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Innate immunity against HIV-1 infection

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During acute HIV-1 infection, viral pathogen-associated molecular patterns are recognized by pathogen-recognition receptors (PRRs) of infected cells, which triggers a signaling cascade that initiates innate intracellular antiviral defenses aimed at restricting the replication and spread of the virus. This cell-intrinsic response propagates outward via the action of secreted factors such as cytokines and chemokines that activate innate immune cells and attract them to the site of infection and to local lymphatic tissue. Antiviral innate effector cells can subsequently contribute to the control of viremia and modulate the quality of the adaptive immune response to HIV-1. The concerted actions of PRR signaling, specific viral-restriction factors, innate immune cells, innate-adaptive immune crosstalk and viral evasion strategies determine the outcome of HIV-1 infection and immune responses.

The immune response to virus infection starts in the infected cell with the processes of pathogen sensing and innate immune signaling (reviewed in refs. 1,2). The sensing of pathogen-associated molecular patterns (PAMPs) in viral products by pathogen-recognition receptors (PRRs) of the host cell initiates a cell-intrinsic innate immune response that directs antiviral defenses and virus restriction[1]. This response also produces cell-mediated and soluble factors including type I and type III interferon (IFN), as well as proinflammatory cytokines and chemokines that recruit and activate innate immune cells, including macrophages, NK cells and dendritic cells, to control virus spread and to activate and modulate the adaptive immune response[3]. For HIV-1 infection, pathogen sensing and innate immune induction typically occur in $CD4^+$ target cells of infection, including innate immune cells and $CD4^+$ T cells. Virus-host interactions at mucosal sites of virus exposure and in lymphoid tissues mediate innate immune activation to determine outcomes of immune responses, virus control, inflammation and immune pathology, including the death of $CD4^+$ cells. Early studies revealed innate signaling programs in the immune system and antiviral effector genes and restriction factors that impart innate immunity to HIV[4,5]. Here we discuss developments in the arena of innate immunity to HIV to provide new insights regarding the virus-host interface that is central in determining the outcomes of HIV infection and immune responses.

Sensing of HIV-1 through IFI16

Several host proteins have been identified as PRRs for HIV PAMPs, including various Toll-like receptors (TLRs) and the RIG-I–like receptors. Each likely has a role in inducing, amplifying or differentiating the innate immune response and immune activation to HIV (reviewed in refs. 4–6). Recent studies of the infection and replication cycle of HIV (**Box 1**) showed that HIV infection is sensed in infected cells through the recognition of viral reverse transcriptase products early in the viral replication cycle by at least two additional intracellular PRRs, interferon inducible protein 16 (IFI16) and cyclic GMP-AMP synthase (cGAS) (**Fig. 1**), and that HIV-1 replication is highly sensitive to restriction by innate immune actions of the host cells[7–10].

IFI16 is one of hundreds of IFN-stimulated genes (ISGs) whose expression is induced or increased in response to treatment with IFN. IFI16 is both a nuclear and a cytosolic protein. It mediates protein-protein interaction via a pyrin domain and can bind to DNA through a hin domain, which defines it as a member of the pyhin family of proteins[11]. IFI16 recognizes and can physically bind to DNA products of HIV reverse transcription, including truncation products, which present specific PAMP motifs of non–self-discrimination by IFI16 (refs. 7,12). A DNA segment of the HIV-1 long terminal repeat region was described as a potent PAMP for IFI16 recognition and binding[7]. Studies of ectopic expression and epistasis showed that IFI16 colocalizes with HIV-1 DNA in transfected cells and signals through the IFN-stimulatory DNA response dependent on the adaptor STING, the protein kinase TBK1 and the transcription factors IRF3 and IRF7. Consistent with a role in the innate immune response, knockdown of IFI16 expression in target cells hosting productive infection results in increased permissiveness toward HIV-1 infection and enhancement of virus replication[7]. Remarkably, IFI16 drives inflammasome activation and inflammatory cell death (or 'pyroptosis') of $CD4^+$ T cells that are nonpermissive toward productive HIV-1 infection (described below)[13]. Other studies show a role for IFI16 in DNA sensing and as an antiviral factor in cytomegalovirus infection in fibroblasts and epithelial cells. Thus, IFI16 is an HIV PRR and viral-restriction factor[14,15]. It remains unclear whether the PRR and restriction-factor functions of IFI16 are exclusive or linked and how each function is programmed in distinct cell types, such as $CD4^+$ myeloid cells versus permissive and nonpermissive $CD4^+$ T cells, to mediate HIV sensing, viral restriction or pyroptosis. Answers to these questions could come from an understanding of how IFI16 is differentially driven to interact with STING versus the inflammasome and from work defining the nature of DNA ligands that are actual PAMPs stimulating IFI16 in antiviral versus proinflammatory signaling during HIV

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Box 1 Infection and replication cycle of HIV

HIV enters target cells by binding to the CD4 receptor, an action mediated by viral surface glycoproteins. HIV interacts with the coreceptors CCR5 and CXCR4 to facilitate the entry process. The lipid envelope of the virus then fuses with the membrane of the host cell to deposit the viral capsid (CA) containing the two copies of viral genome RNA. Reverse transcription of the viral RNA into a cDNA copy is mediated by a CA-associated reverse transcriptase within a complex formed by the CA and the cellular chaperone protein cyclophilin A (CypA). The production of HIV cDNA comprises a variety of steps that involve extension of a tRNA primer on the HIV RNA via nucleotide incorporation, followed by degradation of the RNA template through the specific RNase activity of the reverse transcriptase. Further activity generates a dsDNA 'provirus' of the HIV genome. 'Strong stop' truncation products of reverse transcription are produced during this process through depletion of nucleoside pools by the cellular protein SAMHD1, possible RNA-DNA hybrid molecules of incomplete processing, other cDNA truncation products and dsDNA, each of which might serve as a PAMP to be detected by cellular PRRs. The CA-CypA interaction masks the detection of HIV-1 PAMPs by host PRRs. It is unclear whether CA is then degraded to allow the HIV dsDNA access to the host cell nucleus or whether it docks near the nucleus to deposit the HIV provirus for integration into the host chromosomal DNA. The HIV enzyme integrase forms a complex with the proviral DNA and facilitates integration into the host cell chromosome. Nonintegrated provirus DNA that is left in the cytoplasm is degraded and cleared out by the cellular Trex1 nuclease, which limits HIV-1 DNA PAMP-PRR interactions. After chromosomal integration, HIV-1 proteins are expressed through the transcriptional activity of host RNA pol II with viral and cellular transcriptional cofactors. Translation of viral mRNA produces new viral proteins. HIV particles assemble after viral protein accumulation and packaging of two copies of the HIV genome with reverse transcriptase and tRNA primers. HIV is then released from the cell by budding from the plasma membrane[1,4,6].

infection, as well as information about the host protein cofactors of IFI16 that might contribute to these differential outcomes through various programming functions during infection.

Sensing of HIV-1 through cGAS

cGAS has been identified as a cytosolic DNA–binding protein and a PRR for HIV and other retroviruses[9]. cGAS is a bifunctional protein that contains amino-terminal DNA-binding domains followed by a nucleotidyltransferase domain. In response to binding to double-stranded DNA (dsDNA), cGAS produces a dinucleotide product, cyclic GMP-AMP (cGAMP). cGAS and cGAMP were both discovered through the use of biochemical approaches to identify factors inducing a response to IFN-stimulatory DNA[10,16]. cGAS can bind to dsDNA, including cytosolic DNA of host origin, to produce cGAMP[10,16]. cGAMP then functions as a second messenger to bind STING, thereby activating TBK1 and downstream IRF3 and IRF7 to drive the cell-intrinsic innate immune response (**Fig. 1**)[17]. Moreover, cGAMP can transfer to neighboring cells through gap junctions, driving a paracrine signaling response between the infected cell, where the response is initiated, and bystander cells[18]. Production of cGAMP in response to HIV infection is suppressed when cells are treated with a reverse transcriptase inhibitor, but not when they are treated with inhibitors of later stage HIV-1 enzymatic activities, which indicates that DNA products of HIV-1 reverse transcriptase serve as PAMPs for cGAS binding and activity[9]. However, the specific HIV-1 DNA ligands of cGAS are not yet defined.

Further validation of the role of cGAS in HIV-1 recognition was demonstrated in comparative and mutational studies of infection by HIV-1 and HIV-2 (ref. 19). HIV-2 is similar to HIV-1, but it is less pathogenic[20]. In particular, dendritic cells are readily infected and activated by HIV-2, but not by HIV-1 (ref. 21). In dendritic cells, the host cell restriction factor SAMHD1 normally depletes cellular pools of deoxynucleoside triphosphates to suppress HIV-1 reverse transcription, but HIV-2 uniquely encodes the protein Vpx, which

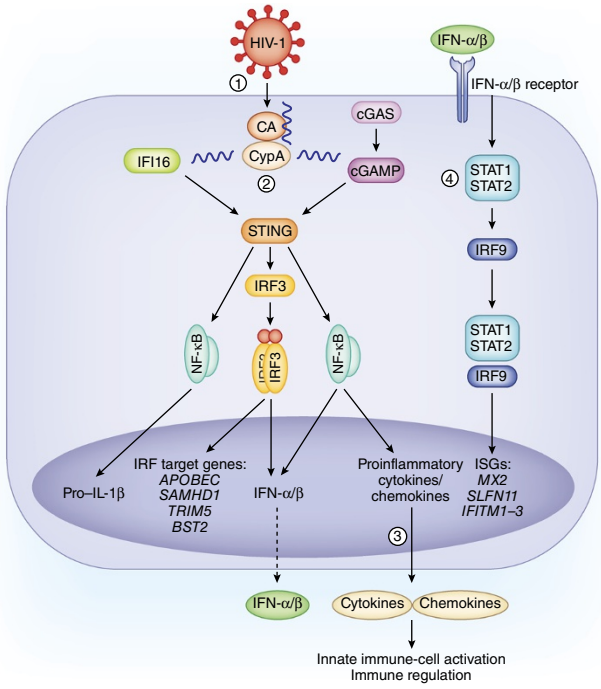

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Figure 1 PAMP-PRR interaction in the innate immune response to HIV-1 infection. CD4+ cell infection by HIV-1 is followed by reverse transcription (RT) of viral RNA into DNA, during which RT products of DNA are produced, including truncation products of RT 'strong stop' regulation (1). The viral capsid (CA), in complex with the chaperone protein CypA, protects RT products from recognition by PRRs, but mutations that alter CA-CypA interactions unmask the HIV DNA products, making it possible for PRRs in the cytosol to recognize them. IFI16 and cGAS recognize and bind to cytosolic HIV DNA (2). cGAS produces a cGAMP second messenger that binds to the STING adaptor protein while activated IFI16 mediates direct STING binding or signals to STING through an intermediate. STING is activated to recruit signaling cofactors such as TBK1 and IKK-α/β (not shown) to activate the IRF3 and NF-κB transcription factors and induce target gene expression including HIV restriction factors, pro–IL-1β, type I IFN and proinflammatory cytokines and chemokines (3). IFN signals back in infected cells and bystander cells through binding to the IFN-α/β receptor to drive signaling through the Jak-STAT pathway and activate ISGF3, the transcription factor complex STAT1-STAT2 and IRF9 to induce expression of ISGs, including anti–HIV-1 ISGs (4).

degrades SAMHD1, rendering dendritic cells and other myeloid cells increasingly permissive to HIV-2, but not HIV-1 (refs. 22,23). This differential permissiveness toward HIV-2 versus HIV-1 facilitates cellular PRR recognition of HIV-2 PAMPs that are produced during infection and can then drive PRR signaling to mediate the activation of dendritic cells[22,23]. However, the process by which PRRs sense HIV is differentially controlled between HIV-1 and HIV-2 through varied affinity of the viral capsid protein for binding to the cellular CypA protein, a protein-folding chaperone[24]. The capsids of HIV-1 and HIV-2 form a complex with CypA, and the interaction of CypA with the HIV-1 capsid masks viral nucleic acid from interaction with PRRs[21]. In contrast, interaction of the HIV-2 capsid with CypA allows for sensing of viral PAMPs in the cytosol by cGAS before integration. Mutations of the HIV-1 capsid that confer altered structure and affinity for CypA binding lead to recognition of HIV-1 complementary DNA (cDNA) in the cytosol of dendritic cells at the preintegration step, in a manner that is also dependent on cGAS[19]. Of note, knockdown of cGAS, but not of IFI16, prevents dendritic cells from sensing HIV-1 under these conditions. Thus, the differential affinities of HIV-1 and HIV-2 capsids for binding to CypA are a major determinant in the regulation of HIV PAMP sensing by cGAS. Dendritic cells seem fully capable of sensing HIV-1; however, the capsid interaction with CypA induces masking of or evasion by viral PAMPs, reducing PAMP sensing by cGAS, whereas SAMHD1 effectively suppresses HIV-1 replication and the subsequent activation of dendritic cells.

Remarkably, when monocyte-derived dendritic cells from HIV-1–infected people were exposed to capsid-mutant HIV-1 particles that unmasked viral PAMPs and expressed HIV-2 Vpx to suppress the action of SAMHD1 and enhance viral sensing, the dendritic cells were fully capable of responding to the HIV-1 PAMPs, activating innate immune signaling and stimulating autologous T cells for anti–HIV-1 effector responses[19]. The distinct capsid-CypA interactions of HIV-1 and HIV-2 explain in part why HIV-2 is less pathogenic than HIV-1 and initiates a more robust and even protective immune response compared to that induced by HIV-1, through effective dendritic cell activation. Moreover, these studies provide a foundation for vaccine design or therapeutic strategies to induce or 'recover' the innate immune actions of dendritic cells against HIV-1 through natural stimulation of cGAS by PAMPs.

It is not clear why two STING binding proteins serve as PRRs in HIV infection, nor is it understood whether IFI16 and cGAS have differential or cell-specific, perhaps exclusive, roles in sensing HIV. It is possible that cGAS and IFI16 have tandem roles in sensing HIV infection, such that one senses early or specific reverse transcription products and the other senses accumulated cDNA products, perhaps later in the reverse transcription process. Of note, the genes encoding both cGAS and IFI16 are themselves ISGs—expression of these proteins increases in response to the IFN exposure that follows PAMP-induced PRR signaling[25]. Thus temporally distinct signaling by one over the other may serve to increase the threshold of each for sensing of HIV infection. In particular, cGAS was identified for its role in sensing dsDNA independently of virus infection in studies using dsDNA-transfected cells[10]. Thus, in addition to sensing viral cDNA, cGAS, and possibly IFI16, might contribute to cellular sensitivity to self-DNA ligands that aberrantly show up in the cytosol.

Related to this, the cellular DNA exonuclease Trex1 is a major negative regulator of the response to dsDNA. Trex1 is a 3′-5′ DNA exonuclease that catalyzes the removal of DNA within the cell cytoplasm. An inactivating mutation of Trex1 is linked to Aicardi-Goutières syndrome, a severe neuroinflammatory disease (reviewed in ref. 26), whereas normal Trex1 activity is essential for processing of DNA fragments from endogenous retro-elements and protection against the development of an autoimmune state and Aicardi-Goutières syndrome. Owing to its role as a DNA exonuclease, knockdown of Trex1 greatly enhances the recognition of HIV-1 PAMPs in $CD4^+$ cells, which helps drive robust IFN-stimulatory DNA signaling that limits HIV-1 infection[27]. Thus, although Trex1 primarily mediates innate immune-checkpoint control against the recognition of self-DNA PAMPs by removing DNA from the cell cytoplasm, its exonuclease actions also clear out HIV DNA PAMPs, preventing host cell recognition of these viral products. In this respect, the induction of cGAS and IFI16 signaling by HIV DNA PAMPs is governed indirectly by Trex1 endonuclease activity that removes cytosolic DNA PAMPs from infected cells. Virus and host regulation of Trex1 activity might lead to differential outcomes of HIV sensing and innate immune control over the course of HIV infection, linking self-DNA metabolism with cellular permissiveness to HIV-1 infection.

PRR crosstalk during innate immune sensing

Sensing of HIV is operational around and beyond early events related to the recognition of reverse transcription products and occurs in response to the interaction between the whole virion and the cell, to capsid interactions and to interactions of viral genome RNA with various PRRs. TLRs are cell membrane–associated PRRs that are present on the cell surface or within endosomes that recognize diverse PAMPs[28]. The HIV envelope glycoprotein gp120 can be recognized by TLR2 and TLR4 on the surface of mucosal epithelial cells[29]. Although epithelial cells are not themselves targets of HIV infection, the virion-induced gp120-TLR interaction results in signaling in epithelial cells that triggers proinflammatory cytokine and chemokine production to activate nearby innate immune cells and recruit immune cells to the site of virus encounter. Moreover, HIV-1 genomic RNA is recognized by endosomal TLR7 and TLR8, similar PRRs with cell-specific expression patterns, which program plasmacytoid dendritic cells and specific myeloid cells, respectively, to respond to HIV infection[30]. The cytosolic PRR and RNA helicase RIG-I[31] can also recognize HIV genomic RNA and induce innate immune signaling in HIV target cells[32,33]. During late-stage HIV replication, new virions are produced that bud from the plasma membrane and are released to infect new target cells. As a result of early PRR signaling after HIV infection, innate immune activation produces a local environment rich in IFN and other cytokines that induce ISG expression. This response increases the abundance of the aforementioned PRRs and produces an inflammatory state that is amplified by additional rounds of PRR signaling actions. Tetherin (also known as BST2 or CD137)[34] is an ISG product expressed on the surface of cells in response to IFN. As suggested by its name, tetherin 'tethers' newly produced HIV virions to the cell surface to abrogate virus release and the cell-to-cell spread of infection[35,36]. After interaction with HIV-1, tetherin also acts as a PRR and initiates an intracellular signaling cascade downstream that activates the transcription factor NF-κB and drives proinflammatory cytokine production[37]. Similarly, IFI16 serves as a PRR for HIV and drives the production of type I IFN and inflammatory cell death or pyroptosis of $CD4^+$ cells[11,13], possibly through nuclear localization and modulation of the transcriptional activity of factors binding to the IFN-α promoter[7].

Inflammasome signaling and $CD4^+$ T cell depletion

The inflammasome is a multicomponent protein complex that catalyzes the activation of caspase-1 and processing of pro–interleukin 1β (IL-1β) into mature IL-1β. IL-1β is a major proinflammatory cytokine that initiates inflammatory cascades including rounds of cytokine

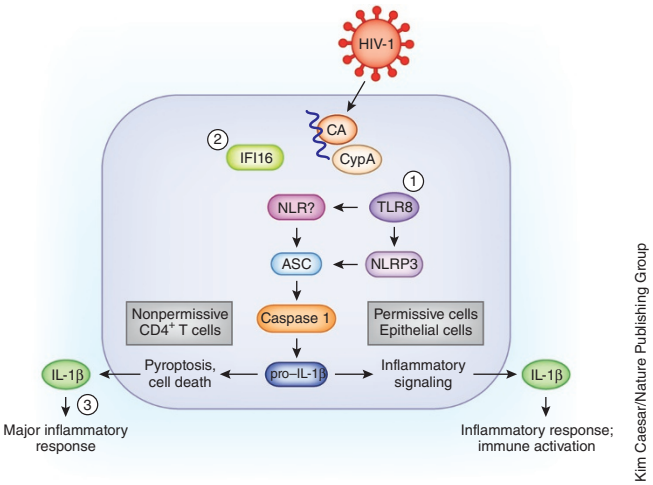

Figure 2 HIV induction of the inflammatory response and linkage with depletion of CD4+ T cells. In innate immune cells permissive to HIV-1 infection, TLR8 signaling can stimulate NLRP3 inflammasome-dependent activation of caspase 1, pro–IL-1β processing and the release of mature, active IL-1β (1). In CD4+ T cells nonpermissive to HIV-1 infection, IFI16 signals inflammasome activation directly through ASC or via an unknown NOD-like receptor (NLR), resulting in activation of caspase 1, processing of pro–IL-1β and pyroptosis that releases mature IL-1β to drive inflammatory signaling (2). Pyroptosis is reported to occur in CD4+ T cells that are nonpermissive to HIV infection but is not known to occur in other target cells of infection. Pyroptosis results in cell death and may explain the loss of CD4+ T cells that is a defining characteristic of AIDS. IL-1β is a potent inducer of proinflammatory cytokines that serve to activate innate immune cells, including dendritic cells, macrophages and NK cells, and to attract immune cells to the site of infection (3).

and chemokine production and immune-cell recruitment and activation[38]. Inflammasomes are defined by their PRR initiator protein, such as NLRP3, and they often operate through two signals to induce component and pro–IL-1β expression (signal 1) and to assemble the inflammasome and activate caspase-1 (signal 2)[39]. HIV infection can trigger inflammasome induction in various cell types, including CD4+ cells that are differentially permissive toward infection. TLR8 sensing of HIV[40] and activation of the NLRP3 inflammasome trigger the release of IL-1β and IL-18 from blood monocytes[41]. Resting, nonactivated CD4+ T cells are not permissive toward HIV infection because HIV requires that a T cell be activated in order for the full infection cycle to proceed. These cells can be infected by HIV-1, but infection stalls, in part because the cellular protein SAMHD1 depletes nucleoside pools in the resting cells to interfere with reverse transcription. This process results in an abortive infection in nonpermissive or 'bystander' CD4+ T cells. Importantly, this process triggers cell death, and depletion of CD4+ T cells is a hallmark of AIDS. These bystander CD4+ T cells undergo pyroptotic cell death characterized by the activation of caspase-1, which leads to swelling and bursting of cells and the release of mature IL-1β and cellular contents, driving a massive inflammatory response[42]. IFI16 was identified as the PRR that initiates inflammasome signaling and T cell pyroptosis, likely through sensing of accumulated reverse transcriptase truncation products within the cell cytoplasm[13]. Crosstalk signaling of antiviral and inflammatory mediators is an underlying feature of innate immune activation in HIV infection (**Fig. 2**). In the case of IFI16, the induction of immune antiviral restriction or pyroptosis could be linked to the nature of the infected cell. Thus, IFI16 signaling in activated, permissive CD4+ T cells can induce an IFN-dependent antiviral state, whereas IFI16 activation in resting, nonpermissive T cells induces abortive HIV infection and drives IFI16 inflammasome activation and pyroptosis[13,42]. These outcomes might be predicated on differential PAMP signaling and/or IFI16 interactions with specific signaling proteins.

Restriction factors limit HIV replication and spread

PRR signaling serves to activate downstream transcription factors, including IRF3, IRF7 and NF-κB, to drive the induction of antiviral and inflammatory effector genes, including those encoding IFN. Activation of IRF3 and IRF7 induces the direct expression of many antiviral effector genes, and signaling of type I IFN further induces hundreds of ISGs, including many with antiviral actions[1,4,5]. Among the proteins encoded by these antiviral genes, or 'restriction factors', are APOBEC3, TRIM5a, SAMHD1 and tetherin, which limit HIV replication and spread. These antiviral genes are direct targets of IRF3 as well as ISGs. Each has been heavily studied, and they are reviewed in detail elsewhere and in this Focus[4–6,43].

More recently, HIV restriction factors have been found to include the proteins Schlafen11 (SLFN11), IFITM and MX2 (**Fig. 3**)[44–48]. Each is expressed in low amounts or not at all in resting cells, but high expression is induced in response to IFN. SLFN11 is part of a protein family with structural similarity to RNA helicases. It has antiviral action at a late stage of HIV infection that serves to suppress viral protein production[44]. SLFN11 binds to tRNA to counter viral-directed changes in the pool of tRNAs available for HIV protein synthesis. This creates a potent blockade to HIV-1 protein synthesis, such that high expression of SLF11 in CD4+ T cells is associated with elite control of chronic HIV-1 infection[49]. IFITM1–3 are transmembrane proteins that restrict HIV by inhibiting virus entry[50,51]. They operate early in the HIV infection cycle to restrict virus production. IFITM proteins inhibit a range of viruses by interfering with viral entry processes, likely at the level of viral fusion[52]. When expressed in cells undergoing productive HIV-1 replication, IFITM proteins colocalize with the HIV-1 proteins Env and Gag and are incorporated into new viral particles, which allows them to limit HIV-1 entry into new target cells[50,51]. MX2 (also known as MXB) is a GTPase that suppresses the replication of primate lentiviruses before proviral integration into the host cell chromosome[46–48]. The HIV-restriction activity of MX2 is targeted to the viral capsid, is dependent on CypA and is mediated through an MX2-CypA interaction in which MX2 binds the HIV capsid in a manner that disrupts the viral uncoating process[53].

HIV regulation of NF-κB and IRF3 activation

A major feature of PRR signaling is the convergence of pathways to activate the transcriptional activity of NF-κB and IRF3, each of which has a wide variety of target genes[1]. Like NF-κB, IRF3 is broadly and constitutively expressed. In contrast, the expression of IRF7, a close relative of IRF3, is induced by IFN signaling in most cells, except plasmacytoid dendritic cells and other immune cells, including T cells, where it is constitutively expressed[54]. Following PRR signaling, NF-κB is activated after the phosphorylation and degradation of its inhibitor IκB, whereas IRF3 is activated by direct phosphorylation via TBK1 or IKK-ε protein kinases. After its induction by IFN, IRF7 is similarly activated through PRR-induced direct phosphorylation[54]. There are several mechanisms by which HIV antagonizes its restriction factors, and this antagonism confers enhancement of HIV cellular tropism, replication and virus spread (**Fig. 3**) (reviewed in ref. 6). Among these evasion strategies is HIV-1's ability to differentially regulate NF-κB and IRF3 to suppress IFN induction and the expression of ISGs

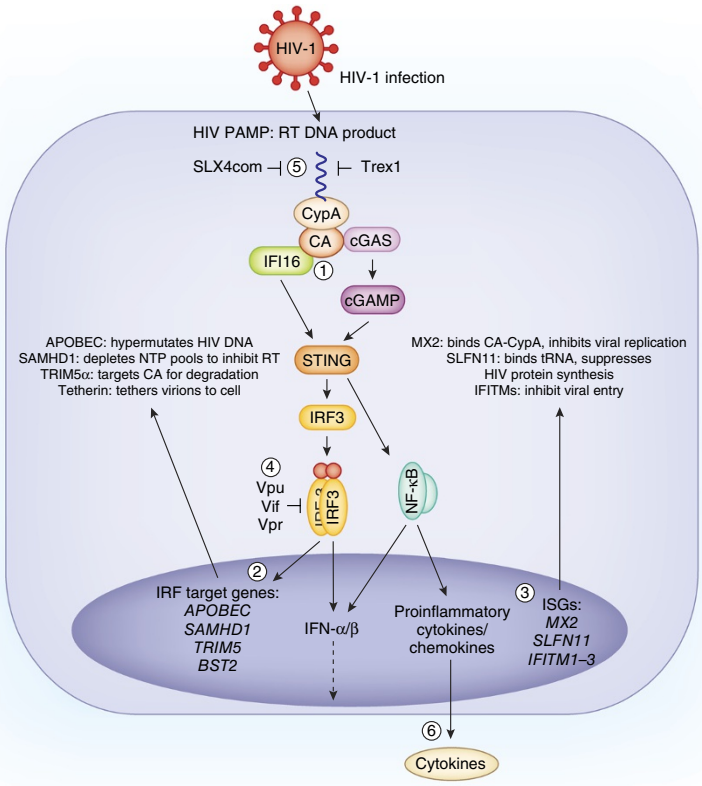

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Figure 3 Regulation of IRF3 activation and the expression of HIV restriction factors. cGAS or IFI16 detects HIV-1 PAMPs and signals through STING to drive downstream activation of IRF3 and NF-κB, resulting in the production of type I IFN (1). IRF3 can directly drive the expression of a set of HIV restriction factors (2), and ISGs induced by IFN signaling also represent HIV restriction factors (3). The synthesis of HIV accessory proteins Vpu, Vif and Vpr marks the onset of IRF3 evasion through Vpu- and Vpr-directed IRF3 suppression (4) and the recruitment of SLX4com to degrade HIV PAMPs (5). Trex1 nuclease actions also reduce the intracellular load of HIV cDNA to block PRR-dependent induction of IFN and the expression of restriction factors. The production of proinflammatory cytokines serves to activate innate immune cells, including dendritic cells, macrophages and NK cells, and to attract immune cells to the site of infection (6). CA, capsid; NTP, nucleoside triphosphate; RT, reverse transcription.

and restriction factors in specific target cells[1,55,56]. HIV-1 accessory proteins can mediate the degradation or cleavage and inactivation of IRF3 (refs. 57–59). Among these viral factors are Vpu, which directs the lysosome-mediated degradation or the caspase-mediated cleavage and inactivation of IRF3 (refs. 57,58), and Vpr, which induces the specific ubiquitination of IRF3, thereby marking it for proteosomal degradation[59]. Vpr can also mediate cell-cycle arrest before mitosis at the G2-M transition[59]. Vpr binds the protein complex SLX4-MUS81-EME1 (SLX4com), a regulator of structure-specific endonucleases that destroys site-specific DNA elements to facilitate the repair of DNA breaks occurring during DNA replication or homologous recombination[60]. Mutations in SLX4 (also known as FANCP) are associated with Fanconi anemia, which manifests with elevated expression of IFN and ISGs, among other abnormalities (reviewed in ref. 61). During HIV-1 infection, Vpr induces the activation of SLX4com to mediate cell-cycle arrest. Importantly, Vpr activation of SLX4com results in reduced innate immune activation of THP-1 monocytes after HIV-1 infection *in vitro*[60], and this reduction is associated with the Vpr-directed binding and degradation of HIV-1 reverse transcriptase product cDNA by SLX4. Overall, this process allows HIV-1 to evade detection and prevents the triggering of an innate immune response. The role of the cell-cycle arrest in HIV-1 infection remains unclear, as does whether the arrest is just a side effect of the Vpr-directed viral immune evasion. The outcome of the Vpr-SLX4com interactions would be suppressed activation of IRF3 and the attenuation of virus-induced expression of ISGs and restriction factors, a theme shared among pathogenic viruses[1]. In the case of HIV-1, viruses lacking Vpu[62] or Vpr[59,60] induce high expression of host cell ISGs and enhanced HIV restriction[62]. These observations suggest that the virus-host interface that controls the outcome of PRR signaling could offer therapeutic targets for enhancing the PRR-IRF3 axis and controlling HIV-1 infection.

Induction and expansion of antiviral innate effector cells

Overall, the sensing of HIV-1 infection by PRRs results in the innate immune activation of both infected cells and bystander cells, accompanied by the induction and production of proinflammatory cytokines and chemokines. This leads to the consecutive activation of innate immune cells, starting with macrophages and dendritic cells, as noted above, and progressing to activation of NK cells. NK cells represent an innate subset of antiviral effector cells with cytotoxic and immune regulatory functions[63]. A number of cytokines produced during the initial phase of HIV-1 infection[64], including IL-12, IL-15 and IL-2, serve as potent activators of NK cells. Although NK cells also express some PRRs, it seems that the activation of NK cells by virus-encoded PAMPs depends on both the presence and the activation status of macrophages and/or dendritic cells[65]. The NK cell population in a given individual is a very heterogeneous subset of cells that differ in their expression of activating and inhibitory receptors[66]. The differential expression of these NK cell receptors determines the ability of NK cells to respond to stimulation and to virus-infected target cells. NK cells express several activating receptors, including NKG2D receptors, which can sense stress ligands on the surface of virus-infected cells, and the family of natural cytotoxicity receptors that have been suggested to directly sense viral peptides expressed on infected cells[63]. The highly polymorphic activating and inhibitory killer immunoglobulin-like receptors (KIRs) also have a critical role in determining NK cell function through their interactions with distinct families of human leukocyte antigen (HLA) class I molecules during NK cell development, in addition to modulating the activity of NK cells against HIV-1–infected cells[67]. The roles of different KIR-encoding alleles in determining HIV-1 disease outcome is discussed by McLaren and Carrington[68] elsewhere in this issue. Here we review the role of KIR-HLA interactions in the expansion of NK cells during primary HIV-1 infection.

During NK cell development, binding of inhibitory KIRs to their respective HLA class I ligands is required in order for KIR$^+$ NK cells to become functionally active—a process termed NK cell licensing or

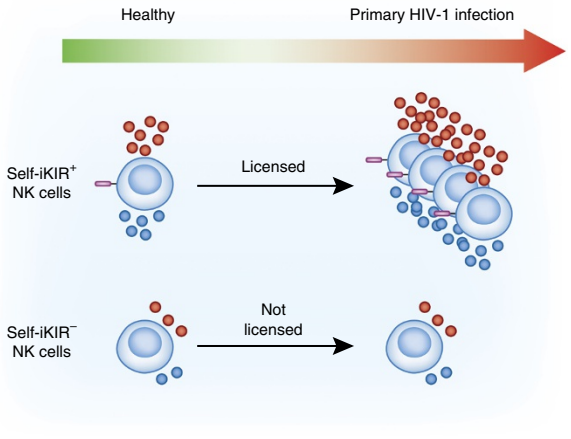

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Figure 4 Population expansion and stronger functionality of licensed NK cells in primary HIV-1 infection. NK cells that express an inhibitory KIR (iKIR) interacting with self-HLA class I are licensed during NK cell development. These licensed NK cell populations expand greatly during an acute viral infection and have higher functionality compared to nonlicensed NK cells that are negative for iKIRs interacting with self-HLA class I.

arming[69]. Licensing is critical in preventing autoimmunity mediated by NK cells: each licensed NK cell expresses at least one inhibitory KIR that interacts with self–HLA class I expressed on normal cells, thereby preventing NK cells from killing these cells. The licensing process is also important in determining the ability of NK cells to respond to HIV-1 infection. Licensed NK cells have stronger antiviral effector functions against HIV-1 *in vitro*, including antibody-dependent cellular cytotoxicity (ADCC)-mediated killing and direct killing of infected cells[70–74]. Furthermore, recent *ex vivo* studies showed that populations of licensed NK cells expressing KIR2DL1, KIR2DL2 or KIR2DL3 are preferentially expanded during primary HIV-1 infection as compared to unlicensed NK cells in the same individual[75]. Relative to other NK cells, KIR2DL3$^+$ NK cells circulate at significantly higher frequencies in the peripheral blood of individuals who also express the alleles encoding HLA-C of the HLA-C group 1 family (including HLA-Cw2, -Cw4, -Cw5 and -Cw6), members of which serve as ligands for KIR2DL3, but not in individuals homozygous for alleles encoding HLA-C group 2 proteins, which do not interact with KIR2DL3. In contrast, populations of NK cells expressing KIR2DL1 or KIR2DL2, which interact with proteins from HLA-C group 2, were significantly expanded during primary HIV-1 infection in individuals who expressed the alleles encoding these proteins, but not in individuals homozygous for the alleles encoding HLA-C group 1 proteins[75]. Furthermore, this preferential population expansion of licensed NK cells was associated with higher functionality, as determined by cytokine production after stimulation (**Fig. 4**). These data provide some functional correlates for the recent observation that HIV-1–infected individuals carrying alleles associated with high surface expression of HLA-C molecules exhibit slower HIV-1 disease progression than infected individuals without such alleles[76]. In individuals expressing high amounts of HLA-C, KIR2DL$^+$ NK cells might be better licensed, as the licensing process might be affected by the level of expression of MHC class I molecules. Further studies are required for a better understanding of the molecular mechanisms that regulate the expansion of individual NK cell subpopulations in response to HIV-1 and the antiviral activity of these cells contributing to the control of HIV-1 infection.

Mechanisms of antiviral activity mediated by NK cells

NK cells can recognize and kill virus-infected cells through a number of different mechanisms, including direct recognition of viral proteins or virus-induced stress ligands by the activating NK cell receptors, the loss of inhibitory signals resulting from virus-mediated downregulation of HLA class I molecules (which serve as ligands for inhibitory KIRs), and ADCC[63]. HIV-1 infection results in increased expression of stress ligands on infected cells and reduced expression of some HLA class I molecules[77,78], rendering infected cells more susceptible to NK cell–mediated lysis. Killing of infected cells by KIR$^+$ NK cells is mediated by the secretion of perforin and granzyme, and KIR$^+$ NK cells can impose immune pressure in HIV-1–infected individuals, resulting in the selection of viruses containing KIR-escape mutations[79]. The precise molecular mechanisms by which KIR$^+$ NK cells mediate immune-selection pressure on the virus, and how viruses can evade this, remain unclear. However, in addition to its role in the functional licensing of NK cells, the binding of KIR to HLA class I is modulated by the sequence of the peptide presented by HLA class I[80,81]. As viral infections result in a dramatic change in the HLA class I–restricted peptide repertoire on infected cells[82], these changes might reduce the binding of inhibitory KIRs to infected cells, which would result in the disinhibition of NK cells and killing of the infected cells. It was shown that differences in the sequence of HLA class I–presented HIV-1 epitopes indeed modulate the binding of inhibitory KIRs and recognition and lysis by KIR$^+$ NK cells[83–85]. These studies, however, focused on individual KIR-HLA-peptide interactions, and additional studies are required to elucidate the consequences of changes in the overall peptide repertoire presented on HIV-1–infected cells for recognition by KIR$^+$ NK cells.

NK cells regulate adaptive immunity

In addition to their antiviral activity, NK cells have a critical role in immune regulation. Several studies, generally using mouse models of viral infection, have demonstrated that NK cells can regulate the function of dendritic cells and T cells[86–88]. The precise mechanisms of this crosstalk are not fully understood and might differ between models of viral infection[89]. In general, stronger NK cell activity during viral infection has been associated with the elimination of dendritic cells and killing of virus-specific T cells (**Fig. 5**). In mice infected with lymphocytic choriomeningitis virus, killing of virus-specific CD4$^+$ helper T cells by NK cells was identified as an underlying mechanism resulting in reduced T cell help for antiviral CD8$^+$ T cells[86]. These observations suggest that NK cells might be able to regulate adaptive antiviral T cell responses either directly, by killing infected cells, or indirectly, through the modulation of T cell priming by dendritic cells. Interestingly, IFN-α production during viral infections has a critical role in protecting virus-specific CD8$^+$ T cells from NK cell–mediated elimination during infection with lymphocytic choriomeningitis virus[90]. The direct relevance of these observations to HIV-1 needs to be elucidated further. Initial studies have suggested that HIV-1–associated changes in dendritic cell maturation and NK cell function can lead to dysregulation of the crosstalk between these two cell types, potentially resulting in impaired antiviral T cell function[91–93]. Taken together, these data suggest that therapeutic targeting of NK cell activity during primary viral infections or vaccinations can modulate the induction and quality of adaptive immunity. These processes should be expected to have important implications for the outcome of HIV-1 infection and the efficacy of HIV-1 vaccines currently under development.

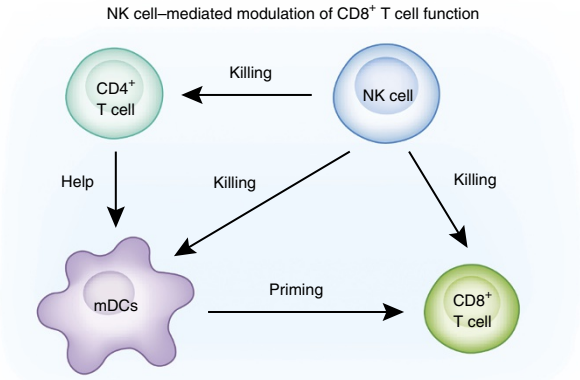

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Figure 5 NK cell–mediated modulation of antiviral CD8+ T cell function. NK cells have a central role in modulating the strength and function of the antiviral CD8+ T cell response. NK cells can kill virus-specific CD4+ helper T cells, and thereby reduce the amount of T cell help required for the priming of CD8+ T cells, or kill myeloid dendritic cells (mDCs). NK cells can also directly kill activated virus-specific CD8+ T cells.

Innate immunity to HIV modulates adaptive immunity

In addition to the immune-regulatory role of NK cells, innate immune activation in general has a critical role in determining the function of the subsequent adaptive immune response. This connection has been best demonstrated in the setting of viral vaccines using systems biology approaches[94]. The activation of several innate immune pathways has been associated with stronger adaptive immune responses, and with antibody production and T cell activity, induced by vaccination. These observations have allowed for the optimization of adjuvants used to enhance immunogenicity of viral vaccines. The consequences of innate immune activation for the induction of adaptive immunity against HIV-1, and subsequent HIV-1 disease outcome, are less well understood. However, several studies suggest a critical role of the very initial immunological events during acute infection for the subsequent course of the disease. Studies from the beginning of the HIV-1 epidemic, before the availability of antiretroviral therapy, demonstrated that both the severity and the duration of the primary infection syndrome that is observed in the majority of HIV-1 infected individuals were associated with the speed of CD4+ T cell loss and death from AIDS[95]. Furthermore, HIV-1–infected individuals that encoded for HLA-B57, a protective HLA class I allele in HIV-1 infection associated with significantly slower disease progression, presented significantly less frequently with primary HIV-1 infection syndromes and showed controlled viremia very early in infection[96]. These clinical data suggested that the very early events during HIV-1 infection have important consequences for disease outcome. This concept is supported by recent studies in SIV-infected rhesus macaques demonstrating that blocking of the IFN receptor during acute SIV infection resulted in a significantly accelerated depletion of CD4+ T cells and faster progression to AIDS[97]. Furthermore, differences in the induction of cytokines and chemokines during primary HIV-1 infection in humans have also been associated with later disease events, such as the kinetics of CD4+ T cell decline and disease progression[98,99]. Future studies are warranted to better understand the mechanisms that are involved in this innate modulation of adaptive immunity during primary HIV-1 infection. Of particular interest for HIV-1 vaccine development is to identify the early innate factors that might differ between the small subset of HIV-1–infected individuals able to generate broadly neutralizing antibodies directed against HIV-1 and those that do not mount these protective antibody responses.

Although the ability of innate immunity to regulate adaptive immune responses has been extensively studied in different models, the consequences of adaptive immune responses, and in particular the virus-specific responses of helper T cells, for the quality of the innate immune response are less well understood. Several recent studies in mice, nonhuman primates and humans have shown that antigen-specific CD4+ helper T cells have a central role in the regulation of innate immunity to a number of infectious agents, including fungi, malaria, influenza A virus and simian immunodeficiency virus[100–104]. Cytokines produced by antigen-specific CD4+ T cells, in particular IL-2 and IL-12, seem to directly affect NK cell function in these disease models. Furthermore, the induction of antigen-specific helper T cells by vaccination can modulate or even reconstitute NK cell responses to pathogens[103,104]. In the context of HIV-1 infection, during which the virus-specific responses of CD4+ helper T cells are lost early in infection, therapeutic immunization can result not only in the reconstitution of HIV-1–specific T cell help, but also in an enhancement of NK cell responses against HIV-1. It will be important to further evaluate the therapeutic potential of immunizations targeted at enhancing virus-specific NK cell function in individuals infected with HIV-1, in particular in the context of recent approaches aimed at reducing the HIV-1 reservoir in infected individuals.

Conclusions

The innate immune response, from cell-intrinsic innate immune defenses to innate immune-cell activation and NK cell effector actions, has a major role in the control of HIV-1 infection. At best, the effective induction of the innate immune response will induce host restriction factors that suppress the replication and spread of HIV-1 and will activate innate immune cells for HIV-1 control. Among the processes of innate immune activation, the effective licensing of NK cells is essential to facilitate killing of HIV-1–infected cells. At worst, the innate immune response will promote CD4+ T cell death and chronic immune activation linked with HIV-1 disease progression. Thus, defining the regulatory mechanisms of innate immune activation and response regulation is paramount for developing strategies to therapeutically leverage the innate immune response for the control of HIV-1 infection. Moreover, there is still no clear knowledge of the nature of HIV-1 PAMPs beyond reverse transcription products involved in PRR signaling in innate immunity, or of how viral evasion of PRR signaling and IRF3 actions affects the outcome of HIV infection and regulation of the global immune response to infection. It is necessary to define the mechanisms of innate immune control in HIV infection in order to inform approaches to enhance anti-HIV immunity, and to provide effective adjuvants targeting innate immunity to improve protective vaccines against HIV infection.

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