HIV-1 Transmission Biology: Selection and Characteristics of Infecting Viruses

Manish Sagar
Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts

Individuals with recent human immunodeficiency virus type 1 (HIV-1) acquisition are likely to be a major source for other new infections because they have a high level of plasma virus, and the circulating virions possess unique properties that are highly suited for transmission. The acute infection period, however, presents a unique “window of opportunity,” because there are a limited number of genetic variants. Studies aim to elucidate the nature of the transmitted viruses and understand the mechanisms that inhibit the majority of variants present in the chronically infected partner from establishing a productive infection in the naive host. Greater understanding of these issues may open promising new ways to effectively block HIV-1 transmission.

IMPORANCE OF SOURCE SUBJECT IN THE BIOLOGY OF TRANSMISSION

In the absence of a highly effective human immunodeficiency virus type 1 (HIV-1) vaccine, interventions to reduce HIV-1 transmission have been primarily targeted at seronegative subjects. For instance, persons without HIV-1 infection have been counseled to abstain from risky behavior, remain in monogamous relationships, and regularly use condoms. Already-infected subjects, who serve as the source of the virus for all incident infections, are also the focus of secondary prevention, primarily through education and counseling strategies. A recent innovative mathematical model proposes that universal testing and immediate treatment could dramatically reduce HIV-1 incidence [1]. Because studies have concluded that HIV-1 transmission is directly correlated with the level of virus circulating in the source subject, it has been hypothesized that mass treatment would reduce viral replication and thus lower the rate of HIV-1 transmission [2, 3].

The success of these types of public health interventions, however, is highly dependent on identifying and characterizing the source subjects. One important issue is whether source subjects are primarily in the acute, asymptomatic chronic, or late stage of disease when they transmit their virus. Because subjects with recently acquired HIV-1 are both unlikely to be aware of their infection and have infection that is more difficult to diagnose than prevalent infection, acutely infected subjects are less likely to be under medical care. Therefore, further transmission from these subjects cannot be prevented by counseling, education, and/or highly suppressive antiretroviral therapies. Furthermore, recently infected subjects have a high level of virus replication and thus are potentially more infectious than infected individuals in other phases of disease. Therefore, besides the importance of primary HIV-1 infection in secondary prevention strategies, the acute infection period holds special significance for HIV-1 transmission biology.

Various studies using diverse methods suggest that anywhere from 5% to 50% of new HIV-1 infections are acquired from newly infected subjects [4–11]. Differences among the population under study are partially responsible for the drastically varied estimates. The proportion of new infections due to transmission from acutely infected subjects varies among different cohorts because it depends on the prevalence of HIV-1 infection...
and other sexually transmitted diseases, the predominant route of HIV-1 acquisition, the frequency with which subjects engage in risky behavior, and many other population-specific factors. Inherently, however, an acutely infected individual must have ≥2 separate relatively concurrent partners to transmit HIV-1 to a naive subject. Interestingly, incorporating data about concurrency of sexual partnerships among subjects in Zimbabwe, Goodreau et al suggested that the HIV-1 infection epidemic may extinguish if all relationships occur sequentially rather than concurrently [12]. In addition to highlighting the importance of the acute infection period for sustaining the HIV-1 epidemic within the Zimbabwe cohort under study, this modeling again suggests that the early infection phase harbors special biological significance for HIV-1 transmission.

ROLE OF PLASMA VIRUS LEVELS AND VIRUS PHENOTYPE IN HIV-1 TRANSMISSION

The importance of the acute infection period for subsequent transmission events is related to both the level of plasma virus during primary disease and potentially virus-specific properties. Immediately after acquiring HIV-1, individuals have a burst of virus replication. Because the rate of HIV-1 transmission is proportionally related to the level of circulating plasma virus [2, 3], it has been surmised that this virus peak during primary HIV-1 infection is responsible for the estimated increased infectiousness of acutely infected subjects [6]. During primary infection, however, the level of infectiousness per potential transmission event is much higher than would be predicted from the high plasma virus levels alone. In other words, the estimated transmission frequency is much greater than would be expected as a function of the number of circulating viruses alone [7]. These calculations have suggested that other factors beside the high level of virus replication early after HIV-1 acquisition are responsible for the increased ability to transmit HIV-1 during primary infection.

Nonhuman primate studies have further bolstered this conclusion. In rhesus macaques, simian immunodeficiency virus (SIV) virions isolated during acute infection are more infectious than those from the chronic phase of infection. Naive macaques (SIV) virions isolated during acute infection are more infectious than those from the chronic phase of infection. In rhesus macaques, simian immunodeficiency virus (HIV-1) during primary infection. Acute-stage viruses are different was implied in some landmark studies conducted >15 years ago [14–16]. By examining virus sequences from heterosexual, homosexual, mother-to-child, and percutaneous transmission pairs, these studies elucidated the nature of the transmitted viruses compared with those present in the source partner. Collectively, they showed that a newly infected subject acquires a limited number of the variants circulating in the transmitting donor. In fact, these diverse studies concluded that often new HIV-1 seroconverters were productively infected with a single virus. Furthermore, the transmitted virus was often a minor variant among the quasispecies present in the source subject. Follow-up investigations showed that newly infected subjects do not always have only a single HIV-1 strain early in infection, although viral genotypic diversity is still limited during the acute phase of the disease [17] (Figure 1). Investigations of a larger number of subjects suggest that anywhere from 10% to 60% of newly infected individuals acquire multiple variants [18–21]. The differences in these percentages can be explained by the fact that biological cofactors such as sexually transmitted infections and hormonal contraceptive use affects the diversity of the infecting virus population [20, 22]. In aggregate, these more recent investigations have further solidified the conclusion that although a source partner may have diverse viruses, naive subjects are productively infected by a small number of variants, often only one.

The easiest explanation for these observations could be that

**Figure 1.** Examples of predicted amino acids for the hypervariable V3 loop sequences from a subject infected with 1 HIV variant (A) and an individual who acquired ≥2 viruses (B), are shown. Dots, the same predicted amino acid among the various sequences in the alignment; *stop codon. The proportion of clones with a given sequence is indicated to the right of the sequence. Adapted from Poss et al [17].
the bottleneck during transmission is a stochastic event. In other words, from the quasispecies circulating in the chronically infected host, one variant or a small number of variants successfully establish an infection in a naive host by random chance alone. The infecting virus, however, is often different from the predominant variant present in the transmitting partner, which argues against this random model of transmission. The notion that chance alone may dictate which variants establish a beachhead in a naive subject, however, cannot be easily dismissed only by this observation, because sequences present in the source for the virus (eg, breast milk, semen) are often not examined, and sampling does not occur at the exact time of transmission. Because HIV-1 diversifies at a rapid rate, the virus being sampled weeks to months after the acquisition event may not reflect what was present at the time of transmission within the source partner.

Other genotypic and phenotypic evidence, however, bolsters the hypothesis that active selection of specific variants occurs during HIV-1 transmission. One early piece of evidence was the observation that although source subjects may harbor viruses that can use both chemokine (C-C motif) receptor 5 (CCR5) and CXC chemokine receptor 4 (CXCR4) receptors for host cell entry, variants that use CCR5 (R5) are nearly exclusively acquired by a newly infected subject [16, 23, 24]. R5 viruses predominate early in infection during all modes of transmission, even among routes that present minimal barriers against acquisition, such as injection drug use [25]. This argues that CXCR4-using variants (X4) are counterselected during transmission. Furthermore, among the diverse R5 viruses circulating in the index subject, variants with sequences closer to the ancestor virus are found in newly infected individuals [26–28]. Over the course of infection, viruses diverge away from the infecting founder virus population [29], but during transmission, variants more closely related to the ancestral sequence are acquired, suggesting preferential selection and acquisition of a minority as opposed to the predominant variants from a chronically infected host. The greater likelihood of acquiring variants with more similarity to the ancestral sequence again suggests that evolutionary changes within a host decrease transmission fitness.

More support for an active selection model was provided by studies showing that newly acquired variants often have shorter and/or less glycosylated envelope glycoproteins than those present in the transmitting partner or among chronically infected subjects [26, 27, 30, 31]. Although this has not been observed in all HIV-1 subtypes or among all routes of HIV-1 acquisition [30, 32], the predominance of compact and less glycosylated envelopes early in infection argues that these envelope genotypic properties confer fitness for transmission. After acquisition over the course of infection, glycosylation sites increase in length and/or number in some HIV-1 subtypes [33, 34]. During the next transmission event, however, envelope length and level of glycosylation reset close to the original baseline. This observation, along with the finding that newly acquired viruses are more closely related to ancestral sequences compared with the predominant variants circulating in a chronically infected host [26–28], further implies that adaptation within a host is counterproductive for transmission efficiency. One way compact and less glycosylated envelopes could be favored for transmission is by possessing higher affinity or requiring a lower concentration of the host cell receptor for productive infection. Thus, viruses with these envelope properties could be favored for transmission at the site of invasion if the number of target cells or the receptor density is limited in the initial susceptible cells.

To date, investigations have failed to demonstrate any clear evidence that early infection variants are better at infecting certain cell types or have a greater ability to use cells with low CD4 or CCR5 densities, compared with chronic-phase isolates, such as those present in the transmitting partner [35–37]. In fact, early viruses are more susceptible to CCR5 receptor and fusion inhibitors than chronic-stage viruses [36, 38], suggesting that the acquired variants have a greater CCR5 receptor requirement and slower fusion kinetics. In aggregate, the observations cited here suggest that specific variants are favored for acquisition, but identification of a viral phenotypic property that confers an advantage during transmission would provide the greatest support for a biological model of active selection during transmission.

**BIOLOGICAL MECHANISMS FOR SELECTION DURING TRANSMISSION**

Acquisition of a limited number of viruses during transmission could potentially occur by various nonexclusive mechanisms. Source fluid has been examined from transmission pairs to discover the origin of the newly acquired HIV-1, which could provide important insights into what contributes to the bottleneck present during transmission. Butler et al suggest that, in 6 homosexual couples examined to date, the newly infected subject’s HIV-1 originates from the viruses within the cell-free seminal plasma and not the cells present in the donor’s semen [39]. This finding, however, has not been corroborated by other investigators who have also examined various cell-free and cell-associated genital fluids from sexually active transmitting couples [40]. As noted above, results from these types of investigations are complicated because of issues regarding the timing of the sampling. Furthermore, extrapolation from diverse clinical, animal, and in vitro studies suggests that cell-associated virus is more relevant during transmission than cell-free virus [41]. In culture, HIV-1 dissemination occurs more efficiently during cell-to-cell contact than during cell-free spread [42–44]. Cell-to-cell transfer may enhance HIV-1 transmission by con-
centrating the relevant receptors and accelerating the rate-limiting step of infection, namely, entry within the host cell [45–47]. Thus, cell-associated virus in various infectious fluids may have higher transmission capacity than cell-free virus.

Interestingly, recent statistical estimations suggest that multiple viruses are not acquired as independent low-probability events but rather that acquisition of one virus is linked to the other infecting variants [48]. Transmission from a cell that harbors multiple HIV-1 variants could be an explanation for this finding [49]. Examination of cells and cell-free fluid from semen, cervical samples, and breast milk, however, has failed to consistently demonstrate that these potential transmission sources harbor primarily R5 viruses with limited genetic diversity, whose sequences are closely related to the ancestor strains [17, 50–54]. Because transmission fluids do not harbor a predominance of variants with properties observed among the newly acquired viruses, the bottleneck during transmission probably does not occur in the transmitting partner. It may be possible that a distinct compartment within a donor’s infectious fluid contains predominantly CCR5-using viruses with low genetic diversity and sequence identity close to that of ancestor strains, but this compartmentalized infectious source has not been identified to date.

The bottleneck during transmission could also occur if virus acquisition or generalized dissemination is restricted at the site of invasion. For mucosal transmission, HIV-1 must cross an epithelial barrier and then find, attach to, and invade a susceptible target cell. The infecting variant must then replicate in the infected cell and disseminate from the initial site of attack to create a systemic generalized infection. Selection for specific HIV-1 variants during transmission could occur at any of these challenging barriers. Unfortunately, our basic knowledge about how the virus overcomes these hurdles is quite limited. Intuitively, it seems that the virus would have the easiest time reaching susceptible cells at places with minimal barriers, such as the endocervix, the transformation zone between the ecto- and endocervix, or the rectal mucosa, where a single epithelial layer separates the luminal surface from the potential target cells. On the other hand, the vaginal mucosa is covered by multiple layers of squamous epithelia, which provide a greater impediment in accessing susceptible cells. This idea may underlie the increased rate of HIV-1 acquisition observed in women with cervical ectopy, in which single epithelial layers replace the normally multilayered cervical ectocervix [55]. Similarly, the foreskin’s relatively thin layer of keratinization may provide a lower barrier to infection than the glans penis or the outer foreskin [56]. The decreases in HIV-1 transmission observed in the male circumcision trials may, in part, relate to the elimination of this easily breached pathway [57–59]. Furthermore, microulcerations from syphilis and herpes simplex virus infection may compromise the physical barrier between the virus and the initial target cells, which may be the basis for the both the increased transmission frequency and the higher likelihood that a newly infected subject will acquire multiple HIV-1 variants in the presence of a sexually transmitted disease, as opposed to just one [20, 22, 60]. Although the ability of different mucosal surfaces to limit access to susceptible cells is probably an important component of the genetic restriction observed early in infection, lack of a cervix, use of a diaphragm, or circumcision do not provide complete protection against HIV-1 acquisition [57–59, 61–63]. Therefore, HIV-1 can reach susceptible cells through less permeable barriers. Furthermore, it should be noted that severely restricted viral genetic diversity has been demonstrated in subjects infected through injection drug use [25, 64, 65] and transfusion of infected blood products [14]. Thus, it is unlikely that the mucosa alone prevents the majority of variants circulating in a chronically infected index subject from infecting a naive individual.

The availability and infectability of early target cells may also be important in maintaining the severe genetic restriction during all forms of HIV-1 transmission. T cells, macrophages, and/or cells of the dendritic lineage, such as Langerhans cells, are likely to be the first target cells encountered by the virus in HIV-1 sexual transmission [66–71]. The density and spatial distribution of these cell types are quite varied in different anatomic tissues, and the ability of the virus to interact with the appropriate cell type may restrict early infection events [41]. For instance, a recent study demonstrates that HIV-1-susceptible cells persist close to the epithelium even after treatment has healed herpes simplex virus–associated ulcers [72], which provides a potential mechanism for the inability of acyclovir treatment to decrease the rate of HIV-1 acquisition [73]. After physical contact between the virus and an early target cell, productive infection may be limited owing to specific viral or host factors. For example, transmitted viruses are poor at entering and replicating in macrophages, suggesting that the early infection envelope glycoproteins are unable to initiate entry with the number or types of cellular receptors present on these cell types [35, 36, 74]. Furthermore, mucosal immune defenses, such as microbicidal defenses, antimicrobial peptides, secretory leukocyte protease inhibitor, and others, restrict the ability of the virus to infect early target cells [75, 76]. Estrogen and progesterone influence both the mucosal defense strategies and susceptibility of potential target cells [77–80], which may explain how menstrual cycle changes and exogenous hormone use affect HIV-1 acquisition, replication, and the bottleneck during transmission [22, 81].

INVESTIGATIONS OF EARLY EVENTS DURING HIV-1 INFECTION

To investigate questions about the early events in mucosal HIV-1 acquisition, researchers have often performed in vitro ex-
Limited foci of simian immunodeficiency virus (SIV)–positive cells in >40 endocervical sections 4 days after intravaginal inoculation. Black-silver grains denote SIV-positive cells in an endocervical section (A) (magnification: main image, ×10; inset, ×40). Adapted from Li et al [88].
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