Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 25–29, January 1993
Medical Sciences

Quantitation of zidovudine-resistant human immunodeficiency virus type 1 in the blood of treated and untreated patients

HIROSHI MOHRI*, MANDALESHWAR K. SINGH*, WENDELL T. W. CHING†, AND DAVID D. HO*‡

*Aaron Diamond AIDS Research Center, Department of Medicine and Microbiology, New York University School of Medicine, 455 First Avenue, New York, NY 10016; and †University of California, Los Angeles, School of Medicine, Sepulveda Veterans' Administration Medical Center, 16111 Plummer Street, 00P-R, Sepulveda, CA 91343

Communicated by H. Sherwood Lawrence, September 23, 1992 (received for review February 14, 1992)

ABSTRACT **A nonselective *ex vivo* assay was used to directly detect and quantify zidovudine (AZT)-resistant human immunodeficiency virus type 1 (HIV-1) in the blood of treated and untreated patients. In contrast to previous reports, drug-resistant virus was detected in peripheral blood mononuclear cells of a few of the patients who had never received AZT. The AZT resistance of HIV-1 isolates from one untreated individual was confirmed by further susceptibility studies *in vitro* and by the finding of a characteristic mutation (Lys → Arg at codon 70) in the reverse transcriptase. In patients who were clinically stable while on AZT, HIV-1 titers in plasma and mononuclear cells were generally low but resistant viruses already predominated. In those individuals who were deteriorating despite AZT administration, high levels of viremia were observed, and the resistance phenotype was nearly universal. These findings serve to emphasize the magnitude of the AZT-resistance problem in patients on drug treatment.**

The human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of the acquired immune deficiency syndrome (AIDS). In 1985, zidovudine (3′-azido-3′-deoxythymidine; AZT) was found to inhibit the HIV-1 reverse transcriptase (RT) and to block viral replication *in vitro* at a concentration of 0.1 μM or higher (1). A promising phase 1 study (2) led to a multicenter collaborative trial that showed that AZT decreased the number of adverse clinical events and prolonged survival in patients with AIDS or AIDS-related complex (ARC) (3). Subsequently, additional studies have found that AZT slowed disease progression in seropositive asymptomatic persons and early ARC patients (4, 5). AZT is one of only three licensed antiretroviral drugs in the United States and is now widely used.

The clinical benefit of AZT is often limited due to its adverse side effects such as gastrointestinal intolerance and hematoxicity (6). Moreover, the emergence of AZT-resistant HIV-1 in patients on prolonged therapy has been described by Larder *et al.* (7). In addition, they found that the resistance phenotype is correlated with distinct genotypic changes in the RT gene, including mutations at codons 67, 70, 215, and 219 (8). These studies, however, were performed on HIV-1 isolates that had been propagated in transformed cell lines, a highly selective situation. Given that HIV-1 exists in each patient as a population of diverse yet related viruses (9, 10), biases introduced by *in vitro* selection can lead to results that poorly reflect the situation *in vivo*. Thus, studies on viruses cultivated *in vitro* can only reveal the "tip of the iceberg" of AZT resistance *in vivo*. We therefore attempted to detect and quantify AZT-resistant HIV-1, without the artifact of *in vitro* selection, in the plasma and peripheral blood mononuclear cells (PBMCs) of 24 AZT-treated and 14 untreated patients in various stages of HIV-1 infection.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

METHODS

Patients. Informed consent was obtained from each of 38 HIV-1-seropositive subjects. Ten had asymptomatic infection, 8 had ARC, and 20 had AIDS. For data analysis, these study subjects were also divided into three groups as shown in Table 1: patients who never received AZT, patients who were clinically stable while on AZT, and patients who were worsening while on AZT and were eligible for dideoxyinosine because of AZT failure. The clinical and treatment information for each of the patients is presented in Table 2.

Quantitation of AZT Resistance in Plasma and PBMCs *ex Vivo*. The quantitation of AZT-resistant HIV-1 in the plasma and PBMCs of patients was performed using the end-point-dilution culture method (11). Plasma was obtained from heparinized blood samples by centrifugation, and PBMCs were obtained by Ficoll/Hypaque density gradient centrifugation. Decreasing inocula of plasma (1000, 100, 10, 1, and 0 μl) and PBMCs (10^6, 10^5, 10^4, 10^3, 10^2, and 0 cells) were each cultured with 2×10^6 phytohemagglutinin-activated PBMCs from healthy blood-bank donors in 1.5 ml of RPMI 1640 medium containing 20% (vol/vol) fetal bovine serum and interleukin 2 (10 units/ml) but no AZT. Additional aliquots of each sample were similarly titered in the presence of increased concentrations of AZT (1, 5, or 25 μM). All cultures were washed three times with medium 24 h later. Subsequently, the cultures were maintained for 3 weeks with twice weekly medium changes, during which the appropriate concentrations of AZT were added back to the cultures. HIV-1 expression was then detected by assaying the supernatant for p24 core protein production by using a commercial immunoassay (Abbott). A culture was considered positive if the concentration of p24 exceeded 1000 pg/ml on a single determination or >200 pg/ml on two or more determinations. The lowest number of PBMCs or the smallest volume of plasma required to produce a positive culture was taken as the end point, and the titers of infectious HIV-1 were then expressed as <1, 1, 10, 100, 1000 and ≥10,000 tissue culture infective doses (TCID) per 10^6 PBMCs or <1, 1, 10, 100, and ≥1000 TCID/ml of plasma for each concentration of AZT. For example, if the culture well inoculated with 10 μl of plasma in the presence of 5 μM AZT was the positive end point, then the patient has HIV-1 at 100 TCID/ml of plasma that is resistant to AZT at 5 μM.

Determination of AZT Resistance of Select Patient Isolates of HIV-1 *in Vitro*. Select HIV-1 isolates obtained from the *ex vivo* assays were further studied *in vitro* to verify their AZT sensitivity or resistance. These assays were performed using 2×10^6 normal phytohemagglutinin-stimulated PBMCs in 1.5 ml of RPMI 1640 medium containing interleukin 2 (10 units/

Abbreviations: AZT, zidovudine; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; ARC, AIDS-related complex; PBMC, peripheral blood mononuclear cell; TCID, tissue culture infective dose(s); ID_{90}, 90% inhibitory dose(s).

‡To whom reprint requests should be addressed.

Table 1. Summary of the study population

	Never treated, no.	Treated, no.		Total, no.
		Stable	Unstable	
Asymptomatic	7	3	0	10
ARC	2	3	3	8
AIDS	5	5	10	20
Total	14	11	13	38

ml). The viral inocula, in the form of supernatant fluids, were added to cultures containing 0, 0.04, 0.2, 1.0, or 5.0 μM AZT. The cultures were washed three times 24 h later to remove the viral inocula, and the appropriate concentration of AZT was added back. Expression of HIV-1 was detected by measuring supernatant p24 antigen production on days 3–7 of culture.

PCR Amplification and Nucleotide Sequencing. An HIV-1 infectious clone, obtained by limiting dilution from the plasma of patient 10, was found to be AZT-sensitive. Another clone was obtained by limiting dilution of the same patient's PBMCs and was found to be AZT-resistant. DNA was extracted from normal PBMCs infected with these viral clones and subjected to a PCR (8) for 30 cycles to amplify the RT genes. Each cycle consisted of 30-sec steps of melting at 94°C, annealing at 50°C, and extension at 72°C. The 1.7-kilobase PCR product was cut with *Xba* I and *Eco*RI, separated by 1% agarose gel electrophoresis, and ligated into M13mp19 vector. Nucleotide sequencing was carried out using the M13 universal primer, a set of five primers in the RT gene, and the Sequenase kit (United States Biochemical).

RESULTS

Fig. 1 summarizes results from 14 patients who reportedly never received AZT on detailed questioning. The lack of AZT use was confirmed by normal mean corpuscular volumes in all patients and by the absence of detectable AZT in the serum of patients 9–11 (data not shown). Eight of these patients had detectable plasma viremia. The total HIV-1 titer in plasma ranged from <1 to 100 TCID/ml with a mean value of 17 TCID/ml. However, within the limits of detection of our assay, HIV-1 with resistance to ≥1 μM AZT was not detected in the plasma of any of these patients. HIV-1 was recovered from the PBMCs of all 14 patients with titers from 1 to 1000 TCID per 10^6 cells and a mean titer of 195 TCID per 10^6 cells. In contrast to the situation in plasma, HIV-1 resistant to 1 μM AZT was detected in 11 of 14 patients with titers from 1 to 100 TCID per 10^6 cells. Furthermore, 4 patients had HIV-1 that was resistant to ≥5 μM AZT, and in 3 patients (patients 9–11) highly resistant virus accounted for most of the total viral burden.

Fig. 2 sums up findings on 11 patients who were clinically stable while receiving AZT. The mean HIV-1 titer in plasma

Table 2. Clinical and treatment information for the patients

Patient	Sex	Risk factor	Stage	T4 cells, no./mm^3	History of AZT use
1	M	Homosexual	Asx	NA	None
2	F	Heterosexual	Asx	NA	None
3	M	Unknown	Asx	NA	None
4	M	Homosexual	Asx	NA	None
5	M	Homosexual	Asx	NA	None
6	M	Homosexual	Asx	NA	None
7	M	Unknown	Asx	NA	None
8	M	Homosexual	ARC	476	None
9	M	Homosexual	ARC	NA	None
10	F	Heterosexual	AIDS	85	None
11	M	Unknown	AIDS	147	None
12	M	Homosexual	AIDS	NA	None
13	M	Homosexual	AIDS	NA	None
14	M	Homosexual	AIDS	320	None
15	M	Homosexual	Asx	282	1200 mg/d for >1.5 yr
16	M	Homosexual	Asx	223	1200 mg/d for >1 yr
17	M	Homosexual	Asx	113	1000 mg/d for >2 yr
18	M	Homosexual	ARC	89	600–1200 mg/d for >1.5 yr
19	M	Homosexual	ARC	280	1200 mg/d for 4 mo
20	M	Homosexual	ARC	NA	Dose and duration unknown
21	M	Homosexual	AIDS	NA	1200 mg/d for 8 mo
22	M	Homosexual	AIDS	72	300–1200 mg/d for >1.5 yr
23	F	Needle stick	AIDS	NA	Dose unknown for 1 yr
24	M	Homosexual	AIDS	57	300–1200 mg/d for >2.5 yr
25	M	Homosexual	AIDS	177	200–1200 mg/d for >3 yr
26	M	Transfusion	ARC	60	600–1200 mg/d for 6 mo
27	M	Unknown	ARC	NA	Dose and duration unknown
28	M	Unknown	ARC	NA	Dose and duration unknown
29	M	Homosexual	AIDS	196	600–1200 mg/d for >2.5 yr
30	M	Homosexual	AIDS	30	600–1200 mg/d for >1.5 yr
31	M	Transfusion	AIDS	54	600–1200 mg/d for 2.5 yr
32	M	Homosexual	AIDS	24	1000–1200 mg/d for >2 yr
33	M	Homosexual	AIDS	95	600–1200 mg/d for 1 yr
34	M	Homosexual	AIDS	146	300–1200 mg/d for 9 mo
35	M	Homosexual	AIDS	175	500–1200 mg/d for 3 yr
36	M	Homosexual	AIDS	5	300–1200 mg/d for >2 yr
37	M	Homosexual	AIDS	10	300–1200 mg/d for >1.5 yr
38	M	Homosexual	AIDS	62	600–1200 mg/d for >1 yr

M, male; F, female; NA, not available; Asx, asymptomatic; d, day(s); yr, year(s); mo, month(s).

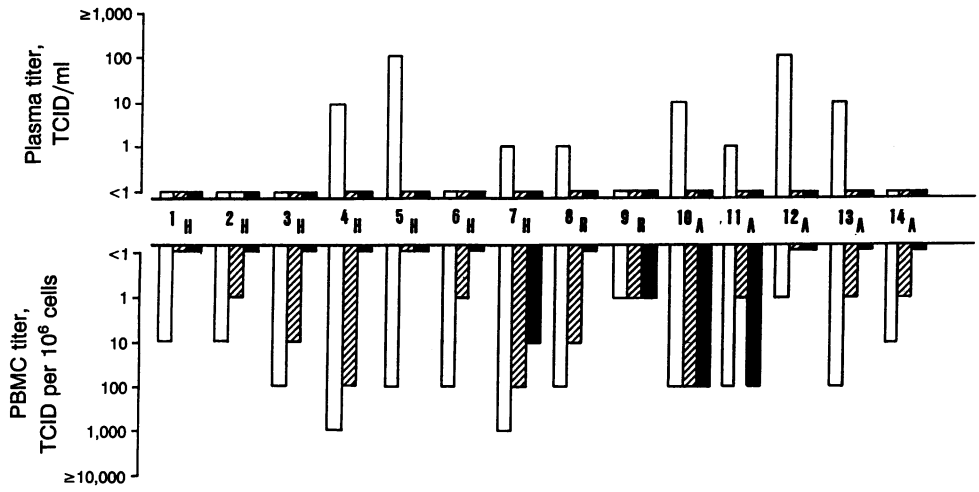

FIG. 1. Quantitation of total and AZT-resistant HIV-1 in the plasma (upper portion) and PBMCs (lower portion) of patients who never received AZT. Open bars, titers of virus determined in the absence of AZT; hatched and solid bars, respectively, titers obtained in the presence of 1 μM and 5 μM AZT. Each patient is represented by a number. H, asymptomatic; R, ARC; A, AIDS.

was relatively low (13 TCID/ml), with 4 patients showing no detectable plasma viremia. In the 7 patients with detectable plasma viremia, resistance to 1 μM AZT was found in 6, and resistance to $\geq$5 μM AZT was observed in 3 patients. In one patient (patient 18), a large fraction of his cell-free virus was highly resistant to AZT. In PBMCs, HIV-1 titers were again relatively low (1–100 TCID per 10^6 cells; mean, 26 TCID per 10^6 cells). However, AZT-resistant HIV-1 was detected in 10 of 11 patients. Although they were clinically stable, resistant virus predominated in these AZT-treated patients as shown in the lower portion of Fig. 2.

A dramatic difference is seen in patients who were worsening while on AZT as shown in Fig. 3. All 13 patients had detectable plasma viremia with a high mean titer of 425 TCID/ml. Titers were similarly high in PBMCs, and the mean value was 917 TCID per 10^6 cells. Every patient had AZT-resistant virus in both plasma and PBMCs. In four plasma and six PBMC cultures, most of the total HIV-1 burden was found to be highly AZT-resistant.

To verify the findings of the *ex vivo* assays described above, six of the HIV-1 isolates were chosen for further characterization *in vitro*. Three were obtained from cultures showing only AZT-sensitive viruses, and three were derived from cultures containing AZT-resistant viruses. As shown in Fig. 4, HIV-1 isolates from the plasma of patient 4, PBMCs of patient 5, and plasma of patient 10 exhibited sensitivity to AZT *in vitro* with 90% inhibitory doses (ID_{90}) of <0.2 μM. These results are consistent with those generated in the *ex vivo* assays (Fig. 1). By contrast, viral isolates defined as AZT-resistant in the *ex vivo* assay (from PBMCs of patients 10, 11, and 25) demonstrated moderate to marked resistance to AZT *in vitro* (Fig. 4). Thus, the findings from these *in vitro* assays appear to validate

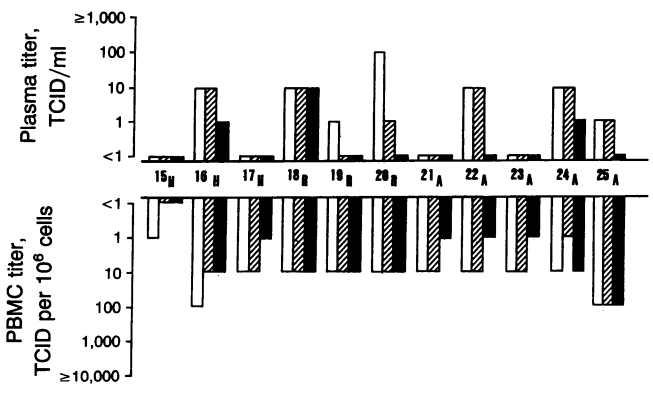

FIG. 2. Quantitation of total and AZT-resistant HIV-1 in the plasma and PBMCs of patients who were clinically stable while receiving AZT. Bars and abbreviations are described in Fig. 1.

the results obtained in the *ex vivo* assays. In particular, the resistance phenotype of HIV-1 isolates (PBMCs of patients 10 and 11) from patients who never received AZT has also been confirmed by the *in vitro* assay.

To define genetic changes responsible for AZT resistance in one untreated patient, the nucleotide sequences of RT genes from one AZT-sensitive plasma virus and one AZT-resistant PBMC virus from patient 10 were determined (Fig. 5). Both RT genes show a full-length open reading frame. The RT of the AZT-resistant clone showed three amino acid substitutions in the polymerase domain (codons 1–266) when compared with that of the AZT-sensitive clone, including the Lys $\rightarrow$ Arg mutation at codon 70 shown to be associated with AZT resistance in treated patients (8). The other two substitutions are in positions where there is significant variability among sensitive HIV-1 isolates (18) and, therefore, are less likely to be involved in drug resistance. Six amino acid differences were found in the tether domain (codons 266–347), and three were noted in the RNase H domain (codons 348–559) (12). These changes, however, are unlikely to affect AZT sensitivity.

When the data from Figs. 1–3 are summarized according to AZT use and nonuse, patients who never received AZT have only sensitive HIV-1 in plasma, although resistant virus can be detected in the PBMCs of many. In contrast, the resistant phenotype is nearly universal in patients treated with AZT.

DISCUSSION

We have found that AZT-resistant HIV-1 can be detected in the PBMCs of infected persons who have never received the drug (Fig. 1). That the resistant virus is not found in the plasma of these individuals suggests that the AZT-resistant HIV-1 is largely latent within their PBMCs, although it is possible that resistant viruses are difficult to detect in these cases due to the low levels of viremia. Of the four patients who had highly resistant virus, three (patients 9–11) contracted HIV-1 in the pre-AZT era. In contrast, one (patient 7) is a recent seroconvertor, and it is possible that he acquired the AZT-resistant HIV-1 from a contact who received the drug. Given that HIV-1 exists *in vivo* as a population of diverse yet related viruses (9, 10), we believe that resistance develops *de novo*, perhaps due to the error-prone reverse transcription step. Initially, the fraction of drug-resistant HIV-1 is probably small. As viral replication increases with disease progression (11), the chance of having AZT-resistant virus also increases. However, if treatment with AZT is started, the resistant population will then have a growth advantage and will thus proliferate to become the dominant population.

Our finding of AZT-resistant HIV-1 in untreated patients is in distinct contrast to published reports from several groups (7, 8, 11, 13–16), although acyclovir-resistant herpes simplex virus has been described in untreated patients (17). The

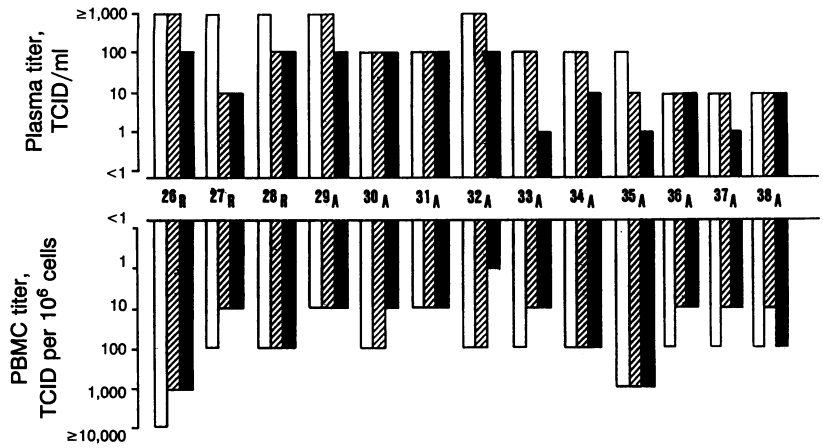

FIG. 3. Quantitation of total and AZT-resistant HIV-1 in the plasma and PBMCs of patients who were deteriorating while receiving AZT. Bars and abbreviations are described in Fig. 1.

explanation, we believe, lies in the differences in the techniques used to study the problem. The other investigators examined the *in vitro* AZT sensitivity of HIV-1 isolates that have been propagated in tissue cultures, often multiple times and in abnormal cell lines. Since Meyerhans *et al.* (9) have shown that to culture is to disturb or select, it is likely that the prior studies examined only a minor fraction of the viruses that are harbored *in vivo* and, therefore, are unlikely to detect AZT-resistant viruses unless they represent a large fraction of the total viral burden *in vivo*. On the other hand, our approach examined *ex vivo* the susceptibility of all infectious HIV-1 populations in plasma and PBMCs directly without preselection. Therefore, it should be more sensitive for detecting AZT-resistant viruses. Moreover, our direct assay has been validated by additional confirmatory studies. When viruses that were scored as resistant in the *ex vivo* assay were tested for AZT sensitivity *in vitro*, the same phenotype was found (Fig. 4). Furthermore, we have shown in patient 10 that the AZT resistance detected in the *ex vivo* assay is associated with one of the genotypic changes characteristic of drug-resistant isolates from treated patients. We thus believe that the *ex vivo* approach is valid and provides information that more accurately reflects the situation *in vivo*.

AZT-treated patients who are clinically stable have relatively low HIV-1 titers in plasma and PBMCs, perhaps reflecting the *in vivo* beneficial effect of the drug (Fig. 2). However, the resistance phenotype already predominates in these patients. It is possible that, as the AZT-resistant HIV-1 proliferates, the situation depicted in Fig. 3 eventually develops along with clinical deterioration. HIV-1 titers return to

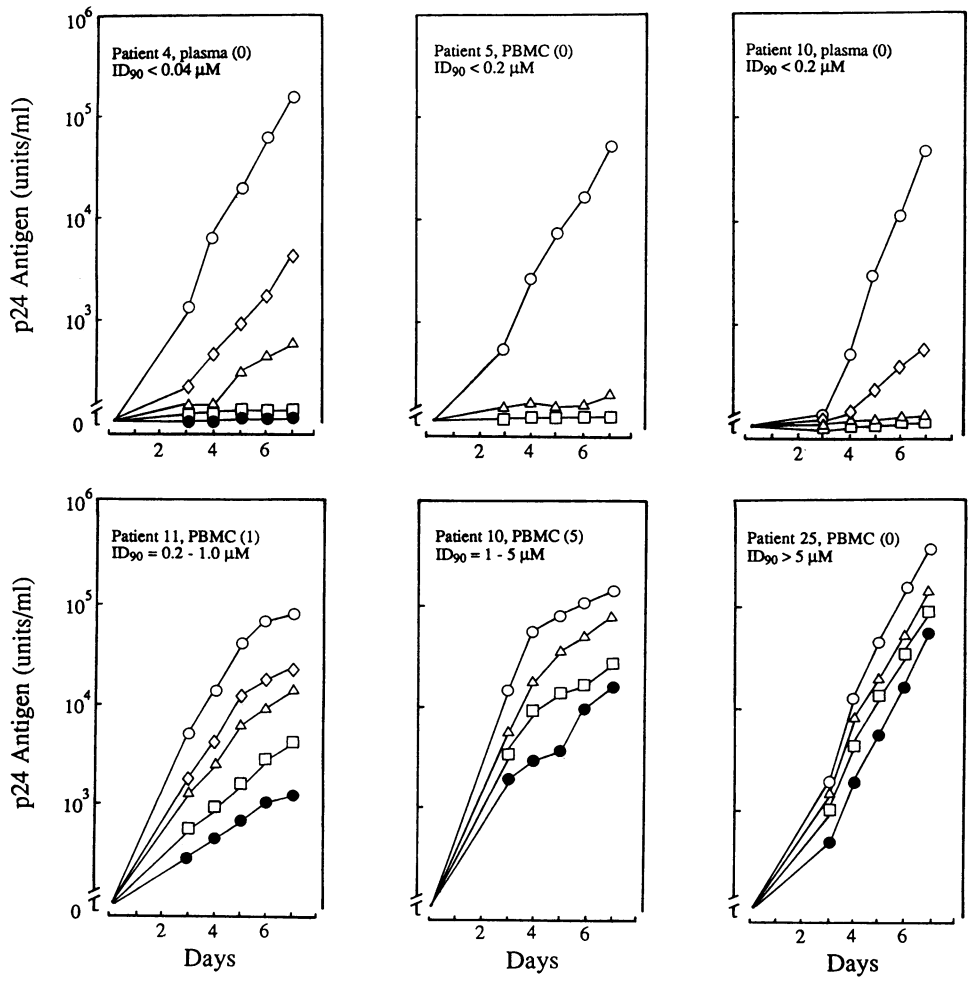

FIG. 4. Characterization of AZT sensitivity of six patient isolates of HIV-1 *in vitro*. Supernatant p24 antigen concentrations were determined on days 3–7 for cultures containing 0 (○), 0.04 (◇), 0.2 (△), 1.0 (□), or 5.0 (●) μM AZT. The HIV-1 isolates examined were obtained from the plasma or PBMCs of patients as indicated. The number in parentheses denotes the AZT concentration in the *ex vivo* culture from which the viral isolate was obtained. Ninety percent inhibitory dose (ID_{90}) values were based on p24 antigen results on day 4 culture supernatant. In these assays, 1 unit of p24 antigen equals ≈0.2 pg.

```
AZT-S   PISPI  ETVPV  KLKPG  MDGPK  VKQWP  LTEEK  IKALV  EICTE  MEKEG  KISKI
AZT-R   -----  -----  -----  -----  -----  -----  -----  -----  -----  -----
51      GPENP  YNTPV  FAIKK  KDSTK  WRKLV  DFREL  NKRTQ  DFWEV  QLGIP  HPAGL
        -----  -----  -----  ----R  -----  -----  -----  -----  -----  -----
101     KKKKS  VTVLD  VGDAY  FSVPL  DEDFR  KYTAF  TIPSI  NNETP  GIRYQ  YNVLP
        -----  -----  -----  -----  -----  -----  -----  -----  -----  -----
151     QGWKG  SPAIF  QSSMT  KILEP  FRKQN  PDIVI  YQYMD  DLYVG  SDLEI  GQHRT
        -----  -----  -----  -----  -----  --M--  -----  -----  -----  -----
201     KIEEL  RQHLL  RWGLT  TPDKK  HQKEP  PFLWM  GYELH  PDKWT  VQPIV  LPEKD
        -----  -----  ---F-  -----  -----  -----  -----  -----  -----  -----
251     SWTVN  DIQKL  VGKLN  WASQI  YPGIK  VRQLC  KLLRG  TKALT  EVIPL  TEEAE
        -----  -----  -----  -----  -----  -K---  -----  -----  ---Q-  -----
301     LELAE  NREIL  KEPVH  GVYYD  PSKDL  IAEIQ  KQGQG  QWTYQ  IYQEP  FKNLK
        -----  -----  R----  -----  -----  V----  -----  -----  -----  -----
351     TGKYA  RMRGA  HTNDV  KQLTE  AVQKI  TTESI  VIWGK  TPRFK  LPIQK  ETWET
        -----  -----  -----  -----  -----  -----  -----  I----  -----  ----A
401     WWTEY  WQATW  IPEWE  FVNTP  PLVKL  WYQLE  KEPIV  GAETF  YVDGA  ANRET
        --I--  -----  -----  -----  -----  -----  -----  -----  -----  -----
451     KLGKA  GYVTN  KGRQK  VVPLT  NTTNQ  KTELQ  AIHLA  LQDSG  LEVNI  VTDSQ
        -----  -----  -----  --S--  D----  -----  -----  -----  -----  -----
501     YALGI  IQAQP  DKSES  ELVSQ  IIEQL  IKKEK  VYLAW  VPAHK  GIGGN  EQVDK
        -----  -----  -----  -----  ---E-  -----  -----  -----  -----  -----
551     LVSAG  IRKV
        -----  ----
```

FIG. 5. Comparison of the deduced amino acid sequences of RTs from a plasma-derived AZT-sensitive clone and a PBMC-derived AZT-resistant clone from patient 10. Dashes denote amino acid identity.

high levels, and the resistant phenotype is almost universal. The data contained in Fig. 3 suggest, but do not prove, that AZT-resistant HIV-1 is pathogenic and is responsible for drug failure and disease progression.

Studies have suggested that patients early in the course of HIV-1 infection should be started on AZT because of short-term benefits (4, 5). However, the overall recommendation of early treatment with AZT should take into consideration the magnitude of the resistance problem as illustrated by our quantitative results. It is possible that the reported short-term benefits may eventually be offset by the earlier development of drug-resistant viruses and that with widespread use of AZT, the modest beneficial effect of the drug may diminish in time. This concern emphasizes the urgent need to quickly develop other anti-HIV drugs for clinical use.

We are indebted to E. Daar, J. Chia, H. Chang, and S. Liu for clinical or laboratory assistance and W. Chen for preparation of the manuscript and illustrations. This study was supported by grants from the National Institutes of Health (AI 25541, AI 28747, AI 27742, and AI 24030), the Ernst Jung Foundation, and the Aaron Diamond Foundation. We also acknowledge the core services provided by the Center for AIDS Research at New York University School of Medicine. H.M. was a recipient of a grant from the Japan Foundation for AIDS Prevention.

1. Mitsuya, H., Weinhold, K. J., Furman, P. A., St. Clair, M. H., Nusinoff, S., Gallo, R. C., Bolognesi, D., Barry, D. W. & Broder, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7096–7100.
2. Yarchoan, R., Klecker, R., Weinhold, K. J., Markham, P. D., Lyerly, H. K., Durack, D. T., Gelman, E., Lehrman, S. N., Blum, R. M., Barry, D. W., Shearer, G. M., Fischl, M. A., Mitsuya, H., Gallo, R. C., Dollins, J. M., Bolognesi, D. P., Myers, C. E. & Broder, S. (1986) *Lancet* **i,** 575–580.
3. Fischl, M. A., Richman, D. D., Grieco, M. H., Gottlieb, M. S., Volberding, P. A., Laskin, O. L., Leedom, J. M., Groopman, J. E., Mildvan, D., Schooley, R. T., Jackson, G. G., Durack, D. T. & King, D. (1987) *N. Engl. J. Med.* **317,** 185–191.
4. Volberding, P. A., Lagakos, S. W., Soch, M. A., Pettinelli, C., Myers, M. W., Booth, D. K., Balfour, H. H., Jr., Reichman, R. C., Bartlett, J. A., Hirsch, M. S., Murphy, R. L., Hardy, W. D., Soeiro, R., Fischl, M. A., Bartlett, J. G., Merigan, T. C., Hyslop, N. E., Richman, D. D., Valentine, F. T., Corey, L. & The AIDS Clinical Trials Group of The National Institute of Allergy and Infectious Diseases (1990) *N. Engl. J. Med.* **322,** 941–949.
5. Fischl, M. A., Richman, D. D., Hansen, N., Collier, A. C., Carey, J. T., Para, M. F., Hardy, W. D., Dolin, R., Powderly, W. G., Allan, J. D., Wong, B., Merigan, T. C., McAuliffe, V. J., Hyslop, N. E., Rhame, F. S., Balfour, H. H., Spector, S. A., Volberding, P., Pettinelli, C., Anderson, J. & The AIDS Clinical Trials Group of The National Institute of Allergy and Infectious Diseases (1990) *Ann. Intern. Med.* **112,** 727–737.
6. Richman, D. D., Fischl, M. A., Grieco, M. H., Gottlieb, M. S., Volberding, P. A., Laskin, O. S., Leedom, J. M., Groopman, J. E., Mildvan, D., Hirsch, M. S., Jackson, G. G., Durack, D. T., Nusinoff-Lehrman, S. & The AZT Collaborative Working Group (1987) *N. Engl. J. Med.* **317,** 192–197.
7. Larder, B. A., Darby, G. & Richman, D. D. (1989) *Science* **243,** 1731–1734.
8. Larder, B. A. & Kemp, S. D. (1989) *Science* **246,** 1155–1158.
9. Meyerhans, A., Cheynier, R., Albert, J., Seth, M., Kwok, S., Sninsky, J., Morfeldt-Manson, L., Asjo, B. & Wain-Hobson, S. (1989) *Cell* **58,** 901–910.
10. Saag, M. S., Hahn, B. H., Gibbons, J., Li, Y., Parks, E. S., Parks, W. P. & Shaw, G. M. (1988) *Nature (London)* **334,** 440–444.
11. Ho, D. D., Moudgil, T. & Alam, M. (1989) *N. Engl. J. Med.* **321,** 1621–1625.
12. Jacobo-Molina, A. & Arnold, E. (1991) *Biochemistry* **30,** 6351–6361.
13. Larder, B. A., Kellam, P. & Kemp, S. D. (1991) *AIDS* **5,** 137–144.
14. Land, S., Treolar, G., McPhee, D., Birch, C., Doherty, R., Cooper, D. & Gust, I. (1990) *J. Infect. Dis.* **161,** 326–329.
15. Richman, D. D., Grimes, J. M. & Lagakos, S. W. (1990) *J. Acquired Immune Defic. Syndr.* **3,** 743–746.
16. Boucher, C., Tersmette, M., Lange, J. M., Kellam, P., De-Goede, R. E. Y., Mulder, J. W., Darby, G., Goudsmit, J. & Larder, B. A. (1990) *Lancet* **ii,** 585–590.
17. Parris, D. S. & Harrington, J. E. (1982) *Antimicrob. Agents Chemother.* **22,** 71–77.
18. Myers, G., Rabson, A. B., Berzofsky, J. A., Smith, T. F. & Wong-Staal, F. (1991) *Human Retroviruses and Acquired Immune Deficiency Syndrome* (Los Alamos National Lab., Los Alamos, NM).