Chasing the Infecting HIV-1

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(See the article by Rieder et al, on pages 1271–9.)

In the absence of an effective prophylactic vaccine and successful viral eradication strategies, the burden of human immunodeficiency virus type 1 (HIV-1) infection and disease continues to grow worldwide. The events characterizing primary HIV-1 infection and the early stages of the infection have been the focus of extensive research addressing the modalities of HIV-1 transmission. A thorough understanding of the early viral and host interactions leading to the establishment of productive infection will provide the necessary evidence base for developing successful prophylactic and therapeutic tools, including drug regimens, vaccines, and microbicides, and for guiding clinical management and the optimal time for the initiation of antiretroviral therapy.

From a global perspective, among the various possible modes of acquisition, the majority of new HIV-1 infections occur through sexual transmission across mucosal barriers. Early after infection, HIV-1 strains appear to be genetically homogenous but rapidly diversify into a swarm of related but genetically divergent variants that compose the viral quasispecies. The highest degree of diversification is observed in the envelope glycoprotein 120 (gp120), the target of strong immune responses, including T-cell–mediated and neutralizing antibodies [1, 2]. There is substantial evidence to indicate that the diverse quasispecies replicating in chronically infected individuals is forced through a genetic “bottleneck” at the time of transmission. As a result, the majority of transmission events leading to productive infection appear to be associated with 1 homogenous founder virus, whereas transmission of multiple divergent species is regarded as much less frequent. One possible explanation is that transmissible HIV-1 strains show certain unique characteristics that allow them to overcome host defenses. The mechanisms by which this selective process leads to the transmission of a homogenous founder virus, and the role of virus and host determinants, however, remain largely unknown.

To gain entry into target cells, HIV-1 envelope glycoproteins must interact with the CD4 receptor and 1 of several possible coreceptors on the host cell membrane. The most common coreceptors used by HIV-1 are CCR5 and CXCR4. Viral strains using either of the 2 coreceptors or both are referred to as R5, X4, and dually tropic viruses, respectively. Most of the viral determinants of coreceptor use appear to be located within the hypervariable region V3 of gp120. One interesting feature of the early phases of HIV-1 infection is the predominance of R5 viruses within the replicating quasispecies detected in plasma. It is when disease progression and profound immune compromise occur that CXCR4–using variants start to emerge. Again, the events leading to the predominance of CCR5–using strains in the initial phases of HIV-1 infection remain incompletely understood, and both virus- and host-related factors are speculated to play a role [3].

Various rates of viral diversity early after infection have been previously described according to the mode of transmission and type of viral clade, both of which are considered to be important influencing factors. Viral diversity is, however, not limited to certain modes of transmission or types of clades [4]. In heterosexual cohorts, heterogeneous variants have been described after transmission with different HIV-1 clades in 10%–24% of participants. Data on men who have sex with men (MSM) report a higher rate of diversity, up to 36% in a study by Li et al [5]. In the case of injection drug users (IDUs), Bar et al have described multivariant transmission in 60% of participants, which is a higher proportion than that observed after sexual transmission, suggesting that mucosal
factors are important in the selection of viruses that establish productive infection [6].

In this issue of Clinical Infectious Diseases, a study by Rieder et al [7] uses molecular techniques, phylogenetic analyses, and bioinformatic tools to attempt to shed light on 2 issues: the degree of viral diversity characteristic of early HIV-1 infection and the preferential coreceptor use of the early replicating strains. The authors take advantage of a well-characterized cohort of patients with primary HIV-1 infection or recent HIV-1 infection based in Zurich. The cohort has prospectively enrolled 145 participants with a majority (73%) of MSM. Peripheral blood sampling was performed within 6 weeks (range, 2–18 weeks) and 12 weeks (range, 2–24 weeks) after the estimated time of infection in primary HIV-1 infection and recently infected participants, respectively. Although a variety of HIV-1 clades were represented, the majority (80%) of participants harbored a clade B virus.

HIV-1 diversity was studied by phylogenetic analysis of cloned gp120 envelope sequences derived from the first available plasma sample, focusing on 1 conserved and 2 hypervariable regions of the protein (C2–V3–C3). The median viral diversity was 0.39% (range, 0.04%–3.23%) but was higher (>1%) in 16 participants (11%). As could have been predicted, diversity increased with time since infection. No association was observed between increased diversity and plasma HIV-1 RNA load, the latter a marker of both the levels of virus replication as a driver of virus diversification and the extent to which the cloning technique was likely to provide a good representation of the quasispecies. No association was also detected between increased diversity and parameters known to potentially influence HIV-1 transmission, such as sex, presence of active genital infection, or mode of exposure. Of note, however, the number of participants with diversity >1% was too small to allow extensive statistical analyses of such associations. HIV-1 tropism was predicted from the V3 envelope sequences using multiple bioinformatic tools and, in a subset of patients, by phenotypic analysis of both plasma and cellular virus. There was good, although not complete, correlation between the 2 methods, as previously observed [8]. Concordant with the prevailing view, most participants harbored R5 strains, and only a small percentage of CXCR4-using viruses within a mixed population were detected in this cohort.

Most of the previously published data on viral diversity after transmission have come from smaller cohorts enrolling 10–102 persons with various risk factors for HIV-1 acquisition or from data pooled from independent cohorts. The Zurich cohort is a large single center–based primary HIV-1 infection observational study group, characterized by a detailed, prospective collection of a homogenous set of clinical and laboratory data and a rich sample repository. This has allowed the identification of a relatively large number of persons presenting with primary HIV-1 infection or recent infection, based on the review of both laboratory and clinical data, which improves the accuracy of estimations of the stage and timing of infection. This is a crucial requirement when comparing relatively small differences in genetic diversity. An adequate assessment of the duration of infection for these persons is of obvious importance to decrease the possibility that any substantial viral diversification simply reflects time since infection, which would prevent firm conclusions about the type of transmitted virus. In this study, the best data come from the 125 participants with acute infection, for whom the opportunities for miscalculating the timing of infection were minimized, although not abrogated.

Data from the Zurich cohort are largely representative of MSM infected with subtype B HIV-1. Overall, the proportion (11%) of participants with high diversity was lower than that recently reported in a similar cohort [5]. The data suggest the possibility of transmission of diverse viral variants through mucosal surfaces. However, certain caveats remain present. We cannot, for example, have absolute certainty about the modality of transmission, because this is generally self-reported and does not necessarily exclude other risk factors, such as associated IDU, which may facilitate transmission of a more heterogeneous virus population. Conversely, direct comparisons with IDU cohorts are complicated by the consideration that sexual transmission remains a strong possibility in IDU cohorts, as suggested by the high rate of associated sexually transmitted infections [9]. Technical issues should also be considered. There are recognized limitations to assessing viral diversity by clonal analysis, including in vitro–induced errors and, most important, incomplete representation of the quasispecies if only a few clones are sampled. Thus, further studies are needed using techniques, such as ultradeeep sequencing and other new-generation sequencing platforms, to obtain a more sensitive representation of the quasispecies (reliably to ~1% with ultradeeep sequencing). The number of clones analyzed in this article did not allow such a degree of sensitivity, and indeed, sampling of the dominant quasispecies was most likely with the methodology chosen. The fact that a subset of participants was also investigated by single genome sequencing with a good reproducibility of results, compared with cloning, means that the likelihood of in vitro–induced errors was low, but it does not imply that the representation of the quasispecies was wider. Prospective sampling would also have shed light on the rate of viral diversification characteristic to individual patients, whereas immunological studies coupled with virological observation are required to link the rate of viral diversification with immunological pressure.

The data provided in this article highlight how informative primary HIV-1
infection cohorts can be in terms of helping determine the degree of viral diversification at transmission, if sampling is started early enough after transmission, which remains challenging. The data underscore the need for further studies on the mechanisms of HIV-1 transmission. Meanwhile, increased awareness of clinical symptoms and use of appropriate diagnostic tools are required to improve the diagnosis of primary HIV-1 infection, thereby enabling the recruitment of large cohorts that may be followed up prospectively. In doing so we will be able to further elucidate the very early transmission mechanisms; monitor viral diversification and host factors, such as immune responses known to drive viral diversification; and translate this knowledge into preventative and therapeutic strategies.

**Note**

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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