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INVITED REVIEW

# Variation of Macrophage Tropism among HIV-1 R5 Envelopes in Brain and Other Tissues

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**Abstract** Human immunodeficiency virus (HIV)-positive individuals frequently suffer from progressive encephelopathy, which is characterized by sensory neuropathy, sensory myelopathy, and dementia. Our group and others have reported the presence of highly macrophage-tropic R5 variants of HIV-1 in brain tissue of patients with neurological complications. These variants are able to exploit low amounts of CD4 and/or CCR5 for infection and potentially confer an expanded tropism for any cell types that express low CD4 and/or CCR5. In contrast to the brain-derived envelopes, we found that envelopes from lymph node tissue, blood, or semen were predominantly non-macrophage-tropic and required high amounts of CD4 for infection. Nevertheless, where tested, the non-macrophage-tropic envelopes conferred efficient replication in primary $CD4^+$ T-cell cultures. Determinants of R5 macrophage tropism appear to involve changes in the CD4 binding site, although further unknown determinants are also involved. The variation of R5 envelopes also affects their sensitivity to inhibition by ligands and entry inhibitors that target CD4 and CCR5. In summary, HIV-1 R5 viruses vary extensively in macrophage tropism. In the brain, highly macrophage-tropic variants may represent neurotropic or neurovirulent viruses. In addition, variation in R5 macrophage tropism may also have implications (1) for transmission, depending on what role macrophages or cells that express low CD4 and/or CCR5 play in the establishment of infection in a new host, and (2) for pathogenesis and depletion of $CD4^+$ T cells (i.e., do highly macrophage-tropic variants confer a broader tropism among $CD4^+$ T-cell populations late in disease and contribute to their depletion?).

**Key words** HIV-1 R5 · macrophage-tropism · CCR5 · brain · neuroaids · macrophages · CD4+ T-cells

## Introduction

Individuals infected with human immunodeficiency virus type 1 (HIV-1) frequently suffer from progressive encephelopathy, which is characterized by sensory neuropathy, sensory myelopathy, and dementia. The most severe dementias occur in about 30% of AIDS patients. The development of neuropathogenesis is instigated by HIV replication in brain tissue. However, it is unclear whether neurotropic and/or neurovirulent HIV-1 variants evolve and are involved in neuropathogenesis. In the macaque model, both neurotropic and neurovirulent forms of $SIV_{MAC}$ have been described and these represent precedents for HIV (Flaherty et al. 1997; Mankowski et al. 1994, 1997; Sharma et al. 1992). The mechanisms that result in dementia are also unclear but likely involve the disruption of normal neurological functions by toxic factors that are up-regulated either as a consequence of inflammatory processes or as a direct result of HIV replication (Gonzalez-Scarano and Martin-Garcia 2005; Kaul et al. 2001, 2005).

HIV-1 entry into cells is triggered by virion envelope glycoproteins binding to the receptor CD4 and coreceptors,

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either CCR5 or CXCR4. HIV-1 cell tropism is therefore predominantly limited to cells that express CD4 and either CCR5 or CXCR4 (or both) coreceptors. CCR5 is expressed on memory T cells (Blaak et al. 2000; Bleul et al. 1997), macrophages, and other cells of monocyte lineage. CCR5-using (R5) viruses are generally transmitted and predominate in the asymptomatic stage of infection. CXCR4-using (X4) variants can be isolated from about 50% of AIDS patients and are associated with a more rapid loss of $CD4^+$ T cells and faster disease progression (Asjo et al. 1986; Connor and Ho 1994; Scarlatti et al. 1997; Tersmette et al. 1989). The wider expression of CXCR4 (compared with CCR5) on $CD4^+$ T cells, including naïve T cells (Blaak et al. 2000; Bleul et al. 1997), provides CXCR4-using viruses with a broader T-cell tropism when they emerge (Blaak et al. 2000; Ostrowski et al. 1999). Nevertheless, CD4 T cell depletion and AIDS occur in patients from which only CCR5-using viruses can be isolated (Cecilia et al. 2000; de Roda Husman et al. 1999).

$CD4^+$ $CCR5^+$ perivascular macrophages represent a major reservoir of HIV-1 in the brain (Fischer-Smith et al. 2001, 2004; Gonzalez-Scarano and Martin-Garcia 2005; Takahashi et al. 1996). Microglia (also of monocyte/macrophage lineage) are resident in the brain (Williams and Hickey 2002) and are also infected (Cosenza et al. 2002; Fischer-Smith et al. 2001, 2004). A hallmark of HIV-associated neuropathology is the presence of multinucleated giant cells (MNGC) in brain tissue (Bell 1998; Gonzalez-Scarano and Martin-Garcia 2005). MNGCs express the monocyte/macrophage marker CD68 (An et al. 1999) and are believed to result from HIV-induced fusion of infected and uninfected perivascular macrophages. Several studies also support astrocyte infection (An et al. 1999; Ranki et al. 1995; Takahashi et al. 1996) particularly in pediatric cases (Saito et al. 1994; Sharer et al. 1994; Tornatore et al. 1994). In addition, Ranki et al. (1995) reported that the detection of astrocytes expressing nef and rev correlated with dementia. These reports suggest that astrocytes *in vivo* support early stages of replication but produce few virus particles. V3 loop sequences amplified from astrocytes dissected from brain sections were distinct from those amplified from neighboring macrophages or MNGCs (Thompson et al. 2004), suggesting that distinct HIV-1 variants may infect astrocytes. In contrast, neurons are rarely infected (Gonzalez-Scarano and Martin-Garcia 2005).

The brain is colonized early after infection (Davis et al. 1992). Nevertheless, proviral DNA is difficult to detect in brain tissue during the asymptomatic phase of infection (Bell et al. 1993; Donaldson et al. 1994; Gosztonyi et al. 1994; Teo et al. 1997). The exact mechanisms of entry into the brain are unclear, although virus must penetrate through the protective blood–brain barrier (BBB) or via the choroid plexus and cerebrospinal fluid (CSF). A "Trojan horse" mechanism of entry has been favored whereby infected monocytes carry the virus through the BBB and into the brain (Nottet and Gendelman 1995). Once in the brain, the infected monocytes are believed to differentiate into mature perivascular macrophages (Williams and Hickey 2002) and actively support HIV replication (Ancuta et al. 2006). *In situ* hybridization and immunohistochemistry approaches have detected HIV infection and accumulation in macrophages surrounding blood capillaries (Glass et al. 1995; Lane et al. 1996; Williams et al. 2001). T cells also traffic through brain tissue at low levels (Hickey 1999) and are a potential carrier of HIV. Occasional infection of endothelial cells in the BBB would also introduce HIV into the brain. Regardless, it has been proposed that the brain is reseeded by infected, activated monocytes that enter brain tissue late in disease (Gartner 2000) when breaches in the BBB may occur (Toborek et al. 2005). The presence of an increased percentage of activated, more mature monocytes in blood (that express CD16) was reported to correlate with AIDS dementia (Pulliam et al. 1997). These activated monocytes are believed to traffic through the BBB into the brain (Fischer-Smith et al. 2004; Fischer-Smith et al. 2001) in response to up-regulation of the chemokine fractalkine (Dunfee et al. 2006b; Pereira et al. 2001; Tong et al. 2000). In addition, a polymorphism in the gene of the chemokine, *MCP-1*, was reported to be associated with enhanced expression of MCP-1 and an increased incidence of dementia (Gonzalez et al. 2002). MCP-1 is a chemoattractant for the CCR2-expressing subset of monocytes that express high levels of CD14 (Weber et al. 2000) and may also contribute to monocyte accumulation in brain tissue. Consistent with this model, Liu et al. (2000) showed that HIV envelope sequences in deep white matter of brain were more closely related to envelope sequences recovered from blood monocytes than to sequences from other tissues (Gartner 2000).

In this article, we review data from our laboratory and from others on the detection of highly macrophage-tropic variants of HIV-1 in brain tissue and their relevance for dementia and AIDS.

## The detection of highly macrophage-tropic variants in brain tissue

Several groups have described HIV-1 brain isolates prepared by cocultivating brain tissue with peripheral blood mononuclear cells (PBMC) or CD8-depleted PBMCs (Cheng-Mayer et al. 1989; Gorry et al. 2001; Li et al. 1999; Smit et al. 2001). These isolates are generally macrophage-tropic (Cheng-Mayer et al. 1989; Gorry et al. 2001; Li et al. 1999; Smit et al. 2001). However, Li et al. (1999) reported that brain isolates were among the most

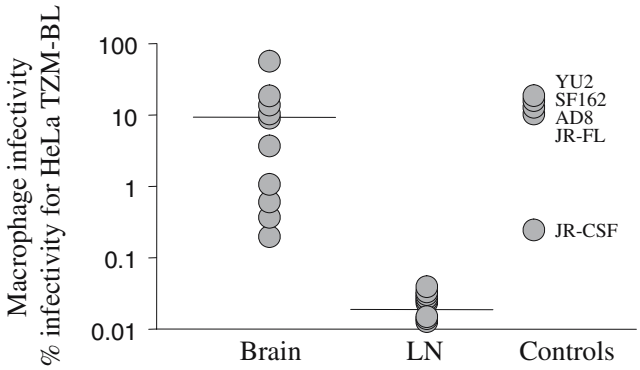


**Fig. 1** HIV-1 envelopes amplified from brain tissue of AIDS patients with neurological complications are more macrophage-tropic than those from LN. Means are marked by a line. Control envelopes are also shown. A Mann-Whitney analysis indicated that the difference in macrophage tropism between brain and LN envelopes is highly significant ($P = 0.0005$).

macrophage-tropic in a comparison with blood isolates. Moreover, Gorry et al. reported that isolates from brain tissue of two individuals were highly fusigenic and efficiently induced large syncytia in primary macrophage cultures. Gorry et al. also showed that the highly macrophage-tropic brain isolates were able to exploit low levels of both CD4 and CCR5 for infection. Nevertheless, isolation of HIV from brain tissue into PBMCs is likely to be highly selective, perhaps favoring variants that replicate most efficiently in T cells and potentially leaving behind quasispecies specifically adapted for replication in brain cells. We therefore used nested PCR to amplify complete envelope genes from brain and lymph node (LN) tissues before cloning into an expression vector to produce $env^+$ pseudovirions. This procedure enables the testing of envelope properties (including tropism and receptor usage) without viral replication. Using this approach, we reported that CCR5-using envelopes amplified from brain tissue of five patients with neurological complications (including dementia and encephalitis) were significantly more macrophage tropic compared with CCR5-using envelopes amplified from LN tissue of three of the same patients (Fig. 1). In addition, several envelopes conferred enhanced macrophage tropism compared with well-characterized, macrophage-tropic envelopes e.g., SF162, YU2, JRFL, and AD8. We were surprised that (in the context of $envelope^+$ pseudovirions) none of the envelopes amplified from LN tissue conferred above background infection of macrophages.

## Role of coreceptors in R5 macrophage tropism

CCR5-using or R5 HIV-1 isolates have generally been described as macrophage-tropic. However, R5 viruses infect $CD4^+$ memory T cells in addition to macrophages and brain-derived microglia (also of monocyte/macrophage lineage). CXCR4-using or X4 variants have been called T-cell tropic and preferentially infect naïve T cells over memory cells. Nonetheless, CXCR4 is expressed at low levels on macrophages matured from blood monocytes (Di Marzio et al. 1998; Lee et al. 1999; McKnight et al. 1997; Naif et al. 1998; Zaitseva et al. 1997) and most primary X4 isolates are dualtropic, infecting both macrophages and T cells *in vitro*, although macrophage infection is usually less efficient than for many R5 viruses (Lathey et al. 2000; Simmons et al. 1996; Valentin et al. 1994; Yi et al. 1999). R5X4 viruses are also usually dualtropic. However, R5X4 strains preferentially exploit CXCR4 to infect T cells (Yi et al. 2005), whereas different strains predominantly use CCR5, CXCR4, or both for macrophage infection (Simmons et al. 1998; Yi et al. 2005).

R5 viruses are believed to predominate in brain tissue (Albright et al. 1999; Li et al. 1999; Peters et al. 2004, 2006; Smit et al. 2001). However, R5X4 isolates from brain that are highly macrophage-tropic have also been reported (Gorry et al. 2001, 2002), whereas a highly macrophage-tropic X4 virus was isolated from the central nervous system (Yi et al. 2003). It is curious that several CXCR4-using viruses have been isolated from brain when analyses of envelope sequences present in brain tissue indicate that R5 viruses predominate. One explanation is that isolation procedures may select for rare CXCR4-using variants if they are present in the brain.

All the envelope genes that we amplified from the brain of AIDS patients were R5, and all but one from LN tissue were also R5 (Peters et al. 2004). Although both brain and LN envelopes were R5, together they conferred a wide range of infectivity for macrophages. In addition, most of our envelopes also used CCR3 efficiently. However, three highly macrophage-tropic brain envelopes from one patient were CCR5 specific, indicating that CCR3 use was not universal among highly macrophage-tropic brain envelopes. In our study, we noted that one patient (NA20) was heterozygous for the Δ32 CCR5 mutation. Brain envelopes from this patient used CCR3 in addition to CCR5. However, one envelope could use a wider range of minor coreceptors to infect $CD4^+$ indicator cells expressing different coreceptors including CCR8, GPR1, CXCR6, and GPR15. Regardless, the use of additional minor coreceptors, including CCR3, did not predict or correlate with macrophage tropism observed for envelopes amplified by PCR from brain and LN tissue.

## Macrophage tropism correlates with the ability to use low CD4 and CCR5 concentrations

Macrophages express low amounts of CD4 (Bannert et al. 2000; Lee et al. 1999; Mori et al. 2000) and moderate

amounts of CCR5 (Lee et al. 1999). Brain microglia express low CD4 and lower levels of CCR5 compared with macrophages (Albright et al. 1999, 2001; Flynn et al. 2003; Wang et al. 2002). Both macrophages (Lee et al. 1999; McKnight et al. 1997) and microglia (Albright et al. 1999; Flynn et al. 2003) express low levels of CXCR4. In addition, it should be noted that levels of CD4, CCR5, and CXCR4 on macrophages and microglia vary depending on donor.

Highly macrophage-tropic brain isolates were reported to exploit low amounts of CD4 and/or CCR5 for infection (Gorry et al. 2002; Martin-Garcia et al. 2006). We used HeLa cell clones that express different amounts of CD4 and CCR5 to confirm that highly macrophage-tropic brain envelopes could exploit low amounts of CD4 for infection. Generally, we found that macrophage tropism of patient envelopes correlated tightly with the capacity to exploit low CD4 levels (Peters et al. 2004, 2006). Moreover, the most macrophage-tropic strains were also able to confer infection and cell/cell fusion of a T cell line, MOLT4#8, via CCR5 levels that are barely detectable (Dejucq et al. 1999; Peters et al. 2004). Together, these observations suggest that highly macrophage-tropic envelopes from the brain may confer a broader tropism for any cell type that expresses low levels of CD4 and/or CCR5, including T cells.

## Impact of macrophage tropism on infection of $CD4^+$ T cells

To investigate whether envelope variation in macrophage tropism influenced the capacity to infect primary $CD4^+$ T cells, we used replication-competent viruses that were made by cloning highly macrophage-tropic and non-macrophage-tropic envelopes into pNL4.3. These viruses were then titrated on primary PHA/IL-2-stimulated PBMCs or $CD4^+$ T cells enriched by negative selection from blood. Flow cytometry analysis showed that enriched $CD4^+$ T cells expressed high levels of CD4. Figure 2a shows that both highly macrophage-tropic and non-macrophage-tropic envelopes conferred high levels of infectivity for both PBMCs and enriched $CD4^+$ T cells. When infectivity for macrophages was plotted as a ratio with infectivity for HeLa TZM-BL, PBMCs, or $CD4^+$ T cells, it was clear that non-macrophage-tropic envelopes were severely curtailed for replication in macrophages compared with T cells (Fig. 2b). However, these assays do not discern whether highly macrophage-tropic envelopes have a broader tropism among different T-cell populations present in PBMC or enriched $CD4^+$ T-cell cultures. Further investigation using immunostaining to detect specific T-cell types that are infected will be required to evaluate whether highly macrophage-tropic envelopes infect a higher percentage of $CD4^+$ T cells in these cultures. It should also be noted that pseudovirions carrying non-macrophage-tropic LN envelopes usually failed to infect macrophages. However, when such envelopes are present in a full-length, replication-competent viral clone, 50- to 100-fold more virus infectivity is recovered following transfection of 293T cells compared with pseudovirions. These higher levels of infectivity allow detection of some infection of macrophages. However, infectivity is still as much as 1,000-fold less efficient compared with highly macrophage-tropic envelopes (Fig. 2).

## Sensitivity to CCR5 and CD4 ligands

The wide variation of R5 envelopes in their capacity to exploit low levels of CD4 and/or CCR5 for infection would be expected to affect their sensitivity to inhibition by ligands that bind these receptors. We found that the extent of macrophage infection by brain and LN envelopes correlated with the level of resistance to a CD4-specific antibody that binds to domain 1 of CD4 and blocks infection (Peters et al. 2004). This result is consistent with Martin-Garcia's observations using envelopes from brain and spleen of a single individual (Martin-Garcia et al. 2006).

Gorry et al. reported that highly macrophage-tropic HIV-1 isolates from brain were more resistant to inhibition by CCR5 antagonists TAK779 (Baba et al. 1999) and SCH-C (Strizki et al. 2001) compared with other R5 isolates. This observation makes sense because brain isolates could exploit low levels of CD4 and/or CCR5 for infection, whereas CCR5 inhibitors effectively reduce the amount of CCR5 available for envelope interactions. However, in contrast to Gorry et al.'s study, we did not find a clear correlation between increasing macrophage tropism of brain envelopes and enhanced resistance to the CCR5 antagonist, TAK779 (Peters et al. 2004 and unpublished data). For two patients, macrophage-tropic brain envelopes were more sensitive to TAK779 compared with most non-macrophage-tropic LN envelopes (data not shown). These results were exemplified by the highly macrophage-tropic brain B59 envelope from patient NA20, which is very sensitive to TAK779 inhibition yet able to exploit trace levels of CCR5 for infection of MOLT4#8 T cells. How B59 can exploit low levels of CCR5 and remain very sensitive to TAK779 inhibition is curious and as yet unclear. Martin-Garcia et al. (2006) also failed to detect a significant difference in TAK779 sensitivity between brain- and spleen-derived envelopes from a single patient. However, these authors were unable to detect a difference in the capacity of brain and spleen envelopes to exploit low CCR5 levels. Together, these results do not show a clear relationship between the extent of R5 macrophage tropism and sensitivity to CCR5 inhibitors. Importantly, these observations do not indicate

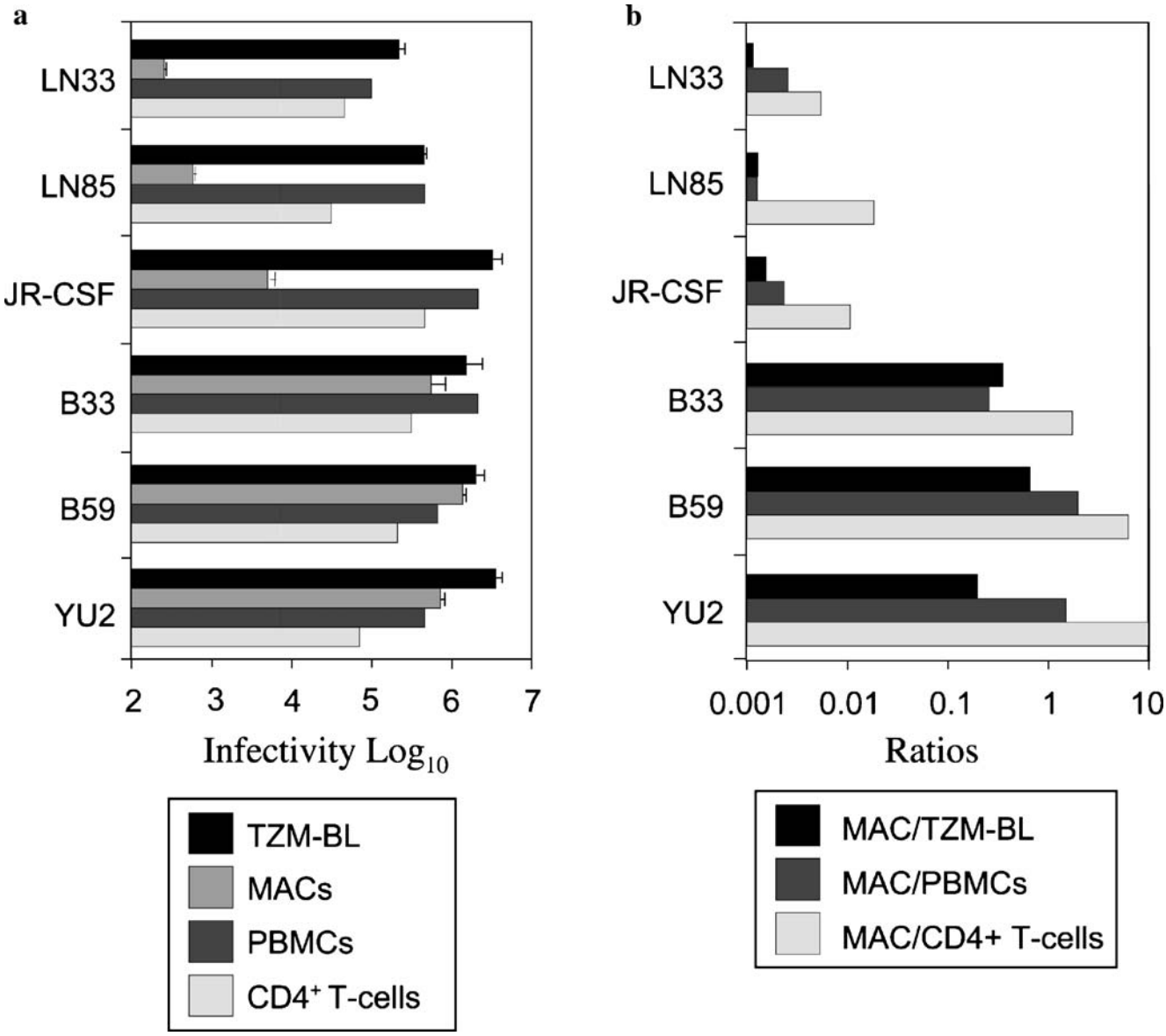


**Fig. 2** Infectivity of replication-competent viruses carrying highly macrophage-tropic and non-macrophage-tropic envelopes evaluated on HeLa TZM-BL, primary macrophages, PBMCs, and $CD4^+$ T cells. (a) Infectivity titers of replication-competent viruses carrying non-macrophage-tropic (NA118 LN33, NA420 LN85 and JR-CSF) or macrophage-tropic (NA420 B33, NA20 B59 and YU2) envelopes. Infectivity for HeLa TZM-BL and macrophages was estimated by focus-forming unit (FFU) assays and results shown represent averaged FFU for duplicate wells from one of several experiments. Infectivity for PBMCs and $CD4^+$ T cells was estimated by $TCID_{50}$ assays using replicates of six wells and is representative of at least two experiments. (b) Ratios of infectivity for macrophages compared with $CD4^+$ T cells, PBMCs, and HeLa TZM-BL. These results show that non-macrophage-tropic envelopes amplified from LN tissue replicated efficiently in primary PBMCs and $CD4^+$ T cells despite inefficient replication in primary macrophages.

that highly macrophage-tropic variants in the brain will generally carry increased resistance to CCR5 inhibitors.

## Highly macrophage-tropic envelopes and sites outside the brain

In our studies on HIV-1 envelope phenotypes in brain tissue, we used nested PCR to amplify complete envelope genes for investigation. This approach convincingly showed that highly macrophage-tropic R5 envelopes are present in brain (Peters et al. 2004, 2006). However, we were surprised that all R5 envelopes amplified from LN tissue of three patients were not macrophage-tropic. These observations prompted us to evaluate whether macrophage-tropic R5 envelopes could be detected in blood or in semen where the tropism of R5 viruses could effect the efficiency of transmission. We investigated whether envelopes ampli-

fied from proviral DNA present in adult blood (six envelopes) and semen (five envelopes) of three patients were able to confer infection of macrophages. Only one envelope derived from semen conferred significant macrophage infection (Peters et al. 2006). This result suggests that HIV-1 variants predominant in semen will confer inefficient infection of macrophages. The capacity of such variants to transmit must depend on the role of macrophages (or cells that express low CD4 and/or CCR5 levels) in establishing infection in a new host. The presence of one envelope in semen that conferred more efficient macrophage infection indicates that further such variants are likely to be identified as more semen samples are analyzed. Clearly, this area requires further study to fully define the phenotype of R5 viruses present in semen and to establish whether the variation in macrophage tropism described here impacts on transmission.

## Determinants of macrophage tropism

Dunfee et al. (2006a) reported that HIV-1 envelopes present in brain tissue of patients suffering from HIV-associated dementia frequently carried an asparagine residue at position 283 in the C2 part of the CD4 binding site, whereas T283 was predominant in nonbrain envelopes. Dunfee et al. (2006a) also reported that N283 was present in 41% of envelope sequences derived from brain tissue of demented patients but in only 8% of envelopes from nondemented patients. In an elegant experiment, Dunfee et al. showed that recombinant gp120 carrying N283 bound CD4 with an increased affinity probably due to the more efficient formation of a hydrogen bond between N283 and glutamine Q40 on CD4 (compared with T283).

Of the envelopes that we amplified from brain tissue of five individuals with neurological complications, 9 of 11 contained N283, whereas only 1 of 9 from LN had N283. Of 32 envelopes amplified from adult blood and semen and from plasma of pediatric patients, none carried N283; however, only three conferred significant macrophage infection. It should be emphasized that N283 only partly explains the difference in macrophage tropism between brain and LN envelopes. For example, one LN envelope from patient NA20 contained N283, but was not macrophage tropic (like other LN envelopes). In addition, we identified several envelopes from within and outside the brain that conferred macrophage infection but did not carry N283. Nevertheless, the observations described above show a clear association of N283 with increased macrophage tropism although further unidentified envelope determinants must also contribute.

## Discussion

HIV-1 R5 viruses are frequently described as macrophage-tropic. However, several studies from different laboratories indicate that the capacity of such strains to infect macrophages is highly variable (Gray et al. 2005; Li et al. 1999; Peters et al. 2004; Peters et al. 2006; Simmons et al. 1996; Tuttle et al. 2002). Such variation is likely to affect various aspects of HIV-1 infection, including transmission and pathogenesis. In clade B infections, R5 viruses predominate in the asymptomatic stages of disease, then CXCR4-using variants emerge in about 50% of AIDS cases and herald a more rapid decline in $CD4^+$ T cells and disease progression (Connor and Ho 1994; Koot et al. 1993). This is logical because CXCR4 is more widely expressed on T-cell populations compared with CCR5, and CXCR4-using variants have an expanded tropism for naïve as well as memory $CD4^+$ T cells (Blaak et al. 2000; Bleul et al. 1997). Yet, in the absence of detectable CXCR4-using variants, infected individuals still progress to AIDS and die. Several groups have reported that R5 virus isolates with increased tropism for macrophages can be isolated from patients with AIDS (Gray et al. 2005; Li et al. 1999; Tuttle et al. 2002). In our study, none of the envelopes amplified from LN tissue were highly macrophage-tropic although such envelopes were amplified from brain tissue. Our data suggest that highly macrophage-tropic variants are either absent or less prevalent in LN tissue compared to non-macrophage-tropic strains. However, the nested PCR approach that we used for envelope amplification targeted proviral DNA, which represents both nonreplicating archived forms and actively replicating virus. If non-macrophage-tropic viruses archived during the asymptomatic phase are predominant, than actively replicating variants that are highly macrophage-tropic (if present) may have been missed by our PCR approach. If highly macrophage-tropic variants do evolve late in disease, their capacity to exploit low levels of CD4 and/or CCR5 may confer a broader tropism among $CD4^+$ T-cell populations that express low levels of CCR5. If true, then highly macrophage-tropic variants may play a role in the depletion of $CD4^+$ T cells late in disease.

Why are cultured R5 virus isolates frequently macrophage tropic, whereas the PCR-derived envelopes that we obtained from LN are not? If macrophage-tropic R5 variants have a replicative advantage during culture-based isolation procedures, they may be preferentially isolated even if they represent minor populations *in vivo*. Increased fitness could be conferred by more efficient envelope interactions with CD4 and CCR5 and enhanced by a broader tropism among $CD4^+$ T-cell populations present in PBMCs. Moreover, if few envelope changes are required to confer macrophage tropism and an enhanced fitness for $CD4^+$ T cells, then macrophage-tropic variants may also rapidly evolve during the isolation procedure.

The association of N283 with macrophage-tropic envelopes in brain and with dementia could indicate that such

strains are neurovirulent. However, it is unclear whether such strains actually cause neurological damage or whether their replication stems from an increase in activated macrophages in already damaged brain tissue in demented individuals (Fischer-Smith et al. 2001, 2004).

Finally, the selection pressures *in vivo* that select for highly macrophage-tropic and non-macrophage-tropic R5 phenotypes are unknown. It could simply be an adaptation for replication in T cells or macrophages. However, the role of neutralizing antibodies in LN and other immune tissues in driving the evolution of envelopes into a structure that protects against antibody neutralization is also a possibility. For example, the envelope has evolved a "glycan shield" formed by extensive glycosylation, which protects against antibody binding and neutralization (Derdeyn et al. 2004). The V1V2 loop is highly variable in sequence and in length and has also been shown to act to protect critical envelope sites, including the CD4 binding sites from antibody neutralization (Pinter et al. 2004). Mechanisms that prevent antibody access to the CD4 binding site may also reduce the efficiency of binding to CD4 and restrict replication in immune tissue to cells expressing high levels of CD4. In contrast, the brain is protected by the BBB and usually excludes antibodies. In this environment, HIV variants that bind CD4 efficiently may evolve enabling infection of cells that express low amounts of CD4, e.g., macrophages and microglia.

In summary, the variation in macrophage tropism of HIV-1 R5 viruses *in vivo* raises important questions. For example, do highly macrophage-tropic R5 variants that are detected in late disease contribute to $CD4^+$ T-cell depletion particularly in individuals from whom CXCR4-using variants cannot be isolated? Variation in macrophage tropism could also have a profound effect on the efficiency of HIV-1 transmission depending on the role of macrophages or cells that express low CD4 and/or CCR5 for establishing infection in a new host. Finally, the brain may act as a sanctuary site where persistent replication of highly macrophage-tropic viruses during highly active antiretroviral treatments may result in the evolution of drug-resistant variants. If highly macrophage-tropic variants have increased fitness for $CD4^+$ T cells, they may then recolonize immune tissue and disseminate drug resistance mutations.

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