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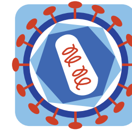
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Macrophage signaling in HIV-1 infection

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Abstract

The human immunodeficiency virus-1 (HIV-1) is a member of the lentivirus genus. The virus does not rely exclusively on the host cell machinery, but also on viral proteins that act as molecular switches during the viral life cycle which play significant functions in viral pathogenesis, notably by modulating cell signaling. The role of HIV-1 proteins (Nef, Tat, Vpr, and gp120) in modulating macrophage signaling has been recently unveiled. Accessory, regulatory, and structural HIV-1 proteins interact with signaling pathways in infected macrophages. In addition, exogenous Nef, Tat, Vpr, and gp120 proteins have been detected in the serum of HIV-1 infected patients. Possibly, these proteins are released by infected/apoptotic cells. Exogenous accessory regulatory HIV-1 proteins are able to enter macrophages and modulate cellular machineries including those that affect viral transcription. Furthermore HIV-1 proteins, e.g., gp120, may exert their effects by interacting with cell surface membrane receptors, especially chemokine co-receptors. By activating the signaling pathways such as NF-kappaB, MAP kinase (MAPK) and JAK/STAT, HIV-1 proteins promote viral replication by stimulating transcription from the long terminal repeat (LTR) in infected macrophages; they are also involved in macrophage-mediated bystander T cell apoptosis. The role of HIV-1 proteins in the modulation of macrophage signaling will be discussed in regard to the formation of viral reservoirs and macrophage-mediated T cell apoptosis during HIV-1 infection.

Introduction

HIV-1 infection is characterized by sustained activation of the immune system. As macrophages, along with other cell types, are permissive to HIV-1 infection, they may be infected by the virus, resulting in signaling modulation [1]. Even uninfected macrophages may be activated by the soluble gp120 HIV-1 protein, or gp120 virion, via several signaling pathways. Additionally, soluble HIV-1 proteins such as Nef, Tat, and Vpr have been detected in serum of HIV-1 infected patients, possibly released by infected/apoptotic cells. Soluble exogenous HIV-1 proteins are able to enter macrophages and modulate both cellular machinery and viral transcription. Deciphering the signaling pathways involved in the activation of macrophages in HIV infection is critical to a better understanding of AIDS pathogenesis as this could lead to innovative therapeutic approaches.

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HIV-1 Proteins and Macrophage Signaling

Nef

Nef is a 27-kDa myristylated protein which is expressed early in the virus life cycle. Nef down-regulates the cell surface expression of CD4, CD28, and MHC class I [2]. Nef also modulates several signaling pathways [3-8]. While Nef is not considered to be a secreted protein, exogenous Nef has been detected in the sera of AIDS patients and in cultures of HIV-1-infected cells [9]. There is increasing evidence of the ability of extracellular Nef to activate signaling pathways in uninfected cells [9-13]. Indeed, Nef is internalized by MDMs and dendritic cells, but not by T cells [14], when added to cell cultures [14-16]. Recently, Qiao *et al.* [11] reported that Nef was internalized in B cells *in vitro*, thereby suppressing CD40-dependent immunoglobulin class switching. The presence of Nef in the sera of HIV-infected patients at concentrations ranging from 1 to 10 ng/mL has also been described [9]. This concentration may be higher in the lymphonodal germinal centers where virion-trapping dendritic cells, as well as virion-infected CD4+ T cells and macrophages, are densely packed [17,18]. Infected cells may release Nef through a non-classical secretory pathway or after lysis. Following this, bystander cells may internalize Nef via endocytosis, pinocytosis or other yet-

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unknown mechanisms. Regarding intracellular signaling induced by Nef treatment of MDMs, it has been reported that Nef modulates the expression of a significant number of genes as early as 2 hours after treatment [19]. This suggested that a prompt transcriptional cell reprogramming induced by Nef leads to the synthesis and the release of pro-inflammatory cytokines/chemokines, which in turn, activate STAT1 and STAT3 signal transducers and transcription activators [20,21]. In line with these results, Nef treatment of MDMs was reported to induce rapid activation of IKK/NF-kB, MAPK and IRF-3 signalling pathways. Nef induces prompt phosphorylation of three MAPKs, i.e., ERK1/2, JNK, and p38 [13,22,23]. A Nef treatment as short as 15 minutes is able to induce p38 phosphorylation, most likely due to rapid recruitment and activation of p38 signaling upstream intermediates. Exogenously added Nef induces rapid phosphorylation of the transcription factor IRF-3, the main regulator of IFN-β gene expression [24-26]. It has also been shown to induce tyrosine phosphorylation of STAT2, well known to be induced by type I IFN signaling, at an early infection stage (8 to 16 h) [22].

Macrophage activation and production of pro-inflammatory cytokines by Nef involves NF-κB activation, especially its p50/p50 homodimeric and p65/p50 heterodimeric forms. This event leads to sustained LTR activation [13,19,27]. The activation of NF-κB in macrophages treated with exogenously added Nef occurs as early as 2 hours after treatment [13,28]. NF-κB activation in primary macrophages treated with recombinant Nef is mediated via the canonical pathway, primarily involving IKKβ phosphorylation [28]. Furthermore, many of the transcripts induced in macrophages treated by Nef are encoded by genes regulated by κB-like responsive elements [19] (Figure 1). Therefore, there is evidence that exogenously added Nef plays a critical role in "hijacking" the NF-κB signaling pathway, most likely upstream of IKK, as observed after endogenous expression in macrophages [29]. This observation is in line with the role of Nef-mediated activation of NF-κB, which promotes HIV-1 replication via both direct and cytokine-mediated effects [13]. Thus, in monocyte-derived macrophages, recombinant Nef enhances the production of cytokines such as macrophage inflammatory protein-1 alpha (MIP1α), MIP1β, TNFα, IL-1β and IL-6 involved in the inflammatory response (Figure 1). Additionally, features observed in promonocytic cells and primary macrophages following exposure to recombinant Nef are very similar to those observed following TNFα treatment [30]. Both recombinant Nef and TNFα activate NF-κB, AP-1 and JNK. That recombinant Nef and TNFα activate these signaling pathways suggests the two events might modulate the cellular machinery in a similar way. Therefore, they may have the same effects on HIV-1 replication in

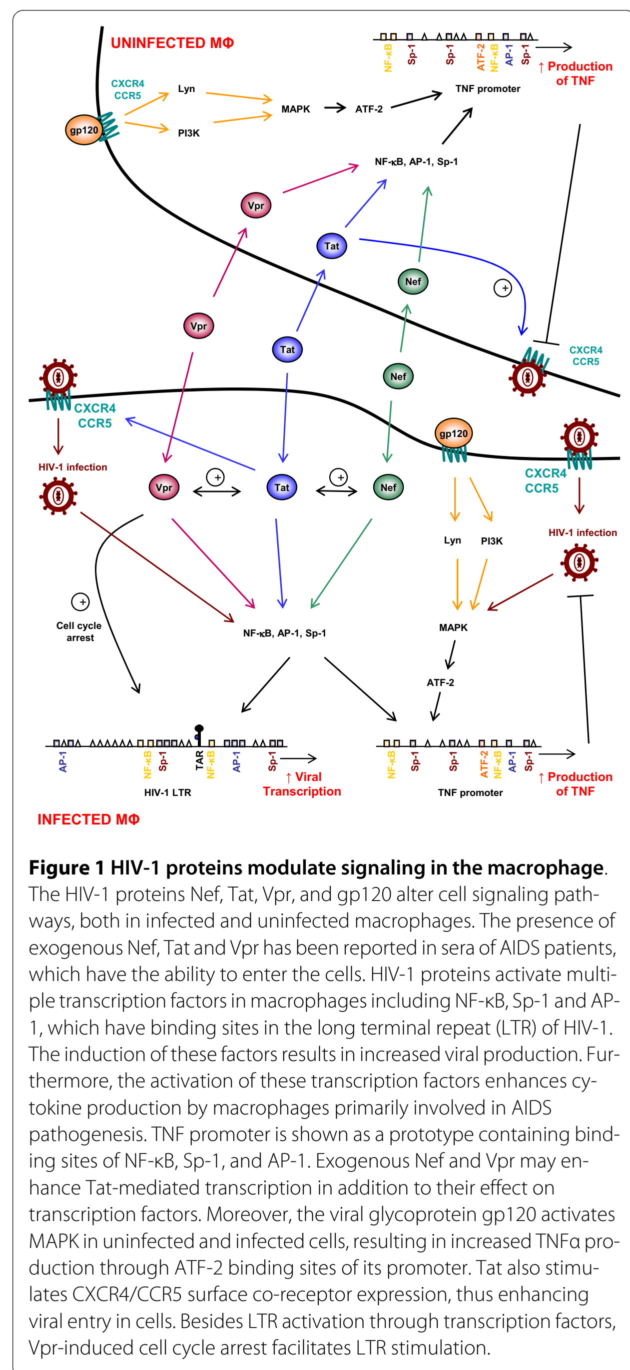

Figure 1 HIV-1 proteins modulate signaling in the macrophage. The HIV-1 proteins Nef, Tat, Vpr, and gp120 alter cell signaling pathways, both in infected and uninfected macrophages. The presence of exogenous Nef, Tat and Vpr has been reported in sera of AIDS patients, which have the ability to enter the cells. HIV-1 proteins activate multiple transcription factors in macrophages including NF-κB, Sp-1 and AP-1, which have binding sites in the long terminal repeat (LTR) of HIV-1. The induction of these factors results in increased viral production. Furthermore, the activation of these transcription factors enhances cytokine production by macrophages primarily involved in AIDS pathogenesis. TNF promoter is shown as a prototype containing binding sites of NF-κB, Sp-1, and AP-1. Exogenous Nef and Vpr may enhance Tat-mediated transcription in addition to their effect on transcription factors. Moreover, the viral glycoprotein gp120 activates MAPK in uninfected and infected cells, resulting in increased TNFα production through ATF-2 binding sites of its promoter. Tat also stimulates CXCR4/CCR5 surface co-receptor expression, thus enhancing viral entry in cells. Besides LTR activation through transcription factors, Vpr-induced cell cycle arrest facilitates LTR stimulation.

mononuclear phagocytes [28]. Exogenous Nef may modulate intracellular signaling pathways downstream of the TNFα receptors (TNFRs), and thus mimic the effects of TNFα on primary macrophages [13].

Tat

HIV-1 Tat is a virally encoded transactivating protein which plays a critical role in viral replication and is conserved in genomes of primate lentiviruses [31,32]. Tat is a HIV-1 protein reportedly detected in the sera of infected

patients as well as in the media of infected cells [33]. This suggests that it might have a role both as endogenous modulator of cellular functions within infected cells and act on bystander cells. Tat activates monocytes, macrophages, and microglial cells.

Tat Action on monocytes, macrophages, and monocytic cell lines

The HIV-1 Tat protein is essential for efficient transcription of viral genes and for viral replication. It also regulates the expression of several cellular genes and interferes with intracellular signaling [34,35]. The mature protein has a variable size, ranging from 86 to 101 amino acids. It is organized in functional domains required for transactivation activity. The C-terminus contains an RDG motif which mediates cell adhesion and Tat binding to integrin receptors [36]. Specific Tat binding has been reported for at least three cell surface molecules including heparin sulfate, beta-integrin and chemokine receptors. Tat as well as peptides spanning its cysteine-rich region compete with cognate ligands to bind CXCR4, CCR2, and CCR3 chemokine receptors in primary human monocytes and PBMCs. Tat has also been reported to trigger Ca^{2+} mobilization in macrophages in a concentration-dependent manner through CCR2 and CCR3 [37,38]. Moreover, Tat induces the expression of CCR3, CCR5 and CXCR4 in monocytes/macrophages in a concentration-dependent manner, possibly promoting HIV-1 infection [39]. Finally, Tat has been shown to serve as chemoattractant for monocytes, and pretreatment with Tat enhanced the monocyte invasive properties [40,41].

Functional consequences of Tat activation include TNFα release from macrophages, monocytes and THP-1 monocytic cell lines [42]. Tat-induced TNFα release was dependent on NF-κB activation and mediated through the activation of protein kinase A, phospholipase C (PLC) and protein tyrosine kinase pathways [43]. Transient $[Ca^{2+}]$i release was observed in macrophages through IP3 receptor-regulated intracellular Ca^{2+} stores [43]. This Tat-induced $[Ca^{2+}]$i elevation was not dependent on extracellular Ca^{2+} or caffeine-sensitive ryanodine receptor-regulated intracellular Ca^{2+} stores but rather on the PLC, protein kinase C (PKC) and Gi/0 protein pathways. Tat-induced calcium signaling in macrophages leads to the production of pro-inflammatory cytokines and chemokines, possibly contributing to inflammation and HIV-1 neuropathogenesis.

Thus, Tat displays biological activities mimicking those mediated by TNFα [28]. HIV-1 Tat may induce the expression of TNFα and various cytokines, including IL-6, TNFβ and TGFβ as well as the expression of cytokine receptors such as the IL-4 receptor [44-49]. Like TNFα, Tat may activate NF-κB, AP-1 and MAPK, including c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) [50]. Tat activates NF-κB, JNK, and AP-1, but not MEK [50]. These results suggest that HIV-1 Tat and TNFα act through different mechanisms and that HIV-1 Tat does not activate all of the kinases involved in TNFR signaling [51]. In short, like Nef, Tat mimics the effects of TNFα resulting in the enhancement of viral replication via activation of NF-κB, AP-1, JNK, and MAPK.

Action of Tat on microglia

Tat protein is actively produced and released in the central nervous system (CNS) by infected cells [52]. Elevated Tat mRNA levels have been detected in the brain of AIDS patients [53], where Tat is believed to play a significant role in the pathogenesis of HAD through not only its direct neurotoxicity, but also through the release of deleterious products in microglial cells [54]. Although they act as CNS macrophages, microglia cells differ in many aspects from peripheral macrophages. Their morphological and functional specificity responds to cell-cell contacts and secreted factors from surrounding astrocytes and neurons. The strict separation of microglia cells from blood components is due to the blood brain barrier (BBB). This results in a down-regulated "surveillance" phenotype [55]. Microglial cells are nevertheless able to undergo activation and acquire typical macrophage functions such as phagocytosis of microbes or apoptotic bodies and the secretion of inflammatory or anti-inflammatory mediators [56,57].

Tat activates microglia and impairs major molecular mechanisms that normally prevent or shorten microglial activation. As is the case in macrophages, Tat increases microglial production of free radicals as well as pro-inflammatory cytokines and chemokines [42,43,58,59]. Tat induction of NO and inducible NO synthase (iNOS) is enhanced by IFN-γ [60]. This suggests that Tat and IFN-γ cooperatively contribute to the severity of brain damage observed in brain tissues from AIDS patients and animal HAD models.

The transcription factor NF-κB plays a central role in the regulation of inflammatory gene expression and is involved in most Tat-induced effects in microglial cultures [61]. In surveillance microglia, signals provided by astrocytes actively contribute to NF-κB down-modulation [62]. Elevated immunoreactivity for p50/p65 heterodimer subunits was found in microglia and brain macrophages of children with HIV encephalitis [63] despite repression by the surrounding cells. Likewise, nuclear staining for NF-κB in the perivascular microglia/macrophages of deep white matter and basal ganglia correlated with the severity of HIV-associated dementia in AIDS patients [64]. Interestingly, Tat-induced formation of free radicals in microglial cells occurs independently from NF-κB activation [65,66], as lipid peroxidation and oxidative stress still occur in microglial cultures exposed to Tat in the presence of NF-κB inhibitors [65]. Likewise,

the pro-oxidant activities of Tat in the N9 microglial cell line depend on MAP kinase activation [66]. Additionally, antioxidants abrogate oxidative stress rather than the other Tat-induced functions such as IL-1β, NO, and TNF-α production or IkBα degradation [65]. Thus, Tat-induced NF-κB activation in microglia may not require the formation of free radicals, although oxidative stress is contributive to its activation [67].

In different cell types, including macrophages and microglia, Tat influences cell function by modifying Ca^{2+} homeostasis [43]. Indeed, Tat possesses a cysteine-cysteine-phenylalanine domain, enabling Tat to mimic beta chemokine effects on both Ca^{2+} movements and chemotaxis [38]. In microglia, Ca^{2+} mobilization and cell migration by Tat are sensitive to pertussis toxin (PTX), but not cholera toxin. This observation supports the involvement of Gi rather than Gs type proteins, as expected for chemokine receptor stimulation [37]. Furthermore, cross-desensitization studies revealed CCR3 receptor involvement. Similar to findings in monocytes, Tat-induced Ca^{2+} signals in human microglia are characterized by rapid desensitization [68].

Nanomolar concentrations of recombinant Tat have been shown to decrease in a dose- and time-dependent manner, cAMP accumulation induced in microglial cultures by the β-adrenergic receptor agonist isoproterenol, or by forskolin, an activator of adenylyl cyclase [69]. In microglia, increased cAMP accumulation lowers potentially neurotoxic pro-inflammatory molecules [70-76] and promotes the production of neuroprotective or immunosuppressive substances [70]. Thus, Tat may interfere with cAMP's control on microglial activation.

Among the ion channels expressed by microglial cells, there are two major classes of K+-permeable channels: the delayed-outward-rectifying (Kdr) and the inward-rectifying (Kir) channels. Their expression differs in macrophages and microglia. Their expression is finely modulated by both activation and differentiation [77-81]. Chronic microglial cell treatment with high Tat concentration (≥ 100 ng/mL) up-regulates Kdr currents due to NF-κB-dependent increase in channel expression without a significant increase in Kdr currents [82]. Therefore, the hyperpolarization thus induced by Tat may has several consequences. Ca^{2+} influx depends on a hyperpolarized membrane potential and Tat's β-chemokine mimicry may thus be favored by Kdr currents. Kdr currents may also modulate the microglial respiratory burst and the transport of amino acids through voltage-dependent transporters. The latter is likely to modify the availability of amino acids for protein synthesis [83,84], as well as the dynamics of glutamate exchange between intracellular and extracellular pools. This may affect the regulation of both extracellular glutamate concentration in the vicinity of glutamate-sensitive neurons and glutathione synthesis rate in microglia [85-87].

Vpr

Vpr is a 96 amino acid-long virion-associated protein located in the cytoplasm and nucleus of HIV-infected cells [88-93]. Vpr is not essential for viral replication in T cells, but critical for HIV replication in non-dividing cells such as macrophages [94-99]. Vpr has pleiotropic effects on viral replication, cellular proliferation and differentiation, cytokine production, NF-κB-mediated transcription and apoptosis [100-103].

Vpr has been shown to induce cell cycle arrest at the G2 cell cycle phase [104-107]. G2 cell cycle arrest correlates with the inhibition of Cdc2 activity and parallels enhanced viral replication [108-110]. G2 cell cycle arrest is followed by apoptosis in HIV-infected and Vpr-expressing cells [111]. Apoptosis is mediated through the interaction of Vpr with the mitochondrion permeability transition pore. This interaction opens the pore, causing mitochondrial swelling, release of cytochrome C as well as caspase 9 and caspase 3 activation [111]. p53 tumor suppressor protein may be implicated in cell cycle arrest and apoptosis mediated by Vpr in certain cell types [107].

Vpr transactivates the viral promoter and HIV-1 LTR resulting in increased viral replication. The G2 cell cycle arrest is concomitant with high levels of viral replication in primary human CD4+ T cells. An interaction between Vpr, Sp1 and TFIIB transcription factors is required for Vpr-mediated transcriptional enhancement of HIV-1 LTR [112,113].

Vpr-mediated transactivation necessitates intact NF-κB sites and depends on Vpr's ability to stimulate p300/CBP coactivator function, which promotes cooperative interaction between the RelA subunit of NF-κB and the cyclin B1Cdc2 [114]. A structural and functional interaction between Vpr and Tat has been reported, synergistically enhancing the transcriptional activity of the HIV-1 LTR [114].

The activity of recombinant Vpr (rVpr) in macrophages has been investigated. High concentrations of rVpr as well as the carboxy-terminal Vpr peptide are cytotoxic to macrophages. However, at low concentrations rVpr was shown to enhance the activity of several transcription factors including AP-1, c-Jun, and, NF-κB [115]. Amino- and carboxy-terminal Vpr peptides retained transcription factor activation properties, albeit to a lesser extent than with the full-length rVpr. Similarly to Vpr expressed in infected cells, rVpr stimulated HIV-1 replication in acutely infected primary macrophages. Furthermore, reduced p24 production by macrophages infected with Vpr-deficient virus could be rescued by adding rVpr to culture medium [116]. Exposure to rVpr also increased transcription and p21/waf1 levels in macrophages [117].

These Vpr effects on macrophages may reflect the mechanisms by which Vpr activates the HIV-1 LTR and enhances virus replication in acutely and latently infected cells [88]. Although primarily considered to be a regulator of viral promoter transactivation, transcription factor activation may have significant effects on macrophage cellular functions [117,118]. Additionally, macrophages and PBLs produce less chemokines following recombinant Vpr treatment. This observation suggests that Vpr modulates cytokine production by interfering with NF-κB-mediated transcription [119,120].

gp120

HIV-1 infects human T cells and monocytes/macrophages through the interaction of gp120 with CD4 and the CXCR4 or CCR5 co-receptor, which determines the cellular tropism [121-131]. HIV-1 gp120 down-regulates CD4 expression in primary human macrophages through induction of endogenous TNFα [121,132-136]. Actually, TNFα down-regulates both surface and total CD4 expression in primary human macrophages at the transcription level [134,137-140]. TNFα inhibits R5 and R5/X4 HIV-1 entry into primary macrophages via downregulation of both cell surface CD4 and CCR5 and via enhanced secretion of CC-chemokines, MIP-1α, MIP-1β and RANTES [129,137,141-146]. An iterative pretreatment of primary macrophages with TNFα prior to HIV infection inhibits HIV-1 replication in primary macrophages [142]. The inhibition of HIV-1 entry into primary macrophages following TNFα pretreatment involves TNFR2 and is mediated by the secretion of CC-chemokines such as RANTES, MIP-1α, and MIP-1β[140,141]. TNFα induces the production of RANTES, MIP-1α, and MIP-1β, which in turn down-regulate cell surface CCR5 expression on primary macrophages, resulting in the inhibition of R5 HIV-1 entry [147-151]. In agreement with this observation RANTES inhibits HIV-1 envelope-mediated membrane fusion in primary macrophages [152] and inhibits the activity of the RANTES promoter containing four NF-κB binding sites which is up-regulated by TNFα [153].

Many studies conducted over the past two decades have shown that besides infection, exposure of macrophages to intact virions or soluble gp120 may exert various functional effects on macrophages, including cytokine secretion activation [121,135,154]. However, the specific pathways involved in gp120-induced responses have only been defined recently. The presence of non-infectious virion particles in excess of infectious virus, the ability of gp120 to dissociate from the transmembrane gp41 portion of Env as well as detection of circulating gp120 in infected patients [155] have raised the question of what biological activities this protein is involved in aside from mediating infection. Such studies have demonstrated the ability of gp120 to activate intracellular signaling in multiple cell types as a result of its binding to receptor/co-receptor complex. Although gp120-induced signaling has been extensively investigated in CD4+ T cells, gp120 has also been reported to activate intracellular signals in macrophages [156].

In primary human macrophages, both R5 and X4 gp120 induce calcium mobilization, although R5 gp120 elicited higher peaks and more sustained elevations than X4 gp120 [157,158]. Single-cell patch-clamp recording combined with pharmacological antagonists and current reversal potential analysis identified the ion channels associated with CCR5 and CXCR4 activation: chloride, calcium-activated potassium, and non-selective cation (NSC) channels [157]. These responses to HIV-1 gp120 were mediated by chemokine receptors, but not by CD4, since the responses to R5 Env were absent in macrophages from patients lacking cell surface CCR5 expression (CCR5Δ 32); responses to X4 gp120 were inhibited by a small molecule CXCR4 antagonist [157,159]. While R5 and X4 gp120 generally induced similar signals through CCR5 and CXCR4, respectively, certain differences were noted. R5 Env opened the calcium-activated outward K^+ channels more frequently than X4 gp120, and induced Cl- currents of greater amplitude. Gp120, instead of CXCR4 or CCR5 binding chemokines, activated the NSC channel [160].

In addition, gp120 has been shown to activate all three MAPK family members (ERK1/2, JNK, and p38) in macrophages. R5 gp120 triggered macrophage release of MIP-1, MCP-1, and TNFα. The secretion of these products was blocked by small molecule inhibitors of ERK1/2 and p38 MAPKs [39,161].

The src kinases Lyn and Hck are highly expressed in macrophages, and recent *in vitro* kinase assays demonstrated that R5 gp120 and MIP-1β activated Lyn in macrophages [162]. Neither R5 gp120 nor MIP-1β activated Lyn in macrophages derived from CCR5Δ 32 donors or in cells treated with a small molecule CCR5 inhibitor, indicating that Lyn activation was elicited through CCR5 receptor. Unlike Lyn, Hck activation did not occur in response to gp120 or chemokine stimulation [162,163]. Both a Lyn-specific peptide pseudo-substrate inhibitor and PP2, a broad src family kinase inhibitor, suppressed gp120-induced TNFα production. These results are suggestive of a signaling cascade initiated by gp120 through CCR5, involving Lyn activation of the MAPK pathway, resulting in gp120-induced TNFα release.

Several lines of evidence indicate that HIV-1 gp120/chemokine receptor interactions activate PI3K in macrophages [39,164]. This finding is based upon R5 gp120 activation of protein kinase B (PKB), a downstream target for class I PI3K and a useful indirect indicator of its activation. Furthermore, several small molecule PI3K inhibi-

tors blocked gp120-induced CCR5-mediated ERK1/2 and p38 phosphorylation, as well as TNFα release. These results not only suggest a role for PI3Ks in CCR5 signaling but also indicate that, like Lyn, PI3K acts upstream of MAPKs in the regulation of cytokine production through this pathway [39]. It is unclear which PI3K isoform is involved in these R5 gp120-induced signals, and the relationship between PI3K and Lyn remains to be determined.

Besides chemokine receptors, interactions between HIV-1 gp120 and CD4 stimulate signal transduction pathways, such as activation of PKC, generation of PKC-dependent phosphorylation of CD4, and activation of the ERK/MAPK pathway, which in turn stimulates transcription factors such as NF-kB, AP-1, and Elk-1, as well as induction of cytokine and chemokine gene expression [115,165-172]. Early inflammatory gene products such as TNFα, may stimulate HIV-1 replication in the absence of HIV-1 Tat protein. Thus, the activation of cellular signaling pathways leading to the production of cytokine and chemokine genes by HIV-1 gp120 could facilitate viral replication in the early phases of the viral life cycle [50].

Proline-rich tyrosine kinase 2 (Pyk2) activation has been suggested as a critical signalling mechanism for integrin-mediated formation of adhesion contacts in macrophages known as podosomes. Pyk2 is known to be activated by chemokines, triggering cell migration [173,174]. CCR5 and CXCR4 are both linked to Pyk2, which is activated by R5 gp120 and MIP-1β as well as X4 gp120 and SDF-1α [161]. Recently, a functional role for Pyk2 in the migration of macrophages has been demonstrated using Pyk2 knockout mice [175], suggesting that gp120 may be involved in macrophage migration.

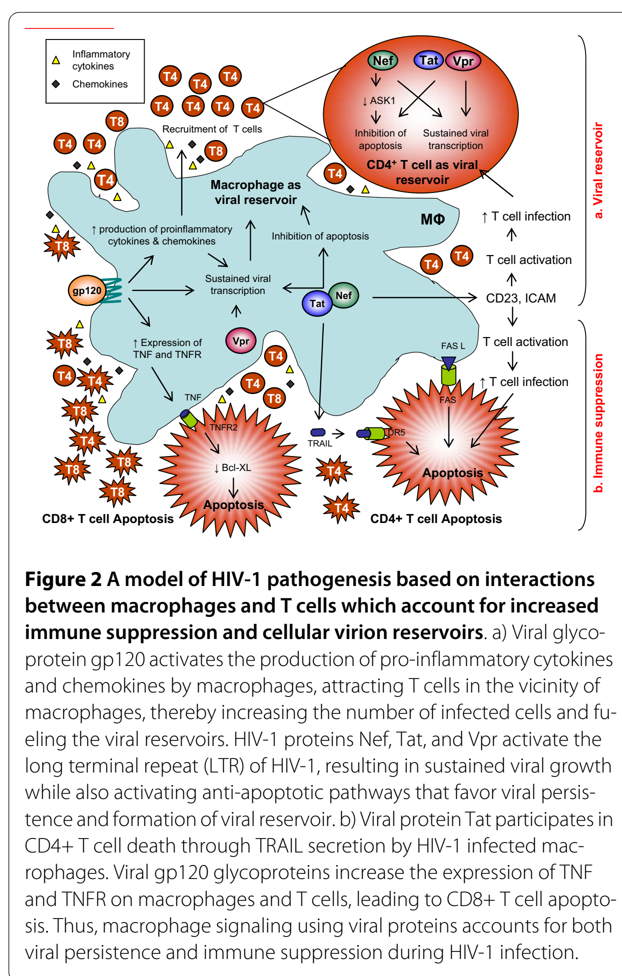

Figure 2 A model of HIV-1 pathogenesis based on interactions between macrophages and T cells which account for increased immune suppression and cellular virion reservoirs. a) Viral glycoprotein gp120 activates the production of pro-inflammatory cytokines and chemokines by macrophages, attracting T cells in the vicinity of macrophages, thereby increasing the number of infected cells and fueling the viral reservoirs. HIV-1 proteins Nef, Tat, and Vpr activate the long terminal repeat (LTR) of HIV-1, resulting in sustained viral growth while also activating anti-apoptotic pathways that favor viral persistence and formation of viral reservoir. b) Viral protein Tat participates in CD4+ T cell death through TRAIL secretion by HIV-1 infected macrophages. Viral gp120 glycoproteins increase the expression of TNF and TNFR on macrophages and T cells, leading to CD8+ T cell apoptosis. Thus, macrophage signaling using viral proteins accounts for both viral persistence and immune suppression during HIV-1 infection.

Macrophage Signaling and HIV-1 Pathogenesis

In this section, we report that several HIV-1 proteins may modulate the macrophage signaling pathway resulting in T lymphocytes depletion and viral cellular reservoir formation, especially in macrophages [176].

Macrophage signaling and T cell apoptosis

Increased spontaneous and activation-induced apoptosis of peripheral CD4+ T cells from HIV-infected patients is observed *ex vivo* in lymph nodes of HIV-infected patients and of SIV-infected macaques [177-180]. Deciphering the molecular mechanisms involved in CD4+ T cell apoptosis in HIV-infected patients is critical to understanding HIV pathogenesis.

In macrophages, Nef has been shown to activate multiple cellular pathways, possibly leading to increased infection of adjacent T cells through bystander mechanisms involving T cell activation (Figure 2). It has been shown that Nef-expressing macrophages enhance resting CD4+ T cell permissiveness through a complex cellular and soluble interaction involving macrophages, B cells, and CD4+ T cells [29]. Nef expression within macrophages via adenoviral vectors has been shown to induce the secretion of soluble CD23 and ICAM, resulting in up-regulation of costimulatory B cell receptors, including CD22, CD54, CD58, and CD80. This leads to T cell activation upon interaction with B cells via these costimulatory receptors, thus enabling the generation of non-productive or productive reservoirs, depending on the interactions [29].

Furthermore, Nef has been reported to prevent Fas- and TNF-receptor-mediated deaths observed in HIV-infected T cells via interaction with the apoptosis signal regulating kinase-1 (ASK-1). Nef inhibits ASK-1, caspase 3 and caspase 8 activation, resulting in apoptosis blockade in HIV-infected cells [181-184]. Apoptosis was measured in productively infected CD4+ T lymphocytes using a reporter virus and a recombinant HIV infectious clone expressing the green fluorescent protein (GFP) in the presence and absence of autologous macrophages. The survival of productively infected CD4+ T lymphocytes has been shown to require Nef expression and acti-

vation by TNFα expressed on macrophage surface, thereby participating in the formation and maintenance of viral reservoirs in HIV-infected patients [184].

In addition to the macrophage-mediated formation of T cell reservoirs, *in vitro* culture models demonstrate that uninfected CD4+ T cells undergo apoptosis upon contact with HIV-infected cells; for example mononuclear phagocytes [180]. Macrophages play a major role in this process, suggesting that apoptosis-inducing ligands expressed by macrophages mediate apoptosis of susceptible CD4+ T cells [159,185-187]. Activated macrophages produce TNFα following HIV infection *in vitro* [135]. TNFα is released as a soluble factor or expressed on the surface of macrophages under a membrane-bound form that primarily targets TNFR2 rather than TNFR1 [188,189]. TNFR2 stimulation may trigger T cell apoptosis, especially in CD8+ T cells [188]. TNFα and TNF receptors are increased in HIV-infected patients and inversely correlated with CD4+ T cell counts [190]. TNFα is expressed on the surface of activated macrophages, and cell surface TNFR2 is not increased on CD4+ infected T cells. Therefore, for the most part, the apoptosis of CD4+ T lymphocytes is mediated via Fas/Fas ligand interaction [185,186,191]. TNFα causes death at a later stage than Fas and may be transduced through TNFR2, which does not contain homology to the Fas death domain and uses different signaling pathways than TNFR1 [115,185]. Recently, Tat has been reported to induce secretion of soluble TNF-related apoptosis-induced ligand (TRAIL) in human macrophages, leading to the death of bystander CD4+ T lymphocytes [73]. Thus, the production of TRAIL by Tat-stimulated monocytes/macrophages is likely to be an additional mechanism by which HIV-1 infection destroys uninfected bystander cells.

CD8+ T cell apoptosis during HIV infection has been shown to result from the interaction between membrane-bound TNFα expressed on the surface of activated macrophages and TNFR2 expressed on the surface of activated CD8+ T cells [158]. Both membrane-bound TNFα and TNFR2 are up-regulated on macrophages and CD8+ T cells, respectively, following CXCR4 stimulation by HIV gp120. However, CCR5 may also play a role, albeit minor [158]. TNFR2 stimulation of T cells results in decreased intracellular levels of apoptosis protective protein Bcl-XL, a member of the Bcl-2 family [192]. Impaired induction of Bcl-XL has been observed in PBMC isolated from HIV-infected patients [193]. Therefore, TNFR2 stimulation of CD8+ T cells by membrane-bound TNFα expressed on the surface of macrophages might decrease the intracellular levels of anti-apoptotic proteins resulting in CD8+ T cell death.

Additionally, chemokines and activated macrophages have been reported to play a role in HIV-1 gp120-induced neuronal apoptosis [194,195].

Macrophage signaling and formation of viral reservoirs

Whereas CD4+ T cells die within a few days after becoming infected with HIV, infected macrophages seem to persist for months, continuing to release viruses. Several reasons may explain why macrophages are a major cellular reservoir of virions during infection (Figure 2). Macrophages are more resistant than T cells to HIV-induced apoptosis and therefore allow for sustained viral production without fatal cell death. Persistent HIV infection of macrophages results in increased NF-κB levels, involved in the resistance to TNFα-induced apoptosis. Macrophages release CC-chemokines which have the ability to attract CD4+ and CD8+ T lymphocytes in their vicinity [196]. They may also block the entry of R5 HIV-1 virions into CD4+ target cells [122]. CC-chemokine production is often associated with that of pro-inflammatory cytokines, such as TNFα and IL-1β, which stimulate the transcription of HIV LTR via activation of NF-kB [197,198]. Additionally, TNFα may block entry of R5 HIV-1 strains into macrophages via a decreased expression of CCR5 on cell surfaces [137,141,142,147]. Thus, CC-chemokines and pro-inflammatory cytokines facilitate the recruitment and productive infection of CD4+ T lymphocytes via increased viral transcription, while regulating the entry of virions into macrophages, thereby preventing macrophage superinfection. Additionally, apoptosis inhibition in HIV-1 infected T cells enhances virus production and facilitates persistent infection [199]. HIV-1 proteins, by modulation of the TNFR signaling pathway, lead to the formation of viral reservoirs, especially in primary macrophages [50]. Altogether, the data indicate that both viral and cellular factors are involved in the controlled and sustained production of virions in infected CD4+ T lymphocytes and macrophages, thereby expanding the viral reservoir which fuels disease progression.

Conclusion

The macrophage is essential in the loss of T lymphocytes and formation of viral reservoirs; it plays a critical role in HIV-1 disease progression. Several HIV-1 proteins modulate signaling in infected and bystander macrophages, thereby facilitating disease progression. A better understanding of the manner by which HIV-1 modulates signaling in macrophages may be instrumental in the development of new therapeutic approaches that may ultimately restrict or decrease the size of cellular virion reservoirs in HIV-1-infected patients.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
GH was responsible for drafting and revising the manuscript as well as organizing the content. GG was responsible for drafting and revising the section

"Action of Tat on microglia". KAK created Figures 1 and 2. WA assisted in revising the manuscript.

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