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Nevirapine Resistance Mutations of Human Immunodeficiency Virus Type 1 Selected during Therapy

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Drug susceptibility and mutations in the reverse transcriptase (RT) gene were analyzed with 167 virus isolates from 38 patients treated with nevirapine, a potent nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) RT. Resistant isolates emerged quickly and uniformly in all patients administered nevirapine either as monotherapy or in combination with zidovudine (AZT). Resistance developed as early as 1 week, indicating rapid turnover of the virus population. The development of resistance was associated with the loss of antiviral drug activity as measured by CD4 lymphocyte counts and levels of HIV p24 antigen and RNA in serum. In addition to mutations at amino acid residues 103, 106, and 181 that had been identified by selection in cell culture, mutations at residues 108, 188, and 190 were also found in the patient isolates. Sequences from patient clones documented cocirculating mixtures of populations of different mutants. The most common mutation with monotherapy, tyrosine to cysteine at residue 181, was prevented from emerging by coadministration of AZT, which resulted in the selection of alternative mutations. The observations documented that, under selective drug pressure, the circulating virus population can change rapidly, and many alternative mutants can emerge, often in complex mixtures. The addition of a second RT inhibitor, AZT, significantly altered the pattern of mutations in the circulating population of HIV.

Nevirapine (BI-RG-587) potently inhibits the reverse transcriptase (RT) activity and replication of human immunodeficiency virus type 1 (HIV-1) (22). The drug binds noncompetitively to conserved tyrosines at residues 181 and 188 of the p66 subunit of the RT in a pocket largely defined by two β-sheets composed of amino acid residues 100 to 110 and 180 to 190 (18, 32, 35). Nevirapine is synergistic with zidovudine (3′-azido, 3′-deoxythymidine [AZT]) and is active against AZT-resistant mutants (26). After relatively few passages in cell culture, nevirapine readily selects for virus with greater than 100-fold reductions in susceptibility (29). This resistant virus contains a tyrosine-to-cysteine mutation at residue 181 and is cross-resistant to other nonnucleoside RT inhibitors (29).

In clinical studies, nevirapine is well tolerated, with a high level of oral bioavailability and a long half-life (5). Two phase I-phase II trials, one of nevirapine monotherapy and one of combination therapy with AZT, demonstrated reductions in the level of p24 antigen in serum and elevations in CD4 cell counts as early as 7 days, which returned to baseline values in a matter of weeks (4) after the initiation of therapy. This rapid loss of activity was associated with the emergence of nevirapine-resistant virus and corresponding mutations in the RT gene of virus isolates from all patients. Similar observations have been made with another nonnucleoside RT inhibitor, L-697,661 (30). These observations provided compelling evidence that the loss of antiviral drug activity was due to the emergence of drug resistance. Evaluation of higher doses of nevirapine suggested that higher drug concentrations in plasma could produce sustained reductions of p24 antigen and HIV RNA in serum for 6 months or longer, perhaps as a result of achieving drug levels that exceeded the susceptibility of drug-resistant virus (14).

In this report, the phenotypic and genotypic characteristics of 167 virus isolates from 38 patients treated with nevirapine are described. The different patterns of mutation that appeared in isolates from patients with nevirapine monotherapy or with concomitant AZT therapy are also analyzed.

MATERIALS AND METHODS

Study subjects. Sequential cohorts of HIV-infected patients received daily doses of 12.5, 50, 200, or 400 mg of nevirapine either as monotherapy (AIDS Clinical Trials Group study 164) or the three lowest doses in combination with AZT (200 mg every 8 h [AIDS Clinical Trials Group study 168]). The protocol design, patient characteristics, and therapeutic responses have been reported previously (4, 14). The data reported here describe isolates from 33 patients from the University of California, San Diego, and 5 patients from the University of Massachusetts, Worcester, enrolled in these studies.

Virology. Virus stocks from 167 isolates of HIV-1 were prepared by cocultivation of peripheral blood mononuclear cells (PBMCs) of 38 patients with stimulated PBMCs from seronegative donors (27). Susceptibility assays only were performed with 42 isolates, sequencing only was performed with 15 isolates, and both were performed with 110 isolates. Assays for susceptibility to nevirapine and AZT were performed with CD4 cells expressing HeLa (HT4-6C) cell monolayers for

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syncytium-inducing isolates and by p24 inhibition in PBMC microtiter cultures for non-syncytium-inducing isolates (27). Fifty percent inhibitory concentrations (IC_{50}s) determined for 13 syncytium-inducing isolates evaluated with both susceptibility assays did not differ significantly. The IC_{50}s of pretreatment isolates ranged from <0.01 to 0.075 μM. Reduced susceptibility (resistance) was defined as an IC_{50} of >0.1 μM.

RNA extraction, reverse transcription, PCR amplification, and direct population sequencing of the RT gene. RNA was extracted from the supernatants of low-passage PBMC cocultures with phenol-chloroform. Genetic sequencing of viral RNA was performed to directly correlate phenotypic and genotypic changes. Reverse transcription and PCR amplification of RNA were performed with 0.2 mM (each) deoxynucleoside triphosphate (dNTP), PCR buffer (consisting of 50 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$, and 0.01% gelatin), and 0.25 μg of HIV-specific oligomers RT 1 (5'-GGA AGA AAT CTG TTG ACT CAG ATT GGT-3') and RT 2 (5'-ACC CAT CCA AAG GAA TGG AGG TTC TTT C-3'). The oligonucleotide primers were purified by reverse-phase high-performance liquid chromatography with a C_{18} column (Genosys, Woodlands, Tex.). The samples were incubated for 90 s at 65°C and then were incubated for 60 s at 42°C. One hundred units of Moloney murine leukemia virus RT (GIBCO BRL) was added, and incubation continued at 42°C for 30 min. After reverse transcription, 2.5 U of native *Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn.) was added, and amplification was carried out in a thermal cycler (Perkin-Elmer Corp.) for 1 cycle at 94°C for 5 min and then for 35 cycles with the following parameters: denaturation for 20 s at 94°C, hybridization for 60 s at 60°C, and extension for 60 s at 72°C with a final extension of 12 min. The PCR product obtained was applied onto a 3% low-melting-point agarose gel. After electrophoresis, the band (752 bp) was excised and purified with the Magic PCR Prep kit (Promega). The direct population sequencing was carried out with 1 μg of target DNA in sequencing buffer (50 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM $MgCl_2$, and 0.01% gelatin) with 62.5 μM (each) dNTPs, 3.3 μM [^{32}P]dCTP (specific activity, 3,000 Ci/mmol [10 mCi/ml]) and one of the following dideoxynucleoside triphosphates (ddNTPs) (100 μM ddG, 300 μM ddA, 300 μM ddT, or 500 μM ddC). Native *Taq* DNA polymerase (2.5 U) and RT primer 2 were added at 0.5 μg per reaction mixture, and the amplification was carried out as described previously. Three microliters of each reaction mixture was applied to a 6% acrylamide sequencing gel. The gel was dried and subjected to autoradiography (Kodak X-Omat).

Sequencing of cloned DNA. DNA was extracted with phenol-chloroform from the first or second passage of cocultivation in PBMCs from patient 453. Total nucleic acids containing HIV-1 sequences were amplified with the 5' primer CKS 116 (5'-CUACUACUACUAGGAATTGGAGGTTTTATCAAA G-3') and the 3' primer CKS 117 (5'-CAUCAUCAUCAU CTACTTGTCCATGCATGGCTT-3'). These primers span the entire coding region of HIV-1 RT protein, including the RNase H domain (from nucleotides 1755 to 3751 of HIV-1 BH10). Amplifications were performed for 40 cycles in a Perkin-Elmer thermal cycler with the following parameters: initial denaturation for 4.5 min at 95°C, followed by 40 cycles consisting of a denaturation step at 96°C for 1 min, a 90-s annealing step at 55°C, and primer extension at 70°C for 3.5 min, with a 5-s increase in extension time per cycle. After completion of the extensions, the amplifications were incubated at 72°C for an additional 10 min and then were held at 4°C.

Products of amplification reactions were separated on agarose gels and purified with a GeneClean kit as specified by the manufacturer (Bio 101, Inc.)

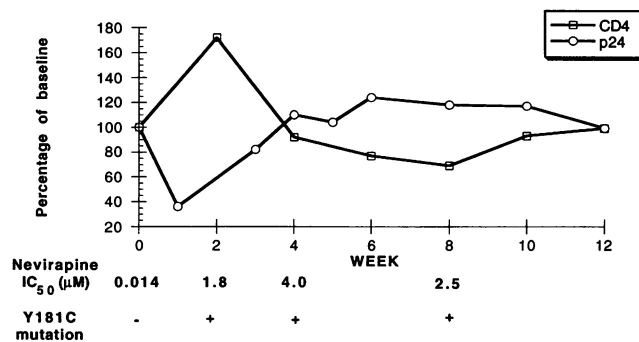

FIG. 1. Effect of nevirapine therapy on surrogate markers and drug resistance. Patient 154 was administered 12.5 mg of nevirapine daily. The responses at the indicated weeks with regard to CD4 lymphocyte count, HIV p24 antigen level in serum, nevirapine susceptibility, and the Y181C resistance mutation of the patient's virus isolate are depicted.

Purified PCR products with uracil-containing primers were treated with uracil DNA glycosylase in the presence of vector DNA containing complementary ends (pAMP 1; Bethesda Research Laboratories, Inc.) for 30 min at 30°C in PCR buffer. Aliquots of these reaction mixtures were then used to transform competent *Escherichia coli* DH5α directly by the protocol obtained from the supplier (Bethesda Research Laboratories, Inc.).

Plasmid DNA containing HIV-1 RT sequences was purified by Qiagen columns from bacterial lysates according to the procedure supplied by the manufacturer. Sequencing of the cloned sequences was performed with *Taq* DyeDeoxy terminator sequencing kits (Applied Biosystems, Inc.) as specified by the manufacturer. Sequencing primers were situated every 200 to 300 bases apart on both strands. Sequencing reactions were purified by Sephadex G-50 spin columns (Boehringer-Mannheim, Quick-Spin), dried under vacuum, and electrophoresed in denaturing polyacrylamide gels in an Applied Biosystems model 373A automated sequencing machine. Data were analyzed with Seqed 373A v1.0.3 software (Applied Biosystems, Inc.).

Statistical analysis. The deduced amino acid sequences were subdivided between those of patients receiving nevirapine alone and those of patients receiving nevirapine in combination with AZT. Baseline amino acid consensus sequences were obtained for both groups prior to initiation of therapy. The posttherapy sequences (subdivided by isolates obtained between weeks 1 through 6, weeks 8 to 12, and more than 13 weeks after initiation of therapy) were aligned with a multiple alignment with hierarchical display and compared with their respective baseline sequences, using the Fisher exact test (7).

RESULTS

Emergence of nevirapine resistance. Treatment of patients with nevirapine rapidly selected for resistant isolates of HIV-1. As exemplified by a representative patient who received the lowest daily dose (12.5 mg) of nevirapine monotherapy (Fig. 1), concentrations of p24 antigen in serum diminished and numbers of circulating CD4 T lymphocytes increased within 7 days of initiating therapy. HIV RNA levels in serum, determined with the Chiron branched-chain assay, paralleled the p24 antigen results (data not shown). These encouraging

TABLE 1. Emergence of isolates of HIV with reduced susceptibility and resistance mutations with nevirapine therapy[a]

Wk of therapy	Cumulative proportion with:		
	Reduced susceptibility	Known resistance mutation[b]	Either
1	3/3		3/3
2	12/14	6/8	12/15
4	23/26	18/21	24/26
8	32/32	30/30	33/33
≥12	38/38	38/38	38/38

[a] Table combines results from 24 patients receiving nevirapine monotherapy and 14 patients receiving combination therapy with AZT.
[b] Mutations identified: K103N, V106A, V108I, Y181C, Y181S, Y188L, Y188H, Y188D, G190A, G190S, G190L. The effects of some allelic variations (i.e., 188D, 190S, and 190L) on the susceptibility to nevirapine have not been fully characterized by site-directed mutagenesis.

indicators of antiretroviral drug activity promptly reverted towards the baseline within 4 weeks, with the emergence of virus that was over 100-fold less susceptible to nevirapine. Viral isolates from this patient contained a tyrosine-to-cysteine mutation at residue 181 (Y181C).

Isolates with greater than 100-fold reductions in nevirapine susceptibility uniformly emerged within 8 weeks of therapy in all patients and at all doses tested (Table 1). Of the 96 resistant isolates, all but 4 had an IC_{50} of >0.5 μM, and all but 9 had an IC_{50} of >1.0 μM. These isolates with lower levels of resistance were seen in the first 8 weeks of treatment. Mutations that have been demonstrated by site-directed mutagenesis to confer resistance were identified in all patients examined, concordant with the development of the resistant phenotype. Resistance developed quickly whether patients received nevirapine monotherapy (24 patients) or combination therapy with AZT (14 patients). Of note, three patients who discontinued nevirapine therapy retained their resistant isolates for at least 14 weeks (data not shown). Mutations in at least six residues have been shown to reduce the susceptibility of HIV to nevirapine (Table 1) (1–3, 19, 23, 29, 31). In fact, one of these, G190A, had not been observed after in vitro selection with any nonnucleoside RT inhibitor but was confirmed by mutagenesis following its repeated identification in these patients (1). Of note, mutations at residues 98, 100, 101, and 236 which confer resistance to other nonnucleoside RT inhibitors were not observed in any of the sequences from patients treated with nevirapine (3, 9).

Genotypic mixtures. Although genetic heterogeneity within individuals infected with HIV-1 is well documented, limited genetic variation in this region of the RT gene was also seen in patient isolates obtained before therapy by direct population sequencing with asymmetric PCR. In contrast, mixtures of wild-type and mutant virus and mixtures of different mutants were frequently seen after the initiation of therapy. Of note, no known mutations conferring resistance to nonnucleoside RT inhibitors were detected in baseline specimens.

For example, patient 453, with only 17 CD4 cells per mm^3 and a history of 52 weeks of AZT therapy prior to the addition of 12.5 mg of nevirapine daily, had a baseline RT sequence that already included two AZT resistance mutations, M41L and T215Y (17, 21). After a single passage of virus isolated from this patient in stimulated PBMCs, the RT sequence was obtained both from the RNA population in the cell supernatant by asymmetric RT PCR and from four clones from independent PCRs from the cell pellet DNA (Table 2). A number of observations could be made. First, with only a few

TABLE 2. Amino acid sequences deduced from nucleotide sequencing of portions of the RT gene of sequential isolates of HIV-1 from patient 453 treated with AZT and nevirapine[a]

Isolate	Amino acid residue															
	41	43	44	67	70	75	103	106	135	181	188	190	210	215	219	254
Consensus amino acid	M	K	E	D	K	V	K	V	I	Y	Y	G	L	T	K	V
Wk 0																
Clone 1	L	—	—	—	—	—	—	—	T	—	—	—	W	Y	—	—
Clone 2	L	—	—	—	—	—	—	—	T	—	—	—	W	Y	—	—
Clone 3	L	Q	—	—	—	—	—	—	T	—	—	—	W	Y	—	—
Clone 4	L	Q	—	—	—	—	—	—	T	—	—	—	W	Y	—	—
Population sequence	L	Q/K[b]	—	—	—	—	—	—	T	—	—	—	W	Y	—	
Wk 4 population sequence	L	Q	—	—	—	—	N	—	T	—	—	—	W	Y	—	
Wk 16																
Clone 1	L	Q	—	—	—	—	—	A	T	—	—	—	W	Y	—	—
Clone 2	L	Q	—	—	—	—	N	—	T	—	—	—	W	Y	—	—
Clone 3	L	Q	—	—	—	—	—	A	T	—	—	—	W	Y	—	—
Clone 4	L	Q	—	—	—	—	—	A	T	—	—	—	W	Y	T	—
Population sequence	L	Q	—	—	—	—	—	V/A[b]	T	—	—	—	W	Y	—	
Wk 32																
Clone 1	L	Q	—	—	—	—	—	—	T	—	—	A	W	Y	—	G
Clone 2	L	—	D	N	—	M	N	—	T	—	—	A	W	Y	—	—
Clone 3	L	—	D	N	—	M	N	—	T	—	—	A	W	Y	—	—
Clone 4	L	—	D	N	—	M	N	—	T	—	—	A	W	Y	—	—
Population sequence	L	Q	—	—	—	—	—	—	T	—	—	A	W	Y	—	

[a] Amino acid abbreviations: A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; —, consensus amino acid.
[b] Mixture.

TABLE 3. Effect of concomitant therapy with AZT on the pattern of nevirapine resistance mutations

Treatment	No. of patients examined	% of patients with isolates developing mutations at the indicated residue of RT:					
		103	106	108	181	188	190
Nevirapine monotherapy	24	33	0	8	79	8	17
Nevirapine plus AZT	14	57	14	0	0	50	50

exceptions, the population sequence for the supernatant RNA reflected the sequences of clones in the cell pellet DNA. Second, even with the baseline specimen, a swarm or genetic mixture of virus was isolated from the patient, as indicated by residue 43. Third, a nevirapine resistance mutation (K103N) emerged within only 4 weeks of nevirapine therapy. Fourth, at 16 weeks, two different populations of nevirapine-resistant virus were cocirculating (K103N and V106A). Fifth, at 32 weeks, nevirapine-resistant virus with a third mutation had emerged (G190A) in the K103N background sequence but not in the V106A portion of the population. The G190A mutation also appeared in the previously circulating virus with neither the K103N nor the V106A mutation. The population containing both the K103N and G190A mutations was observed on a background of signature amino acids at residues 44, 67, and 75 distinct from any viruses previously sequenced. Of note, D67N represents an additional AZT resistance mutation to M41L and T215Y (21). Sixth, drug susceptibility values for both nevirapine and AZT generated with independent isolates at each time point showed a much greater variability than usually found (data not shown). These genetic sequence data would explain this great phenotypic variability.

Effect of concomitant AZT therapy on the pattern of nevirapine mutations. Although concomitant AZT therapy did not delay the emergence of isolates of HIV with reduced susceptibility to nevirapine, the mutations responsible for this resistance were affected (Table 3). The nevirapine resistance mutations present in the last sequenced isolate that was examined for each patient were tabulated because transient appearances and disappearances of mutations (as in patient 453, Table 2) were common. The mean durations of therapy for this isolate were 20 weeks for the monotherapy patients and 32 weeks for the combination therapy patients. The tyrosine-to-cysteine mutation at residue 181 was most common with nevirapine monotherapy (Table 3). Concomitant therapy with AZT appeared to prevent the emergence of the Y181C mutation and increased the appearance of mutations at residues 103, 106, 188, and 190. No Y181C mutation was seen in any of the 44 isolates from 14 patients with combined therapy, while 30 of 58 isolates from the 24 patients receiving monotherapy contained Y181C (Fisher exact test, $P < 10^{-11}$) (Fig. 2A). With combination therapy, one isolate mixture had a Y181Y-Y181S mixture and one had a Y181Y-Y181H mixture. In addition, nine isolates with monotherapy had Y181Y-Y181C mixtures, and four had mixtures of Y181Y and Y181S. Subset analysis of the four monotherapy doses and three combination therapy doses of nevirapine generated group sizes too small to discern statistically meaningful differences in mutations in relation to drug dose.

Shift of mutations over time. Examination of nevirapine resistance mutations in sequential virus isolates demonstrated shifting patterns of mutations over time as exemplified by patient 453 in Table 2. Figure 2 depicts the probability that a different amino acid would emerge at a defined position compared with the background sequences of the RT with monotherapy (Fig. 2A) or with combination therapy (Fig. 2B) by the Fisher exact test. This analysis compared the sequences from all isolates after the initiation of treatment with pretreatment sequences. Several points can be made. First, mutations (or alternative amino acid utilization) occur frequently throughout the RT. Second, drug-related sequence variation occurs, as supported by two lines of evidence. (i) Mutations emerge at residues that have been proven by site-directed mutagenesis to confer changes in drug susceptibility (e.g., 103, 106, 108, 181, 188, 190). (ii) Changes occur at residues in a sufficient number of patients to be unlikely statistically to have emerged without the selective pressure of drug therapy. Statistically significant probability requires either a very frequent occurrence (e.g., Y181C) or very large numbers of patients and isolates. Such analysis may not detect mutations that are less frequent but biologically significant (e.g., changes at residues 106 and 108). Figure 2 suggests that the probability of a residue 181 mutation may diminish slightly with time, while mutations at residue 188 may become more probable.

Effect of concomitant therapy on the emergence of AZT resistance mutations. Although all patients on the combination protocol evaluated had received between 3 and 117 weeks of AZT therapy, 6 of 14 had baseline isolates with no recognized AZT resistance mutations (17, 21). Such mutations appeared at week 2 in one patient (T215Y), week 4 in one patient (T215F), week 28 in one patient (D67N, K70R, T215F, and K219Q), week 32 in one patient (M41L and T215Y), and week 40 in one patient (T215Y). No AZT mutations were detected in one patient for at least 56 weeks of concomitant therapy. In these six patients, nevirapine did not appear to significantly delay the appearance of AZT resistance mutations.

DISCUSSION

In patients administered nevirapine monotherapy or combination therapy, reduced drug susceptibility and mutations associated with it appeared quickly and uniformly. These phenotypic and genotypic changes with the nonnucleoside RT inhibitors nevirapine and L-697,661 were associated with the loss of drug activity, as measured by CD4 lymphocyte counts or levels of HIV p24 antigen and RNA in serum, indicating the clinical significance of these changes (4, 14, 30).

The speed of the appearance of these changes was remarkable. The viral isolates from three patients tested after receiving 1 week of nevirapine therapy all had altered phenotypes and genotypes. This observation is consistent with the concept that HIV infection is a dynamic infectious process with high levels of virus replication (10, 24, 25). The introduction of the selective genetic pressure of a potent drug thus resulted in a dramatic turnover of this large viral population after only a week of drug exposure.

The fitness of this genotypically altered virus selected by drug pressure is indicated by the observation that three patients who were withdrawn from nevirapine therapy retained their drug-resistant virus for at least 14 weeks. Thus, the wild-type phenotype and genotype did not quickly reemerge after the withdrawal of the selective pressure of nevirapine therapy, similar to in vitro observations (29). In addition, the pathogenicity of the altered virus was evident by the immunologic and clinical deterioration that occurred in patients with resistant virus (4).

Although reversion to wild-type virus did not readily occur

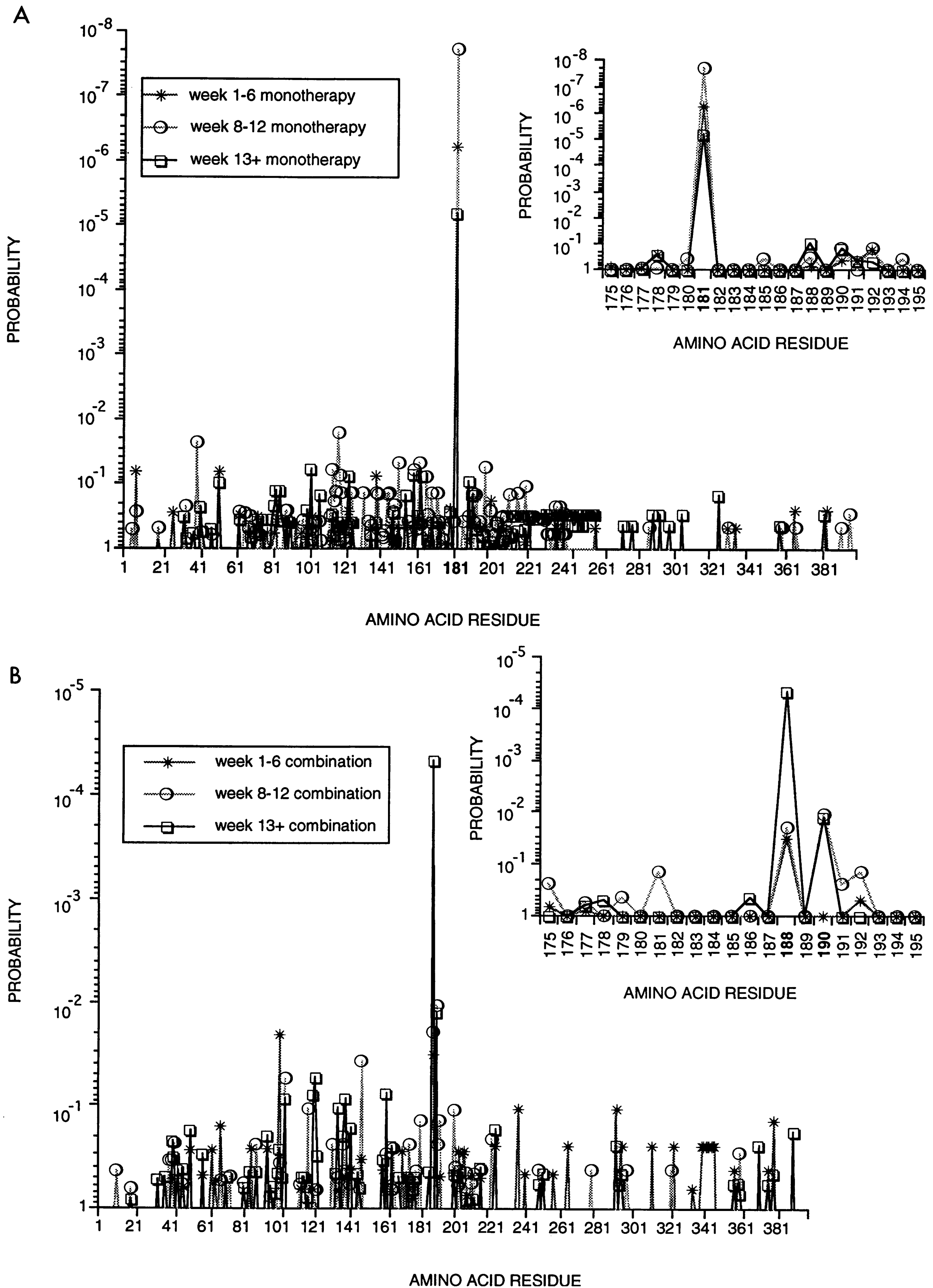

FIG. 2. Probability of changes in amino acid position of HIV-1 RT after therapy. The sequences of 98 isolates obtained after the initiation of therapy were compared with those of 28 pretherapy isolates. The probability of a different amino acid appearing at each residue was calculated by using the Fisher exact test. (A) AIDS Clinical Trials Group 164, monotherapy patients. (B) AIDS Clinical Trials Group 168, combined AZT-nevirapine patients.

after drug discontinuation, the virus population was not static after the first mutant appeared. The virus population evolved under selective drug pressure. The data presented here indicated that populations or genetic variants were in constant flux, with the frequent appearance of additional and different mutations. Examination of the sequences from various patients suggested that the greatest number of mixtures and the highest frequency of new mutations occurred within the first 12 weeks of therapy, after which time a more stable population appeared to establish itself. This observation is consistent with general principles of RNA virus population genetics in which a master sequence (the most fit member of a mutant spectrum) emerges under selective pressures to generate a new consensus sequence (15). Figure 2 also indicates the high basal rate of genetic variation in this region of the HIV genome that must be considered when interpreting mutations relevant to drug resistance.

HIV appears to utilize Y181C as the preferred mutation in the face of nevirapine monotherapy. This preference may be based on the magnitude of change in susceptibility, the ease of acquisition of this mutation, and the apparent lack of impairment of RT enzyme activities that results from this mutation. However, concomitant AZT therapy clearly changes the virus' evolutionary options. The addition of the selective pressures of concomitant AZT therapy to nevirapine increased the relative advantage of alternative mutations. The basis for the disadvantage of Y181C probably relates to the observation that Y181C resensitizes HIV that contains the AZT resistance mutation at residue 215 to AZT (19). Even a slight diminution in fitness has been calculated to confer significantly diminished survival advantage in a frequently replicating population (6). In viral isolates from patients receiving AZT, other nevirapine mutations emerged that do not impair viral resistance to AZT (Table 3). Mutations at residues 181 and 215 are not incompatible, however. Isolates containing Y181C mutations were obtained from six patients on nevirapine monotherapy who had preexisting T215Y mutations (data not shown). In fact, one of these patients (patient 166) who had both the M41L and T215Y AZT mutations and the L74V ddI resistance mutation (34) acquired, in addition, the Y181C mutation within 2 weeks of nevirapine monotherapy, demonstrating the viability of virus resistance mutations to three drugs. The influence of L74V on the coexistence of Y181C with M41L and T215Y is not known, although selection for triply resistant virus in vitro has been described (11, 20).

The failure of combined antiretroviral therapy to prevent the emergence of resistance should not be surprising. The traditional rationale for combined chemotherapy is to prevent the emergence of resistant mutants; however, this approach succeeds only when the combination regimen effectively suppresses replication. The RT inhibitors have all been shown to reduce markers of virus replication by about 90% (4, 8, 25, 33). With levels of HIV RNA in the plasma alone at 10^4 to 10^7 copies per ml (25) and with enormous quantities of virus in lymphoid tissues of even asymptomatic patients (10, 24), significant levels of replication persist in the presence of current chemotherapy with RT inhibitors, thus providing the opportunity for emergence of drug-resistant mutants. In fact, under conditions that permit high levels of continued virus replication in vitro, mutants resistant to multiple antiretroviral drugs readily emerge (2, 11, 19, 20). Similar in vivo observations have been made with the combination of AZT and ddC (28). The potential advantage of combination therapy with our current drugs must thus be based on the additive or synergistic activity of the compounds either in the same cells (12, 16, 26) or in different populations of host cells (13) to provide a beneficial activity not based on a delay in the emergence of drug resistance.

The study of viral drug resistance in clinical isolates exemplifies principles of viral evolution and population genetics (6, 15). The question arises as to whether we can exploit these observations to design more effective drugs and strategies of drug administration.

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