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Gaining a foothold: how HIV avoids innate immune recognition

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During the first week after sexual exposure to HIV, HIV infection does not appear to trigger a strong innate immune response. Here we describe some recent studies that show that HIV may avoid triggering antiviral innate immune responses by not replicating efficiently in dendritic cells and by avoiding detection in infected CD4 T cells and macrophages by harnessing a host cytoplasmic DNase TREX1 to digest nonproductive HIV reverse transcripts.

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The innate immune response alerts the host to infection before an adaptive immune response has a chance to develop. Innate immunity is triggered by the recognition of pathogen-associated molecular patterns (PAMP) that distinguish foreign components from host molecules by structure or intracellular location. Innate immunity protects the host by inducing expression and secretion of antipathogenic mediators, such as type I interferons (IFNs); recognizing and eliminating infected cells; recruiting leukocytes to sites of pathogen invasion; enhancing antigen-presenting cell function; and enhancing the adaptive response to foreign antigens. Antiviral type I IFNs are not usually activated during acute HIV infection, which suggests that the virus avoids triggering innate immunity. Here we review some recent studies that begin to describe how HIV interacts with the innate immune system.

A central question of HIV pathogenesis is what regulates the host's ability to recognize and respond effectively to acute infection. The patient response to HIV is heterogenous. A small number of highly exposed individuals resist infection. Biallelic mutations of *CCR5*, the HIV coreceptor used in HIV transmission, block transmission, but additional mechanisms for resisting infection are likely. Another small group of 'elite controllers' become infected but control the infection and avoid T cell depletion and immunodeficiency without antiviral drugs. Some of these less susceptible individuals may be able to mount an effective innate immune response to HIV.

HIV transmission

HIV sexual transmission, which potentially involves multiple CD4+ cell types in genital tissue (Langerhans cells (LC), dendritic cells (DC), macrophages, and T cells) [1–5,6••], is inefficient. It takes on average hundreds of unprotected encounters to become infected [7]. Of the swarms of virus in the semen, only a single virion usually establishes a foothold in a new host [5,8–13]. HIV is localized to the genital tissue for about a week and is clinically silent until the virus disseminates. This 'eclipse phase of infection', before adaptive immunity develops, provides a window of opportunity for intervening to prevent transmission [6••]. The first week not only determines whether transmission occurs, but likely also sets the equilibrium between the virus and host immunity, which could have a lifelong effect on disease course.

Because the initial phase of infection is asymptomatic, studies of human acute infection usually do not begin until after viral dissemination. Therefore our models of what happens locally during transmission, when innate immunity plays a critical role, are largely based on experiments in Rhesus macaques infected intravaginally with Simian Immunodeficiency Virus (SIV) [10,14–24]. During the first week of macaque infection, a small focus of infection begins and amplifies in CD4 T cells in the subepithelial cervicovaginal mucosa [22,24]. Although initial studies suggested that the first infected cell might be a LC or intraepithelial DC, HIV-infected T cells are detected within a day, but infected DCs are only detected beginning about 4 days after exposure. The current consensus is that DCs, in which viral replication is very inefficient, mostly transmit the infection to T cells by a process termed 'trans-infection' that occurs via an 'infectious or virological synapse' that resembles the immunological synapse between a T cell and a DC [25–28]. DCs may be more important in viral dissemination to draining lymph nodes than in viral amplification within genital tissue. In fact, rather than being the first cell to replicate HIV, LCs may actually serve as sentinels to protect against infection by degrading virions [29]. However, this protective role is compromised during coinfection or inflammation, because of increased HIV replication and trans-infection by activated LCs [30–32].

In the critical first few days, type I IFNs are not induced [23]. HIV infection does not trigger a cell-autonomous IFN or inflammatory response in CD4 T cells and macrophages, its primary target cells [33]. Plasmacytoid dendritic cells (pDC), the main source of type I IFNs in chronic infection, are not present in uninflamed genital tissue. Trafficking of immune cells to nonlymphoid tissues is tissue-specific, depending on selective expression of subtypes of integrins and chemokine receptors [34–36]. Although homing to the gut and skin has been well studied [36–38], not much is known about the molecules that direct leukocytes into and out of the genital mucosa. Proinflammatory cytokines, such as GM-CSF, IL-1, IL-6 and IL-8, produced by genital epithelial cells, recruit a broad array of leukocytes (including lymphocytes, DCs and macrophages) to sites of local inflammation. Inflamed genital epithelia, also produce chemokines, such as MIP-3α, that recruit immature DC [39–41]. MIP-3α expression is strongly upregulated by IL-1. MIP-3α and IL-8 are rapidly upregulated in the vaginal epithelium after SIV infection. The inflammatory infiltrate includes subepithelial pDCs that produce IFNs and MIP-1α and MIP-1β, which can recruit additional CD4 T cells [24]. In one study, a small-molecule inhibitor of the IL-8 receptor CXCR2 decreased HIV replication in cervical explants [42].

SIV infection is confined to genital tissue for at least a week, while the virus amplifies mostly in CD4 T cells to generate virus to seed secondary lymphoid organs. It is not known whether dissemination occurs primarily by migrating cells or by the release of infectious virions into lymphatics or blood. Within two weeks, the infection explodes in LN, gut-associated lymphoid tissue and other secondary lymphoid tissues, leading to T cell depletion. At this time, SIV/HIV virions are easily detected in extracellular fluids, and the adaptive immune response begins to kick in. Although the expansion of HIV-specific T cells coincides with a dramatic reduction of systemic viral burden, the adaptive immune response is unable to prevent the establishment of viral reservoirs and eliminate the infection.

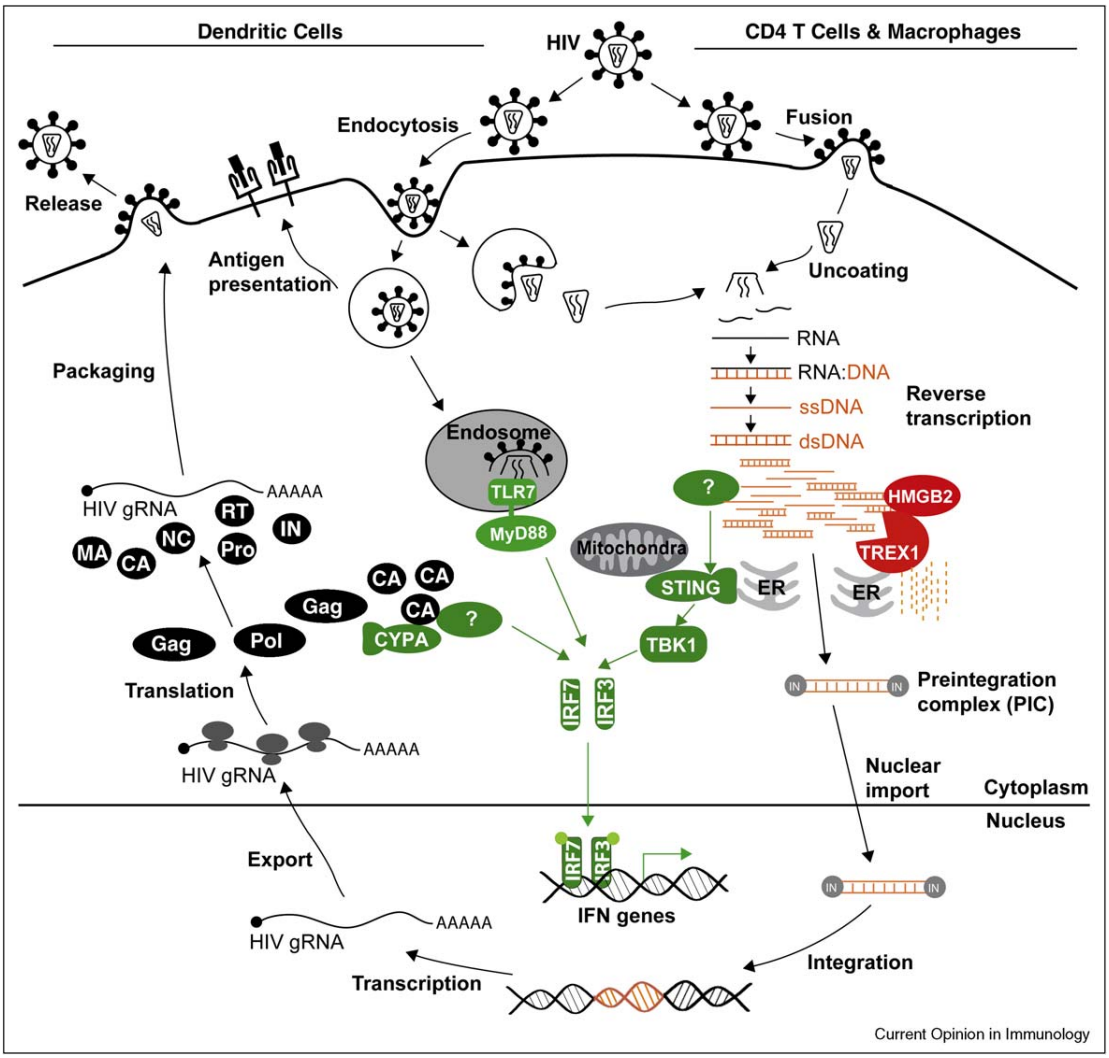
The HIV life cycle

HIV enters T cells and macrophages by its envelope gp120 protein binding to CD4, which enables the membrane proximal portion of the envelope gp41 subunit to bind to the CCR5 or CXCR4 coreceptor on the target cell membrane, triggering viral envelope fusion with the plasma membrane (Figure 1). HIV can also bind to cell surface lectins and enter cells by endocytosis. Endocytosed virus is released into the cytosol by viral membrane fusion with the endosomal membrane or destroyed when lysosomes fuse with endosomes. Endocytosis is the predominant mode of entry into DCs and also occurs in macrophages. Once the viral core is released into the cytosol, HIV reverse transcriptase converts RNA into DNA within the reverse transcription complex (RTC). The RTC matures into the preintegration complex (PIC), where the viral integrase prepares DNA ends for integration by removing a GT dinucleotide in a step called 3′-processing. However, reverse transcriptase is a sloppy enzyme that produces many incomplete reverse transcripts that cannot bind integrase or continue the viral life cycle. 3′-Processing makes the viral DNA vulnerable to autointegration in which the reactive ends attack sites within the viral DNA [43,44]. Autointegration is mechanistically analogous to chromosomal integration, but is a suicidal side pathway [44–47]. The PIC delivers reverse-transcribed HIV DNA to the nucleus for chromosomal integration. Few copies of HIV DNA integrate, leaving behind HIV DNA in the cytosol to be cleared by host enzymes. Once the viral genomic DNA is integrated into a host chromosome, viral transcription is activated by the same transcription factors that activate the host cell with the assistance of HIV tat. HIV produces three major transcripts, 2 kb (spliced), 4.3 kb (partially spliced) and 9.2 kb (unspliced), all capped and polyadenylated, like host RNAs. Alternately spliced transcripts are translated into both structural and regulatory viral proteins. The unspliced RNA is both translated to generate Gag-Pol gene products and incorporated as genomic RNA into nascent virions at cell membrane sites where the envelope and capsid proteins assemble before budding.

Immune detection of HIV RNA and DNA

HIV infection of pDCs leads to IFN production, even though in these cells infection is inefficient [48] and viral replication is limited (1–2 logs lower than in T cells [49]). IFN stimulation in pDCs is triggered mostly by TLR7 recognition of endocytosed viral genomic RNA within endosomes [50••]. Productive HIV infection of macrophages and T cells, however, involves viral membrane fusion with the cell membrane and direct uncoating of the viral capsid into the cytosol, bypassing the endosomal compartment and TLR signaling. It is unknown whether genomic RNA within the capsid is accessible to host RNA sensors, but reverse-transcribed HIV DNA is accessible to exogenous and endogenous nucleases [51••,52–54]. In recent years, a handful of intracellular innate immune DNA sensors have been described, including endosomal TLR9 and cytosolic DAI, POL III/RIG-I, LRRFIP1, IFI16, and HMGB proteins [55–60]. The cytosolic SET complex, which contains three nucleases (Ape1, NM23-H1, and TREX1) and the HMGB protein HMGB2, binds to HIV DNA, but not RNA, in the cytosol and protects HIV DNA from autointegration [51••,52]. TREX1, the most abundant cellular exonuclease, also inhibits the innate immune response to HIV DNA in T cells and macrophages by digesting excess HIV DNA [51••]. Although ubiquitous, *TREX1* is especially highly expressed in lymphocytes. In *Trex1*$^{-/-}$ mouse cells and human CD4+ T cells and macrophages in which *TREX1* expression was suppressed by RNA interference, cyto-

Figure 1



Innate immune detection of HIV PAMPs. Model of the HIV life cycle and known interactions between HIV and innate immunity. HIV RNA and nascent capsid protein (CA) can be detected in dendritic cells (left) by TLR7 and an unknown sensor, respectively. HIV DNA can be detected in CD4 T cells and macrophages (right) by an unknown cytosolic DNA sensor that signals through STING and TBK1. The host factor TREX1 inhibits innate immune detection of HIV DNA by metabolizing nonproductive RT products. Detection of HIV by any of these three innate immune pathways activates IFN genes.

solic HIV DNA accumulates, and HIV infection induces type I IFNs that inhibit HIV replication and spreading. IL-6, but not IL-1, expression is also activated. Whether other proinflammatory cytokines are stimulated by the accumulation of cytosolic HIV DNA has not been examined. HIV DNA activates IFN through a TLR-independent and NLR-independent cytosolic pathway that involves a yet to be identified DNA sensor, the adaptor STING, protein kinase TBK1 and IRF3. The known DNA sensors DAI, POL III, or LRRFIP1 are not involved. HMGB2 facilitates TREX1's digestion of HIV DNA and also inhibits the activation of the IFNβ promoter. The type I IFNs expressed and secreted in TREX1-deficient cells inhibit multiple steps of HIV replication [51••]. Therefore HIV protects itself from the antiviral effects of IFNs by harnessing the TREX1 DNase to evade recognition by an unknown DNA sensor of innate immunity. It remains to be seen whether HIV DNA triggers the same signaling in pDCs when *TREX1* is inhibited. It is uncertain whether a host RNase in T cells and macrophages inhibits innate immune detection of HIV genomic RNA or whether HIV genomic RNA is shielded within the RTC. Digestion of HIV genomic RNA by the RNase H activity of the viral reverse transcriptase may circumvent detection by cytosolic RNA sensors.

TREX1 — a link between HIV infection, innate immunity and autoimmunity

TREX1, isolated nearly a decade ago as a 3′–5′ exonuclease from mammalian cell extracts [61,62], is expressed in all tissues. Its exonuclease motifs are closely homologous to *E. coli* DNA polymerase (DnaQ/MutD). Recombinant TREX1 metabolizes a variety of DNA substrates and acts as a proofreading nuclease for repairing oxidative DNA damage via base excision repair *in vitro*. TREX1 more efficiently digests single-strand DNA (ssDNA) and DNA containing mismatched 3′ termini [61,62]. However, *Trex1*$^{-/-}$ mice do not exhibit increased cancer incidence or DNA mutations [63]. Instead, *Trex1*$^{-/-}$ mice develop inflammatory myocarditis and die of heart failure, perhaps before they would have a chance to show a predilection to cancer [63]. *Trex1*$^{-/-}$ cells accumulate cytosolic ssDNA enriched for sequences of endogenous retroelements [64••,65••]. Therefore TREX1 likely digests DNA generated by reverse transcription of endogenous retroviruses as well as by pathogenic lentiviruses. Accumulation of endogenous retroelement DNA may trigger IFN induction in TREX1-deficient mice and humans. TREX1 might also protect against innate immune triggering by other RNA or DNA viruses whose life cycle involves cytosolic DNA, but this has not been investigated. *TREX1* mutations in humans are associated with autoimmune and inflammatory diseases, including Aicardi-Goutieres syndrome (AGS, a severe neurological brain disease that mimics congenital viral infection [66•,67]), systemic lupus erythematosus (SLE) and familial chilbain lupus (FCL) [68•,69•]. Some *TREX1* mutations associated with these diseases interfere with nuclease activity or result in protein mislocalization from the endoplasmic reticulum (ER). Excess IFN is the hallmark of many of these diseases, suggesting that inappropriate innate immune activation because of inadequate DNA digestion is an important contributor to the pathogenesis of lupus-like autoimmune disease. Of note, there are hints that lupus patients may be underrepresented in HIV infection cohorts [70]. It will be worthwhile to investigate whether polymorphisms in *TREX1* or other genes linked to autoimmunity or clinical autoimmune syndromes are overrepresented in groups of highly exposed, but uninfected, patients or elite controllers.

TREX1 is also known as DNase III. DNase I and II clear extracellular DNA and engulfed DNA from dying cells in macrophage lysosomes, respectively. Mutations in *DNASEI* are associated with SLE, and *Dnase1*$^{-/-}$ mice develop lupus-like disease [71,72]. Knockout of *Dnase2*$^{-/-}$ is embryonically lethal due to excessive IFN expression and can be rescued by *Ifnαr1* deficiency [73]. Mortality of *Trex1*$^{-/-}$ mice can also be rescued by *Irf3*, *Ifnαr1* or *Rag2* deficiency, confirming that excessive IFNs underlie the pathogenesis of *TREX1* deficiency or mutation [64••].

TREX1 is a component of the SET complex, which associates with the ER, but translocates to the nucleus during oxidative stress [74]. In addition to its three DNases, the SET complex contains the chromatin-modifying proteins, SET, HMGB2, and pp32 [75]. SET inhibits TREX1's nuclease activity. TREX1 is thought to anchor the SET complex to the ER by its transmembrane domain. The SET complex is responsible for DNA damage activated by granzyme A in killer cells [74]. Some *TREX1* mutations render cells resistant to granzyme A-mediated cell death, which might affect the cytolytic function of innate immune NK cells. The SET complex may also activate DNA damage in some forms of caspase-independent neuronal cell death [76]. This other function of TREX1 might affect AGS neurological symptoms. TREX1 functions in multiple cellular processes besides digestion of cytosolic DNA, including DNA damage repair and DNA degradation during caspase-independent programmed cell death. These other functions likely also play roles in autoimmunity and the innate immune response to viral infection.

Innate immune detection of HIV proteins

The nascent HIV capsid (CA) protein interacts with host cyclophilin A (CYPA) to trigger IFN via an IRF3-dependent pathway in monocyte-derived DCs (MDDCs) [77••]. For these experiments, IFN induction required productive infection of MDDCs, which do not normally efficiently replicate HIV. This was artificially achieved by coinfection with VSV-G pseudotyped HIV-GFP and SIV viral-like particles to enhance HIV replication in DCs [78]. CYPA was previously found to act as a proviral host factor that binds a proline-rich loop on the surface of HIV CA [79]. Studies of the CYPA–CA interaction led to the codiscovery of TRIM5 [80,81], an intrinsic antiretroviral host factor that regulates uncoating of the incoming virion core [82]. The CYPA interaction with newly synthesized CA, which occurs late in the viral life cycle when new viral proteins are synthesized, inhibits HIV replication by increasing DC activation and inducing type I IFN expression [77••]. Although innate immune signaling in response to CA is IRF3-dependent, it is unclear how the CA is recognized and what other innate immune factors are involved. This innate immune detection of nascent CA appears to be DC-specific and does not occur in CD4+ T cells. Therefore, another way that HIV manages to avoid triggering innate immunity is by not replicating efficiently in DCs. HIV infection of DCs may become more efficient during chronic infection, when proinflammatory cytokines are elevated. More studies are needed to elucidate the innate immune pathway that detects HIV capsid and to understand how CYPA could have opposing proviral and antiviral roles during early and late stages of the HIV life cycle.

HIV infection downregulates *IRF3* expression

HIV also inhibits the innate immune response by suppressing *IRF3* expression [83•]. HIV_{LAI} infection reduces IRF3 protein levels by 92% in a human T cell line and by 26% in peripheral blood mononuclear cells after two days.

IRF3 downregulation in HIV-infected cells correlates with increased viral replication. Because of the delay in IRF3 decline (compared to reverse transcription which happens within hours of infection), this phenomenon is unlikely to be the main mechanism of viral evasion of innate immunity, particularly in T cells, which die about two days after infection. Because IRF3 is essential for many innate immune signaling pathways, inhibiting *IRF3* expression could render infected cells vulnerable to coinfection with other pathogens. However, since the frequency of HIV-infected cells is extremely low, even in end-stage AIDS patients, it remains to be seen whether reduced IRF3 expression in HIV-infected cells has a significant effect on susceptibility to other pathogens at the organismal level.

Concluding remarks

Here we describe several ways by which HIV may gain a foothold during transmission by avoiding triggering the innate immune alarm system. The published studies are just the beginnings of research in this arena. Not much is known about the role of NK cells, the cytotoxic arm of innate immunity, during HIV transmission. Polymorphisms in killer-cell immunoglobulin-like receptor (KIR) genes that regulate NK cell activity have been linked to favorable disease outcome, suggesting that NK cell recognition of HIV-infected cells affects transmission [84,85•]. The interaction of HIV with innate immunity will undoubtedly be complex. During chronic infection circulating blood cells show the signature of increased expression of type I IFN-regulated genes [86,87]. This could be due to circulating IFNs produced by activated pDCs or to chronic activation of overlapping proinflammatory pathways. The effect of systemic, chronic IFN induction on the immune system likely has pleiotropic effects on the ability of the host to handle HIV. Although IFNs and innate immune responses inhibit viral replication and promote antigen-specific immunity, they also lower the threshold for CD4 T cell activation, thereby enhancing the pool of susceptible cells. Viral RNA triggering of TLR8 in conjunction with another signal from gp120 binding to DC-SIGN on DCs activates NF-κB and enhances viral transcription in DCs [88••]. Nonetheless, in a recently published clinical study, IFNα treatment, given with or without AZT during the pre-HAART era, significantly reduced viral load, suggesting that even systemic type I IFNs might be beneficial overall [89].

Some important questions that remain are: Which HIV nucleic acids generated in the cytoplasm during infection can be sensed? What are the sensors for HIV DNA and capsid? Are there additional mechanisms HIV uses to avoid innate immunity? Could sexual transmission be blocked by inducing IFN during acute infection? Are individuals who have chronic innate immune activation less susceptible to transmission? We look forward to seeing these questions answered.

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