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HIV Transmission Biology: Translation for HIV Prevention

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Abstract

Rigorous testing of new HIV prevention strategies is a time-consuming and expensive undertaking. Thus, making well-informed decisions on which candidate prevention approaches are most likely to provide the most benefit is critical to appropriately prioritizing clinical testing. In the case of biological interventions, the decision to test a given prevention approach in human trials rests largely on evidence of protection in pre-clinical studies. The ability of pre-clinical studies to predict efficacy in humans may depend on how well the model recapitulates key biological features of HIV transmission relevant to the question at hand. Here, we review our current understanding of the biology of HIV transmission based on data from animal models, cell culture, and viral sequence analysis from human infection. We summarize studies of the bottleneck in viral transmission; the characteristics of transmitted viruses; the establishment of infection; and the contribution of cell-free and cell-associated virus. We seek to highlight the implications of HIV transmission biology for development of prevention interventions, and to discuss the limitations of existing pre-clinical models.

Keywords

HIV transmission; HIV prevention; Early viral events; Transmission bottleneck; Transmitted viruses

Introduction

Rational design of biomedical HIV prevention interventions relies on detailed understanding of the mechanisms of HIV transmission. In order to be efficacious, such interventions must target critical vulnerabilities of the virus(es) that seed initial infection, at the site where initial infection occurs, and during the window between exposure and establishment of the viral reservoir when infection can be prevented. Defining these targets poses significant methodological challenges, because studying the earliest events between HIV exposure and infection at the portal of entry has not been possible in humans. Rather, the best we can do in humans is study ‘early/acute’ HIV infection once the initial founder virus is acquired, amplified and disseminated systemically and readily detected in blood. By this time, given HIV’s rapid spread within the host and sequence evolution – estimated at up to 1% per year

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in some gene regions [1,2] – seeding of lymphoid tissues and viral sequence evolution has already occurred. Therefore, much of our understanding of the earliest events in infection comes from experimental studies in the macaque model, analysis of virus replication and spread in cell culture and tissue explant models, and sequence and biological studies of early HIV variants and the inferred transmitted/founder virus. Each of these types of studies has inherent strengths and limitations, which are often not clearly delineated or even agreed upon. Consequently, translating data from studies in model systems in order to evaluate the promise of a novel preventative strategy in humans, while essential for prioritizing candidates for clinical testing, can be difficult.

In this review, we discuss our understanding of the biology of HIV transmission and its implications for HIV prevention research, considering some of the key features of existing model systems that must be borne in mind when interpreting pre-clinical data.

Initial interactions and early events in transmission

Much has been learned about the earliest events in HIV infection by experimental infection of macaques with SIV or chimeric SIV/HIV viruses (SHIVs) encoding HIV envelope. Unlike human infection, this model allows virally infected cells to be detected *in situ* within the first days of infection, providing insight into the anatomical sites and cell types where infection is seeded, and therefore where prevention approaches should target. Most SIV/ macaque studies have focused on cervicovaginal transmission, and have implicated memory CCR5+ CD4+ T-cells in the lamina propria of the endocervix as the earliest target cells of SIV infection [3-5]. Productively infected cells can be detected at this site within 2-3 days of virus challenge and local foci of infection soon thereafter. From there, virus dissemination occurs to the regional lymph nodes, which spread the virus to secondary lymphoid tissues including gut-associated lymphoid tissue (GALT).

A recent study suggested that early target cells may be more broadly distributed in the female genital tract than previously appreciated [6]. In this study, rather than using SIV, a SHIV was used to infect macaques, and in these animals, cells in the ectocervix and vagina were the prominent site of infection. However, foci of viral infection were also detected across various tissues in the female genital tract including the ovaries, endocervix and local draining lymph nodes. A potential implication of these observations is that prevention efforts must target cells throughout the female reproductive tract. However, this study also serves to highlight a key limitation in extrapolating from non-human primate (NHP) SIV/ SHIV models to HIV infection in humans: the degree to which the experimental viral strain faithfully recapitulates the characteristics of circulating HIV. Rational development of SHIVs that accurately mimic HIV transmission in humans has been challenging, because circulating HIV variants don't use the macaque CD4 (mCD4) receptor for entry [7,8]. Therefore, most commonly used SHIVs are derived using adapted HIV envelope variants, passaged in culture and in animals, in order to permit entry using mCD4 [9,10]. For example in the study by Stieh et al., the envelope used to generate the SHIV was a lab-adapted HIV isolate (JRFL) from brain that shows broad tropism. Adapted viruses are biologically and antigenically distinct from circulating transmitted variants that establish infection in humans [11] (see the discussion of the characteristics of transmitted variants below). It is therefore

difficult to know whether HIV transmission is best captured using SHIVs based on HIV envelopes that have been adapted to macaques or using a more distantly related virus such as SIV. Furthermore, most of the data from studies involving macaques infected with SIV or SHIVs comes from findings with one SIV strain. This strain, SIVmac251 [12], and a cloned virus derived from it SIVmac239 [13], became the focus of early studies largely because it was pathogenic, rather than because it embodies any particular features of HIV spread in humans. Given the similarities in the overall disease course between macaques and humans, there are likely to be several aspects of early infection that are similar despite these sources of variability. But different findings related to early events in SIV and SHIV infection also serve as a reminder that there may be unique features to each virus/host interaction and, unfortunately, there is no way to know exactly which findings translate from macaques to humans.

The relevant target cells in non-cervicovaginal forms of transmission are less well defined, although more studies are being developed in this area [4,14-16]. It is therefore important to remember when evaluating pre-clinical data that findings from one mode of transmission may not be generalizable to other modes.

One theme that has emerged from these studies is how rapidly virus disseminates from the initial site(s) of infection. Within a week of SIV challenge, virus can be detected in secondary lymphoid organs and distal tissues, and by two weeks the virus is fully disseminated to various tissues and can be detected in blood, signaling the end of the eclipse phase and establishment of systemic infection [3,17-19]. Importantly, a recent study of SIV infected macaques that initiated antiretroviral treatment as early as 3 days after infection suggests that the viral reservoir may be at least partially established within this 3-day period in this model [20]. These findings suggest that the reservoir may be established even earlier than appreciated in humans [21,22], where it has not been possible to intervene so soon after infection to more precisely define this window. While the kinetics of infection are somewhat more rapid in the SIV/macaque model than in HIV infection in humans [23], this nonetheless implies that the reservoir is established very rapidly, before virus can be detected in blood. Thus, there is a narrow window after exposure, before the reservoir is established, to prevent infection.

The HIV transmission bottleneck

Studies of the genetic and phenotypic properties of transmitted viruses have shed light on early events in HIV transmission, based on the premise that these properties likely indicate specific interactions or selective forces during the transmission process. Studies of HIV sequences during acute/early infection suggest that there is a bottleneck in the virus population during transmission. The transmission bottleneck has been observed among different cohorts and for all routes of HIV transmission [23,24]. These studies show that in contrast to the extensive sequence diversity present during chronic infection, the sequence diversity in acute infection is much more limited: typically only one or a few genetic variants of HIV can be detected [23,24].

In most new infections, just one genetic variant is detected but, in a subset of individuals, infection is established by more than one variant from the source partner and this correlates with the presence of sexually transmitted infections (STIs) and use of hormonal contraceptives (HCs) [25,26]. Because both STIs and HC use are associated with increased risk of HIV acquisition [27-29], these findings suggest that the factors that increase overall susceptibility to infection also increase the likelihood of transmission of multiple viruses. Since the prevalence of these factors varies between populations, the fraction of people infected with more than one virus is also variable. It may also depend on whether transmission is primarily driven by newly infected donors, who themselves harbor a single virus variant, or people with well-established infections, who harbor more diverse viruses. Estimates from larger studies are that 24-63% of individuals are infected with multiple viruses, depending on the cohort [30,31]. On the basis of these findings, some studies have used the number of genetically distinct viral lineages detected soon after infection as a marker of susceptibility to infection, or efficacy of an intervention, although the validity of this approach has yet to be well established.

A limitation of sequence studies of early viruses from infected humans is that it is difficult to determine whether the viral bottleneck that occurs during transmission reflects selection during infection of the first target cell (i.e. one cell is infected and the virus(es) produced by this founder cell goes on to seed the infection), or whether the selection is primarily applied during the process of viral dissemination (multiple target cells become initially infected, but selection for one or a few of these variants occurs during virus amplification and dissemination to distal tissue). It may be possible to distinguish these models in experimental infections of non-human primates with a known mixture of variants, or in humans by using deep sequencing methods applied to genital and blood samples collected from cohorts with very frequent monitoring to detect new infections.

Understanding the nature and determinants of transmission bottleneck is important for HIV prevention efforts because if the bottleneck simply reflects the low probability of transmission, there may be nothing particularly special about the transmitted viruses. However, if the transmission bottleneck is shaped by selection pressure that determines the probability of transmission occurring, then this may present places to target biological interventions. Furthermore, defining the bottleneck can inform development of more faithful pre-clinical model systems. For example, in many macaque studies, a high dose inoculum is used because it is critical that a high percentage of animals become infected upon virus challenge. However, using a high dose inoculum results in transmission of multiple viruses. In contrast, a repeated low dose inoculum results in establishment of infection with a single or few transmitted viruses thus limiting the number of infected animals. However, even the low dose challenge studies do not accurately recapitulate the lower inoculum and much lower transmission rate observed in humans [23,32]. Similarly, macaque models do not typically model infection in the presence of drivers of HIV transmission in humans, such as STIs, genital inflammation and ulceration, cervical ectopy and use of HCs. However, they do often involve the use of progesterone to reduce thickness of cervicovaginal epithelium and thereby enhance susceptibility in macaques [33], which may be a relevant cofactor depending on the population targeted by the intervention [28].

Characteristics of transmitted viruses

Given the low probability of HIV transmission per exposure, it seems reasonable to assume that stochastic effects may contribute to the transmission bottleneck. However, there are a number of studies that suggest that there is also selection for viruses with particular features. Persistent infection is associated with rapid viral sequence diversification, driven in part by escape from immune pressure [34]. This evolutionary process has been associated with the emergence of more pathogenic viruses in the SIV/macaque model [35]. If these later-stage, more pathogenic variants are more readily transmitted, each successive transmission event would be expected to lead to a more compressed disease course. However, trends in virulence over the course of the epidemic have been less marked than early NHP studies predicted [36]. This has led to the suggestion that transmission ‘resets’ the virus population to the less pathogenic, transmissible form [37]. This hypothesis is supported by observations that transmitted viruses are closer in sequence to the donor’s computationally inferred ancestral sequence [24] or donor sequences earlier in infection [38] than to the donor sequences detected near the time of transmission. Indeed, these findings may explain results from prior studies suggesting that a minor variant from the donor at the time of transmission established the new infection [26,39-42]. In this case, the transmitted variant could be one that was archived from early infection and largely outgrown by variants that evolved within the host in response to immune and other pressures, although this has not been shown directly.

Studies of sequence differences between individuals and at the population level also suggest selection for transmission of ancestral viruses. There are more similarities between sequences from different individuals early in infection than there are later in infection [43]. In addition, a recent study of a large collection of transmission pairs found that transmitted viruses preferentially encode amino acids matching the cohort consensus and confer increased fitness in the recipient host, again supporting the concept that transmission selects for viruses with features that enhance host-to-host spread, features that are distinct from those selected for during chronic infection [44].

Studies examining sequence patterns of transmitted versus chronic stage viruses provide evidence for selection for viruses with particular signatures that could drive biological fitness. For example, variants with less glycosylation and shorter variable loops are selected during transmission and this has now been shown in multiple studies, although it has been less consistently seen in subtype B infections [24,45-51]. More recent studies of larger sequence collections have given a more granular view by identifying specific glycosylation positions that are less common in transmitted viruses [50]. These findings also support the concept of a reset in the virus at transmission compared to chronic infection because it has been shown that viruses with more glycosylation dominate chronic stages of infection due to their potential to allow escape from neutralizing antibody pressure [52-55]. While these more glycosylated viruses increase fitness in the presence of antibody, they may be inherently less fit in the absence of antibody, thus giving advantage to less glycosylated forms of the virus during transmission. This fitness bottleneck model would be particularly applicable to sexual and parenteral transmission because HIV-specific antibodies are not present and thus not exerting selection pressure in the recipient. This contrasts with mother-to-infant transmission, where passive antibodies are present in the exposed infant.

Nonetheless, there is evidence to suggest that infant viruses are also less glycosylated than maternal (donor) viruses [48], suggesting other factors may also be at play. One mechanism that has been proposed to explain selection for less glycosylated viruses is that they may bind better to mucosal CD4⁺ T cells expressing integrin $\alpha 4\beta 7$, leading to increased efficiency of infection [56]. In support of this role, a recent study suggested that administration of an anti- $\alpha 4\beta 7$ antibody could protect macaques from intravaginal SIV challenge [57]. However, not all studies have found that transmitted virus have unique interactions with $\alpha 4\beta 7$ [58,59] and the generalizability of this phenomenon will determine if the HIV envelope- $\alpha 4\beta 7$ interaction will be a good target for prevention interventions in humans.

Direct study of the infection and replication properties of transmitted variants in cell culture and/or in tissue explant models has also helped define the properties of transmitted viruses that may contribute to the transmission bottleneck. Collectively, these studies have implicated a variety of biological differences that differentiate transmitted from chronic stage viruses, although very few have yielded consistent results across studies, likely in part due to small sample sizes, the use of unpaired early and chronic viruses and variations in the cell systems or assays used. The one biological feature characteristic of transmitted viruses where studies agree is that these viruses invariably use CCR5 as the coreceptor for entry. This general concept was suggested by studies of cell tropism that predated the discovery of CCR5 as a receptor [42,60] and was then shown directly soon after the discovery of CCR5, including for all the circulating subtypes, subtype A [61], B [31,62-64], and C [65,66] infections. The reasons for CCR5-tropic variants are not entirely clear, and may involve multiple selection forces [24,67]. However, even absent a clear mechanistic underpinning, the finding that CCR5 viruses are favored at transmission was important for prevention because it suggested that CCR5 inhibitors could be effective against transmission [68] if used as pre-exposure prophylaxis (PrEP), an approach that is currently under development.

One of the most interesting recent observations regarding transmitted viruses is that they appear to be less sensitive to inhibition by type 1 interferon (IFN) than chronic stage viruses [69,70]. Several studies have shown that transmitted viruses are less sensitive to IFN inhibition in cell culture than chronic stage viruses and, notably, that they are less sensitive than viruses isolated 6 months later from the same individual [70]. It remains unclear whether this difference is common across populations, as not all studies have observed this characteristic of transmitted viruses [71,72]. However, a role for IFN in selection is biologically plausible, particularly during early virus dissemination, because IFN is rapidly triggered by initial infection specifically to block virus spread. If transmitted viruses are generally less sensitive to IFN, this finding would support the idea that selection occurs at least in part during virus dissemination, after there has been time for the initial infected cell to induce the IFN response. This finding would also open potential doors to discover new targets for blocking early virus spread if the specific IFN-stimulated protein(s) that drive selection of less sensitive variants can be identified.

Overall, while some intriguing possibilities have been suggested for why certain variants are favored for transmission, the biological drivers for selection of transmitted viruses are still not well defined. It is possible that some of the complexity reflects the fact that these drivers

may not be the same for all modes of transmission or for infection in the presence and absence of particular cofactors (e.g. STIs, HCs). Identifying the deterministic aspects of the selective bottleneck may open new avenues for targeted prevention approaches. While we await further clarity on the mechanistic aspects, an important take home message from the studies of transmitted viruses is that they are likely to be a subset that are selected for certain fitness qualities. Critically, if transmitted viruses represent a distinct subset with higher fitness for host-to-host spread, cell culture and NHP studies of potential biological prevention approaches should use these viruses.

Role of cell-free vs. cell-associated virus during transmission

Virtually all of the data from NHP models and most of the cell culture data that has informed the biology of HIV transmission comes from studies of cell-free virus infection. However, HIV can also spread directly by cell-to-cell contact of an infected cell and an uninfected cell (cis infection) [73], often resulting in transmission of viruses at higher multiplicity. In cell culture, cell-to-cell transmission is much more efficient than cell-free virus spread [74]. Virus infection may also involve virion capture by mucosal Langerhans cells and submucosal immature dendritic cells with subsequent transfer to CD4+ T cells (trans infection), a process that may protect and shield the virus for effective dissemination to lymphoid tissues [75]. This form of infection may be particularly important in early virus spread in the genital mucosa. Although there is evidence that both cell-free and cell-associated SIV can establish a new infection in macaques, there is little clarity on which is more efficient in animal models at this point [76].

There are limited data that provide insight on the role of cell-free versus cell-to-cell infection in humans. Many studies have shown that the level of viral RNA in donor plasma, i.e. cell-free virus, correlates with transmission [24], suggesting cell-free virus may be an important source of transmitted virus. However, the levels of cell-free virus and cell-associated virus (measured as proviral DNA) are highly correlated, making it unclear if viral RNA is really the biological driver underlying the observed associations. Some studies have measured both forms of the virus so that these variables can be considered together. For example, it has been shown that mothers who transmit via breastfeeding have higher levels of cell-associated breast milk HIV than non-transmitting mothers, even when controlling for cell-free breastmilk virus and cell-free plasma virus levels [77]. Conversely, there was no significant correlation between cell-free virus and transmission when controlling for cell-associated virus. These findings, among others, suggest that cell-to-cell virus spread may be important in the context of breastmilk transmission [78], but it is important to remember that these data may not be generalizable to other modes of transmission.

The source of transmitted virus has also been examined by analyzing both RNA and DNA sequences in transmission pairs to see which sequence in the donor most closely resembles the transmitted virus. These sequence studies have found that sometimes the virus is closest in sequence to the cell-associated DNA provirus and other times it is closest to cell-free RNA [26,40,41,79]. The simplest interpretation of this data is that the source of virus is variable from one transmission pair to another and both cell-free and cell-to-cell transmission may play a role under different settings. However, it is important to emphasize

the complexity of these studies and their limitations because they rely on sampling a very dynamic, rapidly evolving virus. Thus, unless the donor sequences are sampled very close to when transmission occurs, they will not accurately represent the relevant virus population.

For example, it is possible that what was a dominant virus in the RNA pool at the time of transmission became rare in the RNA pool but remained dominant in the DNA pool a month or two later. Thus, if the donor was sampled at this later time point, one could reach the erroneous conclusion that the transmitted virus is closest in sequence to the most prevalent cell-associated virus when in fact it was closest to the cell-free form when transmission actually occurred. Thus, it is important to consider when donor sequences were sampled in relation to transmission, and how precisely the time of transmission was defined, in interpreting such data. More sequence studies of this type with samples closely bracketing the time of transmission could help clarify the source of transmitted virus.

In terms of prevention, why does it matter if virus spread is cell-free or cell-to-cell? Several studies have suggested that HIV antiretrovirals (ARTs) have reduced efficacy against cell-to-cell transmission compared to cell-free spread, at least in cell culture [80-82]. This difference has been attributed to increased local virion concentration in cell-to-cell spread requiring higher amounts of ARTs. Interestingly, some ARTs, including several NRTIs in common use globally (AZT and TFV), are much less potent against cell-to-cell spread, whereas other NRTIs, NNRTIs and PIs show minimal differences in efficacy between the two modes of spread. Furthermore, when two NRTIs such as AZT and NVP are combined, their efficacy against cell-to-cell virus spread is restored to levels comparable to cell-free infection [82]. These studies suggest the intriguing possibility that the class and combination of ARTs may impact efficacy against cell-to-cell transmission. However, an important caveat to most of these studies is that they employ lab-adapted viruses, including a highly adapted, CXCR4 variant, NL4-3, with markedly different biology than circulating HIV variants. Indeed, a limited comparison of how ART inhibits replication of NL4-3 versus a more biologically relevant transmitted virus suggested that the use of this lab-adapted virus may magnify the difference in ART efficacy for cell-free versus cell-to-cell spread [82]. This again highlights the limitations of lab-adapted viruses for understanding questions relevant to HIV transmission. Studies with biologically relevant viruses, including transmitted viruses, are needed to better understand the potential limitations of specific ARTs for preventing cell-to-cell transmission. The fact that ARTs do prevent transmission in the context of PrEP implies that they are efficacious against the form of virus that is most relevant to transmission. But whether lack of efficacy of PrEP in some settings using particular formulations is in part due to relatively limited protection against cell-associated virus is an intriguing and unexplored possibility.

There is also evidence that neutralizing antibodies (Nabs) may differ in their potency against cell-free versus cell-associated virus, although early studies reached variable conclusions on this [83]. A more recent study that carefully separated these modes of transmission demonstrated that some Nabs show dramatic differences in efficacy against cell-to-cell compared to cell-free infection [84]. The relative efficacy of Nabs for different modes of transmission varied depending on the Nab and its cognate epitope. Nabs directed to epitopes in the transmembrane domain, gp41, showed equal potency against cell-to-cell and cell-free

infections. By contrast, Nabs that target epitopes in the extracellular surface protein, gp120, showed as much as 10-100-fold reduction in potency against cell-to-cell compared to cell-free infection. Nabs directed to the CD4 binding site showed the greatest difference in efficacy. Interestingly, this study noted that of the entry inhibitors tested in clinical trials, those that retain cell-to-cell efficacy, such as Maraviroc and T20, have proven useful in the clinic, whereas those with reduced efficacy against cell-to-cell transmission in culture, such as CD4-IgG, were not effective as therapies. These are a small number of examples and other host and viral variables could be at play. Nonetheless these findings are of high relevance to planned studies of preventative or therapeutic use of Nabs, particularly those directed to the CD4 binding site.

Conclusions

A wealth of data exists pointing to specific biological interactions that drive HIV transmission and present potential targets for prevention interventions. In this review, we have summarized some of the data that have shed light on where, when and which viruses must be targeted in order to prevent infection. However, many findings have not been consistent across studies, so the biological drivers of transmission are not yet well understood. It is clear that initial establishment of HIV infection is shaped by both permissive and selective interactions with host cells. Further studies are needed, either in humans or in biologically relevant pre-clinical model systems, to identify the sites, cell types and molecular interactions that shape these critical events and constitute targets for prevention. Better definition of the time window during which HIV infection can be prevented, rather than cured, is also needed. Studies in macaques suggest this window may be very short, calling for better definition of the timing of viral reservoir establishment in humans. Finally, the relative importance of cell-free and cell-associated virus in transmission and/or early viral dissemination remains unclear. The answer may come from studies in NHP or humans where observed results are not predicted by culture models using cell-free virus.

In reviewing these findings, it is important to remember that, given the challenges presented by directly studying early events in HIV transmission in humans, most of what we know is inferred from imperfect model systems: lentiviral infection of NHPs, infection of cells *in vitro* and *ex vivo*, or analysis of transmitted viral variants in humans. All models have strengths and limitations, faithfully recapitulating some aspects of HIV transmission while making imperfect assumptions about others. Some key considerations we have highlighted in this review include the choice of viral variants and inoculum size in experimental infections, the timing of sampling in longitudinal sequence studies, and the modeling of host susceptibility factors.

While these limitations make translation of pre-clinical data challenging, it is in integrating findings from multiple well-defined model systems that we refine our understanding of HIV transmission, test candidate interventions and, critically, improve the models we use in future studies. We therefore propose that progress in translational HIV prevention research will be facilitated by clearer articulation of assumptions and limitations of the model systems used.

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Figure 1.
Schematic of aspects of HIV transmission biology and their implications for pre-clinical evaluation of candidate prevention strategies