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

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Abstract

The human immunodeficiency virus (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 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.

Keywords

Human immunodeficiency virus; cell-to-cell transmission; virological synapse; antiretroviral therapy

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

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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 inhibition of HIV cell-to-cell transmission by tetherin [10, 31, 33–35], the level of inhibition is lower compared to 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 tissue from untreated infected patients found that infected cells can carry multiple proviruses [40, 41]. In 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 pre-integration 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 appears 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 isolates. Interestingly, when two NRTIs were combined, the combination became highly efficient compared to the each inhibitor alone [23]. Since common ART regimens include a combination of multiple inhibitors [2 NRTI and 1 NNRTI or PI or integrase inhibitor (http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf)], these results reaffirm the effectiveness of ART *in vivo* even if cell-to-cell transmission would occur in patients. These data are also consistent with observations that ART is effective in suppressing CD4+ T cell depletion [75]. The ineffectiveness of some NRTIs but the effectiveness of NNRTIs against cell-to-cell transmission also matches the signature phenotypes during bystander cell death by pyroptosis [59, 60].

Some contradiction still remains on how resistant HIV cell-to-cell transmission is to antiretroviral inhibitors. While most reports agree that NRTIs such as zidovudine and tenofovir are less effective, the effectiveness of NNRTIs, such as nevirapine and efavirenz, has been debated [23, 61, 63, 65]. This may in part be explained by variable levels of infection achieved when directly comparing both modes of viral transmission. This point is a critical issue with respect to the experimental design in studying HIV cell-to-cell transmission. In our own study, we opted for matching the inoculum for comparing cell-free and cell-to-cell transmission, e.g. to ~10% infection. The efficiencies of HIV transmission for both modes are far apart and their linear ranges do not overlap, which often results in an apparent resistance to inhibition by the more efficient cell-to-cell transmission. By matching the level of infection after both modes of transmission, the only variable present is the effect of drug on the mode of viral transmission. Our results suggest that NNRTIs, even though can be somewhat less effective against cell-to-cell transmission, are far more effective than NRTIs, consistent with recent work by Titanji, *et al.* [65].

Reverse transcriptase and HIV cell-to-cell transmission

The inability of some NRTIs, but not other drug classes, to suppress HIV cell-to-cell transmission suggested that some activity of reverse transcriptase must be involved in this phenomenon. HIV reverse transcriptase (RT) has nucleotide excision activity [76–79] (Figure 4A). When RT incorporates a nucleoside analog, it can excise the incorporated nucleotide analog in an ATP-dependent fashion [76–79]. Interestingly, when an HIV mutant defective in its ability to excise the nucleoside analog zidovudine was tested [80, 81], this drug was now able to effectively block HIV infection regardless of the mode of transmission. In a similar fashion, NRTI combinations can block either mode of viral transmission by significantly reducing the level of drug excision [23, 82]. In light of these observations, we propose that RT-mediated nucleotide excision activity in combination with low effective drug concentration due to high viral MOI contributes to the apparent failure of some NRTIs to suppress HIV cell-to-cell transmission. We predict a scenario in which the drug concentration is just insufficient to keep up with the rate of drug excision as the

number of incoming viral particles increases (Figure 4B, C). In contrast, combinations of NRTIs or a single excision-resistant NRTI can suppress RT-mediated nucleotide excision thereby increasing the effectiveness of the treatment against HIV cell-to-cell transmission (Figure 4D).

Therapeutically blocking cell-to-cell transmission of HIV

These experimental observations favor the development of NRTIs that resist excision, such as apricitabine, that are currently in clinical trials [83–85]. Moreover, we predict that allosteric drug inhibitors, with similar mechanism of action as NNRTIs and PIs, will be effective against HIV cell-to-cell transmission on their own. For instance, attachment inhibitors, maturation inhibitors and second generation integration inhibitors with clear allosteric and even dominant-negative effects are predicted to be effective [23, 86]. Thus, powerful allosteric inhibitors that allow logs of HIV depletion are predicted to remain effective because the mass action effect alone is likely to be too small to affect drug activity [87, 88].

These considerations have also raised the question if therapies could be developed that specifically inhibit HIV cell-to-cell transmission. This task has proven more difficult than initially thought because all the steps of the viral life cycle are identical between cell-free and cell-to-cell transmission [3, 20]. HIV cell-to-cell transmission differs from cell-free spread in that HIV Env is bound to receptor CD4 in the cell-cell interface, thus exposing distinct HIV Env epitopes [27, 89]. While the secretion of virus particles to the virological synapses and the uptake of particles by endocytosis in the target cell have been proposed [17, 90, 91], it must be taken into consideration that the display of just Env and ICAM-1 in a planar lipid bilayer is sufficient to induce the formation of a virolocial synapse on a target cell [13]. Thus, the isolation of cellular targets specifically associated with HIV cell-to-cell transmission may inherently be limited. Most cellular barriers affect the transmission of cell-free virus more than they affect cell-to-cell transmission [10]. Even virus mutants that prevent the spread as cell-free virus, can still be salvaged by the more efficient transmission at sites of cell-cell contact, which may contribute to the ability of HIV to survive tough evolutionary bottle necks [36]. Therefore, finding inhibitors that block both modes of transmission efficiently is likely more feasible than finding inhibitors that specifically inhibit HIV cell-to-cell transmission [23, 92, 93].

Concluding remarks

Collectively, ART likely remains effective against HIV cell-to-cell transmission and should be able to suppress HIV spread. Thus, it will be important to directly test whether HIV spreads, at least in part, by cell-to-cell transmission *in vivo* (Box 1). *In vivo* imaging of lymphocytes infected by the murine leukemia virus clearly documented the existence of long-lived virological synapses within lymph nodes of mice [94]. However, the visualization of HIV-infected T cells within lymph nodes of humanized mice revealed shorter adhesive contacts between infected and uninfected cells [95]. Further application of intra-vital imaging and explant studies will be required to resolve the relative contribution of cell-free and cell-to-cell transmission to HIV spreading *in vivo*.

In conclusion, current experimental data suggest that commonly used ART regimens are effective in suppressing HIV cell-to-cell transmission and preventing CD4+ T cell depletion. However, they do not solve the problem that HIV-infected patients require life-long chemotherapy, which results in many short and long-term side effects. Progress towards developing better treatments and a cure requires that the mechanisms of HIV pathogenesis are well understood *in vivo*. Although there is renewed interest in the search for a cure, perhaps drug discovery should focus, not necessarily on intensifying current therapies, but on developing smarter therapies that can alleviate the treatment burden of people living with HIV.

References

1. Sattentau Q. Avoiding the void: cell-to-cell spread of human viruses. Nat. Rev. Microbiol. 2008; 6:815–826. [PubMed: 18923409]
2. Zhong P, et al. Cell-to-cell transmission of viruses. Curr. Opin. Virol. 2013; 3:44–50. [PubMed: 23219376]
3. Alvarez RA, et al. Unique Features of HIV-1 Spread through T Cell Virological Synapses. PLoS Pathog. 2014; 10:e1004513. [PubMed: 25522148]
4. Chen P, et al. Predominant Mode of Human Immunodeficiency Virus Transfer between T Cells Is Mediated by Sustained Env-Dependent Neutralization-Resistant Virological Synapses. J. Virol. 2007; 81:12582–12595. [PubMed: 17728240]
5. Jolly C, et al. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. J. Exp. Med. 2004; 199:283–293. [PubMed: 14734528]
6. Phillips DM. The role of cell-to-cell transmission in HIV infection. AIDS. 1994; 8:719–731. [PubMed: 8086128]
7. Dimitrov DS, et al. Quantitation of human immunodeficiency virus type 1 infection kinetics. J. Virol. 1993; 67:2182–2190. [PubMed: 8445728]
8. Carr JM, et al. Rapid and efficient cell-to-cell transmission of human immunodeficiency virus infection from monocyte-derived macrophages to peripheral blood lymphocytes. Virology. 1999; 265:319–329. [PubMed: 10600603]
9. Sourisseau M, et al. Inefficient human immunodeficiency virus replication in mobile lymphocytes. J. Virol. 2007; 81:1000–1012. [PubMed: 17079292]
10. Zhong P, et al. Cell-to-Cell Transmission Can Overcome Multiple Donor and Target Cell Barriers Imposed on Cell-Free HIV. PLoS One. 2013; 8:e53138. [PubMed: 23308151]
11. Martin N, et al. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. J. Virol. 2010; 84:3516–3527. [PubMed: 20089656]
12. McDonald D, et al. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science. 2003; 300:1295–1297. [PubMed: 12730499]
13. Vasiliver-Shamis G, et al. Human immunodeficiency virus type 1 envelope gp120-induced partial T-cell receptor signaling creates an F-actin-depleted zone in the virological synapse. J. Virol. 2009; 83:11341–11355. [PubMed: 19710135]
14. Vasiliver-Shamis G, et al. Human immunodeficiency virus type 1 envelope gp120 induces a stop signal and virological synapse formation in noninfected CD4+ T cells. J. Virol. 2008; 82:9445–9457. [PubMed: 18632854]
15. Jolly C, et al. Adhesion molecule interactions facilitate human immunodeficiency virus type 1-induced virological synapse formation between T cells. J. Virol. 2007; 81:13916–13921. [PubMed: 17913807]
16. Rudnicka D, et al. Simultaneous cell-to-cell transmission of human immunodeficiency virus to multiple tragets through polysynapses. J. Virol. 2009; 83:6234–6246. [PubMed: 19369333]

17. Jolly C, et al. The regulated secretory pathway in CD4(+) T cells contributes to human immunodeficiency virus type-1 cell-to-cell spread at the virological synapse. PLoS Pathog. 2011; 7:e1002226. [PubMed: 21909273]
18. Hubner W, et al. Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. Science. 2009; 323:1743–1747. [PubMed: 19325119]
19. Llewellyn GN, et al. Nucleocapsid promotes localization of HIV-1 Gag to uropods that participate in virological synapses between T cells. PLoS Pathog. 2010; 6:e1001167. [PubMed: 21060818]
20. Monel B, et al. HIV cell-to-cell transmission requires the production of infectious virus particles and does not proceed through env-mediated fusion pores. J. Virol. 2012; 86:3924–3933. [PubMed: 22258237]
21. Li P, et al. Synthesis of human immunodeficiency virus DNA in a cell-to-cell transmission model. AIDS Res. Hum. Retrovir. 1992; 8:253–259. [PubMed: 1540411]
22. Li P, et al. De novo reverse transcription is a crucial event in cell-to-cell transmission of human immunodeficiency virus. J. Gen. Virol. 1992; 73:955–959. [PubMed: 1378883]
23. Agosto LM, et al. Highly active antiretroviral therapies are effective against HIV-1 cell-to-cell transmission. PLoS Pathog. 2014; 10:e1003982. [PubMed: 24586176]
24. Gupta P, et al. Cell-to-cell transmission of human immunodeficiency virus type 1 in the presence of azidothymidine and neutralizing antibody. J. Virol. 1989; 63:2361–2365. [PubMed: 2704079]
25. Ganesh L, et al. Infection of specific dendritic cells by CCR5-tropic human immunodeficiency virus type 1 promotes cell-mediated transmission of virus resistant to broadly neutralizing antibodies. J. Virol. 2004; 78:11980–11987. [PubMed: 15479838]
26. Malbec M, et al. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. The Journal of experimental medicine. 2013; 210:2813–2821. [PubMed: 24277152]
27. Abela IA, et al. Cell-Cell Transmission Enables HIV-1 to Evade Inhibition by Potent CD4bs Directed Antibodies. PLoS Pathog. 2012; 8:e1002634. [PubMed: 22496655]
28. Massanella M, et al. Antigp41 antibodies fail to block early events of virological synapses but inhibit HIV spread between T cells. AIDS. 2009; 23:183–188. [PubMed: 19098487]
29. McCoy LE, et al. Neutralisation of HIV-1 cell-cell spread by human and llama antibodies. Retrovirology. 2014; 11:83. [PubMed: 25700025]
30. Coleman CM, et al. Tetherin does not significantly restrict dendritic cell-mediated HIV-1 transmission and its expression is upregulated by newly synthesized HIV-1 Nef. Retrovirology. 2011; 8:26. [PubMed: 21504576]
31. Jolly C, et al. Cell-cell spread of human immunodeficiency virus type 1 overcomes tetherin/BST-2-mediated restriction in T cells. J. Virol. 2010; 84:12185–12199. [PubMed: 20861257]
32. Richardson MW, et al. Mode of transmission affects the sensitivity of human immunodeficiency virus type 1 to restriction by rhesus TRIM5alpha. J. Virol. 2008; 82:11117–11128. [PubMed: 18768965]
33. Casartelli N, et al. Tetherin restricts productive HIV-1 cell-to-cell transmission. PLoS Pathog. 2010; 6:e1000955. [PubMed: 20585562]
34. Kuhl BD, et al. Tetherin restricts direct cell-to-cell infection of HIV-1. Retrovirology. 2010; 7:115. [PubMed: 21184674]
35. Giese S, et al. Tetherin can restrict cell-free and cell-cell transmission of HIV from primary macrophages to T cells. PLoS Pathog. 2014; 10:e1004189. [PubMed: 24991932]
36. Brandenberg OF, et al. Partial rescue of V1V2 mutant infectivity by HIV-1 cell-cell transmission supports the domain inverted question marks exceptional capacity for sequence variation. Retrovirology. 2014; 11:75. [PubMed: 25287422]
37. Del Portillo A, et al. Multiploid inheritance of HIV-1 during cell-to-cell infection. J. Virol. 2011; 85:7169–7176. [PubMed: 21543479]
38. Duncan CJ, et al. High-multiplicity HIV-1 infection and neutralizing antibody evasion mediated by the macrophage-T cell virological synapse. J. Virol. 2014; 88:2025–2034. [PubMed: 24307588]
39. Russell RA, et al. Multiple proviral integration events after virological synapse-mediated HIV-1 spread. Virology. 2013; 443:143–149. [PubMed: 23722103]

40. Gratton S, et al. Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. Proc. Natl. Acad. Sci. U. S. A. 2000; 97:14566–14571. [PubMed: 11121058]
41. Jung A, et al. Recombination: Multiply infected spleen cells in HIV patients. Nature. 2002; 418:144. [PubMed: 12110879]
42. Josefsson L, et al. Single cell analysis of lymph node tissue from HIV-1 infected patients reveals that the majority of CD4+ T-cells contain one HIV-1 DNA molecule. PLoS Pathog. 2013; 9:e1003432. [PubMed: 23818847]
43. Josefsson L, et al. Majority of CD4+ T cells from peripheral blood of HIV-1-infected individuals contain only one HIV DNA molecule. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:11199–11204. [PubMed: 21690402]
44. Iwasaki A. Innate immune recognition of HIV-1. Immunity. 2012; 37:389–398. [PubMed: 22999945]
45. Holm CK, et al. Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. Nat. Immunol. 2012; 13:737–743. [PubMed: 22706339]
46. Kane M, et al. Innate immune sensing of retroviral infection via Toll-like receptor 7 occurs upon viral entry. Immunity. 2011; 35:135–145. [PubMed: 21723157]
47. Browne EP. Toll-like receptor 7 controls the anti-retroviral germinal center response. PLoS Pathog. 2011; 7:e1002293. [PubMed: 21998589]
48. Gringhuis SI, et al. HIV-1 exploits innate signaling by TLR8 and DC-SIGN for productive infection of dendritic cells. Nat. Immunol. 2010; 11:419–426. [PubMed: 20364151]
49. Lepelley A, et al. Innate sensing of HIV-infected cells. PLoS Pathog. 2011; 7:e1001284. [PubMed: 21379343]
50. Pertel T, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature. 2011; 472:361–365. [PubMed: 21512573]
51. Ganser-Pornillos BK, et al. Hexagonal assembly of a restricting TRIM5alpha protein. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:534–539. [PubMed: 21187419]
52. Solis M, et al. RIG-I-mediated antiviral signaling is inhibited in HIV-1 infection by a protease-mediated sequestration of RIG-I. J. Virol. 2011; 85:1224–1236. [PubMed: 21084468]
53. Gao D, et al. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science. 2013; 341:903–906. [PubMed: 23929945]
54. Yan N, et al. The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. Nat. Immunol. 2010; 11:1005–1013. [PubMed: 20871604]
55. Monroe KM, et al. IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. Science. 2014; 343:428–432. [PubMed: 24356113]
56. Ryoo J, et al. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. Nat. Med. 2014; 20:936–941. [PubMed: 25038827]
57. Cooper A, et al. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. Nature. 2013; 498:376–379. [PubMed: 23739328]
58. Rasaiyaah J, et al. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature. 2013; 503:402–405. [PubMed: 24196705]
59. Doitsh G, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature. 2014; 505:509–514. [PubMed: 24356306]
60. Doitsh G, et al. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. Cell. 2010; 143:789–801. [PubMed: 21111238]
61. Sigal A, et al. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature. 2011; 477:95–98. [PubMed: 21849975]
62. Mathez D, et al. Infectious amplification of wild-type human immunodeficiency virus from patients' lymphocytes and modulation by reverse transcriptase inhibitors in vitro. Antimicrob. Agents Chemother. 1993; 37:2206–2211. [PubMed: 7504908]
63. Duncan CJ, et al. High multiplicity HIV-1 cell-to-cell transmission from macrophages to CD4+ T cells limits antiretroviral efficacy. AIDS. 2013; 27:2201–2206. [PubMed: 24005480]
64. Permanyer M, et al. Antiretroviral Agents Effectively Block HIV Replication after Cell-to-Cell Transfer. J. Virol. 2012; 86:8773–8780. [PubMed: 22696642]

65. Titanji BK, et al. Protease inhibitors effectively block cell-to-cell spread of HIV-1 between T cells. Retrovirology. 2013; 10:161. [PubMed: 24364896]

66. Gulick RM, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N. Engl. J. Med. 1997; 337:734–739. [PubMed: 9287228]

67. Perelson AS, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature. 1997; 387:188–191. [PubMed: 9144290]

68. Hammer SM, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N. Engl. J. Med. 1997; 337:725–733. [PubMed: 9287227]

69. Laskey SB, et al. A mechanistic theory to explain the efficacy of antiretroviral therapy. Nat. Rev. Microbiol. 2014; 12:772–780. [PubMed: 25263222]

70. Patterson KB, et al. Differential penetration of raltegravir throughout gastrointestinal tissue: implications for eradication and cure. AIDS. 2013; 27:1413–1419. [PubMed: 23945503]

71. Patterson KB, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. Science translational medicine. 2011; 3:112re114.

72. Fletcher CV, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc. Natl. Acad. Sci. U. S. A. 2014; 111:2307–2312. [PubMed: 24469825]

73. Evering TH, et al. Absence of HIV-1 evolution in the gut-associated lymphoid tissue from patients on combination antiviral therapy initiated during primary infection. PLoS Pathog. 2012; 8:e1002506. [PubMed: 22319447]

74. Josefsson L, et al. The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. Proc. Natl. Acad. Sci. U. S. A. 2013; 110:E4987–E4996. [PubMed: 24277811]

75. Guihot A, et al. Immune reconstitution after a decade of combined antiretroviral therapies for human immunodeficiency virus. Trends Immunol. 2011; 32:131–137. [PubMed: 21317040]

76. Meyer PR, et al. A mechanism of AZT resistance: an increase in nucleotide-dependent primer unblocking by mutant HIV-1 reverse transcriptase. Mol. Cell. 1999; 4:35–43. [PubMed: 10445025]

77. Meyer PR, et al. Unblocking of chain-terminated primer by HIV-1 reverse transcriptase through a nucleotide-dependent mechanism. Proc. Natl. Acad. Sci. U. S. A. 1998; 95:13471–13476. [PubMed: 9811824]

78. Arion D, et al. Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. Biochemistry. 1998; 37:15908–15917. [PubMed: 9843396]

79. Acosta-Hoyos AJ, et al. The Role of Nucleotide Excision by Reverse Transcriptase in HIV Drug Resistance. Viruses. 2010; 2:372–394. [PubMed: 20523911]

80. Boyer PL, et al. The M184V mutation reduces the selective excision of zidovudine 5'-monophosphate (AZTMP) by the reverse transcriptase of human immunodeficiency virus type 1. J. Virol. 2002; 76:3248–3256. [PubMed: 11884549]

81. Gotte M, et al. The M184V mutation in the reverse transcriptase of human immunodeficiency virus type 1 impairs rescue of chain-terminated DNA synthesis. J. Virol. 2000; 74:3579–3585. [PubMed: 10729133]

82. Feng JY, et al. The triple combination of tenofovir, emtricitabine and efavirenz shows synergistic anti-HIV-1 activity in vitro: a mechanism of action study. Retrovirology. 2009; 6:44. [PubMed: 19439089]

83. Frankel FA, et al. Kinetics of inhibition of HIV type 1 reverse transcriptase-bearing NRTI-associated mutations by apricitabine triphosphate. Antivir. Chem. Chemother. 2007; 18:93–101. [PubMed: 17542154]

84. Michailidis E, et al. 4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) Inhibits HIV-1 Reverse Transcriptase with Multiple Mechanisms. J. Biol. Chem. 2014; 289:24533–24548. [PubMed: 24970894]

85. Wong A. The HIV pipeline. Nat. Rev. Drug. Discov. 2014; 13:649–650. [PubMed: 25145593]
86. Jurado KA, et al. Allosteric integrase inhibitor potency is determined through the inhibition of HIV-1 particle maturation. Proc. Natl. Acad. Sci. U. S. A. 2013; 110:8690–8695. [PubMed: 23610442]
87. Komarova NL, et al. Synaptic transmission and the susceptibility of HIV infection to anti-viral drugs. Sci. Rep. 2013; 3:2103. [PubMed: 23811684]
88. Shen L, et al. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. Nat. Med. 2008; 14:762–766. [PubMed: 18552857]
89. Durham ND, et al. Neutralization resistance of virological synapse-mediated HIV-1 Infection is regulated by the gp41 cytoplasmic tail. J. Virol. 2012; 86:7484–7495. [PubMed: 22553332]
90. Dale BM, et al. Cell-to-Cell Transfer of HIV-1 via Virological Synapses Leads to Endosomal Virion Maturation that Activates Viral Membrane Fusion. Cell Host Microbe. 2011; 10:551–562. [PubMed: 22177560]
91. Sloan RD, et al. Productive entry of HIV-1 during cell-to-cell transmission via dynamin-dependent endocytosis. J. Virol. 2013; 87:8110–8123. [PubMed: 23678185]
92. Swartz TH, et al. P2X-selective purinergic antagonists are strong inhibitors of HIV-1 fusion during both cell-to-cell and cell-free infection. J. Virol. 2014; 88:11504–11515. [PubMed: 25031337]
93. Costantino CM, et al. Cannabinoid receptor 2-mediated attenuation of CXCR4-tropic HIV infection in primary CD4+ T cells. PLoS One. 2012; 7:e33961. [PubMed: 22448282]
94. Sewald X, et al. In vivo imaging of virological synapses. Nat. Commun. 2012; 3:1320. [PubMed: 23271654]
95. Murooka TT, et al. HIV-infected T cells are migratory vehicles for viral dissemination. Nature. 2012; 490:283–287. [PubMed: 22854780]
96. Miyauchi K, et al. HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes. Cell. 2009; 137:433–444. [PubMed: 19410541]
97. Herold N, et al. HIV-1 entry in SupT1-R5, CEM-ss and primary CD4+ T-cells occurs at the plasma membrane and does not require endocytosis. J. Virol. 2014; 88:13956–13970. [PubMed: 25253335]
98. Platt EJ, et al. Reversible and efficient activation of HIV-1 cell entry by a tyrosine-sulfated peptide dissects endocytic entry and inhibitor mechanisms. J. Virol. 2014; 88:4304–4318. [PubMed: 24478426]
99. van Wilgenburg B, et al. The productive entry pathway of HIV-1 in macrophages is dependent on endocytosis through lipid rafts containing CD4. PLoS One. 2014; 9:e86071. [PubMed: 24465876]
100. Bosch B, et al. A clathrin-dynamin-dependent endocytic pathway for the uptake of HIV-1 by direct T cell-T cell transmission. Antivir. Res. 2008; 80:185–193. [PubMed: 18602423]
101. Blanco J, et al. High level of coreceptor-independent HIV transfer induced by contacts between primary CD4 T cells. J. Biol. Chem. 2004; 279:51305–51314. [PubMed: 15371410]
102. Heil F, et al. Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. Science. 2004; 303:1526–1529. [PubMed: 14976262]
103. Chattergoon MA, et al. HIV and HCV activate the inflammasome in monocytes and macrophages via endosomal Toll-like receptors without induction of type 1 interferon. PLoS Pathog. 2014; 10:e1004082. [PubMed: 24788318]
104. Melikyan GB. HIV entry: a game of hide-and-fuse? Curr. Opin. Virol. 2014; 4:1–7. [PubMed: 24525288]
105. Stremlau M, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:5514–5519. [PubMed: 16540544]
106. Refsland EW, et al. The APOBEC3 family of retroelement restriction factors. Curr. Top. Microbiol. Immunol. 2013; 371:1–27. [PubMed: 23686230]
107. Sze A, et al. SAMHD1 host restriction factor: a link with innate immune sensing of retrovirus infection. J. Mol. Biol. 2013; 425:4981–4994. [PubMed: 24161438]
108. Goujon C, et al. Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. Nature. 2013; 502:559–562. [PubMed: 24048477]

109. Kane M, et al. MX2 is an interferon-induced inhibitor of HIV-1 infection. Nature. 2013; 502:563–566. [PubMed: 24121441]

Highlights

- Cell-to-cell transmission may contribute to pathogenesis seen in HIV-infected individuals.
- Effective antiretroviral therapy suppresses both cell-free and cell-cell transmission of HIV.

Box 1. Outstanding questions

- What is the role of cell-cell contact in the depletion of CD4+ T cells *in vivo*?
- What is the role of cell-cell contact in the transmission and spread of HIV *in vivo*?
- Is the innate sensing of HIV in various cell types dependent on the number of incoming HIV particles?
- What other mechanisms affect the efficiency of antiretroviral inhibitors during HIV cell-to-cell transmission?
- Can inhibitors that specifically target HIV cell-to-cell transmission be developed?

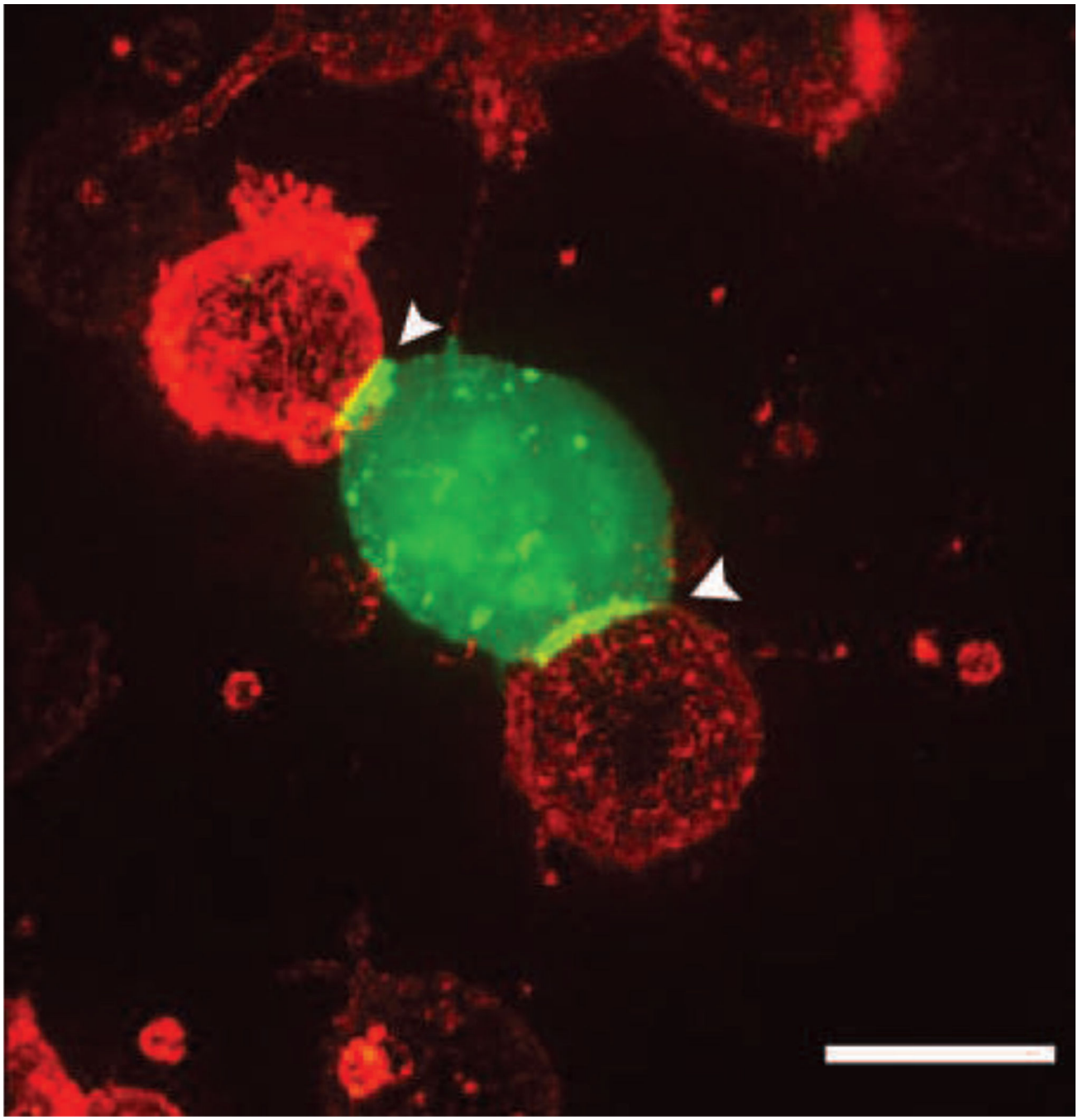

Figure 1. Virological synapses are characterized by the polarization of viral assembly and the accumulation of viral particles at the site of cell-cell contact

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.

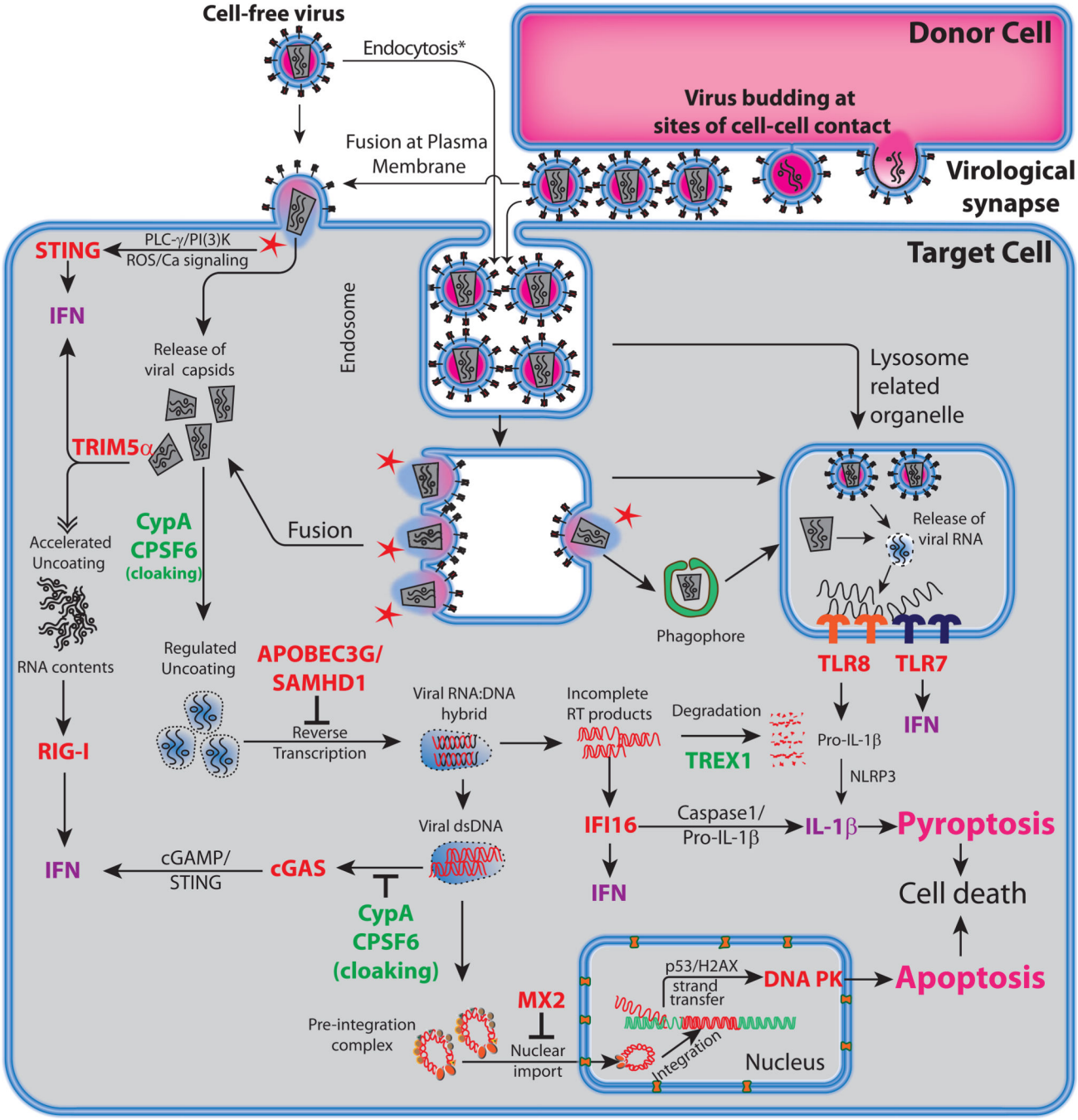

Figure 2. Potential pathways for innate immune sensing of HIV during the early stages of the virus life cycle

In contrast to cell-free virus infection, the virological synapse can promote the entry of multiple HIV virions into target cells [1, 2, 18, 37]. Virus fusion events can be sensed as danger signals (depicted here as red stars) due to the release of reactive oxygen species (ROS) and the activation of phospholipase C gamma 1-phosphoinositide 3-kinase pathway (PLC-γ–PI(3)K pathway), which stimulates the release of Ca^{2+} culminating in stimulator of interferon genes (STING)-dependent Type I interferon (IFN) production [45]. *While the contribution of endocytosis to productive HIV entry remains highly controversial [96–98], it

has been best documented in studies of HIV transmitted via cell-cell contacts [90, 91, 99–101]. Endocytosis of virions, their transfer to lysosome related organelles and subsequent lysis, releases viral RNA contents that can be sensed by TLR7 and TLR8 [102]. TLR7- and TLR8-sensing results in the synthesis of IFN and the inflammatory cytokine IL-1β respectively [46, 47, 103]. Fusion at the plasma membrane or from mature endosomes releases viral capsids into the host cytosol [97, 104]. The released capsids can also be delivered to the lysosome-related organelle by autophagy to be sensed by TLR7/8 [44]. In addition, TRIM5α in the host cytosol senses viral capsid as 'foreign', inducing IFN and accelerating capsid disassembly [50, 105]. In contrast, cyclophilinA (CypA) and cleavage and polyadenylation specific factor 6 (CPSF6) may act as 'cloaking' factors to prevent premature exposure and sensing of viral RNA and reverse transcription (RT) products [58]. Premature disassembly of viral capsids can release the RNA contents of the virus and can be potentially sensed by the cytoplasmic RNA sensor retinoic acid inducible gene-1 (RIG-I) to elicit IFN production [52]. The next step of reverse transcription of viral RNA is repressed by APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G) packed within the incoming virions or SAM domain and HD domain-containing protein 1 (SAMHD1) present in the host cytosol [106, 107]. SAMHD1 is thought to deplete the nucleotide pool and elevates the generation of incomplete RT products. SAMHD1 was also recently reported to have direct RNase activity against incoming HIV RNA [56]. The DNA sensor, IFI16 can sense abortive RT products to induce IFN as well as activate caspase-1 and IL-1β, triggering pyroptosis and cell death [55, 59, 60]. In contrast, the exonuclease TREX1 can degrade excess RT products and prevent sensing of viral DNA [54]. Viral DNA can also be sensed by cyclic GMP-AMP synthase (cGAS) to generate cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) that activates the STING-dependent IFN pathway [53]. Pre-integration complexes (PICs) generated at the end of RT reaction are transported into the nucleus through nuclear pore complex, a step that is inhibited by IFN-inducible factor MX2 [108, 109]. PICs mediate integration of HIV DNA into the host chromosome. Multiple double stranded DNA breaks and strand transfer reactions into the host chromosomal DNA can elicit a DNA damage response pathway that leads to phosphorylation of gamma histone 2AX (H2AX) and activation of p53 and DNA-dependent protein kinase (DNA-PK) to trigger apoptosis and cell death [57].

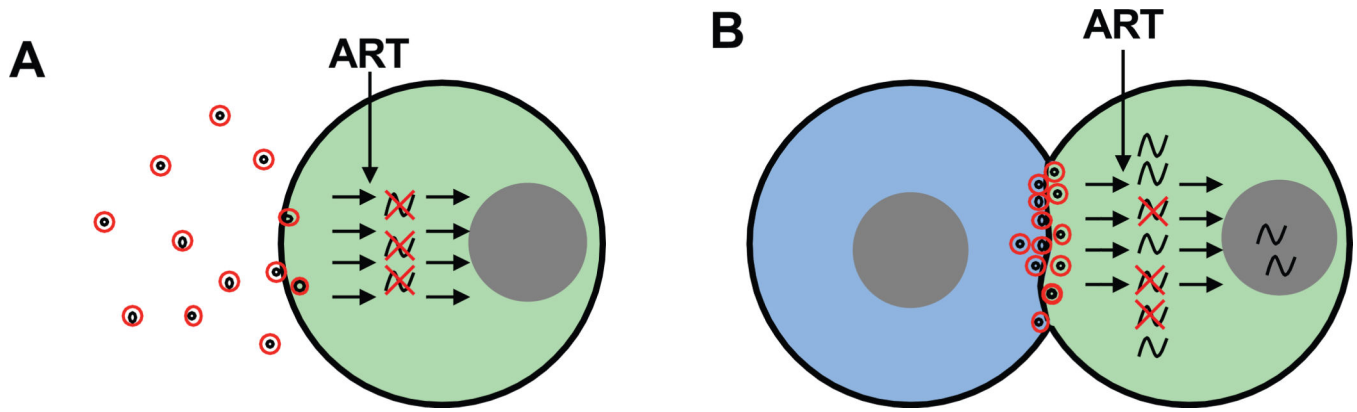

Figure 3. Mechanisms of how HIV cell-to-cell transmission can overcome inhibition by antiretroviral inhibitors

(A) The number of particles that infect a target cell during cell-free HIV infection is small and is inhibited by antiretroviral inhibitors. **(B)** However, during cell-to-cell transmission via virological synapses, the number of particles can be high, thereby overwhelming the drug concentration.

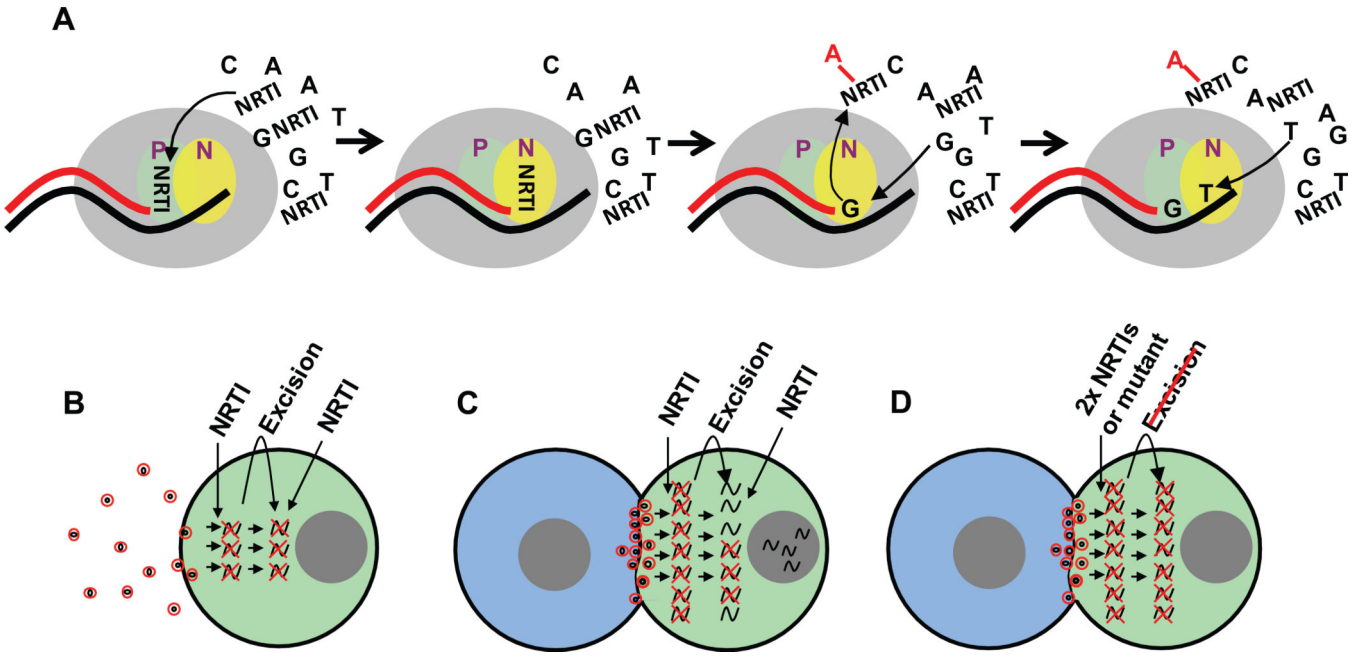

Figure 4. Mechanisms of how RT nucleotide excision and HIV cell-to-cell transmission can overcome inhibition by antiretroviral inhibitors

(A) A simplified model of nucleotide excision by HIV RT. When HIV RT incorporates a nucleotide analog (NRTI) in the growing reverse transcript, RT can return the analog from the chain-terminated primer site back to the nucleotide binding site (shown as green and yellow ovals labeled P and N respectively). Once in the N site, a reaction thought to be pyrophosphate or ATP-dependent (red A), mediates the release of the NRTI from the reverse transcript. Upon freeing the N site, RT can resume normal reverse transcription. **(B, C)** While cell-free spread of HIV may not benefit sufficiently from nucleotide excision activity, delivery of large numbers of viruses across virological synapses can overwhelm the active concentration of intracellular NRTIs, thus increasing the likelihood of reverse transcripts escaping inhibition. **(D)** This resistance to individual NRTIs can be prevented by combining NRTIs, by excision-resistant NRTIs or by mutations in HIV RT.