

Strategies for Preventing Mucosal Cell-Associated HIV Transmission

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Human immunodeficiency virus (HIV) may be transmitted through either cell-free virions or leukocytes harboring intracellular HIV in bodily fluids. In recent years, the early initiation of combination antiretroviral therapy leading to virological suppression has resulted in decreased HIV transmission to uninfected partners. Additionally, the efficacy of primary chemoprophylaxis with oral or topical antiretroviral regimens containing tenofovir (with or without emtricitabine) has been demonstrated. However, the efficacy of these approaches may be compromised by suboptimal adherence, decreased drug concentrations in mucosal compartments in women, and genital inflammation. Furthermore, in vitro studies on the effects of tenofovir on cell-associated HIV transmission have produced conflicting results. Preclinical studies suggest that combination preventive approaches may be most effective in stopping the transmission of HIV after mucosal exposure. Since the development of antibodies were found to correlate with protection in the only effective HIV vaccine trial, the administration of preformed mucosal and systemic antibodies may inform the development of safe and effective antibody-based oral, topical, and/or systemic preexposure prophylaxis agents and provide guidance in the development of HIV vaccines that effectively block cell-associated HIV transmission.

Keywords. antiretrovirals; HIV vaccines; cell-associated transmission; antibodies.

Despite an increasing number of human immunodeficiency virus (HIV)-infected people receiving antiretroviral therapy, the HIV epidemic continues to grow. At present, there are 35 million people living with HIV, less than one third of whom are receiving antiretroviral therapy. There still are around 2 million new HIV infections per year [1]. Since individuals may remain vulnerable to cell-associated HIV transmission despite current approaches to HIV prevention, this mode of transmission should be addressed in the development of emerging strategies. Potential agents to block cell-associated HIV transmission include membrane disrupters, acidifying agents, entry inhibitors, virologic synapse inhibitors, reverse transcriptase inhibitors, and other antiretroviral

agents. A number of these approaches have been evaluated for their ability to block cell-associated HIV transmission in animal models and in vitro assays [2]. Unfortunately, none of the nonspecific approaches (membrane disrupters and acidifying agents) have demonstrated efficacy in human clinical trials, and the level of protection by antiretroviral drugs against cell-associated transmission is uncertain. Further, few candidate HIV vaccines have been designed to block cell-associated transmission. This review will focus on antiretroviral and antibody-based strategies to prevent HIV infection, with a specific focus on cell-associated HIV transmission.

ANTIRETROVIRALS

Animal studies have long suggested that administration of systemic or topical antiretrovirals shortly before or after an animal is exposed to a retroviral challenge results in protection [3]. The highest levels of protection were afforded when antiretroviral medication was administered prior to exposure, so that there would be sufficient time to have the agent achieve high intracellular

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levels [3]. Recent clinical studies have demonstrated that the oral administration of antiretrovirals for primary [4] or secondary prevention [5–8] may make HIV transmission less likely between serodiscordant intimate partners. The first proof of the efficacy of oral chemoprophylaxis study was from the iPrEx study, which enrolled men who have sex with men and transgender women in the United States, Peru, Ecuador, Brazil, Thailand, and South Africa and found a 44% decrease in HIV acquisition among those participants who had been randomly assigned to receive oral tenofovir/emtricitabine (TDF/FTC) on a daily basis [5]. Subsequent studies demonstrated the efficacy of oral TDF/FTC in heterosexual serodiscordant couples in Kenya and Uganda [6], young heterosexual adults in Botswana [7], as well as Thai injection drug users [8]. Two other studies did not demonstrate decreased transmission in female participants assigned to receive tenofovir-based chemoprophylaxis [9, 10]. However, subsequent analyses of drug levels among participants in these studies showed a clear dose-response relationship: participants who had drug levels consistent with daily medication use were most likely to be protected against HIV acquisition [11].

Because decreased efficacy was correlated with low levels of daily medication adherence in several studies in high-risk populations, researchers have begun evaluations of whether longer-duration agents may be beneficial. Studies are currently evaluating a vaginal ring that can be inserted once monthly and contains dapivirine, a nonnucleoside reverse transcriptase inhibitor, with or without maraviroc, a CCR5 inhibitor [12]. There are 2 efficacy studies underway in Africa to see whether this approach may provide a higher level of protection for women than agents relying on daily or pericoital pill use [13]. Two long-acting antiretroviral drugs, a nanosuspension of rilpivirine and a new integrase inhibitor, GSK744, are being evaluated for the possible use as injectable chemoprophylactic agents, which might be able to be administered as infrequently as every 3 months [14].

Despite the efficacy of many antiretroviral compounds studied for chemoprophylaxis, their level of virological suppression may not be as effective in preventing cell-to-cell HIV transmission, compared with their inhibition of cell-free virus transmission. It appears that some of these compounds have differential activity in specific types of genital tract cells and secretions, with the most dominant cells in semen and cervicovaginal secretions being monocyte/macrophages [2]. One study, which used coculture, found that TDF was not as effective in protecting against cell-to-cell HIV transmission as it was against cell-free challenges [15]. Another study [16], found that the protease inhibitors lopinavir and darunavir had equal potency in inhibiting cell-free and cell-associated virus but that the reverse transcriptase inhibitors TDF and nevirapine were not as effective. Other groups have found that antiretroviral drugs were able to block viral DNA production and virus replication during cell-to-cell

viral transmission with efficacy comparable to that of their inhibition of cell-free virus infection [17]. A recent study found that combinations of antiretrovirals offered higher levels of protection than single agents against cell-cell HIV transmission [18], suggesting that combination preventive approaches may deserve further study to optimize the preventive efficacy of chemoprophylaxis [19].

The first human study that demonstrated the efficacy of pre-exposure antiretroviral chemoprophylaxis was the CAPRISA 004 study of almost 900 at-risk South African women that demonstrated that pericoital use of topical TDF gel decreased HIV acquisition by 39% [20]. However, the activity of various antiretroviral medications in the genital tract may be influenced by a variety of different factors, ranging from the acidic pH of the vagina, if commensal lactobacilli dominate, to the large number of genital tract leukocytes that are present in most HIV-infected and at-risk individuals, which may serve as reservoirs or targets for cell-associated HIV transmission [21]. Additionally, it is possible that certain drugs may have a lower or higher threshold for the potential emergence of resistance because of the concentrations that they achieve in different mucosal cells [22]. For example, TDF achieves high tissue concentrations in rectal mucosa after ingestion, but concentrations are not as high in cervicovaginal tissues [23]. Thus, women might need to have a higher level of product adherence to achieve a comparable level of protection when taking this drug orally for chemoprophylaxis. Maraviroc achieves a high level of tissue penetration in cervicovaginal and rectal secretions [23] and is currently under study by the HIV Prevention Trials Network (HPTN069) to see whether it may be an alternative chemotherapeutic agent, used alone or in combination with other antiretrovirals. HIV coreceptor antagonists that have been studied to date have failed to inhibit cell-to-cell HIV transmission via virological synapses, but new agents could potentially affect cell-associated transmission through other mechanisms, such as interfering with CCR5-mediated chemotaxis. Among the integrase inhibitors, raltegravir has been shown to have a higher concentration in mucosal tissues, compared with blood plasma [22, 23]. Integrase inhibitors could block cell-to-cell HIV transmission by inhibiting HIV integration into target cells.

Since local factors in the genital tract milieu may affect HIV transmission dynamics, they need to be addressed as new preventive modalities are developed [21]. It has been demonstrated that individuals who are receiving highly effective antiretroviral therapy may continue to express HIV in genital tract secretions even after experiencing virological suppression for long periods [24]. In one prospective study of 25 men who achieved virological suppression for at least 16 weeks, HIV RNA was detected in semen in almost half (48%), and high levels of HIV RNA (>5000 copies/mL) were detected in 16%, despite HIV not being detected in blood. In addition, several individuals' who had undetectable plasma HIV RNA had seminal specimens

that grew infectious virus [24]. These findings were corroborated by a French study that followed 304 men over several years and found that the overall rate of detection of seminal HIV RNA was 6.6% [25]. Another study from the United States followed a cohort of 101 highly active antiretroviral therapy (HAART)-receiving HIV-infected men who have sex with men, and one quarter who had virological suppression in the blood had HIV detected in semen. Multivariate analyses found that individuals who have sexually transmitted infections were 30 times as likely to have HIV detected in semen despite having virological suppression in the blood, and high levels of polymorphonuclear leukocytes and tumor necrosis factor (TNF) in semen were associated with HIV detection even in the absence of documented sexually transmitted infections. This study detected HIV-infected cells and free HIV virions in semen from men whose virus was systemically suppressed during HAART [26]. Last, it is possible that in addition to an inflammatory milieu causing increased HIV expression in the genital tract of a potential infecting partner, inflammation may also increase susceptibility of HIV-uninfected individuals to HIV acquisition [21]. In the CAPRISA 004 study, independent of other factors (including random assignment to the TDF gel arm of the trial), women who had high systemic levels of TNF- α (a proinflammatory cytokine), interleukin 2, interleukin 7, and interleukin 12p70 and activated natural killer cells were much more likely to become HIV infected than those without genital tract inflammation [27]. So, although suboptimal adherence may explain some of the suboptimal efficacy seen in studies of TDF-based preexposure prophylaxis, it is also conceivable that limited activity against cell-associated HIV transmission was responsible for some of the new infections among participants assigned to TDF-containing arms. Thus, the efficacy of antiretrovirals for chemoprevention may be attenuated by systemic and/or local mucosal inflammation (see paper by Anderson in this series).

VACCINES

For several decades, investigators have attempted to develop safe and effective anti-HIV vaccines, but no major vaccine efficacy trials have yet shown a sufficiently robust protective effect. The RV144 study used a combination of a canarypox virus vector boosted by antigens designed to generate neutralizing antibodies (ALVAC HIV and AIDSVAX B/E) and was conducted among >18 000 Thais recruited from the general population [28]. The vaccine combination resulted in a 31% decrease in HIV acquisition [28], which was a statistically significant result but not at a sufficient level to warrant immediate licensure and wider use. Subsequent analyses of the immune correlates of protection in the RV144 trial identified features of the vaccine-induced immune response that were associated with differential risks of infection or protection, including a beneficial effect of

antibody responses targeting the V1/V2 region of the HIV envelope [29]. The protective effect of RV144 was also associated with the selective induction of antibodies of the immunoglobulin G3 (IgG3) subclass, which mediate multiple functions (ie, antibody-dependent cellular cytotoxicity [ADCC], antibody-dependent cellular phagocytosis [ADCP], and antibody-mediated release of cytokines/chemokines) that are effective against infected cells [30, 31]. Measures of vaccine protective efficacy that move beyond antibody concentration alone and assess variables such as antibody subclass and functional activity may provide critical new insights into the potential antiviral activity of antibodies that extend beyond virus neutralization [30]. Most vaccine studies in animal models and human clinical trials have not been focused on blocking cell-associated HIV transmission, so the antibody mechanism of protection at the site of infection remains unclear. Studies of passive immunization with mucosal and systemic antibodies may provide guidance in the development of vaccines that block cell-associated HIV transmission.

PASSIVE IMMUNIZATION

Several anti-HIV IgG monoclonal antibodies have prevented new infections in macaques when systemically administered before a cell-free SHIV mucosal challenge [32–34]. An injected immunoglobulin A (IgA) version of a broadly neutralizing antibody prevented mucosal transmission of HIV in humanized mice [35], and a dimeric IgA1 (which captures virus particles and prevents their transcytosis across mucosal cells) has shown rectal protection in macaques [36]. However, only a subset of HIV broadly neutralizing antibodies (bnAbs) can efficiently prevent HIV type 1 (HIV-1) cell-to-cell transmission [37]. The concentrations required to inhibit cell-to-cell transmission are often 10–20-fold higher than for cell-free HIV. Similarly, the serum concentrations of bnAbs required to inhibit infection in mouse or monkey models of HIV-1 infection are also 1–2 logs higher than in cell-free assays. Studies of cell-to-cell transmission may therefore provide a reliable method to predict the potency of bnAbs in vivo. Antibodies that target either the CD4-binding site (eg, NIH45-46 and 3BNC60) or the glycan/V3 loop (eg, 10-1074 and PGT121) on HIV-1 gp120 and that act at low concentrations by inhibiting multiple steps of viral cell-to-cell transmission are effective at blocking cell-associated viral transmission [37] (Table 1).

Antibodies to host cell antigens, such as CCR5 and CD4 receptors, may affect cell-associated HIV transmission and are currently in clinical trials. The intravenous use of the anti-CD4 antibody ibalizumab has demonstrated safety and efficacy for treatment of HIV-1 infection (clinical trials registration NCT00784147). A phase 1 study of ibalizumab given by subcutaneous injection to healthy volunteers has been completed (clinical trials registration NCT01292174). To further enhance

Table 1. Potential Antibody Targets to Block Cell-Associated Human Immunodeficiency Virus (HIV) Transmission

Target Class	Specific Targets (Antibodies)
HIV-specific antigens	CD4 binding site (NIH45-46, 3BNC60, VRC01) glycan/V3 loop (10–1074, PGT121) gp41 (10E8, 4E10)
HIV binding sites on CD4 ⁺ T cells	CD4 (ibalizumab) CCR5 (PRO140)
CD4-negative cell-bound virus	CD18
Host derived antigens on both free virus and cells	CD36 LFA-1/CD11a (MEM30) TSG101 (CB8-2) GM3 (DH2) ICAM-1
Uninfected dendritic cells	CD169
Reproductive-tract-coating antigens	SAGA-1, male-tract-specific glycoform of CD52 (H6-3C4, S19)

the efficacy and acceptability of ibalizumab, variants are being evaluated that have increased ADCC activity and longer systemic residence time. The systemic half-life of antibodies can be increased to 3 months via point mutations to the Fc region. This results in increased affinity for the neonatal FcRn receptor, which protects IgG from catabolism in endothelial cells [38]. A phase 2a, randomized, double-blind, placebo-controlled study of PRO140 (targeting CCR5) by subcutaneous administration in HIV-infected subjects has been completed (clinical trials registration NCT00642707). A phase 2b trial of PRO140 as an adjunct to a new, optimized oral antiretroviral regimen is planned (clinical trials registration NCT01272258). Although VRC01 (targeting gp120) was found to be only partially effective in blocking cell-cell transmission in one study [37], this bnAb is currently in phase 1 trials to study its safety and pharmacokinetics when administered subcutaneously or intravenously to healthy adults (clinical trials registration NCT01993706).

While the use of antiretrovirals to prevent mother-to-child transmission (MTCT) of HIV is effective, implementation is challenging in resource-constrained epidemic epicenters. Additional modalities are urgently needed, especially in the developing world, where breast-feeding is critical for neonatal health. As described in the review article by Milligan and Overbaugh in this supplement, cell-associated HIV transmission may play a major role in MTCT of HIV via infected cells in the maternal genital tract or breast milk. VRC01 is currently under consideration for trials to prevent MTCT of HIV [39], but this approach might benefit from the use of a combination of antibodies, including those that strongly inhibit cell-to-cell HIV transmission.

Vectored immunoprophylaxis (VIP) has been developed with the use of systemic adeno-associated virus (AAV)-vectored antibodies [40]. A single intramuscular injection of an

AAV-vector containing an anti-HIV antibody gene resulted in long-lasting and high expression of the antibody and protected humanized mice against intravenous challenge with cell-free HIV. By use of similar technology, anti-HIV antibody fragments were produced in cervicovaginal epithelial stem cells and were protective against cell-free HIV in vitro [41]. Recently, it was demonstrated that VIP is also capable of protecting humanized mice from vaginal challenge with diverse HIV strains despite repeated exposures [42]. To determine whether the VIP approach is effective against cell-associated HIV transmission, the currently used antibody/vector system could be used in humanized mouse or macaque models of cell-associated HIV transmission (as described in review articles by Moench and Le Grand et al in this issue of the JID supplement). For better efficacy against cell-associated HIV transmission, antibodies with strong inhibitory activity in cell-to-cell HIV transmission assays [37] could be incorporated into AAV vectors and evaluated in animal models of cell-associated HIV transmission.

Antibodies differ from other therapeutic and prophylactic modalities for HIV in several important respects [43] since they can neutralize the pathogen directly and have the potential to clear the virus and infected cells through engagement of innate effector responses. Immune complexes produced by passively transferred antibodies may stimulate enhanced immunity to HIV-1, and antibodies have longer half-lives (21 days for IgG₁) in serum than commercially available antiretroviral drugs. In addition, multipurpose prevention technologies [44] can be created using antibody combinations to target an array of sexually transmitted pathogens and sperm [45].

Despite the central importance of Env in mediating HIV-1 binding and fusion, there are on average only 8–10 irregularly spaced Env spikes on the surface of a virion [46]. This low Env density results in a virion surface that is largely composed of host-derived molecules and opens up the possibility that non-virally encoded factors may also play important roles in virus interactions with target cells. The viral machinery exploits the host cell to facilitate many aspects of the viral life cycle. In doing so, these host molecules are manipulated in a manner that differs remarkably from their normal function in noninfected cells. Host antigens found on both cell vectors and free virus [47] may be targets in evaluating antibodies that block cell-associated transmission. For example, anti-CD36 (a member of the scavenger receptor family of cell surface proteins) antibodies inhibit release of virions from HIV-1-infected macrophages and the transmission of virus to CD4⁺ T cells [48]. Pretreatment of cells with an anti-LFA-1/CD11a chain antibody that blocks binding to ICAM-1 prevented HIV-1 transmission [49]. HIV bound to CD4⁻ cells was more infectious than the same amount of cell-free virus for T cells in cocultures, but CD18 (integrin beta-2) antibody reduced virus replication in T cells, suggesting blocking during cell-cell adhesion [50]. TSG101 (a cytoplasmic molecule that functions as a component

of the vesicular protein-sorting machinery and is co-opted by the HIV-1 Gag protein to facilitate virus assembly) is exposed on the outer membrane of cells that have been infected with HIV; TSG101-specific antibodies can reduce virus production in infected cells [51]. GM3 is a host-derived glycosphingolipid responsible for mediating the Env-independent interaction between HIV-1 and mature dendritic cells (DCs) [52]; increasing amounts of anti-GM3 Fab competitively inhibited mature DC capture of HIV particles.

As described in the review article by Gummuluru in this supplement, preexposure to a CD169 (siglec-1; sialoadhesin) antibody blocked virus capture and transinfection of autologous CD4+ T cells by immature and mature DCs, while a DC-SIGN antibody had modest inhibitory effects in immature DCs and failed to block HIV-1 capture by mature DCs [53]. Antibody to ICAM-1 can disrupt cell-associated HIV-1 transmission across the cervical epithelium in mouse models [54].

Antibodies to surface-coating antigens on seminal cells may trap cell-associated HIV by coagglutination with sperm and by so-called muco-trapping (ie, preventing HIV from entering potential host cells but binding it in mucus) [55, 56]. An antibody against a unique glycoform of CD52 found only in the human male reproductive tract, SAGA-1 [57–58], has been shown to coagglutinate 100% of human sperm and other seminal cells (eg, white blood cells) in <30 seconds at 100 µg/mL [59].

SUMMARY AND CONCLUSIONS

Antiretrovirals have been demonstrated to decrease HIV transmission and acquisition in diverse populations when used orally or topically for chemoprophylaxis. However, protection has not been perfect in these studies, with optimal outcomes mitigated by inadequate medication adherence, suboptimal medication penetration into key mucosal target cells, and, possibly, the presence of genital tract inflammation in high-risk participants. Data on efficacy of these approaches against cell-associated HIV transmission is inconclusive. More-effective future HIV prevention approaches may benefit from combinations of antiretrovirals and immunoprophylactic agents to improve efficacy.

Nonneutralizing antibodies against HIV were found to correlate with protection in the only (partially) effective HIV vaccine trial, suggesting that antibody-dependent cellular mechanisms such as ADCC and ADCP, which target HIV-infected cells, are critical components of HIV vaccine efficacy [60]. Although several antibodies to HIV and host antigens have been shown to block cell-cell HIV transmission in vitro and ex vivo (Table 1), it is essential to evaluate these vaccine candidates for efficacy in relevant animal models that are vaginally or rectally challenged with cell-associated virus, to ensure their effectiveness at sites of transmission [2, 61]. Although less advanced than antiretroviral strategies, antibody-based strategies to block cell-associated

HIV transmission could target HIV antigens (eg, the glycan/V3 loop on gp120), host antigens on infected cells (eg, TSG101), host antigens on uninfected cells (eg, CD4 binding site or LFA-1/ICAM-1 adhesion molecules involved in the formation of virological synapses), or cell-coating antigens in semen (eg, CD52 glycoform). Moreover, bioengineered broadly neutralizing antibodies may be able to be administered monthly or quarterly, obviating the need for daily medication adherence. Further studies may lead to the development of safe and effective antibody-based oral, topical, and/or systemic preexposure prophylaxis agents and to more-effective HIV vaccines.

Notes

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