Transmission of the X4 Phenotype of HIV-1: Is There Evidence Against the “Random Transmission” Hypothesis?

Charlotte Hedskog,1 Mattias Mild,1 and Jan Albert1,2

1Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 2Department of Clinical Microbiology, Karolinska University Hospital, Solna, Stockholm, Sweden

The links between human immunodeficiency virus type 1 (HIV-1) biological phenotypes, coreceptor use, transmission and disease progression have been the focus of intense research for 25 years. In 1986, Fenyo and coworkers [1] described an association between the biological properties of HIV-1 isolates and the severity of disease of the infected individual. They showed that primary HIV-1 isolates differ in their capacity to replicate in peripheral blood mononuclear cells (PBMCs) and tumor cell lines. Isolates that failed to infect cell lines generally replicated slowly in PBMCs and were named “slow/low,” whereas isolates that replicated efficiently in both cell lines and PBMCs were called “rapid/high.” Persons in the early, asymptomatic stages of the disease usually had slow/low HIV-1 isolates, whereas patients with AIDS often had rapid/high isolates. Tersmette et al [2] confirmed these findings and introduced an alternative terminology (syncytium-inducing and non-syncytium-inducing) to describe the same biological characteristics of HIV-1 isolates. The Dutch group also developed the MT-2 cell line assay as a convenient method to distinguish between the 2 biological phenotypes of HIV-1 [3]. Subsequent studies showed that MT-2–positive isolates occasionally are observed in patients with primary HIV infection, even though most asymptomatic patients have MT-2–negative isolates [4–7]. Furthermore, a switch from MT-2–negative to MT-2–positive isolates occurs in approximately 50% of patients during progression toward end-stage AIDS and is associated with an increased rate of CD4 cell decline [6, 7]. These findings were interpreted to suggest that MT-2–positive isolates are more virulent in vivo, and their appearance is both a cause and a consequence of disease progression.

The underlying cause for the phenotypic differences among HIV-1 isolates was elucidated by the identification of the coreceptors for HIV-1 entry into target cells (reviewed in [8, 9]). It was shown that MT-2–negative isolates exclusively use the CCR5 coreceptor (R5 phenotype), whereas MT-2–positive isolates use CXCR4 alone (X4 phenotype) or in combination with CCR5 (R5X4 phenotype) [8, 9]. R5X4 viruses are also called dual/mixed to signify that some assays do not distinguish between viruses consisting of truly dual tropic clones and those with mixtures of R5 and X4 clones [8]. Subsequent studies confirmed that X4/R5X4 variants are rare in early stages of disease but emerge during disease progression in approximately 50% of patients [8]. This coreceptor switch is associated with an accelerated rate of disease progression [8, 10]. The proportion of patients with X4/R5X4 variants during primary HIV infection (PHI) has been reported to be low (<10%) in most relatively large studies, based on phenotypic assays [11–14]. The rarity of X4/R5X4 variants during PHI has been interpreted by some researchers to indicate that (1) “only R5, and in a few instances R5X4 variants, but not X4 HIV-1 are transmitted” [9] and (2) “it is therefore reasonable to suggest a ‘gatekeeper’ that nearly always selects for transmission of R5 over X4 HIV-1” [9]. Thus, many studies have tried to find a biological bottleneck or “gatekeeper” (reviewed in [9]), but Schuitemaker et al [8] have argued that, in reality, no conclusive evidence has been provided to indicate that X4/R5X4 variants are less transmissible.

In recent years, studies on the coreceptor phenotypes of HIV-1 have been spurred on by the introduction of antiretroviral drugs that block the entry of R5 viruses through the CCR5 coreceptor (eg, maraviroc). Furthermore, new methodologies with high sensitivity and throughput provide novel means of studying HIV-1 populations and coreceptor use. These methods include ultradeep pyrosequencing (UDPS) to dissect the composition of
HIV-1 populations in patients [15–18]; recombinant virus assays for efficient determination of HIV-1 coreceptor use [15, 19–22]); and bioinformatic tools to predict coreceptor use from sequences of the V3 region of the HIV-1 envelope gene [23, 24]. Finally, there has been rapid development in phylogenetic methods that can be used to study HIV-1 transmission [25]. Despite these advances, there are still many unanswered questions concerning the transmission characteristics of X4/R5X4 HIV-1 variants.

In this issue of the Journal, Chalmet et al [26] provide valuable new information that is of relevance for understanding the transmission of X4/R5X4 variants. In a comprehensive study, they investigated samples from 593 patients and have conclusively shown that X4/R5X4 viruses can be transmitted. They use phylogenetic analyses to reveal epidemiological links between their study subjects and document 7 clusters of genotypically predicted X4 transmission. They documented the relatedness of the viruses in these clusters, as well as the clinical characteristics of the patients, and show that some X4/R5X4 transmissions are closely linked and probably represent direct transmission. Reports of such transmission pairs with X4 viruses are very rare. Importantly, Chalmet et al [26] have confirmed their genotypic coreceptor predictions with phenotypic testing on selected samples. This is highly relevant, because they find that only 4 of the 7 X4/R5X4 transmissions that were predicted using V3 sequences and the Geno2Pheno tool could be confirmed as X4/R5X4 by phenotypic testing. This finding is not entirely surprising because Geno2Pheno, as well as other bioinformatic approaches, is known to have a certain false-positive rate [11, 19, 22, 27].

In Geno2Pheno, the false-positive rate can be adjusted by the user and is typically set to 5.75% or 10%. These rates of false-positive X4/R5 calls are acceptable, and even advisable, in the setting of screening prior to maraviroc use [28], but they are problematic when Geno2Pheno is used to search for rare cases of X4/R5X4 transmissions. Consequently, studies of X4/X4R5 transmissions that are based on genotypic predictions should be interpreted with caution. The fact that Chalmet et al have identified the probable source of a few X4/R5X4 transmissions is also important, because it essentially rules out the possibility that the detection of X4/R5X4 variants is due to an early coreceptor switch in viruses that initially were transmitted with an R5 phenotype. Thus, Chalmet et al [26] have provided strong evidence for transmission of X4/R5X4 variants and have also shown that transmitting patients with X4/R5X4 virus regularly transmit X4/R5X4 virus, rather than R5 virus.

We feel that it is justified and important to challenge the widespread contention that there is selection against X4/R5X4 variants during HIV-1 transmission. We will do so by formulating a null hypothesis that states that transmission of R5 or X4/R5X4 variants occurs randomly from the pools of variants that are present in relevant body fluids of transmitting patients (in most cases genital secretions). Is it expected that a low proportion of X4/R5X4 transmissions will be observed under this "random transmission" hypothesis, or will it be necessary to invoke a "gatekeeper" of some type? First, we need to consider the patients who are the sources of new infections. Available data indicate that a substantial proportion of transmissions occur from source patients with PHI [29, 30]. These patients rarely have X4/R5X4 variants and therefore are unlikely to transmit such variants [4, 5, 11–14]. Remaining transmissions occur from chronically infected patients who might be in earlier or later stages of their disease, but it could be argued that the former patients probably are a larger source of new infections because they are more likely to be unaware of their infection. Analyses with sensitive phenotypic assays show that <10% of patients in earlier stages of the disease (CD4 ≥200 cells/µL) have virus with X4/R5X4 phenotype.

In late stages of the disease (CD4 <200 cells/µL) this fraction increases to 25%–50% [22]. Thus, most source patients do not have the possibility to transmit X4/X4R5 variant because they have only R5 viruses. Second, we also need to consider the proportion of X4/R5X4 variants relative to R5 variants in the body fluids of patients who have detectable levels of X4/R5X4 variants. This should be done in light of recent data that strongly indicate that most HIV-1 infections are established by 1 or a small number of infectious particles [13, 31, 32]. This means that according to the "random transmission" hypothesis, X4/R5X4 viruses will likely only be transmitted if they dominate the virus population of the donor. Several studies, which have employed highly sensitive methodology (ie, phenotypic assays and UDPS), show that R5 variants often outnumber X4/R5 variants by a factor of 5–10 in patients who test positive for X4/X4R5 virus in bulk testing [16, 19–21, 27]. However, it should be acknowledged that almost nothing is known about the proportions of R5 and X4/R5X4 variants in genital secretions. Given the facts mentioned above, it is hardly surprising that X4/R5 variants are rarely detected in patients with primary HIV infection. Thus, there is insufficient evidence to reject the "random transmission" hypothesis, and available data, in fact, lend support to the hypothesis. However, rigorous hypothesis testing is necessary and should, ideally, involve sensitive and accurate phenotypic determination of proportions of R5 and X4/R5X4 variants in genital secretions of transmitting patients. We would be surprised if such analyses provide evidence against the "random transmission" hypothesis.

References