HIV Transmission

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HIV-1 is transmitted by sexual contact across mucosal surfaces, by maternal-infant exposure, and by percutaneous inoculation. For reasons that are still incompletely understood, CCR5-tropic viruses (R5 viruses) are preferentially transmitted by all routes. Transmission is followed by an orderly appearance of viral and host markers of infection in the blood plasma. In the acute phase of infection, HIV-1 replicates exponentially and diversifies randomly, allowing for an unambiguous molecular identification of transmitted/founder virus genomes and a precise characterization of the population bottleneck to virus transmission. Sexual transmission of HIV-1 most often results in productive clinical infection arising from a single virus, highlighting the extreme bottleneck and inherent inefficiency in virus transmission. It remains to be determined if HIV-1 transmission is largely a stochastic process whereby any reasonably fit R5 virus can be transmitted or if there are features of transmitted/founder viruses that facilitate their transmission in a biologically meaningful way. Human tissue explant models of HIV-1 infection and animal models of SIV/SHIV/HIV-1 transmission, coupled with new challenge virus strains that more closely reflect transmitted/founder viruses, have the potential to elucidate fundamental mechanisms in HIV-1 transmission relevant to vaccine design and other prevention strategies.

HIV-1 transmission results from virus exposure at mucosal surfaces or from percutaneous inoculation. Because such exposures in humans are inaccessible to direct analysis, our understanding of the transmission event must necessarily come from insights gleaned from studies of HIV-1 epidemiology, viral and host genetics, risk factor and behavior analyses, animal models, human explant tissues, and in vitro studies of virus-target cell interactions. In this article, we explore themes that connect these varied aspects of HIV-1 infection with the

ultimate goal of understanding the molecular basis of HIV-1 transmission.

EPIDEMIOLOGY OF HIV-1: IMPLICATIONS FOR TRANSMISSION BIOLOGY

At the broadest level, HIV-1 transmission must be viewed in the context of the global pandemic. Population level phylogenetic patterns of endemic, epidemic, and pandemic strains of HIV-1 can provide insight into clinically relevant aspects of virus transmission. HIV-1 is

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classified phylogenetically into groups M, N, O, and P, each reflecting a separate introduction of simian immunodeficiency viruses (SIVs) from naturally infected great apes into humans (see Sharp and Hahn 2011). Of these, only group M underwent pandemic spread. Viral and host factors that may have influenced the relative transmissibility of the different HIV-1 groups are discussed elsewhere (Malim and Bieniasz 2011; Sharp and Hahn 2011). For group M viruses, mathematical modeling of virus diversification suggests a most recent common ancestor near 1910–1930 (Korber et al. 2000; Worobey et al. 2008), followed by subclinical endemic spread of the virus in human populations in West Central Africa. There, largely as a consequence of founder effects and viral population bottlenecks, HIV-1 emerged as early as the late 1950s as phylogenetically distinct subtypes, of which nine (subtypes A, B, C, D, E, G, H, J, K) are now recognized as contributing to the global pandemic (Taylor et al. 2008).

The extraordinary sequence diversity within and among these different HIV-1 group M subtypes, which can reach 25%–35% in *env*, has made possible a precise tracking of HIV-1 transmission within and between populations on a global scale (Gilbert et al. 2007; Taylor et al. 2008) and between individuals on a micro scale (Derdeyn et al. 2004; Haaland et al. 2009). A global survey of the distribution of HIV-1 subtypes and intersubtype circulating recombinant forms (CRFs) in 2004 revealed that subtype C accounted for 50% of infections worldwide, with subtypes A, B, D, and G accounting for 12%, 10%, 3%, and 6%, respectively, and subtypes E, H, J, and K together accounting for 1% (Hemelaar et al. 2006). The global distribution and genetic complexity of HIV-1 subtypes and CRFs have continued to evolve (Taylor et al. 2008), and with this has come the potential for virus evolution, adaptation, and altered transmissibility. Despite this fertile environment for mutation, there is little evidence for meaningful differences among HIV-1 subtypes in their patterns or efficiencies of transmission. The best example of differences in transmission probability is for CRF01_AE in injection drug users (IDUs) in Thailand, which

increased substantially in prevalence between 1995–1998 compared with subtype B viruses. Yet, it is unclear whether epidemiological factors, host factors, or viral properties were responsible (Hudgens et al. 2002). There is also an epidemiological observation that subtype D viruses from Uganda and neighboring regions may show R5/X4 dual tropism more commonly than do other HIV-1 subtypes, but this may have a greater effect on viral pathogenesis than on transmission per se (Church et al. 2010). Thus, unlike influenza virus in which ongoing genetic mutation dramatically impacts the frequency and patterns of virus transmission, such is not the case for pandemic HIV-1 group M viruses, which have been remarkably consistent in their transmissibility over expanses of time, geography, and target populations (Taylor et al. 2008) and as the virus moved between individuals and groups of individuals with widely different risk behavior and virus transmission routes (Kouyos et al. 2010).

TRANSMISSION ROUTES AND RISKS FOR HIV-1 INFECTION

In 2009, an estimated 2.6 million people globally became newly infected by HIV-1 (UNAIDS 2010). This represents a reduction in new infections by 21% compared with 1997 when incident infections peaked. But declining HIV-1 incidence rates have not been uniform across all regions and risk groups, highlighting the importance of different transmission routes and risk behaviors in facilitating HIV-1 transmission. The most extreme example is in Eastern Europe and Central Asia where HIV-1 prevalence tripled between 2001–2009 as a consequence of concentrated epidemics associated with sex work, drug use, and men who have sex with men (MSM) (UNAIDS 2010). Table 1 summarizes the risks for HIV-1 transmission associated with different transmission routes and their relative contributions to HIV-1 prevalence worldwide. What is evident from these estimates is that heterosexual transmission is responsible for nearly 70% of HIV-1 infections worldwide with the remainder largely attributable to MSM, maternal-infant infection, and

Table 1. Transmission routes and risks for HIV-1 infection

HIV invasion site	Anatomical sublocation	Type of epithelium	Transmission medium	Transmission probability per exposure event	Estimated contribution to HIV cases worldwide
Female genital tract	Vagina	Squamous, nonkeratinized	Semen; blood	1 in 200–1 in 2000	12.6 million
	Ectocervix	Squamous, nonkeratinized			
	Endocervix	Columnar, single layer			
	Other	Various			
Male genital tract	Inner foreskin	Squamous, poorly keratinized	Cervicovaginal and rectal secretions; blood	1 in 700–1 in 3000	10.2 million ^a
	Penile urethra	Columnar, stratified			
	Other	Various			
Intestinal tract	Rectum	Columnar, single layer	Semen; blood	1 in 20–1 in 300	3.9 million ^b
	Upper GI tract	Various	Semen; blood	1 in 2500	1.5 million
Placenta	Chorionic villi	Two-layer epithelium (cyto- and syncytiotrophoblast)	Maternal blood, genital secretions (intrapartum)	1 in 5–1 in 10	960,000 ^c
			Breast milk	1 in 5–1 in 10	960,000 ^c
			Maternal blood (intrauterine)	1 in 10–1 in 20	480,000 ^c
Bloodstream			Blood products, sharps	95 in 100–1 in 150	2.6 million ^d

Adapted from the 2010 UNAIDS/WHO AIDS epidemic update and Hladik and McElrath (2008).

^aIncludes men having sex with men (MSM), bisexual men, and heterosexual men.

^bIncludes MSM, bisexual men, and women infected via anal receptive intercourse.

^cMother-to-child transmission.

^dMostly intravenous drug use, but includes infections by transfusions and health-care-related accidents. GI, gastrointestinal.

injection drug use. This is the case despite the fact that transmission probability per coital act is lowest for heterosexual exposures (1 in 200–1 in 3000) (Hladik and McElrath 2008; McElrath et al. 2008). However, two recent meta-analyses of HIV-1 incidence and prevalence data (Powers et al. 2008; Boily et al. 2009) suggest a far wider range in HIV-1 transmission risk for heterosexual exposures depending on confounding risk factors such as genital ulcer

disease, male circumcision, HIV disease stage, and exposure route. For example, penile-vaginal transmission of HIV-1 was reported at a frequency as high as 1 in 10 exposures and penile-anal transmission as high as 1 in 3 depending on confounding risk cofactors (Powers et al. 2008). Thus, the commonly quoted risk estimate for heterosexual HIV-1 acquisition of 1 in 1000 exposures must be considered a lower bound.

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Socioeconomic factors can also influence HIV-1 transmission indirectly. El-Sadr and colleagues have suggested that in the United States, the risk of acquiring HIV-1 infection is defined more by a person's "sexual network" than by their individual risk behaviors, with the former influenced primarily by socioeconomic factors (El-Sadr et al. 2010). On a global basis, HIV-1 transmission estimates per coital act vary significantly between high-income countries, where male to female (MTF) transmission estimates are 0.08% and female to male (FTM) rates are 0.04%, and low-income countries, where MTF and FTM transmission rates are 0.38% and 0.3%, respectively (Powers et al. 2008; Boily et al. 2009). These differences may in part reflect the relative frequency of HIV serodiscordant couples in the two settings since it has been estimated that they represent the source of a majority of adult infections in many low-income countries (Dunkle et al. 2008).

Viral load (vL) in the transmitting partner plays a major role in determining the risk of HIV-1 transmission from one individual to another. Although vL likely affects all modes of transmission, it has been best characterized in HIV-1 discordant couples, in which as much as a 2.5-fold increase in transmission was observed for every 10-fold increase in vL (Quinn 2000; Fideli et al. 2001). Moreover, although vL in genital secretions may not correlate directly with that in the blood, partners with plasma vL less than 1000 rarely transmitted to their partners (Quinn et al. 2000; Fideli et al. 2001). The recent observation (HIV Prevention Trials Network Study 052, unpubl.) that antiretroviral treatment of the positive partner of discordant couples can result in a 96% reduction in transmission is consistent with this finding (Cohen et al. 2011b). The clinical stage of infection (acute vs. middle vs. late) in the transmitting partner can also play a key role in defining the efficiency of transmission, with the risk of infection from individuals with acute or early infection being higher than that in established infection (Wawer et al. 2005; Brenner et al. 2007; Powers et al. 2008; Miller et al. 2010). This likely reflects the high vLs observed in acute infection, a lack of neutralizing antibodies that

may inactivate circulating virus in established infection, and clonal amplification of highly fit viruses in acute infection that are especially suitable for initiating productive infection (Richman et al. 2003; Wei et al. 2003; Wawer et al. 2005; Keele et al. 2008; Cohen et al. 2011a). Indeed, in the Indian rhesus macaque model of SIV transmission, SIV in the plasma from animals in the acute stage of infection had a specific infectivity up to 750 times greater than that of virus in the plasma from chronically infected animals (Ma et al. 2009).

The efficiency of HIV-1 transmission can be modulated by still other factors, including sexually transmitted diseases, particularly those that result in genital inflammation and ulcers, which can elevate HIV shedding into the genital tract and can increase infection susceptibility by two- to 11-fold (Galvin and Cohen 2004); pregnancy, during which a greater than twofold increase in HIV acquisition risk has been observed (Gray et al. 2005); and circumcision, which in a series of clinical trials has been shown to decrease transmission acquisition risk in the male partner by 60% (Auvert et al. 2005; Bailey et al. 2007; Gray et al. 2007; Quinn 2007).

THE CLINICAL TRANSMISSION EVENT AND ACUTE HIV-1 INFECTION

Despite the many routes by which HIV-1 can be transmitted from one individual to another, there is generally an orderly and reproducible appearance in the blood of viral and host markers of infection following a clinically productive transmission event (Fig. 1). This sequential appearance of laboratory markers of new HIV-1 infection was systematically evaluated by Fiebig and colleagues who devised a laboratory staging system for acute and early infection (Fiebig et al. 2003). The initial period between the moment when the first cell is infected and when virus is first detectable in the blood is termed the eclipse phase. The duration of this period has been estimated to be approximately 7–21 d, based on clinical histories of high risk exposure events (Gaines et al. 1988; Clark et al. 1991; Schacker et al. 1996; Little et al. 1999; Lindback et al. 2000a,b) and

Figure 1. Laboratory staging and natural history of acute and early HIV-1 infection. The average durations and 95% confidence intervals (parentheses) of the eclipse phase and Fiebig stages (Fiebig et al. 2003) of acute infection are shown in the inset.

mathematical modeling of early HIV-1 replication and diversification (Keele et al. 2008; Lee et al. 2009). During the eclipse phase, which is clinically silent, virus is propagated in $CD4^+$ T cells in mucosa, submucosa, draining lymphatics, and perhaps to a modest extent in gut-associated lymphoid tissue (GALT) and systemic lymphatic tissues (Haase 2010). Once virus becomes detectable in blood plasma, it increases exponentially (Ribeiro et al. 2010) as a consequence of explosive replication in GALT and peripheral lymphoid tissue compartments (Veazey et al. 1998; Guadalupe et al. 2003; Brenchley et al. 2004; Mehandru et al. 2004; Li et al. 2005b; Mattapallil et al. 2005; Haase 2010). Fiebig stages I–VI reflect the ordered appearance in the plasma of HIV-1 viral RNA (Fiebig I), viral p24 antigen (Fiebig II), and virus-specific antibodies detectable first by recombinant protein-based enzyme-linked immunosorbent assay (Fiebig III), and then by Western immunoblotting (indeterminant banding pattern: Fiebig IV; diagnostic banding pattern but missing p31 reactivity: Fiebig V;

diagnostic banding pattern with p31 reactivity: Fiebig VI). The average durations of the eclipse phase and Fiebig stages along with estimated 95% confidence intervals are shown in the inset of Figure 1. Diagnosing HIV-1 infection as soon as possible after the transmission event is important clinically to ensure the safety of blood for transfusion and of body organs for transplantation. It is also important for preventing forward transmission of the virus from index patients to their contacts. Current HIV-1 nucleic acid tests based on different amplification platforms can detect viral sequences qualitatively at 1–5 copies per milliliter of plasma (Palmer et al. 2003; Nugent et al. 2009) and quantitatively at levels exceeding 50 copies per milliliter plasma (Damond et al. 2010). Alternatively, a recently approved fourth-generation combined p24 antigen-antibody test can detect HIV-1 at viral loads exceeding 10,000 virions per milliliter plasma (Eshleman et al. 2009; Cohen et al. 2011a). Thus, commercially available p24 antigen tests can detect HIV-1 infection approximately 1 wk before the first detection of

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anti-HIV antibodies, and vRNA tests can detect HIV-1 infection approximately 1 wk before that (Fig. 1). There are well-documented examples of HIV-1 antibody seroconversion as late as 6 mo or more after an exposure event but such cases are rare (Ridzon et al. 1997); what is more remarkable is the consistency in the timing of appearance of viral and host markers of infection (Fig. 1) despite the different cells and tissues that are involved in the transmission process at the different sites of HIV-1 invasion (Table 1).

A POPULATION BOTTLENECK TO HIV-1 TRANSMISSION

The concept that transmission of HIV involves a population bottleneck, in which a limited number of variants from a genetically diverse virus quasispecies in the transmitting partner establishes productive infection in the newly infected partner, was first established nearly two decades ago (Wolfs et al. 1992; Wolinsky et al. 1992; Zhang et al. 1993; Zhu et al. 1993). These early findings were of particular interest to investigators involved in HIV-1 vaccine research and to those interested in HIV-1 transmission biology, pathogenesis, and natural history because they raised the possibility that selective pressures could be at play in virus transmission. This idea was strengthened by the subsequent discovery of CCR5 and CXCR4 as obligate coreceptors for HIV-1 entry into cells, by the finding of a strong preference for CCR5 use by HIV-1 strains from acute and early infection, and by the observation that humans who are homozygous defective for CCR5 expression are protected from HIV-1 infection (see Berger et al. 1999; Wilen et al. 2011b). Subsequent studies continued to explore the quasispecies complexity and genetic and biologic composition of HIV-1 in acutely infected individuals in an attempt to better define the features of transmitted viruses and cofactors that enhance the risk of transmission (Poss et al. 1995; Zhu et al. 1996; Long et al. 2000; Learn et al. 2002; Derdeyn et al. 2004; Grobler et al. 2004; Ritola et al. 2004; Sagar et al. 2004, 2009). The results of all of these studies, together

with the earlier work, led to the concept that whereas chronically infected subjects were invariably infected by a genetically diverse viral quasispecies, acutely infected individuals could be grouped into those with relatively “homogeneous” infections, presumably resulting from transmission and productive clinical infection by one or few closely related viruses, and those individuals with more “heterogeneous” infections that resulted from transmission of multiple viruses with greater diversity. This distinction between homogeneous and heterogeneous virus diversity in acute HIV-1 infection was for the most part a qualitative description, which was further confounded by the suggestion that acutely infected individuals could harbor genetically diverse HIV-1 genotypes that in the weeks following transmission underwent purifying selection so as to give the appearance of a homogeneous acute infection (Learn et al. 2002). Thus, although it was clear that there was a population bottleneck to HIV-1 transmission, a quantitative and molecularly precise description of this bottleneck was not possible. This led to more controversy than consensus regarding the multiplicity of HIV-1 infection in different patient populations with distinct demographic and behavioral risk profiles as well as the genetic and biological properties of such viruses.

The stimulus to a more focused examination of the genotype and phenotypic properties of the viruses that initiate new HIV-1 infections was prompted by a report by Derdeyn and colleagues (2004), which combined an extensive phylogenetic comparison of viral *env* sequences in chronically infected subjects and their newly infected sexual partners with a phenotypic analysis of viruses pseudotyped by the encoded chronic and acute Env proteins. This study suggested that one or a few virus variants initiated infection in most of the cases examined and that such viruses differed in a consistent way from the bulk of the viruses in the chronically-infected partner’s viral quasispecies. With an obvious need to further clarify the molecular and biological features of transmitted viruses, Keele and colleagues (2008) devised a novel experimental sequencing strategy to enable a more

precise molecular identification and enumeration of transmitted HIV-1 genomes. This new approach was based on single-genome amplification (SGA) of endpoint-diluted plasma vRNA/cDNA or peripheral blood mononuclear cell (PBMC) DNA followed by direct population sequencing of the uncloned DNA amplicon. Sequences were then analyzed phylogenetically in the context of a mathematical model of exponential virus growth and random virus evolution so as to identify actual transmitted/founder viral genomes that were responsible for productive clinical infection (Keele et al. 2008; Salazar-Gonzalez et al. 2008; Lee et al. 2009). This SGA approach had several key advantages over previous methods: First, SGA-direct sequencing eliminates *Taq* polymerase errors in finished sequences because such base substitutions are essentially random in distribution and any one substitution is present in exceedingly low proportions in the uncloned amplified product; because the amplified product is directly sequenced, such mutations are not evident on sequence chromatograms unless they occur in the initial polymerase chain reaction (PCR) amplification cycles, in which case they are identified as “double peaks” and the sequence is disregarded. Second, SGA eliminates both template switching (recombination) between genetically distinct viral genomes and template resampling, because amplification is initiated from single genomes. Third, SGA-direct sequencing avoids misrepresentation of target sequence frequencies because of unequal cloning.

SGA-direct amplicon sequencing was thus applied in rapid succession to a large number of clinical cohorts of individuals acutely infected by HIV-1 subtypes A, B, C, D, CRF01_AE, and others. In the first large cohort study of 102 subjects with acute HIV-1 subtype B infection, SGA-direct amplicon sequences of 3449 complete *env* gp160 genes from plasma vRNA/cDNA were generated (Keele et al. 2008). A key feature of this study was the categorization of study subjects by Fiebig staging (Fig. 1), which allowed for a systematic analysis of HIV-1 sequence diversification in the earliest phases of acute infection. Sequences showed discrete low-diversity lineages representing the progeny

of distinct transmitted viral genomes. Sequences generally showed a Poisson distribution of mutations and star-like phylogeny that coalesced to unambiguous transmitted/founder genomes at or near the moment of virus transmission. This was true for subjects who acquired their infection by any of several routes including vaginal, rectal, penile, or intravenous transmission. Sequences evolved over time consistent with the Poisson distribution and a model that accounted for exponential virus growth, reproductive ratio, virus generation time, and reverse transcriptase error rate estimated for HIV-1 (Lee et al. 2009). This, in turn, allowed for estimates of time to a most recent common ancestor for all sequences. By this approach, a precise molecular identification and enumeration of transmitted/founder virus genomes was obtained for 98 of 102 consecutively studied subjects. Seventy-eight of 102 subjects had evidence of productive clinical infection by a single virus, and 24 subjects had evidence of acute infection by a minimum of two to five genetically distinct viruses from a single partner. Since this study identified the sequences of full-length transmitted/founder *env* gp160 genes, these sequences could be molecularly cloned and characterized biologically. The key difference between this approach and previous strategies for analyzing cloned *env* genes is that in the SGA-direct amplicon sequencing method the cloning step is done *after* an exact and unambiguous sequence determination of the transmitted/founder *env* gene has been made. This allows the cloned version of the transmitted/founder *env* to be matched exactly with the inferred *env* sequence of the transmitted/founder virus. This same SGA approach was used in subsequent studies to clone full-length transmitted/founder HIV-1 genomes (Salazar-Gonzalez et al. 2009; Li et al. 2010). Thus, in the initial Keele study (Keele et al. 2008), 55 *env* genes whose sequences matched exactly with transmitted/founder genes were molecularly cloned, expressed in 293T cells, and used to pseudotype in *trans* Env-deficient HIV-1 viruses. All Envs were biologically functional with respect to virus entry and all were CD4 dependent. Fifty-four of 55 Envs were CCR5-tropic and

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one was CCR5/CXCR4 dual-tropic. Transmitted/founder Envs showed neutralization sensitivity profiles typical of tier 2 or 3 primary virus strains (Li et al. 2005a). Thus, the findings from this study provided new clarity to the quantitative and molecular aspects of HIV-1 transmission.

Based on the empirical findings described by Keele et al. (2008) and the mathematical model of early HIV-1 replication and diversification developed by Korber, Perelson, and colleagues (Keele et al. 2008; Lee et al. 2009), a conceptual framework for describing HIV-1 transmission and early diversification was developed (Fig. 2). Direct evidence in support of this transmission model and the notion that actual transmitted/founder viral genomes could be identified unambiguously by SGA-direct

amplicon sequencing came from three additional sets of studies: Indian rhesus macaque monkeys inoculated intra-rectally with low-dose SIVmac251 in which viruses in the inoculum and in newly infected animals were found to be identical in *env* gene sequences (Keele et al. 2009); human transmission pairs in which HIV-1 *env* and full-length viral genomic sequences were found to be identical in donors and recipients (Haaland et al. 2009; J. Salazar-Gonzalez and G. Shaw, unpubl.); and 454 deep sequencing of tens of thousands of plasma vRNA/cDNA HIV-1 genomes from three acutely-infected human subjects, demonstrating productive clinical infection by the same transmitted/founder viruses as identified by SGA-direct amplicon sequencing (Fischer et al. 2010). Of note, the terms “transmitted/founder sequence”

Figure 2. HIV-1 transmission model. HIV-1 virions that breach the mucosa may have different fates. Empirical measurements of virus replication and diversification, together with a mathematical model of random virus evolution, allow for a precise molecular identification of transmitted/founder viruses that are responsible for productive clinical infection (Keele et al. 2008; Lee et al. 2009). R_0 is the reproductive ratio. $R_0 > 1$ leads to productive clinical infection, whereas $R_0 < 1$ results in an extinguished infection.

and “transmitted/founder virus” are used to designate the coalescent or consensus sequence of a low-diversity virus lineage in acutely infected humans or monkeys because such sequences correspond literally to founder sequences that are responsible for establishing productive clinical infection. In most instances, the founder sequence corresponds to the actual transmitted sequence. Exceptions are likely uncommon and include founder sequences that differ from the actual transmitted sequence by one or a few nucleotides because of reverse transcriptase errors in the initial one or two replication cycles or, more substantially, because of recombination between copackaged heterozygous RNA genomes during reverse transcription in the first infected cell (Lee et al. 2009).

Additional studies of acutely infected human cohorts using SGA-direct amplicon sequencing provided further insights into the HIV-1 population bottleneck. These studies focused on heterosexual transmission in sub-Saharan Africa (Abrahams et al. 2009; Haaland et al. 2009; J Baalwa and G Shaw, unpubl.), MSM transmission in the United States (Kearney et al. 2009; Li et al. 2010), injection drug use transmission in Canada, Russia, and Thailand (Bar et al. 2010; Masharsky et al. 2010; K Bar and G Shaw, unpubl.), and most recently, maternal-infant transmission in Malawi (Russell et al. 2011). Each of these studies provided unambiguous molecular identification of transmitted/founder viral genes or genomes and precise estimates of the numbers of transmitted/founder viruses responsible for productive clinical infection. Two studies used SGA-direct amplicon sequencing to identify full-length transmitted founder genomes (Salazar-Gonzalez et al. 2009; Li et al. 2010). A summary of all of these studies, which included over 300 acutely infected subjects, indicated that approximately 80% of heterosexual subjects are productively infected by a single viral genome (Keele et al. 2008; Abrahams et al. 2009; Haaland et al. 2009) and approximately 60% and 40% of MSM and IDUs, respectively, are productively infected by single genomes. The range in transmitted/founder genomes that resulted in productive clinical infection in these studies

was 1–16, and not surprisingly, the highest numbers were in IDUs (Bar et al. 2010; Li et al. 2010; Masharsky et al. 2010; K Bar and G Shaw, unpubl.). For maternal-infant transmissions, 68% were productively infected by a single virus and this varied depending on intra-uterine versus intrapartum transmission routes (Russell et al. 2011). The observed differences in the multiplicity of viral infection associated with different routes of transmission roughly parallels the relative risk of clinical infection on a per event basis (Table 1). As predicted for transmitted/founder viruses, transmitted/founder *env* genes were invariably biologically functional and full-length genomes were invariably replication competent (Keele et al. 2008; Salazar-Gonzalez et al. 2009; Li et al. 2010; C Ochsenbauer, J Kappes, and G Shaw, unpubl.). Interestingly, in studies of primarily heterosexual transmission (Keele et al. 2008; Abrahams et al. 2009), in those individuals in which multiple viruses initiated infection, the number of infecting variants did not follow a Poisson distribution. This finding is inconsistent with each variant being transmitted independently and with low probability and suggests that transmission cofactors such as sexually transmitted infections (STIs) or hormonal contraceptives lower the barrier to transmission (Abrahams et al. 2009; Haaland et al. 2009). This interpretation is consistent with the model portrayed in Figure 2, in which virus transmission can fail at multiple steps following inoculation onto a mucosal surface. Thus, STIs including genital ulcer disease may abrogate the barrier imposed by an intact mucosa by inducing breaks in the epithelial lining, thereby allowing more virus variants to initiate infection in the mucosal tissue. Alternatively, inflammation induced by STIs could increase the availability of activated CD4 cells required to initiate a spreading infection, in this way allowing infections that would have failed because of lack of available target cells to expand. Finally, recent SGA comparisons of the genetic diversity of HIV in the genital tract of the transmitting partner to the transmitted/founder virus in the acutely infected partner argue against genital compartmentalization of specific viral

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variants in the donor as being responsible for origin of the population bottleneck, consistent with the model presented in Figure 2 (D Boeras, and E Hunter, unpubl.).

GENETIC SIGNATURES AND PHENOTYPES OF TRANSMITTED/FOUNDER VIRUSES

The identification and enumeration of transmitted/founder viruses in acutely infected heterosexuals, MSM, IDUs, and infants born to infected mothers provided an opportunity to explore such viruses for genetic signatures and phenotypic properties that might distinguish them from other viruses. Such studies had been undertaken previously with early but not transmitted/founder viruses, and they yielded intriguing findings, but the molecular identification of transmitted/founder viruses allowed for the first time such studies to be undertaken with far greater precision. From earlier studies, it was well established that the transmission of HIV-1 in humans selected for CCR5 tropic viruses, and that R5 viruses could be distinguished from CXCR4-tropic viruses (X4 viruses) genotypically by amino acid “signatures” primarily in the V3 region of Env (Resch et al. 2001; Jensen et al. 2003). But the important question now raised was if there were additional genetic signatures and corresponding phenotypic properties that distinguish transmitted R5 from others. Put differently, is HIV-1 transmission essentially a stochastic process in which any reasonably fit R5 virus can be transmitted, or are there critical properties of viruses that are transmitted that distinguish them from the innumerable variants that circulate in every chronically infected individual? If such signatures and phenotypes could be identified, they could conceivably represent targets of rational vaccine or drug design.

The first suggestion that HIV-1 transmission might select for traits other than coreceptor usage came from a genetic comparison of the viral *env* sequences from eight subtype C HIV-1 transmission pairs (Derdeyn et al. 2004). This study was conducted prior to reports describing SGA and thus carries the caveat that molecular clones of *env* could have

contained in vitro generated artifacts from *Taq* polymerase misincorporation or recombination. Nonetheless, in each subject pair, regardless of whether virus was transmitted MTF or FTM, the newly transmitted viruses encoded statistically shorter and less glycosylated V1–V4 regions than did *envs* from the chronically infected partner. This finding raised the possibility that more compact Env glycoproteins might interact more efficiently with relevant target cells in the genital mucosa. This observation was subsequently confirmed using SGA methods in an independent cohort of 10 subtype C transmission pairs (Haaland et al. 2009). Similar results, albeit with non-SGA methods, were obtained in studies comparing Envs from subtype A HIV-1 acutely infected sex workers to a database of matched chronic virus sequences (Chohan et al. 2005) and 13 subtype D and A transmission pairs from the Rakai district of Uganda (Sagar et al. 2009). Interestingly, such differences in acute versus chronic Envs were not seen in studies of recently transmitted subtype B HIV-1 heterosexual or MSM infections, suggesting that subtype differences in the virus or maturity of the epidemics may influence the contribution of such a phenotype to HIV-1 transmission (Chohan et al. 2005; Frost et al. 2005; Wilen et al. 2011a). Most recently, Korber and colleagues (Gnanakaran et al. 2011) compared over 7000 SGA-derived *env* gp160 sequences from 275 acutely or chronically infected subtype B subjects and observed statistically robust signatures comprising single amino acids, glycosylation motifs, and multisite patterns of clustered amino acids. These included signatures near the CCR5 coreceptor binding surface, near the CD4-binding site, in the cytoplasmic domain of gp41, and in the signal peptide. The motif with highest statistical significance was at amino acid position 12 in the signal peptide, which may affect Env expression and incorporation of Env into virions (Asmal et al. 2011). The second most significant signature was at amino acid position 413–415, which affected a glycan involved in escape from antibody neutralization. How these changes might affect HIV-1 transmission is currently unknown.

A second approach to analyzing transmitted HIV-1 genomes for distinguishing properties is by phenotypic characterization. It has been difficult to link sequence differences observed between chronic and acute Envs, even within transmission pairs, to a distinct phenotypic property that might influence transmission other than CCR5 tropism. In an analysis of Zambian subtype C transmission pairs (Derdeyn et al. 2004), it was found that Envs from the newly transmitted viruses retained sensitivity to neutralization by the partner's antibodies and in fact were more sensitive on average than Env variants derived from the transmitting partner (Derdeyn et al. 2004). This characteristic coupled with shorter variable loops in early viruses led to the hypothesis that transmissibility was linked to loss of Env modifications required for neutralization resistance in the chronically infected host but dispensable in the immunologically naïve partner. Despite this, the acute Envs were not generally more sensitive to neutralization by either pooled HIV-1-positive sera or to a majority of broadly neutralizing antibodies (Derdeyn et al. 2004; Li et al. 2006). Moreover, the acute and chronic Envs had similar requirements for high CD4 and CCR5 levels on target cells and they showed comparable utilization of CCR5 chimeric proteins and alternative coreceptors (Isaacman-Beck et al. 2009; Alexander et al. 2010). Also, following up on genetic signatures of virus transmission identified by Korber and colleagues (Gnanakaran et al. 2011), Asmal et al. (2011) characterized the phenotypic effects of the position 12 polymorphism in the Env leader sequence, which was found to be an enriched motif in transmitted/founder viruses. Experiments showed an association between a positive amino acid (histidine) at position 12 and higher Env expression, higher virion Env incorporation, and higher virion infectivity compared with control viruses.

The first biological analysis of complete HIV-1 subtype B transmitted/founder *env* genes (Keele et al. 2008), and of subtype B and C transmitted/founder full-length viral genomes (Salazar-Gonzalez et al. 2009; Li et al. 2010), showed these viruses to be uniformly CD4 dependent and CCR5 or CCR5/CXCR4

dual tropic. These findings thus revealed that CCR5 tropism is a property of the transmitted virus itself and not a phenotype that evolves in the initial days and weeks of infection. Similarly, transmitted/founder viruses were found to show neutralization sensitivity patterns typical of tier 2 or 3 primary virus strains (Li et al. 2005a) with V3 and coreceptor binding surface regions well protected from binding by neutralizing monoclonal or polyclonal HIV-1 antibodies (Keele et al. 2008). Again, these were properties of the transmitted/founder virus at or near the moment of virus transmission and were not properties that evolved in early infection. Subtype B and C transmitted/founder genomes encoded viruses that replicated efficiently in primary human CD4⁺ T cells but much less well in monocyte-derived macrophages (Salazar-Gonzalez et al. 2009; Li et al. 2010; C Ochsenbauer, J Kappes, and G Shaw, unpubl.), consistent with results obtained with Env pseudotyped viruses (Isaacman-Beck et al. 2009). These observations were extended to subtype A transmitted/founder viruses, which replicated efficiently in primary human CD4⁺ T cells but very poorly in monocyte-derived macrophages. Interestingly, a substantial proportion of subtype D transmitted/founder viruses replicated efficiently in both CD4⁺ T cells and macrophages (J Baalwa and G Shaw, unpubl.), which may correlate with enhanced neuropathogenesis of subtype D viruses more generally (Sacktor et al. 2009). Again, these findings described features of transmitted/founder viruses at or near the moment of transmission and not virus properties that evolved in the newly infected host. Thus, these findings have particular relevance to rational vaccine design efforts and studies of virus transmission in general. With respect to animal models and human tissue explant studies of HIV-1 transmission, the results suggest that tissue macrophages may not play a significant role in HIV-1 transmission and that prototypic macrophage-tropic HIV-1 strains such as BaL, ADA, and YU2 may not be best suited as challenge viruses.

Swanstrom and colleagues (Russell et al. 2011) recently examined the genetic and biological

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basis of the HIV-1 population bottleneck in mother-infant HIV-1 transmission in subtype C infections in Malawi. They used SGA techniques to characterize 19 transmission pairs, of which 10 involved intrauterine transmission and nine intrapartum transmission. There was a stringent transmission bottleneck in each case. Thirteen of 19 transmissions were estimated to be from a single virus. Intrapartum (but not intrauterine) transmissions were characterized by transmitted/founder Envs that were shorter and had fewer potential amino-linked glycans in V1–V5. Mother and infant viruses were similar, however, in their sensitivity to soluble CD4, a panel of neutralizing monoclonal antibodies, and to autologous and heterologous polyclonal antibody neutralization. Thus, a distinguishing transmission phenotype was not evident.

The most comprehensive and systematic assessment thus far of the biology of transmitted/founder Envs compared with chronic HIV-1 Envs has been conducted by Doms and colleagues (Wilén et al. 2011a). These investigators compared the biological activity of 24 clade B transmitted/founder Envs with that of 17 chronic controls. To increase the likelihood of an intact mucosal barrier in the acutely infected recipients and thus the likelihood of identifying phenotypic properties associated with mucosal transmission, only transmitted/founder Envs from individuals productively infected by a single virus were enrolled in the acute infection arm of the study. Env pseudotyping was used to assess envelope function in single-round infectivity assays to compare coreceptor tropism, CCR5 utilization, primary CD4⁺ T-cell subset tropism, dendritic cell *trans*-infection, Env fusion kinetics, and neutralization sensitivity between acute and chronic Envs. Transmitted/founder and chronic Envs were phenotypically equivalent in most assays, although transmitted/founder Envs were slightly more sensitive to neutralization by the CD4-binding-site antibodies b12 and VRC01 and by pooled human HIV hyperimmune immunoglobulin (HIVIG). These findings were independently validated using a panel of 14 additional chronic HIV-1 Env controls.

With a relatively large number of transmitted/founder and acute Envs now having been examined across a number of different studies and by different investigative groups, it seems that no single major genetic or phenotypic signature is required for transmission beyond the use of CCR5. Current data suggest that an array of genetic traits, including but not limited to shorter variable loops and reduced numbers of amino-linked glycosylation sites is associated with enhanced virus transmission at least for subtypes A, C, and possibly B. The structural implications of these signatures and of the modestly enhanced neutralization sensitivity of subtype B viruses are not yet understood. However, the possibility exists that relatively subtle alterations of Env structure and function in the context of the native Env trimer could provide sufficient selective advantage during the eclipse phase of HIV-1 transmission to result in preferential transmission of such viruses. The recent finding that HIV-1 gp120 binds to the CD4⁺ T-cell gut homing integrin $\alpha 4\beta 7$ has raised the possibility that this capacity is important to an infecting virus by targeting cells capable of trafficking to the gut-associated lymphoid tissue (Arthos et al. 2008). It has been reported that $\alpha 4\beta 7$ highly expressing CD4⁺ T cells are more susceptible to productive infection by HIV-1 than those expressing low levels of the integrin, in part because this subset is enriched for activated CD4⁺ cells, and in part because $\alpha 4\beta 7$ hi cells express high levels of CCR5 and low levels of CXCR4 (Cicala et al. 2009). Interestingly, a small sample of acute subtype A and C virus Envs bound $\alpha 4\beta 7$ with high affinity, and in some cases, later virus strains showed significantly reduced binding (Nawaz et al. 2011), consistent with an early requirement for infection of $\alpha 4\beta 7$ -expressing cells that is dispensable once infection in the gut mucosa has been established. Given that HIV-1 transmission is inherently inefficient and likely represents the most vulnerable point in the natural history of HIV-1 infection, identifying unique properties and potential vulnerabilities of transmitted/founder viruses remains an important objective.

MODELS OF HIV-1 TRANSMISSION

Because the eclipse phase of infection and mucosal invasion sites invariably obscure the earliest virus-host interactions, investigators have turned to models of HIV-1 transmission to study the earliest events that initiate productive clinical infection. Figures 1 and 2 illustrate early HIV-1 replication dynamics and diversification along with initial host antibody responses for which there is now substantial direct experimental validation in humans. Figure 3 illustrates early virus-host interactions, many of which are not amenable to direct analysis in humans but which have been studied in human tissue explants and in the primate SIV model of HIV-1 transmission. Importantly, experimental data supporting all three models of HIV/SIV replication, diversification, and transmission biology are largely internally consistent and mutually reinforcing and have begun to provide a window on the HIV-1 transmission process not previously possible.

Mathematical Models

Central to the development and validation of a strategy to identify transmitted/founder virus genomes by SGA, direct amplicon sequencing, and phylogenetic analysis was the development of a mathematical model to describe early virus replication and diversification. This model was developed and refined by Korber, Perelson, and colleagues (Keele et al. 2008; Lee et al. 2009) and used previously estimated parameters of HIV-1 generation time (2 d), reproductive ratio ($R_0 = 6$), and reverse transcriptase error rate (2.16×10^{-5}) and assumed that initial virus replicates exponentially infecting R_0 new cells at each generation and diversifying under a model of evolution that assumes no selection. Viruses were predicted to show a Poisson distribution of mutations and a star-like phylogeny. This model led Keele and colleagues (2008) to posit that the progeny of individual transmitted/founder viruses that establish productive clinical infection could thus be identified in acute infection as distinct low-diversity genetic lineages and that the consensus sequence,

or coalescent, of each lineage would correspond to actual transmitted/founder viruses. These hypotheses were proven to be valid in both acute HIV-1 and SIV infection (Keele et al. 2008, 2009; Li et al. 2010; Liu et al. 2010; Stone et al. 2010). More recently, Perelson and coworkers developed a stochastic model of the early HIV-1 infection (Pearson et al. 2011), as opposed to deterministic models, to probe the earliest virus-host events following virus inoculation. They distinguish virus that is released from cells continuously versus in a burst and show that these different mechanisms of virus production lead to substantially different early viral dynamics and different probabilities of virus extinction. The stochastic model moves us a step closer to what is believed to be the most vulnerable point in HIV-1 infection, in the hours and days immediately following virus exposure, and provides insights relevant to vaccine and therapeutic interventions at this critical period in the transmission process of HIV-1.

Primate-SIV Infection Models

The primate-SIV infection model captures many of the essential elements of virus-host interactions illustrated in Figures 1–3. Because more data are available for the primate cervico-vaginal infection model, our discussion will be limited to it, although recent work with a penile SIV infection model is promising (Ma et al. 2011; Yeh et al. 2011). Although the eclipse phase of mucosal infection by SIVmac251 or SIVsmE660 is generally shorter than the 7–21 d reported for HIV-1 infection of humans, early SIV replication kinetics in acute SIV infection of macaques is quite comparable with HIV-1 infection of humans (Liu et al. 2010; Stone et al. 2010). In fact, Barouch and colleagues showed that in a low-dose rectal mucosal infection model of Indian rhesus macaques, the eclipse phase lengthens to approximately 7 d as the multiplicity of infection approaches one virus (Liu et al. 2010). Moreover, the early diversification of mucosally transmitted SIV in macaques is virtually indistinguishable from HIV-1 in humans (Keele

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Figure 3. Model of cervicovaginal infection by HIV-1. Preferential R5 HIV-1 transmission is illustrated along with potential roles for Langerhans cells, dendritic cells, and tissue macrophages. Most HIV-1 transmitted/founder viruses replicate efficiently in CD4⁺ T cells but not in monocyte-derived macrophages (Salazar-Gonzalez et al. 2009; Li et al. 2010), raising questions about the role of macrophages in HIV-1 transmission. Virus-host cell interactions in the initial days of infection have been elucidated primarily in the SIV-Indian rhesus macaque infection model (Haase 2010) and in human tissue explants (Hladik and McElrath 2008). (Adapted from Pope and Haase 2003.)

et al. 2008, 2009; Liu et al. 2010; Stone et al. 2010). The virus-host cell interactions believed to underlie HIV-1 transmission based on human tissue explant studies and in vitro analyses of virus-host cell interactions are also generally similar to SIV infection of macaques. Figure 3 represents a mucosal infection model based on a large amount of SIV–rhesus macaque cervicovaginal transmission and infection data, human tissue explant findings, and in vitro

analysis of HIV–host cell interactions. SIV can cross mucosal epithelial surfaces within hours (Hu et al. 2000) and infects small numbers of cells productively (Zhang et al. 1999; Miller et al. 2005). In a high-dose mucosal challenge model, the numbers of viruses that cross the epithelial surfaces are great, as are the numbers of initially infected cells (Haase 2010; B Keele, J Estes, and J Lifson, unpubl.). In low-dose mucosal challenge models, these numbers are

much smaller and more characteristic of human mucosal infection in which the numbers of transmitted/founder viruses are typically one or few (Keele et al. 2008, 2009; Abrahams et al. 2009; Haaland et al. 2009; Li et al. 2010; Liu et al. 2010; Stone et al. 2010). In macaques, the initial founder populations of productively infected cells undergo rapid expansion and within 1 wk or less, SIV disseminates widely to secondary lymphoid organs including GALT, in which a self-propagating systemic infection is established (Veazey et al. 1998; Miller et al. 2005). Although these events likely have close parallels in human HIV-1 infection, a key difference is that in high-dose mucosal SIV infection, every animal becomes productively infected by generally large numbers of transmitted/founder viruses following a single virus inoculation. In low-dose mucosal SIV infection, only a fraction of animals becomes productively infected following a single inoculation, and then generally by one or a few viruses. In uncomplicated human vaginal exposure to HIV-1, the virus inoculum is effectively still lower, resulting in productive clinical infection in one in every 200–2000 exposed women, and then by a single virion in 80% of cases (Table 1) (Keele et al. 2008; Abrahams et al. 2009; Haaland et al. 2009). Thus, it is unclear at the present time how closely the in situ virus-host interactions described thus far for the earliest phases of SIV infection of macaques (Haase 2010) correspond to eclipse phase events in human HIV-1 infection. Beyond these earliest events, SIV generally becomes detectable in the blood by the second week of infection and increases exponentially at a rate comparable or even faster than that of HIV-1 in humans (Lifson et al. 1997; Nowak et al. 1997; Ribeiro et al. 2010).

SIV and HIV-1 presumably invade the new host where mucosal surfaces can most easily be breached, and for HIV-1 this can be aided by microabrasions caused by the trauma of sexual intercourse or associated with sexually transmitted diseases or genital ulcerative disease (Abrahams et al. 2009; Haaland et al. 2009). It is of note that hepatitis C virus (HCV) generally circulates at even higher titers in human plasma than does HIV-1, yet it is exceedingly rare for

it to be transmitted by penile-vaginal intercourse—so rare that the U.S. Department of Health and Human Services and Centers for Disease Control do not recommend barrier protection (condoms) to prevent heterosexual transmission of HCV in discordant couples. This suggests that the mechanism of transmission of HIV-1 across the vaginal and cervical mucosa is fundamentally different from a simple exchange of virus-contaminated blood or blood plasma across an abraded or otherwise traumatized mucosal surface. A single layer of columnar epithelium covers the endocervix and part of the transformation zone between endocervix and ectocervix. The endocervix and transformation zone appear to be preferential but not exclusive sites of HIV-1 entry and early replication (Li et al. 2009a). The stratified squamous epithelium of the vagina contains Langerhans cells whose extensions may reach the luminal surface. Langerhans cells are infectable by HIV-1 and can traffic to the submucosa. The submucosa of both the vagina and cervix contain dendritic cells, which also can become infected or transmit HIV-1 to CD4⁺ T cells (Boggiano and Littman 2007; Boggiano et al. 2007; Hladik et al. 2007).

In macaques, the earliest focal collections of productively infected cells in cervical mucosa and submucosa are CD4⁺ T cells that show a largely “resting” phenotype and low-level CCR5 expression (Zhang et al. 1999, 2004; Li et al. 2005b). In acute HIV-1 infection of humans, “resting” CD4⁺ T cells also play a substantial role in supporting high-level virus replication (Zhang et al. 1999; Schacker et al. 2001). It is believed that such cells are memory CD4⁺ T cells that have reverted to a largely resting phenotype but have retained sufficient CCR5 and activation pathway molecules to support SIV/HIV replication. This may be accentuated in GALT, in which $\alpha 4\beta 7$ expression on helper CD17⁺/CD4⁺ T cells may facilitate virus infection and replication (Arthos et al. 2008; Kader et al. 2009). Interestingly, tissue macrophages are not a primary target of early SIV infection, consistent with the failure of most transmitted/founder HIV-1 genomes to replicate efficiently in this cell type in vitro

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(Salazar-Gonzalez et al. 2009; Li et al. 2010; C Ochsenbauer, J Kappes, and G Shaw, unpubl.).

Perelson has suggested that mucosal infections by HIV-1, and by analogy SIV, may be extinguished because of stochastic events in the transmission process (Pearson et al. 2011). This could be caused by limitations in target cell availability that may be more significant in cervicovaginal mucosa and submucosa than in rectal tissue (Edwards and Morris 1985; Ma et al. 2001; Zhang et al. 2004; Pudney et al. 2005; Miyake et al. 2006). A complex interplay of early innate host defenses may contribute to early virus containment or elimination, but paradoxically such responses can facilitate transmission by recruiting new potential target cells for infection (Li et al. 2009a; Haase 2010). Once infection disseminates to locoregional lymphatics, the GALT, and other secondary lymphoid tissues, massive depletion of memory $CD4^{+}$ T cells in the lamina propria ensues (Clayton et al. 1997; Veazey et al. 1998; Guadalupe et al. 2003; Brenchley et al. 2004; Mehandru et al. 2004; Mattapallil et al. 2005; Li et al. 2005b). Massive immune activation, further loss of $CD4^{+}$ T cells, and an acute enteropathy commonly follow (Kotler et al. 1984; Heise et al. 1994; Li et al. 2008). Defining critical steps in the mucosal transmission of SIV and HIV-1, including those most vulnerable to therapeutic or immunologic modulation or intervention, remains a high priority. Modulation of immune activation in the SIV–rhesus macaque infection model by glycerol monolaurate represents proof-of-concept of such an approach to prevention (Li et al. 2009a). Passive immunization with neutralizing monoclonal antibodies including 2F5, 4E10, 2G12, and B12 in a SHIV infection model provides another important proof-of-concept of the vulnerability of HIV-1 at this critical juncture (Mascola 2002; Hessel et al. 2007, 2009a,b).

Human Tissue Explant Models

An extensive body of literature describes human tissue explant models and their application to the analysis of HIV-1 and SIV transmission. Much of this has been summarized in recent

reviews (Hladik and McElrath 2008; Wira and Veronese 2011). Although increasing attention in recent years has been paid to penile (Fischetti et al. 2009; Dinh et al. 2011) and gastrointestinal (Fletcher et al. 2006; Grivel et al. 2007; Shen et al. 2009, 2010) explant models, most information (Collins et al. 2000; Greenhead et al. 2000; Hu et al. 2004; Maher et al. 2005; Hladik et al. 2007; Saba et al. 2010; Merbah et al. 2011), can be correlated with in situ studies of primate infection by SIV (Haase 2010). Hladik and colleagues (2007) described a human vaginal organ explant culture system that involved the physical separation of vaginal epithelium from underlying stroma. This allowed for an identification of HIV-1 infection of cells exclusively in the epithelium and tracking of these cells as they exited the epithelium into surrounding culture medium. By using confocal fluorescent microscopy, virions were observed to bind and productively infect $CD4^{+}$ $CCR5^{+}$ $CD45RO$ (memory) T cells within 2 hours of virus exposure to the intact epithelial surface. Both binding and infection of these cells was dependent on $CD4^{+}$ $CCR5^{+}$ engagement by the viral Env glycoprotein and could be blocked by receptor antibodies. HIV-1 also bound to Langerhans cells in the epithelium and localized to a perinuclear intracellular compartment but without evidence of productive infection. Antibody blocking of $CD4^{+}$ and $CCR5^{+}$, and mannan inhibition of C-type lectin binding by Env, did not inhibit Langerhans cell uptake of HIV-1, suggesting alternative pathways of virus binding and uptake by Langerhans cells. As long as 60 h posttransmission, HIV-1 could be identified in vacuolar compartments of Langerhans cells, which were frequently observed to associate with $CD4^{+}$ T cells. Whether Langerhans cell $CD4^{+}$ T-cell associations represented an “immunological synapse” active in virus transmission was not determined. This study thus showed rapid productive infection of human memory $CD4^{+}$ T cells consistent with in situ findings in the SIV–macaque infection model (Haase 2010), and internalization and persistence of HIV-1 in Langerhans cells in association with $CD4^{+}$ T cells (Fig. 3). Studies by Saba et al. (2010) in a cervicovaginal explant

model also observed that CCR5⁺ CD4⁺ T cells with the effector memory phenotype are a primary target for infection. Human cervicovaginal tissue ex vivo was found to preferentially support productive infection by R5 HIV-1 rather than by X4 HIV-1 despite ample expression of CXCR4. Productive infection by R5 HIV-1 occurred preferentially in activated CD38⁺ CD4⁺ T cells in association with activation of HIV-1–uninfected (bystander) CD4⁺ T cells that may amplify viral infection. That CXCR4-tropic HIV-1 replicated only in the few tissues that were enriched in CD27⁺ CD28⁺ effector memory CD4⁺ T cells, if translatable to tissue in vivo, could in part explain the selection of R5 viruses during transmission. Still other human cervical explant studies were conducted by Shattock and colleagues (Greenhead et al. 2000). This work characterized cellular factors involved in HIV-1 entry (Hu et al. 2004) and identified potential therapeutic agents that block infection (Fletcher et al. 2005; Cummins et al. 2007; Buffa et al. 2009). In this model, blockade of CD4 or CCR5/CXCR4 prevented localized mucosal infection and trafficking by dendritic cells.

An extensive ex vivo tissue explant literature thus describes virus–host cell interactions in female and male genital mucosa that may contribute to natural HIV-1 transmission. This work includes descriptions of cell targets, cell surface molecules that mediate virus attachment or infection, and cell signaling pathways that facilitate virus infection and transmission (Hladik and McElrath 2008). However, a key question still unanswered by in vitro cell culture analyses, ex vivo tissue explant studies, or in vivo SIV-macaque infection studies is what is the contribution of Langerhans cells, dendritic cells, or macrophages, compared with CD4⁺ CCR5⁺ T cells alone to mucosal transmission of HIV-1 (Fig. 3). As this question is revisited, attention to the biological relevance and authenticity of the challenge viruses used in the model systems is warranted. The macrophage-tropic HIV-1 BaL, ADA, and YU2 strains, and the T-cell line-adapted NL4.3 and HXB-2 strains, have been used extensively in previous transmission model studies, yet none of these

viruses appears to faithfully reflect the properties described thus far for transmitted/founder viruses from acutely infected humans (Keele et al. 2008; Salazar-Gonzalez et al. 2009; Li et al. 2010; Wilen et al. 2011a). Future mucosal transmission studies may benefit from using virus challenge strains that more closely reflect actual transmitted/founder viruses.

CONCLUSIONS

Interrupting HIV-1 transmission by vaccination, microbicides, pre- or postexposure antiviral drug prophylaxis, or by any of a number of other prevention strategies is of paramount importance in curbing the HIV/AIDS pandemic. Elucidation of molecular viral–host interactions responsible for virus transmission and productive clinical infection can be instrumental in achieving this end. A new strategy for the molecular identification of transmitted/founder HIV-1 genomes, together with improved tissue explant and in vivo models of HIV-1 transmission, brings the possibility of elucidating critical HIV-1 transmission events and vulnerabilities within reach. Future studies can benefit from building on what has become an increasingly firm foundation in our knowledge of HIV-1 transmission.

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