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Perspective

Chemotherapeutic Approaches to the Treatment of the Acquired Immune Deficiency Syndrome (AIDS)

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Acquired immunodeficiency syndrome (AIDS) was first recognized in 1981 as a distinct clinical entity,[1] and soon after the infectious nature of the disease became apparent, human T-cell lymphotropic virus type III (HTLV-III), also referred to as lymphadenopathy-associated virus (LAV),[2] was identified as the putative causative agent of AIDS.[3] At present, there is little doubt that HTLV-III/LAV [or AIDS-associated retrovirus (ARV)[4]] is indeed the primary cause of a spectrum of immunological disorders, with AIDS as the most severe clinical manifestation.[5]

The natural history of HTLV-III/LAV infection is depicted in Figure 1, which clearly illustrates the variety of clinical conditions that may be associated with AIDS.[6] For an unknown time period, the infected person may remain antibody-negative and perfectly healthy. A certain percentage of these people (up to 38% of the high-risk group members, i.e., homosexual men, parenteral drug users, and hemophiliacs[7]) may then seroconvert, thus become antibody-positive, and of the seropositive subjects, again a certain percentage (up to 34% for some cohorts of homosexual men[7]) may go on to develop AIDS. A larger portion of antibody-positive subjects, however, would not develop full-fledged AIDS, but either subclinical signs of immune deficiency, persistent generalized lymphadenopathy, or both (Figure 1).[6] The term "lesser AIDS" or "AIDS-related complex (ARC)" may be coined for AIDS-related illnesses of lesser clinical severity.

Clinical signs indicative of AIDS are weight loss (>10% of body weight), chronic diarrhea (>1 month), prolonged fever (>1 month), oral candidiasis, and generalized lymphadenopathy; but the definition of AIDS as such is based on the occurrence of *Pneumocystis carinii* pneumonia (PCP) or other life-threatening opportunistic infections, Kaposi's sarcoma, or malignant (non-Hodgkin's) lymphoma. Opportunistic infections (OI), besides PCP, that are indicative of AIDS include cryptosporidiosis, strongyloidosis, toxoplasmosis, candidiasis, cryptococcosis, as well as bacterial (i.e., *Mycobacterium avium-intracellulare*) and viral (i.e., cytomegalo, herpes simplex, Epstein-Barr virus) infections.[8] HTLV-III/LAV has a specific tropism for T-helper/inducer ($OKT4^+$ or $LEU3^+$) lymphocytes, while leaving the T-suppressor/cytotoxic ($OKT8^+$ or $LEU2^+$) subset unaffected.[9,10] This leads to an inversion of the T4/T8 or helper/suppressor ratio, a characteristic feature of AIDS and AIDS-related illnesses. The selective depletion of the T-helper lymphocytes results in a profound cellular immunodeficiency, which, in turn, renders the patient highly vulnerable to opportunistic infections and neoplasia.

In principle, chemotherapeutic approaches toward AIDS could be based on at least three strategies: (i) treatment of the intercurrent neoplasia (Kaposi's sarcoma) and/or opportunistic infections,[8] (ii) reconstitution of the cellular immune status, or, if this would not prove feasible, enhancement of the immune functions through the use of biological response modifiers,[11] and (iii) therapeutic measures directed against the etiologic agent, HTLV-III/LAV, and aimed at blocking one or another step in the replicative cycle of the virus.[12] Obviously, the HTLV-III/LAV virus

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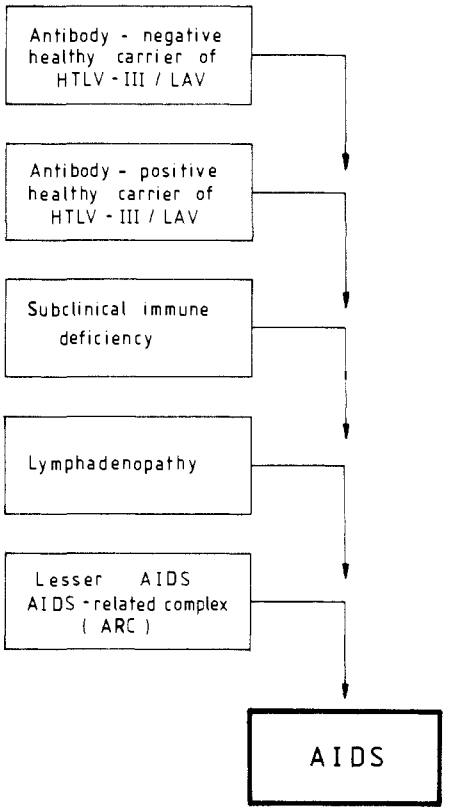

Figure 1. Natural history of HTLV-III/LAV infection. Available data suggest a number of intermediate outcomes. Although these outcomes represent stages of a progressive immunoblative process, the sequence of events is not invariable. According to Blattner et al.[6] (slightly modified).

may be considered as the most attractive target in the development of an adequate therapy for AIDS, as it is the *primum movens* in the pathogenesis of the disease, and, if it could be eliminated or suppressed in time, would make further interventions in terms of immune reconstitution, immune enhancement, or other chemotherapeutic measures of lesser importance or necessity. The fact that HTLV-III/LAV is a retrovirus with a unique genomic structure coding for unique viral proteins such as reverse transcriptase (RNA-directed DNA polymerase) and TAT [*trans*-acting "transcriptional" (actually translational)] activating protein, makes a chemotherapeutic approach directed against the causative agent all the more attractive.

HTLV-III/LAV Genome

The genome of HTLV-III/LAV is similar to that of the usual retroviruses in that it contains *gag*, *pol*, and *env* genes, encoding for the group-specific antigens, reverse transcriptase (plus protease and endonuclease) activities, and envelope proteins, respectively (Figure 2).[13,14] Starting at the 5′ end of the genome, following the 5′ long terminal repeat (LTR) is the *gag* gene, which encodes a polyprotein of approximately 55 kilodaltons (kd). This polyprotein is subsequently cleaved to form p17, p24 (the major structural protein of the mature virions), and p15. The second known gene of the virus, *pol*, would encode for the reverse transcriptase as well as a protease (at its 5′-terminus) and an endonuclease (at its 3′-terminus). The reverse transcriptase activity would, according to recent evidence,[13b] reside with the highly immunogenic p66/p51 product of HTLV-III/LAV. The third gene contains a short open reading (*sor*) frame, also called orf-1, of unknown function; it is unique for HTLV-III and does not occur in HTLV-I or HTLV-II. The fourth gene, designated *env*, corresponds to a large open reading frame that encodes for a protein of about 90 kd (in its nonglycosylated form). This protein is heavily glycosylated to a glycoprotein of about 160 kd (designated gp 160): it gives rise to the gp120 and gp41 glycoproteins,[15] the latter being the transmembrane glycoprotein also encountered with other retroviruses. The glycoproteins gp160 and gp120 represent the antigens most consistently recognized by antibodies found in patients with AIDS or ARC.[16,17] Hence, the *env*-encoded glycoproteins are the most immunogenic of the HTLV-III/LAV proteins. They also correspond to the sites where individual HTLV-III isolates show the greatest polymorphism.[18,19] The fifth gene, or fifth open reading frame (3′-orf), also called orf-2, extends into the 3′ long terminal repeat (LTR) and encodes a myristilated protein of about 27 kd.[20]

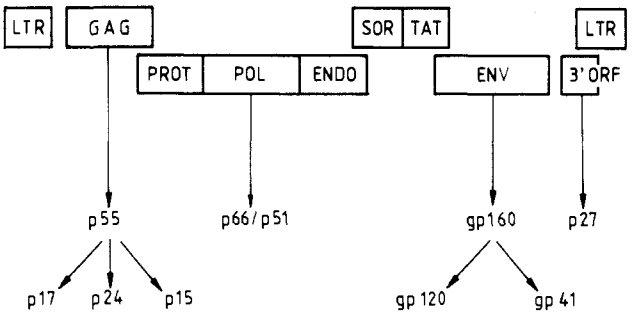

Figure 2. Schematic representation of the HTLV-III/LAV genome, with the major antigens that induce antibodies in exposed humans. LTR: long terminal repeat. GAG: group-specific antigen genes [encoding for the core proteins (p)]. POL: polymerase gene (encoding for the reverse transcriptase). PROT: part of the *pol* gene that encodes for a protease. ENDO: part of the *pol* gene that encodes for an endonuclease. SOR: short open reading frame (also called ORF-1). TAT: *trans*-acting "transcriptional" (actually translational) activating gene. ENV: envelope gene [encoding for the envelope glycoproteins (gp)]. 3′ORF: 3′ open reading frame (also called ORF-2) (encodes for a 27kd protein). According to Essex et al.,[13a] di Marzo Veronese et al.,[13b] and Wong-Staal and Gallo[14] (slightly modified).

Of prime importance is the ability of HTLV-III/LAV to exhibit TAT activation (originally defined as "*trans*-acting transcriptional" activation). Responsible for this phenomenon is a *trans*-acting factor that would be capable of activating the expression of genes at a distance from the *tat* gene, i.e., LTR-linked genes. It is speculated that the

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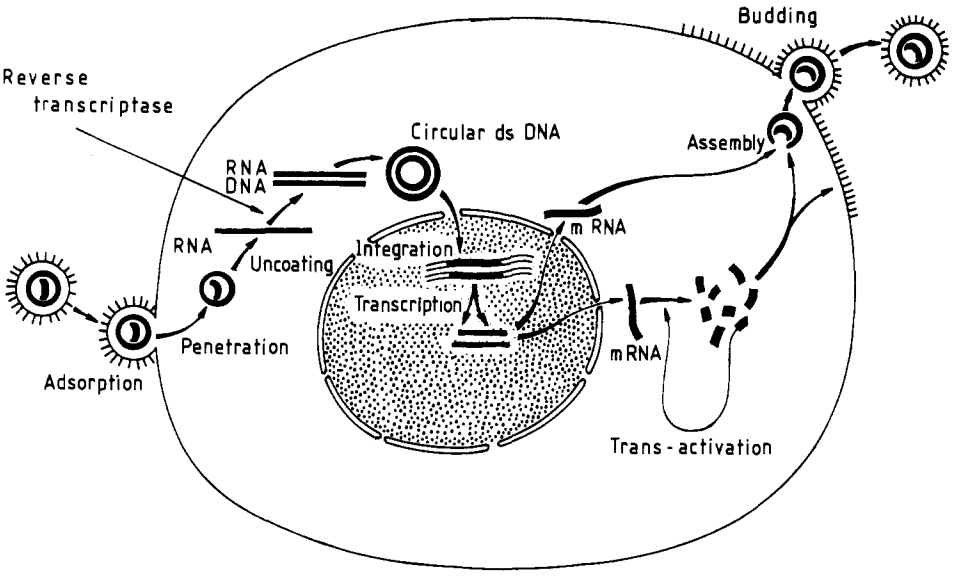

Figure 3. Replicative cycle of HTLV-III/LAV. According to Hirsch and Kaplan[12] (modified).

tat gene product(s) would mediate the cytopathic effects of HTLV-III/LAV. The major functional domain of the HTLV-III/LAV *tat* gene[21,22] has been mapped to a region immediately before the *env* gene (Figure 2). The transcription of the *tat* gene to mRNA would involve two splicing events, thus bringing together three exons, the first being located in the nontranslated leader sequence of the *gag* gene, the second corresponding to the region immediately adjacent to the 5′ end of the *env* gene, and the third encompassing the 3′ part of the *env* gene up to the 3′ end of the genome. TAT activation appears to be a common feature of several retroviruses: i.e., HTLV-I, HTLV-II, HTLV-III, bovine leukemia virus (BLV), visna virus, equine infectious anemia virus, and caprine arthritis and encephalitis virus.[14] However, whereas the *trans*-activating proteins of HTLV-I and HTLV-II may turn on genes that promote cell growth, the HTLV-III *trans*-activating protein may turn off such genes or, at least, alter cellular gene expression such that cell growth is disrupted.[23]

HTLV-III/LAV Replication

The fundamental difference between the retrovirus replication cycle and the "classical" virus replicative cycle resides in the ("reverse") transcription of the viral RNA genome to (proviral) DNA and the subsequent integration of the proviral DNA into host chromosomal DNA. The retrovirus replication cycle (Figure 3) starts with the adsorption of the virus particle to a specific cell-surface receptor.[24,25] This receptor, termed T4 or CD4, corresponds to a glycoprotein of 62 kd[25] (or 58 kd),[26] which would specifically interact with the viral glycoprotein gp120 (gp110),[26] and this binding may thus be a major factor in the tropism of HTLV-III/LAV for T-helper/inducer lymphocytes. After penetration, the virus is uncoated in the cytoplasm and the viral RNA is transcribed by reverse transcriptase with use of a host lysyl transfer RNA as primer. The reaction proceeds via the formation of an RNA·DNA hybrid intermediate, of which the RNA part is degraded and the DNA duplicated and circularized to double-stranded proviral DNA, which may then be integrated into host chromosomal DNA. The sequence of events thus initiated by the HTLV-III/LAV reverse transcriptase would be quite similar to that of other retrovirus reverse transcriptases.[27]

To what extent and at which site(s) the proviral DNA is integrated, and whether it has to be integrated into the host chromosomal DNA, are so many issues that remain to be resolved. Expression of the viral genes would follow the usual flow of transcription, posttranscriptional processing, translation and posttranslational modifications. During the posttranscriptional processing, the viral mRNAs are subject to splicing, 5′ end capping, and 3′ end polyadenylation. As mentioned above, the *tat* mRNA is generated by a double-splice mechanism.[14] The retroviral mRNAs are translated into viral-precursor proteins, which are further processed by either proteolytic cleavage or glycosylation, or both. The proteases involved in the proteolytic cleavage may be of either viral or cellular origin. The glycosylation process is exclusively dependent on cellular enzymes. The glycoproteins (gp) are all specified by the *env* gene (see Figure 2), synthesized and glycosylated in the rough endoplasmic reticulum, and transported through the Golgi apparatus to the plasma membrane. Particularly gp120 (gp110),[26] the major glycoprotein of the viral envelope, is heavily glycosylated, with >20 potential glycosylation sites.[14] Glycoprotein gp41, the transmembrane envelope glycoprotein, is assumed to play an important role in the immunosuppressive action of HTLV-III/LAV.[28] The virus particles are assembled and released by budding through the plasma membrane at areas where the viral glycoproteins have congregated (Figure 3).

The HTLV-III/LAV replication cycle thus offers a wealth of potential targets for antiviral agents: i.e., adsorption, penetration, uncoating, reverse transcription, proviral DNA circularization and integration, viral mRNA transcription, maturation (capping, splicing, poly-

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Table I. Inhibitors of Reverse Transcriptase

I. Enzyme-Binding Compounds
rifamycins, streptovaricins, pyran copolymer, calcium elenolate, suramin,[32] Trypan Blue, Evans Blue, Direct Yellow 50 (and other anionic dyes),[33] pyridoxal 5′-phosphate,[34] aurintricarboxylic acid (and other triphenylmethane dyes),[35,36] gliotoxin analogues,[37] tungstoantimoniates,[38] i.e., HPA-23 $[(NH_4)_{18}(NaW_{21}Sb_9O_{86})_{17}]$, HPA-39 $[K_{18}(KW_{21}Sb_9O_{86})_{17}]$

II. Template/Primer-Binding Compounds
Actinomycin D, daunomycin, adriamycin, distamycin A, proflavin, ethidium bromide, tilorone, diaryl amidine and -imidazoline derivatives,[39] fagaronine, bleomycin, fluoranthene derivatives, 5-(*p*-chlorobenzyl)-6-aminouracil[40]

III. Substrate or Product Analogues
ara-CTP (arabinocytidine 5′-triphosphate), ddNTP (2′,3′-dideoxynucleoside 5′-triphosphates),[41] PAA (phosphonoacetate), PFA (phosphonoformate)[42]

IV. Template Analogues
2′-substituted polynucleotide derivatives: i.e., poly(2′-azido-2′-deoxyuridylic acid) $[(dUz)_n]$, poly(2′-azido-2′-deoxycytidylic acid) $[(dCz)_n]$,[43] poly(2′-azido-2′-deoxyadenylic acid) $[(dAz)_n]$[44]
2′-substituted polyinosinic acid $[(I)_n]$ and polyadenylic acid $[(A)_n]$ derivatives: i.e., poly(2-methylthioinosinic acid) $[(ms^2I)_n]$,[45] poly[2-(methylthio)adenylic acid] $[(ms^2A)_n]$, poly[2-(ethylthio)adenylic acid] $[(es^2A)_n]$,[44] poly(2-fluoroadenylic acid) $[(fl^2A)_n]$, poly(2-chloroadenylic acid) $[(cl^2A)_n]$, poly(2-bromoadenylic acid) $[(br^2A)_n]$, poly(2-iodoadenylic acid) $[(io^2A)_n]$[46]

V. Divalent-Cation-Binding Agents
O-phenanthroline, thiosemicarbazones

adenylation), and translation, viral protein precursor cleavage and glycosylation, virus assembly and release (budding). In addition, the *trans*-activator protein or *tat* product may also be viewed as an interesting target for antiviral chemotherapy. According to recent evidence,[29] the *tat* protein would greatly elevate the expression of HTLV-III/LAV gene products through a stimulatory effect at the translational level. Thus, the *trans*-acting factor would work to speed up production of viral products, thereby contributing to the cytopathic effects of the virus. Because of the autostimulatory role of the *tat* protein in the replication cycle of HTLV-III/LAV, chemotherapeutic devices aimed at blocking either the expression or action of the *tat* protein should have a decisive effect on the virus replication cycle.

The production of new HTLV-III/LAV virions puts a heavy burden on the cell's supply of basic precursors for the biosynthesis of nucleic acids, proteins, or polysaccharides, and it can be foreseen that, if one of the intermediary substances involved in the biosynthesis of these macromolecules would be shut off, this may well slow down, if not stop, the production of new virus particles. Such situation could, theoretically, arise, if, for example, transmethylation reactions (required for the 5′ end capping and maturation of viral mRNA) are inhibited by adenosine analogues, which interfere with the hydrolysis of *S*-adenosylhomocysteine, and thereby enable *S*-adenosylhomocysteine to accumulate and to suppress the methyl transfer from *S*-adenosylmethionine. Vulnerable spots may also be situated in the de novo pathways leading to the biosynthesis of purine and pyrimidine nucleotides, and as the replication of viruses in general is quite readily disturbed by nucleoside analogues interfering with one or another step of purine or pyrimidine nucleotide biosynthesis, i.e., IMP dehydrogenase, OMP decarboxylase, dTMP synthetase, CTP synthetase, it is likely that any interaction at one of these steps may also affect HTLV-III/LAV replication.

Retrovirus Reverse Transcriptase Inhibitors

Because of its unique association with retroviruses, the reverse transcriptase has since long been considered as an attractive target, and pending elucidation of the role of the reverse transcriptase and retroviruses in any human pathology, numerous inhibitors of reverse transcriptase were described in the 1970s.[30,31] These inhibitors could be classified as either enzyme-binding compounds, template/primer-binding compounds, substrate or product analogues, template analogues, and divalent-cation-binding agents (Table I). From the compounds that were evaluated in our laboratory as reverse transcriptase inhibitors, i.e., suramin,[32] gliotoxin analogues,[37] diaryl amidine derivatives,[39] 2′-substituted polynucleotide derivatives $[(dUz)_n, (dCz)_n, (dAz)_n]$,[43,44] 2-substituted polynucleotide derivatives $[(ms^2I)_n, (ms^2A)_n, (es^2A)_n, (fl^2A)_n, (cl^2A)_n, (br^2A)_n, (io^2A)_n]$,[44-46] emerged the 2-fluorinated $(A)_n$ analogue as by far the most potent inhibitor.[46]

The reverse transcriptase inhibitors listed in Table I cannot be considered as truly specific; on the contrary, they may be expected to inhibit DNA polymerases other than reverse transcriptase. For example, suramin is equally inhibitory to HTLV-III/LAV reverse transcriptase and cellular DNA polymerase α,[47] while DNA polymerases β

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and γ and terminal deoxynucleotidyl transferase are relatively resistant to the drug.[48] For most of the inhibitors listed in Table I, however, the relative sensitivities of the different DNA polymerases have not even been studied. Such studies require, in fact, that the enzymes first be isolated, purified, and then evaluated under optimal working conditions qua template/primer, divalent cation, pH, and other requirements. The differential effects that different inhibitors may achieve under these experimental conditions do not necessarily bear on the in vivo situation, since, in intact cells, the optimal requirements for enzymatic activity may be totally different from those exhibited by the cell-free enzyme, particularly because certain cofactors normally associated with the enzyme, may be lost during the isolation and purification procedure. Thus, inhibitor studies with cell-free reverse transcriptase may provide adequate indications as to the potency of the inhibitors but do not allow prediction concerning the specific effects these inhibitors may have on the enzymes in their natural environment.

HTLV-III/LAV Reverse Transcriptase Inhibitors

The HTLV-III/LAV reverse transcriptase is not fundamentally different from the reverse transcriptase of other retroviruses. For optimal activity it prefers Mg^{2+} over Mn^{2+} as the divalent cation[49] and poly(C)·olido(dG) over poly(A)·oligo(dT) as the template/primer.[50] If, however, poly(Am)·oligo(dT) or poly(Cm)·oligo(dG) is used as the template/primer, it prefers Mn^{2+} over Mg^{2+}. The optimal conditions for detection of HTLV-III/LAV reverse transcriptase have been described by Hoffman et al.[49] Upon electrofocusing the enzymatic activity resolves into two peaks with a pH of 5.75 and 6.25, respectively.[50] The meaning, if any, of this heterogeneity is not clear.

Because of the functional resemblance of HTLV-III/LAV reverse transcriptase with the retrovirus reverse transcriptases in general, it is not surprising that several of the compounds listed in Table I as reverse transcriptase inhibitors have proven effective in inhibiting HTLV-III/LAV reverse transcriptase activity. This has been demonstrated directly for suramin,[32,51] phosphonoformate,[52,53] and the ammonium 21-tungsto-9-antimoniate (HPA-23)[54] and indirectly, on the basis of their inhibitory effects on the replication of HTLV-III/LAV, for a number of other compounds such as Evans Blue,[33] aurintricarboxylic acid,[55] 3′-azido-2′,3′-dideoxythymidine (AZT),[56] and 2′,3′-dideoxynucleosides (ddCyd, ddAdo, ddIno, ddGuo, and ddThd).[57] As it is further documented in the next section, all these compounds (Chart I) are selective inhibitors of HTLV-III/LAV replication, and they may owe this selectivity to either (or both) of the following factors: (i) greater affinity for the HTLV-III/LAV reverse transcriptase than for the cellular DNA polymerases α, β, and γ or (ii) easier accessibility to the HTLV-III/LAV reverse transcriptase (localized in the cytosol) than to the cellular DNA polymerases (localized in the nucleus and mitochondria).

The precise mechanism(s) by which suramin and its congeners (Chart I) inhibit HTLV-III/LAV reverse transcriptase remains to be clarified. For suramin, Evans Blue, and aurintricarboxylic acid, one may postulate that they

Chart I. Structures of Some Selective Inhibitors of HTLV-III/LAV Replication

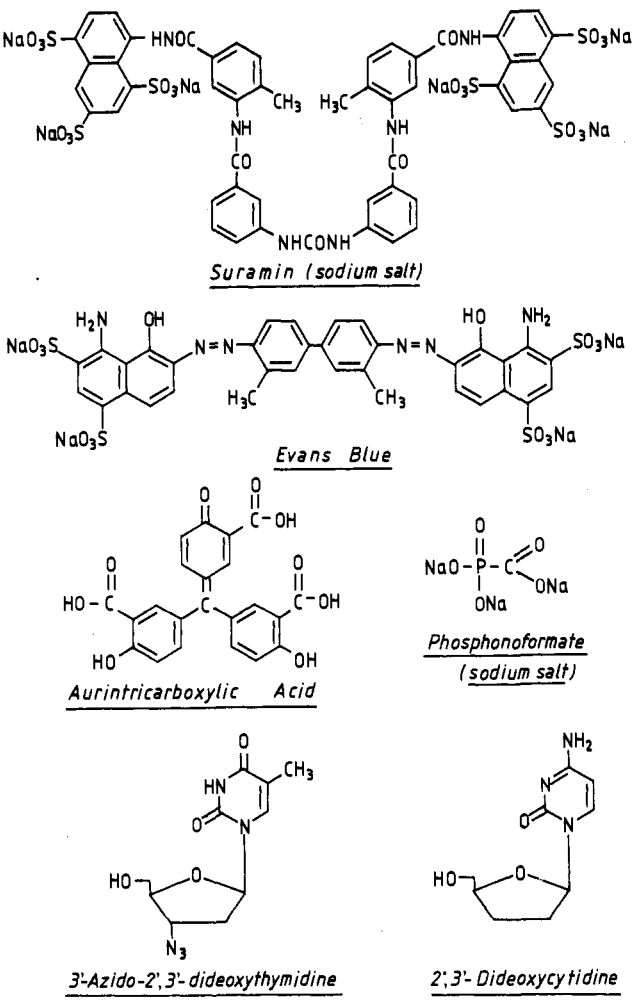

Table II. Comparative Potency and Selectivity of Inhibitors of HTLV-III/LAV Replication in Vitro

compound	MIC,[a] μM	selectivity index[b]	ref
suramin	35	5	33
Evans Blue	26	>4	33, 55
aurintricarboxylic acid	47	>2.5	55
phosphonoformate	350	>1	33
3′-azido-2′,3′-dideoxythymidine	1	>50	56
2′,3′-dideoxycytidine	0.5	50	57
ribavirin	>400	<0.3	33
HPA-23	>15	<0.1	33

[a] Minimum inhibitory concentration, achieving a *complete* protection of ATH8 cells against the cytopathic effect of HTLV-III/LAV. [b] Ratio of compound concentration required to reduce the growth of normal uninfected ATH8 cells by 50% to the compound concentration (MIC) required for achieving full protection of ATH8 cells against HTLV-III/LAV.

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directly bind to the template/primer-binding site of the enzyme. For AZT, ddCyd, and the other 2′,3′-dideoxynucleosides, a competitive action with respect to the normal substrates for the reverse transcriptase (dTTP, dCTP, dATP, dGTP) may be envisaged. If any of these 2′,3′-dideoxynucleosides were incorporated onto the 3′ end of the growing DNA chain, then further elongation would be prevented, as the compounds act as chain terminators.

HTLV-III/LAV Replication Inhibitors

Suramin (Chart I) has been the first compound to be recognized as a selective inhibitor of HTLV-III/LAV replication in vitro:[51,58] it inhibits the cytopathogenicity of HTLV-III/LAV for ATH8 cells (an immortalized OKT4$^+$ T-cell clone) *gag* p24 protein expression at concentrations well below the cytotoxicity threshold.[33] Yet, the safety margin (toxicity/activity ratio) of suramin is rather narrow: 5 at best (Table II). Evans Blue, which is structurally related to suramin (Chart I), inhibits the cytopathic effect of HTLV-III/LAV for ATH8 cells with a similar potency and selectivity as suramin[33,55] (Table II).

Aurintricarboxylic acid (Chart I) represents another class of anionic compounds that offer promise as HTLV-III/LAV inhibitors. Aurintricarboxylic acid (ATA) inhibits reverse transcriptase by a similar mechanism as suramin, that is, by reducing the affinity of the enzyme for the template/primer molecule.[36] ATA completely protects ATH8 cells against the cytopathogenicity of HTLV-III/LAV at a concentration of 47 μM and thus is about equipotent as suramin[55] (Table II).

Phosphonoformate (Chart I) represents yet another anionic compound, which is thought to interact with DNA polymerases at the site where pyrophosphate is split off during the DNA polymerization process. Phosphonoformate (PFA) is a rather potent inhibitor of HTLV-III/LAV reverse transcriptase (50% inhibitory dose: <2 μM).[52,53] To achieve an inhibition of HTLV-III/LAV replication, much higher PFA concentrations (>100 μM) are required, however, and to confer full protection of the ATH8 cells against the cytopathic effect of HTLV-III/LAV,[33] the PFA concentration must be increased to 350 μM (Table II).

The nucleoside analogues 3′-azido-2′,3′-dideoxythymidine (AZT)[56] and 2′,3′-dideoxycytidine (ddCyd)[57] (Chart I) appear to be more potent and more selective inhibitors of HTLV-III/LAV replication than suramin, Evans Blue, ATA, or PFA (Table II). AZT was originally synthesized by Horwitz et al.[59] and later by Lin and Prusoff[60] and Colla et al.[61] Its inhibitory effect on retrovirus (i.e., Friend lymphatic leukemia helper virus) replication was first reported by Ostertag et al.;[62a] but, as mentioned by De Clercq et al.,[62b] the compound is also inhibitory to normal cellular DNA synthesis, as monitored by the incorporation of [*methyl*-^{3}H]dThd, at a concentration of 20–30 μM. To protect ATH8 cells against the cytopathic effect of HTLV-III/LAV, a concentration of 1 μM AZT suffices[56] (Table II), and this concentration is at least 50-fold lower than the concentration at which AZT interferes with the growth of the uninfected host cells.

Of the 2′,3′-dideoxynucleoside series,[57] ddCyd is the most potent inhibitor of HTLV-III/LAV: at 0.5 μM it confers full protection of ATH8 cells against the cytopathogenicity of HTLV-III/LAV while it is not toxic for the host cells up to a concentration of 25 μM (Table II). As mentioned above, ddCyd and the other 2′,3′-dideoxynucleosides and their derivatives, i.e., AZT, are assumed to be targeted at the HTLV-III/LAV reverse transcriptase. To this end, the compounds must first be converted intracellularly to the corresponding 5′-triphosphates (ddNTPs)[63] before they could interact with the reverse transcriptase. Uninfected and virus-infected cells would not differ in their ability to phosphorylate the 2′,3′-dideoxynucleosides,[64] which means that the reason(s) for the selectivity of the compounds against HTLV-III/LAV have to be based on their interaction with the reverse transcriptase, and, as mentioned above, HTLV-III/LAV reverse transcriptase may be more vulnerable a target than the cellular DNA polymerases α, β, and γ because it is either more susceptible to the inhibitory effects of the ddNTPs or more accessible, or both.

In addition to the compounds depicted in Chart I, several other substances have been described as inhibitors of HTLV-III/LAV infectivity in vitro: i.e., ribavirin,[65] human interferon (HuIFN-αA),[66] dithiocarb,[67] AL-721,[68] rifabutin,[69] and D-penicillamine.[70] For none of these substances has the mechanism of action been determined, but it is quite likely that, being a rifamycin derivative (see Table I, class I) and a chelating agent (see Table I, class V), respectively, rifabutine and D-penicillamine may be expected to interact with HTLV-III/LAV reverse transcriptase.

Although ribavirin has been reported to inhibit the replication of HTLV-III/LAV in human adult T lymphocytes at a concentration of 50 μg/mL,[65] it does not appear to protect ATH8 cells against the cytopathic effect of HTLV-III/LAV even at a concentration of 100 μg/mL (400 μM), while being toxic for the host cells at a concentration as low as 10 μg/mL[33] (Table II). The latter findings indicate that ribavirin is neither active nor selective as an anti-HTLV-III/LAV agent in vitro.

The tungstoantimoniate (HPA-23) has been claimed to inhibit the growth of HTLV-III/LAV in patients.[71] Yet, no experimental evidence has ever been reported as to an inhibitory effect of HPA-23 on the replication of HTLV-III/LAV in cell culture, and, as a matter of fact, HPA-23 fails to protect ATH8 cells against the cytopathogenicity by HTLV-III/LAV at a concentration of 100 μg/mL (15

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μM; the highest concentration tested), while being toxic for the uninfected host cells at a concentration of 1 μg/mL[33] (Table II). Thus, HPA-23 is, just like ribavirin, negatively selective in its anti-HTLV-III/LAV activity in vitro.

Interferon, dithiocarb, AL-721, rifabutine, and D-penicillamine represent interesting leads in the search for effective anti-HTLV-III/LAV agents.[66-70] Interferon (HuIFN-αA) inhibits the replication of HTLV-III/LAV in normal peripheral blood mononuclear cells at concentrations (4–1024 units/mL) that are not toxic to the cells and that are readily achievable in blood after parenteral administration.[66] It would now seem mandatory to examine whether other types of interferon (i.e., HuIFN-β or -γ) are equally active and whether combinations of different interferon types may yield even better effects.

While the mode of action of dithiocarb (diethyl dithiocarbamate sodium salt), which is also known as imuthiol and generally regarded as an immunomodulator, can only be speculated upon,[67] rifabutine[69] and D-penicillamine[70] may well achieve their inhibitory effects on HTLV-III/LAV through an inhibition of the virus-associated reverse transcriptase (as indicated above). AL-721, however, appears to be distinct from the classic reverse transcriptase inhibitors. It is a lipid mixture composed of neutral glycerides, phosphatidylcholine, and phosphatidylethanolamine in a 7:2:1 ratio; it is not inhibitory to the reverse transcriptase but would have a direct virucidal effect on HTLV-III/LAV.[68]

Clinical Studies with HTLV-III/LAV Inhibitors in AIDS (or ARC) Patients

Although clinical trials have been initiated or advocated for most of the compounds that were found effective against HTLV-III/LAV in vitro, only few reports have so far appeared in the literature.[71-76] These have indicated the following. If administered intravenously to AIDS or ARC patients at a dose of 6.2 g over a 5-week period, suramin attains plasma levels greater than 100 μg/mL,[74] which is well above the minimum inhibitory concentration (50 μg/mL) required to completely block HTLV-III/LAV replication in vitro (Table II). Such short-term treatment regimen proved efficient in suppressing virus replication (Figure 4), but, despite this in vivo virustatic effect, no clinical or immunological improvement was observed.[73] Other studies suggest, however, that clinical and immunological improvement may be observed upon a more intensive dosage regimen of suramin consisting of 20 mg/kg of body weight, every 5 days, for 35 days.[72] Also, long-term maintenance therapy may be required in order to obtain favorable clinical and immunological responses.

From Figure 4 it is also evident that, as long as sufficiently high plasma drug levels are maintained, virus replication is suppressed, but that, as soon as these drug

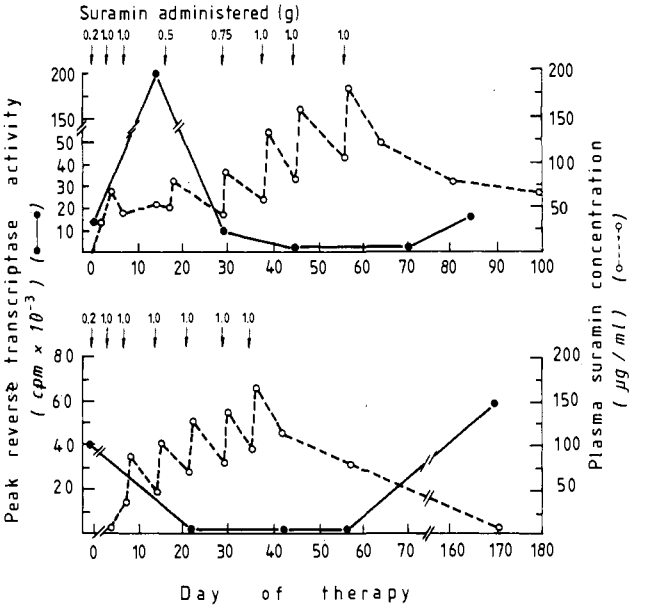

Figure 4. Peak reverse transcriptase activity in cultures of peripheral blood lymphocytes (●) and plasma suramin concentrations (○) in two patients who had detectable HTLV-III/LAV virus before suramin therapy was started. According to Broder et al.[73] (modified).

levels fall below the activity threshold, virus replication resumes. This means that the virustatic effect of suramin is reversible, that the virus cannot be eliminated or eradicated form its target or reservoir cells, and that for keeping virus replication under control long-term therapy may be essential.

With AZT, given intravenously at 2.5 or 5 mg/kg every 4 h for 2 weeks and then orally for 4 weeks at twice the intravenous dose, matters look perhaps more encouraging than with suramin, in that some clinical and immunological improvements were noted during the 6-week treatment period,[75] but whether these improvements will be sustained, whether AZT can be tolerated over a prolonged time, and whether AZT would favorably affect the ultimate outcome of the disease remain issues that can be resolved only by properly controlled long-term studies. Only at the highest AZT dosage regimen, peripheral blood mononulcear cells became negative for HTLV-III/LAV,[75] and it is likely that, as noted for suramin, prolonged treatment with AZT may be required to keep the virus under control.

Thus, the clinical data gathered so far from AIDS or ARC patients treated with the HTLV-III/LAV inhibitors suramin and AZT indicate that these compounds are able to suppress virus replication in vivo and that this virustatic effect may be accompanied by some clinical and immunologic improvement (as demonstrated in particular for AZT). Little, if any, information is available on the clinical prospects of the other compounds. For ribavirin, no effect whatsoever was noted in the treatment of HTLV-III/LAV infection of infants,[76] which appears to be consistent with our in vitro observations.[33]

Possible Strategies and Approaches for the Chemotherapy of AIDS

Considering the importance of HTLV-III/LAV replication in the pathogenesis of AIDS and the stringent dependence of HTLV-III/LAV replication on the virus-associated reverse transcriptase, the latter can be considered as the prime target in the design of chemotherapeutic agents against AIDS. There are, however, various other targets that could be envisaged, and in principle, all major steps [i.e., adsorption, penetration, uncoating, early

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Table III. Possible Targets and Approaches in the Development of Chemotherapeutic Agents against AIDS

target	approach
reverse transcriptase	reverse transcriptase inhibitors (see Table I)
virus adsorption	polyanionic substances
T4 (CD4) receptor	blocking or modification of T4 receptor
TAT protein	inhibitors of expression or action of the TAT protein
viral genome expression	anti-sense RNA
viral mRNA translation	complementary oligonucleotides
proviral DNA transcription	specific oligopeptides
splicing of viral pre-mRNA	branched ribonucleotides
cleavage of viral precursor proteins	protease inhibitors
viral glycoproteins	glycosylation inhibitors

("reverse") transcription, circularization, integration, DNA replication, late transcription, translation, assembly, and release (budding)] of the virus replicative cycle (Figure 3) could serve as points of attack for antiviral drugs.[77]

More specifically for HTLV-III/LAV one might entertain the possibility of aiming chemotherapeutic agents at the following targets (Table III).

Virus adsorption and/or penetration: polyacrylic acid, polymethacrylic acid polyvinyl sulfate, dextran sulfate, and polyphloroglucinol phosphate are all examples of polyanionic substances known to interfere with the virus adsorption process,[78,79] and being an hexasulfonic acid derivative, suramin might also effect virus adsorption and so might other polyanionic compounds related in suramin.

T4 (or CD4) receptor: through its glycoprotein gp120 (gp110)[26] HTLV-III/LAV interacts with this receptor, and while this gp110-T4 interaction would explain the tropism of HTLV-III/LAV for T-helper/inducer cells, it may also prove useful in developing therapeutic or preventive measures for AIDS, i.e., based on monoclonal antibodies directed against T4 antigens and/or chemical modifications in either the T4 or gp110 glycoproteins.

tat protein: since TAT activity is essential for virus production,[29] inhibitors that specifically block the expression of the *tat* gene or the action of the *tat* product may yield great promise for the chemotherapy of AIDS; as putative inhibitors of the *tat* protein action, translational inhibitors should be pursued, as the *trans*-activation phenomenon seems to be based on translational rather than transcriptional events.

Viral genome expression, i.e., expression of the *tat* gene, may be achieved by using anti-sense (= complementary) RNA. Such anti-sense RNA may be expected to hybridize to the target mRNA and thereby prevent its translation.[80-82] This approach works remarkably well upon microinjection of the anti-sense RNA into frog oocytes[81] and *Drosophila* embryos,[82] but whether it could be developed to the stage that it would be therapeutically applicable in AIDS patients is another matter.

Viral mRNA translation may also be prevented by using specific oligodeoxynucleotides that are complementary to the 5′ end of the mRNA. This approach has been introduced by Zamecnik and Stephenson,[83] but has so far not led to any concrete therapeutic results. Of critical importance is undoubtedly the accessibility of the oligodeoxynucleotide to its target sequence. It would also help if the affinity of the oligodeoxynucleotides for their target could be increased, and this may be achieved by modifying the phosphate backbone to phosphonate or by covalently linking an intercalating agent, i.e., acridine, to the 3′ end of the oligonucleotide.[84]

Transcription of proviral DNA may be too difficult a target, but, in principle, it should be possible to suppress transcriptional processes by hypermethylating the DNA or shielding it with specific DNA-binding proteins. Novel oligopeptides, related to netropsin and distamycin, which bind to specific DNA sequences, have been recently described by Lown et al.[85]

Splicing of viral pre-mRNA seems to be an attractive target, since two such events are involved in the transcription of the *tat* gene.[21,22] During the splicing process a lariat is formed with 2′–5′ and 3′–5′ phosphodiester bonds joined to a single adenosine residue ("branched adenylate").[86-88] Branched trinucleotides have now been obtained synthetically by Sekine and Hata,[89] and it would seem interesting to see whether such branched trinucleotides may interfere with the splicing process.

Cleavage of the viral precursor proteins is a crucial step in the maturation of most, if not all, viruses and HTLV-III/LAV is no exception (Figure 2). This proteolytic cleavage may be achieved by either cellular or viral proteases. Pending the specificity in the substrate affinity of the viral proteases, it should be possible, at least in principle, to design specific inhibitors for these proteases and for the viral functions they are entangled with,[77] but so far this goal has not been accomplished.

Viral glycoproteins, in particular the heavily glycosylated gp120 (gp110) of HTLV-III/LAV, and their complementary cell surface glycoproteins may seem interesting targets for glycosylation inhibitors, such as 2-deoxy-D-glucose and β-hydroxynorvaline, and these compounds indeed decrease the infectivity of HTLV-III/LAV (and other enveloped viruses),[90] but they only do so at very high concentrations (5–10 and 0.5–5 mM, respectively). Future attempts should be directed at developing more potent and more

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specific glycosylation inhibitors.

An important strategy in the development of an adequate treatment for AIDS should not go unnoticed. That is the potential benefit that may ensue from combination chemotherapy: combination of two or more inhibitors of reverse transcriptase exhibiting different pharmacokinetics (i.e., suramin + AZT); combination of reverse transcriptase inhibitors with antiviral drugs acting at other targets; combination of reverse transcriptase inhibitors (or other antiviral drugs) with so-called immunomodulators or biological response modifiers[91] (i.e., interleukin-2) or other measures aimed at immunologic reconstitution (i.e., bone marrow transplantation).[11] An antiviral agent or combination of antiviral agents may not work without additional immunostimulatory or reconstitutive therapy, and, vice versa, stimulation of T-cell multiplication without concomitant antiviral therapy may also prove unsuccessful, or even counterproductive, as it leads to an increased number of target cells for HTLV-III/LAV replication.[12] Thus, combined treatment with antiviral agents and immunostimulators may be necessary for keeping both the virus and the immune status under control.

There are some substances, however, that seem to be endowed with both functions, i.e., antiviral and immunostimulatory. A classical example is interferon, which is not only active as an antiviral (and antitumor) agent but, depending on the experimental conditions, also capable of either suppressing or enhancing the host's immune response. Other examples of substances with joint antiviral/immunostimulatory functions are polymethacrylic acid, which, on the one hand, inhibits virus adsorption[78,79] and, on the other hand, mobilizes lymphocytes from their reservoirs;[92] phosphonoformate, which is an effective inhibitor of HTLV-III/LAV replication[52,53] and, in addition, may also allow T-cell colonies to grow and expand;[93] and, finally, suramin, which, in addition of being inhibitory to HTLV-III/LAV in vitro[51] and in vivo,[73] has both suppressive[94,95] and stimulatory[96] effects on the immune system.

Conclusion

Provided the HTLV-III/LAV genome is integrated as proviral DNA into the host-cell chromosomes, it may seem too formidable a task to try to eradicate the virus from its target or reservoir cells, a situation that is reminiscent of recurrent herpes virus infections, where it is common knowledge that in its latent phase the virus is not amenable to antiviral chemotherapy.[97]

Thus, none of the reverse transcriptase inhibitors currently pursued in the chemotherapy of AIDS may be expected to definitely eliminate the virus from the organism. Nor might any of the additional approaches discussed above be expected to do so. Furthermore, patients with full-blown AIDS may not benefit much from HTLV-III/LAV inhibitors as in the advanced stage of the disease HTLV-III/LAV replication may have ceased or slowed down. Why then should our efforts be directed at developing inhibitors of HTLV-III/LAV replication?

The use of such inhibitors would seem justified from a number of viewpoints: (i) to interrupt the virus replicative cycle so as to prevent repeated rounds of infection within the same cell or spread of the virus to other cells; (ii) to counteract the T4 cell immunosuppressive effects associated with continued virus replication.

The central nervous system may well provide a sanctuary for HTLV-III/LAV, and here again, progression of the disease (neurological disorders) may depend on continued virus replication.[98-100] As continued synthesis of new virus particles or antigens may be promoted by the immunosuppression and as, in turn, HTLV-III/LAV aggravates the immunodeficiency, it would seem of paramount importance to break this vicious cycle; and the anti-HTLV-III/LAV agents may be particularly efficient in this regard.

Admittedly, their usefulness may be greatest in the initial stage of the disease, before the patient has become immunodeficient, but even after installment of the immunosuppression, HTLV-III/LAV inhibitors should be considered, as at this stage they may be useful, possibly in conjunction with immunostimulants, in restoring the host's immunity.

It is at present premature to speculate on the possibility of a cure for AIDS. Surely, the more selective the inhibitory effect on HTLV-III/LAV, the greater the likelihood the compound may be efficacious in the treatment of AIDS. However, unequivocal answers as to the clinical efficacy of any of the drugs currently pursued or yet to be initiated in the therapy of AIDS must await the results of properly controlled long-term studies, monitored for as many parameters (virological, immunological, clinical) of the disease as possible.

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