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Review

HIV-Macrophage Interactions at the Cellular and Molecular Level

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Abstract. Macrophages, centrally involved in both the innate and adaptive arms of the immune system are not only the chief target of the human immunodeficiency virus (HIV), but also its main reservoir and vehicle of transmission. Macrophage-tropic (M-tropic) viruses are responsible for the initial infection, predominate in the asymptomatic phase, and persist throughout infection, even after the emergence of preferential T cell- and/or dual-tropic HIV-1 variants. Functional impairment of HIV-infected macrophages plays a role in the immune dysregulation characteristic of acquired immunodeficiency syndrome (AIDS). Efforts directed at understanding the cellular and molecular mechanisms underlying HIV-macrophage interactions remain the basis for devising novel and efficacious therapeutic strategies against HIV ant the AIDS epidemic.

Key words: macrophages; HIV reservoir; immune dysregulation; AIDS.

Introduction

Macrophages belong to the mononuclear phagocyte system and exhibit a marked heterogeneity of phenotype in various tissues, reflecting localised, inter-cellular interactions (reviewed by GORDON[54]). This cell type is relatively resistant to the cytopathic effects of HIV infection, and occurs at tissue sites that could facilitate viral transmission and contribute to AIDS pathogenesis (e.g. AIDS dementia)[13].

Burgeoning research on the mechanism of HIV entry into, and replication within, host target cells and the generation of HIV-specific immunity has opened avenues to study viral transmission and disease progression, collectively called HIV pathogenesis. However, the basis for HIV pathogenesis remains one of the central challenges facing AIDS research. The immunological deficit characteristic of HIV disease is most simply described as a progressive depletion of $CD4^+$ T lymphocytes, and it was this observation that led to the discovery that CD4 is the primary receptor for HIV (reviewed by SATTENTAU and WEISS[104]). This description is, however, insufficient because it overlooks a multifactorial process that starts at the point of infec-

Abbreviations used: AIDS – acquired immunodeficiency syndrome; CD4bs – CD4 binding site; D-tropic – dual tropic; GAGs – glycosaminoglycans; GM-CSF – granulocyte macrophage colony stimulating factor; HAART – highly active anti-retroviral therapy; HIV – human immunodeficiency virus; hu-PBL – human peripheral blood lymphocytes; IL-12 – interleukin 12; LAM – lipoarabinomannan; MAC – *Mycobacterium avium* complex; MDM – monocyte-derived macrophages; MIP – macrophage inflammatory protein; M-tropic – macrophage tropic; NK-κB – nuclear factor – κB; OI – opportunistic infections; PBMC – peripheral blood mononuclear cells; RANTES – regulated-upon activation, normal T cell expressed and secreted; SCID – severe combined immunodeficiency; TCL-tropic – T cell line tropic; TGF-β – transforming growth factor β; TNF – tumor necrosis factor.

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tion. Thus, any substantial loss of $CD4^+$ T cells is preceded by a series of events that includes repeated cycles of target cell activation and subsequent immune dysregulation[22, 79]. Despite the intense focus on HIV infection of the T cell compartment, a growing body of evidence suggests that macrophage-tropic (M-tropic) HIV variants are critical for AIDS pathogenesis[50, 76, 82, 98, 109, 110, 137]. Although the appearance of T lymphocyte HIV strains heralds a more rapid decline in $CD4^+$ T cells, they are unable to establish the primary infection, as evidenced by the resistance to HIV infection of individuals homozygous for inactivating mutations in the CCR5 gene[102, 138]. The discovery of HIV coreceptors provided fundamental insights into the mechanism of viral entry and evolution, making the virus-coreceptor interaction an attractive target for anti-HIV drugs. This article therefore reviews our current understanding of HIV-macrophage interactions at both the entry and post-entry level, with particular emphasis on HIV coreceptors and their physiological ligands, and the immune dysregulation during HIV infection.

HIV Cellular Tropism

Until recently, one of the less comprehensible behaviors of HIV-1 was its cell tropism. Despite the fact that the CD4 clearly is the primary cellular receptor for HIV-1, not all CD4-expressing human cells are readily infected by all strains of HIV-1. Some strains, particularly those isolated early in infection, only infect macrophages and primary activated T lymphocytes, and are called M-tropic strains. Other strains, isolated later during infection and associated with AIDS progression, readily infect both primary activated T lymphocytes and transformed T cell lines but not macrophages, and are called T-tropic strains or more aptly, T cell line--tropic (TCL-tropic) strains. Some of the latter isolates, besides evolving towards TCL-tropism, also retain their M-tropism and are denoted as dual-tropic (D-tropic) viruses[11, 41]. Thus, in about half of all HIV-1 infections there is viral evolution towards D- and TCL-tropism that is prognostic for accelerated AIDS progression. Nonetheless, many individuals progress to AIDS without detection of TCL-tropic virus, showing that M-tropic virus may be sufficient for AIDS pathogenesis[82].

Coreceptor Utilization in HIV Infection of Macrophages

The CD4 surface receptor is necessary, but not sufficient for HIV entry into target cells, hence, coreceptors were hypothesized long before their identification. The 1996 identification of the CCR5 and CXCR4 chemokine receptors as the major HIV-1 coreceptors advanced our understanding of HIV pathogenesis[6, 19, 34, 40, 42, 100, 123]. Utilization of CCR5 and CXCR4 coreceptors explains HIV-1 tropism to a great extent (reviewed in[8, 38, 43, 80]). Prototype TCL-tropic strains use CXCR4, M-tropic strains use CCR5, and D-tropic strains use both chemokine receptors, culminating in the latest nomenclature X4-, R5- and R5X4-tropic strains, respectively[7]. While differential use of CXCR4 or CCR5 by HIV strains has been observed, the striking differences seen appear to be in how R5 and R5X4 viruses utilize the CCR5 coreceptor. Generally, R5-tropic viruses are particularly well adapted for CCR5 use[19, 34, 42], and can tolerate deletion or substitution of the CCR5 amino-terminal domain[59, 130]. R5X4 viruses, however, are much more sensitive to mutations in CCR5, particularly in the amino-terminal domain[40]. Thus, there is a cost associated with the benefit of using dual chemokine receptors, exhibited by R5X4-tropic strains having more stringent requirements for CCR5 usage[38]. The third hypervariable loop (V3) on the gp120 survace glycoprotein is a critical determinant of coreceptor utilization and HIV tropism[69, 99, 133].

Macrophages constitutively express low levels of CD4 and CXCR4, whereas CCR5 expression is inducible, and R5 and R5X4 HIV isolates readily infect macrophages. The extent to which primary X4 isolates can infect macrophages through the CXCR4 is highly contentious. Some studies suggest that all or most primary X4 isolates can infect macrophages, and that CXCR4 may be the sole macrophage coreceptor for these isolates[106, 114, 127]. In contrast, other reports show little or no infection of macrophages by X4 isolates[26, 135, 136]. Thus the CXCR4 receptor expressed on macrophages is thought to function as a coreceptor for entry by R5X4-, but not X4-tropic HIV-1 isolates, and macrophage resistance to X4-tropic strains does not result from a lack of the CXCR4 coreceptor[26].

It is postulated that contradictory reports on the utilization of the CXCR4 on macrophages by X4 HIV isolates reflect different methods of macrophage culture and levels of expression of CXCR4[44]. Almost all *in vitro* studies have used monocye-derived macrophages (MDM), a macrophage model unlikely to represent all macrophage populations *in vivo*. At least one study suggests that there are differences in post-entry events in MDM and alveolar macrophages that could influence the ability of X4 viruses to replicate in these cells[60]. Although CXCR4 is also expressed on MDM, it is present in a functionally restricted form, hence CCR5 is

preferentially utilized and serves as the major coreceptor on macrophages for HIV[84, 126, 136]. Additionally, a post-entry block due to defects in nuclear translocation may further limit productive infection of macrophages by X4 strains[61, 127]. Thus, coreceptor selectivity influences post-entry events in HIV replication within macrophages. Finally, a panoply of alternative coreceptors, including CCR2b, CCR3, CCR8 and a growing list of known or putative chemokine receptors, has been postulated to support virus infection *in vitro*[41, 78, 100]. Generally, these receptors have been identified in heterologous transfection-based system and are used inefficiently relative to CCR5 or CXCR4. Just like the extent to which X4 strains colonize macrophages *in vivo*, the role these alternative coreceptors play in infection of macrophages remain uncertain.

Mechanism of HIV-1 Entry into Macrophages

The viral envelope glycoprotein (gp120 and gp41) encoded by the *env* gene, mediates the binding, fusion and entry of HIV into macrophages using the CD4 and chemokine receptors (for review see[80, 134]). Elucidation of the three-dimensional structure of a derivative of gp120, in which the variable loops were truncated to allow crystallization[69], and site-directed mutagenesis studies[29, 67, 86, 99, 112] provided a milestone in mapping those regions of gp120 critical to its function. Upon binding the target cell, the gp120 assumes a transiently exposed conformation following the displacement of the V1/V2 hypervariable loops that normally mask the CD4 binding site (CD4bs) on the Env protein[103]. This transient conformation allows the exposed CD4bs on gp120 to bind to domain 1 of the CD4 surface receptor. Binding of gp120 to CD4 exposes the V3 loop and unmasks the cryptic binding site for the chemokine coreceptor, usually CCR5 for macrophages (Fig. 1). The first clue as to the interactive mechanism was the observation that β-chemokines (RANTES, MIP-1α and MIP-1β) competitively inhibited HIV gp120-mediated entry[42, 132], suggesting that an HIV component was likely to bind directly to the CCR5 molecule. Interaction with the CCR5 coreceptor induces further conformational changes in the envelope protein, resulting in gp41 activation. This leads to insertion of the hydrophobic fusion domain of the gp41 sub-unit into the target cell membrane and the formation of a coiled coil between 3 copies of the gp41 fusion domain that snaps the virus and cell membranes into close apposition, followed by HIV fusion and entry through poorly defined mechanisms[15, 103, 131].

Dichotomy between CCR5 Utilization and Macrophage Infection

Several studies have challenged the notion that CD4 and CCR5 expression are sufficient for productive MDM infection[17, 37, 62]. For instance, CHENG-MAYER and her co-workers[17] reported that chimeric viruses carrying the SF2 envelope, sufficient to allow CCR5 use, did not productively infect MDM. Recently, HUNG et al.[61] published similar results using a panel of chimeric viruses dervied from R5 and R5X4 isolates that could effectively utilize CCR5 for entry. HIV strains that can use the CCR5 coreceptor yet fail to infect macrophages have also been isolated[37]. In contrast, there are overwhelming independent findings that CCR5 is the most important coreceptor for high-efficiency infection of macrophages[3, 19, 34, 41, 42]. Particularly fascinating was the observation that HIV entry function by R5-, but not X4-tropic strains, was potently suppressed by the β-chemokines RANTES, MIP-1α and MIP-1β[23], and that these CC chemokines were natural ligands for the CCR5 coreceptor[97, 101]. To complete the circle, individuals who are homozygous for a 32-base deletion (Δ32) resulting in non-functional CCR5 protein are highly resistant to HIV infection[10, 85, 120]. Macrophages from these individuals are resistant to *in vitro* infection by CCR5-restricted viruses, but not by viruses that use the CXCR4 coreceptor[27, 96], further implicating CCR5 as the paramount coreceptor for macrophage infection. Taken together, these data clearly show a dichotomy between CCR5 utilization and macrophage infection. The data also suggest that other undetermined cellular factors may mediate infection of macrophages by some HIV isolates and that CCR5 usage might not be sufficient for macrophage infection and/or R5 tropism. A number of explanations that could account for the dichotomy between CCR5 utilization and macrophage infection have been advanced. One possible explanation independently reported by POOTS et al.[95] and SCHUITEMAKER et al.[111] is that terminally differentiated *in vitro* MDM are completely resistant to HIV infection. Thus, variations in MDM isolation, culture methods and duration may have different effects on CCR5 utilization and infection with HIV. The expression of CCR5 on adult monocytes increases during monocyte differentiation to MDM, and is up-regulated over the 5 days of maturation and correlates approximately with susceptibility to HIV infection[84, 126]. This suggests that differences in the surface expression levels of both CD4 and CCR5 on MDM may be critical for susceptibility to infection by some HIV isolates. Different HIV isolates exhibit differences in the threshold requirements

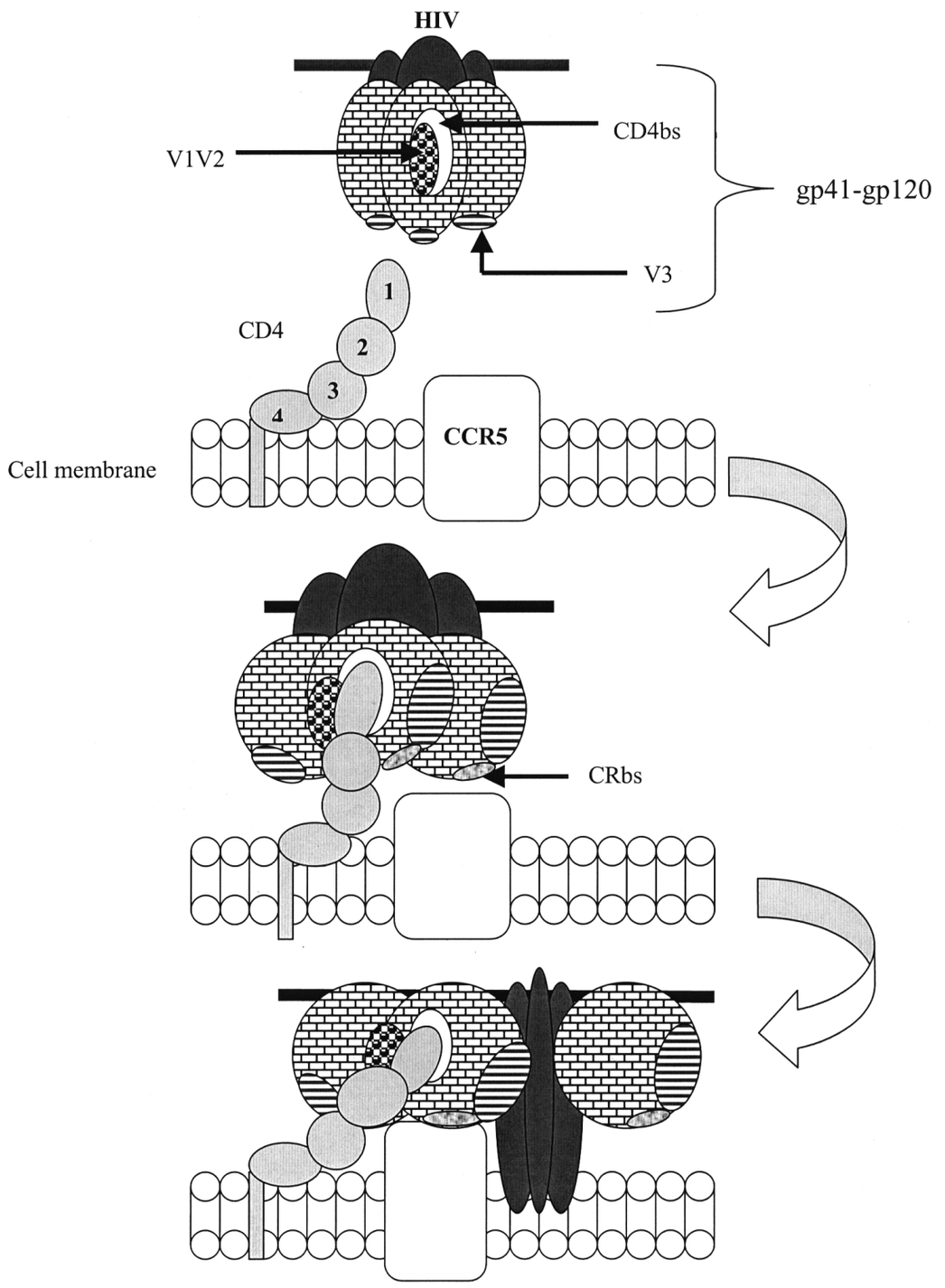

Fig. 1. A model for gp120-mediated CD4-dependent entry of HIV into macrophages. A trimer of the gp41-gp120 (Env) heterodimer binds to the CD4 after transient exposure of the CD4 binding site (CD4bs) subsequent to displacement of the V1/V2 variable loops. CD4 binding induces conformational changes on the gp120, including a shift on the V3 loop resulting in increased exposure of the conserved CCR5 binding site (CRbs) on the gp120. CCR5 binding induces further conformational change that results in gp41 activation and insertion of the exposed fusion peptide into the target cell membrane and the formation of a coiled coil that brings the virus and cell membrane into close apposition, allowing fusion and viral entry

for surface expression of CD4 and/or CCR5[68, 94]. Relatively low levels of CCR5 are required for HIV infection and less for laboratory-adapted strains than for primary clinical isolates[31]. CCR5 is also expressed in different conformational forms, which may be accounted for by posttranslational modification[58]. Thus, natural variation in conformation, level of expression and threshold requirements of CCR5 by different R5 and R5X4 isolates may account for *in vitro* differences in the utilization of the coreceptor to infect macrophages.

β-Chemokines and Their Effects on Productive HIV Infection of MDM

Conflicting results have also been reported regarding the effects of the CCR5 chemokine ligands (RANTES, MIP-1α and MIP-1β) on the replicative capacity of HIV in macrophages. Some groups have reported the expected inhibition of macrophage infection by R5 viruses[3, 14, 24], while others did not observe this effect[42, 81, 107], instead, they found them to act as stimulators, enhancing viral infection of MDM[32, 53, 124]. The exact mechanisms underlying the differential effects of β chemokines on HIV infection and replication within MDM have not been fully established. However, the cognate chemokines can inhibit or enhance HIV infection of MDM in several ways *in vitro*. A popular hypothesis is inhibition of HIV entry because of competition between the virus and the chemokines for binding sites on the common CCR5 receptor[5, 87, 113, 125]. Receptor down-regulation in response to chemokine binding and signaling can also interfere with HIV entry by reducing the density of available CCR5 receptors on the cell surface[4, 73]. In contrast, stimulatory effects of β chemokines are dependent on cell signaling events via pertussis toxin-sensitive G protein-linked pathways[64]. Thus, β chemokines may stimulate a number of intracellular mechanisms in macrophages as in human T cells, which may lead to increased HIV replication[116, 117]. Furthermore, β chemokines may activate macrophages, resulting in a subsequent increase in HIV replication[64]. GORDON et al.[53] reported that the CC chemokine RANTES can enhance HIV infection of macrophages and other target cells in a manner that is independent of CCR5 or any other known coreceptor, and even independent of the normal route of virus entry. Later, the same group[124] studied the activities of RANTES on HIV infectivity when present at concentrations above physiological levels *in vitro*. Their findings suggested that RANTES cross-links HIV to cells via glycosaminoglycans (GAGs) such as heparan sulfate present on both cells and HIV, and that RANTES directly interacts with cell-surface GAGs to transduce a signal which eventually renders the cells more permissive to HIV infection. These results, however, contradict a suggestion by ORAVECZ et al.[88] that HIV inhibition by RANTES is facilitated by chemokine interaction with GAGs, and that this interaction occurs minimally with macrophages, consistent with the resistance of these cells to the antiviral effects of chemokines.

The effect of CC chemokines on virus infection and replication within MDM is also reported to be highly dependent on concentration and the time of addition relative to virus. Stimulation occurs when they are added before infection, while inhibition occurs when they are added during or after infection[64]. Thus, when cells were exposed to β chemokines before HIV infection, subsequent HIV replication in both MDM and monocytes was enhanced by up to 800% of untreated controls, and this enhancement was seen across a broad concentration range from 10 to 500 ng/ml[64]. These data corroborate earlier findings that β chemokine expression is strongly enhanced in the lymph nodes of HIV patients[118]. Thus, cells recruited to the lymph nodes of HIV patients are likely to interact with the chemokines prior to HIV exposure and infection, given the primary physiological role of chemokines to recruit cells to sites of inflammation[1]. The inhibitory effects on HIV replication that occurred when cells had been exposed to β chemokines either simultaneously with or after HIV infection were observed at concentrations of 100 ng/ml and above[64], and not below 100 ng/ml as previously reported by another group[65]. Generally, it therefore seems that the exact effects of β chemokines in HIV infection of macrophages voth *in vitro* and *in vivo* are yet to be fully delineated, and until then treatment advances with these molecules or their derivatives should proceed with caution.

Coreceptors as a Determinant of HIV Pathogenesis

HIV pathogenesis frequently corresponds to the evolution of coreceptor phenotype. D-tropic (R5X4) viruses have been proposed as the transitional phenotype during the evolution of coreceptor usage[25, 41]. Thus, when the phenotype switch occurs, there is an initial evolution from viruses using CCR5 to those that can use both CCR5 and CXCR4[28]. To investigate the impact of coreceptor usage on HIV pathogenesis, PENN et al.[91] and SCHRAMM et al.[108] exploited an *ex vivo*

human lymphoid histoculture system which recapitulates the virus/host interaction integral to disease progression *in vivo*[51]. Their data showed that coreceptor preference influences the pathogenic potentials of HIV, with CXCR4 utilization strongly associated with an aggressive depletion of $CD4^+$ T cells in this human lymphoid model. The same group[52], using the lymphoid culture model, also found that viruses using CCR5 efficiently infected both macrophages and CCR5-bearing T cells[12], thereby leading to robust replication within macrophages and preferential depletion of the CCR5 T cells, GRIVEL and MARGOLIS[55] reported similar findings, namely that R5 isolates are highly cytopathic for their $CCR5^+$ T cell targets in lymphoid tissue *ex vivo*. The R5 viral isolates were also found to exhibit high levels of replication and to cause severe depletion of activated human $CD4^+$ T cells in human peripheral blood lymphocyte SCID (hu-PBL-SCID mice) compared with X4 strains[45]. In an independent cross-sectional study, LI et al.[72] examined the tropism and coreceptor usage of primary HIV isolates collected from the blood of individuals at different stages of HIV infection and pathogenesis and from tissue-derived isolates. Their study indicated that in the late stage of HIV pathogenesis, with increased viraemia and the viral quasispecies, CCR5 utilization and M-tropism persist in blood and tissues and the replicative ability increases in macrophages. These findings in general may explain why many individuals succumb to AIDS, with severe depletion of $CD4^+$ T lymphocytes, without a switch from R5- to X4-tropic viruses[105, 119]. GRIVEL and MARGOLIS[55] postulate that these individuals might have an unusually high proportion of $CCR5^+/CD4^+$ T cells, because of the effects of CCR5 promoter polymorphism[74, 75]. These data also delineate specific interactions with coreceptors that influence target cell specificity, viral replication within macrophages, and disease progression within the host[52, 72].

Immune Dysregulation: Opportunistic Infection and Impaired Cytokine Production in HIV-Infected Macrophages

Progression from acute HIV infection to AIDS is often accompanied by macrophage-mediated immune dysregulation and susceptibility to opportunistic infections (OI). Infection of macrophages with HIV impairs a number of effector functions normally carried out by these cells, resulting in defective phagocytosis and subsequent killing of opsonized pathogens, and dysregulation of cytokine production[9, 16, 20, 30, 36, 48]. Disseminated *Mycobacterium avium* complex (MAC) is one of the most frequent OI occurring in HIV-infected patients, causing significant morbidity and mortality[46]. Reciprocally, OI augment HIV replication within macrophages. In HIV-infected individuals, clinical co-infection with *Mycobacterium tuberculosis*, MAC, herpesvirus type 1, and *Pneumocystis carinii* have all been shown to correlate with increases in levels of circulating HIV[36, 39, 89, 129]. *M. tuberculosis*, through its lipoarabinomannan, selectively induces macrophage secretion of the anti-inflammatory cytokine transforming growth factor β, indirectly supporting HIV infection and replication within macrophages[33]. The MAC up-regulates CCR5 expression and increases levels of tumor necrosis factor α (TNF-α) in macrophages[128]. Increased CCR5 expression, together with the up-regulation of TNF-α, a reported promoter of HIV replication[47, 77, 90], by OI facilitates the entry of HIV and its replication in macrophages, contributing to the viremia that occurs at the onset of AIDS[128]. In systems where it has been analyzed, the mechanism of TNF-α up-regulation of HIV appears to involve NF-κB[49, 90], and OI-induced TNF-α may promote increased transcription of viral long-terminal repeat via this pathway[2, 121]. Infection with MAC and other opportunistic pathogens also appears to protect macrophages from apoptosis, thereby maintaining the pool of infected and infectable targets. It therefore follows that treating opportunistic pathogens in HIV patients may not only inhibit the OI-induced pathology, but also the viral burden. Promising results have recently been reported that granulocyte-macrophage colony stimulating factor, a cytokine regulating proliferation, differentiation, and function of granulocytes as well as macrophages, can restore phagocytosis in MDM and augment phagocytosis of MAC by HIV-infected macrophages both *in vitro* and *in vivo*[63].

The underlying mechanisms of waning immunity and function of HIV-infected macrophages are not restricted to OI, but include a number of factors associated with inflammation and cytokine dysregulation. HIV is capable of tipping the cytokine production balance in its favor by either inhibiting the production of proinflammatory cytokines or augmenting the production of anti-infflammatory ones[83]. A proinflammatory cytokine, interleukin 12 (IL-12), which is central to the orchestration of both innate and adaptive immune response (reviewed in[122]), is one of the cytokines dysregulated in HIV-infected macrophages. Peripheral blood mononuclear cells, which include MDM from HIV-infected individuals, show attenuated IL-12 production in response to bacterial stimuli[16, 20]. Alveolar macrophages from HIV patients also secrete less IL-12

compared with those from normal donors in response to *Staphylococcus aureus*[35]. These data point to a likely role for defective IL-12 production in the immune dysregulation associated with HIV infection of macrophages. The mechanisms responsible for HIV-related IL-12 suppresion are not understood, but a number of hypotheses have been advanced and are reviewed in[83]. Other properties of HIV-infected macrophages include an alteration of the antigen-presenting function and stimulation of interferon response in adjacent T cells[66].

Another macrophage-mediated immune dysregulation during HIV infection includes apoptosis of $CD8^+$ T cells[56, 57]. $CD8^+$ T lymphocytes play an important role in the control of infection by HIV as a result of their cytotoxic activity and by releasing soluble factors[23]. Apoptosis of $CD8^+$ T cells isolated from HIV-infected patients is mediated by macrophages through the interaction between TNF-α bound to the membrane of macrophages (mbTNF) and a receptor on $CD8^+$ T cells called TNF receptor II (TNFII)[56]. Thus, the HIV gp120 envelope protein activates CXCR4 on macrophages and $CD8^+$ T cells, which increases the expression of two cell proteins, mbTNF and TNFII involved in apoptosis induction. The HIV accessory gene product Nef also results in macrophage-mediated immune dysregulation[115]. HIV Nef induces the production of MIP-1α and MIP-1β by HIV-infected macrophages, thereby mediating lymphocyte chemotaxis and activation, permitting productive HIV infection.

Macrophages as HIV Reservoir

Macrophages serve as a potentially important reservoir and as vehicles for dissemination of HIV in different tissues[50, 70, 93]. HIV-infected macrophages are found in the brain, lungs, lymph nodes, skin, bone marrow and blood of seropositive individuals[18, 50]. Thus, HIV harbored in macrophages may escape immune surveillance and anti-viral therapy. Although highly active anti-retroviral therapy (HAART) significantly suppresses viral replication[92], ongoing viral replication and dissemination has been noted during HAART, especially in macrophages compared with resting T cells[92], although HIV that persists in the latter, re-emerges quickly after discontinuing HAART[21]. Macrophages can therefore play a key role in regulating the intensity and progression of disease in HIV infection even during therapy, and their secretory products have been implicated in the pathogenesis of AIDS dementia complex[71].

In conclusion, we reiterate that M-tropic HIV strains are the central villain in AIDS pathogenesis, and that other viral variants are less crucial. Thus, macrophages provide an important target for efficacious anti-HIV drug development. A critical focus for future work might therefore, amongst others, be directed towards answering the following questions:

1) What is the viral selective advantage, or mechanisms underpinning the prevalence of R5 strains during the establishment of initial infection and their persistence throughout the asymptomatic phase of the disease?

2) Do macrophages have additional receptors or molecular components/pathways for HIV fusion and/or entry?

3) How many members of the coreceptor repertoire on macrophages, in addition to CD4, must be blocked to achieve therapeutic effect?

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