Early Events in Sexual Transmission of HIV and SIV and Opportunities for Interventions

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Keywords
microbicides, glycerol monolaurate (GML), target cell availability

Abstract
To constrain the growth of the HIV/AIDS pandemic and ultimately end it, effective measures must be developed to prevent sexual mucosal transmission, the major route by which new infections are acquired. I review sexual mucosal transmission of HIV and SIV, with a focus on vaginal transmission in the SIV rhesus macaque animal model, and the evidence for small founder populations of infected cells and the local expansion at the portal of entry necessary to establish systemic infection. These early events represent windows of maximum opportunity for interventions to prevent systemic infection. I highlight the paradoxical role the innate immune response plays in actually facilitating transmission, and a novel microbicide strategy that targets this innate response to prevent systemic infection, and I conclude with an agenda for future research that emphasizes mucosal immunology, virology and pathogenesis studies at each anatomic site of entry.
INTRODUCTION

Despite the great advances in and benefits of antiretroviral therapy (ART), it is clear that the HIV/AIDS pandemic will continue to grow until treatment can be combined with effective means of preventing transmission by its principal genital and rectal routes (1, 2). Thus, there is a great and continuing effort to develop effective vaccines and other measures to prevent HIV infection, with a mixed but largely unsuccessful record to date. The first two vaccine candidates tested in human trials did not provide protection, but in the recent trial in Thailand in a low-risk population, a combination of vaccine components that elicit antibodies and a cellular immune response to HIV prevented acquisition in ∼30% of the vaccines (3). Microbicides to prevent vaginal transmission have thus far proved ineffective or even enhanced transmission in human trials (4), but there is hope that in the VOICE trial (5), microbicides and pre-exposure prophylaxis with antiretrovirals that specifically target HIV replication will prove efficacious, and male circumcision has provided remarkably robust protection by as yet unknown mechanisms (6–8).

In this personal perspective and interpretation of the literature, I review what we know about early events in sexual mucosal transmission of HIV and SIV that might provide clues to enable design of more effective vaccines and microbicides. I primarily focus on the expanding epidemic in women (9) and the SIV rhesus macaque animal model of vaginal transmission (10). I conclude, as I have elsewhere (11), that the greatest opportunities for successful interventions to prevent transmission are in the initial stages of infection, where there are the greatest host advantages and viral vulnerabilities at the portal of entry.

HIV ECLIPSE AND WINDOW OF OPPORTUNITY FOR INTERVENTIONS

Following sexual mucosal exposure to HIV, viral RNA is not detectable (at 100 copies of viral RNA per milliliter of plasma) in the circulation for about 10 days. This time frame has been referred to as the eclipse phase (reviewed in 12) by analogy to viral life cycles, where eclipse refers to the period from virus entry into a cell to production of new virions (Figure 1). Following eclipse, virus levels increase exponentially to peak around 21–28 days, at which time patients may present with symptoms of the acute phase of infection.

Pathogenesis of vaginal transmission and early infection, interventions and in vivo viral growth curves, eclipse phases, and windows of opportunity in tissues and blood in SIV and HIV infections. Virus crosses the mucosal epithelial barrier to establish a small founder population that then expands locally using the influx of new target cells recruited through outside-in signaling. The local expansion disseminates via lymphatic drainage to genital lymph nodes where expansion quickly produces more virus, which spreads via the thoracic duct and then hematogeneously to the lymphatic tissues. Virus production in the lymphatic tissues with rising titers of virus in circulation inaugurate and define the systemic phase of infection. Vaccine-induced neutralizing antibodies or antibodies that mediate antibody-dependent cellular cytotoxicity (ADCC) with natural killer (NK) cells and macrophages can prevent or partially control infection at each site, as can cytotoxic T lymphocytes (CTLs). These mechanisms are positioned at the portal of entry where they are most likely to be effective. Microbicides that lyse virus in the lumen or prevent fusion, entry, and reverse transcription, applied topically or given as pre-exposure prophylaxis (PrEP), could limit local propagation and expansion to prevent transmission and systemic infection. Glycerol monolaurate (GML) may prevent transmission by blocking signaling and the influx of target cells.

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a) Pathogenesis

- Viral lysis
- Binding
- Fusion
- Inhibitors
- ART
- PrEP

b) Interventions

- Microbicides
  - Viral lysis
  - Inhibitors
  - ART
  - PrEP

- Vaccines
  - Neutralization
  - ADCC
  - Cell-mediated killing
  - Neutralization
- Microbicides
- Inhibitors
- ART
- PrEP

- Lymphatic dissemination
- Draining lymph node
- Thoracic duct
- Gut
- Lymph nodes
- Spleen

- Hematogenous dissemination

- New target cells
- GML?
- Local expansion
- Outside-in signaling
- Cervix
- Vagina

- Small infected founder population

- Local propagation and expansion

- Entry
- Eclipse
- SIV DNA+
- SIV DNA-

- Time (d)
- Levels of viral RNA

- Window of opportunity
- Days following SIV exposure

- Window of opportunity
- Days following SIV exposure

- Window of opportunity
- Days following HIV-1 transmission

- Levels of viral RNA

- SIV
- HIV
- Peripheral blood
- Lymphoid tissues (LT)

- Transmission
retroviral syndrome. By this time there has already been substantial depletion of CD4 T cells, particularly in gut (13, 14); the lymphatic tissue reservoir, where virus is produced and persists in latently infected cells, has already been established (15–17); and the immune system has been activated, with adverse consequences I describe below in greater detail.

Clearly the window of opportunity for early interventions should precede the CD4 T cell depletion, establishment of the lymphatic tissue reservoir, and adverse consequences of immune activation just described. Windows of opportunity are also defined by interventions and the goals of those interventions. In the case of HIV, the window of opportunity for a vaccine to prevent systemic infection might be bracketed between the time of exposure and the time of detection of systemic infection (eclipse) as shown in Figure 1, or it might extend (indicated by the arrow and question mark in the figure) to peak viremia, where a recall response might provide better control of infection but not clear it. However, if the goal of a vaccine, microbicide, or other strategy is to prevent acquisition, the window of opportunity will be narrower—closer to the time and location of exposure and prior to systemic infection. To examine these opportunities, we must turn to a nonhuman primate model.

**SIV ECLIPSES AND WINDOWS OF OPPORTUNITY**

The SIV rhesus macaque model (10) of vaginal transmission of HIV enables investigation of virus-host interactions at the portal of entry and/or genital draining lymph nodes, and prior to establishment of systemic infection. This highly relevant animal model may make it possible to design and assess strategies to prevent acquisition (11). In this model, animals are exposed to high doses of SIV to insure that (a) most animals will be infected; (b) infection will proceed relatively synchronously; and (c) key events in the virus-host interaction will be frequent enough to be detected in the tissue samples obtained at daily intervals during the first two weeks following exposure (18). Under these conditions, tissue samples obtained from relevant compartments at very early times after exposure, in a way that obviously would not be possible in HIV infection, provide glimpses of the rapidly unfolding events that precede systemic infection and the earliest adverse consequences of systemic infection once established.

In the in vivo growth curve shown in Figure 1 for cervical/vaginal tissues, reconstructed from these tissue analyses (18), there are striking parallels to in vitro viral growth curves in cultured cells. In the phase labeled Entry in Figure 1, there is a rapid fall in detectable viral RNA in the tissues compared to the inoculum. This is followed by a true eclipse phase in which SIV DNA is detectable in the tissues, but viral RNA, representing progeny virions, is not. This eclipse phase of 3–4 days is followed by a phase of rapid local expansion and exponential replication to peak levels at 10–14 days after exposure, and then gradual decline.

**SMALL INFECTED FOUNDER POPULATIONS IN MUCOSALLY TRANSMITTED SIV AND HIV INFECTIONS**

The huge reduction from the level of viral RNA in the inoculum to levels of tissue-associated viral RNA in the entry phase suggests that virus may gain access initially to only a relatively small number of susceptible target cells, and that therefore the founder populations of infected cells at the portal of entry may be quite small. The detection of a cluster of only 40–50 cells with SIV RNA 3 and 4 days post vaginal exposure is consistent with this conclusion (18, 19). These clusters are consistently found in the endocervix and transformation zone (the junction between endo- and ectocervix), sites where virus may most easily find breaks (20) in a region of the mucosal barrier lined by a single layer of columnar epithelium and a region of high target cell density and turnover (21), where initially small foci will be established and expand through accretion of new infections (22).
It is likely that target cell availability plays some role in the preferential infection of “resting” CD4 T cells, as they outnumber other potential targets—macrophages and dendritic cells—by 4 to 5:1. However, target cell availability alone cannot account for the other cell type initially infected, activated CD4 T cells, which resting CD4 T cells outnumber by 70:1 (23).

Infected founder populations and host cell tropisms have not been directly observed in HIV infections, but they can reasonably be inferred from recent studies by single genome amplification, deep sequencing studies, and mathematical modeling in blood samples obtained in the very early stages of infection. The conclusion from these studies is that in heterosexual transmission, infection is acquired from a single virus genotype (or infected cell) in 80% of cases, and the transmitted variant is an R5-tropic virus that replicates well in CD4 T cell cultures, but not in monocyte-derived macrophage cultures (24, 25). Although the genetic bottleneck and host cell tropisms could be determined by a number of mechanisms and different anatomic sites, the results are certainly consistent with the small-founder-population concept and host tropisms observed in the SIV rhesus macaque model.

**EARLIEST OPPORTUNITIES FOR INTERVENTIONS TO PREVENT ACQUISITION**

Given the small founder populations documented in SIV mucosal transmission, and inferred in HIV transmission, the first week of infection is a time of greatest vulnerability of virus and maximal opportunity for interventions that decrease the basic reproductive rate below one, and thereby eliminate infection at the portal of entry. Microbicides, or pre-exposure prophylaxis with antiretroviral drugs such as tenofovir, are in clinical trials (5) to see if preventing virus propagation at the portal of entry can prevent acquisition (Figure 1). Mucosal antibodies, ideally broadly neutralizing antibodies, could prevent establishment of the small founder population, as has been shown in a proof of principle in rhesus macaques protected with a microbicide containing high concentrations of neutralizing monoclonal antibody (26). Perhaps even just binding antibodies could prevent establishment of the small founder population of infected cells, and/or its expansion, through antibody-dependent cellular cytotoxicity (ADCC). Also, a resident or rapidly responding mucosal virus-specific cytotoxic T lymphocyte (CTL) response would be working at a favorable ratios of immune effectors to infected cell targets (E:T ratios) against small founder populations to eliminate infection at the portal of entry.

**Local Expansion, Lymphatic Dissemination, Wave-Particle Analogy**

During the first week of infection, virus must not only establish a small founder population of infected cells at the portal of entry but also expand that local infection to continue to disseminate virus and infected cells via lymphatic drainage to establish a self-propagating infection in the genital draining lymph nodes. Thus, in the SIV rhesus macaque model, although there is evidence that virus within a day of exposure may reach this and even more distal sites by unknown routes and mechanisms, eclipse, here defined as detection of virus production, is about one week (18). This apparent paradox might be understood by analogy to the wave-particle duality in physics, where the particles are virions (or infected cells) that are disseminated as a wave via the lymphatics. The amplitude of the wave is inversely proportional to the distance from the source, and must exceed two thresholds to “call” productive infection in the draining lymph nodes: one threshold of sufficient numbers of infected cells to sustain a self-propagating infection in the lymph node; and a second threshold of sufficient virus production to be detectable experimentally.

The expansions that occur locally and in the draining lymph nodes first reached by virus and infected cells represent a second window of opportunity for intervention. I describe microbicide strategies directed to expansion at

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**“Resting” CD4 T cells:** CD4 T cells that lack detectable markers of activation but support replication. In tissue culture, HIV and SIV replicate in CD4 T cells that are activated. Surprisingly, in vivo, “resting” CD4 T cells are the major cell type first infected.

- **R5-tropic:** HIV and SIV enter cells by binding to CD4 and a coreceptor, CCR5 or CXCR4. Viruses that use CCR5, which initiate most infections, are referred to as R5-tropic strains.
- **ADCC:** antibody-dependent cellular cytotoxicity.
- **E:T ratio:** ratio of immune effectors to infected cell targets.
Viral load: level in copies of viral RNA per milliliter or microgram of tissue RNA

the portal of entry, but there is also a slightly longer time frame for a vaccine-induced recall response that might still operate at relatively favorable E:T ratios at either the portal of entry or draining lymph nodes.

Role of the Mucosal Epithelium in Local Expansion of Infection

Mapping numbers and locations of infected cells in tissue sections has shown that the local expansion of the infected founder population (Figure 1) results from growth of the initial cluster by accretion of new infections recruited by a mucosal epithelium outside signaling mechanism (22). Exposure to the virus (or other component in the inoculum) immediately increases MIP3-α/CCL20 expression in the endocervical epithelium. This recruits CCR6+ plasmacytoid dendritic cells (pDCs), which in turn recruit T cells and macrophages via chemokines such as MIP1-β. This process largely transforms the local submucosal environment by day 4 post exposure to a CD4 T cell–rich environment, creating favorable conditions for growth of the infected founder population. The innate and inflammatory cell influx also facilitates local spread beyond the initial cluster through increased target cell availability in the cellular infiltrates.

PARADOXICAL ILL EFFECTS AND INEFFECTIVENESS OF THE INNATE RESPONSE

The innate response initiated by the mucosal epithelium thus has the paradoxical ill effect of providing new target cells to facilitate local propagation and spread, a specific instance of the more general concept of target cell availability as a critical determinant of transmission (23). This concept accounts for the failure of treating HSV-2 infections to decrease HIV acquisition (29, 30).

In addition to these ill effects, the innate immune response is surprisingly ineffective. The pDCs in the endocervical mucosa are expressing high levels of interferons and interferon-stimulated genes, which, along with the recruited cells that express antiviral chemokines such as MIP1-β, would be expected to inhibit viral replication. Perhaps this innate response does protect the pDCs and some other target cells such as macrophages but not the preferred target, CD4 T cells, which are relatively resistant when clustered (31) as they are in the expanding founder population; or, like HIV, SIV might disrupt IRF-3 signaling in CD4 T cells to blunt interferon-mediated effects in vitro (32).

In the systemic stages of acute SIV and HIV infection, it also appears that a robust innate immune response may or may not provide some measure of control of viral replication, but through recruitment of target cells it could also have the paradoxical ill effect of facilitating infection and the immunopathological effects of immune activation. Thus, in early systemic SIV infection, expression of the interferon system and of chemokine and proinflammatory genes increases proportional to viral load (33), and in acute HIV infection there is a cytokine “storm” that exceeds cytokine levels in other viral infections (reviewed in 12). Microarray transcriptional profiling also revealed stage-specific “signatures” with upregulated expression of >300 genes, many related to innate defenses, chemokines and cytokines involved in lymphocyte trafficking, inflammation and immune activation (34). These genes are downregulated in the transition to the presymptomatic stage of HIV infection, and the cytokine storm recedes in parallel with decreasing viral loads, presumably reflecting host mechanisms to mitigate the immunopathological effects of immune activation.

THE GML MICROBICIDE STORY

If the innate and inflammatory response to exposure and early infection actually facilitates
local expansion and spread through recruiting target cells, it should be possible in principle to limit local expansion and thereby block systemic infection by moderating this response. This was the rationale for studies of glycerol monolaurate (GML) as a microbicide (22).

Generally recognized as safe by the U.S. Food and Drug Administration, GML is a fatty acid monooester that has long been used extensively in the food and cosmetics industries as an emulsifier and antimicrobial agent (35), which at high concentrations will inhibit the replication of many viruses, including the prototypic lentivirus visna (36, 37). At lower concentrations, GML blocks many toxins such as toxic shock syndrome toxin-1 of Staphylococcus aureus. It also blocks T cell activation, probably through interfering with signaling by stabilizing or “freezing” mobility of cell membrane components (38, 39).

This latter property suggested that GML might prevent the initial mucosal epithelial signaling responsible for recruiting target cells. GML did in fact decrease MIP3-α/CCL20 levels in cervical/vaginal fluids of rhesus macaques, and protected animals against acute systemic infection (22) from as many as four vaginal exposures to $10^5$ TCID$_{50}$, doses of SIV that far exceed the highest exposures to HIV during the acute stage of infection in humans (40). The study is noteworthy in pointing to potential new mechanisms to prevent systemic infection with an agent in widespread use that is both cheap and likely to be safe, based on the reported safety profile in rhesus macaques (41).

**HEMATOGENEOUS DISSEMINATION AND SYSTEMIC INFECTION: LOST OPPORTUNITIES AND ADVERSE CONSEQUENCES**

From estimates in the SIV rhesus macaque model of the “age” of infection (18), the small founder population is established and expands locally during the first week of infection to spread via lymphatic drainage to establish infection in the draining lymph nodes. Shortly thereafter, infection spreads hematogenously to establish systemic infection throughout the secondary lymphatic tissues—spleen, gut-associated lymphatic tissue, and peripheral lymph nodes—and detectable virus in the circulation. The eclipse phase for systemic infection is about 7 days post exposure in the animal model and perhaps 10 days in HIV infection (12).

In this systemic phase, it is already unlikely that infection can be eradicated because of the occult and latent infections documented respectively in SIV and HIV infections (16, 17, 42–44); opportunities for control of infection are quite limited because of supply-side considerations in E:T ratios discussed below; and there are immediate adverse consequences and pathological processes set in motion that impair host defenses and facilitate infection. These adverse consequences include the following:

1. Massive depletion of gut lamina propria CD4 T cells (13, 14, 45–49) mediated by the direct effects of infection and indirect apoptotic mechanisms (49).
2. Damage to gut epithelium through apoptosis and other mechanisms (50–52) that initiate increased microbial translocation. This contributes in later stages of infection to immune activation (53), which in turn will provide new targets for virus replication and deplete CD4 T cells.
3. A T regulatory response to counter immune activation and pathology, which suppresses the host’s immune response (54).
4. A pathological process of collagen deposition, which disrupts lymphatic tissue architecture to contribute to CD4 T cell depletion and limits immune reconstitution with ART (55, 56).

**GML: glycerol monolaurate**

**Microbial translocation:** entry of gut bacteria and associated products such as lipopolysaccharides into the systemic circulation without overt bacteremia

**In Vivo E:T Ratios and Control of Early Viral Infection: Supply-Side Immunology and the Red Queen Problem**

The cellular immune response to SIV infection has been described as “too little and too late”
CTL “exhaustion”: dysfunctional CTLs that proliferate, kill, and release cytokines poorly in chronic viral infections

PD-1: programmed death-1, a CD28 family member and negative regulator of T cells. Increased expression induces CTL exhaustion

because it is not detectable until after peak viral replication and the immediate adverse consequences of the systemic phase of acute infection, particularly the massive depletion of CD4 T cells in the gut (57). Nonetheless, the immune response clearly has a positive impact on partially controlling viral replication in both SIV and HIV infections, e.g., the immune response temporally coincides with a reduction in viral loads in blood and lymphatic tissues; and the selective pressures imposed by the immune response are reflected in the rapid emergence of antigenic escape variants recognized by immunological assays and by sequence changes inscribed in the generally monophyletic genotypes of the transmitted founder populations (12, 25).

What are the determinants of the timing, magnitude, location, and effectiveness of the immune response? First, the advent of a detectable immune response offset from peak viral replication implies that antigen-driven expansion and a threshold of virus production are required to elicit an immune response. Second, the magnitude and effectiveness of the immune response are location/tissue-compartment specific and related to in vivo E:T ratios. It is now possible to determine E:T ratios by combining in situ tetramer staining and hybridization. In situ tetramer staining can determine in tissue compartments the numbers of tetramer$^+$ CD8$^+$ T cells specific for immunodominant epitopes in Gag and Tat, which comprise 70% of the immune response in early SIV infection; in situ hybridization can determine the numbers of SIV RNA$^+$ cells in the tissue sections and the spatial relationships of these targets to virus-specific CTLs (58).

These measurements of both sides of the equation—infected cells and immune effectors—have revealed the importance of the E:T ratio and the tissue compartment–specific effectiveness of the immune response. The most significant reductions from peak viral loads are in the cervical/vaginal tissues where infection began, where E:T ratios of 50–100:1 or greater result in commensurate reductions in viral loads 3–4 weeks post exposure. By contrast, in systemic lymphatic tissues there are comparable numbers of tetramer$^+$ cells, and they are close to their infected targets, but because there are also large numbers of targets, the E:T ratios are only $\sim$2:1 with comparably modest reductions in viral loads from peaks.

The parallel increases in targets with antigen-driven expansion of effectors point to a problem I refer to as supply-side immunology and the Red Queen problem. In Lewis Carroll’s *Through the Looking-Glass* (59), the Red Queen tells Alice, “Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!” In SIV and HIV infections, immune activation and the cytokine storm in early infection provide virus with large numbers of activated targets and a favorable milieu for replication in lymphatic tissues, so that the immune response is continually in a catch-up position where only the most robust responses will provide excellent control of replication. Similarly, in lymphocytic choriomeningitis virus (LCMV) infections, strain Armstrong virus infections are quickly cleared whereas clone 13 infections are not because the extended host range of clone 13 enables continuing infection of macrophages and the fibroreticular cells that provide the anatomical and functional network for T cell migration and survival. Consequently, the E:T ratio is only $\sim$0.2:1 (58). Thus, when target cells are not limiting, there is an ongoing and unresolved battle between virus and host defenses, the downsides of which, documented in persistent LCMV infections and in the chronic stages of HIV infection, are dysfunctional states of CTL “exhaustion” mediated by PD-1 (60–63).

**Implications of Target Cell–Limited Conditions for Vaccine Design**

The clearance of strain Armstrong LCMV by 8 days post infection, at relatively low E:T ratios of $\sim$4:1 (58), is instructive and reinforces the view that the maximal opportunities for clearance are in the earliest stages of infection under conditions where target cells are limiting. The

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comparable stage in SIV infection is at the portal of entry where a resident or rapidly responding population of virus-specific CTLs would be working against a small founder population of infected cells under initial conditions of small, dispersed populations of susceptible target cells, and just before the influx of new target cells for expansion.

**Current Scorecard for HIV and SIV Vaccines**

Against this background, it is perhaps not surprising that the first CTL-based HIV vaccine failed in human trials (the Step Study) to prevent infection or reduce viral loads. The cellular immune response measured in blood was relatively small compared to levels likely needed at mucosal surfaces according to the above analysis in the SIV rhesus macaque model (64). An earlier human trial with recombinant AIDSVAX envelope gp120 that elicited non-neutralizing antibody also failed to provide protection (65). However, in a recent trial in Thailand in a low-risk population, a canary pox vector expressing gp120, Gag, and Pol was administered to prime immune responses, followed by boosting with AIDSVAX gp120, and there was a modest protective effect against acquisition—approximately 30% compared to the control arm, which was statistically significant in the modified intention-to-treat analysis in which individuals found to be HIV-infected at baseline were excluded (3). This first hint of efficacy was surprising, as neither component alone had elicited a sufficient cellular immune response to proceed to trial, nor conferred protection. The surprising result provides a motive to investigate how the combination may have induced mechanisms of protection such as ADCC that would operate at the portal of entry to prevent acquisition, but would not reduce viral loads, as was observed, once systemic infection was established.

By contrast, the best recent CTL-based recombinant attenuated adenovirus vaccines induce immune responses that reduce viral loads sufficiently to prolong survival of rhesus macaques following challenge, but do not prevent acquisition (66). However, as a proof of principle, attenuated SIV vaccines and a rhesus cytomegalovirus vaccine have been shown to prevent vaginal and rectal transmission of SIV (67–69). Understanding the mechanisms by which these vaccines provide such protection would clearly enable HIV vaccine design, and is thus high on the list of priorities for future vaccine research (see below).

**FUTURE ISSUES**

In seeking a path forward to development of a safe and effective vaccine, microbicide, or other strategy to prevent acquisition of HIV by the main heterosexual and rectal routes, three areas of research deserve high priority:

- *Fundamentals of mucosal immunology and virology at each site.* We clearly have much to learn about the fundamental biology of the mucosal epithelium as the front line of the immune system and the innate and adaptive immune response at the portal of entry—character of the cells, their activation states and functions, residency time, turnover and fates in humans and primates—and we need to be able to use that knowledge to elicit protective immune responses without the immune activation and immunosuppressive features of the natural response that fuel viral replication and impair host defenses. We also need to know at each mucosal site—rectal as well as cervical, vaginal, and penile—the anatomy, physiology, and nature of the virus–cell interactions. These are likely to be different at each site, e.g., the eclipse phase and window of opportunity may be briefer and narrower in rectal transmission because of the greater availability of target cells in the rectum, and thus the more rapid dissemination documented in the SIV animal model (70) and increased probability of transmission per exposure (71).
- **Clues from successes and surprises.** Mechanisms involved in reducing acquisition in the Thai vaccine trial, successes with attenuated SIV and rhesus cytomegalovirus vaccines, protective benefits of circumcision, low viral loads without ART in elite controllers (72), and unexpected defense mechanisms revealed by systems biology approaches to understanding effective vaccines such as the yellow fever vaccine (73), all may provide clues to designing effective preventive measures.

- **Better animal models.** I have described the advantages of the high-dose vaginal-challenge model for the window it has provided to view the earliest events in transmission and infection, but the model also has limitations. The dose of virus used far exceeds the highest human exposures (40), and there are as yet unpublished reports that multiple genotypes are transmitted rather than the single genotypes characteristic of HIV transmission. Thus, efforts should be directed to developing this animal model to better reproduce the features of human vaginal transmission. The challenges are also with cell-free virus, justified by the difficulties in consistently infecting animals with cell-associated virus (74) and by recent evidence that favors human transmissions by virus rather than cells (75). However, some efforts need to continue to explore transmission with infected cells.

The continuing advances from ongoing and these proposed studies, and efforts to design immunogens to elicit broadly neutralizing antibodies, in my view point the way to development of effective measures to stem the growth of this pandemic and ultimately to end it.

### DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

### LITERATURE CITED


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