Review

CellPress

Special Issue: Microbial Translocation

HIV cell-to-cell transmission: effects on pathogenesis and antiretroviral therapy

Luis M. Agosto[1,2], Pradeep D. Uchil[1], and Walther Mothes[1]

[1] Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA
[2] Department of Medicine, Section of Infectious Diseases, Boston University School of Medicine, Boston, MA 02118, USA

HIV spreads more efficiently *in vitro* when infected cells directly contact uninfected cells to form virological synapses. A hallmark of virological synapses is that viruses can be transmitted at a higher multiplicity of infection (MOI) that, *in vitro*, results in a higher number of proviruses. Whether HIV also spreads by cell–cell contact *in vivo* is a matter of debate. Here we discuss recent data that suggest that contact-mediated transmission largely manifests itself *in vivo* as CD4+ T cell depletion. The assault of a cell by a large number of incoming particles is likely to be efficiently sensed by the innate cellular surveillance to trigger cell death. The large number of particles transferred across virological synapses has also been implicated in reduced efficacy of antiretroviral therapies. Thus, antiretroviral therapies must remain effective against the high MOI observed during cell-to-cell transmission to inhibit both viral replication and the pathogenesis associated with HIV infection.

Contact-mediated spread of HIV

Viruses can spread by infecting cells in a cell-free form or via cell–cell contacts. Both modes of transmission offer distinct advantages and disadvantages for viral spread [1–3]. Given the high mutation rate of HIV and the resulting increased capacity to adapt, it is prudent to assume that HIV has found a way to balance out the advantages and disadvantages of either mode of transmission and efficiently spread from cell to cell, tissue to tissue, and person to person. While the contribution of both modes to virus spreading *in vivo* is unknown, there is overwhelming evidence that HIV spreads more efficiently by utilizing direct cell–cell contact *in vitro* [4–9]. In tissue culture, contact-mediated spread of HIV can be orders of magnitude more efficient than cell-free transmission [4,7–11]. This contact-dependent mode of transmission, known as cell-to-cell transmission, involves the formation of a virological synapse between an infected donor cell and an uninfected target cell [5,12]. Virological synapses owe their name due to some similarities with immunological synapses [5,13,14]. In the case of HIV, the formation of virological synapses depends on the interaction between CD4 and HIV envelope glycoprotein (Env) along with several cellular adhesion molecules characteristic of immunological synapses, such as LFA-1 and ICAM-1 [5,15,16] (Figure 1). Once a stable interaction between donor and target cells is established, large numbers of infectious particles can be assembled and released at the sites of cell–cell contacts [17–22].

The efficient coordination of the viral life cycle at virological synapses allows HIV to overcome barriers that would normally hinder the spread of cell-free particles. For example, an infected cell may not express sufficient levels of viral gene products needed for effective assembly and release or the actin cytoskeleton of the target cell may represent a barrier for infection by cell-free virus. Yet both barriers can be overcome when virus assembly and entry are coordinated at virological synapses [10]. Virological synapses have also been observed to provide some level of protection from neutralizing antibodies. This protection depends on whether antibodies are present prior to the formation of virological synapses [11,23–26], the specific epitopes recognized [4,10,27,28], and the type of antibodies used [29]. Furthermore, this mode of transmission has also been observed to lower the effectiveness of innate restriction factors such as rhesus TRIM5α and tetherin [10,30–32]. Although several studies document the inhibition of HIV cell-to-cell transmission by tetherin [10,31,33–35], the level of inhibition is lower compared with conditions when cells do not contact each other [10,31]. The higher efficiency of HIV cell-to-cell transmission also permits the transfer of mutant viruses that are not sufficiently fit to spread as cell-free virions [36]. While all these observations suggest that cell-to-cell transmission may contribute to HIV pathogenesis, it remains to be determined how relevant these observations are *in vivo*.

High MOI in HIV cell-to-cell transmission

The single most important feature of HIV cell-to-cell transmission is likely the generation of a high local multiplicity of infection (MOI) at the site of cell–cell contact that results in the integration of multiple proviruses in target cells *in vitro* [10,23,37–39]. Two studies conducted with splenic

Corresponding authors: Agosto, L.M. (luis.agosto.82@gmail.com); Mothes, W. (walther.mothes@yale.edu).
Keywords: human immunodeficiency virus; cell-to-cell transmission; virological synapse; antiretroviral therapy.

0966-842X/
 http://dx.doi.org/10.1016/j.tim.2015.02.003

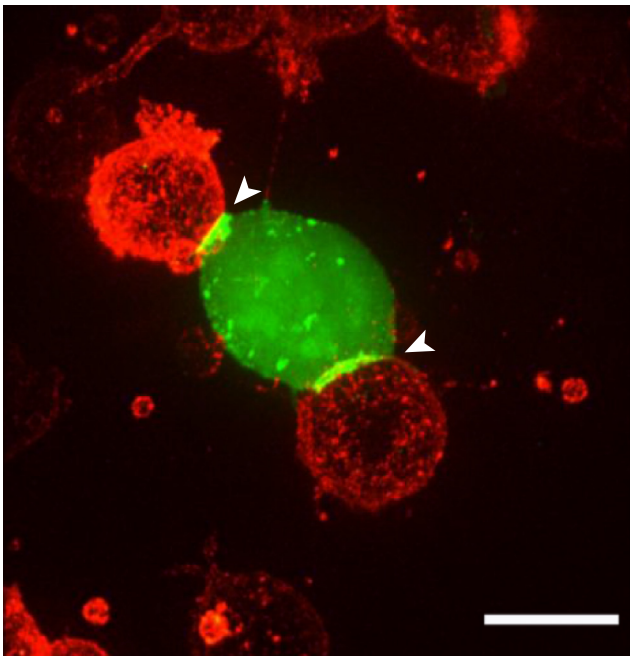

Figure 1. Virological synapses are characterized by the polarization of viral assembly and the accumulation of viral particles at the site of cell–cell contact. An HIV-infected CD4+ T cell (green) accumulates HIV Gag-GFP at the sites of cell–cell contact (arrows) with uninfected target CD4+ T cells (red). The size bar corresponds to 17 μm.

tissue from untreated infected patients found that infected cells can carry multiple proviruses [40,41]. By contrast, Josefsson *et al.* suggests that the majority of lymphocytes in circulating blood and in peripheral lymphoid tissue carry only a single provirus [42,43]. To reconcile the apparent contradictory evidence, it is important to consider the possibility that the observation of a very large number of proviruses per cell may be limited to *in vitro* studies because highly infected cells may die *in vivo*. Many primary cell types, particularly in tissues, contain multiple innate sensing pathways that may be triggered by an assault with a high number of retroviral particles and can lead to the death of the cell. Innate sensing pathways likely detect every step of the retroviral life cycle [44] (Figure 2). A high number of virus fusion events with cellular membranes may already be recognized as a danger signal by the targeted cell [45]. Viral nucleic acids of degraded or defective particles can be sensed by endosomal Toll-like receptors (TLRs) [46–49]. The delivery of many retroviral particles into the cytoplasm may be sensed by cellular factors that recognize 'foreign' patterns associated with retroviral capsids [50,51]. The RNA contents of the virion and complete or incomplete reverse transcribed viral DNA products may be sensed by cytoplasmic nucleic acid sensors [52–56]. Lastly, the invasion into the nucleus by many preintegration complexes and their subsequent integration into chromosomal DNA may trigger the DNA damage response pathway mediated by DNA-PK [57]. As a response to these immune sensing pathways, there is evidence that HIV evolved counter measures to cloak incoming capsids or excess reverse transcribed DNA by recruiting the host factors cyclophilin A and CPSF6, or by exploiting the natural nucleic acid degradative pathway involving TREX-1 [54,58]. Recent work by the Warner Greene and Gary Nabel laboratories suggest that some of these innate sensing pathways can indeed recognize retroviral DNA and trigger cell death by pyroptosis or apoptosis [55,57,59,60]. Human tonsil cells, when infected with an X4-tropic HIV, were observed to undergo caspase-1-dependent pyroptosis [55,59,60]. Given that pyroptosis leads to cell death, as well as a strong inflammatory response, these data suggest that the transfer of a high viral MOI at sites of cell–cell contact can drive the resulting CD4+ T cell depletion and chronic inflammation observed in HIV-infected patients. Thus, innate immune responses may be selecting for target cells that carry a small number of proviruses. It is critical that these proposed models and the relative contribution of apoptosis and pyroptosis to CD4+ T cell depletion be tested directly *in vivo*.

The effectiveness of ART against HIV cell-to-cell transmission

These considerations emphasize that antiretroviral therapies (ART) must remain active against a potentially high MOI during HIV cell-to-cell transmission to not only suppress viral replication but to also effectively suppress HIV pathogenesis. Work from the Baltimore laboratory suggests that a high local number of viral particles requires a higher local concentration of antiretroviral inhibitors [61] (Figure 3). The study showed that the nucleoside analog inhibitor (NRTI) tenofovir and the non-nucleoside analog inhibitor (NNRTI) efavirenz, while potent against cell-free virus infection, were far less effective in suppressing replication of cell-to-cell transmitted HIV due to the transfer of large number of viral particles [61]. These observations have been largely reproduced in several laboratories [23,24,62–65]. However, the data appear to contradict years of clinical observations, which indicate that ART effectively suppresses HIV replication in patients [66–69]. Although the ability of HIV to replicate deep within tissues despite suppressive ART due to incomplete drug penetration remains a topic of debate [70–72], strong evidence from blood and tissues supports that viral replication is mostly suppressed [69,73,74]. If ART is indeed effective against cell-free HIV infection *in vivo*, then the failure of therapy to suppress HIV cell-to-cell transmission *in vitro* must mean that the spread of virus *in vivo* must be solely due to cell-free virus. In other words, if viral spread occurs *in vivo* via cell–cell contacts, ART would fail to suppress HIV in patients. Given the success of ART in patients, some groups, including ours, doubted the accuracy of this interpretation and systematically tested the effectiveness of single and combination therapies against HIV cell-to-cell transmission [23,65]. While the observations by Sigal *et al.* [61] were largely reproduced, this phenomenon appears to apply only to a small number of NRTIs and the integrase inhibitor raltegravir [23,24,62–65]. NNRTIs, protease inhibitors (PIs), and entry inhibitors appear to be more effective in suppressing HIV cell-to-cell transmission compared to NRTIs [23,65]. Furthermore, the resistance of HIV cell-to-cell transmission to some inhibitors was also less apparent when a clinical viral isolate was studied [23], indicating variability among viral