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In vitro selection of human immunodeficiency virus type 1 resistant to 3′-azido-3′-deoxythymidine

Ferdinando Dianzani, Guido Antonelli, Ombretta Turriziani, Guanmu Dong*, Maria R. Capobianchi and Elisabetta Riva

Institute of Virology, University 'La Sapienza', Rome, Italy

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Summary

3′-Azido-3′-deoxythymidine (AZT)-resistant human immunodeficiency virus type 1 (HIV-1) was obtained by growing HTLV-III_B in C8166 cell cultures in the presence of inhibitory concentrations of AZT. The AZT-resistant HIV-1 was capable of replicating, as measured by infectious virus yield, and inducing cytopathic effect in the presence of AZT concentrations able to completely suppress the replication of parental HTLV-III_B. Cloning of the AZT-resistant HIV-1 revealed that a number of different variants of HIV-1 with various degrees of sensitivity to AZT emerged during propagation of HTLV-III_B in C8166 cells in the presence of the drug. PCR experiments performed on DNA extracted from C8166 cells infected with a resistant strain revealed that viral DNA was produced in the presence of inhibitory concentrations of AZT, while viral DNA in C8166 cells infected with the parental virus was drastically inhibited. Reverse transcriptase isolated from the AZT-resistant HIV-1 variant failed to show resistance to AZT 5′-triphosphate.

AZT; C8166 cell; HTLV-III_B; AZT-resistant HIV-1

Correspondence to: Dr. Ferdinando Dianzani, Institute of Virology, University 'La Sapienza', V.le di Porta Tiburtina 28, 00185 Rome Italy.

**Present address:* National Institute for Control of Pharmaceutical and Biological Products, Temple of Heaven, Beijing, China.

Introduction

3′-Azido-3′-deoxythymidine (AZT) is effective against HIV in vitro (Mitsuya et al., 1985) and has been demonstrated to improve the quality and length of life of patients with AIDS (Fischl et al., 1987). It has been recently shown that some isolates of HIV from patients under treatment with AZT are less sensitive to the drug (Larder et al., 1989a; Land et al., 1990; Japour et al., 1991).

Since AZT is known to prevent HIV-1 replication by inhibiting the HIV-1 reverse transcriptase (RT) (Mitsuya et al., 1985), it was tempting to attribute the observed resistance to mutations in the *pol* gene. In fact, it has been shown that multiple RT mutations confer resistance to AZT (Larder et al., 1987; Larder and Kemp, 1989b; Larder et al., 1989c; Japour et al., 1991).

However, the actual mechanism of AZT-resistance naturally acquired by HIV-1 still has to be clarified. In fact, the RT of many AZT-resistant HIV-1 strains isolated from patients (Larder et al., 1989a; Larder and Kemp, 1989b) as well as the RT of drug-resistant feline immunodeficiency virus (FIV) obtained in vitro (Remington et al., 1991), fail to show resistance to AZT 5′-triphosphate in vitro, thus suggesting that the mechanism of such resistance may involve other, at this moment unknown, mechanism(s).

Therefore, in order to gain new insights into the mechanism of AZT-resistance of HIV-1, it would be useful to select drug-resistant strains in vitro. These strains would offer the opportunity to easily obtain homogeneous virus populations which may represent an useful tool to study the mechanism underlying the phenomenon of drug-resistance. So far, attempts to generate drug-resistant lentivirus strains were unsuccessful with the exception of the selection of AZT-resistant FIV, which, however, does not entirely mimic the virus-host cell interactions of HIV-1 (Pedersen et al., 1990).

We have now attempted to select AZT-resistant HIV by propagation of HTLV-III_B in C8166 cells in the presence of high concentrations of AZT. This lymphoblastoid CD4+ cell line, containing the HTLV-I genome and expressing only tax gene (Chapman et al., 1987), has been chosen since it is highly permissive to HIV infection (Dianzani et al., 1988) and particularly useful to determine drug susceptibility of HIV by directly measuring infectious virus yield rather than indirect parameters of viral replication, such as RT activity or viral antigen production (Dianzani et al., 1989).

The results show that it is possible to generate AZT-resistant HIV-1 mutants simply by growing HIV-1 in C8166 cells in the presence of inhibitory concentrations of the drug.

Materials and Methods

Chemical

3′-azido-3′-deoxythymidine (AZT) and 2′-3′ dideoxyinosine (ddI) were

purchased from Sigma Chemical Co., St. Louis, MO; 2'-3' dideoxycytidine (ddC) was kindly provided by Hoffmann-La Roche, Basel, Switzerland. The compounds were dissolved in absolute ethanol, divided in aliquots and kept at −80°C. Further dilution was made in tissue culture medium. AZT 5'-triphosphate (AZT-TP) was purchased from Moravek Biochemicals, Inc., Brea, CA. The compound was dissolved in absolute ethanol. Poly (rA) and oligo (dT) were purchased from Pharmacia, Inc., Uppsala, Sweden and ^{3}H-dTTP was from Amersham Corp., U.K.

Virus and cells

The HTLV-III$_B$ strain was provided as chronically HIV-infected H9 cells by Dr. M. Cloyd (U.T.M.B., TX). Wild type and resistant strains of HIV-1 were grown and maintained in a CD4+ lymphoblastoid cell line, C8166, containing the HTLV-I genome and expressing only tax gene (Clapham et al., 1987). These cells were maintained in RPMI 1640 supplemented with 10% FCS (Flow Lab., Inc., Irvine, U.K.).

Density gradient centrifugation (Ficoll Hypaque, Pharmacia, LKB Biotechnology AB, Uppsala, Sweden) was used to separate peripheral blood mononuclear cells (PBMC) from venous blood of healthy donors.

Selection and cloning of resistant strain

AZT-resistant strains were selected by infection of C8166 cells with HTLV-III$_B$ in the presence of inhibitory concentration of AZT (5 μM). One ml (referred in Table 1 as passage no. 0) of undiluted H9 supernatant, containing $10^{4.8}$TCID$_{50}$/ml, was used to infect 4 × 10^6 C8166 cells. Cultures were maintained for 3 weeks in the presence of 5 μM AZT. Every week, cells and

TABLE 1

Procedure for selection of AZT-resistant strain of HIV-1

No. of passages	Markers of viral replication		
	cpe[d]	Viral antigen (ELISA)	Virus titer (Log TCID$_{50}$/ml)
0 (HTLV-III$_B$)[a]	+	+	4.8
1[b]	−	+	<1.5
2[b]	−	+	<1.5
3[c]	+−	+	1.5
4 (HIV-R1)[a]	+	+	5.0

[a] Passage No. 0 represents the HTLV-III$_B$ strain of HIV-1; Passage No. 4 is the virus obtained after 4 passages of HTLV-III$_B$ in C8166 cells. This virus was called HIV-R1.

[b] Passage performed by splitting infected cultures.

[c] Passage performed by passing virus only. For more details see Materials and Methods.

[d] cpe: + = generalized formation of syncytia (>80%); − = absence of syncytia; +− = low number of syncytia (<10%).

medium were collected and tested for the presence of viral antigens and infectious virus yield (referred to in Table 1 as passage no. 1, 2, 3, respectively). At the same time the culture fluids were replaced with fresh, AZT-containing, medium. After 3 weeks of culture, a significant cytopathic effect (cpe) was detectable. At this time the cultures were freeze-thawed, deprived of cellular debris by centrifugation, and the supernatant was titrated and used to infect fresh C8166 cells in the presence of AZT. One week later a strong cpe developed and the culture was freeze-thawed again to obtain a viral stock, named HIV-R1, and referred to in Table 1 as passage no. 4.

HIV-R1 was cloned by the limiting dilution method following current methodology. Briefly, 0.1 $TCID_{50}$ of HIV-R1 was plated with 5×10^4 C8166 cells in a 96-well microtiter plate. After 4 days of incubation cultures showing only one syncytium were collected and the virus was propagated in fresh C8166 cells to obtain a viral clone seed.

Assay of drug sensitivity

Two hundred thousand cells in 0.2 ml of medium were incubated with $HTLV\text{-}III_B$, HIV-R1, or HIV-R1 clones at approximately the same multiplicity of infection (MOI) (ranging in different experiments between 0.1 and 0.5 $TCID_{50}$/cell). At the same time, dilutions of the proper antiviral agent were added to each tube. One hour later, cells were washed three times, resuspended in 0.2 ml of medium containing the antiviral agent at the appropriate concentration, and incubated at 37°C. Forty-eight hours later the cells were subjected to three cycles of freeze-thawing, cell debris was removed by low speed centrifugation and the supernatants were titrated as described below. This experimental approach was chosen since at any time of the growth curve, with the exception of the peak of the eclipse phase, the titer of cell-associated virus was approximately 2 Logs higher than the titer of the virus released in the medium (Dianzani et al., 1988).

Virus titration

Titration was performed in C8166 cells by the standard limiting dilution method (0.5 Log ratio, 5 replicates per dilution) in 96-well microtiter plates. Infectious titer was determined by scoring syncytia under the microscope after 4 days of culture. Infectious titer, expressed as $TCID_{50}$/ml, was calculated by the method of Reed and Muench.

Antigen detection

Assessment of viral antigen levels in samples was carried out by ELISA (Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions.

Reverse transcriptase (RT) assay

To determine the sensitivity of RT to AZT-TP, virion-associated RT activity was assayed using a minor modification of a technique previously described (Willey et al., 1991). Briefly, 10 μl of solubilized pellets from polyethyleneglycol precipitation (overnight at 4°C) were incubated for 2 h at 37°C with 50 μl of RT cocktail which contained: 60 mM Tris-HCl, pH 7.8, 75 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 4 mM DTT, 50 μCi/ml [^{3}H]-dTTP, 5 μg/ml of poly (rA), 0.031 U/ml of oligo (dT) and AZT-TP (at various concentrations). After 2 h, 10 μl of each sample were transferred onto DE81 paper, washed and dried. After addition of 2 ml non-aqueous liquid scintillation cocktail, the radioactivity was counted in a scintillation counter.

DNA analysis and PCR

C8166 cells, infected with HTLV-III_B or HIV-11H and treated or untreated with AZT, were disrupted after 48 h of culture in 10 mM Tris HCl (pH 7.8), 10 mM EDTA, 1% SDS, followed by digestion with proteinase K. After 1 h at 37°C DNA was extracted with phenol/chloroform and then with chloroform/isoamyl alcohol. After addition of 0.1 M NaCl and 2 volumes of 95% ethanol the samples were centrifuged. Precipitated DNA was dissolved in 1 mM Tris-HCl (pH 7.8), 0.1 mM EDTA and dot blot analysis was performed using two-fold dilutions of the DNA starting from 5 μg of total DNA. HIV-specific DNA was visualized using the biotin-labeled full length HTLV-III_B probe, supplied by Oncor, Gaithersburg, MD, following the manufacturer's instructions.

Cells (10^5) to be examined for HIV-1 DNA by PCR were washed and pelleted in PBS, lysed in 0.001% Triton X-100/0.0001% SDS in TE buffer with 600 μg/ml of proteinase K (Boehringer Mannheim, Milan, Italy) for 1 h at 56°C, followed by 15 min at 95°C. The PCR reaction mixture contained 50 μl of DNA lysate, 25 pmol of each primer, 200 μM each of four deoxynucleotide triphosphates (Boehringer Mannheim, Milan, Italy), 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 50 mM KCl, 0.2% gelatin, and 2 units of Taq DNA polymerase (Perkin Elmer Cetus, Monza, Italy). Primer pairs used in this experiment included SK68/69 (env), SK38/39 (gag) (Synthetic Genetics, San Diego, CA). Amplification was carried out by means of a programmable thermal cycler (Perkin Elmer Cetus, Monza, Italy) with denaturation at 94°C, annealing at 55°C and extension at 72°C for 25 cycles. After amplification, 20-μl aliquots were taken from the sample for agarose gel electrophoresis (1.5% of agarose) to determine if amplification had occurred. Aliquots of 30 μl were taken from the test amplification tubes, mixed with a ^{32}P-adenosine triphosphate (ATP) end-labeled probe (SK70, env; SK19, gag: Synthetic Genetics, San Diego, CA), denatured at 94°C, and hybridized at 56°C for 15 min. The hybridized heteroduplexes were then analyzed on a 10% polyacrylamide gel (Schnittman et al., 1989). Autoradiograms of the gel were obtained by exposure of Kodak XAR film at −80°C for 12–16 h in the presence of an intensifying screen.

To determine the number of copies in HIV-infected C8166 cells, PCR was performed on serial 10-fold dilutions of the cellular lysates in the presence of excess control C8166 cell DNA derived from 10^5 cells. These amplifications were then compared with the PCR performed on serial dilutions of the ACH2 cell line (kindly provided by Dr. G. Pantaleo, NIH, Bethesda, MD), a chronically infected T cell line containing one copy of HIV-1 DNA per cell (Clouse et al., 1989).

Results

Selection of AZT-resistant HIV-R1 mutants

HTLV-III_B was subjected to propagation in C8166 cell in the presence of inhibitory concentrations of AZT, as described in Materials and Methods.

Table 1 shows the results obtained when different viral replication indexes were evaluated during this procedure. It can be seen that viral yield and cytopathic effect (cpe) were undetectable in the first passage but became detectable in the subsequent passages performed in the presence of 5 μM AZT. Virus obtained in this way is referred to as HIV-R1. This virus, whose titer was $10^{5.0}TCID_{50}$/ml, is able to induce cpe in the presence of AZT.

TABLE 2

AZT-resistance of HIV-R1 as based on cpe determinations

Virus	cpe[a] in the presence of AZT (μM)				
	5.0	0.5	0.05	0.005	0
HTLV-III_B	–	–	+	+	+
HIV-R1	+	+	+	+	+

C8166 cells were infected with HTLV-III_B or HIV-R1 at the same MOI in the presence or absence of different concentrations of AZT; 48 h later, cpe was evaluated by means the appearance of syncytia. The same results were obtained in three additional experiments.

[a] cpe: + = generalized formation of syncytia (>80%); – = absence of syncytia.

TABLE 3

AZT-resistance of HIV-R1 as based on viral yield measurements

Virus	Viral yield (Log $TCID_{50}$/ml) in the presence of AZT (μM)				
	5.0	0.5	0.05	0.005	0
HTLV-III_B	<1.5	<1.5	2.8	4.8	5.6
HIV-R1	3.3	4.2	6.1	5.8	6.2

C8166 cells were infected with HTLV-III_B or HIV-R1 at the same MOI in the presence or absence of different concentrations of AZT; viral progeny was measured 48 h later as described in Materials and Methods. Data are reported as means of two samples separately titered. Standard deviations never exceeded 0.5 Log. Similar results were obtained in three additional experiments.

To test the level of AZT-resistance, C8166 cells were infected at the same multiplicity of infection (MOI) with HIV-R1 and HTLV-III_B in the presence of various concentrations of AZT; cpe and viral yield were measured after 48 h of culture. The results are shown in Tables 2 and 3. It can be seen that HIV-R1 is able to induce an evident cpe in C8166 in the presence of 5 μM AZT, a concentration which is 100-fold higher than the concentration required to inhibit the induction of cpe by HTLV-III_B (Table 2). When the viral yield is measured (Table 3), HIV-R1 is not inhibited by 0.05 μM AZT and shows significant replication at 5 μM, while HTLV-III_B replication is inhibited by all AZT concentrations used. In other experiments both cpe and viral yield were examined at 72 h after infection of C8166 cells with HTLV-III_B and HIV-R1 at different MOI (between 1 and 0.001), and essentially the same results were obtained (data not shown).

Levels of viral DNA in AZT-treated cells infected with HIV-R1, or HTLV-III_B

Samples of viral DNA extracted from C8166 cells infected with HIV-R1 or HTLV-III_B and treated or untreated with 0.5 μM AZT, were subjected to dot blot analysis. Figure 1 shows that the level of viral DNA from both HTLV-III_B- and HIV-R1-infected cells are strongly inhibited by AZT, although that

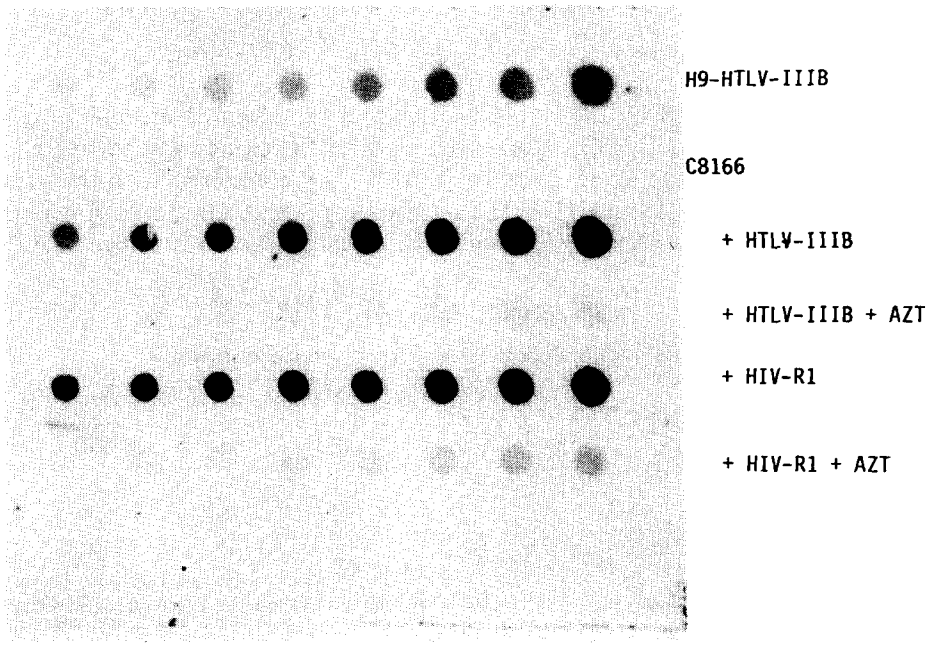

Fig. 1. Dot blot analysis of viral DNA extracted from 20 × 10^6 C8166 cells at 48 h after infection with HTLV-III_B or HIV-R1 (0.5 $TCID_{50}$/cell) in the presence or absence of AZT (0.5 μM). DNA from uninfected C8166 cells H9 cells chronically infected with HTLV-III_B are also shown.

TABLE 4

AZT-resistance measured in terms of viral yield after cloning of HIV-R1

Virus	Viral yield (Log $TCID_{50}$/ml) in the presence of AZT (μM)				Extent of resistance (μM)
	0.5	0.05	0.005	0	
$HTLV\text{-}III_B$	< 1.5	3.5	4.6	5.2	$0.005 < ID_{90} < 0.05$
HIV-R1	4.2	6.2	5.8	6.0	$0.05 < ID_{90} < 0.5$
HIV-8G	4.0	4.6	5.1	5.1	$ID_{90} > 0.5$
HIV-9E	< 1.5	2.8	5.1	5.5	$0.005 < ID_{90} < 0.05$
HIV-6A	3.0	3.6	4.6	4.8	$0.005 < ID_{90} < 0.05$
HIV-11H	5.0	5.2	5.3	5.6	$ID_{90} > 0.5$

C8166 cells were infected with $HTLV\text{-}III_B$, HIV-R1, or different clones derived from HIV-R1 at the same MOI in the presence or absence of different concentrations of AZT. Viral yields were measured at 48 h after infection as described in Materials and Methods. ID_{90} values were calculated on the basis of virus titers obtained in these experiments. Data represent means for two samples separately titered as described in Materials and Methods. Standard deviations never exceeded 0.5 Log. Basically the same results were obtained in two additional experiments.

from HIV-R1-infected cells is inhibited by AZT to a lesser extent. The evidence that viral DNA from cultures infected with HIV-R1, which in terms of viral yield is resistant, is inhibited, may suggest that HIV-R1 does not represent a single strain of HIV-1 but, indeed, may include various variants of HIV-1 with different sensitivities to AZT, some of them being still highly sensitive to AZT.

Cloning of HIV-R1 and measurement of the level of AZT-resistance

HIV-R1 was cloned by limiting dilutions and the clones were individually tested for resistance to AZT. Again, viral yield of the clones was measured in C8166 cells in the presence or absence of AZT, and compared with the yields induced by $HTLV\text{-}III_B$ and HIV-R1. The results are shown in Table 4, where it can be seen that the various clones showed different levels of resistance to AZT,

TABLE 5

Effect of AZT, ddC, and ddI on the replication of different variants of HIV-1

Virus	Viral yield (Log $TCID_{50}$/ml) in the presence of			
	none	AZT	ddC	ddI
$HTLV\text{-}III_B$	5.2	1.5	2.5	4.0
HIV-R1	5.7	5.0	5.2	ND
HIV-11H	5.6	5.0	4.7	5.3
HIV-8G	5.1	4.0	3.7	4.7

C8166 cells were infected with different viruses at the same MOI in the presence or absence of different drugs. Viral yield was measured at 48 h after infection, as described in Materials and Methods. Drugs were used at the following concentrations: AZT, 0.5 μM; ddC and ddI, 5 μM. Data are reported as means of duplicates separately titered as described in Materials and Methods. Similar results were obtained in two additional experiments.

ranging from full sensitivity (as compared to HTLV-III_B) to marked resistance. When expressed as inhibitory dose able to inhibit viral yield by 90% (ID_{90}) the sensitivity of the clones to AZT varied from greater than 0.5 μM (HIV-11H and HIV-8G) to 0.005 μM (HIV-6A). Thus, these data confirm the hypothesis that HIV-R1 is composed of a number virus variants with different sensitivities to AZT, the clones 11H and 8G showing the highest, and 9E and 6A the lowest level of resistance. Basically the same results were also obtained when C8166 cells were infected at different MOI (between 0.1 and 0.001) (data not shown).

The susceptibility of these clones to other antiviral nucleoside analogues was determined by measuring the viral yield and compared with the sensitivity of the wild type virus (Table 5). The data show that the AZT-resistant strains were also less susceptible to ddC and ddI.

Analysis of the mechanism of resistance

To analyze the mechanism of drug-resistance we examined whether the AZT-resistant HIV-11H strain was capable of overcoming the AZT inhibition at the level of reverse transcription. To avoid variability that may occur during multiple replication cycles, viral DNA synthesis was measured at 5 h post-infection in C8166 cells infected at the same MOI with HIV-11H and HTLV-III_B. At the end of the incubation period the number of copies of viral DNA was analyzed by using quantitative PCR using the SK38/39 primer pair with the appropriate probe (Schnitmann et al., 1989). The results are shown in Fig. 2. It can be seen that viral DNA derived from HTLV-III_B infected cells is

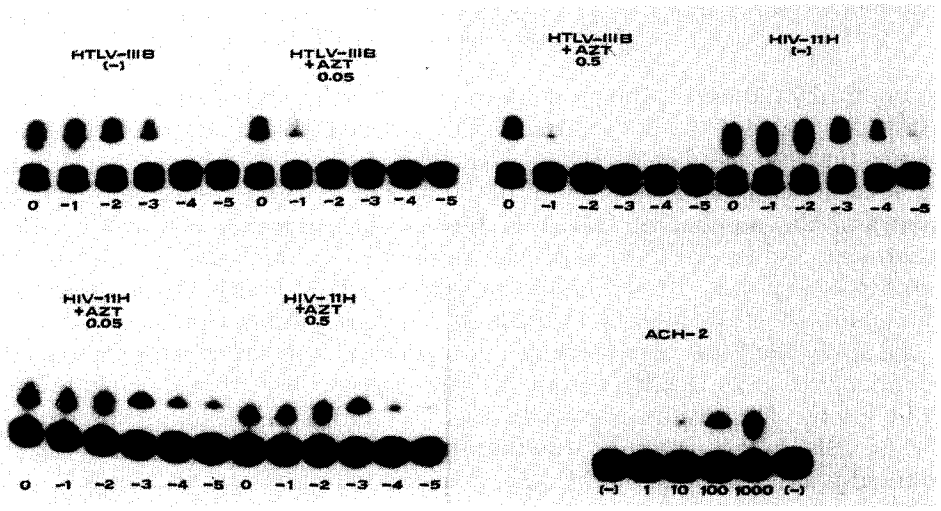

Fig. 2. Log dilutions (starting from 0) of the total DNA derived from the lysis of 10^5 C8166 cells at 5 h after infection with HTLV-III_B or HIV-11H (0.1 $TCID_{50}$/cell) in the presence or absence of AZT (at the indicated concentrations). The DNA was subjected to PCR using the SK38/39 primer pair and hybridized with the labeled probe (SK19). Serial dilutions of DNA extracted from ACH2 cells are also shown.

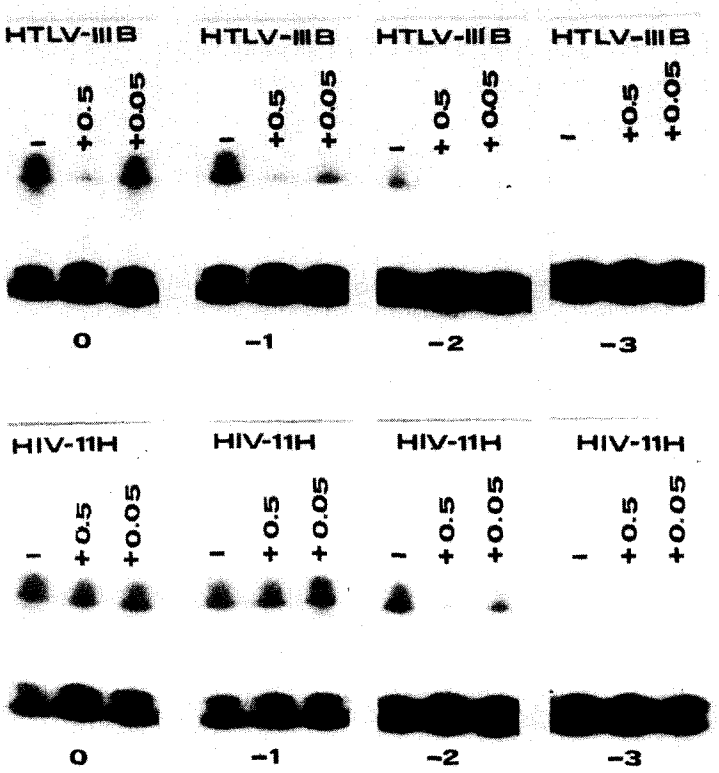

Fig. 3. Log dilutions (starting from 0) of the total DNA derived from the lysis of 10^5 PHA-activated PBMC at 16 h after infection with HTLV-III_B or HIV-11H (0.5 $TCID_{50}$/cell) in the presence or absence of AZT (at the indicated concentrations). The DNA was subjected to PCR using the SK38/39 primer pair and hybridized with the labeled probe (SK19).

dramatically reduced in the presence of AZT. On the contrary viral DNA from HIV-11H is essentially not reduced in the presence of AZT. Particularly, Fig. 2 shows that the number of HTLV-III_B DNA copies was reduced by 99% or 99.9% by treatment with AZT at 0.05 μM and 0.5 μM, respectively. On the contrary, the number of HIV-11H DNA copies was not affected by AZT treatment. Similar results were obtained using the SK68/69 primer pair with the appropriate probes. These findings suggest that HIV-11H is able to overcome the inhibitory effect of AZT at the level of primary transcription.

Basically the same experimental approach was applied to peripheral blood mononuclear cells (PBMC) from healthy donors infected with HIV-11H or HTLV-III_B. The results, shown in Fig. 3, confirm the finding observed in C8166 cells. In fact, it can be seen that the number of HIV-11H copies was not markedly affected by 0.05 μM AZT and only marginally reduced by 0.5 μM AZT treatment.

To test whether any functional alteration of RT from resistant strains could be detected in vitro, virion-associated RT from HTLV-III_B, HIV-R1 and HIV-11H were assayed for the inhibition by AZT-TP. These experiments revealed no

significant differences in the degree of inhibition of RT activity by AZT-TP, suggesting that the RT of resistant variants is not functionally modified in terms of affinity for AZT-TP. Inhibition of RT activity by AZT-TP was observed in the range of 5 μM to 0.005 μM in all viruses tested (data not shown).

Discussion

This paper shows that AZT-resistant strains can be obtained in vitro. Preliminary results indicate that this finding is reproducible also by using other clinical HIV-1 isolates.

Since no data on the sequence of the *pol* gene of HIV-11H are available at the moment, this study does not allow us to conclude whether or not AZT-resistance is due to a mutation of the *pol* gene. On the other hand, like other authors (Larder et al., 1989a; Remington et al., 1991) we failed to show resistance of RT from the resistant strains to AZT-TP. Thus, from our study, no definitive conclusion can be drawn on the mechanism of AZT resistance. However, the fact that total HIV-11H viral DNA is not inhibited by AZT concentrations that fully inhibit viral DNA synthesis in cells infected by HTLV-III_B strongly suggests that the reverse transcription process is involved in the acquisition of resistance, as suggested also by others (Larder et al., 1989c). It should be considered that this finding is not peculiar for HTLV-I transformed lymphoblastoid cells, since the same phenomenon was also observed in PBMC, which may mimic more closely the situation occurring in vivo.

Taken together the data not only show that AZT-resistant strains may be easily obtained in vitro, but also provide the first demonstration that variants resistant to AZT overcome the inhibition at the level of the reverse transcription.

It is not easy to explain why isolation of a resistant strain was relatively easily accomplished in this study while attempts made by others were unsuccessful (Smith et al., 1987; Rooke et al., 1990). A possible reason may be found in the heterogeneity of the viral population that is obtained under selective pressure of AZT. In this study the problem was at least in part solved by clonal selection. The clones thus obtained still revealed a high level of heterogeneity.

Another explanation may be found in the detection methodology; most workers monitor HIV yield by detection of viral proteins, which in our hands (unpublished observations), and as reported by others (McKeating and Moore, 1991; McKeating et al., 1991), do not necessarily reflect the yield of infectious virus or its inhibition. Probably similar considerations apply also to determinations based on detection of cell viability instead of viral yield (Larder et al., 1990). Accordingly, in this study, drug-sensitivity was measured in terms of yield of infectious virus and, for the first time, of DNA synthesis. In

this regard, it is worth noting that our findings are consistent with the isolation of an AZT-resistant FIV mutant using a focal immunoassay which accurately measures FIV infectivity (Remington et al., 1991). The apparent discrepancy among measurements of infectious viral yield and antigen production is not clear and should be further investigated. However, it does not detract from the present findings fully showing virus growth in the presence of high AZT concentrations.

Because of the high mutation rate of HIV-1 (Coffin, 1986; Sagg et al., 1988; Goodenow et al., 1989), the selection of viral clones with variable sensitivity to AZT may be expected. During the selection process at least two highly resistant variants were isolated. The characteristics of resistance of such variants basically parallel those of the HIV-clinical isolates (Larder et al., 1989a; Land et al., 1989). It is worth noting, however, that the AZT-resistant variants thus obtained in vitro are less sensitive also to other nucleotide analogues such as ddC and ddI, while such cross-resistant mutants are seldom found in vivo (Japour et al., 1991). This is not totally unexpected, as in other systems, namely herpes simplex virus type 1, the biological characteristics of acyclovir-resistant mutants selected in vitro do not strictly parallel those of resistant mutants isolated from patients (Collins and Darby, 1991).

In conclusion, the use of a continuous cell line highly susceptible to HIV enabled us to detect AZT- resistant strains of HIV-1. To our knowledge, this is the first report describing the selection of HIV-1 strains resistant to AZT in cell culture as well as demonstrating that HIV-1 resistant to AZT can overcome the inhibitory activity of AZT at the level of DNA synthesis. These mutants may represent an useful tool to further study the emergence and mechanism(s) of drug-resistance. Sequence studies are in progress to establish the site(s) of mutation which confer AZT-resistance and the biological and replicative characteristics of such clones.

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Note added in proof

While this paper was being processed by the editors, a study by Larder, B.A. et al. (J. Virol. 1991, 65, 5232–5236) was published, the conclusions of which basically parallel our findings.