Transmission dynamics of the M184V drug resistance mutation in primary HIV infection

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Objectives: M184V in HIV-1 reverse transcriptase is among the most common mutations in patients failing antiretroviral therapy but is found only rarely in cases of transmitted drug resistance.

Methods: To investigate this apparent paradox, we developed an allele-specific real-time PCR (AS-PCR) assay to determine the transmission of M184V in newly infected individuals.

Results: M184V transmission may occur to a greater extent than previously thought. Persistence of M184V may commonly involve linkage to other drug resistance mutations. The presence of M184V as a single substitution in newly infected individuals was shown to wane over time, as a likely consequence of reversion and overgrowth by more fit wild-type viruses.

Conclusions: The M184V mutation can be documented in newly infected individuals, and the alternative hypothesis that this substitution might impact on the ability of HIV to be transmitted is unfounded.

Keywords: mutational deselection, viral fitness, viral replicative capacity

Introduction

Many studies have shown that the M184V mutation is selected rapidly in individuals who fail lamivudine- or emtricitabine-containing regimens because of the strong selection pressure that is exerted by these drugs.1,2 Since most first-line treatment regimens have long included either lamivudine or emtricitabine, it is not surprising that M184V is among the most frequent substitutions observed when patients who show elevations of plasma viral load are genotyped for the possible presence of drug resistance mutations. It is also well known that drug-resistant variants of HIV-1 can be sexually transmitted,3,4 and many studies have shown that as many as 15%–20% of newly infected individuals may harbour at least one mutation associated with drug resistance. Furthermore, HIV infection in newly infected individuals is dominated by the presence of a single species that only diversifies as a result of mutagenesis and recombination over time.5,6 Curiously, however, the M184V substitution is rarely detected by routine genotyping in the context of new HIV infections, whereas other mutations such as K103N, associated with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), are more frequent in transmitted drug resistance.5–7 This is thought to be both a consequence of the increased use of efavirenz over the past decade and the fact that K103N has very little impact on viral replicative capacity.

Drug resistance is a major limiting factor in the long-term successful use of antiretroviral drugs to treat HIV disease. For this reason, there is broad consensus that all drug-naive patients should undergo an HIV genotype test to reveal the possible presence of drug resistance mutations so as to ensure that all of the components of treatment regimens are active.

Recently, however, it was shown that bulk genotyping methods may not be adequately sensitive to reveal the presence of all drug resistance mutations in a population.6,8 In some instances, the presence of drug resistance mutations at relatively low frequency has been shown to impact on responsiveness to therapy.6,8 The presence of drug resistance mutations at low levels among HIV quasi-species has most often been revealed by either of two ultrasensitive methods, pyrosequencing or allele-specific PCR (AS-PCR).6–8 The former method is expensive, but has the advantage of being able to provide information about the entire viral genome in terms of minority representation of select mutations or sequences. In contrast, AS-PCR assays are relatively inexpensive, but are limited to providing information about one mutation at a time. Here, we have employed both...
bulk sequencing methods and AS-PCR to examine the possible presence of the M184V nucleoside reverse transcriptase inhibitor mutation in chronically treated patients and acutely infected drug-naive individuals.

Our results show that the M184V substitution was present to a greater extent by AS-PCR as opposed to bulk sequencing analysis, providing evidence that viruses of limited replication capacity can, in fact, be effectively transmitted, but that reversions to wild-type will result in overgrowth by viruses that possess superior replication capacity.

Methods

Populations evaluated

We studied a total of 204 individuals, infected for less than 6 months, who were enrolled between 2005 and 2010 in the Montreal primary infection cohort. Routine genotyping was performed by Virco methodology as previously described. All patients provided informed consent. Plasma HIV-1 RNA was measured using the Quantiplex HIV-1 RNA and bDNA systems (threshold, 50 copies/mL; Siemens Diagnostics).

AS-PCR

Plasma viral samples were obtained from newly infected individuals. Samples that lacked either the M184V or the K103N mutations by bulk sequencing were evaluated by AS-PCR to detect these substitutions, as previously described (sensitivity ~1%; sensitivity and specificity were monitored using positive and negative controls as previously described).

Results

The data in Table 1 show the frequencies between 2005 and 2010 of different types of mutations in our treated population versus the primary infection cohort. In individuals infected by drug-resistant viruses, the percentage who carried NNRTI mutations was high (i.e. 61.3%), but relatively few thymidine-associated mutations (TAMs), associated with resistance mutations was important. We observed that M184V was most likely to be detected by conventional genotyping if other drug resistance mutations were also present (i.e. 10 of 204 cases versus 4 of 204 when M184V was found as a single substitution). Moreover, the likelihood of detecting M184V was highest in those individuals who had two or more other transmitted mutations besides M184V (not shown). This is probably because M184V reversions followed by wild-type overgrowth are most likely to occur quickly in cases in which M184V is transmitted as a single substitution rather than when this mutation is linked to others, since the amount of time needed to spontaneously back-mutate a large number of such mutations along a single viral backbone will be greater than would be required for single substitutions. Among drug-naive chronically infected subjects, M184V was detected by conventional genotyping in 11 of 585 cases if other drug resistance mutations were also present versus 0 of 585 cases when they were not. No differences were observed in regard to the likelihood of a linkage association of M184V with mutations of the TAM, NNRTI or PI categories (not shown).

Table 1. Percentages of various types of drug resistance mutations in treated versus acutely infected individuals among people having any drug-resistant viruses as determined by population sequencing and AS-PCR

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Treated individuals (n = 2658)</th>
<th>PHI (n = 204) (population sequencing), %</th>
<th>PHI (n = 204) (AS-PCR), %</th>
<th>PHI (n = 204) (population sequencing), %</th>
<th>PHI (n = 204) (AS-PCR), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TAM</td>
<td>25.0</td>
<td>6.4</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any NNRTI mutation (except K103N)</td>
<td>61.3</td>
<td>69.8</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any PI mutation</td>
<td>41.4</td>
<td>8.3</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M184V</td>
<td>67.5</td>
<td>7.0</td>
<td>23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K103N</td>
<td>49.6</td>
<td>44.2</td>
<td>57.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined.

Sequential sample analysis

If our hypothesis is correct, then reversions of M184V-containing virus to wild-type variants should be an ongoing process and the presence of M184V in patient samples should decline over time, even if M184V detection is performed by AS-PCR. The results in Table 2 show that this is exactly what happened in each of three individuals who apparently possessed only the M184V mutation at baseline and who donated three successive blood samples.
samples over periods of 40, 52 and 61 weeks after the estimated time of initial infection. In all instances, M184V was no longer detectable in the last sample analysed. Four other PHI donors who had tested positive for M184V by AS-PCR were negative for the presence of this mutation by AS-PCR after 48, 60, 40 and 32 weeks, respectively, but intermediate samples were not available from these individuals. In contrast, two individuals who possessed at least two additional mutations besides M184V at baseline were also followed sequentially. The results show that these subjects retained the M184V mutation, as determined by AS-PCR (and routine genotyping) for at least 65 weeks (see above), suggesting that the presence of additional mutations on the viral genome had created a situation in which the need for multiple reversions and outgrowth of wild-type virus would take far longer to occur than would be likely with viruses that contained only M184V as a resistance mutation.

### Discussion

Here, we show that drug resistance mutations that have negative consequences for viral replication probably do not affect HIV-1 transmission efficiency, which is probably determined by such factors as viral load in seminal fluid and blood, and mucosal considerations, rather than by the replication capacity of individual viruses. Our findings show that drug resistance mutations that affect replication capacity may revert or be deselected, a process that is favoured in the absence of antiretroviral drug pressure, followed by outgrowth of more replication-competent wild-type viruses. Although, initially, transmitted viruses that do contain these reversible mutations will likely become archived in lymphocyte reservoirs, the clinical significance of this is not well understood, although they could presumably be amplified under relevant drug pressure. Others have reported that transmission of viruses that contain NNRTI mutations may impact subsequent clinical responsiveness to NNRTI-containing treatment regimens.6–8

Our study shows that higher proportions of M184V mutations were detected among acutely infected individuals by AS-PCR compared with routine genotyping, but it is also interesting that the numbers of such individuals waned over time, probably as a consequence of continuing overgrowth by more replication-competent wild-type viruses. This conclusion is strengthened by the finding that M184V was most often detected by bulk sequencing in cases in which this mutation was simultaneously present alongside other drug resistance mutations, and that reversion of M184V, when simultaneously present with other drug resistance mutations, took longer to occur. Of course, full-length sequencing of individually cloned viruses would be necessary to prove that these various mutations were, in fact, present in simple virus genomes. Nonetheless, these data are consistent with findings that viruses that contain multiple drug resistance mutations, associated with different families of antiretrovirals, can persist as dominant viral species over long periods (i.e. months to years).

The reasons that the M184V mutation can compromise viral replicative fitness are well understood and include diminished reverse transcriptase processivity, diminished capacity to initiate reverse transcriptase reactions and a lessened ability to participate in template switching. Studies are needed to better understand the impact of other drug resistance mutations, including possible compensatory mutations, on the biochemical functions of the enzymes in which they are located.

### Nucleotide sequence accession numbers

The sequences described in this study have been deposited in GenBank under accession numbers EU375800-801 and EU906882-907.

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### Transparency declarations

None to declare.

### References


