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Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for the loss of human immunodeficiency virus type 1 RNA load suppression by zidovudine

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ABSTRACT **The association between human immunodeficiency virus type 1 (HIV-1) RNA load changes and the emergence of resistant virus variants was investigated in 24 HIV-1-infected asymptomatic persons during 2 years of treatment with zidovudine by sequentially measuring serum HIV-1 RNA load and the relative amounts of HIV-1 RNA containing mutations at reverse transcriptase (RT) codons 70 (K → R), 41 (M → L), and 215 (T → Y/F). A mean maximum decline in RNA load occurred during the first month, followed by a resurgence between 1 and 3 months, which appeared independent of drug-resistance. Mathematical modeling suggests that this resurgence is caused by host–parasite dynamics, and thus reflects infection of the transiently increased numbers of $CD4^+$ lymphocytes. Between 3 and 6 months of treatment, the RNA load returned to baseline values, which was associated with the emergence of virus containing a single lysine to arginine amino acid change at RT codon 70, only conferring an 8-fold reduction in susceptibility. Despite the relative loss of RNA load suppression, selection toward mutations at RT codons 215 and 41 continued. Identical patterns were observed in the mathematical model. While host–parasite dynamics and outgrowth of low-level resistant virus thus appear responsible for the loss of HIV-1 RNA load suppression, zidovudine continues to select for alternative mutations, conferring increasing levels of resistance.**

Zidovudine is a nucleoside analogue inhibitor of human immunodeficiency virus (HIV) reverse transcriptase (RT) and forms the mainstay of current antiretroviral treatment strategies. The emergence of HIV type 1 (HIV-1) isolates with reduced susceptibility to zidovudine during treatment is well established (1, 2). Reductions in zidovudine susceptibility are caused by specific amino acid changes in the HIV-1 RT gene that accumulate in an ordered fashion, with a K70R change appearing first but transiently followed by the cumulative acquisition of mutations at codons 215 (T → Y/F), 41 (M → L), 67 (D → N), and 219 (K → Q), respectively, and the reappearance of the codon 70 change (3–5).

Employing quantitative nucleic acid-based amplification techniques to measure viral RNA load in serum or plasma, it became clear that the appearance of drug-resistant virus is associated with a loss of suppression of viral replication by the RT inhibitors nevirapine and 3TC (6, 7). For zidovudine, however, such a relationship between resistance development and HIV-1 RNA load changes has never been demonstrated. Loveday *et al.* (8) reported a resurgence of serum HIV-1 RNA load during the first weeks of zidovudine treatment, which could not be explained by the appearance of resistant viral genotypes. However, a return to pretreatment values was generally not observed within this study, probably because of the relatively short treatment periods studied.

We here investigate whether the ultimate loss of inhibition of viral replication by zidovudine is associated with the appearance of resistant virus variants. Serum HIV-1 RNA load and the relative amounts of HIV-1 RNA containing mutations at RT codons 41, 70, and 215 were assessed sequentially during a 2-year period of zidovudine treatment in 24 previously untreated HIV-1 infected individuals. A mathematical model was developed to explain resistance-independent viral kinetics, as well as differences in viral kinetics between individuals.

MATERIALS AND METHODS

Study Population. Twenty-four Dutch HIV-1-infected homosexual men were selected from two previously described zidovudine efficacy studies (9, 10). Because the syncytium-inducing capacity of HIV-1 seems to influence the response to zidovudine treatment and the rate of development of drug resistance (11, 12), only subjects solely harboring MT2-negative isolates during the course of the study were selected for the purpose of this study. All subjects were asymptomatic or had persistent generalized lymphadenopathy at study entry. The median $CD4^+$ lymphocyte count at baseline was 385 cells/mm^3 (range, 190–710). All subjects were treated with zidovudine at a dosage of 1000 mg/day, following a dose of 2000 mg during the first 4 weeks in four individuals. Mean corpuscular volume values showed an immediate and sustained increase in virtually all subjects, indicating proper compliance to therapy. The dose of zidovudine was lowered to 400–750 mg/day after 27–81 weeks (median, 54 weeks) in six subjects because of side effects. Five individuals admitted irregular intake of zidovudine during the study period, while four of these individuals did not take any medication for extended periods of time (weeks 0–16, 75–115, 97–126, and 110–130 respectively).

Laboratory Evaluation. HIV-1 RNA load was measured in stored serum (−70°C), obtained at baseline, after 1, 3, 6, and 9 months, and after ≈1 and 2 years of treatment. Relative amounts of serum HIV-1 RNA containing mutations at RT positions 41 (M → L), 70 (K → R), and 215 (T → Y/F) were assessed sequentially in reversed order (i.e., starting at 2 years) at the same time points; when two successive assessments showed <5% mutant HIV-1 RNA, no further assessments were performed. In four subjects, direct population sequenc-

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Abbreviations: HIV, human immunodeficiency virus; RT, reverse transcriptase; PMA, point mutation assay.

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ing in serum of the HIV-1 RT gene was performed at baseline and after 3 months of treatment. $CD4^+$ cell counts were measured at every visit (13).

HIV-1 RNA Quantitation. RNA was extracted from 100 μl of serum as described previously and quantified in a previously reported prototype single-tube RT-PCR assay (14, 15).

Relative Amounts of Wild-Type and Variant HIV-1 RNA. The relative amounts of HIV-1 RNA containing RT mutations at positions 41, 70, and 215 were determined using a point mutation assay (PMA) (16). Purified HIV-RNA, obtained from the same extraction as used in the quantitation assay, was either reverse transcribed by using oligonucleotide primer NE1–35 (5′-CCT ACT AAC TTC TGT ATG TCA TTG ACA GTC CAG CT-3′), followed by PCR amplification using primers NE1–35 and RT18 (5′-GGA AAC CAA AAA TGA TAG GGG GAA TTG GAG G-3′), or reverse transcribed and amplified in a single-tube reaction as described (17). This was followed by a second nested amplification by using primers K-RT (5′-CAG GAT GGA GTT CAT AAC-3′) and biotinylated H-34 (5′-biotin CTT CAG GAA GTA TAC TGC ATT TAC CAT ACC TAG T-3′) for the 215 PMA, and primers L34-RT (5′-CCC TGT GGA AGC ACA TTG TAC TGA TAT CTA ATC C-3′) and biotinylated A-35 (5′-biotin TTG GTT GCA CTT TAA ATT TTC CCA TTA GTC CTA TT-3′) for the 41 and 70 PMAs. The PMA was performed as described (16) by using antisense probes pma 41 (5′-AAT TTT CCC TTC CTT TTC CA-3′), pma 70 (5′-TAC CTC TTT TAA TCA TC-3′), and pma 215 (5′-CTG ATG TTT TTT GTC TGG TGT G-3′). Scintillation counts were corrected for differences in incorporation efficiency between each of the four nucleotides by using control samples containing either 100% wild-type or 100% variant amplification products. The performance of the assay was assessed in each experiment by using a mixture of 50% wild-type and 50% variant amplification products in the 70 and 215 PMAs, and a mixture of 50% wild-type, 25% 41 TTG variant, and 25% 41 CTG variant amplification products in the 41 PMA. The lower limit of detection of a virus subpopulation is $\approx$5%.

Direct Population Sequencing RT Gene from Serum HIV-1 RNA. Direct sequencing of serum RNA was performed by using the same preamplification products as used for the PMA. Two separate nested PCR amplifications were performed by using primer pairs RT19 (5′-GGA CAT AAA GCT ATA GGT ACA G-3′) and biotinylated K-RT, and biotinylated RT19 and K-RT. After purification of single-stranded DNA (Dynabeads M-280; Dynal, Oslo), sequencing was done using Sequenase 2.0 (United States Biochemical), with modifications as described previously (18), and sequencing primers K-RT, BR-RT (5′-GGT GAT CCT TTC CAT CC-3′), RT11 (5′-TAT GTA GGA TCT GAC TTA G-3′), and RT19, which enabled sequence analysis of the first 225 residues of RT.

Analysis. Serum HIV-1 RNA load was analyzed after log transformation of absolute copy numbers. Mean changes from baseline in total RNA load, 70 wild-type RNA load, and $CD4^+$ cell counts were analyzed by paired two-tailed *t* tests.

Host–Parasite Model. A mathematical model was designed to explain resistance-independent viral kinetics and differences in viral kinetics between subjects. General assumptions in this model are that before treatment, an equilibrium exists between HIV infection and $CD4^+$ cell renewal, and that, in the short term, the virus load is largely limited by the availability of infectable $CD4^+$ cells (19). Although the immune system may play a role in down-regulating viral load, we assume that the immune response remained relatively constant during the time-course of our experiments. Similar models based on identical assumptions have been employed before by McLean and coworkers (20–22) to study similar, but less detailed data. We have extended these models to allow for a more realistic model of T-cell renewal and HIV-1 turnover.

In our model, we only consider cycling T cells—i.e., we ignore a contribution of the thymus to the adult T cell repertoire. We distinguish resting T cells (T) and activated T cells (T^*). Resting cells become activated by low-level activation and may subsequently proliferate, giving rise to maximally two daughter cells. For simplicity, we assume that the daughter cells require reactivation before they proliferate again.

HIV infection is implemented by considering virus particles V_j and productively infected T cells T_j for each virus strain j. We assume that HIV establishes productive infection in activated T cells only (23). Productively infected T cells produce new virus particles and turn over rapidly (24, 25). HIV can thus be considered as a parasite killing the CD4 host population.

Mathematically, we write that the resting T cells are activated by low level activation at a rate α, they die at a rate d_T, and they appear by proliferation of activated T cells at a maximum rate β:

$$\frac{dT}{dt} = \frac{2\beta\, T^*}{1 + T_{tot}/T_{max}} - (\alpha + d_T)\, T,$$

where the $1/(1+ T_{tot}/T_{max})$ term defines a density dependent regulation of the proliferation rate. The activated T cells (T^*) appear by activation of resting cells, they revert to the resting stage at a rate β, and they are infected by all virus strains at a rate γ weighted by the total RT fitness f_j of the strain—i.e., we write:

$$\frac{dT^*}{dt} = \alpha T - \beta T^* - \gamma\, T^* \sum_j f_j V_j.$$

The fitness f_j of virus strain j combines the RT efficiency in the absence of the drug with the level of drug-resistance in its presence. We scale the RT efficiency of wild-type virus to 1—i.e., we set $e_{\mathrm{wt}} = 1$. All mutant strains should therefore have $e_j < 1$. For the fitness in the presence of the drug we employ 50% inhibitory concentrations (IC_{50}). Thus we write:

$$f_j = \frac{e_j}{1 + D/R_j},$$

where D is the effective drug concentration, and R_j is the IC_{50} of strain j. We again scale $R_{\mathrm{wt}} = 1$, and require for all mutants j that $R_j > 1$.

The productively infected T cells appear by infection of activated T cells by virus. Because infection may involve mutation secondary to a RT error, we implement a mutation matrix (M), which incorporates the estimated mutation frequencies. Although mutation is stochastic, we model it as a continuous process, which implies that mutants are present at low prevalences before treatment (19). Matrix element M_{jl} is the mutation frequency by which strain j appears from strain l. Thus the diagonal elements M_{jj} give the frequency that strain j correctly transcribes itself. Hence we write:

$$\frac{dI_j}{dt} = \gamma\, T^* \sum_l M_{jl} f_l V_l - d_I I_j,$$

where d_I is the turnover rate of infected cells.

Thus the total number of T cells in our model is defined by:

$$T_{tot} = T + T^* + \sum_j I_j.$$

We complete the model by assuming that the virus density is proportional to the density of infected T cells. By appropriate scaling we write $V_j = I_j$, such that we only have differential equations for the T cell variables T, T^*, and I_j.

We let the model describe 1 μl of blood. Whenever possible the parameter setting is based upon published estimates; a full

account will be provided elsewhere (N.I.S., C.A.B.B., M.D.d.J., R. van Leeuwen, R.S., and R.J.d.B., unpublished data). In the latency phase 4% of resting T cells get activated—i.e., $\alpha = 0.04$/day; the healthy CD4 T-cell count is ≈1000 cells, i.e. $T_{max} = 1100$ cells; resting T cells are long-lived (26)—i.e., $d_T = 0.001$/day; activated T cells rapidly revert to the resting stage—i.e., $\beta = 1$/day; the infection rate is arbitrarily set to $\gamma = 0.05$ cells per scaled particle per day; the life-time of infected T cells is 2 days (24, 25)—i.e., $d_I = 0.5$. Setting $\alpha = 0.04$, $d_T = 0.001$, and $\beta = 1$, we obtain exactly the same rates of CD4 cell recovery as were recently reported (24, 25).

The drug resistance of the mutant strains have previously been been estimated to be $R_{41} = 4$, $R_{70} = 8$, $R_{215} = 16$, $R_{41/70} = 9$, $R_{41/215} = 60$, and $R_{70/215} = 6$ (4, 27). We conservatively set the fitness in the absence of the drug to $e_{215} = e_{41/215} = e_{70/215} = 0.99$ for strains involving the 215 mutation. For the other strains we set $e_{41} = e_{70} = e_{41/70} = 0.95$ to prevent a too early appearance of the first mutations in our model (N.I.S. *et al*. unpublished data). The mutation frequencies are estimated by the specific nucleotide changes they involve. While it is well established that G to A changes are preferred over other changes, it appears that transitions are more likely to occur than transversions (28). We therefore set the mutation rate of G to A changes μ_1 to 3×10^{-5} (i.e., the basic error rate of RT *in vivo*) (28), A to G changes to $\mu_1/2$, and other changes to $\mu_1/4$. The rate of double nucleotide mutations is the product of the two particular single nucleotide rates involved. Because RT is 1680 bases long, the probability that no mutations occur is $M_{jj} = \mu_0 = (1 - \mu_1)^{1680} = 0.95$.

RESULTS

CD4$^+$ Lymphocyte Counts. The mean CD4$^+$ cell count increased significantly during the first month of treatment, but returned toward baseline values during subsequent months (Fig. 1*A*). The initial changes in CD4$^+$ cell count mirrored the changes in serum HIV-1 RNA load.

Serum HIV-1 RNA load. Serum HIV-1 RNA was detectable in all subjects at all time points with a mean level at baseline of 4.7 log copies/ml (±0.43) (Fig. 1*B*). A maximum decline of 0.6 log after 1 month was followed by increases during subsequent months. Although still significantly below baseline values after 3 months, mean RNA load reached baseline values within 6 months of treatment.

Relative Amounts of 41, 70, and 215 Mutant Serum HIV-1 RNA. The percentage of 70 mutant RNA could not be measured in one subject, possibly due to sequence variation of HIV-1 RT at the annealing site of the probe. In the remaining 23 subjects, the codon 70 change generally was the first to appear (Fig. 1*C*). After 2 years, the median percentage of 70 mutant RNA had decreased to <5% with a concurrent increase of the percentage of T215Y mutant RNA. The T215F change was not observed within this study. Although appearing in 11 of 24 subjects, the median percentage of 41 mutant RNA remained low throughout the study period.

While Fig. 1*C* represents the general pattern, marked differences between individuals were observed in the rate of development of the 215 codon change (with or without the 41 codon change). Subjects developing the codon 215 change within the first year seemed to have a slightly lower baseline CD4$^+$ cell count, albeit not statistically significant, than those in whom it appeared later or it did not appear during the study period, while baseline HIV-1 RNA levels were similar in the two groups (data not shown). Interestingly, while the latter group generally reached a 100% 70 mutant RNA population at some point, the proportion of 70 mutant virus usually declined before it reached 100% in the group of subjects developing the codon 215 change within the first year (see Fig. 4 *B* and *D*).

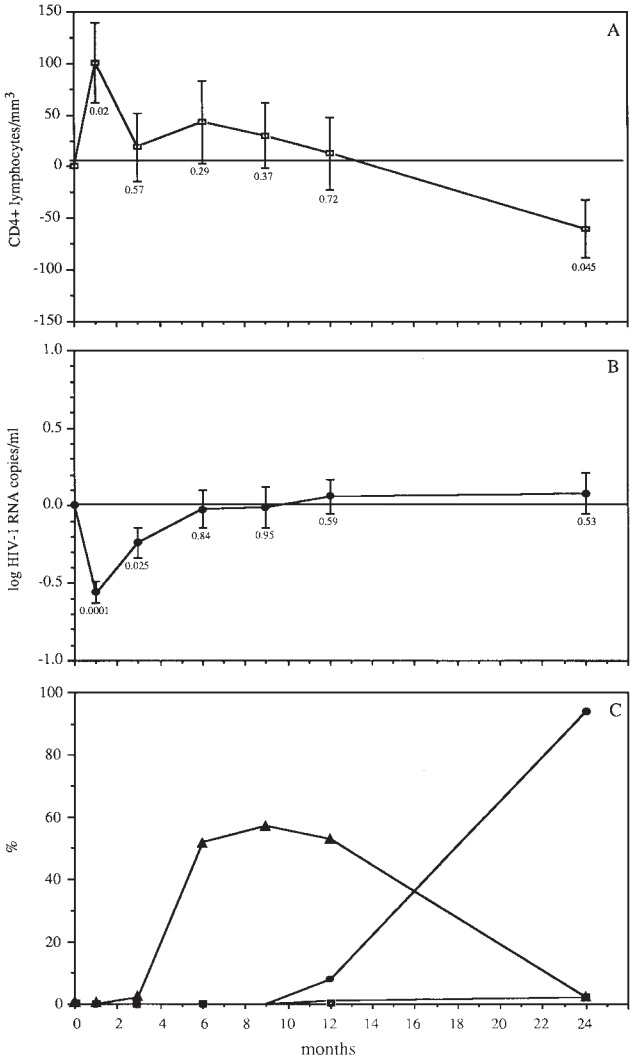

FIG. 1. Mean changes from baseline (±SE) in (*A*) CD4$^+$ cell count (*B*), log HIV-1 RNA load, and (*C*) median percentages of HIV-1 RNA containing a M41L (■), K70R (▲), and T215Y/F (●) amino acid change in HIV-1 RT during 2 years of zidovudine treatment. Numbers underneath error bars indicate the *P* values of changes (paired two-tailed *t* test).

HIV-1 RNA Load Changes and Resistance Mutations. A temporal relationship appeared to exist between the emergence of K70R mutant virus and a return to baseline values of serum RNA load (Fig. 1). Because it was not clear whether the relative amounts of 70 mutant RNA were sufficient to explain the rises in virus load, the absolute copy numbers of 70 wild-type and mutant RNA were calculated for each subject during the first 9 months of treatment, when the other mutations had generally not appeared (Fig. 2).

The initial increase of serum RNA load between 1 and 3 months of treatment was not caused by the K70R amino acid change, because a similar resurgence was observed in 70 wild-type virus. However, the ultimate increase to baseline values between 3 and 6 months was clearly associated with outgrowth of 70 mutant virus; 70 wild-type virus showed declining levels during this period.

Because mutations at codons 215 and/or 41 generally appeared when serum HIV-1 RNA load had already returned to baseline values, these codon changes did not appear to play a role in the ultimate loss of suppression of RNA load by zidovudine. The only exception was the initial appearance of the T215Y change in one subject; calculation of absolute RNA

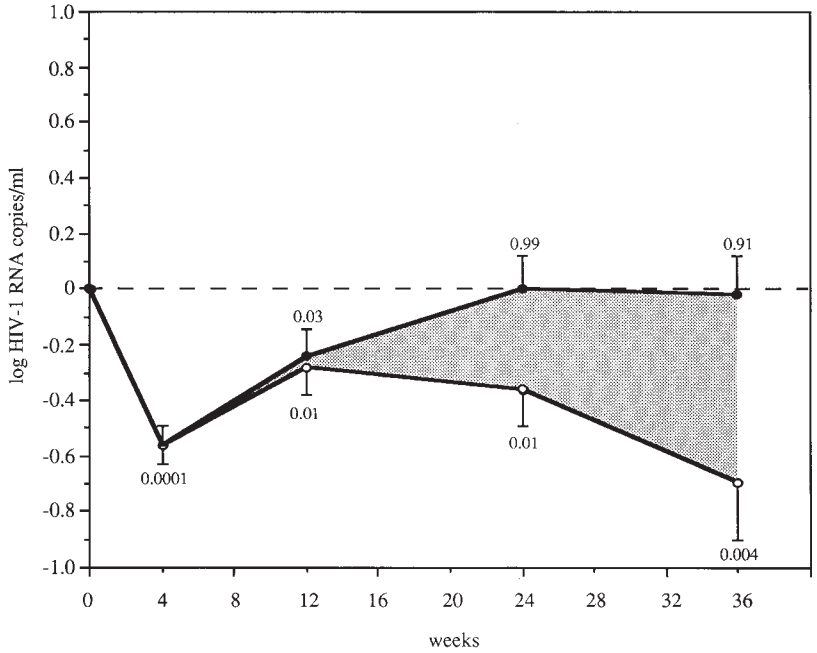

FIG. 2. Mean changes from baseline (±SE) in total HIV-1 RNA load (●), and 70 wild-type HIV-1 RNA (○). Absolute copy numbers of 70 wild-type RNA were calculated from total serum HIV-1 RNA load by using the percentages of 70 wild-type RNA, as detected by the point mutation assay. Numbers above and underneath error bars indicate *P* values of the changes (paired two-tailed *t* test).The shaded area represents the contribution of 70 mutant HIV-1 RNA to changes in total HIV-1 RNA load.

copies showed that outgrowth of 215 mutant virus caused the return to baseline values in this subject (data not shown).

Direct Sequencing Serum HIV-1 RNA. Sequences after 3 months in four subjects were largely conserved with only a small number of inconsistent changes, which argued against the presence of alternative resistance-conferring mutations. Except a mixture of 70 wild-type and mutant sequences in two subjects, no other known zidovudine-resistance-conferring mutations were observed.

Host–Parasite Modeling. Plotting the fractions of the mutants in our model, we found a good correspondence with our *in vivo* observations (Fig. 3). A decline in total virus load again was observed, followed by a resurgence that was independent of the K70R amino acid change. In the model, the virus load also returns to baseline levels upon the development of the codon 70 mutation. We thus conclude that the limited reduction in drug-susceptibility conferred by the K70R change indeed suffices for the return to baseline levels of the HIV-1 RNA load. Similar to our *in vivo* observations, changes in $CD4^+$ cell count mirrored RNA load changes.

In Fig. 4 we compared the percentages of the respective mutants in our model with the *in vivo* data. In Fig. 4*A*, the initial prevalence of all mutants is provided by their equilibrium values before the onset of treatment. For this rigorously derived initial prevalences we found good correspondence with our *in vivo* observations, as shown in Fig. 4*B*, which depicts the percentages of the respective mutants in a representative subject from our study who developed a 100% 70 mutant RNA population within the first year, which disappeared concurrent with the appearance of a 100% 215 and 41 mutant RNA population during the second year of treatment. Thus, in the model, selection for the 215 and 41 mutations also continues while the HIV-1 RNA load had already returned to baseline levels (see Fig. 3).

We can explain the observed differences in the rate of appearance of the respective mutations by changing the initial prevalences of the mutants. In Fig. 4*C*, the initial prevalence of 215 mutant virus is increased 1000-fold. The resulting rapid emergence of 215 mutant virus during treatment appeared to prevent the appearance of a 100% 70-mutant virus population. Again, a striking similarity is observed with a representative subject from our study, who developed 215 mutant virus within the first year of treatment while a 100% 70 mutant RNA population was not observed at any of the time points (Fig. 4*D*).

DISCUSSION

We investigated whether a similar relationship between changes in HIV-1 RNA load and the emergence of genotypically resistant virus, as shown for nevirapine and 3TC, exists

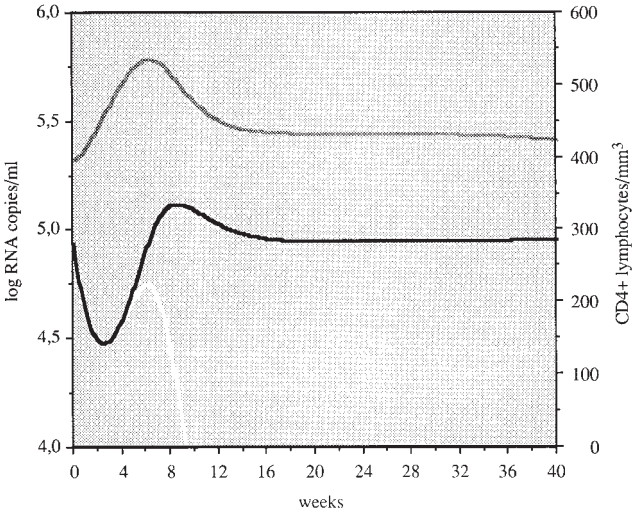

FIG. 3. $CD4^+$ cell counts (grey), total HIV-1 RNA load (black), and wild-type RNA load (white) during the first 40 weeks of treatment, as simulated in our model (see Fig. 2). Zidovudine treatment was simulated by setting $D = 0.5$ at day 0. The $CD4^+$ cell count and HIV-1 RNA load at day 0 correspond to the equilibrium of the model in the absence of drug pressure (i.e., $D = 0$) for the parameter values as described in the text. Because the HIV-1 RNA load in the model is proportional to the number of productively infected cells, we scale toward a realistic RNA load by multiplying V_j by 10^4.

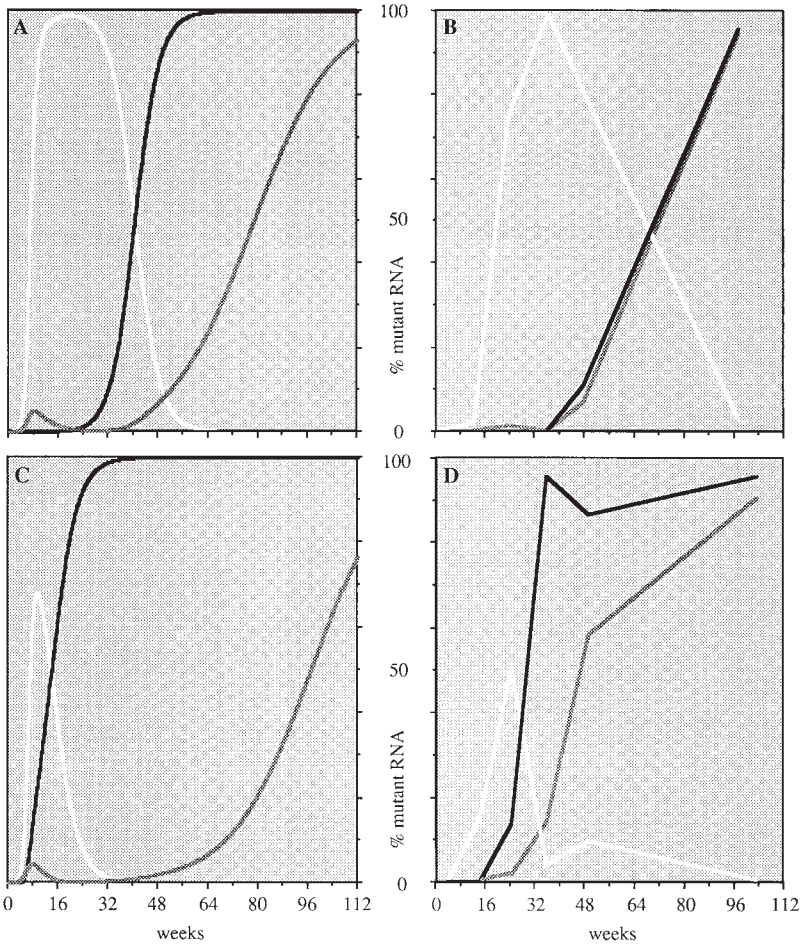

FIG. 4. Percentages of HIV-1 RNA containing mutations at positions 41 (grey), 70 (white), and 215 (black) as simulated in our model (*A* and *C*) and in two representative subjects from our study (*B* and *D*). The model results depicted in *A* represent the same simulation as shown in Fig. 3, whereby percentages are plotted and the model is run for 112 weeks. *C* represents the same simulation with a different initial prevalence of 215-mutant virus—i.e., T_{215} is increased 1000-fold from its equilibrium value.

during treatment with zidovudine (6, 7). A maximum decline in serum HIV-1 RNA load of 0.6 log was observed after 1 month of treatment, which may be an understimation of the actual nadir, since another study showed that maximum suppression occurs during the first 2 weeks (8). After the decline, HIV-1 RNA load resurged, and returned to baseline values within 6 months.

The combined presence of the M41L and T215Y/F amino acid changes in HIV-1 RT appears crucial for the development of high level zidovudine resistance (4). However, resurgences of virus load preceded the appearance of these mutations, indicating that high-level resistance is not required for the loss of inhibition by zidovudine. In our study, the ultimate return to baseline values of serum HIV-1 RNA load between 3 and 6 months of treatment was associated with the emergence of virus containing the K70R change, which is the initially appearing mutation and only confers an 8-fold reduction in sensitivity (5).

However, the return to baseline values was preceded by a resurgence of virus load between 1 and 3 months, which appeared independent of drug resistance. Loveday *et al.* (8) recently reported a similar resistance-independent resurgence of HIV-1 RNA load during early zidovudine treatment. These observations confirm earlier modeling experiments (20–22). Assuming that an equilibrium exists between virus and $CD4^+$ lymphocytes, and that, in the short term, the virus load is largely limited by the number of target cells, one can explain the initial resurgence of virus load by classic host–parasite dynamics. Treatment with zidovudine results in an increase in the $CD4^+$ cell count (host), while it does not result in complete suppression of HIV-1 replication (parasite). In the model, a new equilibrium is set with a higher $CD4^+$ cell count and a slightly reduced wild-type HIV-1 RNA load. In the absence of resistance-conferring mutations, this new equilibrium is attained oscillatory—i.e., the virus load first decreases—but the subsequent increase in target cells allows the virus to rebound until the new equilibrium is instituted. A similar increase would not be observed if HIV-1 replication would be completely inhibited, which no antiretroviral drug to date has been able to achieve. In the model, there exists a critical treatment effect—i.e., fitness reduction—below which wild-type virus will not rebound and the effect of treatment is largely reflected in increased $CD4^+$ cell counts and only marginally in HIV-1 RNA load rises (data not shown). The emergence of drug-resistant mutants enables a return to the pretreatment equilibrium and is thus largely reflected in the $CD4^+$ cell count: when the K70R mutant appears, the $CD4^+$ cell count returns to baseline levels.

Because our model is a simplification of the complex biological system, we cannot exclude that other mechanisms [e.g., decreasing intracellular phosphorylation of zidovudine over time (29)] play a role in the initial rebound of virus load *in vivo*. While our study population solely harbored nonsyncytium-inducing (NSI) HIV-1 isolates, there is no reason to believe that our observations will essentially be different in individuals harboring syncytium-inducing (SI) virus. Although reported differences in the viral replication rate and the rate of resistance development between SI and NSI variants may

result in temporal differences, the observed pattern will most likely be similar (11).

Interestingly, despite the fact that the K70R amino acid change apparently was sufficient to cause a relative loss of replication-inhibition by zidovudine, selection toward additional or alternative resistance-conferring RT mutations continued, eventually replacing virus containing the codon 70 change. This order of events may be explained by the facts that the K70R change is easy to acquire, but only results in a relatively weak selective advantage over wild-type virus during zidovudine treatment. A lysine to arginine change only requires a single A to G nucleotide change, which is obviously acquired with more ease than double nucleotide changes (e.g., T215Y). Of note, the nevirapine resistance-conferring Y181C- and the 3TC-resistance-conferring M184V changes also require single A to G nucleotide changes. However, while the latter two mutations result in >100-fold reductions in drug susceptibility to nevirapine and 3TC, respectively (30, 31), the K70R change only results in an 8-fold reduction of zidovudine susceptibility. Although this relatively small reduction in susceptibility appears sufficient to cause a relative loss of inhibitory activity by zidovudine, the replicative advantage of K70R mutant virus over wild-type virus during zidovudine treatment is only minor. On one hand, this may explain why it takes more than 3 months for significant numbers of 70 mutant virus to appear, contrasting with Y181C and M184V mutants that replace the entire serum virus population within only a few weeks of treatment with nevirapine and 3TC respectively (7, 24). On the other hand, the relatively small reduction in drug susceptibility of K70R mutant virus explains the continued selection of other resistance-conferring mutations, despite the apparent loss of replication inhibition by zidovudine. Continued evolution of unsupressed wild-type and K70R mutant virus will result in alternative or additional mutations, which, although acquired with less ease than the K70R change, confer increasing levels of resistance and thus increasing replicative advantages under zidovudine-pressure (32). The accumulation of RT mutations will continue until ultimately only virus with >100-fold reductions in susceptibility prevails, which requires the accumulation of several RT mutations (3, 4).

Our mathematical model suggested that the maximal proportion of 70 mutant virus achieved during treatment with zidovudine is dependent on the rate of appearance of 215 mutant virus, which is not surprising in the light of the previous discussion. Although the number of time points tested were limited, our *in vivo* observations were strikingly similar to the model. On one extreme, a rapid emergence of 215 mutant virus could prevent the initial appearance of 70 mutant virus, as appeared to be the case in one subject, while on the other extreme, a prolonged presence of a 100% 70 mutant virus population is possible when 215 mutant virus variants do not appear, as was observed in 4 subjects (data not shown).

The individual differences in the rate of appearance of the respective resistance-conferring RT mutations observed in our study could neither be fully explained by differences in the $CD4^+$ cell count or serum HIV-1 RNA load at baseline, nor by differences in syncytium-inducing capacity of the virus. Our mathematical model suggested that the rate and the pattern of appearance of resistance mutations is influenced by the prevalence of resistant virus variants before treatment. Further research is needed to assess the relevance of this variable *in vivo*.

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