Diversity of thymidine analogue resistance genotypes among newly diagnosed HIV-1-infected persons

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The introduction of highly active antiretroviral therapy (HAART) has resulted in a significant decrease in HIV and AIDS-related mortality and morbidity. However, these treatments can select for drug-resistant viruses which are associated with poor virological responses to the antiretroviral therapy and possible loss of clinical benefit. Drug-resistant viruses can also be transmitted between individuals. In the absence of drug pressure, transmitted drug-resistant viruses gradually lose resistance mutations that confer a selective disadvantage as they evolve to more fit viruses. As a result, unusual resistance-related genotypes not commonly seen in treated patients may arise in the population. Viruses with unique patterns of thymidine analogue-associated mutations (TAMs) have now been identified in a substantial proportion of treatment-naive recently diagnosed persons. In this leading article, I discuss these findings and the potential impact of these unique reverse transcriptase (RT) genotypes on evolution of resistance and treatment responses.

Keywords: revertant viruses, fitness, virus evolution

Introduction

Potent antiretroviral therapy with inhibitors of the HIV-1 reverse transcriptase (RT) or protease has significantly reduced the rate of HIV and AIDS-related morbidity and mortality. There are 20 antiretroviral drugs approved by the US Food and Drug Administration (FDA) for the treatment of HIV-1-infected persons including 11 RT inhibitors, eight protease inhibitors (PI), and one fusion inhibitor.1

The selection of a combination regimen that maximally suppresses virus replication is critical for treatment success, since persistent virus replication due to suboptimal therapy may result in the selection of viruses carrying drug resistance mutations. The emergence of drug-resistant viruses during treatment, commonly known as secondary or acquired resistance, is an important cause of treatment failure. Recent estimates of the prevalence of drug resistance during the first years of widespread use of potent antiretroviral therapy have indicated that about 70% of treated adults with detectable viraemia have viruses with drug resistance mutations.2,3

Drug-resistant viruses can also be transmitted between individuals. Primary or transmitted resistance has been documented through vertical, sexual and parenteral routes.4 The proportion of patients newly infected with drug-resistant HIV-1 has increased during the past few years, and current estimates indicate that between 10% and 20% of acutely or recently infected persons in the USA and Europe have viruses that are resistant to one or more drugs.5 Infection with drug-resistant HIV-1 is of clinical and public health concern. Persons infected with drug-resistant viruses show a longer time to viral suppression and a shorter time to virological failure following initiation of antiretroviral therapy compared with patients infected with wild-type viruses.4

To date, more than 90 mutations have been associated with drug resistance. The list of mutations is periodically updated by the International AIDS Society–USA Drug Resistance Mutation Group.6 Mutations are identified by several criteria including in vitro selection with increasing concentrations of the antiviral drug, studies with site-directed mutants, susceptibility testing of laboratory or clinical isolates, selection in persons receiving antiretroviral drugs, and correlation studies between genotype and virological responses.5

Evolution of drug resistance in treated and untreated persons

The emergence of drug resistance during treatment is due to the extensive genetic diversity of HIV-1 and the selection of drug-resistant variants. The genetic variability of HIV-1 is the result of several factors including the high rate of replication and recombination, the low fidelity of HIV-1 RT, and the lack of proof-reading activities during the RNA-dependent DNA synthesis. If an estimated 10^10 virions are produced daily within an individual and each genome contains an average of 1 mutation, every single drug resistance mutation may be present before initiation of antiretroviral therapy and double mutants may also occur commonly.6 Estimates based on the rate of emergence of nevirapine resistance in untreated persons indicate that ~1 in 1000 genomes carry the Y181C
mutation associated with nevirapine resistance before treatment with nevirapine. Therefore, pre-existing viruses with resistance mutations may become rapidly selected by antiretroviral drugs.

Viral adaptation to antiretroviral drug pressure is characterized by the initial selection of deleterious mutations that generally decrease drug susceptibility and viral fitness (Figure 1). Such mutations are commonly known as primary or major and are relatively specific for each drug. The deleterious effect of primary mutations is efficiently reduced by the selection of additional compensatory mutations known as secondary or minor. Such compensatory evolution usually results in a restoration of the structure and/or function of the RT or protease and generally increases the level of drug resistance. For instance, selection of D67N, K219Q and K70R in viruses carrying the T215Y mutation increases the levels of phenotypic resistance to zidovudine and enhances DNA synthesis by mutant RT enzymes. In some cases, selection of compensatory mutations is not associated with increased levels of drug resistance. For instance, PI-resistant mutants carrying M36I, I54V and V82T acquire A71V and K20R to compensate for a reduced protease catalytic activity but show no detectable increases in resistance to ritonavir. Compensatory evolution has also been observed outside the RT and protease gene. Such is the case for mutations at Gag and Gag–Pol protease cleavage sites observed during treatment with protease inhibitors which are associated with improved enzyme kinetics. However, despite the accumulation of compensatory mutations, drug-resistant viruses generally display a reduced fitness compared with wild-type viruses. Reductions in viral fitness and replication capacity of drug-resistant viruses have been associated with sustained immunological responses in patients who fail antiretroviral therapy, suggesting that viruses with low replication capacity might be less pathogenic.

The interruption of antiretroviral therapy in treated patients carrying drug-resistant viruses usually results in the rapid overgrowth of mutant viruses by wild-type viruses from an archived population. Although information concerning the clonality of the transmitted virus population is limited, some studies suggest that HIV-1 transmission may involve infection with an oligoclonal viral population. In this setting and in the absence of treatment, transmitted drug-resistant isolates gradually lose resistance mutations that confer a high fitness cost as they evolve to more fit viruses (Figure 1). The determinants of persistence and pathways of reversion of transmitted resistance mutations are not fully understood. It is generally accepted that mutations that confer a low or moderate fitness cost have the potential to persist for long periods of time as opposed to those that have a high impact on viral fitness. For instance, fit mutants carrying the K103N mutation are able to persist for as long as 3 years compared with the relatively rapid reversion observed in less-fit mutants that have the M184V mutation. However, the rate of reversion when low-fitness mutants are allowed to replicate may also depend on other factors including the characteristics of the original inocula (oligoclonal or polyclonal), immune selective pressures, and characteristics of the infecting virus such as the number and type of resistance mutations.

Thymidine analogue-associated mutations (TAMs)

Zidovudine and stavudine are the two thymidine analogues currently approved for the treatment of HIV-1-infected persons. Mutations associated with thymidine analogue resistance were originally identified by their role in zidovudine resistance, and were initially classified as primary or secondary based on their effect on drug susceptibility. Of the TAMs, K70R and T215Y/F are generally considered primary and cause low or moderate (5- to 15-fold) levels of zidovudine resistance whereas D67N, M41L, L210W and K219Q/E are considered secondary and do not confer resistance by themselves. The emergence of TAMs in treated patients occurs in an orderly manner and their accumulation is associated with increasing levels of resistance.

Although TAMs are usually selected by zidovudine and stavudine-containing regimens, recent studies indicate that these mutations are also associated with phenotypic and clinical resistance to each of the other nucleoside RT inhibitors (NRTIs) with the possible exception of lamivudine. The magnitude of phenotypic and clinical resistance to other NRTIs appears to be related to the number of TAMs. Complete loss of responses to abacavir usually requires the presence of three or more TAMs along with the M184V mutation, and four or more TAMs are needed for a complete loss of virological response to the addition of didanosine to a stable regimen. Specific patterns of TAMs may have a different impact on treatment responses. For instance, responses to tenofovir are less
affected by the combination of D67N, K70R, K219Q/E and T215F than by the combination of M41L, L210W and T215Y. The magnitude of thymidine analogue resistance conferred by TAMs can also be modulated by other nucleoside analogue mutations. Such is the case for the M184V mutation commonly seen in regimens containing lamivudine or emtricitabine which causes high-level resistance to lamivudine and emtricitabine, moderate resistance to didanosine and abacavir, and increases the susceptibility to zidovudine, stavudine and tenofovir.

Reversion of TAMs in transmitted HIV-1 generates new genotypes with distinct properties

Surveillance of transmitted resistance has documented unusual patterns of TAMs in isolates from newly diagnosed persons. The finding that the majority of transmitted HIV-1 isolates with TAMs lack a primary mutation and are phenotypically sensitive to zidovudine is remarkable, and illustrates how the patterns of resistance mutations may differ among the treated and untreated population. The largest proportion of these isolates were characterized by having revertants of the 215Y/F mutations such as 215C, 215D or 215S. These viruses were found in ~3% of recently diagnosed persons and were more frequent than the zidovudine-selected mutants carrying T215Y/F. A second group of isolates was characterized by having only secondary TAMs such as D67N, K219Q or M41L. These unique RT genotypes probably represent revertants of transmitted drug-resistant viruses that lose primary mutations as they evolve to improve viral fitness in the absence of drug pressure. A common characteristic of all these isolates was the efficient replication seen in the absence of drug pressure, and the absence of phenotypic resistance to thymidine analogues. A high fitness in the absence of drug probably explains the persistence of these mutations seen in vivo, and provides opportunities for subsequent secondary transmission, as was noted in a transmission chain of viruses containing 215D.

The establishment of new RT genotypes and the potential for secondary transmission heighten the importance of evaluating the impact of these mutations on resistance evolution. A rapid evolution towards zidovudine or stavudine resistance was first noted in vitro in transmitted isolates carrying 215D/C, and was explained by the need for only one nucleotide change to evolve from 215D/C to 215Y/F, as opposed to the two nucleotides required for wild-type viruses (Figure 2). Clinical studies have also suggested that the presence of the 215D/C substitutions may be associated with an increased risk of virological failure in antiretroviral-naïve adults starting therapy with zidovudine or stavudine. A similar rapid evolution towards zidovudine resistance was recently noted in revertant viruses carrying D67N or K219Q (Figure 2). Interestingly, the rapid selection of zidovudine resistance in these viruses was associated with a high viral fitness in the presence of zidovudine. The high fitness of these viruses with zidovudine suggests a low level of phenotypic resistance that is not detected by the most sensitive phenotypic assays.

The identification of revertant viruses in recently diagnosed persons suggests that these viruses may have originated from viruses that had additional resistance mutations. The loss or reversion of mutations may have occurred in these persons or in a different person before these mutants were transmitted. Both possibilities have the potential to compromise the efficacy of antiretroviral therapy through either a rapid selection of T215Y or K70R or by a selection of an archived zidovudine-resistant virus.

Impact of reversion on surveillance of transmitted resistance

The identification of revertant viruses with distinct virological properties illustrates the expanding diversity of resistance-related genotypes in the untreated population, and heightens the need for surveillance for novel mutations or mutational patterns in persons recently diagnosed with HIV-1 infection. A better understanding of the virological factors that influence the rate of reversion of drug-resistant viruses following transmission is also critical.
for drug resistance surveillance. For instance, the relationship between the fitness cost of mutations and the ability of the virus to persist in vivo is not fully understood, as it is not known how mutational interactions can modulate fitness and persistence (Table 1). These studies will shed light on the mutations that are more likely to be missed in surveillance because they confer a high fitness cost and have a higher potential to revert and become undetectable. The known reversion of drug resistance mutations emphasizes the need for more sensitive assays that can be used for resistance surveillance. Such assays may be useful to identify low levels of mutant viruses that may be undetected by sequencing methods.33

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References

Table 1. Unanswered questions about reversion of drug resistance mutations and its virological implications

| Role of fitness cost of mutations and immunological pressures in persistence of transmitted drug-resistant viruses |  |
| Modulation of fitness of mutant viruses by other resistance mutations or specific genetic backgrounds |  |
| Ability of revertants with compensatory PI or NNRTI mutations to acquire resistance |  |
| Clinical responses to antiretroviral treatment in persons carrying revertant viruses |  |
| Implication of persistence of mutations on drug resistance surveillance. What mutations might be underestimated? |  |

