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HIV DRUG RESISTANCE[1]

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INTRODUCTION

Infection with human immunodeficiency virus (HIV) is a chronic process with persistent, high rates of replication. The virus exhibits high rates of mutation over time within the same individual. It is thus not surprising that drug-resistant mutants of HIV emerge under the selective pressure of prolonged chemotherapy, especially with the knowledge of the clinical emergence of drug-resistant mutants of herpes simplex virus, varicella zoster virus, cytomegalovirus, influenza A virus, and rhinovirus (1).

DIMINISHED SUSCEPTIBILITY TO AZT

The original description of AZT resistance analyzed 46 isolates of HIV from the peripheral blood mononuclear cells from 33 individuals, utilizing a syncytial focus reduction assay in a CD4-expressing HeLa cell line (2). This study generated several conclusions: (*a*) Isolates from subjects not treated with AZT displayed a narrow range of susceptibility to AZT, with the 50% inhibitory concentration (IC_{50}) ranging from .001 to 0.04 μM. (*b*) This narrow range of susceptibility was seen with isolates from subjects at all stages of HIV infection, from asymptomatic through advanced AIDS. (*c*) Isolates from patients with AIDS or advanced AIDS-related complex displayed no detectable reduction in susceptibility during the first 6 months of AZT treatment; almost all isolates from such individuals displayed some reduction in

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susceptibility after 6 months of therapy. (*d*) Sequential isolates from individual patients receiving AZT therapy may display progressive, step-wise increases in resistance. (*e*) Several isolates with > 100-fold increases in the IC_{50} of AZT have been identified. (*f*) AZT-resistant isolates displayed cross-resistance to 3′-azido-2′,3′-dideoxyuridine (AZdU) but not to dideoxycytosine (ddC) or foscarnet.

Subsequent studies have confirmed the emergence of AZT-resistant isolates with prolonged therapy (3, 4). These studies have included isolates from children (5), from plasma of adults (6, 7), and from an AZT-treated individual infected with HIV-2 (8). The observations that cross-resistance occurs with AZdU has been extended to other nucleosides containing a 3′-azido moiety, including 3′-azido-2′,3′-dideoxyguanosine (AZG) and 3′-azido-2′,3′-dideoxyadenosine (AZA) (9; D. D. Richman, unpublished observations). Cross resistance to other nucleosides, including several thymidine analogues, was not documented and cross-resistance to other nonnucleoside reverse transcriptase inhibitors or of compounds acting at other sites of HIV replication has also not been documented (9–11). These observations are encouraging with regard to the use of other nucleosides such as ddC or ddI and of drug combinations. Two of 11 AZT-resistant isolates have been reported to display cross-resistance to didehydrodideoxythymidine (d4T) (12). Further confirmation with characterization of the responsible mutations will be important.

An extension of the original studies to a total of 97 isolates from 73 individuals has provided information regarding the effects of disease stage and drug dose on the rates of emergence of resistance (13). Susceptibilities to zidovudine were determined in 55 isolates from 31 patients receiving

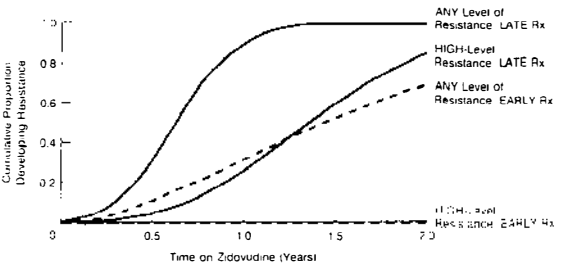

Figure 1 Estimated cumulative proportion of persons developing resistant isolates as a function of time since initiation of AZT by stage of HIV infection. Patients with late-stage infection had less than 200 CD4 lymphocytes and HIV-related symptoms. Patients with early-stage disease had 200–500 CD4 lymphocytes and mild or no symptoms. Any level resistance is defined as an IC_{50} of 0.05 μM. High-level resistance is defined as an IC_{50} of ≥1.0 μM. No high-level resistance was seen in the early-stage patients during this interval but has been observed in the third and fourth years of therapy (14; D. D. R., unpublished observations). Adapted from Ref. (13).

zidovudine (13). Patients with late-stage HIV infection (AIDS or advanced ARC) developed resistance significantly sooner than those with early-stage disease ($p = 0.002$). By 12 months after initiation of AZT therapy, an estimated 89% (95% confidence interval = 64–99%) of persons with late-stage HIV infection have developed resistance, compared with 31% (95% confidence interval = 16–56%) of those with early-stage infection. It is possible that the clinical significance of resistance could be a function of the degree of drug susceptibility. All six subjects who developed highly resistant virus were from among the 14 with late-stage HIV infection. For this population, the estimated proportion that developed highly resistant virus within 1 year after initiation of AZT was 33% (95% confidence interval = 16–59%), whereas no high-level resistance was documented in the first 18 months of therapy among earlier-stage patients (13). High-level AZT resistance has been documented in the third year of therapy for asymptomatic therapy (14). Reversal of resistance occurs over a period of months to years in most but not all patients who are withdrawn from AZT therapy (7; D. D. R., unpublished observations). Reversal appears to occur more readily in patients with virus mixtures (D. D. R., unpublished observations).

Lower initial CD4 lymphocyte counts were also predictive of increased likelihood of the emergence of resistant isolates ($p = 0.004$). The estimated rates of resistance at 1 year were 89%, 41%, and 27% for baseline CD4 cell counts <100, 100–400, and >400 CD4 cells/mm^3 (95% confidence intervals = 63–99%, 18–75%, and 11–59%, respectively).

Development of resistance occurred somewhat sooner among individuals assigned to higher daily doses of AZT (1,200–1,500 mg) than those assigned to lower doses (500–600 mg), although this difference did not attain statistical significance ($p = 0.18$ without controlling for stage and $p = 0.06$ after controlling for stage). Baseline positivity (>37 pg/ml) for serum HIV p24 antigen, which occurred in five of the late-stage subjects and none of the early-stage subjects, was not significantly correlated with the development of resistance ($p = 0.2$). These results are of some importance, because current studies and clinical recommendations utilize a daily dose of 500–600 mg AZT (15–17).

ENZYMATIC AND GENETIC BASIS OF AZT RESISTANCE

The antiviral effect of AZT is conferred by the triphosphate that is generated by anabolic phosphorylation by host cell thymidine kinase and other enzymes (18). AZT triphosphate inhibits the reverse transcriptase of HIV in cell-free enzyme assays and also acts as a terminator of DNA chain elongation because the 3′-azido group prevents the formation of 3′,5′-phosphodiester bonds. It

was not surprising therefore when mutations in the gene for the reverse transcriptase of the resistant virus were documented (see below). What remains puzzling, however, has been the difficulty in demonstrating an enzymologic difference between the mutant and wild-type reverse transcriptases (2). Utilizing enzyme extracted either from AZT-sensitive and resistant virions or prepared from enzyme expressed in *E. coli* after molecular cloning from these viruses, no differences in inhibition by AZT triphosphate has been demonstrated in cell-free enzyme assays. Because the genetics are definitive, these observations would suggest that cell-free enzyme assays do not reflect the mechanism of inhibition of AZT triphosphate upon the transcription complex in the cell.

Sequencing the reverse transcriptase gene of five pairs of isolates that displayed more than 100-fold reductions in susceptibility during the course of therapy documented multiple mutations, four of which appeared common (19). When these four mutations at amino acid residues 67, 70, 215, and 219 were inserted by site-directed mutagenesis into the susceptible infectious molecular clone pHXB2, a greater than 100-fold reduction in AZT susceptibility resulted. Sequential isolates from the same individual that displayed progressive, step-wise increments in resistance were associated with the sequential cumulative acquisition of these four mutations (19). Cumulative mutations thus contribute additively or synergistically to stepwise reductions in susceptibility (14, 19). Mutations at the four identified residues are among the most important, but almost certainly not the exclusive, contributors to the resistance phenotype (14, 20, 21).

More recently, a fifth mutation at residue 41 (methionine to leucine) was demonstrated to contribute to significant reductions in susceptibility to AZT (22). The only definitive method to confirm that a mutation in this highly variable virus actually contributes to reduced susceptibility is to quantitate the reduction in susceptibility of a virus with a defined susceptibility and genetic background after introducing the mutation by site-directed mutagenesis (Table 1). By assessing the impact on AZT susceptibility of these mutations singly or in various combinations, Larder et al have shown that these mutations vary quantitatively in their impact, and that some combinations appear to result in additive, synergistic, or even antagonistic quantitative changes in drug susceptibility (Table 2). These effects appear to correlate with the changes in susceptibilities in sequential isolates obtained from patients on AZT therapy and with the patterns of the appearance and even disappearance of some mutations.

Several investigators have developed assays utilizing sequence amplification methodologies to assay for these point mutations. Boucher and colleagues, utilizing primer pairs for the polymerase chain reaction (PCR) that

Table 1 Mutations in the HIV-1 gene for reverse transcriptase that confer drug resistance as confirmed by site-directed mutagenesis

Drug	Mutations of wild-type codon														Reference
	41M	67D	69T	70K	74L	100L	103K	106V	108V	1351	138E	181Y	188Y	215T	219K	
AZT	L	N		R										Y,F	Q,E	19, 22, 27, 58
ddI					V					V						32, 35
ddC			D													34
TSAO											K					37
L-697,639						I	N		I			C	C			42, 46
nevirapine						I	N	A	I			C	C			43, 47

Amino acid abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; R, arginine; T, threonine; V, valine; Y, tyrosine.

Table 2 AZT susceptibility of HIV variants with defined mutations in RT

HIV variant	Mutations introduced	AZT IC_{50}, μM	Change in susceptibility
HXB2-D		0.01	1
HXB 41L	L-41	0.04	4
HIVRTMF	Y-215	0.16	16
HXB 41L/215Y	L-41, Y-215	0.60	60
HIVRTMC/F	N-67, R-70, Y-215	0.31	31
RTMC/F 41L	L-41, N-67, R-70, Y-215	1.79	179
HIVRTMCY	N-67, R-70, Y-215, Q-219	1.21	121
HIVRTMC	N-67, R-70, F-215, Q-219	1.47	147

The genotypes of HIV variants constructed by site directed mutagenesis are shown. Modified from reference (22)

include the wild-type or mutant sequence for codon 215, demonstrated the appearance of mutant sequence at residue 215 in asymptomatic patients receiving chronic therapy with AZT (23). Mutations gradually appeared over a two-year period with therapy and correlated with the phenotype of resistance. López-Galíndez et al have utilized patterns of susceptibility to ribonuclease A to discriminate wild-type or mutant sequence at residue 215 (24). Although isolates from California and Spain had very distinctive digestion patterns, the pattern characteristic of a mutation at residue 215 occurred only in patients from either area receiving chronic AZT therapy. Richman et al (14) have utilized a PCR technique with primer pairs that generate a 535 base-pair sequence that spans all four residues of interest in the reverse transcriptase. Four pairs of oligonucleotide probes that are specific for wild-type or mutant sequence at each of the residues were then used to characterize the sequence at each residue, utilizing as target either patient peripheral blood mononuclear cells or virus isolates on which AZT susceptibility had been performed. Gingeras et al have utilized an isothermal, single cycle amplification technique (25), termed the self-sustained sequence replication (3SR) system, that can discriminate wild-type or mutant sequence at each of the four residues. Because this amplification method generates a predominance of one strand of single-stranded RNA, direct sequencing is possible, thus permitting additional issues to be investigated (26).

Three hundred and four independent specimens (virus stocks or peripheral blood mononuclear cells) from 168 individuals have been genotypically characterized by the PCR method (14). Sixty-seven specimens have been obtained prior to AZT therapy, all of which have displayed wild-type genotype. Sixty specimens obtained from individuals receiving AZT therapy

have contained a mutant sequence at a single residue: 5 at residue 67, 23 at residue 70, 31 at residue 215, and 1 at residue 219. Thus, a mutation may first appear at any residue but the selection for some mutations occurs far more readily than for others. A similar sequence of the appearance of mutations 215 and 70, then 67, followed by 219 has been demonstrated by selective pressure with AZT in vitro (27). Certain patterns of appearance of mutations appear to be more frequent. Isolates with mutations at residue 70 tend to appear first (14, 28). These mutations are associated with relatively small reductions in susceptibility. As multiple mutations appear, especially at residues 215 and 41, the magnitude of reduction of susceptibility increases significantly, with both perhaps 100-fold (Table 2). Moreover, the appearance of mutations at residue 215 often presages the disappearance of mutations at residue 70, consistent with the relative disadvantage of the two mutations in the presence of the selective pressure of drug therapy (28). The existence of virus mixtures in patients complicates the assessment of the drug susceptibility of isolates, the genetic composition at residues associated with drug resistance and the clinical relevance of drug resistance.

The suggestion of mixed populations of virus with different AZT susceptibilities has been confirmed with genotypic analysis. Boucher et al (23) documented several individuals with the simultaneous presence of both wild-type and mutant sequence at residue 215. López-Galíndez et al (24) and Mayers et al (20) documented by sequencing of clones the simultaneous presence in the same individuals of both the phenylalanine and tyrosine mutations at residue 215. Richman et al (14) have demonstrated mixtures at each of the residues, occasionally several simultaneously, during the transition from pure wild-type to pure mutant sequences in that individual.

Phenotypic mixtures in patients have been confirmed by using the HeLa-CD4 assay (14) or by assaying different virus isolates obtained at one time from patients using lymphocytes and plasma (6). A small proportion of virus isolates have been documented to change susceptibility pattern or genotype with passage in vitro in the absence of drug (14, 26). This phenomenon occurs more frequently if a mixed population is present in the original mononuclear cell specimen. Shifts to either more or less sensitive populations have been documented.

Sequencing studies suggest that the appearance of additional mutations either within the residue in question or in adjacent residues can account for diminished probe affinity (20, 21, 26). This problem will presumably affect most such assays with this highly mutable virus. It is important to appreciate that a mutation in the reverse transcriptase could affect drug susceptibility, have a neutral effect on drug susceptibility, or confound assays to detect other specific sequences.

DIMINISHED SUSCEPTIBILITY TO OTHER NUCLEOSIDES

It has been much more difficult to document the development of diminished susceptibility to ddC or ddI with isolates of HIV from patients on prolonged therapy with these drugs (29; D. D. R., unpublished observations). Quantitative variations among virus isolates in susceptibility to ddI, for example (30), are not sufficient to document drug-induced mutations conferring specific resistance. Criteria to document resistance would include a standardized well-characterized assay, definition of the normal range of susceptibility of isolates from untreated patients, demonstration of a change in susceptibility in sequential isolates from a patient receiving therapy, and transfer of resistance with transfer of specific mutations to a sensitive strain of HIV.

Several of these criteria have now been fulfilled. Four- to tenfold reductions in susceptibility of sequential isolates from patients on prolonged therapy have been reported (31–33). Isolates resistant to AZT at the initiation of ddI therapy exhibit increases in AZT susceptibility in conjunction with reductions in ddI susceptibility (32, 33). St. Clair et al documented a leucine-to-valine mutation at residue 74 of the reverse transcriptase in association with these phenotypic changes. Site-directed mutagenesis of an infectious provirus indicated that this mutation could account for both the diminished susceptibility to ddI (and to ddC) and the increased susceptibility to AZT (32). This mutation that produced partial reversal of AZT resistance had no appreciable effect upon the AZT susceptibility of wild-type (sensitive) virus. This observation supplements the rationale for combination regimens of AZT with other nucleosides. A mutation conferring a reduction of ddC susceptibility of approximately fivefold has been identified and confirmed by site-directed mutagenesis at residue 69 (threonine to aspartic acid) (34). A second mutation in the RT gene (isoleucine to valine at residue 135) was also recently reported to account for diminished susceptibility to ddI following prolonged administration to patients (35).

Derivatives of nucleosides such as thymine with an unusual silicon containing sugar moiety (TSAO, 2′,5′-bis-*O*-(*tert*-butylimethylsilyl)]-3′-spiro-5′′-(4′′-amino-1′′,2′′-oxathiole-2′′, 2′′-dioxide)]) are new HIV-1 specific RT inhibitors (36); one of these has entered clinical trial in Europe. Balzarini recently described the selection in vitro of a mutant with reduced susceptibility with a mutation at residue 138 of the RT (37).

DIMINISHED SUSCEPTIBILITY TO NONNUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

A number of structurally divergent compounds have been described that share some remarkable similarities (11, 38–41). These compounds have the

following characteristics: they do not require cellular metabolism to be active; they are potent inhibitors of HIV-1 replication but relatively nontoxic in vitro; they inhibit reverse transcriptase activity and replication of HIV-1 but not of HIV-2 or animal lentiviruses; and they are equally effective against AZT-sensitive or -resistant isolates (10, 11). This specificity may indicate a potential Achilles heel of these otherwise promising compounds in that lentiviral RT sequences can clearly be fully functional without being susceptible to these compounds. In fact, two recent studies have documented that drug resistance readily emerges with passage of virus in the presence of drug in vitro (42, 43).

Nunberg et al have shown that the Merck pyridinone inhibitors rapidly select for mutants in vitro with 1000-fold reductions in susceptibility (42). This resistance is accounted for by two mutations, lysine to asparagine at residue 103 and tyrosine to cysteine at residue 181. Richman et al have shown that the Boehringer Ingelheim dipyridodiazepinone inhibitor, nevirapine (BI-RG-587), which binds to the tyrosine at residue 181 (44), rapidly selects for mutants in vitro with 100-fold reductions in susceptibility (43). By site-directed mutagenesis this resistance can be accounted for by the tyrosine to cysteine mutation at residue 181. Utilizing the self-sustained sequence replication methodology that has been applied to AZT resistance (26) to assay for this mutation, this mutation can be detected as early as the first passage in vitro (43). The many similarities of the various nonnucleoside reverse transcriptase inhibitors have been unfortunately sustained in that each of the reported mutants displays cross-resistance to the other compounds of this class (42, 43). It is remarkable that the mutant reverse transcriptases selected by the nonnucleoside inhibitors are less susceptible to inhibition in cell-free enzyme assays (42, 43); this difference contrasts to the lack of effects seen with the analogous experiments with AZT.

A 3.5 Å resolution electron density map of the HIV-1 RT was made possible by complexing the RT heterodimer with nevirapine (45). The binding pocket of this drug is comprised of two β-strands from residues 100 to 110 and 180 to 190. That mutations conferring drug resistance occur in residues in these domains provides a gratifying correlation of structure and function that could theoretically lead to the design of better inhibitors.

Phase I/II clinical trials with two nonnucleoside RT inhibitors, L-697, 661 (Merck) and nevirapine (Boehringer Ingelheim), have confirmed in the patient the rapid selection for resistant virus that was seen in cell culture (46, 47). In both studies, an initial reduction in p24 antigen and elevation of CD4 cell counts within a week or two of initiating drug therapy was lost within a month or two. This loss of activity was associated with the emergence of the drug-resistant phenotype and the acquisition of one or more mutations at residues 100, 103, 106, 108, 181, and 188 (Table 1). This resistant virus was

observed consistently after two weeks of therapy, whether or not AZT was co-administered (47).

RESISTANCE INTERACTIONS OF REVERSE TRANSCRIPTASE INHIBITORS

Cross resistance of AZT-resistant isolates has been described to 3′-azido containing nucleoside analogues (2, 9). The ddI-resistance mutation at residue 74 confers cross-resistance to ddC and a compensatory reduction of AZT resistance conferred by residue 215 (32). Nonnucleoside reverse transcriptase inhibitors display cross-resistance, although quantitative differences in the effects of various mutations indicate that the different compounds do not interact with RT identically (42, 43). More recent studies suggest that mutations conferring AZT resistance can be antagonistic, additive, or synergistic (22), or even that AZT resistance can be associated with relatively subtle quantitative reduction in susceptibility to ddI or ddC (48). It has also been hypothesized that mutations conferring resistance to one drug may be incompatible with those to another drug, thus providing a rationale for combination therapy with certain inhibitors of RT (49). Additional studies examining such interactions, correlating these with the crystal structure of RT (45), should provide additional insights into both the mechanisms of resistance and approaches to circumvent resistance.

DIMINISHED SUSCEPTIBILITY TO PROTEASE INHIBITORS

The selection in vitro of a mutant to a protease inhibitor resulting in an eightfold reduction in susceptibility was recently reported (50). The mutant had a single mutation at amino acid residue 82 of the protease, which is situated near the substrate/inhibitor binding pocket of the catalytic site. This raises concerns similar to those raised for the RT inhibitors. Additional data with other inhibitors in vitro and with isolates from clinical trials are eagerly awaited.

ASSAYS OF DRUG SUSCEPTIBILITY

There is a widespread desire and need for a dependable, standardized assay of drug susceptibility. The most commonly used assays for antiviral drugs have measured the inhibition of cytopathology, p24 production, or reverse transcriptase production of a laboratory strain of HIV in a lymphoblastoid cell line. Such assays cannot be readily applied to clinical isolates of HIV. The two most commonly used assays of drug susceptibility of clinical isolates have been the syncytial focus assay in CD4-HeLa cells and inhibition of p24 production in primary peripheral blood mononuclear cells (51).

The first assay has a distinct advantage in generating a monotonic sigmoid curve that is highly reproducible when focus number is plotted against the log of the concentration of drug. The assay thus permits reproducible results, quantitative susceptibilities, easy detection of spurious single values, and the detection of phenotypic mixtures (9, 14). A disadvantage of the assay, however, is that it works well with virus stocks exhibiting the syncytial inducing phenotype that, in practice, can only be obtained from a minority of specimens from seropositive individuals. The assay in peripheral blood mononuclear cells generates less precise quantitation and is expensive but has the advantage of permitting assay for most clinical isolates. No dependable assay yet described has obviated the need of generating an isolate, propagating it, and then quantitating that stock to permit an assay with a standardized inoculum.

CLINICAL SIGNIFICANCE OF DIMINISHED DRUG SUSCEPTIBILITY

The clinical importance of AZT-resistant HIV has been difficult to document for several reasons. First, the development of AZT resistance is not abrupt; it occurs slowly and progressively. The significance of different levels of susceptibility could possibly be quite different clinically. These different levels appear over periods of months to years. Recent observations with assays for the presence of mutations in clinical specimens suggest that mixtures of viruses with different resistance genotypes may be circulating simultaneously in the same individual (14, 23, 24). Moreover, clinical endpoints in HIV disease are often neither clearcut nor the immediate consequence of a change in virus replication, but rather the result of an opportunistic consequence of immunosuppression. It is thus difficult to attribute cause to a prolonged, progressive, complicated, and quantitative event like AZT resistance with an effect that is only indirectly the consequence of diminished control of virus replication. It is proving increasingly difficult to document that a new antiretroviral drug is clinically effective. It should thus be even more difficult to document loss of this efficacy with the emergence of resistance.

Studies to investigate the question of clinical significance are not easy to design. Efforts to correlate HIV drug resistance with poor outcome or virus load or poor immunologic status will inevitably be confounded. Because the rate of emergence of mutations is a function of replicative events (virus load) and because virus load is correlated with poor status and clinical progression, any correlations of resistance with virus load, syncytial inducing phenotype, or with poor immunologic status and clinical outcome should be expected to occur and thus cannot be invoked to address the issue of causality. Well-designed case-controlled studies may control for this problem; however, such studies are not simple because the generation of sufficient data to answer the

question requires large numbers of patients followed for long periods, with many properly collected specimens for the labor-intensive analysis of susceptibility. These studies will also have to control for the syncytium-inducing phenotype, which appears to be more virulent and accelerates clinical deterioration (52, 53).

The most compelling evidence for the clinical significance of HIV drug resistance to date comes from the studies documenting the loss of antiviral and CD4 cell activity with the emergence of resistance during the administration of the nonnucleoside reverse transcriptase inhibitors L-697,661 and nevirapine (46, 47).

ANIMAL MODELS

At least two important questions might be addressed with animal models: the effect of reduced susceptibility on the efficacy of drug therapy; and the impact of drug resistance mutations on virulence. One potentially informative approach is the selection of AZT-resistant mutants of feline immunodeficiency virus (FIV) (54). Resistant mutants of FIV have been readily selected, perhaps because growth and plaquing is possible in a cat monolayer cell culture system. The resistant isolates have cross-resistance to other compounds identical to the resistant isolates of HIV (9). Unfortunately, the process of passage in cat monolayer cells render FIV noninfectious for cats, requiring new approaches to mutate pathogenic virus to permit in vivo studies (T. North, personal communication). Kemp & Larder recently described the preparation of clones of simian immunodeficiency virus (SIV) containing the HIV mutations that confer AZT resistance (55). This may permit investigation of the drug-resistant mutations in the macaque model. A more recent promising animal model is *Macaca nemestrina* in which HIV-1 itself replicates for prolonged periods (56). Studies in this model with well-characterized drug-resistant mutants are warranted when the model becomes further characterized and more readily available.

CONCLUSIONS

The demonstration of AZT resistance in clinical isolates has been extended to the other antiretroviral nucleosides, ddI and ddC. Moreover, the selection for resistance to highly promising nonnucleoside reverse transcriptase inhibitors has been accomplished by in vitro passage before extended clinical use has been initiated. Although the clinical significance of antiretroviral drug resistance has not been easy to demonstrate, most investigators are proceeding under the assumption that effective chemotherapy will have to contend with resistance with regimens of multiple agents in combination.

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