducing (SI) virus (*120*). Whether SI phenotype is more pathogenic or whether it is simply a marker for increased viral replication is not known. Reports have suggested that AZT is only minimally effective against the SI phenotype (*121*). Understanding the process and consequences of the change in phenotype will be very important. In the interim, screening new potential therapies against both NSI and SI types of virus is recommended.

Finally, the processes underlying immune damage, including loss of $CD4^+$ cells and induction of anergy, need to be explored further. Direct killing of cells by HIV may not be the only mechanism of immune damage (*122*), as recently reviewed (*123*). Elucidation of pathogenic mechanisms may not only provide additional targets for intervention but may also guide approaches to augment or restore immune function.

Overall, substantial resources have not been devoted to the pursuit of potential drug targets other than RT and protease, and even fewer resources have been devoted to more innovative and risky approaches to restore immune function. In part, selection of therapeutic approaches has been affected by limitations in basic knowledge and by the existence of technologies suitable for high-throughput screens. Pharmaceutical companies have demonstrated extreme care in managing the overall level of resources devoted to the development of antiviral agents. Most higher risk technologies are supported by venture capital. The success of any particular approach is likely to generate interest from larger companies. In the interim, it is critical that the government continues to support basic research on the pathogenesis of HIV disease and to foster linkages that accomplish a rapid translation of new findings and new technologies into therapeutic gains.

The field of HIV therapeutics would not be where it is today had it not been for previous research on retroviruses that helped identify RT and other viral proteins as targets for therapeutic intervention. The field has progressed to the point where therapies targeted to different stages of replication are in trial and additional mechanism-based targets are in place. It is the view of these authors that ultimate success will depend not only on learning how best to use the drugs that are currently available and in development, but also on improving our understanding of the basic disease process so that all steps of the virus's impact on the host can be identified and countered, if not eliminated.

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