

Human Immunodeficiency Virus Type 1 Tat-Mediated Cellular Response in Myeloid Cells

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Human immunodeficiency virus type 1 (HIV-1)-infected cells respond to the infection with different outcomes depending on their cell type. The interplay of cellular and viral proteins is a key player of differences in virus replication and disease progression. Myeloid cells, including monocytes, macrophages, and myeloid dendritic cells (mDCs) play a crucial role in the transmission and pathogenesis of HIV. The viral protein Tat, which is the viral transcriptional activator, modulates the expression of both HIV and cellular genes in these myeloid cells. This review will focus on recent advances on the interplay between HIV and myeloid cells and will discuss how this interaction may contribute to HIV pathogenesis. A better understanding of the pathogenesis of HIV disease will provide us with the scientific rationale for novel approaches to prevention.

Key Words: Myeloid cells, Dendritic cells, Monocytes-derived macrophages, Tat, Human immunodeficiency virus type 1

Introduction

Human immunodeficiency virus (HIV) infects a variety of cells that are critical components of the immune system and the outcome of the infection varies in the different cell types. Cells like CD4⁺ T cells produce substantial amount of virus and undergo apoptosis, die of cytopathic effect, or become latently infected; macrophages produce substantially less virus than T cells upon infection but are much more resistant to virus-mediated cell death; immature dendritic cells (iDCs) produce even lower amounts of virus than macrophages and are also resistant to virus-mediated death. It is therefore logical to envision that these different cells respond differently to HIV infection and manage to do so by different adjustments of their gene expression program to the viral infection. Myeloid cells, including monocytes,

macrophages, and myeloid dendritic cells (mDCs), are a particularly relevant cell type capable of providing targets for virus infection as well as a source of immunomodulatory cytokines and chemokines (1). HIV-1 expresses a number of proteins that could directly play a role in affecting cellular gene expression at the transcription levels. The transcriptional transactivator Tat regulates HIV and SIV gene expression and interacts with important components of the transcription machinery (2). Here, we review recent literature about the interplay between HIV and myeloid cells, including viral infection, type I interferon signaling, and the contribution of the viral protein Tat to HIV-associated immune activation.

Biology of myeloid cells

HIV causes a generalized infection of the immune system

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and pathogenesis of HIV infection is the result of complex immunologic events triggered by the initial presence of high levels of virus replication and a subsequent dramatic depletion of CD4⁺ T cells. Effects of persistent virus infection of CD4⁺ T cells and, to a lesser extent, myeloid cells induce a systemic increase in the turnover and activation of numerous immune cells. While massive information is available about the dynamics of CD4⁺ T cells during the course of HIV infection, the contribution of myeloid cells to pathogenesis of HIV disease remains relatively unclear. Lentiviruses like HIV and Simian Immunodeficiency Virus (SIV) are known for their ability to infect non-dividing cells such as myeloid-lineage cells. Macrophages and mDCs express the HIV receptor CD4 and coreceptors (CCR5 or CXCR4) as well as C-type lectin receptors and play a critical role in the initial mucosal transmission and dissemination of HIV/SIV as well as in the maintenance of the viral reservoir (3). Their role in HIV pathogenesis has been focused on the target cells for infection. In fact, high levels of virus replication in macrophages are associated with rapid SIV disease progression (4). However, several recent advances indicate other role for monocytes, macrophages, and mDCs in HIV disease via mechanisms independent of their infection status, including their rapid turnover and ability to promote HIV/SIV-associated chronic immune activation (5). Thus, the potential of these cells to both promote and resolve immune activation empowers further investigation of their role in HIV pathogenesis.

Myeloid cells are a family of immune cells comprising monocytes, macrophages, myeloid dendritic cells, granulocytes, and mast cells that originate from a common myeloid progenitor in the bone marrow. The primary function of these cells is to mount innate immune defenses against a wide range of pathogens including phagocytosis, the secretion of immunomodulatory cytokines and chemokines, and the release of an assembly of granule-associated factors (5). However, myeloid cells also stimulate the adaptive immune responses via antigen presentation and the recruitment and activation of lymphocytes, and they are also involved in numerous aspects of tissue function and homeostasis.

Monocytes are precursors of macrophages and mDCs that originate in the bone marrow and circulate in the peripheral blood before homing into tissues. While in the blood, monocytes contribute to innate immune defenses via the ability to detect numerous pathogen-associated molecular patterns (PAMPs), to phagocytose, and to produce reactive oxygen and nitrogen species (Fig. 1) (6). Once present in tissues, monocytes differentiate into macrophages or mDCs depending on signals they receive from the local micro-environment (7). Tissue-based macrophages are critical cells involved in immune surveillances and anti-microbial defenses. Their goal is typically to maintain tissue homeostasis through the ingestion and ultimately the removal of apoptotic and necrotic cells. Macrophages and mDCs express a battery of pattern recognition receptors (PRRs) and when faced with foreign antigens they are able to rapidly phagocytose and kill microbes, and/or to generate numerous cytokines and chemokines to recruit and activate additional immune cells. Moreover, while these cells contribute to inflammation and immune activation, they also likely resolve these responses by producing anti-inflammatory cytokines and suppressing the activity of other immune cells (6).

HIV infection of myeloid cells

Dendritic cells (DCs)

Human mucosal surfaces are populated by dendritic cells, which are among the primary targets of HIV-1 early in infection. Dendritic cells are highly heterogeneous, antigen-presenting cells that initiate acquired immune responses by priming naive, antigen-specific T cells (6). HIV-1 can productively infect immature dendritic cells and use them as vehicles to infect T cells by undergoing a maturation process that facilitates induction of further innate and adaptive immune responses (reviewed in 6). Pathogenic factors that are referred to as pathogen-associated molecular patterns (PAMPs) can promote DCs maturation, a process that involves the production of pro-inflammatory cytokines, increased surface expression of MHC molecules and the co-stimulatory molecules CD80 and CD86, as well as up-regulation of the lymphoid homing marker CCR7 (8). DCs

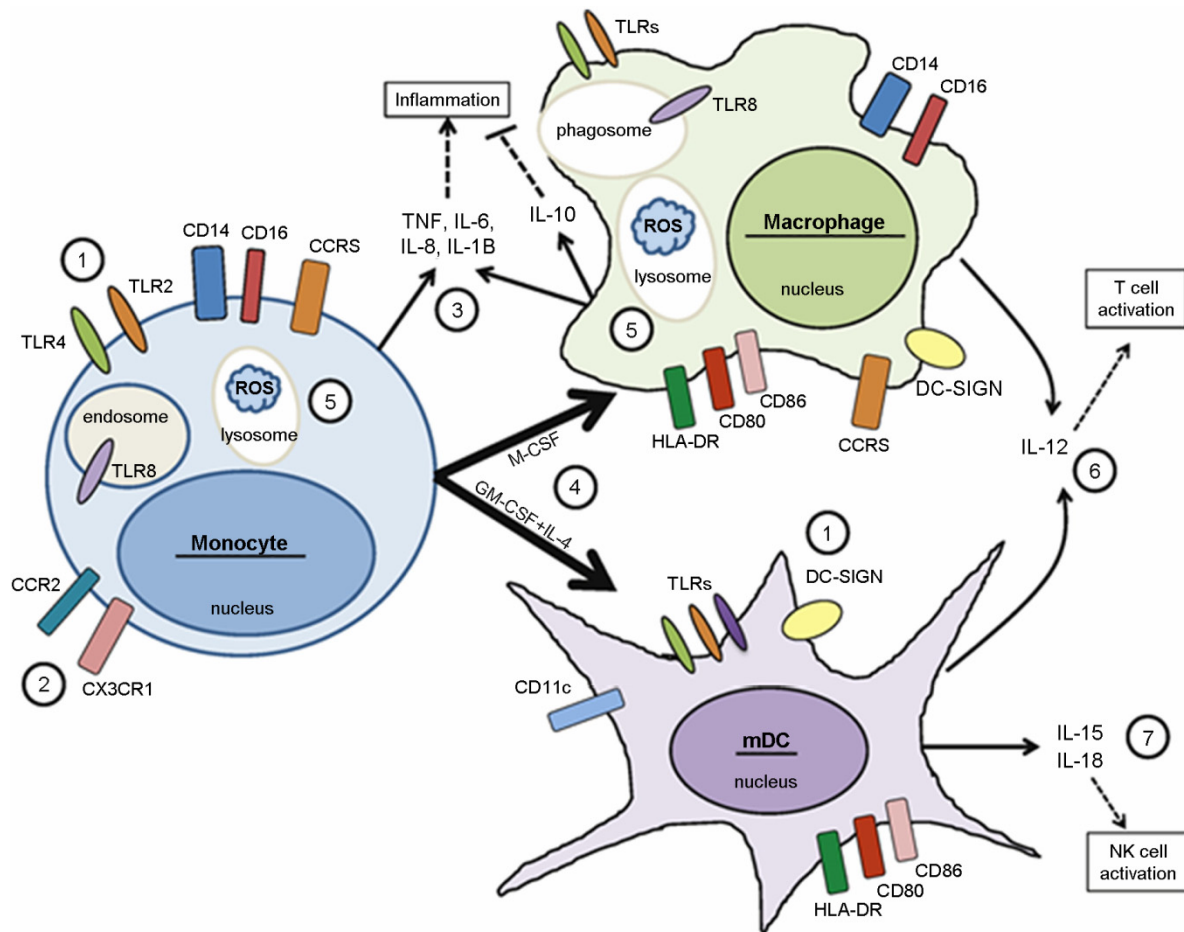


Figure 1. Overview of myeloid cell functions. Monocytes, macrophages, and myeloid dendritic cells (mDCs) express multiple receptors to recognize microbes, including toll-like receptors (TLRs), and c-type lectin receptors such as DC-SIGN (①). Chemokine receptors such as CCR2 and CX3CR1 direct the migration of blood monocytes to sites of inflammation (②), where they contribute to the pro-inflammatory cytokine milieu through the production of $\text{TNF-}\alpha$, IL-6, IL-8, and IL-1 β (③). Once present in tissues, monocytes are influenced by local signals to differentiate into either macrophages or mDCs. *In vitro*, M-CSF directs monocyte differentiation into macrophages while GM-CSF + IL-4 results in differentiation into mDCs (④). Tissue resident macrophages are also capable of producing pro-inflammatory cytokines as well as ample amounts of IL-10, an anti-inflammatory cytokine important for resolving local inflammation (③). Both monocytes and macrophages are capable of phagocytosis and intracellular killing of microbes through the production of reactive oxygen species (ROS) and reactive nitrogen species (⑤). Activated macrophages and mDCs secrete IL-12, a cytokine involved in the activation and differentiation of T cells (⑥). Activated mDCs also stimulate NK cell activation via the production of IL-15 and IL-18 (⑦). Adapted from "The myeloid cytokine network in AIDS pathogenesis", by Mir *et al.*, 2012, Cytokine Growth Factor Rev, 23, 224 as a reference 6 in the text.

express a range of receptors for these PAMPs, including toll-like receptors (TLRs) (9), a family of molecules in which each member recognizes a specific PAMP. For example, lipopolysaccharide (LPS) is a PAMP expressed by gram-negative bacteria. LPS interacts with TLR4, along with the TLR4 co-receptors MD-2 and CD14, on the cell surface and induces a response to the invading bacteria via a complex signaling cascade (10). LPS stimulation causes

DCs maturation, leading to increased DCs migration, decreased DCs endocytosis, and increased expression of co-stimulatory molecules required for interactions with CD4⁺ T cells on the DCs (11). Recent study demonstrates that LPS activation of DCs is a key event because there is an association between gram-negative bacterial translocation and high levels of LPS in the serum and the systemic immune activation observed in chronic HIV-1 infection

(11). Moreover, studies point to a possibility of coinfection with gram-negative bacteria along with HIV-1 infection (12), which may facilitate HIV-1 spread by enhancing LPS-stimulated maturation of DCs and, therefore, DCs-mediated HIV-1 transmission to CD4⁺ T cells.

Myeloid DCs transit HIV-1 to CD4⁺ T cells by two distinct processes; by being directly infected and transmitting newly synthesized virus (cis-infection) or by capturing virions without being infected themselves for subsequent cell-to-cell transfer (trans-infection) (13). This transmission process involves cellular factors that are expressed by the mDCs and interact directly with HIV-1 at initial stages of infection, or during the course of infection. Among those there are dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), CD4, and intercellular adhesion molecules (ICAMs) (reviewed in 13). HIV-1 structural proteins and some accessory proteins have the potential to interact with cellular factors and promote HIV-1 transmission during the initial stages of infection. In addition, HIV-1 proteins produced during replication may promote cell-to-cell transmission after the initial infection. For example, the multifunctional pathogenic accessory protein Nef plays a key role in promoting DCs-mediated HIV-1 transmission to CD4⁺ T cells by activating CD4⁺ T cells and modulating DCs interactions with T cells (14). Viral protein Env is present on the surface of the virion and is able to interact directly with HIV-1 receptors and other cell surface molecules to promote cell-to-cell transmission of the virus and newly synthesized Env can promote CD4⁺ T cell-mediated cell-to-cell transmission (15).

Release of immunological factors, such as pro-inflammatory cytokines, chemokines, and other soluble factors, is an important for preventing spread of infection within the host, as these molecules can act on surrounding naïve cells to promote immune cell activation or to protect surrounding cells by upregulating cellular factors that restrict pathogen spread. In the case of DCs, some immunological factors lead to DCs maturation. In particular, type I interferons (IFN) are antiviral cytokines produced as part of the innate immune response to an infection. The type I IFN

can inhibit the replication of HIV-1 in CD4⁺ T cells, mDCs, and macrophages *in vitro*, the cell-to-cell transmission of HIV-1 between CD4⁺ T cells and DCs-mediated HIV-1 transmission to CD4⁺ T cells (16). These inhibitions of HIV-1 replication in DCs can be relieved by viral factors such as the Vpx proteins from HIV-2 or certain SIV (17), which may allow the identification of type I IFN-inducible HIV-1 restriction factors in DCs. DCs may also act as important HIV-1 reservoir and maintain a significant pool of HIV-1 during long-term viral infection. Given the low levels of HIV-1 replication and high levels of DCs-mediated transmission of HIV-1 observed in some DCs subtypes, it is possible that DCs subtypes, particularly those in the lymph node, may act as significant pools of HIV-1 during long-term HIV-1 infection (18). Furthermore, increasing evidence indicate that HIV-1 and SIV infection lead to alteration of the chemotactic environment in lymphoid tissues through direct and indirect mechanisms. The viral proteins Tat, Nef, and Vpr lead to the production of chemokines by infected DCs or other cells (19). Upregulation of chemokine expression by infected DCs or other cells in lymphoid tissues will indisputably have local and systemic effects that contribute to the development of immunodeficiency. Increased recruitment of target cells to lymphoid tissues through chemokine induction will also contribute to sustained viral replication, even in the face of potent virus-specific immune responses. Another major effect of chemokine dysregulation would be the increased recruitment and potential apoptosis of immune cells from peripheral blood. Models of this ectopic recruitment and cell loss have been proposed for HIV-1 infection in humans and SIV infection in macaques, and have been validated in humans (20).

Macrophages

Macrophages are terminally differentiated, non-dividing cells, derived from circulating monocytes. They represent a distinct population of phagocytes which are found under different names in various tissues. Circulating monocytes can be infected and then migrate to peripheral tissues, including the brain, lung, lymphatic system, bone marrow, and kidney and infected monocytes then differentiate into

monocyte-derived macrophages (MDM) and may form a long-lived reservoir for the virus (21). MDM can also be infected after differentiation and are more susceptible to new infection in comparison to freshly isolated monocytes. It is likely that multiple cellular factors needed for viral replication are present at limiting levels in monocytes, and the program of macrophage differentiation may lead to the up-regulation of these factors. CCR5, the major co-receptor utilized by HIV-1 to enter macrophages, is one such factor. CCR5 is present at very low levels in monocytes and is strongly up-regulated during the first week of macrophage differentiation. Infected MDM seed the periphery with new infectious virus, directly transmit virus to T cells (22), release toxic viral proteins (23), and produce effector functions that contribute to HIV pathogenesis (24). HIV-infected macrophages have been detected in numerous tissues of infected individuals, including macrophages in lymph nodes (25), microglia in brain (26), Kupffer cells in liver (27), and alveolar macrophages in the lung (27). In late-stage AIDS patients in whom the CD4⁺ T lymphocyte population has been depleted, infected macrophages make a much greater contribution to the viral load. In situ hybridization analysis of lymph nodes in late-stage AIDS patients has shown that macrophages can produce high levels of HIV-1, especially in patients with opportunistic co-infections such as *Mycobacterium avium* and *Pneumocystis carinii* (28). While the viral replication cycle is generally rapid and cytopathic in activated CD4⁺ T cells, infected macrophages survive for long periods of time after the infection *in vitro* (29). Thus, infected macrophages are considered to be a significant reservoir of HIV-1 because of their resistance to the cytopathic effects of the virus and their longer half-life as compared to activated T cells (30). Especially long-lived macrophages may therefore harbor the virus for long time periods, thus constituting HIV-1 reservoirs and posing a major obstacle to virus eradication from infected individuals.

Resident macrophages in the mucosa usually do not migrate to lymph nodes. Non-infected macrophages take up and process the virus and present HIV-1 derived peptides via MHC-II to CD4⁺ T cells. They also help to optimize the anti-HIV CTL response due to cross presentation of virus

derived peptides via MHC-I (31). Moreover, recent studies demonstrate that HIV-1 infected macrophages can be killed by CTLs, although HIV-1 has evolved mechanisms to down-modulate MHC-I from the surface of virus infected CD4⁺ T cells and macrophages (32). Thus, macrophages in the mucosa contribute to the humoral and cellular immune response during the acute phase of HIV-1 infection. A significant proportion of macrophages at the mucosal surface is productively infected with HIV-1 (33). Since macrophages secrete cytokines that attract/recruit T lymphocytes to sites of infection, they can support establishment of viral infection by expanding the number of primary target cells. They may transmit the virus to CD4⁺ T cells at the mucosal surface via cell to cell contact during HIV-antigen presentation (34). It is likely that a productively infected macrophage interacts with CD4⁺ T cells as a consequence of MHC class II mediated presentation of HIV-1 antigens and simultaneously transmits the virus to the interacting CD4⁺ T cell, even though this has not been shown experimentally, yet. Thus, macrophages could also form so-called virological synapses to transfer HIV-1 to uninfected macrophages and T cells with features similar to those seen in T cells and DCs for efficient transmission to adjacent cells. Overall, recent evidence clearly establishes that vaginal macrophages are productively infected during sexual transmission of HIV-1. However, these tissue-associated macrophages stay at the mucosal surface and therefore probably do not transport HIV-1 to secondary lymphoid organs. Instead they recruit CD4⁺ T cells and contribute to the establishment of infection at sites of viral entry, i.e. the mucosal barrier.

In recent years a variety of host cell factors suppressing HIV-1 at different steps in the viral replication cycle have been described and are now collectively called HIV-1 restriction factors (Fig. 2) (35). Myeloid cells are more resistant to HIV-1 infection than other cell types such as activated T lymphocytes. Macrophages express high amounts of tetherin and SAMHD1, whereas CD4⁺ T cells express no or only low levels of these restriction factors (36). As a consequence, in *ex vivo* experiments, viral production and release in macrophages is strongly impaired by tetherin and is only partly restored by Vpu nor does HIV-1 Vpr contain

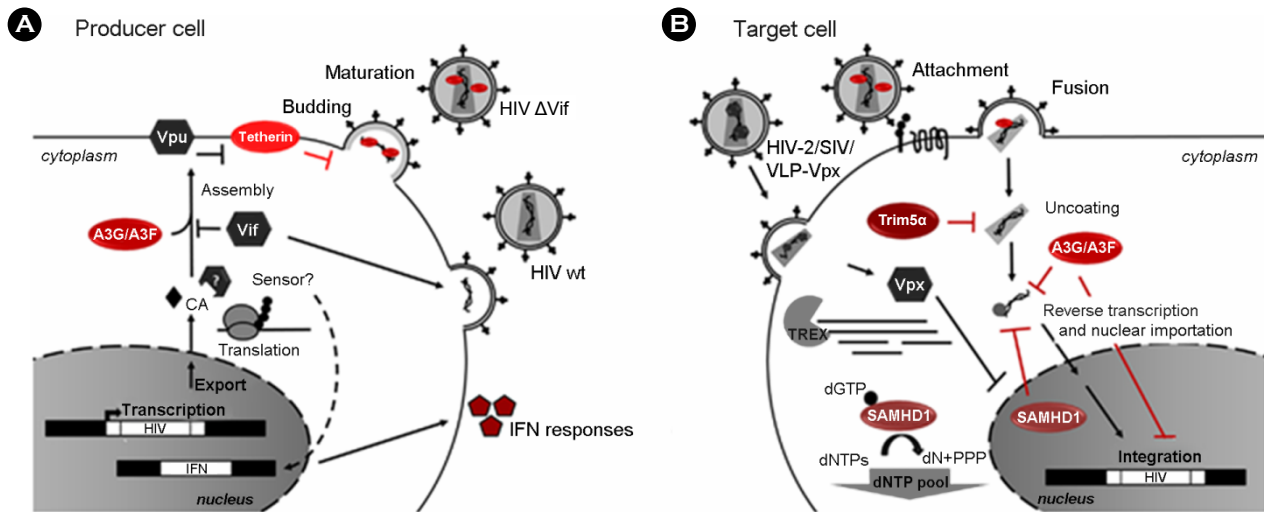


Figure 2. Host restriction factors and their action during HIV-1 replication. Schematic representation of (A) HIV-1-infected producer cell, and (B) HIV-1 target cell. Cellular restriction factors are represented by red ovals, and viral counterparts are represented by gray hexagons. Black arrows represent the course of viral replication and actions. Broken arrows represent inhibition. Question marks (?) represent unresolved questions. Adapted from "Host factors and HIV-1 replication", by Goncalves *et al.*, 2013, Front Immunol, 4, 343 as a reference 35 in the text.

the ability to antagonize SAMHD1, which is recently identified as the cellular SAM (sterile alpha motif) domain and HD (histidine/aspartic acid domain) containing protein 1 (SAMHD1) (37). SAMHD1 is highly expressed and functional in myeloid cells and its role as restriction factor was identified based on studies showing that certain strains of primate lentiviruses that encode the accessory protein Vpx can bypass the block to viral replication in myeloid cells (38). HIV-2, SIV from sooty mangabeys (SIVsmm, and its derivative SIVmac), and one of the mandrill viruses (SIVmnd-1), but not HIV-1, SIV from chimpanzee (SIVcpz), and SIV from African green monkeys (SIVagm) indeed encode Vpx, which provides a replication advantage in human myeloid cells. This Vpx function can be mediated by the Vpr gene in SIVagm (38). Vpx deficient HIV-2/SIVsm viruses are less infectious in myeloid cells than wild-type viruses and delivery of Vpx to monocyte, MDM or DCs increases HIV-1 infectivity (39). Vpx promotes more efficient HIV-1 infection by mediating proteasome-dependent degradation of SAMHD1, which can be restored by treatment with a proteasome inhibitor. The effects of SAMHD1 on HIV-1 reverse transcription are demonstrated by the fact that knock-down of this molecule in THP1

cells, a human monocytic cell line derived from an acute monocytic leukemia patient, and macrophages increased viral DNA levels. Further studies revealed that Vpx expression or SAMHD1 depletion increases the amount of deoxynucleotides tri-phosphates (dNTPs) in phorbol 12-meristate 13-acetate (PMA)-treated THP1 human monocytic cells or purified primary macrophages, and that the addition of dNTPs to primary macrophages enhances HIV-1 infection (39). These studies demonstrate that SAMHD1 functions by reducing intracellular dNTP substrates required for the synthesis of viral DNA.

HIV-induced type I IFN signaling in myeloid cells

Type I interferons (IFN) represent a major defense against viral infections. Sensing of viral nucleic acids by pattern recognition receptors (PRRs) leads to the activation of interferon regulatory factors (IRFs), including IRF-3 and IRF-7, and the downstream synthesis of IFN- β . Secreted IFN- β acts in a paracrine and autocrine manner to elicit a positive feedback loop which culminates in the production of IFN- α and the up-regulation of numerous interferon stimulated genes (ISGs) (40). In the course of chronic viral

infections such as HIV, IFNs act as a two-edged sword in that they restrict viral replication but contribute to the chronic immune activation that is associated with disease progression. The antiviral effect of IFN is mediated by the induction of a large number of ISG, which encode proteins with diverse functions including antiviral properties, proapoptotic functions and modulation of ubiquitination pathways (41). Induction of these genes is initiated upon binding of Type I IFN to its cellular receptor, which initiates receptor-mediated signaling pathways involving the activation of two receptor associated kinases (JAK1 and Tyk2), and the consequent tyrosine phosphorylation of pre-existing signal transducers and activators of transcription (STAT). Upon phosphorylation, STAT1 and STAT2 assemble together with interferon regulatory factor 9 (IRF-9) into a multimeric complex (ISGF3), which interacts with interferon-responsive elements (ISRE) present in the 5' flanking region of ISG and activates their transcription (41). Type I IFN also stimulates the formation of STAT1 homodimers, which bind to the IFN- γ -activated site (GAS), present in the ISG promoters that can be induced both by Type I IFN and IFN- γ . The mechanism of transcriptional regulation of ISG involves different cellular pathways including Janus kinase-STAT (JAK-STAT) and the mitogen-activated protein (MAP) kinase signaling cascades. Although virus infections result in expression of type I IFN, which induces expression of ISG, some ISGs are also directly induced independently of IFN signaling, via TLR activation. IFN-independent expression of some ISG such as IP10 and TRAIL after TLR7 triggering is stimulated by STAT1, phosphorylated via the p38 MAPK pathway (reviewed in 41). Induction of an IFN-like response can establish an antiviral state that may be a key to the lower levels of virus production observed in myeloid cells including iDCs and MDM compared with T cells. Importantly, activation of cytokines that impact T-cell activation can also contribute to the chronic immuno-activation observed in HIV-infected patients.

HIV-1 can be recognized by innate immune sensors; both viral proteins and nucleic acids represent potential targets of recognition. The primary PRRs involved in sensing of HIV in myeloid cells are the TLR8 and retinoic inducible

gene (RIG)-I. In myeloid cells, detection of viruses may occur after viral uptake, in an endosomal compartment, and/or after viral fusion in the cytoplasm of target cells. TLR8 recognizes multiple uridine-rich sequences in the ssRNA of HIV-1 genome resulting in the production of IFN- α , while differentiation into macrophages leads to a loss of HIV-induced IFN-production (42). RIG-I is a ubiquitous cytoplasmic PRR that detects dsRNA and ssRNA with abnormal 5'-triphosphate motifs (43). Both dimeric and monomeric isoforms of genomic RNA from HIV-1 induce RIG-I-dependent type I IFN response, however, following de novo HIV-1 infection of human macrophages, RIG-I and type I IFN response are not observed and macrophages are permissive to HIV-1 replication. HIV-1 uses the viral protease to inhibit the initiation of the RIG-I signaling cascade and IRF-3 activation (43). In addition to HIV-1 RNA, reverse-transcribed HIV-1 DNA may be detected by a yet unidentified cytoplasmic DNA sensor. In macrophages and lymphocytes HIV hijacks the host cytosolic nuclease 3' repair exonuclease1 (TREX1), to digest HIV-1 DNA generated during infection, thus providing another mechanism for the virus to avoid detection by nucleic acid sensors. Consequently, inhibition of TREX1 by RNA-mediated interference allows cytosolic HIV DNA accumulation and type I IFN production, mediated by the STING-TBK1-IRF-3 axis, resulting in the inhibition of HIV replication and spreading (44).

Host restriction factors are active early in virus-cell interactions and directly limit retroviral infection by impairing reverse transcription, correct genome copying, chromosomal integration and viral shedding (45). These factors are constitutively expressed at baseline levels in many cell types, but their expression can be transcriptionally up-regulated by type I IFN through ISRE/IRF-E responsive elements in their promoters. Upregulation of these factors accounts for much of the anti-HIV-1 activity of type I IFN. As cells have evolved a variety of restriction factors that act to inhibit or block viruses, most retroviruses including HIV-1, have acquired accessory proteins that antagonize host restriction factors and facilitate viral immune evasion and replication (45). Among the numerous ISGs induced by IFN-I signaling

are several restriction factors with well-characterized anti-HIV activity, including TRIM5 α , APOBEC3G, tetherin, and SAMHD1. The virus has evolved numerous survival mechanisms for counteracting these factors in CD4⁺ T cells, including capsid mutations which prevent binding of TRIM5 α , Vif-mediated degradation of APOBEC3G, and Vpu-dependent degradation of tetherin (37). However, HIV evasion of myeloid restriction factors does not seem to occur, and HIV-1 is unable to inhibit the activity of SAMHD1 due to the lack of the accessory protein Vpx (36). In addition, HIV-1 infection of macrophages and mDCs results in minimal IFN production. *In vitro* infection of MDM causes very few changes to the cellular transcriptome, including limited activation of NF- κ B and no detectable increase in IFN- β mRNA. A key mediator of the low IFN-I response is the cytosolic exonuclease TREX1, which binds and degrades HIV DNA (44). TREX1 binding to viral DNA prevents recognition by an unidentified viral DNA sensor which signals through STING and IRF-3 to induce IFN-I production. However, pretreatment of macrophages and mDCs with exogenous type I IFN or poly I:C renders them resistant to HIV replication, indicating that myeloid cells are not inherently unable to block infection (42). Consistent with an inhibition of IFN expression by SAMDH1, mutations in SAMDH1 gene can cause the Aicardi-Goutières syndrome, a genetic encephalopathy with symptoms mimicking congenital viral infection, in which excessive production of IFN- α and detrimental immune activation are major pathogenetic factors (reviewed in 45). Interestingly, the host exonuclease TREX1 that inhibits innate immune responses to HIV-1 DNA is also associated with Aicardi-Goutières syndrome. While SAMH1 limits HIV-1 by inhibiting reverse transcription, TREX1 stimulates HIV-1 replication by inhibiting the immune recognition of non-productive products of reverse transcription. Like TREX1, SAMHD1 may limit activation of innate immune sensors by eliminating excess nucleic acids and synthesis of the retroviral cDNA, thus limiting the sensing of HIV-1 intermediates. This observation reveals an intricate relationship between innate immune mechanisms that control the response to self and retroviral pathogens in myeloid cells.

The ISG has been associated with the generalized immune activation that characterizes chronic infection and progression to AIDS, although the qualitative and quantitative association between viral load, IFN and ISG expression remains to be defined. Studies in nonhuman primates indicate that the innate immune response distinguishes non-pathogenic and pathogenic SIV infections. In natural hosts for SIV that do not progress to AIDS, such as sooty mangabeys, type I IFN production and ISG expression is high but transient and declines in the chronic phase, despite high levels of SIV replication. Conversely, ISG expression persists in non-natural hosts (46). Similarly, in humans, ISGs remain elevated in chronic HIV-1 infections and are associated with immune activation in AIDS progressors while rapidly decline in HIV controllers (47). To this point, the mechanisms responsible for this differential regulation of immune activation in pathogenic versus nonpathogenic primate immunodeficiency virus infection are unclear: they could be related to low levels of TLR7/9 signaling, likely mediated by impaired IRF-7 activity, or to an efficient down-regulation of acute type I IFN response (reviewed in 45). Whatever the mechanisms involved in HIV-induced IFN-I signaling in myeloid cells and despite IFN production, the continuous virus replication, innate immune stimulation and multifactorial immunopathology may represent the primary force driving AIDS progression in pathogenic HIV and SIV infections.

HIV-1 Tat-mediated intracellular signaling in myeloid cells

HIV-infected cells respond to viral invasion with various defensive strategies. Conversely, the virus has also developed many offensive tactics to suppress host cellular responses. Among many of the viral offensive tactics, HIV-1 viral proteins such as Tat, Nef, Vif, and Vpr play important roles in the host-pathogen interaction and thus have significant impacts on the outcome of HIV infection. HIV-1 Tat is a multifunctional protein that contributes to several pathological symptoms of HIV-1 infection as well as playing an important role in virus replication. Tat is among the first

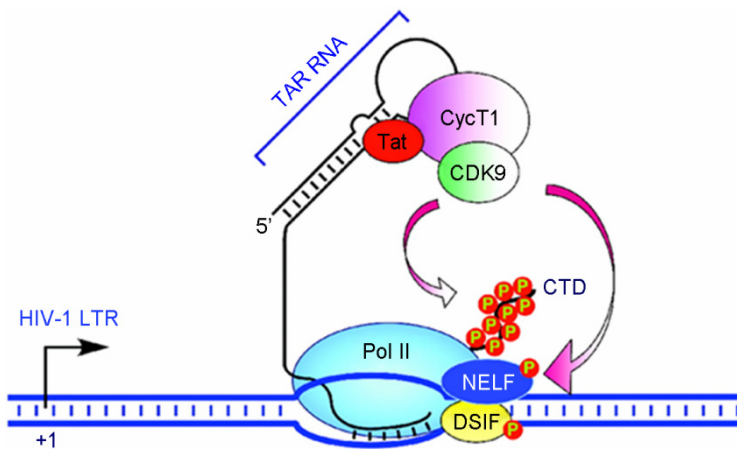


Figure 3. Schematic of the pathway triggered by Tat and leading to activation of ISGs by Tat in APCs infected by HIV. (A) Tat triggers signaling pathways that activate ISGs by associating with MAPK kinases MAP2K3 (MKK3) and MAP2K6 (MKK6), which in turn activate p38 MAPK and STAT1, and with IRF7 in APCs infected by HIV. (B) Activation of MKK3, MKK6, and IRF7 leads to expression of many ISGs (shown here in connection with MKK3, MKK6, and IRF7), whose product can positively impact T-cell immunoactivation and negatively impact virus production. Bold labeling indicates genes found upregulated by HIV and Tat in APC. Adapted from "Tat engagement of p38 MAP kinase and IRF7 pathways leads to activation of interferon-stimulated genes in antigen-presenting cells", by Kim *et al.*, 2013, Blood, 121, 4090 as a reference 53 in the text.

genes expressed during HIV-1 infection and is essential for viral gene expression and virus production. Tat increases HIV-1 gene expression by interacting with the host-cell elongation factor P-TEFb, which phosphorylates the C-terminal domain of the large subunit of RNA polymerase II, which in turn stimulates transcriptional elongation (Fig. 3) (48). Identification of the critical role of Tat in transcription of the HIV-1 genome has provoked a number of studies which focus on Tat as a target for antiretroviral drugs (49). The interaction of Tat with TAR is considered a suitable target for the chemotherapy of HIV infection, because an inhibitor of the Tat-TAR interaction may have the potential to maintain the virus in a latent state. A basic peptide oligomer of nine residues, known as CGP64222, can effectively compete with Tat for binding to TAR and block HIV-1 replication in peripheral blood lymphocytes (49). Several studies have developed short TAR RNA decoy molecules which can compete with TAR for binding to Tat (50). The ability of Tat to cross the cellular membrane has also been addressed as a mechanism to deliver new drugs, polypeptides or antibodies to target cells. Several studies have shown that different substances conjugated to Tat or the nuclear import sequences of Tat can cross the cellular membrane. These findings may have important therapeutic implications for the future.

In addition to the regulatory roles of HIV-1 Tat in the transactivation of virus genes, Tat has been implicated as a modulator of expression levels of a number of cellular genes

(51). For example, Tat is able to stimulate the expression of immunoregulatory cytokines including tumour necrosis factor (TNF), interleukin-2 (IL-2), and transforming growth factor (TGF) as well (reviewed in 51). IL-2 is the most important growth and differentiation factor of T cells and its observed increase might facilitate virus spread from or to T cells. Tat transactivates the human IL-6 promoter in primary monocytes by interacting with a short sequence at the 5' end of the IL-6 mRNA that acquires a stem-loop structure and contains a UCU sequence that is essential for Tat binding. A recent study also demonstrates that subtype B and subtype C Tat protein exert differential cytokine modulating effects. The expression of anti-inflammatory molecules including IL-4 and IL-10 is higher in Tat C-treated compared with Tat B-treated cultures of primary human monocytes. Conversely, subtype B Tat protein shows significant upregulation of pro-inflammatory cytokines, IL-6 and TNF- α , as compared with subtype C Tat protein (52). Deregulated production of IL-6 is implicated in the pathogenesis of several AIDS-associated pathologies, like psoriasis, B-cell lymphoma and Kaposi's sarcoma. Our recent studies demonstrate that HIV infection and Tat expression induces expression of a subset of ISG that encode transcription factors as the IRF7, STAT1, and chemokines that recruit activated T cells and monocyte-derived macrophages by activating p38 MAP kinase and IRF7 pathways via Tat association with the 2 MAPKK and IRF7 promoters (Fig. 4) (53). A species-specific increase of

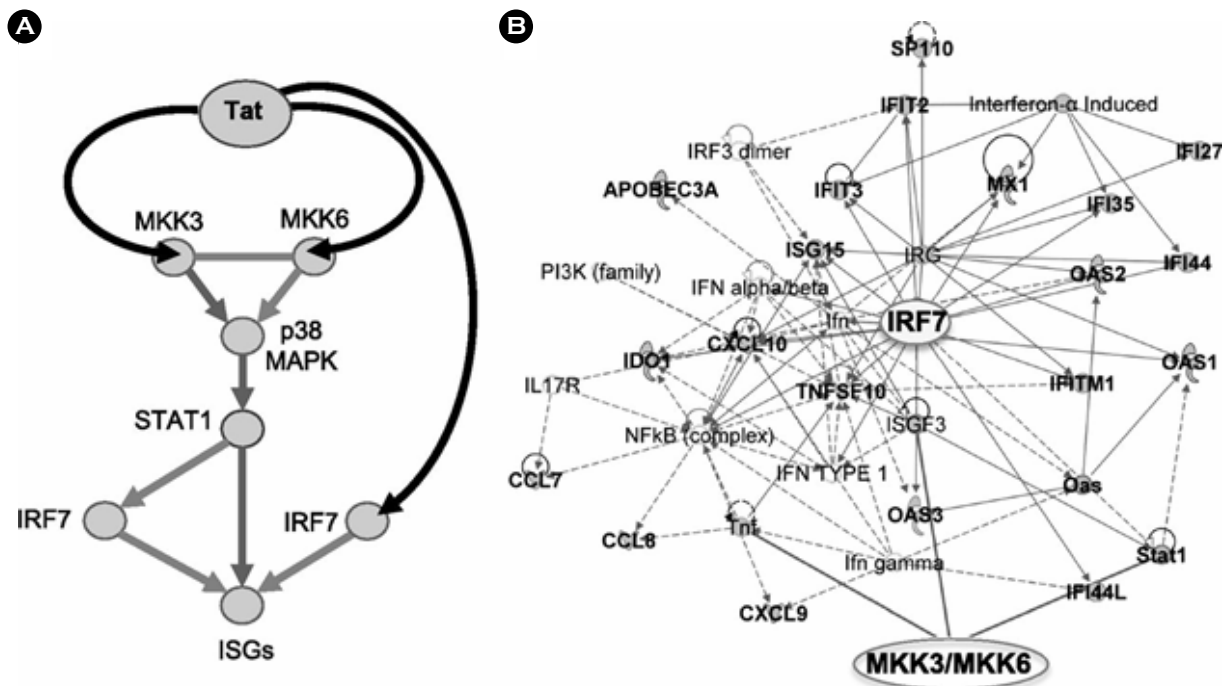


Figure 4. Schematic of the pathway triggered by Tat and leading to activation of ISGs by Tat in APCs infected by HIV. (A) Tat triggers signaling pathways that activate ISGs by associating with MAPK kinases MAP2K3 (MKK3) and MAP2K6 (MKK6), which in turn activate p38 MAPK and STAT1, and with IRF7 in APCs infected by HIV. (B) Activation of MKK3, MKK6, and IRF7 leads to expression of many ISGs (shown here in connection with MKK3, MKK6, and IRF7), whose product can positively impact T-cell immunoactivation and negatively impact virus production. Bold labeling indicates genes found upregulated by HIV and Tat in APC. Adapted from "Tat engagement of p38 MAP kinase and IRF7 pathways leads to activation of interferon-stimulated genes in antigen-presenting cells", by Kim *et al.*, 2013, Blood, 121, 4090 as a reference 53 in the text.

some ISG was observed in human and Rhesus macaque iDCs and MDM but not in the same cells from chimpanzee, sooty mangabeys, and African green monkeys, in which simian immunodeficiency virus infection is asymptomatic or less severe (54). These results establish a correlation between the Tat-mediated differential induction of ISG and species-specific differences in HIV-associated disease susceptibility. This reprogramming in HIV-1-infected cells and the resulting persistent activation of ISG including inflammatory cytokines and chemokines can potentially contribute to the increased immune activation that characterizes HIV infection.

Tat is also capable of inducing the expression of other cellular genes as well, such as various adhesion molecules. In this regard, Tat may play a role in the extravasation of HIV-infected cells (reviewed in 51). Tat upregulates the expression of extracellular matrix proteins fibronectin and

types I and III collagen in a glial-derived cell line. Fibronectin is an extracellular adhesion molecule involved in many cellular processes, including cell migration and adhesion. Tat also downregulates several genes, such as the gene encoding major histocompatibility complex (MHC) class I and matrix metalloproteinases (MMPs) (55). The dysregulation of MMPs activity in HIV-infected individuals is associated with disease progression by influencing the integrity of the extracellular matrix via an NF- κ B-dependent pathway in astrocytes. Additionally, Tat increases the expression of apoptotic factors such as CD95 ligand (CD95L), TNF-related apoptosis-inducing ligand (TRAIL), and TRAIL receptor TRAIL-R1/R2. The activation of extrinsic apoptotic pathway via CD95L and TRAIL may have implications for cell death occurring in noninfected cells. Interestingly, Tat can be found in patients' serum and can cross the cell membrane to enter cells. Thus, also in uninfected cells, Tat

is able to transactivate genes in a paracrine fashion and its action on uninfected cells may have significant consequences for the pathogenesis of HIV-1 infection. Interestingly, production of TRAIL from HIV or SIV iDCs and MDM is restricted to cells from AIDS-susceptible species (54). It will be important to evaluate if production of TRAIL and FasL occurs only in CD4⁺ T cells from AIDS-susceptible and not in nonhuman primates that are natural hosts of primate lentiviruses. Moreover, activation of the PTEN-FOXO3a pathway via Tat association with PTEN and protein phosphatase 2A (PP2A) promoters could be the mechanism by which apoptosis is triggered in HIV-infected and non-infected cells and explain the significant decline of the CD4⁺ T cell memory population in HIV-1-infected individuals (56, 57). Indeed, the levels of phospho-FOXO3a are reduced in HIV-infected individuals and are higher in elite controllers, who control viral replication to undetectable viremia in the absence of therapy (58). FOXO3a activation, which can be achieved via expression of Tat alone, appears to be at the convergence of pathways involved in both DNA repair and apoptosis. It may be also important to investigate what factors or events tip the balance in one of the two directions during HIV infection of CD4⁺ T cells, and whether FOXO3a levels of uninfected memory CD4⁺ T cells can be modulated in vivo by the Tat protein present in the patients' sera.

Among the many pathogenic mechanisms associated with HIV infection, HIV-associated immune activation is a multifactorial phenomenon that involves a persistent and aberrant activation of numerous immune cell types, increased plasma and tissue levels of pro-inflammatory cytokines, and high levels of activation-induced lymphocyte apoptosis. It is not yet fully understood what the causes of the HIV/SIV-associated immune activation are. But, it may contain the direct effects of viral proteins and nucleic acids, the innate and adaptive immune responses to viral antigens, the bystander activation of immune cells by high levels of pro-inflammatory cytokines. Collectively, the modulation of biological pathways can affect many aspects of infected and non-infected cells. Tat could have different effects on different cell types, thereby the modulating effects of Tat

on gene expression need to be addressed in different cells and tissues. It is important to understand how changes in the modulation of biological pathways such as cytokine, chemokine, and IFN networks impact HIV pathogenesis is a critical issue for the development of therapeutic vaccine and eradication strategies.

Conclusion

This review summarizes studies characterizing chronic HIV-1 infection of myeloid cells can lead to physiological changes that contribute indirectly to AIDS-associated disorders and symptoms. The pathogenic HIV infection affects phenotypical and functional changes to various immune cell types. In the case of myeloid cells such as macrophages, mDCs, and their common monocyte precursor, virus infection and host interactions with viral components cause increases in the production of IFNs, pro-inflammatory cytokines and chemokines, as well as perturbations in cell trafficking and turnover. These alterations lead to increased activation of myeloid cells and proliferation of lymphocyte and thus accompanied by increases in viral replication. In other cases, myeloid cells appear to play an immunosuppressive function in favor of overcoming the general inflammation characteristic of pathogenic HIV infection. However, these immune modulatory responses are mostly ineffective, as chronic immune activation persists, and instead may result in undesirable dampening of adaptive immune responses to the virus.

The study on the gene modulation by HIV in myeloid cells provides an incomparable resource for understanding their contributions to viral pathogenesis. However, there are several questions still need to be answered: (1) What is the relative impact of myeloid cells on the chronic immune activation and inflammatory cytokine production during HIV infection? (2) What is the contribution of the level of infection of macrophages to persistent immune activation in HIV-infected individuals receiving anti-retroviral therapy (ART)? (3) What could be the impact of Tat on myeloid innate immune responses to the protection against virus transmission during infection? The answers to these and

other questions will undeniably yield new insights into the cellular contributions to HIV-associated pathogenesis and be critical to identify new targets for therapeutic interventions. In particular, Tat modulation of antigen presenting cells such as myeloid DCs and macrophages appears to have two major consequences. First, Tat acts as an inflammatory cytokine, modulating chemokines and cytokines linked to increased immunoactivation. Second, Tat acts as an antiviral factor via the modulation proteins that reduce retroviral gene expression, facilitating immune evasion from the adaptive response but supporting sufficient virus production to spread to T cells where virus replication is abundant. This dual arrangement may be important to favor both the persistence and high level replication of this pathogen in its infected host.

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