Studies of Human Immunodeficiency Virus Type 1 Mucosal Viral Shedding and Transmission in Kenya

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If human immunodeficiency virus type 1 (HIV-1) vaccines are to be highly effective, it is essential to understand the virologic factors that contribute to HIV-1 transmission. It is likely that transmission is determined, in part, by the genotype or phenotype (or both) of infectious virus present in the index case, which in turn will influence the quantity of virus that may be exchanged during sexual contact. Transmission may also depend on the fitness of the virus for replication in the exposed individual, which may be influenced by whether a virus encounters a target cell that is susceptible to infection by that specific variant. Of interest, our data suggest that the complexity of the virus that is transmitted may be different in female and male sexual exposures.

This review summarizes recent studies from our group that focus on analyses of both the potential reservoir of transmitted variants in the index case as well as the viruses found early in infection in the new host. This is not meant to be a comprehensive review of human immunodeficiency virus type 1 (HIV-1) transmission, and for this reason, many important studies in this area that were performed by other investigators are not included in this short summary. The studies described here specifically focus on individuals in Kenya who are infected with HIV-1 variants from multiple subtypes; the majority of infections are with clade A HIVs, but clade D and C viruses are also present in a significant minority of the population [1] (unpublished data). These HIV-1 clades are common in many areas in sub-Saharan Africa, and thus our studies may be of general relevance for understanding HIV-1 transmission in this region where the HIV-1 pandemic is most severe.

Origin of Transmitted Virus

It is likely that transmission is determined by the genotype and phenotype of infectious virus present in the index case, which in turn will influence the level of virus replication and consequent virus burden. In particular, the qualities and quantity of virus in mucosal secretions could influence the probability of sexual and vertical transmission. The quantity of HIV-1 as measured by analysis of either viral RNA in virions or cells (or both) or cell-associated proviral DNA is often used as a surrogate marker for infectious virus. Proviral DNA provides an archive of successful infection events, whereas cell-free viral RNA measures the pool of viruses that can potentially initiate new rounds of infection. In the case of HIV-1 transmission, it is unclear whether cell-free or cell-associated virus is most important in transmission. For retroviral infections in animal models and for other human retroviral infections, both cell-free and cell-associated virus have been implicated in transmission. Thus, it may ultimately be important to evaluate the association of both cell-free and cell-associated virus with HIV-1 transmission.

HIV-1 infection through breast-feeding remains a major route of vertical transmission, and both HIV-1 infected cells and virion RNA can be detected in breast milk. Studies of viral DNA and RNA in breast milk demonstrate a trend for detection of free virus in breast milk samples that have a higher number of infected cells [2]. Of interest, the number of breast-milk cells that are infected by HIV-1 is as high or higher (up to 1 in 3 infected cells) than in blood [3], whereas the levels of virion RNA are much lower (from ≤240 copies to 8100 copies/mL) than in plasma [2]. This may reflect differences in HIV expres-

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sion in the target cells in which virus is replicating in peripheral blood versus breast milk, or it may reflect the fact that plasma virus may originate from a much larger systemic pool, including lymph node cells. High levels of infected breast-milk cells were correlated with lower CD4-positive T cell numbers, and in immunosuppressed women there was an increased prevalence of HIV-1–infected cells in breast milk when the women were vitamin A deficient [3]. The quantities of both DNA and RNA were higher in mature milk than in colostrum, suggesting that the infant continues to be exposed to significant quantities of HIV-1 while being breast-fed. However, these studies did not attempt an in-depth analysis of the temporal patterns of HIV-1 shedding in breast milk, and this, along with further analyses of the interrelationship of viral DNA, RNA, and other cofactors in breast-feeding transmission, will be important in advancing our understanding of the timing and mechanisms of postpartum HIV-1 transmission.

We have also examined the correlates of cervical and vaginal shedding in both pregnant and nonpregnant women, focusing initially in these studies on shedding of HIV-1 infected cells. There were several correlates of shedding that were similar in genital mucosal secretions compared with breast milk, and women who shed HIV-1–infected cells in mucosal secretions were more likely to shed infected cells in their breast milk. There was also an association between vaginal DNA shedding and vitamin A deficiency in both pregnant and nonpregnant women. For example, in nonpregnant women, severe vitamin A deficiency was associated with a 12.9-fold increased odds of vaginal shedding after adjusting for the CD4 cell count [4]. In both groups, markers for immunosuppression correlated with cervical as well as vaginal shedding [4, 5]. Together, these data suggest that factors such as immunosuppression, which are associated with an increased systemic virus load, are also associated with increased local virus load. In fact, in a small group of 17 women, shedding of infected cells from the genital tract was found to be more common in women with a higher plasma viral RNA level [6]. Thus, replication of virus in the mucosa may be influenced directly by the amount of virus in the periphery, perhaps as a result of continued virus trafficking. Alternatively, there may be higher levels of local virus replication or impaired immune surveillance at mucosal sites in immunosuppressed or vitamin A–deficient hosts.

The presence of other genital tract infections, such as gonococcal cervicitis and vaginal candidiasis, as well as abnormal cervical and vaginal discharge, have been associated with increased shedding of infected cells from the cervix and vagina [4, 5]. Cervical shedding of HIV-1–infected cells has been linked to the use of hormonal contraceptives, including both injectable progesterone and oral contraceptive pills [4]. Of importance, a dose effect was observed in women who used low-dose versus high-dose oral contraceptive pills [4]. Taken together with studies showing an increased susceptibility to infection in high-risk HIV-1–seronegative women using injectable progesterone [7], these data may suggest that female sex steroid hormones promote an environment in the genital mucosa that is more favorable for HIV-1 replication. However, analyses of genital tract shedding throughout the menstrual cycle did not demonstrate a cyclic pattern of HIV-1 shedding in response to normal hormonal fluctuations in women [6]. Instead, this study suggested that there are some women who are more likely to shed HIV-1–infected cells, and these women generally have lower levels of CD4-positive lymphocytes and higher systemic viral load [6].

It remains to be established whether increased amounts of virus or infected cells in mucosal secretions are associated with increased transmission. If systemic virus replication provides a model for mucosal virus replication, then higher levels of virus may reflect emergence of a predominately T cell–tropic virus population in the mucosa. On the basis of current models for transmission to men and infants, such viruses may be predicted to be less efficiently transmitted. However, the predominance of T cell–tropic variants may be counterbalanced by higher quantities of virus, a portion of which perhaps remain macrophage tropic or dual tropic. Moreover, there are little or no data on the phenotype of viruses transmitted to women, and it is possible that T-tropic viruses may contribute to infection in women exposed to HIV-1 through sexual contact.

Thus, despite the intuitive appeal of models linking increased viral shedding with transmission, it will be important to directly examine whether higher levels of virus in mucosal secretions correlate with an increased chance of transmission. Such studies are extremely difficult because sampling of the index case near the time of transmission is generally impractical, especially in the case of sexual transmission. However, this model can, at least in part, be tested in the setting of vertical transmission where the index case can be clearly identified and the timing of transmission can be estimated and samples obtained. In the context of such studies, it will be important to analyze the relationship of viral phenotype and genotype on replication and shedding of HIV-1 and the impact of these, independently and collectively, on transmission.

Studies of Transmitted Viruses

To develop strategies to prevent the spread of HIV-1, it is important to identify and characterize the viruses that are transmitted, particularly in areas of high incidence. These analyses may illuminate the properties of the virus that determine its fitness for transmission, define the earliest target cells for virus replication, and advance our understanding of the mechanisms of transmission. In addition, prototype transmissible isolates and clones of HIV-1 obtained from such studies may provide potentially useful strains for vaccine design.

Much of what is known about transmitted variants has been gleaned from analysis of viruses in persons who present with acute symptoms due to primary viremia or of viruses from

individuals when they first test positive for HIV-1–specific antibodies. From analyses of viruses present at first detection of HIV-1 antibody, we have found that the virus population that is transmitted to women in Africa who acquire HIV-1 through heterosexual contact is typically heterogeneous [8]. A heterogeneous virus population was detected in most of the ~20 women we have analyzed. In contrast, in our studies (unpublished data) and in studies from other groups, only 1 virus genotype has been detected in men infected through sexual contact with men or women and in infants infected vertically. Thus, it appears that women demonstrate more complexity in the virus population that is transmitted, whereas men and infants are similar in being persistently infected by a single virus (figure 1).

Phylogenetic analyses demonstrated that the detection of multiple virus genotypes in women at seroconversion was not the result of reinfection by different partners or dual infection by viruses from different subtypes [8]. We also assessed whether factors that are known to increase the risk of HIV-1 acquisition, such as intercurrent sexually transmitted diseases, might correlate with infection by multiple viruses, but no association was found in the initial study of 6 women or in ongoing studies of a larger sample size [8] (unpublished data). The presence of multiple variants early in infection was not specific to blood: A similar diversity was observed in the viruses from cells present in cervical secretions [8]. However, in individuals chronically infected with HIV-1, there appears to be some compartmentalization of viruses in blood versus the mucosa [9, 10].

It is important to keep in mind that our models of virus transmission are based largely on analyses using samples that likely were obtained from several weeks to several months after the infectious exposure. We infer from studies like these that

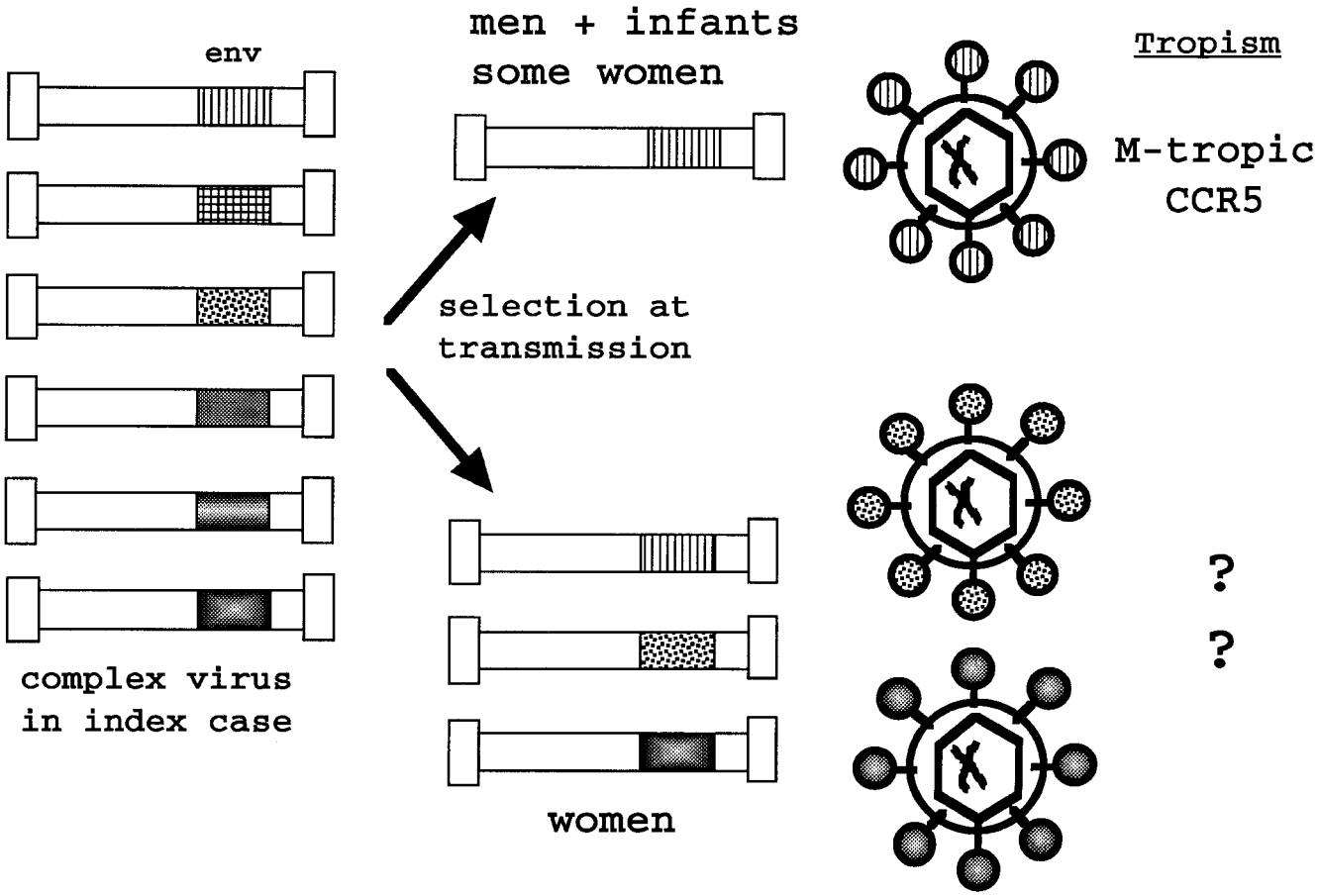

Figure 1. Schematic representation of virus population that is selected for transmission. Different proviral genomes are depicted schematically, and within each provirus, envelope genes (env) are distinguished by different patterns. Envelope is specifically highlighted because most analyses of diversity in viruses that are transmitted have focused on the env gene, in part because it is critical viral determinant of host cell tropism. Index case in this example has complex virus population, depicted as 6 proviral genomes with different env genes. Selection at transmission leads to infection with single variant in men and infants and some women, and this is typically macrophage-tropic (M-tropic) virus that can utilize CCR5 chemokine co-receptor for entry. Question marks indicate that tropism and co-receptor specificity of viruses transmitted to women infected by multiple variants is not known.

the viruses present at documented seroconversion have not varied significantly from the infecting virus, and this has been shown to be the case for studies in macaques infected with simian immunodeficiency virus (SIV) derived from a proviral clone [11]. However, little is known about how the virus populations fluctuate over time if persistent infection is initiated with multiple variants. Thus, it is important to keep in mind the limitations of these studies when developing models for HIV-1 transmission and replication. Studies in the SIV and simian-human immunodeficiency virus (SHIV) macaque models have provided some insights into the early events in the newly infected host, but these studies also have limitations that require that they be interpreted with caution. For example, it is unclear whether the SIV or SHIV strains used for these studies would ever be naturally transmitted during sexual contact. Thus, there remains a gap in our information of the genotype and phenotype that initiate a productive infection in a new host, and it is unclear whether the bottleneck in viral diversity between the index case and the newly infected host occurs during the stage of initiating productive infection of the first target cells in the host or whether the bottleneck occurs in dissemination to the lymphatic system. Differences in fitness at either stage could be responsible for the differences in transmitted variants between men and women.

The observation of viral heterogeneity at seroconversion serves as a reminder that the current models of transmission derived largely from studies of men and infants, which propose that a single macrophage-tropic virus is selectively transmitted from the index case to the new host, must be applied specifically where they are applicable. Indeed, very little is known regarding the viruses that are transmitted to women, and it is important that the paradigms used for designing strategies against HIV-1 infection take into account the differences in the patterns of transmission to women versus men.

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