Replication of human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus strain mac (SIVmac) and chimeric HIV-1/SIVmac viruses having env genes derived from macrophage-tropic viruses: an indication of different mechanisms of macrophage-tropism in human and monkey cells

Jiangli Chen,⁠1 Eiji Ido,⁠1 Minghao Jin,⁠1 Takeo Kuwata,⁠1 Tatsuhiko Igarashi,⁠1 Akiko Mizuno,⁠1 Yoshio Koyanagi¹ and Masanori Hayami¹

¹ Laboratory of Pathogenic Virus, Institute for Virus Research, Kyoto University, 53 Shogoin-kawaracho, Kyoto 606, Japan
² Department of Microbiology, Tokyo Medical and Dental University, Tokyo 113, Japan

To investigate the transferability of macrophage (MΦ)-tropism among primate lentiviruses, we constructed recombinants of human immuno deficiency virus type 1 (HIV-1), simian immunodeficiency virus strain mac (SIVmac) and chimeric HIV-1/SIVmac (SHIV) having env region MΦ-tropic determinants. A recombinant HIV-1 having env partially derived from a MΦ-tropic HIV-1 strain (JR-FL) replicated in human macrophages but not in monkey macrophages. Conversely, a recombinant SIVmac having env from a MΦ-tropic strain (SIVmac316) replicated in monkey macrophages but not in human macrophages. A new SHIV (designated NM-3rN/JRFL) carrying the LTR and gag, pol, vif, vpx and nef of SIVmac and vpr, tat, rev and env of HIV-1 with env partially replaced by that of JR-FL was replication-competent in human macrophages but not in monkey macrophages. These results suggest that the MΦ-tropic determinant is specific to each host species and that the mechanism of MΦ-tropism is different between HIV and SIV.

T-lymphocytes and monocyte-macrophages are the major target cells for human immunodeficiency virus type 1 (HIV-1). Depletion of CD4+ T-lymphocytes in HIV-1-infected-patients eventually results in profound immunosuppression (Fauci, 1988). HIV-1 also causes severe encephalopathy. Since macrophage (MΦ)-tropic HIV-1 isolates were frequently obtained from brain tissues of AIDS patients with subacute encephalopathy (Koenig et al., 1986; Koyanagi et al., 1987, 1988), their role in pathogenesis became an issue of research interest. Several groups have reported that the determinant for MΦ-tropism lies within the env gp120 gene of HIV-1 and particularly within the region that includes the V3 loop (O’Brien et al., 1990; Cheng-Mayer et al., 1990; Shioda et al., 1991; Westervelt et al., 1992).

The similarity of immunosuppression caused by simian immunodeficiency virus (SIV) to that caused by HIV-1 is well-known (Letvin et al., 1985; Desrosiers, 1990). Not only polyclonal SIVmac but molecularly cloned SIVmac239 were shown to cause lethal AIDS in rhesus monkeys (Macaca mulatta) (Kestler et al., 1990). SIVmac239 can replicate productively in T-lymphocytes but not in monocyte-macrophages. However, a neurotropic SIV strain was obtained from brain tissues of macaques which had been inoculated by sequential passages of the lymphotropic parental virus (Sharma et al., 1992). Subsequently, Mori et al. (1992) demonstrated by constructing recombinant SIVs between SIVmac239 and the MΦ-tropic SIVmac316 that the MΦ-tropism of SIV is also determined by env, although determinants are distributed throughout the gene.

We previously reported a series of HIV-1/SIVmac chimeric viruses (SHIV) and showed that the determinant for macaque cell tropism lies within the 5′-half of the SIVmac genome (Shibata et al., 1991). Recently, we generated another series of SHIVs having intact vpr and/or nef from different parental sources (Kuwata et al., 1995). These accessory genes were defective in the former series, but they were thereby reported to be critical for viral pathogenesis in vivo (Kestler et al., 1991; Lang et al., 1993). Using the new SHIVs, we found that one of them [designated NM-3rN (Kuwata et al., 1995), having the LTR and gag, pol, vif, vpx and nef genes of SIVmac and vpr, tat,
rev, vpu and env genes of HIV-1 efficiently infected macaque monkeys. Since this SHIV carries HIV-1 env, we thought it would be useful for investigating whether the determinant for MΦ-tropism in the env region of HIV-1 is functional in monkeys and establishing transferability of MΦ-tropism between HIV-1 and SIV. Thus, we constructed recombinants of HIV-1, SIVmac and SHIV having various MΦ-tropism-determining env regions.

The CD4⁺ human T-lymphoid cell lines M8166 [a subclone of C8166 (Clapham et al., 1987)] and MT-4 (Harada et al., 1985), and a human T-B hybrid cell line, CEMx174 (Salter et al., 1985), were grown in RPMI 1640 medium supplemented with 10% heat-inactivated foetal calf serum (FCS). Cos-1 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% FCS. Human or cynomolgus monkey (Macaca fascicularis) peripheral blood mononuclear cells (PBMC) were prepared as described (Ohta et al., 1988) and maintained in RPMI 1640 medium containing 10% FCS and human IL-2. Primary monocyte-macrophages were obtained from human or cynomolgus monkey PBMC basically according to the protocol for the plastic adherent technique described by Perno & Yarchoan (1991). In brief, PBMC (approximately 3–5 x 10⁶ per well) were cultured in 24-well plastic plates for 5 days in RPMI medium containing 10% FCS, 10% human AB serum (Sigma) and 500 units/ml M-CSF (macrophage-colony-stimulating factor, kindly provided by Morinaga Nyugyo Co. Ltd, Japan), washed twice with RPMI medium, and then adherent cells (approximately 1–2 x 10⁵ per well) were cultured in fresh RPMI medium containing 15% FCS, 10% AB serum and 100 units/ml M-CSF. Ninety-five percent or more of the cells were macrophages based on morphological criteria determined after standard Giemsa and esterase staining.

The molecular clones used for constructing recombinant viruses were: a T-lymphotropic HIV-1 (NL432) (Adachi et al., 1986), a MΦ-tropic HIV-1 (JR-FL) originally isolated from brain tissue of an AIDS patient who had severe encephalopathy (O’Brien et al., 1990), and a T-lymphotropic SIVmac (MA239) (Kestler et al., 1990; Shibata et al., 1991). The genomic organization of these viruses is shown in Fig. 1. A recombinant HIV-1 termed NL432/JRFL was constructed by inserting a StuI–BglII fragment of JR-FL env into the corresponding site of NL432 (nt 6822–7611). A recombinant SIVmac, MA239/
post-infection in all cells except those infected with JR-FL and NL432/JRFL (data not shown). These two viruses were replication-competent only in human macrophages or primary T-cells and there were no other permissive cell lines. Therefore, the supernatants of transduced Cos-1 cells were used as virus stocks for further infection experiments after adjusting the amount of virus based on RT values.

To infect human or monkey PBMC, appropriate volumes of the stocks (2 x 10^5 RT units, which is approximately equivalent to 1 x 10^5 TCID_{50}) were mixed with the cells (1–2 x 10^5 cells per well) in a 24-well plate. To infect human or monkey monocyto-macrophages, appropriate volumes of the stocks (2 x 10^5 RT units) were added to the adherent cells, incubated for 4 h or overnight at 37 °C, and cultured in fresh medium.

Growth kinetics of the recombinant viruses were first examined in human PBMC (Fig. 2 A) and macrophages (Fig. 2 B). The parental NL432 strain, a T-lymphotropic HIV-1, replicated well in human PBMC but not in human macrophages. In contrast, the recombinant virus NL432/JRFL, having env partly derived from a MΦ-tropic strain, JR-FL, replicated well in human macrophages. O’Brien et al. (1990) previously found that the determinant of HIV-1 MΦ-tropism was in a 157 amino acid region (a Stul–MstII fragment, nt 6822–7305) of gp120 by constructing chimeras between NL432 and JR-FL. The minimum determinant was upstream of the CD4-binding domain and included the V3 loop. In this study, however, we used a slightly larger region (a Stul–BglII fragment of JR-FL) to construct NL432/JRFL. This choice was based on the fact that the replicative capacity of a recombinant virus having the Stul–MstII fragment was weaker than that of the parental JR-FL strain, whereas another virus having a larger region (a Stul–XhoI, nt 6822–8887) replicated better in mononuclear phagocytes (O’Brien et al., 1990). We expected that MΦ-tropism would appear more clearly if we used a larger region of JR-FL. In fact, virus NL432/JRFL replicated well in human macrophages. Since NL432 has no replicability in human macrophages, it is obvious that MΦ-tropism was conferred by introducing the segment of JR-FL env.

The SIV strain MA239 was replication-competent in human PBMC, but not in human macrophages. The MA239/316ENV virus, having env of SIVmac316, showed a higher replication-competency in human PBMC than MA239. An important result was that MA239/316ENV showed no indication of replication in human macrophages, suggesting that the MΦ-tropism determinant in the SIV–monkey system is ineffective in the human system.

The chimeric virus NM-3rN, having env of NL432, and its derived virus NM-3rN/JRFL were replication-competent in human PBMC, but only the latter replicated in human macrophages. This result indicates that a portion of JR-FL env conferred a growth capacity to otherwise replication-incompetent viruses in human macrophages. It is noteworthy that the growth capacities of both NM-3rN and NM-3rN/JRFL were weaker than those of NL432 or NL432/JRFL.

316ENV, was constructed by replacing env of MA239 with that of a MΦ-tropic SIVmac316 as described (Mori et al., 1992). A SHIV termed NM-3rN was constructed from NL432 and MA239 (Kuwata et al., 1995). A new SHIV designated NM-3rN/JRFL was constructed by replacing the Stul–BglII fragment of NM-3rN by that of JR-FL.

To examine whether the recombinant proviral plasmids were able to produce infectious viruses, they were introduced into Cos-1 cells by the DEAE-dextran method (Naidu et al., 1988), and culture supernatants were harvested, filtered (0.45 μm pore size) and stored at −80 °C. These supernatants were then used to infect CD4+ human cells, either M8166, MT-4 or CEMx174. The virus-associated reverse transcriptase (RT) activity was measured as described by Willey et al. (1988). Increasing RT activities were detected in all the culture fluids and syncytium formation was apparent at around 7–14 days post-infection in all cells except those infected with JR-FL and NL432/JRFL (data not shown). These two viruses were replication-competent only in human macrophages or primary T-cells and there were no other permissive cell lines. Therefore, the supernatants of transduced Cos-1 cells were used as virus stocks for further infection experiments after adjusting the amount of virus based on RT values.

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observation implies that, in addition to the JR-FL env, some other part(s) of the HIV-1 genome, probably LTR, gag or pol, might be contributing to the productive growth in human macrophages.

Growth kinetics of the recombinant viruses were then examined in PBMC (Fig. 3A) and macrophages (Fig. 3B) from cynomolgus monkeys. MA239 was replication-competent in monkey PBMC, but only weakly so in monkey macrophages (a slight rise in RT values was observed). MA239/316ENV possessed a relatively high replication-competency in monkey PBMC and it showed significantly higher replication in monkey macrophages. The MΦ-tropic property was clearly conferred by env derived from the 316 strain as reported by Mori et al. (1992).

Although we performed the infection experiments with NL432 and NL432/JRFL in monkey PBMC and macrophages, we did not obtain any evidence for their replication (data not shown). It is clear that the segment of JR-FL env which is a MΦ-tropic determinant in the human system did not confer replication-competence to NL432 in the monkey system.

Of particular interest were the growth-potencies of two SHIVs. NM-3rN, having env of NL432, was replication-competent in monkey PBMC, although its apparent RT increase was weak. (RT values were usually lower when the virus-containing Cos-1 supernatants were inoculated.) However, no NM-3rN replication could be demonstrated in monkey macrophages. It should be noted that no replication of NM-3rN/JRFL could be detected in monkey macrophages despite the fact that it had a replication-competence similar to that of NM-3rN in monkey PBMC. Essentially the same results were obtained when PBMC and macrophages from rhesus monkeys were used (data not shown). These results indicate that we could not transfer the MΦ-tropic property to NM-3rN by introducing the MΦ-tropic-determining env region of JR-FL. In other words, the MΦ-tropic determinant for the human system is ineffective in the monkey system. The limitation of the transferability of MΦ-tropicism in the opposite direction was mentioned above. Taken together, we conclude that the viral MΦ-tropic property is confined to each host species and the mechanism of MΦ-tropicism may be different between HIV-1 and SIV. Whether this conclusion can be generalized or is restricted to the strains JR-FL and 316 awaits further investigation using other MΦ-tropic strains.

Recently, the discovery of co-receptors for HIV and SIV infection has been in the spotlight (Clapham & Weiss, 1997). So-called non-syncytium-inducing (NSI) viruses of HIV-1, which are usually MΦ-tropic, use CCR5 as a co-receptor on virus entry. In contrast, syncytium-inducing (SI) viruses, which are typically T-lymphotropic, use CXCR4 in addition to CCR5. SIVmac uses CXCR5 but not CXCR4. Interestingly, SIVmac and NSI HIV-1 appear to commonly use some newly identified co-receptors (for example, Bonzo and BOB). Further studies will be needed to clarify the relationship between such co-receptors and the viral MΦ-tropicism at the molecular level.

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References


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