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Using Nonhuman Primates to Model HIV Transmission

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Abstract

Purpose of Review—One of the major obstacles in fully understanding HIV transmission comes from the impracticality of studying transmission in humans. Because of this encumbrance, the early phases of HIV transmission and systemic dissemination are poorly understood. In order to fully comprehend these critical steps in HIV infection, animal models must be devised to accurately reflect HIV's mode of action. This review seeks to highlight the essential nature of modeling HIV transmission in nonhuman primates.

Recent Findings—Recently it was discovered that HIV infection is established in newly infected recipients by a single or few transmitted/founder variants. This has reshaped how animal modeling is conducted with researchers currently recapitulating a physiologically relevant, low-titer infection. Pertinent animal models have been established for the most common routes of infection, including rectal, vaginal, and penile transmission; models for intravenous and oral transmission are still in developmental stages.

Summary—These limited dose models now accurately reflect HIV transmission in humans, and provide a realistic experimental platform for vaccine development and other intervention strategies which can be used to inform vaccine development in humans. Using information obtained in NHP and human trials, it is conceivable to envision effective prevention modalities in the near future.

Keywords

transmission; NHP models; acute infection; mucosa; SIV

Introduction

HIV transmission in humans is a complex biological process confounded by the difficulty of identifying and characterizing primary HIV infections. These obstacles are further complicated by socioeconomic demographics, stigmatism of sexual practices, and the social fridge nature of these at-risk populations. Nonhuman primate (NHP) models have been developed to examine key features of HIV disease that otherwise would be challenging or impossible to determine in humans. In order to most accurately model HIV transmission in

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Conflicts of interest
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NHP, we must first identify what is known about HIV transmission in humans then determine what key aspects of transmission are or can be faithfully reproduced in this model. NHP species not naturally infected with simian immunodeficiency virus (SIV) have been shown to be an excellent model of HIV disease by accurately reproducing immune activation, CD4 depletion, and significant viral replication when infected with SIV from other naturally infected monkeys. Although this model has been used for years, SIV transmission studies in NHP have recently been modified to incorporate new findings from HIV transmission studies. At its most basic level, HIV transmission is caused by the exposure of mucosal surfaces or the blood compartment to infectious virus. Epidemiological studies have identified a number of key parameters detailing the risks of HIV transmission which include (i) identifying the most probable behaviors conducive to infection and estimating the infection rate per unprotected exposure to be 1:5 to 1:3,000 (depending on behavior and site of exposure) [1], (ii) determining that transmission is more likely to occur from partners with primary HIV infection due to unknown infection status, higher viral load, and potentially a more fit virus [2–4], (iii) identifying preexisting sexually transmitted diseases that increase probability of infection [5–7], and (iv) proving that male circumcision reduces infection rate by over 60% [8–10]. Understanding how humans are exposed to infectious virus and how often these events lead to productive infection is crucial to creating a NHP model that most accurately mirrors HIV infection.

One essential concept in HIV transmission predicted by epidemiological studies and proven by molecular and mathematical studies involves a genetic bottleneck between donor and recipient. In general, a genetic bottleneck is the reduction in genetic diversity of a population with only relatively few lineages surviving some otherwise catastrophic event. While this new population is by definition reduced, the extent or completeness of the reduction is variable. Since HIV is found as a quasi-species with varying degrees of diversity associated with the time since infection, each infected individual represents a population of virus. The genetic bottleneck during transmission from a diverse population has been known imprecisely for years with chronic patients being defined as containing a heterogeneous viral population and most primary infections as being homogeneous. Recently, we and others have discovered that during transmission, genetic diversity is reduced so drastically that the vast majority of infected individuals are productively infected with a single genetic unit [11–15]. Additionally, patients infected with one or few variants represent over 95% of all HIV infections in these studies suggesting a very low infectious dose at time of transmission, a significant host barrier to new infections or both. A key technological advance allowing for the exact enumeration of infecting variants is single genome amplification (SGA), which is a limiting dilution PCR where cDNA or DNA is diluted so that the majority of reactions have only a single template. The benefits of SGA over standard bulk sequencing or cloning and then sequencing includes proportional representation of the viral population, a lack of in vitro recombination, and no Taq induced errors [13,16,17]. This remarkably simple technical advance provided the opportunity to elucidate fundamental questions in viral transmission including: (i) enumerating transmitted/founder variants [11–15], (ii) identifying and molecularly cloning the exact nucleotide sequence of these genomes [13,18], (iii) computationally modeling early viral diversification [19–22] (iv) identifying increased variants in men-who-have-sex-with-men and intravenous-drug-users [14,23–25], (v)

determining the earliest anti-viral responses both innate and humoral [26–28], and (vi) defining the phenotype of transmitted viruses which includes the requirement for CCR5 [13,18,29,30]. However, there are limitations in studying HIV transmission, which include an inability to sample relevant tissues immediately following exposure, an inability to genetically characterize or quantify the donor's virus at the time of transmission, and to unambiguously identify the route of exposure. These obstacles make an accurate NHP model an essential tool to fully understanding HIV transmission (Fig. 1).

NHP Models of Transmission

There are many NHP models available for research. The fundamental criterion for a successful model is whether or not it recapitulates HIV infection. For mucosal infections in rhesus macaques, infection with SIV or chimeric SHIV **can** recapitulate the key features of HIV transmission if investigators are willing to invest time and resources. Various challenge sites and viruses can be used to initiate infection and model viral transmission including the earliest events in establishing infection in a new host. As for the sites of infection, they can be categorized as mucosal or intravenous. While the intravenous challenge model is frequently used for convenience, it is rarely utilized to model intravenous infections in humans. Mucosal transmissions are modeled most frequently using intrarectal or intravaginal challenge. Recently, there have been advances in modeling male genital track infection to better recapitulate heterosexual HIV risk. Single genome amplification has been used as a means to more precisely determine the infectious dose necessary to infect animals with a minimal number of variants, thereby reflecting the most important feature of HIV infection—low infectious titer [31–41]. Furthermore, titrating virus to a limiting dose is possible without the benefits of enumerating founder variants (i.e. using cloned virus stocks) but requires significant animal testing [42]. Finally, using a low-dose, repeated challenge with an infection rate of approximately 20%, allows for a reduced founder population and can be performed in a reasonable time frame [37,41,43]. Regardless of route of challenge, using a single challenge titered-dose provides the opportunity for few but often multiple transmitted/founder variants while a repetitive low-dose challenge often results in productive infection of a single transmitted/founder variant perfectly modeling the vast majority of HIV infections (Table 1).

Rectal

HIV infection resulting from unprotected anal intercourse (UAI) is the most common route of disease transmission in many developed countries and represents the highest risk mode of sexual HIV transmission at 1:20 – 1:300 infections per exposure [1]. The rectum is lined with only a single layer of protective columnar epithelium as a barrier between infected blood or semen and the lymphoid-rich mucosal tissue [44]. Intestinal mucosal tissues contain the largest reservoir of activated $CD4^+$ and $CCR5^+$ cells in the body (particularly in the lamina propria and within lymphoid aggregates) and represents an environment prime for viral infection. Rectal infection is the most commonly utilized NHP model for its consistency and as an authentic site of HIV infection. Previous models of rectal transmission have relied upon high dose challenges in order to ensure infection, yet recent findings have shown that low dose challenges in NHPs provide a more accurate model for the route of

HIV transmission and dissemination throughout the body [31,32]. In studies using a single challenge, the goals are to infect most if not all animals with as few variants as possible. Based on a Poisson distribution, it is apparent that at a dose required to infect all animals in a study, the majority of animals will not be infected with a single variant, but most likely infected with 3–6 unique variants [31]. To ensure a single variant infection, a limited dilution challenge is necessary so that the majority of animals are uninfected (i.e. ~20% infection rate) [41]. These models provided for a limited transmitted/founder population, which revealed a delay in detectable viral load in animals infected with one or few variants. Furthermore, the infecting virus originated in multiple sites within the phylogenetic tree suggesting an unbiased selection of variants. This infection route using single or repetitive titered challenges accurately reflects HIV transmission via UAI and should be used for studies aimed at identifying the key early events surrounding transmission and systemic dissemination.

Female Genital Tract

Heterosexual transmission of HIV via the vagina is the most prevalent mode of disease transmission globally, despite the fact that it has a comparatively low risk per exposure rate estimated at 1:200 – 1:2000 [1]. In female genital tract transmission, virus carried in semen or blood contacts both the vagina and the cervix with a mucosal barrier consisting of a single layer of columnar epithelium (in the endocervix) or squamous nonkeritinized epithelium in the vagina or ectocervix [44]. As with rectal challenges, until recently, vast excess quantities of virus were used to challenge animals vaginally. These studies focused on the endocervix for convenience, but may have been interpreted by others as the major or only site of infection [45]. Recently, a lower dosed challenge model has been adopted which better imitates HIV in animals [33]. In this study, the number of transmitted/founder variants was determined in animals challenges intravaginally at either 10^5 or 10^3 infectious units. Although there was greater variation in the number of variants vaginally compared to rectal challenges, there was clearly a dose effect and some animals at the lower dose showed evidence of productive infection with a single variant. Additional studies using a low, titered dose should provide additional insight into where in the female genital tract infection originates and how infection disseminates systemically.

Male Genital Tract

Penile HIV transmission occurs during homo- or hetero- sexual intercourse during which virus is transferred from infectious cervicovaginal or rectal secretions. Putatively, infection can occur either at the foreskin (which presents an epithelial barrier of squamous, poorly keratinized cells) or in the penile urethra (in which stratified, columnar epithelium acts as a barrier to transmission) [46]. Until recently, this route of transmission as an NHP model was not well developed. A new emphasis on relevant models has led to the development of two NHP models for penile transmission [34,47]. Here researchers expose penis to infectious virus by dipping a flaccid penis into infectious virus or by forming a cup with the foreskin and applying virus. Productive infection was limited to a single viral variant per animal using a repeated challenge model [34]. Recently, this model has been used in an adenovirus-vector based vaccine efficacy study recapitulating a clinical trial humans [48]. Additional

studies are necessary to elucidate the initial sites of productive infection and routes of systemic dissemination following male genital tract exposure.

Oral

Oral transmission of HIV occurs primarily during the exposure to infected breastmilk, maternal vaginal secretions intrapartum, infected blood, or semen. For infants, infection can occur from mouth through the upper gastrointestinal tract. For adults, it is unlikely that infectious virus can survive in an acidic stomach and infection is more likely to occur in mouth or esophagus. Importantly, tonsils are highly active secondary lymphoid tissue and are a likely site of viral infection. Depending on the exact site of viral transmission, there are various barriers to infection and target cell availability. Currently, there are limited NHP models dedicated to oral transmission [49,50]. Additional studies will be needed to expand our understanding of oral transmission.

Non Mucosal Infections

HIV infection via blood contact occurs during direct blood to blood contact (i.e. sharing contaminated needles), or in utero when virus from the mother crosses the placental barrier. Direct intravenous infection is commonly used to infect animals, but not specifically to model this route of HIV transmission. In bypassing a mucosal barrier, intravenous infections eliminate the genetic bottleneck and alters the dynamics of viral replication and systemic dissemination. Little effort is made in this model to limit transmitted/founder variants found in most human patients [23,24], but doing so could provide useful information as to the sites and timing of early viral replication.

Optimally Using NHP Models

Having an authentic model of HIV transmission is useful in understanding transmission itself, but it is also essential for determining the correct method to challenge animals in vaccine trials, therapeutic interventions, passive antibody studies, and in viral reservoir studies. High-dose intravenous challenge is an unrealistic model for vaccine intervention because it excludes the mucosal barrier and alters the dynamics of viral dissemination [32]. Even high-dose mucosal challenges are potentially unnecessarily stringent for vaccine efficacy studies. Many investigators improved the challenge portion of vaccine efficacy studies by challenging animals with a single, limited dose or by repeated exposure at a fraction of that dose [35–41,48,51–58]. For example, Barouch et al. and Hansen et al. utilized a repetitive low-dose intrarectal challenge (described in [31,59]) as a bases for a heterologous challenge in a successful adenovirus-virus-based vaccine study [43] and cytomegalovirus-vector-based vaccine study [52]. Furthermore, Vaccari et al. specifically tested the notion that vaccine efficacy was correlated to challenge dose and found partial vaccine effect in low-dose challenged animals but no vaccine effect in animals challenged at a 10 fold higher dose [39]. It is clear that challenging animals at too high a dose does not allow for accurate assessment of vaccine potential. However, it is uncertain if vaccine strategies that are successful in a low-dose NHP model will translate to humans, but our current opinion is that it will. Furthermore, therapeutic intervention studies and passive antibody protection studies have been used for years, but have recently been altered to limit

founder variants of the chimeric HIVenv with a SIV-backbone (SHIV) virus [60–62]. Additional insights could be obtained using this more relevant transmission model.

Although the transmission event itself could be defined as productive infection of a single cell, transmission typically includes the time of exposure through establishment of productive, systemic dissemination. This period of time is also known as the eclipse phase not because the virus is dormant, but because the virus is undetectable in peripheral blood. Importantly, it is during this time that the infecting virus is at its most vulnerable. Regardless of the route of infection, plasma viremia represents the accumulation of thousands of rounds of exponential replication in a new host starting with as few as one virus to billions of viruses found throughout the host. The length of the eclipse phase is likely dependent on the route of exposure, the replicative capacity of the infecting virus, the host response and the inflammation state of the host. Furthermore, compared to HIV's eclipse phase (7–21 days [13,20]), the time to viremia via mucosal challenges ranges from 3–9 days in nonhuman primates [31,63]. While the actual time to detectable plasma viremia is variable and dependent on dose, route, viral strain, and potentially other non-viral constituents in the inoculum [64], this period represents our best window of opportunity for intervention (i.e. the virus's Achilles heel) because viral reservoirs are not yet fully established and viral replication is anatomically limited. Once viral reservoirs that can persist for the life of the patient are established in sufficient quantities, this small window to interdict is shut. Importantly, it is not well known how rapidly intervention must be initiated to prevent infection. However, early antiretroviral treatment in post-exposure prophylaxis studies and in latency studies suggest that reservoirs establishment sufficient to maintain infection is approximately 3–5 days post exposure [65–71]. Additional studies are necessary to more accurately determining the timing of reservoir establishment in animals infected with a limited number of transmitted/founder variants.

Conclusions

The first tenant of creating any animal model should be *Primum non nocere*, "first, do no harm". Unfortunately some investigators believe that animals models can and often mislead human trials [72]. We submit that authentically reproducing HIV infection comes from robust and thoughtful animal modeling. The most compelling rationale for using models appropriately originates in a NHP study that predicts the STEP trial failure but was unfortunately performed in retrospect [51]. When new evidence is generated by NHP/SIV or human/HIV researchers, it behooves us to adapt and modify as necessary. The NHP/SIV field has actively and successfully incorporated new knowledge obtained in HIV transmission studies into new models of transmission. These improved models allow for productive infection of one or few variants challenged at multiple mucosal sites thereby recapitulating the essence of HIV transmission. We hope that many human/HIV researchers will acknowledge these improved models and allow NHP research to inform human clinical trials. Overall we are in a position to address several fundamental research questions including: (i) identifying the source of transmitted virus (cell-associated or cell-free), (ii) the mechanism for CXCR4-tropic exclusion or CCR5-tropic selection, (iii) the means used to breach the epithelial barrier (transcytosis, breaks or abrasions, or simply navigating between cells), (iv) the amount and inhibitory potential of mucus at various sites of infection, (v) the

number of variants transmitted and replicating only locally versus variants identified systemically, (vi) the dynamics and routes of systemic dissemination, and (vii) the quantity and rapidity of long-term viral reservoir establishment. Greater understanding of these essential questions will require both NHP research and studies in HIV infected humans, and successfully addressing these questions will provide the necessary underpinning for future intervention strategies.

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Key Points

HIV infection is established in newly infected recipients by a single or few transmitted/founder variants.

Animal modeling is conducted with researchers currently recapitulating a physiologically relevant, low-titer infection.

Pertinent animal models have been established for the most common routes of infection, including rectal, vaginal, and penile transmission.

Vaccine and other intervention studies have the modified challenge approach to recapitulate HIV infection.

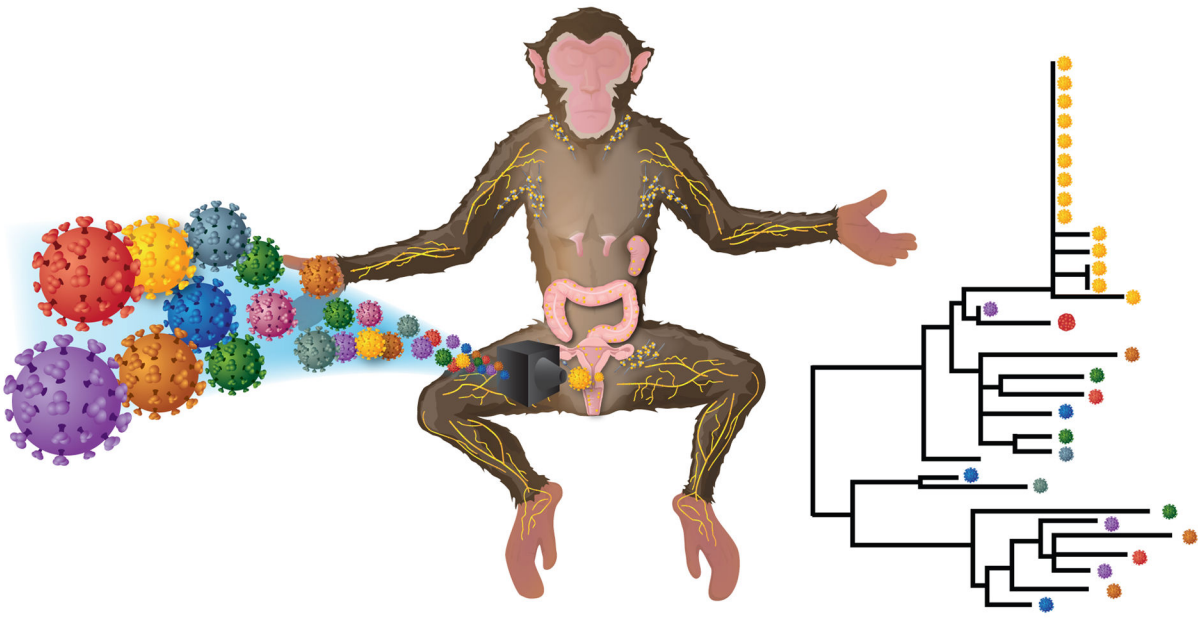

Figure 1. Model of the viral genetic-bottleneck following mucosal challenge

Despite exposing animals mucosally with a large number of genetically distinguishable variants, the systemic dissemination of a single genetic unit can be obtained in NHP models thereby recapitulating HIV-1 infection. Genetic analyses of variants that are transmitted to the inoculum allows for enumerating the number of variants systemically replicating and potentially identifying unique features of these lineages. However, a precise molecular description of how this genetic constraint is accomplished is still largely unknown with the earliest events of viral infection still within a metaphorical black-box. Additional experiments are needed to assess the various contributions of anatomic and other host barriers to infection and current NHP models provide the necessary sensitivity and tissue availability to describe these early events.

Table 1

Characteristics of various modes of transmission

Route of Transmission		Epithelial Barrier	Target Cell Availability	Key Advantages	Limitations	Selected References
Rectal		Columnar	Abundant	Most widely used infection route, consistent infection rate, requires limited dose, primary site of infection in developed countries	Frequently overdosed, atraumatic model only	[31,32,41]
Female Genital Tract	*Vagina/Ectocervix* *Endocervix*	Squamous Columnar	Abundant Limited	Widely used infection route, modeling the most frequent mode of infection globally	Inconsistent infection rate, typically includes monitoring menstrual cycle, requires significant inoculum dose	[33,44,45]
Male Genital Tract	*Foreskin* *Urethra*	Keratinized Squamous Columnar	Limited Limited	Recently developed as a transmission model, frequent site of male infections	Technically challenging to perform, requires significant inoculum dose	[34,46–48]
Oral		Squamous & Columnar	Variable	Understudied, but important site of transmission	Logistically challenging to establish model	[49,50]
Non Mucosal		No Barrier	Abundant	Consistent infection rate, simple to administer	Typically not used to model transmission	--