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Human Immunodeficiency Virus Fitness In Vivo: Calculations Based on a Single Zidovudine Resistance Mutation at Codon 215 of Reverse Transcriptase

JAAP GOUDSMIT,[1]* ANTHONY DE RONDE,[1] DAVID D. HO,[2] AND ALAN S. PERELSON[3]

Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands[1]; Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, New York 10016[2]; and Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545[3]

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We monitored a subject newly infected with a zidovudine-resistant human immunodeficiency virus type 1 strain and found that in the absence of drug, the viral population with the resistance-conferring tyrosine (TAC) codon 215 of reverse transcriptase was gradually replaced. By using standard formulas to model the effects of selection at a single locus in an asexual haploid population, the relative fitness gain of the viral population with a single mutation at codon 215 creating a serine (TCC) was calculated. We concluded that a viral population with a serine at reverse transcriptase codon 215 conferring zidovudine sensitivity was between 0.4 and 2.3% more fit.

As a consequence of human immunodeficiency virus type 1 (HIV-1) dynamics (5, 15, 16), mutations conferring resistance to antiretroviral drugs appear rapidly after initiation of treatment (5, 16). It has been postulated by Coffin (1) that drug-resistant mutants will always replicate more poorly in the absence of drug; otherwise, the mutation conferring drug resistance would be present in the wild type. From the low rate of reversion of zidovudine (AZT) resistance mutations in the absence of drug (10) and from the occasional appearance of these mutations in untreated individuals (10, 12), it was concluded (1) that "at least some of these mutations have very little effect on replication of the virus." However, in untreated individuals, partially AZT-resistant viruses with mutations at codon 70 have been found (10, 12) but no viruses with mutations at codons 41 and 215, conferring almost complete resistance, have been found. This observation suggests that the fitness loss caused by the latter mutations might be substantial and that in the absence of drug, the "master" sequence of a quasispecies containing both drug-resistant and wild-type virus would soon be devoid of the drug resistance-conferring sequence (4). Coffin (1) estimated, as an example, that the selective disadvantage or fitness loss of an AZT-resistant virus would be of the order of 1%. Recently, it has been reported that persons are infected by HIV strains with AZT resistance-conferring mutations. Such cases provide an environment in which the viral target cell population is relatively intact. Data on the viral generation time, defined as the time from release of a virion until it infects another cell and causes the release of a new virion, and the distribution of the different viral species allow calculations of the relative fitness of an AZT-sensitive mutant in an AZT-resistant population. These concepts were applied to calculate the relative fitness of an AZT-sensitive virus mutant with a serine (TCC) at codon 215 of reverse transcriptase in a new infection with an AZT-resistant virus population in the absence of drug.

By using the microtiter format point mutation assay (PMA) (7), we identified an intravenous drug user who was newly infected in 1993 with a virus containing an AZT resistance-conferring mutation at codon 215 (3). Direct sequencing of the HIV-1 RNA in serum at the moment of seroconversion by using nested reverse transcriptase PCR (13) showed a master sequence which contained AZT resistance-conferring mutations at codons 41 and 215 but was wild type at other codons involved in AZT resistance, i.e., 67, 70, and 219 (9). The PCR product from the moment of seroconversion was also used to generate 10 clones. All clones contained a leucine at position 41, and all except one contained a tyrosine (TAC) at position 215. The one exception had a TCC triplet, coding for serine, at position 215. In a wild-type background, a tyrosine at position 215 confers AZT resistance and serine confers sensitivity (8), whereas a threonine (ACC) is the wild-type sequence seen in untreated individuals infected in the pre-AZT era (12). The infected person was monitored for 20 months, and the frequency of the TCC mutant was tested at 5, 9, 12, 16, and 20 months by the PMA. The PMA assessed the frequency distribution of the second nucleotide of codon 215 and detected only the A of the TAC codon at the moment of seroconversion. At 9 months, a C at this position was detected at a level of 13%; this level rose to 49% at 20 months (Fig. 1). Sequence analysis of 10 clones at 20 months indicated that all the codons with a C at the second position were TCC (Ser) and that those with an A were GAC (Asp), a codon whose AZT resistance-conferring properties are unknown.

We calculated the fitness gain of the TCC population by using standard formulas for the effects of selection at a single locus in an asexual haploid population. We used two approaches, one assuming discrete generations of viral replication and the other assuming replication in continuous time. The latter approach might be more realistic, since it does not assume that the virus replicates in discrete, nonoverlapping generations. Both approaches assume that both "wild-type" and mutant populations are present at time zero (seroconversion) but at different proportions and that they grow with different fitnesses. Therefore, a selection process is modelled by ignoring mutational effects like the TAC-to-GAC mutation. In the first approach for two populations, each with constant fitness, that grow with discrete nonoverlapping generations,

* Corresponding author. Mailing address: Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Phone: 31.20.566.4853. Fax: 31.20.691.6531.

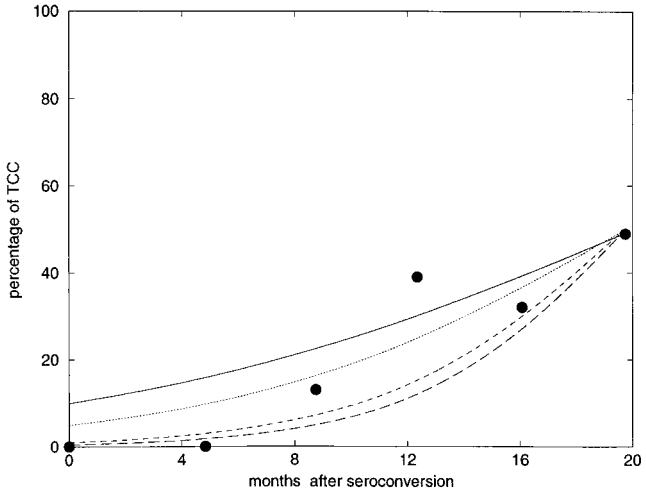

FIG. 1. Percentage of the more fit TCC variant predicted by equation 2, assuming that this variant was initially present at a level of 0.5% (long dashed line), 1.0% (dashed line), 5% (dotted line), or 10% (solid line) and was present at a level of 50% at 20 months. Datum points measured by PMA (7) are also indicated.

the proportion, $p(t)$, of the less fit population changes in time according to

$$p(t) = \frac{p(0)\, q(t)\, (\text{fitness})^t}{q(0)} \quad (1)$$

where q is the proportion of the more fit variant $(1 - p)$, t is the time in generations, and fitness is the relative fitness of the less fit population with respect to the more fit population (11). Using a viral generation time calculated by Perelson et al. (15) of 2.6 ± 0.8 days and assuming that initially 10% (based on the sequences of 10 clones) of the virus was made up of the more fit population, i.e., $q(0) = 0.1$ and $p(0) = 0.9$, the relative fitness calculated from equation 1 was 0.99, implying that the original AZT-resistant variant was 1% less fit than the TCC mutant. If, taking into account the PMA values and a conceivable error in the sampling of 10 clones from a quasi-species, the estimate for the initial more fit population were lowered to 5, 1, or 0.5%, consistent with a homogeneous population at seroconversion, the fitness loss would be 1.3, 2, or 2.3%, respectively. If we call day 266 (when the more fit population was first detected by PMA) time zero, then $p(0) = 0.87$ and $q(0) = 0.13$, and at the end of the observation period (day 601), when 129 generations instead of 230 have passed, the fitness loss would be 1.5%.

For the second approach, solving the problem in continuous time, the corresponding formula is

$$s = \frac{1}{t} \ln \left[\frac{q(t)p(0)}{p(t)q(0)} \right] \quad (2)$$

where s, the selection coefficient, is the fitness difference (6, 11). Using our data again, if $p(t) = q(t) = 0.5$ and $p(0) = 0.9$, $q(0) = 0.1$, and $t = 600$ days, then $s = 0.0036$, corresponding to a fitness loss of approximately 0.4%. If $q(0) = 0.05$, 0.01, or 0.005 and $p(0) = 0.95$, 0.99, or 0.995, the fitness loss becomes 0.5, 0.8, or 0.9%, respectively (Fig. 1). If, again, day 266 is taken as $t = 0$, the fitness loss is 0.6%. A more comprehensive analysis involving nonlinear least-squares fitting of the data to equation 2 gives similar results.

The present study was able to approximate the competitive advantage of a viral population with TCC (Ser) at codon 215 relative to the remainder of the viral population containing TAC (Tyr) or GAC (Asp). The loss of fitness of the tyrosine 215 population conferring complete AZT resistance in combination with leucine 41 cannot be assessed by the experimental data presented and awaits extensive clonal sequence analysis as well as phenotypic analysis, currently under way. The fitness gain of the TCC mutant did not substantially affect the steady-state HIV RNA levels (fluctuating from 0.7×10^5 to 2.0×10^5 copies per ml in the Amplicor Roche assay), in agreement with the limited fitness gain calculated. The current in vivo case involved an intravenous drug user acquiring HIV through parental transmission. This route of transmission involves a relatively large virus population brought into direct contact with the cells best adapted to propagate HIV efficiently (2). In vitro, large population transmissions have been described by Novella et al. (14) for vesicular stomatitis virus, showing exponential increases in replicative fitness. However, reference to these in vitro experiments is probably not possible because of an initially lower viral load in vivo and complicating factors, like a functional immune system and differential cell tropism. Our calculations and our viral load data in this one case do not suggest a large increase in the fitness of the viral population accompanying the outgrowth of the TCC (Ser) mutant. Taken together, our data show a shift in the virus population, with a single mutant growing out with a relative fitness gain over the master sequence of 0.4 to 2.3%.

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