Macrophages and CD4+ T cells are natural target cells for HIV-1, and both cell types contribute to the establishment of the viral reservoir that is responsible for continuous residual virus replication during antiretroviral therapy and viral load rebound upon treatment interruption. Scientific findings that support a critical role for the infected monocyte/macrophage in HIV-1-associated diseases, such as neurological disorders and cardiovascular disease, are accumulating. To prevent or treat these HIV-1-related diseases, we need to halt HIV-1 replication in the macrophage reservoir. This article describes our current knowledge of how monocytes and certain macrophage subsets are able to restrict HIV-1 infection, in addition to what makes macrophages respond less well to current antiretroviral drugs as compared with CD4+ T cells. These insights will help to find novel approaches that can be used to meet this challenge.

HIV-1-infected monocytes and macrophages play crucial roles in establishing the viral reservoir and in the etiology of multiple HIV-1-associated pathologies. In this article, we discuss current knowledge on HIV-1 replication in monocytes and macrophages, with emphasis on macrophage polarization/activation and associated host restriction(s) of HIV-1 replication, and the role of monocytes/macrophages in HIV-1-associated pathologies. We also discuss several genomics studies of HIV-1 replication in monocytes/macrophages that have identified new host factors specifically relevant to HIV-1 replication in these cells. As HIV-1 infection of monocytes and macrophages is one of the barriers to eradication of HIV-1 from the body, the identification of these host factors may direct the development of novel therapeutic strategies.

**Monocytes & macrophages**

Cells from the monocyte–macrophage lineage are critical immune cells responsible for a wide range of both innate and adaptive immune functions. Monocytes derive from a myeloid progenitor cell in the bone marrow and migrate into the blood. In the blood and in tissues, monocytes are exposed to different stimuli and differentiate into different macrophage subpopulations (see later). Three major subpopulations of monocytes have been identified in blood, based on variations in the expression level of surface receptors CD14 and CD16 and cytokine production: classical monocytes (CD14++ CD16−), intermediate monocytes (CD14++ CD16+) and nonclassical monocytes (CD14+ CD16++ [1] and reviewed by [2–4]). Nonclassical monocytes are derived from classical monocytes, are more mature and also express other Fc-receptors (CD32 and CD64). Furthermore, they are potent producers of inflammatory cytokines, such as TNF-α, upon lipopolysaccharide (LPS) stimulation.

**Polarization of macrophages**

Circulating blood monocytes continuously repopulate the macrophage population that resides in the tissues. In tissue, monocytes are exposed to a variety of different cytokines and stimulating factors, such as bacterial products, and depending on the location and the stimuli, polarization occurs. Polarization of macrophages has been the subject of several excellent reviews [5–7]. In this article, we mainly discuss the various polarized subsets and the specific stimuli that induce them, as these specific stimuli have also been studied with respect to their effect on macrophage susceptibility to HIV-1. Polarized macrophages have been broadly divided into two groups based on their function and cytokine production pattern: M1 and M2 macrophages, with M1 macrophages typically producing high levels of IL-12 and low levels of IL-10, and M2 macrophages producing high levels of IL-10 and low levels of IL-12 (Figure 1). M1 macrophages are classically activated by IFN-γ, TNF-α and bacterial products (LPS; reviewed in [8–10]). These cells are potent effector cells that activate Th1 responses to kill microorganisms and produce high concentrations of proinflammatory cytokines (e.g., IL-1, IL-6, IL-12 and TNF-α). Alternative activation of macrophages (M2) results in three functionally classified subsets (Figure 1). M2a (alternative) activation

**Keywords**

- AIDS dementia = genomics
- HIV-1 = host factors
- macrophage = monocyte
- polarization = reservoir
is induced by IL-4/IL-13. These cells promote Th2 and type II inflammation responses, produce high levels of anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist, and are involved in tissue repair. M2b activation is induced by immune complexes and Toll-like receptor antagonists. These cells produce proinflammatory cytokines (IL-1, IL-6 and TNF-α), but in contrast to M1 macrophages, they also produce IL-10. M2b macrophages play a role in Th2 activation and immunoregulation. Finally, an M2c (deactivated) state is induced by IL-10. M2c macrophages are important suppressors and regulators of the immune response and produce IL-10 and TGF-β. Interestingly, the various subpopulations of monocytes and macrophages are not all equally susceptible to HIV-1 infection, and the various blocks to HIV-1 replication in the different subpopulations can teach us many things about the host proteins that either restrict or enable HIV-1 replication. This will be illustrated in the next section.

HIV-1 infection of monocytes/macrophages in vitro

Monocytes
Although freshly isolated monocytes express reasonably high levels of CD4 [11], expression of C-C chemokine receptor 5 (CCR5) is low [12,13]. Nevertheless, HIV-1 is able to efficiently enter the cells. However, the process of reverse transcription is not completed, indicating that the block in virus replication in vitro is at an early post-entry level [14,15]. At present, it is not clear whether monocytes lack cellular factors that are required for virus replication or
express a cellular factor that restricts virus replication (see also below). It has been suggested that the block at reverse transcription is due to the low levels of dNTP in nondividing cells, as was observed in monocyte-derived macrophages (MDM) [16–18]. Others suggested that apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3) proteins might be involved in the resistance of monocytes to HIV-1 infection. APOBEC3 proteins encode cytidine deaminases that can edit the viral genome during the reverse transcription process. The HIV-1-encoded accessory protein Vif is able to neutralize APOBEC3 proteins and allows the virus to replicate in the presence of APOBEC3 [19,20]. Peng et al. observed high levels of APOBEC3A and APOBEC3G in freshly isolated monocytes that decreased during differentiation into macrophages [21]. Moreover, in the CD16+ monocyte subset that is better able to support HIV-1 replication after entry (see later), inactive high-molecular-mass APOBEC3G was observed, while CD16+ monocytes contained active low-molecular-mass APOBEC3G [22,23]. Silencing of APOBEC3A expression in monocytes reverses resistance to HIV-1 infection, suggesting that APOBEC3A is indeed involved in the restriction to infection in undifferentiated monocytes [21]. More recently, it was suggested that anti-HIV microRNAs block infection in freshly isolated monocytes [24], although the relevance of this to the early post-entry restriction is not clear at this stage. Finally, HIV-1 gene expression in undifferentiated monocytes is also impaired owing to repression of cyclin-T1 expression and decreased phosphorylation of cyclin-dependent kinase 9 (CDK9) in these cells [25,26].

**Monocyte-derived macrophages**

As outlined above, in vitro, freshly isolated monocytes are almost refractory to HIV-1 infection and become susceptible to infection when they are allowed to differentiate into macrophages during culture [14,15]. Moreover, HIV-1 susceptibility of macrophages is even more enhanced when exogenous macrophage colony-stimulating factor (M-CSF) or granulocyte M-CSF (GM-CSF) is added [27–32]. The increase in susceptibility of macrophages to HIV-1 infection during differentiation correlates with enhanced CCR5 expression during differentiation, especially in the presence of M-CSF and GM-CSF [12,13,33]. Despite expression of C-X-C chemokine receptor type 4 (CXCR4) on macrophages, these cells are refractory to infection with most CXCR4-using HIV-1 isolates in vitro [34]. This indicates that CCR5 and not CXCR4 is functional as an HIV-1 co-receptor in macrophages, perhaps owing to the lack of an association between CXCR4 and CD4 on the cellular membrane [35]. Moreover, CXCR4-using isolates have been reported to have a higher dependency on CD4 than do CCR5-using isolates, and CD4 expression is lower on macrophages than on CD4+ T cells [36,37]. However, although most macrophage-tropic HIV-1 strains use CD4 as receptor and CCR5 as co-receptor, certain macrophage-tropic strains use CXCR4 instead of CCR5, and not all CCR5-using viruses have the capacity to infect macrophages [38]. Furthermore, not only binding to the co-receptor but also the subsequent intracellular signaling influences the ability of HIV-1 to replicate in macrophages [39–41]. Envelopes derived from CXCR4-using isolates in addition to CCR5-using isolates that did not replicate in macrophages failed to efficiently mobilize calcium.

While unipolarized macrophages become increasingly susceptible to HIV-1 infection during differentiation, upon exposure to polarizing stimuli, the susceptibility of macrophages to HIV-1 infection decreases. HIV-1 replication in polarized macrophages can be blocked at multiple levels, also depending on the polarizing agent, as described in more detail later.

**M1 macrophages**

Although the effect of macrophage polarization on HIV-1 infection has not been studied extensively, it was observed that M1 and M2 polarization of macrophages results in resistance to HIV-1 infection (Figure 1) [42]. Since CD4 and CCR5 expression is strongly downregulated in M1-polarized macrophages and the production of the CCR5-binding chemokines, C-C chemokine ligand (CCL)3, CCL4 and CCL5, is strongly enhanced, HIV-1 infection in M1 macrophages is most likely blocked at the level of virus entry. This is in agreement with the effect of individual M1-polarizing cytokines on HIV-1 infection in macrophages: IFN-γ treatment alone also blocks HIV-1 replication in macrophages, and in most studies, a decrease in proviral DNA is observed [27,43–46]. IFN-γ-polarized macrophages produce CCL3, CCL4 and CCL5 and these cytokines compete with HIV-1 binding to CCR5 and downregulate CCR5 expression, thereby interfering with HIV-1 infection at the level of virus entry [46–48]. Similarly, TNF-α induces increased expression of CCL3, CCL4 and CCL5 and reduced expression of CCR5 expression in macrophages, thus also resulting...
in a block of HIV-1 infection at the level of virus entry [47–49]. However, a stimulatory effect of TNF-α on HIV-1 replication in macrophages has also been reported [50], which could be due to TNF-α-induced enhancement of the HIV-1 long terminal repeats (LTRs)-mediated HIV-1 transcription [51–53]. At this stage, the discrepancy between the observed inhibitory and stimulatory effect of TNF-α cannot be explained.

Other proinflammatory cytokines produced by M1-activated macrophages may also modulate HIV-1 infections in these cells. Studies have shown that HIV-1 replication is inhibited at an early step by IL-6 [54] and IL-12 [55], but is enhanced by C-X-C chemokine ligand 10 [56] and CCL2 [57], at early and late steps in the replication cycle, respectively.

In summary, HIV-1 replication in M1 macrophages is inhibited early, at the level of co-receptor interaction and at later steps, explained at least in part by tripartite motif-containing protein (TRIM)22 (as explained below), but other uncharacterized restriction factors are likely to be involved as well.

M2 macrophages

In M2a macrophages (induced by IL-4/IL-13), a more sustained inhibitory effect at a late stage in the virus life cycle is observed (Figure 1, M2a macrophages) [42]. Treatment of HIV-1-infected MDM with IL-4 alone stimulates HIV-1 reverse transcription, p24 production and HIV-1 transcription by accelerating nuclear import of NF-κB [58–61]. However, treatment of MDM prior to viral inoculation significantly reduces reverse transcription and p24 production [31,62,63], associated with the reduced proliferative capacity, advanced maturation state and levels of cellular cofactors of the IL-4-treated MDM [62,64]. This restriction occurs at a pretranscriptional level [65]. Treatment with IL-13 also inhibits HIV-1 replication, similar to IL-4 [31,63].

Resting macrophages can be activated into Mb2 macrophages by immune complexes that bind to the Fcγ receptor on the cell surface. Using human IgG to cross-link Fcγ receptors on macrophages either before or after viral inoculation strongly inhibits replication of both HIV-1 R5 and X4 strains (Figure 1, M2b macrophages). Although human IgG treatment decreases expression of CD4 and CCR5, inhibition of HIV-1 replication in this subset of macrophages was demonstrated to take place at a post-entry level, more specifically at integration into the host genome [66,67].

Induction of macrophages by IL-10 results in the inhibition of viral replication at a late point during infection (Figure 1, M2c macrophages), probably at the level of viral assembly [68,69]. However, others demonstrate that treatment of infected MDM with IL-10 and TNF-α increases HIV-1 replication, probably owing to a synergistic effect [70].

In summary, restrictions of HIV-1 replication in M2 macrophages occur at multiple post-entry steps. The host factors responsible for these restrictions have not yet been identified, although IL-10-mediated degradation of cyclin-T1 may be responsible for inhibition in M2c macrophages.

HIV-1 infection of monocytes/macrophages in vivo

Initially, it was believed that monocytes in HIV-1-infected individuals were not infected. However, detailed studies have revealed low levels of replication in circulating monocytes and have demonstrated this to be a clinically relevant compartment in vivo as viral quasispecies may evolve independently from the CD4+ T cell-infecting species [71–74]. Thus, there remains an intriguing discrepancy between in vivo and in vitro HIV-1 replication in monocytes, and so far, no experimental work has been published that can explain this difference. Possibly, the observed block at reverse transcription in vitro is simply a result of in vitro conditions that do not sufficiently mimic the in vivo situation. Alternatively, since in the bone marrow, monocytes originate from stem cells that undergo at least three stages of differentiation (i.e., monoblast, promonocyte and monocyte), they could have been infected before they were released into the circulation shortly after the completion of S phase [75,76].

There is evidence that CD16+ monocytes are more likely to be infected by HIV-1 in vivo [23,77]. Moreover, HIV-1 infection induces expansion and permissiveness of this monocyte subset [78]. These CD16+ monocytes can become infected while circulating in the blood and can subsequently migrate to tissues and differentiate into macrophages. At the tissue level, macrophages are exposed to many tissue-specific stimuli and may acquire tissue-specific functions, such as higher levels of scavenger receptors on alveolar macrophages and bone-remodeling functions on osteoclasts. In HIV-1-infected individuals, HIV-1 has been detected in tissue macrophages of nearly all tissues: among others, in brain (microglial...
cells) [79], lung (alveolar macrophages) [80-82], kidney [83], intestine [84,85] and liver (Kupffer cells) [86,87]. Macrophages from different tissues display differences in susceptibility. Intestinal macrophages demonstrated reduced permissiveness to HIV-1 infection owing to differential expression of host proteins required for entry and replication of the virus [88,89], whereas macrophages in the vaginal mucosa are more permissive to HIV-1 infection [90]. The enhancing effects of GM-CSF on HIV-1 replication in macrophages were corroborated in vivo by results showing lower GM-CSF levels in cord blood in HIV-1-exposed, uninfected infants [91]. In addition, viral replication was enhanced in *Mycobacterium tuberculosis/HIV-1-*coinfected cultures, and was associated with increased levels of GM-CSF [92]. The host factors that affect HIV-1 susceptibility and replication will be discussed in the following section.

The infection of tissue macrophages has implications for both the HIV-1 infection-associated pathologies as well as for treatment. For example, the infection of macrophages in the brain is associated with severe neurological pathologies. The clinical consequences of HIV-1 infection of macrophages in specific tissues are also discussed in more detail below.

While certain diseases, such as cancer and obesity, may drive a phenotypic switch in the macrophage population (reviewed in [5]), these data are not readily available for HIV-1 infection in vivo. However, HIV-1 infection has been shown to affect macrophage function: HIV-1-infected MDM have deficiencies in phagocytosis, apoptosis and pathogen recognition, either due to the effect of viral proteins like Nef [93], or alterations in important signaling pathways that are usually triggered by Toll-like receptors [94] or M-CSF [95]. Although the many direct and indirect effects of HIV-1 infection are likely to influence the macrophage population in vivo, no clear pattern has emerged to date (reviewed in [96]).

### The role of host factors in HIV-1 infection of human macrophages

Here, we provide an overview of host proteins that either enable or restrict HIV-1 replication in MDM. To avoid duplication, host factors that are discussed in more detail in other sections of this article (‘HIV-1 infection monocytes/macrophages in vitro’ and ‘Genomics and HIV-1 infection in monocytes/macrophages’), such as APOBEC3G and cystatin B, will only be mentioned briefly.

### HIV-1 dependency factors

HIV-1 requires host proteins for its replication, such as its cellular receptor CD4 and co-receptors CCR5 or CXCR4, for binding and entry. Genetic variation in CCR5 illustrates the profoundness of its dependence on host proteins. Macrophages from donors homozygous for a 32-bp deletion in the gene encoding for CCR5 (CCR5 wild-type [wt]/Δ32 genotype) are less permissive for HIV-1 infection than cells from donors with the wt/wt CCR5 genotype [97,98], whereas the absence of CCR5 on the cell surface (Δ32/Δ32 genotype) results in complete resistance to infection with a CCR5-using virus [98-100]. Furthermore, the CCR5 wt/Δ32 genotype was found to have an effect on disease progression and HIV-1 acquisition [101-104]. Interestingly, the CCR5 Δ32 genotype can only explain part of the large observed variability in the *in vitro* replication of HIV-1 in macrophages [98,105-108]. In addition, experiments using vesicular stomatitis virus glycoprotein-pseudotyped HIV-1 also suggest the presence of other, post-entry, HIV-1 dependency or restriction factors in these cells [98].

Additional HIV-1 dependency factors (HDFs) have been identified by study of the biology of HIV-1 infection in MDM (see Table 1 for all known HDFs in monocytes/macrophages), such as Alix (AIP1) and cyclin-T1. Alix is an endosomal sorting factor involved in the budding and scission of new virions [109]. The cellular protein cyclin-T1 is hijacked by HIV-1 to enhance its replication [110]. Early in infection, mainly non-full-length HIV-1 transcripts are generated, resulting in the translation of the HIV-1 protein Tat. HIV-1 Tat recruits the host proteins cyclin-T1 and CDK9 to form a complex that binds the HIV-1 LTR and enhances elongation of HIV-1 transcripts. Infection with HIV-1 promotes cyclin-T1 protein expression [110], whereas IL-10 induces proteosomal degradation of cyclin-T1, which in turn has a suppressive effect on HIV-1 replication in macrophages [111].

Binding of NL-IL6, a member of the CCAAT enhancer binding protein-β (C/EBPβ) family of transcription factors, to sites in the HIV-1 LTR is required for HIV-1 replication in monocytes and macrophages [112], but not for replication in CD4+ T cells [113,114]. However, the two isoforms that originate from the C/EBPβ gene differ in functionality. While the large isoform functions as transcriptional activator, the small isoform is a dominant negative transcription factor that blocks viral DNA transcription [115-117].
The dependency of HIV-1 on cystatin B has recently been shown by multiple independent proteomic studies, which are discussed in more detail in the proteomics section below.

Hundreds of potential HDFs have been identified in genome-wide siRNA screens using cell lines [118–121]. Although the overlap in the genes identified between the studies was limited, possibly because of differences in experimental conditions and readouts, three genes were found in at least three of the siRNA screens: RELA, MED6 and MED7 [122]. Not all newly identified HDFs will be suitable drug targets, since only a small fraction of the human genome represents druggable targets [123]. Especially host proteins that easily bind small-molecule drugs that can modulate the function of the protein are of particular interest (see Table 1 for the druggability of the HDFs in MDM). Future studies will need to determine the relevance and druggability of these newly identified HDFs to HIV-1 infection of monocytes/macrophages.

### HIV-1 restricting factors

While HIV-1 is apt at using cellular proteins for its own replication, host cells also contain antiviral proteins that restrict its replication. Factors inhibiting HIV-1 at multiple stages of its replication cycle have been identified, such as APOBEC3, tetherin and TRIM proteins. While the relevance of TRIM5α in monocytes/macrophages has not yet been clearly demonstrated, TRIM22 is expressed in macrophages and exhibits anti-HIV-1 activity (see Table 1 for all known restriction factors in monocytes/macrophages). TRIM22 interferes with HIV-1 transcription, most likely by suppression of the activity of the HIV-1 LTR [124,125]. However, inhibition of virus production at a late stage in the replication cycle through disruption of cellular trafficking of viral proteins has also been observed, although not in MDM but in cell lines, which may not accurately reflect processes in primary macrophages [126].

Tetherin, also known as BST2, was recently identified as a restriction factor blocking viral release from the cellular membrane [127]. High expression and inhibitory activity of tetherin was also found in MDM [128]. However, this restriction factor was only found to be efficient in blocking virion release in the absence of sufficient amounts of the HIV-1-encoded accessory protein Vpu [127]. Although the exact mechanism by which Vpu antagonizes tetherin is still unclear, it now seems that Vpu interferes with the surface delivery of the tetherin protein by intracellular sequestration and proteosomal degradation [129].

Two other recently described HIV-1 restriction factors in macrophages are OTK18 and p21. OTK18 (ZNF175) is a DNA zinc-finger protein that can suppress HIV-1 LTR promoter activity by binding to multiple sites in the LTR, and its expression is increased after HIV-1 infection in MDM [130,131]. Knock-down of the cyclin-dependent kinase inhibitor p21 was found to increase HIV-1 replication in MDM and also enhanced reverse transcription and integration [132–134]. However, the precise mechanism by which p21 restricts replication of HIV-1 remains poorly understood.

The protein nicotinamide phosphoribosyltransferase (NAMPT) has been recently identified as a novel restriction factor in MDM. Its

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<th>Protein</th>
<th>HDF/RF</th>
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<tr>
<td>Alix</td>
<td>HDF</td>
<td>Budding</td>
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<td>APOBEC3</td>
<td>RF</td>
<td>Reverse transcription</td>
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<td>C/EBPB (large)</td>
<td>HDF</td>
<td>Transcription</td>
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<td>C/EBPB (small)</td>
<td>RF</td>
<td>Transcription</td>
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<td>CCR5</td>
<td>HDF</td>
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<td>Cystatin B</td>
<td>HDF</td>
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<td>NAMPT</td>
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<td>OTK18 (ZNFI75)</td>
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<td>Tetherin (BST2)</td>
<td>RF</td>
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1 Druggable (Human Druggable Genome siRNA Set V4.0 from Qiagen [289]).
2 Maraviroc (Pfizer) is a CCR5 inhibitor that has been approved by the US FDA [290].
3 Not druggable (Human Druggable Genome siRNA Set V4.0 from Qiagen [289]).

HDF: HIV-1 dependency factor; RF: Restriction factor.
identification and effect on HIV-1 replication are discussed in more detail in the transcriptomics section below.

### Role of macrophages in HIV-1 transmission

Macrophages can transmit HIV-1 to other cells, but it is also believed that macrophages play an important role in the spread of the virus from host to host. Both processes are discussed below.

#### Cell-to-cell transmission

HIV-1-infected macrophages can transmit the virus to uninfected CD4+ T cells. Transmission of the virus from macrophages to CD4+ T cells is accompanied by activation of the T cells and it has been shown that cell-to-cell transmission is more efficient than infection by cell-free virus where there is no contact between the cell surfaces [135–137]. The virological synapse, the structure between the infected cell and an uninfected permissive target cell, is used for the transfer of HIV-1. Earlier work provided evidence that HIV-1 buds from the leading pseudopod of the MDM, possibly to increase the chance that HIV-1 will be transmitted when the macrophage encounters a target cell [140]. Recently, it has been demonstrated that HIV-1 infection of macrophages induces the formation of tunneling nanotubes in these cells. The tunneling nanotubes are hijacked by the virus to spread HIV-1 to the connected cell [141]. In addition, macrophages express mannose receptors that can bind and endocytose HIV-1. This process does not result in HIV-1 infection of the macrophage, but allows subsequent transmission of HIV-1 to CD4+ T cells [142–144]. Recently, expression of mannose receptor on microglia and uptake of HIV-1 via this receptor by these cells has also been demonstrated [145]. The longevity of macrophages and the high frequency at which virus can be transmitted to CD4+ T cells clearly show the importance of macrophages in HIV-1 infection and pathogenesis [138]. In addition, there is evidence from in vitro studies that DC-SIGN on macrophages might play a role in the transmission of HIV-1 from macrophages in colostrum (early breast milk) to recipient cells in the newborn child [146,147].

#### Host-to-host transmission

Macrophages are widely believed to be among the first cells to become infected following exposure to HIV-1, and to be important for the establishment of infection. HIV-1 transmission occurs mainly through mucosal tissue after translocation of the virus across the epithelium. Since macrophages constitute the largest population of immune cells in the subepithelial lamina propria, it is thought that macrophages play a role in the transmission of the virus from host to host [148]. Organ culture systems derived from cervical tissue allowed the identification of CD4+ T cells as the first cells to become infected after contact with cell-free HIV-1 R5 virus, but also helped to identify Langerhans cells and macrophages as early target cells for HIV-1 infection [149]. In addition, it has been demonstrated that macrophages are present in the mucosa and submucosa of the human vagina and foreskin, which are also early sites of HIV-1 replication before the virus disseminates [90,150]. Furthermore, macrophages are long-lived cells that are less susceptible to the cytopathic effects of HIV-1 and that do not migrate out of these tissues. Finally, the predominant infection of macrophages by CCR5-using HIV-1 may be one of the factors contributing to the predominant transmission of these variants from host to host [151,152].

### Macrophages as a reservoir for HIV-1

Despite impressive results obtained with combination antiretroviral therapy (cART), residual viremia can still be detected when using highly sensitive methods [153,154]. Furthermore, an increase in viral RNA derived from an additional source other than resting CD4+ T cells is observed with intermittence of cART [155,156]. There are several cell-specific reasons as to why macrophages facilitate the formation of this viral reservoir: infection of macrophages with HIV-1 is not lytic to these cells [157,158], macrophages are more resistant to the cytopathic effects of HIV-1 infection [159], HIV-1 infection induces antiapoptotic mechanisms in macrophages [160,161] and macrophages are long-lived cells. In addition, the poor tissue penetration of certain antiretrovirals prevents the inhibition of HIV-1 replication in macrophages that are residing in so called ‘sanctuary sites’, such as the testis [162,163], gut-associated lymphoid tissue [164–166] and brain [167]. Results from a recent study by Carter et al. demonstrated that the bone marrow also harbors latently infected cells and forms an HIV-1 reservoir [168]. Thus, HIV-1 infection in macrophages is not lytic and virus may accumulate in intracellular vacuoles, allowing the cells to harbor virus for a prolonged period of time. Indeed, in addition to resting memory CD4+ T cells, HIV-1-infected monocytes/macrophages are...
thought to be an important reservoir for the virus during chronic infection [169–171]. HIV-1 has also been shown to replicate in monocytes/macrophages during cART, even when no virus is detectable in the plasma. Moreover, HIV-1-infected macrophages may also contribute to the rebound in viral load that has been observed in patients upon discontinuation of cART [171–173].

Alternatively, host factors such as C/EBPβ may restrict HIV-1 transcription in infected tissue macrophages, thereby contributing to viral latency in macrophages and extending the macrophage HIV-1 reservoir [174]. Furthermore, it has been demonstrated that HIV-1-infected macrophages render resting T cells permissive to infection [175,176]. This indicates that both the resting T cells and the macrophage reservoirs are connected and are therefore not completely independent.

Exogenous activation of resting T cells in combination with cART has recently been suggested to overcome latent infection of resting T cells [177–181]. An in vitro study with alveolar macrophages demonstrated that latent HIV-1 could be activated again [182], and novel strategies to purge the latent HIV-1 reservoir in macrophages in vivo have been proposed recently [183]. Overcoming viral latency in macrophages has been demonstrated in vivo through the inhibition of C/EBPβ after the contact of latently infected alveolar macrophages with lymphocytes [184,185]. However, these approaches are unlikely to end the residual HIV-1 replication in macrophages/microglia, since this reservoir is not only established through latency (latent reservoir), but also through the poor tissue penetration and reduced efficiency of currently used antiretroviral drugs (anatomical reservoir) [186]. The half-life of HIV-1-infected macrophages is unknown, but the half-life of uninfected macrophages is estimated by some to be half a month [187], although this can be different for HIV-1-infected macrophages and the turnover rate might be highly tissue specific [188]. Specific therapeutic strategies that target HIV-1-infected macrophages are therefore needed. In addition to being a barrier to the complete eradication of HIV-1 from the body, HIV-1-infected macrophages are also associated with specific pathologies in HIV disease.

Pathologies associated with HIV-1 infection of monocytes/macrophages

Macrophages are present in almost every tissue in the human body, and could therefore contribute to a multitude of tissue-specific pathologies. In this article, we have chosen to specifically discuss only those HIV-1-related pathologies for which there is substantial evidence that HIV-1-infected monocytes/macrophages are involved, namely AIDS dementia, AIDS-related lymphomas (ARLs) and cardiovascular disease.

HIV-1-associated neurocognitive disorders

AIDS dementia complex (ADC) is a severe neurological disorder associated with HIV-1 infection. Although much less frequent in the era of cART, it is still observed in a small group of AIDS patients [189]. Furthermore, an increase in milder cognitive disorders is observed, which may be explained by the increased life expectancy of HIV-1-infected individuals on cART [190]. Recruitment of HIV-1-infected monocytes/macrophages to the brain, and dysfunction of perivascular macrophages/microglia as a consequence of the infection, can result in neuron death and is thought to play a crucial role in the pathogenesis of HIV-1-associated neurocognitive disorders (reviewed in [191]).

HIV-1 infection has been demonstrated to induce expression of adhesion molecules on brain endothelium [192], increase susceptibility to LPS-induced disruption of the blood–brain barrier (BBB) [193], increase transmigration abilities [194] and reduce egress of monocytes/macrophages [195,196], resulting in accumulation of perivascular macrophages in the brain of ADC patients [197]. Moreover, higher levels of LPS are associated with ADC [198]. Activation of perivascular macrophages and microglia results in the secretion of chemokines and cytokines, by which an inflammatory and neurotoxic environment is created. The release of the chemokines will again further stimulate the recruitment of circulating monocytes/macrophages. This is the so-called bystander hypothesis for the etiology of HIV-1-associated dementia. In addition, macrophage-derived HIV-1 proteins gp120, gp41 and Tat, and compounds such as glutamate, arachidonic acid and many others (reviewed in [191]) have strong neurotoxic effects, directly contributing to neuronal damage. Since recruitment of monocytes/macrophages to the CNS seems to play such an important role in the onset of ADC, it is not surprising that single nucleotide polymorphism (SNP) in the gene coding for CCL2 (or monocyte chemoattractant protein-1) has been found to be associated with an increased risk of AIDS dementia [199]. In addition, other common genetic variants have been found to be associated with an increased risk...
for developing ADC, such as polymorphisms in CCR5, TNFA and CCL3 among others. However, most of these associations remain to be replicated in other studies.

The major obstacle that hinders effective inhibition of HIV-1 replication in macrophages/microglia in the brain is the BBB. Although systemic HIV-1 viremia can be well controlled by current cART and drug therapy, the drug also often results in undetectable HIV-1 load in cerebrospinal fluid. cART may not always prevent replication of the virus in sanctuary sites, such as the CNS. The increased prevalence of milder but serious neurocognitive pathologies despite the use of cART may indicate that penetration of current antiretrovirals into the CNS is suboptimal. Penetration of the BBB is restricted for many drugs, including antiretrovirals. While protease inhibitors are of particular importance to end ongoing residual replication in macrophages that are already infected, their capacity to penetrate into the brain tissue is rather low and their efficacy in macrophages is reduced as compared with CD4+ T cells. However, even in the time when antiretroviral therapy was not available, not all infected patients suffered from ADC, suggesting that in addition to drug penetration, viral factors (cell tropism and virus load) and host factors are likely to be involved in the development of ADC. A complete understanding of virus characteristics, host genotype and BBB penetration levels of the drug regimen for each patient could help towards preventing HIV-1-related neurological disorders.

AIDS-related lymphomas
In analogy with the bystander effect hypothesis for the etiology of HIV-1-associated dementia where neurons are not directly affected by the virus, macrophages might also indirectly contribute to the onset of AIDS-related cancers. The occurrence of non-Hodgkin lymphomas in HIV-1-infected patients has decreased since cART, but is thought to remain too frequent to only be associated with poor immunity caused by the virus. As a possible cause for macrophage-related lymphomas, overproduction of cytokines by macrophages has been proposed. The excessive cytokine production could result in the overstimulation of B cells with subsequent DNA modifications, resulting in malignant B cells. Furthermore, the recent finding that HIV-1-infected macrophages form tunneling nanotubes that can connect to B cells was suggested to play a role in the formation of ARL. HIV-1 has not been found in malignant B cells, but was present in tumor-associated macrophages present in the stroma. Upon transfer into immunodeficient mice, these HIV-1-infected tumor-associated macrophages were associated with the occurrence of murine lymphomas, while transferred HIV-1-infected CD4+ T cells or uninfected macrophages were not. Since B-cell immortalization and proliferation could also be due to opportunistic viral infections such as human herpesvirus-8 or the Epstein–Barr virus, ARL tissues from 60 patients were screened for p24 (HIV-1) and CD68 (macrophage) expression. A total of 40% of the ARL tissues were found to harbor HIV-1-infected macrophages, whereas 35% of the tissues were Epstein–Barr virus positive and none were human herpesvirus-8 positive. These findings suggest that HIV-1-infected macrophages may play a role in the pathogenesis of AIDS-related lymphomas.

Cardiovascular disease
HIV-1-infected patients have a greater risk for developing cardiovascular disease, independent of the use of cART or of dyslipidemia. Multiple processes are thought to contribute to the increased risk environment for cardiovascular disease in HIV-1 infection. Several studies have demonstrated a direct effect of HIV-1 on plasma lipid levels, resulting in an atherogenic lipoprotein profile (e.g., high levels of low-density cholesterol) and CD68 (macrophage) expression. HIV-1-induced systemic inflammation and expression of cytokines recruit monocytes/macrophages to the damaged arterial wall. External stimuli subsequently promote local differentiation and activation of the macrophages, resulting in increased uptake of lipids by these cells. Excessive uptake of cholesterol by macrophages is prevented by either efflux of cholesterol, often to high-density lipoprotein, or the storage of cholesterol as lipid droplets inside the cell. These droplets, however, gradually fill the cytoplasm, which will affect normal cellular metabolism, transforming them into foam cells. Plaque is formed at the inner lining of the inflamed artery by the accumulation of leukocytes (mainly foam cells), cholesterol, connective tissue and cell debris. Decreased mobility of these foam cells and the expression of adhesion molecules reduce emigration from the formed plaque. It was demonstrated that HIV-1 infection reduced macrophage reverse transendothelial migration in vitro. Foam cells can also secrete chemokines that will increase migration of...
Figure 2. Contribution of HIV-1 to the etiology of cardiovascular disease.

Thick red arrows indicate the direct effects of HIV-1 infection, whereas the thin white arrows indicate HIV-1-independent effects. Infection with HIV-1 results in changes of lipid levels in the blood: increased LDL and decreased HDL cholesterol. Oxidized LDL can damage the vascular wall, resulting in recruitment of monocytes/macrophages to the damaged site. HIV-1 requires cholesterol for its replication; therefore, HIV-1 Nef inhibits cholesterol efflux and stimulates the production and uptake of cholesterol. Cholesterol accumulates in HIV-1-infected macrophages, turning them into foam cells. Excessive intracellular cholesterol levels and reduced emigration of the infected macrophages will result in cell death and will further contribute to the plaque formation and subsequent cardiovascular disease.

LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TEM: Transendothelial migration.

Genomics & HIV-1 infection in monocytes/macrophages

Technological advances have resulted in high-throughput analysis methods such as high-throughput mass spectrometry, microarray-based gene-expression profiling and genome-wide SNP genotyping. The use of these technologies results in the generation of a vast amount of data, and may help to better understand the life cycle of HIV-1 in monocytes and macrophages, as well as the effect of viremia on the uninfected monocyte/macrophage population. Genetic variation in the human population can affect protein function in many ways, which may also affect the interaction between HIV-1 and host proteins. Indeed, there is great diversity in HIV-1 replication kinetics in MDM from different healthy blood donors [98,105–108].

This suggests that genetic variants, such as insertions, deletions, SNPs and copy number variation influence the replication of HIV-1. These polymorphisms can be exploited in large-scale genetic studies to identify genomic regions that affect HIV-1 infection and disease. Several genome-wide SNP analyses have already been performed using DNA from HIV-1-infected patients with known viral, immunological or clinical end points, and have identified SNPs in both known and novel host proteins that affect viral load or disease progression in vitro [257–264], in vivo [257–264], and in vivo [257–264].

One in vitro study has tested genome-wide polymorphisms for their effect on HIV-1 replication in CD4+ T cells [265], but to date, such an analysis has not been published specifically for HIV-1 replication in monocytes/macrophages. By contrast, results from studies employing other genomic technologies, such as gene-expression studies and proteomic analyses in monocytes/macrophages have already identified genes and proteins in monocytes/macrophages that may serve as novel targets for antiretroviral drugs. In addition, many of these studies can be used to generate novel hypotheses to be tested. This unbiased approach may help us in finding genes or proteins and identifying pathways that we have not previously associated with dysfunction of, or HIV-1 replication in, monocytes/macrophages. Results from gene-expression and proteomic studies that specifically focused on HIV-1 infection and monocytes/macrophages are discussed in the final two sections of this article.
Transcriptomics
Initial microarray studies with monocytes/macrophages have shown that in vitro infection of macrophages with HIV-1 results in modulation of genes, leading to cellular support of viral replication ([266–271] and reviewed in [272]). Modulation of proinflammatory cytokines, signaling and cell cycle genes prevents cell death in macrophages, unlike in CD4+ T cells, natural killer cells and B cells, and additionally facilitates HIV-1 infection and replication in nondividing, differentiated cells that are otherwise not susceptible to lentiviral infection. HIV-1 infection of MDM itself did not result in changes of the inflammatory cytokine response, both at the transcriptional and protein level, despite attenuation of the NF-κB activation pathway [273]. In addition, genome-wide expression profile analyses have been applied to better understand the effect of HIV-1 viremia on monocytes/macrophages, for example by studying therapy-naive HIV-1-infected patients, treated patients and controls [125,274–276]. One of the major pathways shown to be affected is apoptosis (Table 2) [274] and the differential expression of transcripts between cells from therapy-naive patients and those on cART led to the identification of NAMPT as a host factor that interferes with early events in the HIV-1 life cycle [276]. NAMPT concentrations were increased in HIV-1-infected therapy-naive patients, and treating MDM with NAMPT resulted in less integrated proviral DNA. As referred to above, the restriction of HIV-1 replication in M1 macrophages was investigated by comparing the gene expression profiles of IFN- or LPS-stimulated and control macrophages, thus identifying TRIM22 (also known as STAF50) as a novel host factor that restricts HIV-1 replication in M1 macrophages [125].

Proteomics
Studies performed by Luo et al. [277] and Wojna et al. [278] were among the earlier HIV-1-oriented proteomic studies and specifically focused on the monocyte/macrophage proteome. The MDM protein profile could distinguish between seronegative and HIV-1-infected patients and those with and without cognitive impairment. Several new proteomic studies related to HIV-1 replication in monocytes/macrophages have been performed in recent years [279–286], all with their own unique research question. Unfortunately, there was little overlap in the proteins identified, which could be because of the rather specific subjects addressed (Table 2) or as a result of differences in the methodology. In all studies, sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE)
Future perspective
The increased life expectancy of HIV-1-infected individuals in developed countries may be accompanied by increased manifestations of disorders associated with HIV-1 infection of monocytes/macrophages, such as neurological disorders, cardiovascular disease and lymphomas, emphasizing the need for strategies to eradicate HIV-1 from its monocyte/macrophage reservoir. At present and in the near future, high-throughput technologies will yield a large number of candidate host proteins that may enhance or restrict HIV-1 replication. The challenge of the future will be to translate these findings into increased understanding of the processes involved and the identification of interactions that can be targets for new therapeutic approaches.

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Macrophage polarization influences HIV-1 infection
- HIV-1 replication is restricted in monocytes at an early post-entry level. This restriction is relieved upon differentiation into macrophages.
- Polarization into M1 or M2 macrophages by different stimuli results in restrictions of HIV-1 replication at multiple levels; only a few of the restriction factors responsible have been identified.

Macrophages are involved in cell-to-cell and host-to-host transmission of HIV-1
- HIV-1 is efficiently transmitted from macrophages to uninfected CD4+ T cells, either through the virological synapse or via tunneling nanotubes formed by the macrophages.
- Macrophages contribute to the spread of HIV-1 from host to host, because these cells are present in large numbers at sites that are important for transmission and less susceptible to the viral cytopathic effect.

HIV-1-infected macrophages constitute a long-lived reservoir
- Macrophages contribute to both the anatomical and latent HIV-1 reservoir because these cells are long lived and relatively resistant to the cytopathic effects of the virus, infection is not lytic and induces antiapoptotic events, antiretroviral drugs penetrate tissues less efficiently and the efficacy of protease inhibitors is reduced and proviral transcription can be repressed.

Host proteins can both enable & restrict HIV-1 infection
- HIV-dependency factors are host proteins required for HIV-1 replication, such as Alix, C/EBPβ (large isoform), cyclin-T1 and cystatin B in monocytes/macrophages.
- Restriction factors such as C/EBPβ (small isoform), NAMPT, APOBEC3, OTK18, p21, TRIM22 and tetherin inhibit HIV-1 replication in monocytes/macrophages.

HIV-1-infected tissue macrophages are associated with tissue-specific pathologies
- HIV-1 infection of tissue-specific monocytes/macrophages play a critical role in the pathology of HIV-1-associated neurocognitive disorders, HIV-1-related lymphomas and cardiovascular disease.

Genomic technologies have identified novel host proteins affecting HIV-1 replication in macrophages
- Gene-expression profiling has identified several genes associated with HIV-1 replication in monocytes/macrophages, such as the restriction factors NAMPT and TRIM22.
- Proteomic studies have identified multiple proteins associated with HIV-1 infection, although most remain to be replicated. However, several groups have shown cystatin B to be required for efficient replication of HIV-1 in macrophages.
- While several genome-wide association studies of HIV-1 infection have been published, none have been reported for monocytes/macrophages to date.
- Each newly identified target will need to be evaluated for its potential as a therapeutic target.

Bibliography
Papers of special note have been highlighted as:
- of interest
- of considerable interest


HIV-1 & the macrophage

Review


The authors show that HIV-1 infection of macrophages affects the expression of host proteins contributing to productive HIV-1 infection. For example, the expression of DC-SIGN, a carbohydrate-binding agent, inhibits HIV-1 infection in macrophages. The authors also report that the expression of the restriction factor, tetherin, is required for HIV-1 replication in macrophages. The authors suggest that future research should focus on the development of strategies to inhibit the expression of these host proteins and thus prevent HIV-1 infection of macrophages.

The authors also show that HIV-1 infection of macrophages induces the formation of tunneling nanotubes. The authors report that tunneling nanotubes are formed by HIV-1 infection of macrophages and that the formation of tunneling nanotubes is inhibited by the expression of tetherin. The authors suggest that future research should focus on the development of strategies to inhibit the formation of tunneling nanotubes and thus prevent HIV-1 infection of macrophages.

The authors conclude that HIV-1 infection of macrophages affects the expression of host proteins and induces the formation of tunneling nanotubes. The authors suggest that future research should focus on the development of strategies to inhibit the expression of these host proteins and thus prevent HIV-1 infection of macrophages.
First paper describing that a portion of the hematopoietic stem cells can get infected with HIV-1, in vivo as well as in vitro.
HIV-1 & the macrophage

Review


Paper showing that HIV-1 Nef inhibits efflux of cholesterol in macrophages.


Proteomic study showing that expression of the newly identified HIV-1 dependency factor cystatin B is reduced in placental macrophages.


