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Alessia Verani, Gabriel Gras, Gianfranco Pancino

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Alessia Verani[1], Gabriel Gras[2] and Gianfranco Pancino[3]*

[1] Human Virology Unit, DIBIT, San Raffaele Scientific Institute, Milan, Italy; [2] CEA, Service de Neurovirologie, DSV/DRM, Centre de Recherches du Service de Sante des Armees, EPHE, IPSC, Fontenay aux Roses, France ; [3] Unité de Biologie des Rétrovirus, Institut Pasteur, Paris, France.

Corresponding author:
Gianfranco Pancino
Unité de Biologie des Rétrovirus - Département de Virologie
Institut Pasteur
25, rue du Dr. Roux -75724 Paris cedex 15
Fax: 33 (0)1 45 68 89 57, e-mail : gpancino@pasteur.fr

Abstract

HIV-1, like the other lentiviruses, has evolved the ability to infect nondividing cells including macrophages. HIV-1 replication in monocytes/macrophages entails peculiar features and differs in many respects from that in CD4 T lymphocytes. HIV-1 exhibits different tropism for CD4 T cells and macrophages. The virus can enter macrophages via several routes. Mitosis is not required for nuclear import of viral DNA or for its integration into the host cell genome. Specific cellular factors are required for HIV-1 transcription in macrophages. The assembly and budding of viral particles in macrophages take place in late endosomal compartments. Viral particles can use the exosome pathway to exit cells. Given their functions in host defence against pathogens and the regulation of the immune response plus their permissivity to HIV-1 infection, monocytes/macrophages exert a dual role in HIV infection. They contribute to the establishment and persistence of HIV-1 infection, and may activate surrounding T cells favouring their infection. Furthermore, monocytes/macrophages act as a Trojan horse to transmit HIV-1 to the central nervous system. They also exhibit antiviral activity and express many molecules that inhibit HIV-1 replication. Activated microglia and macrophages may also exert a neurotrophic and neuroprotective effect on infected brain regulating glutamate metabolism or by secretion of neurotrophins. This review will discuss specific aspects of viral replication in monocytes/macrophages and the role of their interactions with the cellular environment in HIV-1 infection swinging between protection and pathogenesis.

Introduction

Lentiviruses can infect and replicate in non-dividing cells, including cells of the monocyte/macrophage lineage. Some non-primate lentiviruses, such as caprine arthritis and encephalitis virus (CAEV) and Maedi-Visna virus, which cause chronic inflammatory diseases, exhibit a restricted tropism for monocytes/macrophages. Conversely, lentiviruses, such as the feline, simian and human immunodeficiency viruses (FIV, SIV and HIV, respectively), have acquired a wider tropism and an expanded range of target cells, primarily CD4+ T lymphocytes. Lentiviruses may cause CD4+ T cell depletion and immunodeficiency. The capacity of lentiviruses to infect macrophages and other antigen-presenting cells (APC) plays a determinant role in the establishment, persistence and pathogenesis of infection. Macrophages greatly contribute to innate responses to pathogens, and are at the interface between innate and adaptive immunity. Thus, they play a central role in defence and in the control of infections, either by directly destroying invading pathogens or by secreting cytokines able to inhibit their replication or to activate other arms of the innate or adaptive immune responses. The infection of macrophages by intracellular pathogens, including lentiviruses, may impair their functions and alter the cytokine production pattern, resulting in chronic inflammation and tissue damage. Unlike T cells, HIV-infected macrophages appear to be resistant to the cytopathic effects of the virus and thus serve as a reservoir for persistent infection. The capacity of monocytes and macrophages to migrate in organs and to survive in tissues makes them potential conveyors of HIV-1 infection. Their interplay, as APCs or a source of chemotactic cytokines, with CD4 T cells may favour intercell virus transmission. Therefore, monocytes/macrophages are thought to play an ambiguous, dichotomous role in lentiviral infections, acting either as an antiviral defence system or a virus target, a host cell guardian or a Trojan horse (Herbein et al., 2002). There is increasing interest in several aspects of macrophage infection by HIV, including the mechanisms of HIV replication in

monocytes/macrophages, and the roles of these cells in the control of the infection, the pathogenesis of disease and viral escape from antiretroviral therapy. A considerable number of recent reviews have addressed many of these points (Freedman et al., 2003; Gras et al., 2003; Kedzierska et al., 2003a; Kedzierska et al., 2003b) and articles contained in the special issue of November 2003 of J. Leukoc. Biol., n° 74). Thus, this review will summarise current knowledge of some characteristics of HIV infection of macrophages: a) peculiar features of HIV replication in macrophages, including viral entry, nuclear import of viral DNA, and virus assembly and release, b) interactions of HIV-1-infected macrophages with the cellular environment, c) the molecular interactions of brain macrophages with the central nervous system in HIV infection and d) the range of inhibitory activities exerted by macrophages on HIV replication.

Peculiar features of HIV-1 replication in macrophages.

The HIV-1 life cycle in macrophages differs in many respects from that in CD4 T lymphocytes (Fig. 1). Indeed, different HIV-1 isolates show different tropism for CD4 T cells and macrophages (Cheng-Mayer et al., 1988; Fenyö et al., 1988; Wu et al., 1997). Unlike T cells, productive HIV-1 infection occurs independently of cellular DNA synthesis in macrophages (Weinberg et al., 1991). The assembly and budding of viral particles take place in cytoplasmic vacuoles in macrophages but not in T cells (Orenstein et al., 1988). Furthermore, HIV-1 accessory genes may have distinct effects in primary macrophages and lymphocytes (Sherman et al., 2002; Subbramanian et al., 1998; Swingler et al., 2003). Some cellular factors required for HIV-1 transcription, such as GATA-3, ETS-1, LEF-1 and NF-ATc, are lymphoid or T cell-specific (Kinoshita et al., 1997; Yang and Engel, 1993), whereas others, such as CCAAT/enhancer binding protein β (C/EBPβ), are necessary for HIV-1 replication in macrophages but not in T cells (Henderson and Calame, 1997; Lee et al., 2002).

HIV-1 entry in human macrophages

In recent years, there has been much debate regarding the ability of HIV-1 strains that use CXCR4 as a co-receptor to enter target cells (X4 viruses) to infect macrophages. Indeed, although CXCR4 is expressed in both monocytes and mature macrophages (Naif et al., 1998; Valentin et al., 2000; Verani et al., 1998), it is unclear whether X4 viruses can productively infect macrophages. It is generally agreed that most T-cell line-adapted laboratory (TCLA) strains of HIV-1, such as the IIIB strain, infect macrophages at best inefficiently. However, conflicting results have been obtained about primary isolates. In particular, some authors observed that macrophages are refractory to X4 HIV-1 isolates (Yi et al., 1998), whereas others found that X4 strains can efficiently enter macrophages but subsequently remain blocked at a post-entry level (Schmidtmayerova et al., 1998). In contrast, several groups have reported that primary isolates that use CXCR4 can enter macrophages and replicate efficiently (Simmons et al., 1998; Valentin et al., 2000; Verani et al., 1998). This is supported by the rigorous demonstration that all the relevant viral isolates are selective CXCR4 users, by the demonstration that CXCR4 is functional in an independent assay (i.e., chemotaxis), and most importantly, by the ability of different ligands for CXCR4, including stromal cell-derived factor (SDF)-1, anti-CXCR4 mAb and the bicyclam derivative AMD3100, to prevent HIV-1 infection. Finally, an HIV-1 primary strain isolated from the central nervous system of an individual with AIDS that is restricted to CXCR4 and induced neuronal apoptosis was reported to replicate efficiently in macrophages (Yi et al., 2003).

The abovementioned discrepancies were probably caused by differences in experimental conditions, such as macrophage isolation and culture methods, assays for measuring co-receptor function or HIV-1 infection, and temporal modulation of CD4 and CXCR4 levels during cell culture. Indeed, the capacity of X4 strains to replicate in macrophages depends

largely on how these cells were cultured; different culture conditions may produce macrophages at different stages of activation, which affects the pattern of molecules expressed on the cell surface including proteoglycans and, most importantly, the profile of released cytokines and chemokines (Bakri et al., 2001).

However, peculiar molecular features also contribute to differences between X4 and R5 HIV-1 replication in macrophages. Indeed, western blot analysis of surface proteins from monocytes, macrophages and T cells demonstrated differences in the biochemical properties of CXCR4 molecules in different cell types. CXCR4 was found to be mainly a monomer in monocytes and T cells, but was principally a species of higher molecular weight on the surface of macrophages (Lapham et al., 1999). CD4 co-precipitated with CXCR4 monomers but not with the high molecular weight form, suggesting that this CXCR4 form cannot associate with CD4 (Lapham et al., 1999). In addition, CCR5 and CXCR4 interfere with each other during viral fusion and entry (Lee et al., 2000). This interference is due to competition for limiting CD4 molecules. Interestingly, in cells with low surface density of CD4, co-expression of CCR5 and CXCR4 significantly reduces their susceptibility to infection with X4 viruses (Lee et al., 2000).

Horizontal transmission of X4 HIV-1 is believed to occur very rarely. Indeed, examination of the phenotypes of HIV-1 strains sampled at different times during the course of infection revealed that recently infected individuals predominantly harbour R5 isolates (Koot et al., 1996; Zhang et al., 1998). Moreover, selective suppression of X4 viruses has been reported in some cases of *in vivo* transmission of a heterogeneous HIV-1 population (Cornelissen et al., 1995). Furthermore, individuals homozygous for a 32-bp deletion in the CCR5 gene, which abrogates CCR5 expression, are resistant to HIV-1 infection (Samson et al., 1996). These observations emphasise the pivotal and unique role of CCR5 in the *in vivo* transmission of HIV-1 and imply the existence of negative selective forces acting against X4 variants, the

nature of which is still unknown. The observations that CXCR4 is expressed on macrophages and mediates the cell-free entry of X4 viruses rule out the possibility that this co-receptor is not available in cells that act as initial targets for infection, such as mucosal macrophages or Langerhans cells, and suggest that other types of antiviral mechanism selectively target X4 variants. The CXCR4 ligand, SDF-1, is a suitable candidate as it is constitutively and strongly expressed in the epithelium of the genital mucosa, which could favour the preferential transmission of R5 isolates in this zone (Agace et al., 2000).

Although HIV-1 enters target macrophages mainly by the binding of the viral glycoprotein gp120 to CD4 and a chemokine coreceptor and fusion with the cell membrane, electron microscopy revealed virions in several locations shortly after viral exposure (Maréchal, 2001). This suggested the existence of alternative entry routes. Macropinocytosis has been shown to be involved in the entry of HIV-1 into macrophages (Maréchal, 2001). Macropinocytosis is an endocytic process that does not require any binding at the cell surface, as the extracellular fluid is engulfed by cellular ruffles (Amyere et al., 2002). Shortly after exposure of macrophages to HIV-1, and irrespective of gp120-receptor interactions, viral particles are visible in intracellular vesicles named macropinosomes. Most virions are subsequently degraded. However, a fraction of the virions that are internalised by intracellular vesicles escapes destruction and leads to productive infection (Maréchal, 2001). Virions can also interact with macrophages in several other ways. Various molecules expressed on cell surface bind the virus without leading to productive infection. These molecules concentrate virus particles on the target cell surface or transfer the virus to $CD4^+$ cells. Receptors for the Fc portion of immunoglobulins or for complement are involved in the entry and infection of macrophages by antibody and complement opsonized viruses (reviewed in (Montefiori, 1997). Initial virus-cell interactions may involve non-specific binding of gp120 with cell surface heparan sulphate proteoglycans (Mondor et al., 1998;

Roderiquez et al., 1995; Saphire et al., 2001). The carbohydrate moieties of gp120 may also play an important role. In this context, dendritic cell-specific intercellular adhesion molecule (ICAM)-3 grabbing non-integrin (DC-SIGN), a C-type lectin specifically expressed by dendritic cells (DC), mediates the capture and transmission of HIV-1 from DC to $CD4^+$ T cells (Geijtenbeek et al., 2000). DC-SIGN-bound virus is more infectious and has a longer half-life than free virus. As DC-SIGN mediates the binding of R5 and X4 strains of HIV-1, HIV-2 and SIV, it may function as a general lentiviral attachment factor (Baribaud et al., 2001; Geijtenbeek et al., 2000). However, despite the marked up-regulation of DC-SIGN by type 1 (IFN-γ) and type 2 cytokines (IL-4 and IL-13) in macrophages, no association was observed between DC-SIGN expression and the amount of HIV-1 transmission to $CD4^+$ T cells (Chehimi et al., 2003). Furthermore, there is evidence suggesting that DC-SIGN is not the only adhesion molecule that can bind and transmit HIV. It was recently suggested that additional C-type lectin receptors play a role (Turville et al., 2003). In particular, as about half of the carbohydrates on gp120 are terminally mannosylated, the role of the macrophage mannose receptor (MMR) on primary macrophages has been investigated (Nguyen and Hildreth, 2003). The MMR is an innate immune receptor expressed on macrophages and DC, the function of which is to recognise patterns of terminal mannosylation on foreign particles (Stahl and Ezekowitz, 1998). Approximately 60% of the initial association of HIV with macrophages is mediated by MMR, as evidenced by the inhibitory effect of mannan, D-mannose and soluble mannose-binding lectin, but not D-galactose. Moreover, macrophages are able to mediate the transmission of bound HIV to co-cultured T cells and up to 80% of this transmission is blocked by inhibitors of MMR binding (Nguyen and Hildreth, 2003).

It is important to identify the attachment factors on DC and macrophages. DC in the mucosal epithelium and macrophages located in the submucosal tissues may be initial targets for HIV-

1. Thus, these data further suggest that the capture of HIV in these zones and the subsequent transport of infectious viral particles by DC and macrophages to the lymph nodes, where virus replication can be amplified, could strongly facilitate virus dissemination.

Nuclear import

The capacity to replicate in non-dividing cells is a specific feature that distinguishes lentiviruses from murine and avian retroviruses, which require nuclear membrane breakdown during mitosis for viral cDNA integration (Lewis et al., 1992; Roe et al., 1993). Some studies have suggested that only macrophages that maintain proliferative capacity can support productive HIV-1 infection (Kootstra and Schuitemaker, 1998; Schuitemaker et al., 1994). This was attributed to the need for cellular activation to complete the reverse transcription. However, mitosis is not required for HIV-1 provirus integration in macrophages or for the establishment of productive infection (Schuitemaker et al., 1994). Nevertheless, integration of viral DNA requires access to chromosomal DNA. In non-dividing cells, a large nucleoprotein complex, the preintegration complex (PIC), which contains the linear viral DNA and the viral and cellular proteins needed for integration, must pass through nuclear pores to reach the nucleus (Miller et al., 1997). HIV-1 PIC was first suggested to have a diameter of 56 nm. However, more recent studies suggested that it is even larger (McDonald et al., 2002; Nermut and Fassati, 2003), even though the nuclear pore channel allows the passage of smaller molecules (<40-45 kDa). PIC is transported through nuclear pores via an active, energy-dependent mechanism (Bukrinsky et al., 1992; Depienne et al., 2001; Gallay et al., 1997). This property of lentiviruses has major consequences in the establishment of infection and in pathogenesis, and has been exploited to generate vectors for the delivery of genes to non-dividing cells. However, the ability of lentiviral vectors to transduce monocytes or macrophages can differ, suggesting that nuclear import efficiency varies with cell differentiation and/or activation. Neil *et al*. reported that an HIV-1-derived vector remained

blocked at the level of nuclear translocation in freshly isolated monocytes, but not in macrophages (Neil et al., 2001). HIV-1 nuclear import is down-regulated in lipopolysaccharide (LPS)-stimulated monocyte-derived macrophages, due to the activation of p38 kinase (Zybarth, 1999). Despite numerous studies, the factors involved in the nuclear transport of the lentiviral PIC and the underlying mechanisms are still unclear and a matter of debate.

After reaching the cytoplasm of the infected cell, HIV-1 reverse transcription takes place in the reverse transcription complex (RTC), constituted by virion core proteins, cellular proteins and the RNA genome (McDonald et al., 2002; Nermut and Fassati, 2003). Following the completion of DNA synthesis, the RTC matures in the PIC. The double-stranded viral DNA is synthesised within a few hours in T cells but is slower (36 to 48 h) in macrophages, for unknown reasons (Collin et al., 1994; O'Brien et al., 1994). RTCs migrate towards the nucleus, probably by using cellular motors, such as dynein, to move along the cell cytoskeleton (Bukrinskaya et al., 1998; McDonald et al., 2002). At the nuclear membrane, the viral DNA assembled in the PIC has to deal with nuclear pore complexes before it can reach the nucleus. A peculiar feature of the lentiviral genome, the central DNA flap, is involved in the nuclear import of PIC. The central DNA flap is a stretch of triple-stranded DNA that is formed due to the presence in lentiviruses of two initiation sites for the plus strand DNA synthesis, the 3’ terminal and the central polypurine tracts (PPT). During the plus strand DNA synthesis, the two nascent DNA fragments overlap at the central PPT (cPPT) region. In HIV-1, the central DNA flap is a 99-nucleotide overlap. This structure enhances the nuclear import of the PIC, increasing the transduction efficiency of lentiviral vectors (Sirven et al., 2000; Zennou et al., 2000). The increased efficiency of cPPT-containing vectors has been attributed at least in part to enhanced nuclear import (Van Maele et al., 2003; Zennou et al., 2000). However, some studies have shown that cPPT mutant HIV-1 can replicate in non-dividing

cells including primary macrophages, albeit less efficiently than the parental virus (Dvorin et al., 2002; Limon et al., 2002b).

Viral proteins are also involved in the nuclear transport of HIV-1 PIC (Fouchier and Malim, 1999). HIV integrase (IN) and matrix (MA) proteins carry sequences that may serve as nuclear localisation signals (NLS) for the recognition and targeting of the PIC to the nuclear pore by the importin-α/β pathway (Gallay et al., 1997; Gallay et al., 1996). However, IN NLS mutants cannot replicate in either dividing and non-dividing cells, suggesting that IN NLS is involved in steps of replication other than nuclear import (Limon et al., 2002a; Petit et al., 2000; Tsurutani et al., 2000). IN has intrinsic karyophilic properties and may play a key role in nuclear import, independent of NLS (Bouyac-Bertoia et al., 2001; Depienne et al., 2001; Devroe et al., 2003). HIV-1 MA contains two sequences that could act as an NLS (Bukrinsky et al., 1993; Haffar et al., 2000). MA NLS mutants were found to be unable to replicate in macrophages in some studies but not in others (Fouchier et al., 1997; Reil et al., 1998; von Schwedler et al., 1994). The third viral protein involved in nuclear import of HIV-1 PIC is the viral protein R (Vpr) (Heinzinger et al., 1994; Popov et al., 1998) which is present only in lentiviruses. Vpr contains two non-canonical NLS that may contribute to nuclear transport of the PIC (Jenkins et al., 1998), and interacts with cellular import machinery through importin α and the nucleoporins (Popov et al., 1998; Vodicka et al., 1998). A study based on the overexpression of Vpr has suggested that PIC enters the nucleus by inducing herniations and partial breaking of the nuclear envelope (de Noronha et al., 2001). However, the role of Vpr in the nuclear import of PIC in macrophages is still controversial (Bouyac-Bertoia et al., 2001; Gallay et al., 1997). Vpr and MA may cooperate for the nuclear import of the PIC (Haffar et al., 2000; Popov et al., 1998). However, viruses lacking MA NLS and Vpr (ΔVpr.ΔNLS) are able to replicate in macrophages, albeit less efficiently than the wild-type virus (Fouchier et al., 1997; Kootstra and Schuitemaker, 1999). This suggests that Vpr and

MA-NLS are not essential for HIV-1 replication in primary macrophages. Nevertheless, several studies have suggested that Vpr confers an advantage for HIV replication in macrophages (Balliet et al., 1994; Connor et al., 1995). A Vpr-deficient R5 virus efficiently infected T lymphocytes in tonsil cell cultures, but was severely affected in its ability to infect macrophages (Eckstein et al., 2001). This was potentially explained in recent experiments using a Vpr mutant that does not affect nuclear import but that impairs the nuclear export of Vpr and its incorporation into virions. The efficient nuclear export of Vpr enhances viral replication in cultured macrophages (Sherman et al., 2003). HIV-2 and SIV contain two related genes, *vpr* and *vpx*. In these viruses, Vpx performs the functions of HIV-1 Vpr associated with replication in macrophages and nuclear targeting (Fletcher et al., 1996). It is noteworthy that a Vpx mutant SIV that was unable to replicate in macrophages did not disseminate in infected macaques and did not cause disease (Hirsch et al., 1998).

The cellular machinery required for the nuclear import of proteins is also involved in HIV-1 PIC import (Fouchier et al., 1998; Gallay et al., 1997; Gallay et al., 1996; Vodicka et al., 1998). However, it is not clear whether nuclear import receptors (karyopherins or importins) are required for the nuclear transport of IN: the nuclear accumulation of IN has been shown to be independent of importins and of the associated GTPase Ran activity (Depienne et al., 2001). Using purified PIC, Fassati *et al*. have recently reported that importin 7, a member of the importin β superfamily, plays an important role in the nuclear import of HIV-1 PIC (Fassati et al., 2003). Competition with the histone H1, which binds the imp7-impb heterodimer, blocks nuclear import of HIV-1 PIC in primary macrophages. Suppression of imp7 by siRNA reduces HIV-1 infection of HeLa cells. Fassati *et al*. propose that imp7 mediates nuclear import through the NPC after binding to IN and/or other basic viral proteins in the PIC (Fassati et al., 2003).

Finally, nuclear import and integration of HIV-1 vectors can also occur independently of mitosis in cycling cells (HeLa) (Katz et al., 2003). If confirmed in natural target cells, such as CD4+ T cells, this would suggest that a biological feature of lentiviruses that allows infection of non-dividing cells without the need for mitosis-dependent integration could also favour HIV-1 replication in cycling cells.

Viral transcription in macrophages

HIV-1 transcription is differentially regulated in macrophages and in T cells (reviewed in (Rohr et al., 2003)). Transcriptional modulation of HIV-1 replication in monocytes/macrophages has been related to the expression of different isoforms of the transcription factor C/EBPβ. Different isoforms of C/EBPβ are generated by alternative translational initiation. The 30-37-kDa activating isoform is required for HIV replication in monocytes/macrophages but not in CD4+ T lymphocytes (Henderson and Calame, 1997). Conversely, the small (16-23-kDa) isoform is dominant negative and its induction by INF-β in differentiated THP-1 macrophages inhibits HIV-1 transcription (Honda et al., 1998). *Mycobacterium tuberculosis* or LPS also induce the expression of the inhibitory C/EBPβ isoform, possibly through INF-β induction, and inhibit HIV-1 replication (Honda et al., 1998; Weiden et al., 2000). GM-CSF treatment during the differentiation of monocytes into macrophages strongly decreases the susceptibility of macrophages to R5 viruses in comparison with macrophages differentiated in the presence of M-CSF. Viral suppression in GM-CSF-induced macrophages is related to the expression of the inhibitory C/EBPβ isoform (Komuro et al., 2003). Treatment of GM-CSF-induced macrophages with antisense oligonucleotides for C/EBPβ decreases the amount of the inhibitory isoform produced and increases HIV-1 production (Komuro et al., 2003). This study also showed the inverse regulation of the Src-like tyrosine kinase Hck and suggested that the heterogeneity in macrophage susceptibility to HIV-1 infection was related to distinct regulation of Hck and the

inhibitory isoform of C/EBPβ . GM-CSF-induced macrophages present similar morphological and functional features to alveolar macrophages (Komuro et al., 2001). The low level of susceptibility of alveolar macrophages to HIV-1 infection may be related to the expression of the dominant inhibitory C/EBPβ isoform (Honda et al., 1998). Activated allogeneic lymphocytes reduce the expression of the C/EBPβ isoform in differentiated THP-1 and alveolar macrophages, increasing susceptibility to HIV-1 (Hoshino et al., 2002). This mechanism may operate in pathological conditions such as tuberculosis, which causes inflammation of the lungs (Orenstein et al., 1997).

Virus assembly and release

New virions are generated by the assembly of HIV genomic RNA and viral proteins, and by the release of viral particles by budding from the infected cell. This process is promoted and regulated by the Gag polyprotein, which is targeted to membranes by the myristylated NH_2-terminus of MA (Freed, 1998). Viral components are assembled in late endosomes/multivescicular bodies (MVB) in macrophages (Pelchen-Matthews et al., 2003; Raposo et al., 2002). Gag targeting and HIV-1 assembly have been suggested to occur in late MVB in macrophages and in other cells including CD4+ T lymphocytes (Nydegger et al., 2003). However, other authors suggested that the targeting of Gag to the plasma membrane in T cells or to MVB in macrophages involves different signals (Ono and Freed, 2004). In T cells and HeLa cells, mutations in MA retarget HIV-1 Gag from the plasma membrane to MVB. In contrast, in macrophages, both MA mutants and wild-type viruses are targeted to MVB (Ono and Freed, 2004). Gag drives HIV-1 budding through the Gag p6 late domain (L domain). L domain binds the tumour susceptibility gene 101 (TSG101), hijacking the host's MVB vesicle formation machinery, and recruits proteins of the ESCRT (endosomal sorting complexes required for transport) family, which regulate MVB biogenesis and cellular vacuolar protein sorting pathways (Pornillos et al., 2003; Strack et al., 2003; von Schwedler et

al., 2003). The release of HIV-1 also seems to differ in T cells and in macrophages. In T cells, the nascent HIV-1 particle buds from the cell membrane at the sites of cholesterol- and sphingolipid-rich regions known as lipid rafts (Esser et al., 2001; Nguyen and Hildreth, 2000). Late endosomes also fuse to the plasma membrane and may release virus particles into the external medium (Nydegger et al., 2003). In macrophages, viruses can also bud to the lumen of endocytic organelles (Pelchen-Matthews et al., 2003). It has long been known that HIV-1 particles accumulate in intracellular vacuoles in macrophages (Orenstein et al., 1988). Recent studies using immuno-electron microscopy identified these vacuoles as the major histocompatibility complex class II (MHC class II) late endocytic compartment (Raposo et al., 2002). A combination of immuno-ultrastructural studies on HIV-1-infected macrophages and biochemical analysis of the released virions confirmed that the virus-containing compartments have markers and topology consistent with late endosomes or MVBs, and that the viral membrane contains late endosomal proteins (Pelchen-Matthews et al., 2003). MVBs contain intraluminal vescicles that can be released as exosomes from the cell by fusion of MVB with the plasma membrane. According to the Trojan exosome hypothesis (Gould et al., 2003), HIV-1 (and other retroviruses) exits infected cells via the exosome pathway. Immunochemical studies showed that infectious viruses released from macrophages contain low amounts of cell surface markers and lack some lipid-raft-associated proteins, such as CD14, but share similar protein patterns with macrophage-derived exosomes (CD63, MHC class II, Lamp 1) (Nguyen et al., 2003; Pelchen-Matthews et al., 2003; Raposo et al., 2002). These data are consistent with previous studies showing different markers in macrophage- and T cell-derived viruses (Esser et al., 2001; Frank et al., 1996) and suggest that HIV-1 budding and release differ in macrophages and in T cells. The differential inclusion of cellular proteins in viral particles may be relevant for virus transmission and replication. The CD63 molecule may be involved in R5 HIV-1 infection of macrophages but not of T cells (von Lindern et al.,

2003). In macrophages, budding into endosomes may render the virus inaccessible to antiviral inhibitors, including RNAi, and enable the virus to persist in macrophages, which function as viral reservoirs. Viral exosomes may further escape immune surveillance during cell to cell transmission (Gould et al., 2003).

Interactions between macrophages and the cellular environment in HIV-1 infection

Functional defects in HIV-1-infected macrophages

Functionally defective monocytes have been described in HIV-1-infected individuals for many years (Miedema et al., 1988). Monocyte/macrophage functions that are essential for adequate innate and adaptive responses to pathogens, such as antigen presentation, intracellular pathogen killing or phagocytosis, are affected by HIV-1 infection (Biggs et al., 1995; Kumar et al., 1999; Polyak et al., 1997; Yoo et al., 1996); revewed in (Kedzierska et al., 2003a). Phagocytosis is mediated by a number of phagocytic receptors expressed on monocytes/macrophages, including complement receptors (CR) and receptors for the Fc portion of immunoglobulins (FcR) (Aderem and Underhill, 1999). CR and FcR mediate phagocytosis of pathogens opsonised with antibodies and complement. FcRs that are able to induce phagocytosis in human macrophages include FcγRI, FcγRIIA and FcγRIII. FcγR aggregate after binding to immune complexes, this induces signalling through the phosphorylation of a cytoplasmic activating motif (immunoglobulin gene family tyrosine activation motif, ITAM). FcγRIIA bears an ITAM in its intracytoplasmatic tail, whereas FcγRI and FcγRIII associate with γ subunits containing ITAMs. Kedzierska *et al*. recently showed that HIV-1 infection of macrophages at a high m.o.i. affects FcγR-mediated phagocytosis by inhibiting the phosphorylation of Src kinases and other proteins involved in the early steps of FcγR signalling, such as Hck, Syk and paxillin (Kedzierska et al., 2002). This defect was attributed to the down-regulation of the intracytoplasmic expression of the γ signalling subunit, probably at the posttranscriptional level (Kedzierska et al., 2002).

Surprisingly, the defect of γ subunit expression did not alter the surface expression of FcγRs in HIV-1-infected macrophages. Defective phagocytosis and γ chain down-regulation are independent of Nef expression (Kedzierska et al., 2002; Kedzierska et al., 2001).

HIV-1 infection in macrophages results in impaired FcγR signalling and phagocytosis, whereas macrophage stimulation through FcγRs potently inhibits HIV-1 replication (Perez-Bercoff et al., 2003). To determine the effect of FcγR-mediated stimulation by immune complexes on HIV-1 infection, monocyte-derived macrophages were exposed to human IgG immobilised on culture plates. FcγR cross-linking by immobilised hIgG suppresses the replication of both R5 and X4 strains of HIV-1. Although FcγR cross-linking by immobilised IgG induces the secretion of several cytokines and chemokines, including tumour necrosis factor α (TNF-α), macrophage colony-stimulating factor (M-CSF) and macrophage-derived chemokine (MDC), HIV-1 suppression is not dependent on the soluble factors released into culture supernatants. Neither HIV-1 entry nor reverse transcription appear to be affected in FcγR-stimulated macrophages. In contrast, PCR signals for integrated proviral DNA were weaker and 2-LTR signals were stronger, indicating a restriction of viral integration in the host genome (Perez-Bercoff et al., 2003). Aggregation of the activator receptors FcγRI, FcγRIIA and FcγRIII on macrophage surface by specific Fab or $(Fab')_2$ cross-linking also inhibits HIV-1 replication (David et al., 2003). Circulating immune complexes (CIC) may be abundant in chronic infections, which are common in developing countries where HIV/AIDS is highly prevalent. Moreover, since the very beginning of the AIDS pandemic it has been known that the levels of circulating immune complexes (including HIV-specific CIC) are elevated in HIV-positive patients, independent of other infections (Carini et al., 1987; Tausk et al., 1986). HIV-1 immune complexes may, however, facilitate HIV-1 entry through FcRs or complement receptors (Homsy, 1989; Takeda et al., 1990). Thus, the stimulation of

monocytes and resident macrophages by immune complexes through FcγR might modulate HIV-1 infection and spread in opposite directions.

Macrophage-T cell interplay and HIV-1 activation

HIV-1 infection differentially activates T cells and macrophages. In macrophages, early activation is due both to gp120 signalling through CD4 and CCR5 molecules at viral entry and to activation of innate defence mechanisms (cytokines, chemokines, proteases) (Freedman et al., 2003; Lee et al., 2003; Wahl et al., 2003). After a quiescent phase of a few days, viral replication results in further changes in gene expression (Wahl et al., 2003). HIV-1-infected macrophages can interact with other immune system cells, including CD4+ and CD8+ T lymphocytes, resulting in either cell activation or death by apoptosis (recent reviews in (Herbein et al., 2002; Mahlknecht and Herbein, 2001). Among the viral proteins implicated in macrophage activation, Nef appears to play a prominent role. Nef is a 27-kDa protein that is produced early during infection and exerts multiple functional activities. The expression of Nef induces the secretion of CC-chemokines MIP-1α and MIP-1β by infected macrophages (Swingler et al., 1999). These chemokines may help to recruit T lymphocytes at the site of infection by chemotaxis. Moreover, T lymphocytes are activated by a soluble factor released by Nef-expressing macrophages, favouring HIV-1 replication (Swingler et al., 1999). Further studies by the same laboratory showed that CD40L stimulation of macrophages also leads to the release of a factor that induces T lymphocytes to support the replication of an X4 strain of HIV-1 without any other extrinsic stimulation (Swingler et al., 2003). Both Nef and the CD40L-dependent ability to induce T cell permissivity to HIV-1 is blocked by inhibiting NF-κB activation in macrophages. Indeed, T cell activation is mediated by B lymphocytes through the CD22, CD54 CD58 and CD80/CD86 receptors induced by Nef-expressing macrophage supernatants. Cross-linking experiments suggested that T cells are stimulated by the interaction of the CD22 and CD58 ligands, CD45 and CD2, and that CD54 increased the

stimulatory effect by interacting with the CD11a/CD18 integrin. Ligation of the CD80/CD86 ligand, CD28, induces T cells to enter the cell cycle, which in turn allows productive HIV-1 infection. Two soluble mediators released from Nef-expressing macrophages, sCD23 and sICAM, are involved in the induction of the expression of B cell receptors (Swingler et al., 2003). It was thus proposed that Nef intersects the CD40 signalling pathways in macrophages to induce the release of sCD23 and sICAM. Hence, Nef could promote T cell infection by activating signals on antigen-presenting cells that activate resting T lymphocytes in lymphoid organs (Swingler et al., 2003). Although most studies mainly concerned endogenously expressed Nef, some recent reports suggested that exogenous Nef, which is found in the serum of AIDS patients at ng levels (Fujii et al., 1996), also contributes to macrophage activation. Exogenous Nef can be internalised by macrophages. Exogenous Nef induces the expression of genes coding for inflammatory cytokines in monocytic cells and macrophages, and the secretion of CC-chemokines, IL-6 and TNF-α. The secretion of inflammatory chemokines and cytokines is associated with the activation of the transcription factors NF-κB and AP-1 (Olivetta et al., 2003; Varin et al., 2003). The release of soluble factors, including CCL3, induced by exogenous Nef is also involved in the activation of STAT elements 1 and 3, which may contribute to the deregulation of signalling pathways associated with cell survival or the IFN response (Federico et al., 2001; Percario et al., 2003).

Macrophages express CD40, which binds CD40L (CD145) expressed on activated CD4+ T cells and other immune cell types. The CD40/CD40L interaction plays a central role in the activation and regulation of immune responses (van Kooten and Banchereau, 2000) (Chougnet, 2003). As discussed above, the activation of macrophages through CD40L up-regulates the expression of co-stimulatory molecules and leads to the production of inflammatory cytokines and chemokines that contribute to T cell activation at the site of inflammation. As a consequence, the CD40/CD40L interaction can activate X4 HIV-1

replication in CD4+ T cells (Chougnet et al., 2001; Kutsch et al., 2003; Swingler et al., 2003). Moreover, activation through CD40 also directly stimulates the replication of X4 HIV-1 in macrophages (Bakri et al., 2002). Conversely, CD40L stimulation of macrophages prevents the replication of R5 strains of HIV-1 (Bakri et al., 2002; Cotter et al., 2001; di Marzio et al., 2000; Kornbluth et al., 1998). Supernatants from CD40L-activated macrophages also inhibit R5 HIV-1 infection of CD4+ T cells and macrophages. The inhibitory activity is associated with the secretion of β-chemokines and TNF-α, and with the down-regulation of the surface expression of CCR5 and CD4 (Bakri et al., 2002; Cotter et al., 2001; di Marzio et al., 2000; Kornbluth et al., 1998). The CD40L-induced secretion of β-chemokines is mediated by the rapid activation of MAPK signalling pathways (Bakri et al., 2002; Cotter et al., 2001; di Marzio et al., 2000; Kornbluth et al., 1998). When monocytes differentiate in the presence of CD40L an opposite effect is described, leading to the up-regulation of CD4 and CCR5 and to an increase in HIV-1 entry, but not to an increase in total virus production (Bergamini et al., 2002). Thus, CD40L activation of macrophages may lead to different and even opposite outcomes depending on the viral tropism and the cellular environment. Accordingly, the CD40/CD40L interaction occurring between macrophages and T lymphocytes at the site of infection and in lymphoid organs may either inhibit viral replication in macrophages and protect surrounding T cells from R5 strains of HIV-1 or favour the replication of X4 viruses.

The particular relationships between brain macrophages/microglia, HIV and the central nervous system environment: some keys to neurone survival and death in neuro-AIDS.

The invasion of the brain by HIV probably involves activated monocytes crossing the blood brain barrier (BBB) (Fig. 2 A) (Nottet, 1999; Nottet and Gendelman, 1995). Monocytes/macrophages may also contribute to neuro-invasion by producting TNF-α, which alters the BBB structure (Fiala et al., 1997), possibly mediated by enhanced local gelatinase expression (Johnston et al., 2001; Lévêque et al., 2004). The role of mononuclear phagocytes

in HIV transmission into the brain further concerns the constant input of $CD14^+/CD16^+$ HIV-infected monocytes (Crowe et al., 2003; Fischer-Smith et al., 2001) and the associated inflammatory activation that last throughout the course of the disease. Indeed, infiltrating macrophages also contribute to the overproduction of β-chemokines. These β-chemokines keep the invasion going directly through their own protein production (Persidsky et al., 2000) and via inflammatory stimulation of astrocytes to high macrophage chemotactic protein-1 (MCP-1) release (Weiss et al., 1998).

It is now accepted that infection and activation of macrophages and microglial cells play a key role in HIV-induced neurotoxicity. They can act directly through the production of viral neurotoxins. However, the main pathway leading to neuronal death involves immune activation and the subsequent release of neurotoxic factors. The mechanism of neuronal damage is not fully understood but probably involves glutamate-related exotoxicity and oxidative stress resulting in neuronal apoptosis (for review, (Kaul et al., 2001). Nevertheless, intense microglial activation, mainly thought to be associated with neurotoxin production, occurs early in the disease, even in the asymptomatic pre-AIDS stage (An et al., 1996; Sinclair et al., 1994), whereas neuronal loss occurs during the end stage of the disease (for review, see reference (Everall et al., 1993). This discrepancy may be related to the neuroprotective and neurotrophic aspects entailed by microglial activation, provided that activated macrophages and microglial cells (AMM) express neurotrophins, high affinity glutamate transporters and glutamine synthetase.

Activated macrophages and microglia express the molecular effectors of the glial glutamate-glutamine cycle.

Since 1995, several groups have demonstrated that primary microglia (Kondo et al., 1995; Lopez-Redondo et al., 2000; Nakajima et al., 1998; Noda et al., 1999; Rimaniol et al., 2000; Swanson et al., 1997), as well as resident spleen macrophages and monocyte-derived

macrophages (MDM) (Rimaniol et al., 2000), express the two main glial high affinity glutamate transporters — excitatory amino acid transporter (EAAT)-1 and -2 — when activated (Fig. 2, B,C). Macrophages and microglial cells also express glutamine synthetase *in vitro* (Bode et al., 2000)and in SIV infection (Chrétien et al., 2002). Co-expression of EAATs and glutamine synthetase is striking, as the neuroprotective and neurotrophic properties of astrocytes are associated with the same expression pattern.

These effectors are not expressed in normal brains (Lehre et al., 1995), and are highly dependent on cell activation and differentiation *in vitro* (Rimaniol et al., 2000). This strongly suggests a compensatory mechanism that responds to depressed astrocytic function. Indeed, substances released by infected AMM, such as gp120 (Vesce et al., 1997) TNF-α (Fine et al., 1996), PAF (Nishida et al., 1996) and TGF-ß (Chao et al., 1992; Toru-Delbauffe et al., 1990), inhibit glutamate uptake by astrocytes and glutamine synthetase activity and induce glutamate release (Bezzi et al., 2001). Conversely, TNF-α induces EAAT function in differentiating monocytes (Rimaniol et al., 2000).

This expression pattern of glutamate transporters and glutamine synthetase is also found in human AIDS and its macaque model. Neuropathological examinations showed that microglia and brain macrophages from asymptomatic SIVmac251-infected macaques express EAAT-2 and GS, whereas those from uninfected animals do not (Chrétien et al., 2002). EAAT-2 is expressed in both perivascular macrophages and parenchymal ramified and non-ramified microglia. Most perineuronal microglia strongly express EAAT-2 and GS. Likewise, in study on 12 HIV-infected humans at different stages of the disease and three HIV-negative controls, EAAT-1 expression by AMM increased with the disease stage in the white matter, whereas it was strong in perineuronal microglia only in subjects without HIV encephalitis (Vallat-Decouvelaere et al., 2003). These data are in keeping with the previous finding that AMM may express EAATs when glutamate uptake by astrocytes is impaired. Several *in vitro* studies

have shown that EAAT expression and function in astrocytes are reduced by HIV, probably due to the effects of inflammatory mediators and viral proteins (Dreyer and Lipton, 1995; Fine et al., 1996; Kort, 1998; Patton et al., 2000; Vesce et al., 1997). These findings (Vallat-Decouvelaere et al., 2003) support the hypothesis that in HIV infection, besides their classical neurotoxic properties involving glutamate-related excitotoxicity (Jiang et al., 2001) and oxidative stress (Mollace et al., 2001; Shi et al., 1998), activated microglia play a counterbalancing neuroprotective role by clearing extracellular glutamate and producing the anti-oxidant glutathione (Rimaniol et al., 2000; Rimaniol et al., 2001).

Neurotrophin expression defines complex interactions between brain macrophages and microglia, and the brain environment.

Brain macrophages and microglia express a variety of neurotrophins and neurotrophin receptors (Barouch et al., 2001; Elkabes et al., 1998; Gilad and Gilad, 1995; Miwa et al., 1997). The expressed repertoire varies with the cell type, the activation state and the location *in vivo*. These molecules interact closely with inflammatory factors. For example, IL-1β, TNF-α and LPS induce or increase the expression of the nerve growth factor (NGF) by microglia through a NFκB-dependent mechanism (Heese et al., 1998a; Heese et al., 1998b; Lindholm et al., 1987; Mallat et al., 1989; Nakajima et al., 2001), whereas the complement component C3a has the same effect independently of NFκB activation (Heese et al., 1998b). Likewise, LPS and C8 ceramide induce microglia to release brain-derived neutrophic factor (BDNF), the latter acting even in the absence of tumour necrosis factor α (TNF-α) (Nakajima et al., 2001; Nakajima et al., 2002). Interleukin-1β (IL-1β) is a key regulator of ciliary neurotrophic factor (CNTF) production by astrocytes (Herx et al., 2000). This trophic factor is chemotactic to macrophages (Kobayashi and Mizisin, 2000) and an enhancer of CD4, CR3 and p75NTR production by microglia (Hagg et al., 1993). The effects of neurotrophins on macrophages and microglia are not clearly understood. Nevertheless, NGF has been reported

to have anti-inflammatory activities. For example, it can decrease the expression of activation molecules (Nakajima et al., 1998; Wei and Jonakait, 1999) and responsiveness to LPS (Nakajima et al., 1998). Moreover, the phagocytosis of apoptotic cells by microglia induces the already described anti-inflammatory activation profile with prostaglandin E2 (PGE2) and transforming growth factor β (TGFβ) induction, as well as the repression of TNF-α production. This peculiar activation of microglial cells also entails the production of NGF (De Simone et al., 2003), suggesting that it has anti-inflammatory properties.

In HIV-infected patients with neurological impairment (HIV encephalitis and/or AIDS dementia complex), activated microglia overproduce BDNF (Soontornniyomkij et al., 1998), and NGF is found in excess in perivascular macrophages (Boven et al., 1999). These data strongly suggest that AMM produce neurotrophins in the infected brain, thereby limiting inflammation and its deleterious effects on neurones. Together with the low level of virus replication in the central nervous system (CNS), probably due to the repressive effect of astrocytes (Hori et al., 1999; Nottet et al., 1995), this may participate in the lack of neuronal death in HIV infection before very late stages. The relationships between AMM and the neurotrophin family may also be critical for the establishment and long-term persistence of the virus reservoir in the brain. Indeed, HIV infection *per se* induces NGF expression in macrophages (Garaci et al., 1999), leading to an autocrine loop involving NGF and its high affinity receptor TrkA for macrophage survival despite massive virus production. This survival effect involves bcl2, bcl-xL and bfl1 anti-apoptotic molecules (la Sala et al., 2000) and is abrogated by NGF neutralisation leading to p75NTR-mediated apoptosis (Caroleo et al., 2001; Garaci et al., 1999). This could be a powerful mechanism for maintaining viral replication (Garaci et al., 2003), especially in the restrictive CNS milieu, and illustrates exactly how HIV uses endogenous molecules for its own benefit.

Macrophage antiviral factor, "MAF"

It is well-known that $CD8^+$ T cells actively contribute to host defences against HIV infection via two different mechanisms. The first involves the lysis of infected cells in an antigen-specific, HLA-restricted fashion (Yang and Engel, 1993) and the second is a suppressive activity that occurs in the absence of cell killing. This non-cytolytic antiviral activity is mediated primarily by the release of soluble suppressive factor(s), termed "CAF" (CD8 antiviral factor) (Levy et al., 1996; Walker et al., 1986). Seventeen years after the first phenomenological description of "CAF", it is becoming increasing clear that no single protein can account for all the complex activities documented in different experimental systems. Indeed, "CAF" appears to be constituted by numerous factors (Baier et al., 1995; Cocchi et al., 1995), some of which are still unknown.

A wide variety of stimuli have been found to suppress HIV strongly through macrophage activation, pointing towards a macrophage-associated antiviral activity. Several results corroborate this hypothesis. Urokinase-urokinase receptor interactions (Alfano et al., 2003), CD40-CD40L interactions (Cotter et al., 2001; Marzio et al., 2003), stimulation with bacterial LPS (Verani et al., 1997) and stimulation through FcγR (Perez-Bercoff et al., 2003), have all been reported to inhibit HIV-1 replication in macrophages. Protection from productive infection is frequently mediated by the release of soluble factors. As with "CAF", macrophage antiviral factor ("MAF") appears to be a complex cocktail of different proteins (Agace et al., 2000; Agerberth et al., 2000; Cotter et al., 2001; Perez-Bercoff et al., 2003; Verani et al., 1997). Some of the soluble suppressive factors have been extensively studied and coincide with the main component of the "CAF" activity, notably the CCR5-binding chemokines RANTES, MIP-1α and MIP-1β. In addition, several macrophage-derived cytokines, including type-I interferons (i.e., IFN-α and IFN-β), MDC, TNF-α, IL-10, and leukemia inhibitory factor (LIF), have been reported to suppress HIV-1 replication, at least in some experimental systems.

IFN-α and -β have long been known to inhibit HIV-1 replication by suppressing reverse transcription and preventing transcription of the integrated provirus (Honda et al., 1998; Kornbluth et al., 1989; Tissot and Mechti, 1995). IL-10 and TNF-α have been shown to have different effects on HIV replication in macrophages depending on the experimental conditions. Indeed, treatment of primary macrophages with IL-10 abrogates HIV-1 infection by inhibiting the production of both spliced and unspliced HIV-1 RNA transcripts and by interfering with protein processing (Kootstra et al., 1994; Naif et al., 1996). However, another study found that IL-10 promotes the productive infection of monocytes by the R5 virus by selectively upregulating CCR5 and increasing viral entry (Sozzani et al., 1998). The effect of TNF-α on HIV-1 replication depends on the timing of exposure of mononuclear phagocytes to this cytokine. Endogenous TNF-α released by infected macrophages may protect uninfected monocytes and macrophages from infection with HIV-1 (by stimulating CCR5 ligand production) (Lane et al., 1999). However, following integration of the virus into the cellular genome, the continual production of TNF-α may enhance HIV-1 replication (Duh et al., 1989; Osborn et al., 1989). MDC is a β-chemokine purified from the supernatant of immortalised $CD8^+$ T cells of HIV-seropositive individuals. It has been reported to inhibit different HIV and simian immunodeficiency virus strains regardless of their co-receptor usage (Cota et al., 2000; Pal et al., 1997). Nevertheless, the inhibitory activity of MDC is still controversial, and the mechanism through which MDC inhibits both R5 and X4 isolates also remains elusive (Lee, 1998; Perez-Bercoff et al., 2003). Finally, LIF, a factor that can be released by $CD4^+$ T cells, $CD8^+$ T cells (Metcalf et al., 1990) and monocytes/macrophages (Anegon et al., 1991), inhibits HIV-1 in a tropism-independent manner and is produced at higher concentrations in placentae from non-transmitting HIV-infected women than in placentae from transmitting women (Patterson et al., 2001). Nevertheless, others factors undoubtedly constitute another important component

of “MAF” activity. This is supported by data on LPS stimulation. Indeed, soluble factor(s) released by macrophages upon LPS treatment inhibit infection with X4 viruses in both macrophages and T lymphocytes (Verani et al., 2002). HIV-1 suppression is independent of SDF-1, the only known natural ligand of CXCR4, and unrelated to the release of IFN-α/β, MDC, LIF or TNF-α. Even more importantly, infection of both cell types appears to be blocked primarily at the level of viral entry. For this reason, LPS-induced suppressive factor(s) appears to be distinct from the as yet unidentified “CAF”, which inhibits the replication of X4 strains of HIV-1 at the level of viral transcription by suppressing long terminal repeat-driven viral expression (Mackewicz et al., 1995). These results reveal the existence of potent HIV-1 inhibitory factor(s). These uncharacterised factors are released by activated cells of the mononuclear phagocytic system. This suggests that further knowledge of the mechanisms of macrophage activation may lead to novel therapeutic and preventive strategies for the control of HIV disease.

Conclusions

Several lines of evidence show that macrophages play a pivotal role in HIV-1 persistence and pathogenesis. Macrophages may serve as sites for virus replication at late stages of AIDS when $CD4^+$ T cells are markedly depleted or following withdrawal of viral inhibitor treatment. Indeed, the presence of persistently infected macrophages in the body represents a key challenge for therapeutic efforts to eradicate HIV infection by eliminating all cells harbouring the viral genome and/or sustaining virus replication for a long period of time (Collman et al., 2003).

However, the role of macrophages in HIV-1 infection is appearing more complex as the result of studies highlighting their potential role in the protection against HIV-1 infection either through the production of inhibitory factors or through the direct control of HIV-1 replication. Macrophages may also exert neurotrophic and neuroprotective activities in CNS infection.

Improvements in our knowledge of the features of viral replication that are peculiar to macrophages will provide us with a better understanding of HIV-1 transmission and pathogenesis. Furthermore, they may lead to the design of more refined and selective antiviral strategies.

Abbreviations:

AMM: activated macrophages and microglial cells, APC: antigen-presenting cells, BBB: blood brain barrier, CAF: CD8 antiviral factor, C/EBPβ: CCAAT/enhancer binding protein β, CNF: central nervous system, cPPT: central polypurine tract, EAAT: excitatory amino acid transporter, FcR: Fc receptor, GM-CSF: granulocyte macrophage-colony stimulating factor, HIV: human immunodeficiency virus, IN: integrase, ITAM: immunoglobulin gene family tyrosine activation motif, LIF: leukemia inhibitory factor, LPS: lipopolysaccharide, MA: matrix, MCSF: macrophage-colony stimulating factor, MDC: macrophage derived factor, MMR: macrophage mannose receptor, MVB: multivescicular bodies, Nef: negative early factor, NGF: nerve growth factor, NLS: nuclear localisation signal, PIC: preintegration complex, RTC: reverse transcription complex, SDF-1: stromal cell-derived factor, TNF-α: tumour necrosis factor α, Vpr: viral protein R

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Figure legends

Figure 1. Peculiar features of HIV-1 replication in macrophages. Differences between the HIV-1 life cycle in macrophages and CD4 T cells: - Distinct tropism of HIV-1 isolates: inefficient replication of TCLA X4 HIV-1 in macrophages. - Involvement of different surface molecules in the binding of HIV-1 to macrophages and alternative entry by macropinocytosis. - PIC nuclear import through an intact nuclear membrane. – Macrophage-specific regulation of transcription: role of C/EBPβ. – Viral assembly and budding in MVB. - Exosomal viral exit.

Figure 2. A : Perivascular infiltrate of macrophages in an asymptomatic SIVmac251-infected macaque. Stained with CD68 mAb. **B**: high level of EAAT-2 expression in a perivascular macrophage in an asymptomatic SIVmac251-infected macaque. Stained with anti-EAAT-2 polyclonal Abs. **C**: high level of EAAT-2 expression in a parenchymal microglial cell in an asymptomatic SIVmac251-infected macaque. Stained with anti-EAAT-2 polyclonal Abs.