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HIV Transmission: Time for Translational Studies to Bridge the Gap

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Abstract

More than 7000 new HIV infections are documented each day worldwide. In this Perspective, we dissect new results from a large clinical trial showing that genital and plasma HIV-1 RNA loads predict the risk of heterosexual transmission and that genital tract viral RNA load does so independently of plasma viral load. Furthermore, beyond its defined study end points, this well-conducted trial identified new research directions that should be pursued in smaller intensive basic and translational studies.

The research article by Baeten *et al.* in this issue of *Science Translational Medicine* (1) clearly reveals that prevention of human immunodeficiency virus-1 (HIV-1) infection remains a global imperative as the number of people living with HIV and AIDS continues to grow. The new work also pointedly identifies the critical need for fundamental translational research to enable advances on the public health stage. Worldwide, more than 7000 new infections are documented each day among those being tested. This number is undoubtedly an underestimate of the problem, as large numbers of sexually active individuals are unaware of their infection and may be active transmitters fueling the epidemic (2, 3).

Treatment advances have brought astounding benefits: The mean life span of HIV-infected individuals with full access and adherence to antiviral therapy and supportive medical care nearly equals that of HIV-uninfected people. This was unthinkable 20 years ago and is a remarkable achievement that reflects the combined efforts of science and public health. However, HIV prevention has met with more limited success. Education and condom use continue to be our greatest arsenal in prevention, with more recent work demonstrating significant benefits from male circumcision in reducing female-to-male transmission (4–7).

The field experienced a palpable move forward with the recent successes of the CAPRISA 004 (8) and iPrEx (9) clinical trials. CAPRISA 004 involved ~900 women in South Africa, in which 1% tenofovir gel was applied topically as pre-exposure prophylaxis (PrEP) to the vagina in a coitally dependent manner and demonstrated a 39% reduction in new HIV-1 infections (8). The iPrEx trial involved 11 sites in 4 countries and enrolled 2500 HIV-seronegative men who have sex with men (MSM); these individuals were randomized to daily (that is, independent of sexual activity) oral PrEP with Truvada—a combination therapy that contains the antiretroviral drugs tenofovir and emtricitabine—or a placebo; an

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~42% decrease in new infections was observed in participants who received Truvada compared to the placebo (9). These two trials not only provided proof of concept that transmission and consequent infection could be proactively reduced by the use of antiviral drugs but also set the course for consideration of U.S. Food and Drug Administration–approval for these prevention strategies. These studies were remarkable in their recruitment and retention of large numbers of participants with detailed demographic, behavioral, clinical, translational, and basic research data acquired. They also served to validate earlier mathematical models that predicted that even moderately effective interventions used by fractions of the targeted populations could dramatically impact and, eventually, reduce the AIDS epidemic (10, 11, 12).

But within the successes was robust evidence that confirmed the need for ongoing intensified efforts to promote adherence to therapies and to better characterize the mechanisms that drive HIV transmission and infection. Subset analyses in both trials suggest that topical and oral PrEP could be more effective in reducing transmission risks if greater adherence could be achieved. Researchers and policy-makers are addressing the difficulties inherent in adhering to a topical regimen, in part through the development of alternative delivery systems, such as intravaginal rings for vaginal PrEP (13), and through behavior-related public policy efforts to retain newly and chronically HIV-infected individuals within medical care. This includes strategies to identify and provide treatment for those HIV-infected persons who are “highly connected,” a term describing newly infected individuals with high viral loads who continue the rapid spread with their high-frequency encounters (14).

The PrEP (topical and oral) studies also highlight the need for better biomarkers that reliably predict pharmacokinetics (PK), pharmacodynamics (PD), tissue efficacy (the ability of a drug to protect a target tissue from infection), and safety. For topical PrEP, safety measures must include a rigorous assessment of the impact of the agents on the mucosal barrier and innate host defenses that protect against HIV infection (15, 16). In addition, biomarkers of adherence (those that ensure that prevention therapies are actually used as directed) within clinical trials are critical. These biomarkers will become more urgently as partially effective prevention modalities (such as those from the CAPRISA and iPrEx trials) are advanced into clinical practice. These approvals will render future placebo-controlled trials unethical. More importantly, the results underscore the requirement for strategies that promote real-world adherence, in particular because oral PrEP is now being advocated and prescribed in high-risk settings (17, 18). Identification of these new research directions is what well-designed, well-conducted large clinical trials offer beyond their defined study end points: guidance for the field to address and answer specific research questions with smaller intensive studies.

EARLY EVENTS IN TRANSMISSION

Progress in the field of HIV prevention continues to be restrained by the lack of refined information regarding specific behavioral acts involved in sexual transmission as well as a paucity of human data defining the mechanisms that underlie mucosal infection (15, 19). The uncertainty of factors that promote infection within tissue compartments as well as the target cell phenotypes for founder virus infection and propagation limit strategic development of effective large-scale interventions. Behavioral programs, circumcision, and test-and-treat policies can go only so far (Fig 1). Now that we have proof of concept that pharmacological interventions reduce HIV transmission in specific study cohorts, furthering these milestones requires more focused understanding of the biology of sexual transmission at the virus, cell, and tissue-compartment levels. These translational research study designs

need to progressively incorporate clinically relevant factors present in real-life, human sexual contexts (20, 21).

Enormous resources have been committed toward characterizing the frequencies of transmission in large cohorts, often based on serodiscordant couples (those in which only one partner is HIV-positive) (22–24), and incorporating treatment as a prevention tool for seropositives identified (for example, the Botswana MP3, HPTN065, POPART, and PepFAR programs). These studies provide the opportunity to assess the benefits and feasibility of more widely deployed test-and-treat programs (25). However, to maximize the impact of these programs, the next steps require essential input from translational studies. These studies will provide a greater understanding of the driving forces that enable the virus to establish an infectious nidus in a previously uninfected human host. Such advances necessitate the expansion of more conventional, controlled experimental in vitro and animal studies by progressively adding the clinically relevant features of insertive and receptive (both vaginal and anal) human sexual intercourse (20). Many of these features can now be assessed and parameters characterized in human clinical studies or with human tissue and other biologically relevant samples. These findings can then be translated back to animal models (humanized mice and nonhuman primates) to address more focused interventional, longitudinal, and pharmacological consequences of infection, in preparation for early-phase human trials.

COMPARTMENT CHARACTERISTICS

Over the past decade, there has been a welcome and essential paradigm shift to focus on tissue and secreted fluids in HIV pathogenesis studies that investigate the mechanisms and dynamics of sexual transmission (15, 19). This has resulted in further clarification of the important role played by compartmental viral burden (tissue or fluid within the female and male genital tracts, the lower gastrointestinal tract, and the anal area), as well as by the number and type of target cells at higher risk for infection within each of these compartments. These sites differ dramatically with respect to the number and activation status of vulnerable, activated target immune cells that express many of the co-receptors needed for HIV to enter the cell; there are far more in the gut compared to the cervicovaginal tissue. There are marked physical barriers to viral mucosal transmission: In the gut, the epithelium is simple, single-cell columnar, which is easily disrupted by trauma and irritating enemas or lubricants, in contrast to the vagina and ectocervix, with multi-layered, stratified, squamous epithelium, which is more resistant to breakdown (Fig. 2). Transmission is also affected by the viral load (cell-free or cell-associated) within the relevant fluid compartment (semen and genital tract secretions). Although the compartmental viral load usually parallels that in the blood, when the values are not concordant, it is usually the plasma concentrations of virus that are undetectable, with intermittent virus shed detected within local tissue reservoirs (26).

Semen is the most important vector for HIV transmission, yet few studies have examined how the sexual act and semen alter the mucosal environment (19, 21). Postcoital studies with samplings of vaginal and rectal fluid, ejaculate, and tissue biopsies may provide critical insights into factors that promote or prevent HIV transmission and their impact on the efficacy and safety of topical PrEP. Understandably, most microbicide safety and PK studies are conducted in the absence of coitus. However, semen and the physical act of sex may modulate efficacy by interfering with a drug's activity (27), altering PK through leakage or dilution effects, or changing the drug's distribution within the genital tract (28, 29). In addition, certain properties of semen, such as a recent report of the presence of amyloid fibrils, may actually promote HIV infection (30). Moreover, semen neutralizes the protective effects of the acidic pH of vaginal fluid, stimulates the release of inflammatory cytokines

(31), and promotes the influx of leukocytes and Langerhans cells, targets for HIV infection, into the genital tract (32)—factors that may also promote HIV transmission.

Contributing to the innate host defense against HIV transmission are the anatomical barriers provided by the multilayered squamous penile, vaginal, and ectocervical epithelia and the single-layer columnar colon and rectal epithelia; the pH of the endocervical epithelial compartment; microflora; contractility (especially of the colorectal compartment); mucus; and genital tract secretions. Consistent with the potential role of soluble mediators in contributing to host defense is the observation from several groups that genital tract secretions provide significant but variable protection against HIV infection *in vitro*. However, the molecules that contribute to this host defense (which include the defensin family of antimicrobial peptides, cytokines, chemokines, and others) and their biological regulatory mechanisms have not been well defined. Investigation is needed into the relative importance of each of the candidate host defenses in protecting against HIV infection, as preserving or augmenting these innate mechanisms may further limit HIV transmission.

The distinction between transmission (or exposure) and actual infection merits further clarification. Not every exposure results in an established infection, a point that underscores the necessity of understanding constitutively expressed and effective innate defenses so that these are maintained or enhanced when adding extrinsic agents. The differences between exposure and infection are further demonstrated by awareness of the relative risks of HIV infection observed for insertive partners compared to their receptive partners (vaginal and/or rectal). This relative-risk concept is based on mucosal features of both the insertive and receptive compartments. And yet, it is telling that more than 30 years into this epidemic, we still do not know, with confidence, the viral inoculum needed to transmit infection or the phenotype of the first cell(s) targeted by HIV in the vaginal, rectal, or male genital tract compartments. Importantly, we also do not know the tissue and cellular kinetics of the spread of the mostly homogeneous founder virus populations to generate the genetic diversity observed after widespread mucosal and systemic infection (33).

CURRENT CLINICAL CONTRIBUTIONS

The new work by Baeten *et al.* (1) describes a subset of the largest, longitudinal prospective study of HIV-1 transmission within a cohort of serodiscordant couples in 7 African countries, who participated in a randomized, placebo-controlled clinical trial to determine whether daily acyclovir suppressive therapy could reduce HIV transmission from HIV⁺/HSV⁺ individuals to their HIV-seronegative partners (34). Of the 3408 HIV-serodiscordant couples in the trial, 2521 provided genital samples (endocervical or semen) for HIV-1 RNA quantification; HIV-infected partners were seen monthly and uninfected partners quarterly, with HIV status assessed by measuring antibodies to HIV in plasma. Transmission events were linked within couples by viral sequence analysis using the most proximate samples when same-day samples were not available (35). While more frequent concordant sampling is always desired, the size of the study group buffers this limitation and was addressed with subset analyses by the authors in which they demonstrated that the statistically significant findings remained when they used only the samples that were collected on the same day.

Their results confirm in a prospective, longitudinal manner what has long been presumed and demonstrated primarily in cross-sectional studies: Higher viral loads in one person (usually measured by plasma viral loads) predict transmission and subsequent infection in a previously uninfected person. Also, the paper importantly documented that genital tract viral load predicted transmission risk independently of plasma viral load. While higher plasma HIV-1 RNA concentrations were also associated with increased HIV-1 transmission risk, curiously, only the effect on female-to-male-transmission was statistically significant. This

finding highlights the utility of focusing on the most proximal site where transmission occurs.

However, the study also identified a subset of 11 new infections from partners with undetectable genital tract viral loads while having detectable plasma HIV-1 RNA concentrations (at the visit closest to the collection of the genital sample). These events included 7 female-to-male transmissions of 46 reported (15%) and 4 male-to-female transmissions of 32 reported (12.5%). This observation may reflect the infrequent and lengthy time periods between sampling of the seronegative partners as well as intermittent genital tract shedding. But as these were not rare events of all the new HIV infections, it is clear that the field needs to better characterize what controls viral replication within the different compartments and, more specifically, what contributes to viral transmission. The “discrepancy” may also be attributed to sexual behaviors that were not captured in the study, including encounters with other partners (36). Notably, 40% of transmissions did not demonstrate linkage by viral sequences and likely reflect out-of-partnership exposures and infection. In addition, the study did not address receptive anal intercourse, which is being increasingly recognized among heterosexual couplings and is associated with a greater risk of HIV-1 infection.

These findings suggest the need for additional small but more intensive studies to define the stability and variability of genital tract viral loads, similar to what has been conducted for HSV genital tract shedding (37, 38). These HSV studies led to a paradigm shift in the definition of HSV latency. Similar intensive compartmental sampling (endocervical swabs, rectal fluid, and semen) could provide critical insights into the natural history of HIV-1 viral compartmental shedding. In the same manner, knowledge about whether more frequent ejaculations dilute or concentrate transmittable virus (or neither) is important information for study interpretation and may explain the unanticipated, nearly 5 times higher endocervical viral loads compared to ejaculate loads observed in this study. The data presented by Baeten *et al.* support the inclusion of genital tract (and plasma) viral loads as biomarkers of transmission risk in future studies and trials. However, both are imperfect predictors, a recognition that indicates that the other factors discussed above (for example, virulence of the viral strain, cellular targets, host immune status, and sexual behaviors) play important modulatory roles and also should be targets of future studies.

FOILING FALSE STARTS

The HIV prevention field has, by necessity, approached interventions indirectly using in vitro-responsive agents in clinical trials, often with disappointing results, as exemplified by the parent study of the current report (34). In that study, HSV-2 suppressive therapy did not reduce HIV-1 transmission rates, despite an observed average reduction of 0.25 log₁₀ copies/ml in plasma HIV-1 concentrations. These findings confirm that modest reductions in plasma (and presumably genital tract) viral loads are not sufficient to impact transmission.

To provide new insights, next-generation interventional trials must incorporate the observations and data obtained from the current and prior studies and include more intensive and comprehensive sample collection. Results obtained from ancillary basic biological studies performed within clinical trials provide the opportunity to address fundamental questions about modulators of HIV transmission and use these data to advance novel interventions. In addition to viral load, transmission risk is influenced by the type and frequency of sexual encounters, presence of other sexually transmitted infections (STIs), vaginal and/or rectal flora (especially when both compartments are part of the same sexual episode), infectivity of the viral particle (co-receptor usage, fitness), and the number and activation of immune target cells within the tissue. Some of these factors have been the

subject of epidemiological studies prompting public health efforts to reduce transmission rates with variable success. Examples include syndromic management of STI (39), treatment of bacterial vaginosis (BV) (40), acyclovir suppressive therapy (34), and various behavioral interventions. Scant attention, however, has focused on the roles that semen and genital tract secretions play in controlling HIV transmission or on developing strategies to further define and exploit innate defenses in these compartments.

COLLABORATIVE CYCLE

The findings of Baeten *et al.* (1) provide conclusive evidence regarding the relationship between viral load and risk of transmission. The work highlights the importance of reducing viral loads within the transmitting compartment but also underscores that other modulatory factors contribute to the risk of transmission. The prevention field should move forward to address the relevance of these other modulatory factors using focused translational research studies, while at the same time applying the knowledge gained to optimize and advance new strategies for prevention (20). For example, the importance of compartmental viral loads in transmission suggests that selection of antiviral therapy should take into account whether the drugs achieve high concentrations in semen or cervicovaginal fluid. In addition to test-and-treat programs and continued efforts to promote male circumcision and safe sex, future studies must be directed at defining those intrinsic biological and behavioral factors that promote HIV transmission and subsequent infection. Patient-focused efforts would include over-the-counter HIV testing kits in an effort to reduce the numbers of untested, HIV-positive individuals, many of whom are in the early, highly infectious phases of seroconversion. Protective host mucosal defenses (innate immune factors, microflora, mucus, etc.) need to be better defined and preserved, by identifying factors that (i) disrupt the epithelial barrier [for example, lime juice vaginal douches and hyperosmolar rectal douches or gels (41)]; (ii) interfere with soluble mucosal defenses (such as other STIs) (42); or (iii) recruit new HIV targets into the mucosa (inflammation and STIs).

It is precisely with the unique data sets provided by large clinical trials such as the current study (1) that our understanding of the landscape around HIV transmission and mucosal infection will be changed. These new perspectives then require more focused, smaller-scale, proof of concept trials to address the questions raised by the large trials. This interplay, in which the results from clinical studies are then translated back to the laboratory and to smaller clinical studies, illustrates the circular nature of translational research. This approach will advance prevention science by contributing improved interventions to be tested in subsequent larger, interventional trials. This opportunity to build on seminal data to better understand the real-life processes of HIV transmission and infection will enable the field to iteratively make prevention strategies more effective, providing the best products available to a willing, wanting, and hopefully informed public.

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Fig. 1. Intervention for prevention

Phases of intervention to reduce HIV transmission and spread emphasize the need for prevention efforts beginning with any sexual coupling in which one individual is HIV-seropositive and transmits to an uninfected partner. **(A)** Relative risk of infection from a seropositive insertive partner for the cervicovaginal and colorectal compartments is depicted with differently sized boxes. Lighter-shaded replicates fanning out underneath are meant to reflect multiple transmissions and subsequent infections from the primary insertive partner. **(B)** Amplification of HIV transmission and infection from a representative index coupling to within higher-sexually-active cohorts to the local/regional/national levels with global ramifications shown. At each level, education, test-and-treat, and biological or behavioral interventions (prevention) can be applied to curb the epidemic.

Fig. 2. Tracking HIV transmission

Features of sexually exposed mucosal compartments contribute to the relative vulnerability of HIV transmission. Three major anatomical compartments that drive the sexually transmitted HIV-1 epidemic are featured with insets of local histology. **(A)** (Left) Insertive: Penile with urethral, glans, foreskin histology. **(B)** (Top right) Receptive: Lower female genital tract (cervix and vagina) with vaginal wall and endocervical canal histology. **(C)** (Bottom right) Receptive: Colorectum with colorectal histology. Note the generally more vulnerable single-cell epithelia present in the colorectal and endocervical canal as well as the relatively increased area of exposure in the colorectum. Current findings that receptive compartments are at increased risk for HIV infection over insertive tissues are reflected by the relative size of the arrows indicating ejaculate and, in reverse, vaginal or rectal fluid. [Credit: Histology photos used with permission but are owned by Lippincott Williams & Wilkins. From: *Histology for Pathologists*, Third Edition, S. E. Mills, Ed. (Lippincott Williams & Wilkins, Philadelphia, 2007), pp. 633 (colorectum), 966 (glans penis), 970 (foreskin [both inner/outer epi]), 976 (male urethra), 1004 (vaginal mucosa [not wall]), 1022 (cervix-endocervical transition).]