# Resumption of Virus Production after Human Immunodeficiency Virus Infection of T Lymphocytes in the Presence of Azidothymidine

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The new antiviral agent, azidothymidine (AZT; BW A509U), is currently the only successful drug in use for patients with acquired immunodeficiency syndrome. The effect of this thymidine analog, 3'-azido-3'-deoxythymidine, on the replication of the lymphadenopathy-associated virus strain of the human immunodeficiency virus was evaluated by using susceptible H9 and Jurkat cells. Cells were pretreated with concentrations of drug ranging from 0.5 to 100  $\mu$ M, infected, and maintained in medium containing drug. Virus production was assayed by reverse transcriptase assays, and virus-specific DNA was analyzed by Southern blots probed with cloned human immunodeficiency virus sequences. At 4 to 8 days postinfection, infected cells without drug reached a peak of reverse transcriptase activity that was sustained. Increasing concentrations of AZT caused increasing delays in virus production; however, replicate cultures at nontoxic levels of the drug (up to 25  $\mu$ M) eventually produced as much virus as did non-drug-treated infected cells, despite the continued presence of the drug. Levels of intracellular, unintegrated, virus-specific DNA paralleled reverse transcriptase levels. Virus-caused cytopathic effect was likewise delayed in drug-treated cultures. Virus recovered from H9 cultures after 25  $\mu$ M AZT treatment did not appear resistant to AZT.

Current therapies for acquired immunodeficiency syndrome (AIDS) (17, 18) have been directed at the step in the virus life cycle which, to date, is most readily blocked: the transcription of the human immunodeficiency virus (HIV) RNA into its DNA copy by the viral enzyme reverse transcriptase (RT), encoded by the viral pol gene. Attempts to interrupt the virus life cycle are made more complicated by the fact that the virus may remain latent in some cells for long periods, with later reactivation and production of virus by incompletely understood mechanisms (5). It is thought that continued virus infection of newly formed CD4<sup>+</sup> T cells is responsible for the progressive loss of immune function. Thus, ideally, treatment would prevent virus production in diseased patients. Cell-to-cell spread of virus is also possible by fusion of infected cells with susceptible uninfected cells (4).

The thymidine analog azidothymidine (AZT), or 3'-azido-3'-deoxythymidine (BW A509U), blocks expression of the core gag protein (p24) in H9 cells after the cells are exposed to HIV (10). The drug itself cannot inhibit (RT) in vitro; the drug is phosphorylated in the cell to the triphosphate form by host cell enzymes, and this form binds to the HIV RT (6). The drug is capable of inhibiting the replication of HIV in MT4 cells infected with human T-cell lymphotropic virus type I, but has no effect on an already infected HIVproducing cell line (11). In mice infected with Rauscher murine leukemia virus, there were significant survival gains even when the drug was started late in the course of disease (13).

In a phase 1 clinical trial, a majority of patients with AIDS showed increases in numbers of circulating helper T cells (CD4<sup>+</sup>) during therapy, yet virus continued to be recovered despite therapy (19). In a phase 2 efficacy trial of AZT in patients with late AIDS-related complex and AIDS, the drug

reduced both opportunistic infections and death. Some patients have had to reduce the dosage of AZT or discontinue AZT treatment because of drug toxicity leading to, mainly, anemia or neutropenia (D. Barry and S. N. Lehrman, Abstr. UCLA Symp. Mol. Cell. Biol. 1987, PO19, p. 37). It is important to know if patients who fail while on AZT therapy are developing resistant strains of HIV or if the original virus present in the patient is being produced despite drug treatment.

We have examined the ability of AZT to interfere with HIV replication in T cells in culture, confirming that AZT could suppress infection of susceptible cells in culture. However, we have also found, in contrast to previous reports, that concentrations of AZT as high as 25  $\mu$ M were unable to continue preventing the synthesis of virus-specific DNA and subsequent virus production despite constant drug exposure. In preliminary tests, the virus produced in the presence of drug did not develop obvious resistance to AZT.

## **MATERIALS AND METHODS**

Cells and virus strains. H9 cells were kindly provided by Robert Gallo, and Jurkat cells were provided by Kent Weinhold. Both T-cell lines were grown in RPMI 1640 with 10% fetal calf serum. Viability determinations were performed by trypan blue exclusion. The BLAV strain of HIV was obtained from Luc Montagnier, and working stocks were produced in H9 cells. The pBenn2 plasmid, containing a portion of the lymphadenopathy-associated virus (LAV) strain genome, was obtained from Malcolm Martin.

Virus infections. H9 cells ( $8 \times 10^6$ ) or Jurkat cells ( $4 \times 10^6$ ) were pretreated for 4 h in the appropriate concentration of drug, pelleted, suspended in 1 ml of medium, and then mixed with 1 ml of a mixture of virus and DEAE-dextran (final concentration, 2 µg/ml) plus or minus drug. After 1 to 2 h at 37°C, cells were washed twice in medium containing the

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FIG. 1. Toxicity of AZT for uninfected H9 and Jurkat cells. Cells (5 × 10<sup>4</sup>/ml) in triplicate cultures were adjusted to the drug concentrations given. Averages of log of total cells per milliliter from triplicate cultures are plotted versus time in the drug. Drug concentrations: no drug ( $\blacksquare$ ), 0.5 mM (+), 5  $\mu$ M ( $\diamond$ ), 25  $\mu$ M ( $\Delta$ ), and 100  $\mu$ M (×).

appropriate level of AZT and seeded at  $2.5 \times 10^5$  to  $4 \times 10^5$ /ml in medium containing drug. Every time cells were counted, percentage of viability was determined, and cells were pelleted from their medium and replaced at a density of  $2 \times 10^5$ /ml into new medium containing drug. Clarified supernatant fluids were stored at  $-70^{\circ}$ C until experiments were completed, and RT levels were determined in all samples in parallel.

**RT** assay. A variation of the procedure of Poiesz et al. (12) was used to detect the presence of virus-associated RT. Clarified supernatant medium (6.5 ml) was adjusted to final concentrations of 0.1 M NaCl and 9% polyethylene glycol 8000 and kept on ice overnight. Virus was pelleted at 11,950  $\times$  g in a Sorvall SS-34 rotor at 4°C for 10 min. The pellet was suspended in 0.1 M NaCl-0.05 M Tris (pH 7.5) to give a 13× or 26× concentration of the original supernatant fluid. Concentrated virus (30 µl) was added to duplicate reactions containing 5 µCi of [<sup>3</sup>H]TTP (60 to 80 Ci/mmol; Amersham Corp.), final concentrations of 10 mM Tris (pH 7.5), 8 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.5% Triton X-100, 50 µg of poly(A) per ml, and 10 µg of oligo(dT)<sub>12-18</sub> (Pharmacia, Inc.) per ml. After incubation of the reaction was spotted onto DE81

filter paper (Whatman, Inc.) and washed six times in 5%  $Na_2HPO_4$  followed by two washes each of water and ethanol. The dried filters were counted in Betafluor scintillation fluid.

DNA analyses. Cell culture volumes were increased 2 days preceding a harvest of DNA; low-molecular-weight DNA was isolated from cells by the method of Hirt (8). DNA samples were subjected to electrophoresis through 0.8% agarose gels and blotted onto nitrocellulose (Schleicher & Schuell, Inc.) by the method of Southern (14). Filters were hybridized to [32P]dCTP-labeled, nick-translated probes made from the LAV 6.44-kilobase HindIII fragment subcloned into the Bluescribe M13 vector (Vector Cloning Systems). Prehybridization and hybridization solutions were in 50% formamide as previously described (15). After hybridization at 42°C for 18 h, filters were washed in  $2 \times$  SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate at room temperature and in  $0.2 \times$ SSC-0.1% sodium dodecyl sulfate at 65°C and exposed to XAR film (Eastman Kodak Co.) at  $-70^{\circ}$ C with intensifying screens.

## RESULTS

Effect of AZT on growth rates and viabilities of T-cell lines. The growth rates of H9 and Jurkat cells in various concentrations of AZT were determined during a period of logarithmic growth (Fig. 1). Neither cell line showed a decrease in growth rate in concentrations of AZT of up to 0.5  $\mu$ M; with 25  $\mu$ M AZT, the growth rate decreased (Fig. 1). Viability decreased to 26 and 88% for H9 and Jurkat cells, respectively, at day 3 in 25  $\mu$ M AZT. The toxicity of the drug was more evident in our cultures than in drug-treated, virus-infected cultures in which cell concentrations were higher.

Suppression of HIV replication in T-cell lines by AZT. Two T-cell lines susceptible to HIV were used to determine the effects of AZT on HIV replication. Infected H9 cells became continuously productive. Jurkat cells were selected as an



FIG. 2. Production of HIV in H9 cells at various concentrations of AZT. Cells were pretreated with drug or mock treated for 4 h, infected with the LAV strain of HIV, and washed, and the cultures were carried in the presence or absence of drug. Samples of supernatant medium were assayed for RT activity at intervals of a few days; at each assay time, the cells were adjusted to  $2 \times 10^5$ /ml and the medium was completely replaced with fresh medium containing drug where indicated. Drug concentrations: no drug (+), 0.5  $\mu$ M ( $\diamond$ ), 5  $\mu$ M ( $\diamond$ ), 25  $\mu$ M ( $\times$ ), and 100  $\mu$ M ( $\bigtriangledown$ ). Mock infection ( $\blacksquare$ ) is also shown.

alternate system. Cells were pretreated with AZT for 4 h to allow loading of intracellular pools with the drug and were then infected in the presence of drug with a dose of HIV (LAV strain) predetermined to give high RT levels by 1 week postinfection (p.i.). Infections and washings were carried out in the presence of AZT to avoid rapid loss of the drug from the cells (6). Cells were adjusted to  $2 \times 10^{5}$ /ml in fresh medium containing drug twice weekly at the time of sampling for RT activity. Residual AZT from the drug-treated cultures was ignored, as it has been shown that AZT does not interfere with the RT assay in vitro (11). All doses of AZT were inhibitory, delaying the rise in RT levels as well as cytopathic effects. With 25 µM AZT, virus production was largely suppressed for 10 days. For both cell lines, however, in all concentrations of drug except the toxic 100 µM level, virus replication increased to the control infected-culture level by 20 days or sooner, despite continued presence of drug (Fig. 2 and 3).

Levels of unintegrated viral DNA in drug-treated cultures. Samples of Hirt supernatant DNA extracted from the H9 cultures at days 1, 4, and 20 p.i. were subjected to electrophoresis through agarose gels, blotted onto nitrocellulose, and hybridized to a <sup>32</sup>P-labeled DNA probe made to the 6.4-kilobase HindIII internal fragment of HIV. At 1 day p.i., the level of viral DNA in the control infected culture was barely detectable (Fig. 4A); in drug-treated samples, viral DNA was undetectable. At 4 days p.i., the signal from the control infected culture was at least 10 times stronger than that from culture with 0.5 µM AZT (Fig. 4A), parallel to the relative amounts of virus produced on that day (Fig. 2). At 20 days p.i., cultures treated with AZT at concentrations of up to 25 µM contained virus-specific DNA; only about 50% of the cells were still viable in the culture treated with 100  $\mu$ M AZT at day 20. The reproducibility of RT and DNA levels was verified in three additional experiments.

Test of virus produced under drug treatment for resistance to AZT. Since virus was produced at normal levels after a short interval in drug-treated cultures, we decided to determine if resistant virus had been generated or if the originally sensitive virus had broken through the drug treatment. In an experiment similar to that described in the legend to Fig. 2,



FIG. 3. Production of HIV in Jurkat cells at various concentrations of AZT. Cells were infected and carried as described in the legend to Fig. 2. The culture in 100  $\mu$ M AZT was terminated at day 15 when viability had decreased to 16%. Drug concentrations: no drug (+), 0.5 mM ( $\diamond$ ), 5  $\mu$ M ( $\triangle$ ), 25  $\mu$ M ( $\times$ ), and 100  $\mu$ M ( $\nabla$ ). Mock infection ( $\blacksquare$ ) is also shown.



FIG. 4. Detection of unintegrated HIV DNA during the course of HIV infection of H9 cells at various concentrations of AZT. Cultures were expanded to allow the harvest of approximately  $1 \times 10^7$  to  $2 \times 10^7$  cells for Hirt supernatant DNA. DNA (10 [A] or 20 [B] µg) was subjected to electrophoresis in agarose gels, blotted onto nitrocellulose, and probed with <sup>32</sup>P-labeled, nick-translated DNA from the 6.4-kilobase internal *Hin*dIII fragment of LAV. (A) Lanes 1 to 3, Standards corresponding to 100, 10, and 1 copy per cell, respectively, for 20 µg of total cellular DNA; lanes 4 to 9, 1 day p.i.; lanes 10 to 12, 4 days p.i. (B) twenty days p.i. Molecular weight markers (in kilobase pairs) were  $\lambda$  [kbp]) *Hin*dIII fragments run in the adjacent lane. -V, Control, uninfected cultures; +V-AZT, control, infected cultures.

virus samples recovered on days 16 and 21 p.i. from cultures treated with 25 µM AZT were used to inoculate fresh H9 cells, parallel to a parent LAV stock of similar titer (estimated by RT levels), in the presence or absence of 25  $\mu$ M AZT. AZT was present 4 h before infection and throughout the course of infection. Virus samples from 16 and 21 days p.i. of the original experiment gave rise to fully productive infections (RT levels of 500,000 cpm) by day 20, as did the parent stock. All three samples showed a slow increase in RT in the absence of drug, reflecting the low initial dose of virus. The parent LAV stock was diluted such that all three inocula had approximately the same number of RT counts per minute. This method was used to normalize the virus stocks in the absence of a convenient plaque assay. AZT clearly inhibited the virus recovered at both times of the original experiment: i.e., both experimental samples behaved identically to the parent stock in the presence of drug (Fig. 5). Thus, there was no evidence that a significant proportion of the virus produced in the original experiment had developed resistance to AZT.



FIG. 5. Test of breakthrough virus for resistance to AZT. Diluted parent stock virus (A) and two samples of H9 supernatant medium from virus breakthrough at 25  $\mu$ M AZT, from 16 (B) and 21 (C) days p.i., were tested for resistance to AZT. Each sample was used to infect H9 cells either in the presence (+) or in the absence ( $\blacksquare$ ) of 25  $\mu$ M AZT. The rise in RT with time was followed by periodic sampling of the medium. At sampling times, the medium was completely replaced with fresh medium containing AZT.

### DISCUSSION

We have found that HIV infection of two T-cell lines in the continuing presence of highly inhibitory doses of AZT proceeded to a highly productive state after an initial period of inhibition. Others have reported low doses of AZT to be effective at inhibiting HIV replication. Nakashima et al. (11) found 0.1 to 1 µM AZT inhibited HIV-induced cytopathic effect, antigen expression, and PFU production at 3 to 6 days after HIV infection of human T-cell lymphotropic virus type I-infected MT4 cells at a multiplicity of infection of 0.002. Mitsuya et al. (10) found that 5  $\mu$ M AZT could reduce expression of p24 to about 2% of H9 cells as well as protect TM3 and HTLV-I<sup>+</sup> ATH8 cells from HIV-induced killing. They also reported a fourfold decrease in RT levels at 5 days p.i. in HIV-infected peripheral blood mononuclear cells in 0.5 µM AZT. We have used a high multiplicity of infection (as gauged by RT levels) to observe the effect of the drug on a single-step growth curve of the virus, classically used to assay drug effects. In contrast, experiments carried out at a very low multiplicity of infection (e.g., 0.002) depend on many rounds of replication to produce detectable virus, with opportunity for the drug to act at each cycle of replication. In addition, we have used cells which are not quickly killed by the virus, thus allowing the infection and reinfection processes to proceed. Due to the polyethylene glycol concentration step in our RT assay, we were able to detect differences of at least 100-fold in RT levels above the background level (Fig. 2 and 3). These differences in experimental design probably account for the fact that we could detect virus replication in the presence of drug within 7 to 12 days p.i.

Although AZT may be primarily a competitive inhibitor for RT, acts as a chain terminator, and perturbs nucleoside triphosphate pools within the cells (6), our results showed that complete DNA copies of the viral genome were formed in the presence of AZT. Since further steps in the virus life cycle (e.g., production of mRNA and progeny viral RNA) dependent on cellular RNA polymerase were not affected by the drug, virus production could then ensue. These proposed effects of the drug on aspects of the viral replication cycle are supported by a report that virus production is not suppressed in cells already producing HIV (11). Virus spread in the presence of 25 µM AZT may have been at low efficiency. Probably, a majority of the DNA copies initiated by RT in newly infected cells are prematurely terminated, but if at least one complete copy is made, a cell may go on to produce progeny virus. Whether virus spread occurs by cell-free virus or by cell-to-cell contact, cultures treated with 25  $\mu$ M AZT eventually produced as much virus as the non-drug-treated infected cultures. These results were confirmed by the detection of unintegrated viral DNA in drugtreated H9 cultures when they began producing virus at high levels. The unintegrated viral DNA in these drug-treated cultures was present in quantities similar to those in nondrug-treated infected cultures.

In two samples of HIV recovered after replication in the presence of a high concentration of AZT (25  $\mu$ M), we did not see the generation of resistant mutant virus, although our preliminary tests were not sensitive enough to detect small increases in resistance or increases in a very small proportion of the virus population. HIV shows wide variability in nature, in that every strain isolated appears to have many differences in sequences over the entire genome. In addition, changes in sequential isolates from the same patient have been detected (1–3, 7, 16). Thus, it is conceivable that naturally occurring nucleotide changes in the *pol* region could result in a virus with an altered affinity for AZT triphosphate or with other changes, causing an AZT-resistant phenotype. In fact, AZT-resistant RT (in vitro) has been generated by site-directed mutagenesis of the *pol* gene

(9). However, it seems likely that in most cases the drug lessens the likelihood that RT will be able to generate a full-length DNA copy of the incoming viral RNA, but the drug does not completely prevent such generation. Cellular RNA polymerase II is then able to produce mRNA and progeny viral RNA. In addition, once the *tat* gene product has been produced, it is able to increase the level of expression of other viral gene products.

In a phase 2 efficacy trial of AZT in patients with late AIDS-related complex and AIDS, the drug had striking effects in preventing both opportunistic infections and death (17, 18). In the phase 1 trials, virus continued to be recovered despite therapy. After 5 mg of drug per kg of body weight given orally every 4 h, the peak level of AZT in the plasma was reported to be 5  $\mu$ M (19), yet virus was isolated during drug therapy. As there does not exist a good method to determine virus levels in patient serum, it may be argued that the virus isolated during AZT treatment is the result of in vitro release of virus production from in vivo AZT suppression. However, for at least some patients (6 of 20) in the AZT-treated group, there was no change in the number of days required for a positive culture (W. Parks, E. S. Parks, M. Fischal, R. Makuch, M. Leuther, and J. P. Allain, Abstr. UCLA Symp. Mol. Cell. Biol. 1987, PO21, p. 38). It may well be that infected cells in patients with AIDS continue to produce virus at a certain level and that newly made CD4 cells will become infected at low efficiency even in the presence of AZT. It appears that our in vitro system more closely simulates what happens in vivo than do those in vitro systems that simply show inhibition of HIV replication.

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#### ADDENDUM

Since submission of our paper, Olsen et al. (J. C. Olsen, P. Furman, J. A. Fyfe, and R. Swanstrom, J. Virol. **61**:2800–2806, 1987) have reported a dose-dependent inhibition by AZT of avian leukemia virus DNA synthesis in newly infected cells.

#### LITERATURE CITED

- Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo. 1986. Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. Cell 46:63–74.
- Benn, S., R. Rutledge, T. Folks, J. Gold, L. Baker, J. McCormick, P. Feorino, P. Piot, T. Quinn, and M. Martin. 1985. Genetic heterogeneity of AIDS retroviral isolates from North America and Zaire. Science 230:949–951.
- 3. Coffin, J. M. 1986. Genetic variation in AIDS viruses. Cell 46: 1-4.
- 4. Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4(T4)

antigen is an essential component of the receptor for the AIDS virus. Nature (London) **312**:763–767.

- Folks, T., D. M. Powell, M. M. Lightfoote, S. Benn, M. A. Martin, and A. S. Fauci. 1986. Induction of HTLV-III/LAV from a nonvirus-producing T-cell line: implications for latency. Science 231:600–602.
- 6. Furman, P. A., J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. N. Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, and D. W. Barry. 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus with reverse transcriptase. Proc. Natl. Acad. Sci. USA 83:8333–8337.
- Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Stall, R. C. Gallo, E. S. Parks, and W. P. Parks. 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science 232:1548-1553.
- 8. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. 26:365-369.
- Lardner, B. A., D. J. M. Purifoy, K. L. Powell, and G. Darby. 1987. Site-specific mutagenesis of AIDS virus reverse transcriptase. Nature (London) 327:716-717.
- Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder. 1985. 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. USA 82:7096-7100.
- Nakashima, H., T. Matsui, S. Harada, N. Kobayashi, A. Matsuda, T. Ueda, and N. Yamamoto. 1986. Inhibition of replication and cytopathic effect of human T cell lymphotropic virus type III/lymphadenopathy-associated virus by 3'-azido-3'deoxythymidine in vitro. Antimicrob. Agents Chemother. 30: 933-937.
- Poiesz, B. J., F. W. Ruscetti, P. Gazdar, A. Bunn, J. D. Minna, and R. C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA 77:7415-7419.
- Ruprecht, R. M., L. G. O'Brien, L. D. Rossoni, and S. Nusinoff-Lehrman. 1986. Suppression of mouse viraemia and retroviral disease by 3'-azido-3'-deoxythymidine. Nature (London) 323: 467–469.
- Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.
- 15. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA 76:3683-3687.
- Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo. 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science 229:759–762.
- Yarchoan, R., and S. Broder. 1987. Development of antiretroviral therapy for the acquired immunodeficiency syndrome and related disorders. N. Engl. J. Med. 316:557-564.
- Yarchoan, R., and S. Broder. 1987. Strategies for the pharmacological intervention against HTLV-III/LAV, p. 335-360. *In S.* Broder (ed.), AIDS: modern concepts and therapeutic challenges. Marcel Dekker, Inc., New York.
- Yarchoan, R., R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. N. Lehrman, R. M. Blum, D. W. Barry, G. M. Shearer, M. A. Fischl, H. Mitsuya, R. C. Gallo, J. M. Collins, D. P. Bolognesi, C. E. Myers, and S. Broder. 1986. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS/related complex. Lancet i:575-580.