Sequences responsible for efficient replication of simian immunodeficiency virus SIV<sub>MND</sub> in cells of the monocyte/macrophage lineage

Hiroyuki Sakai, Sayuri Sakuragi, Jun-Ichi Sakuragi, Meiko Kawamura, Riri Shibata and Akio Adachi*

Institute for Virus Research, Kyoto University, Kyoto 606, Japan

We determined the susceptibility of monocytic cell lines to infection with viral strains derived from two infectious clones of simian immunodeficiency virus isolated from a mandrill. One of the strains, which replicates poorly in T cell lines, was found to grow more rapidly than the other in these cells. The viral determinant for this property was genetically mapped within the env gene encoding a surface protein. Six amino acid substitutions identified appeared to be located outside of the domains corresponding to human immunodeficiency virus type 1 env functional domains such as the CD4-binding and V3 loop regions.

Human immunodeficiency virus type 1 (HIV-1) infects a number of cell types in vitro and in vivo (Clapham, 1991; Gartner et al., 1986; Koyanagi et al., 1987; Schnittman et al., 1989; Wiley et al., 1986). Among them, CD4-positive T lymphocytes and monocyte/macrophages appear to be the major targets for infection in vivo. CD4-positive T lymphocytes may be the major reservoir for the virus in peripheral blood (Schnittman et al., 1989). Monocyte/macrophage cells are thought to be the primary tissue reservoir (Gartner et al., 1986) and the dominant cell types infected in the central nervous system (Wiley et al., 1986; Koenig et al., 1986). Various HIV-1 isolates have been reported to be T cell- or macrophage-tropic and the viral determinants of tropism have been investigated (Cheng-Mayer et al., 1990, 1991; Hwang et al., 1991; Kim et al., 1990; O'Brien et al., 1990; Pomerantz et al., 1991; Shioda et al., 1991).

Recently, we have constructed and characterized two infectious molecular clones of a simian immunodeficiency virus from an African mandrill (SIV<sub>MND</sub>) (Sakai et al., 1992a, b), which represents a distinct subgroup of the primate lentiviruses (Desrosiers, 1990; Tsujimoto et al., 1988, 1989). Viruses derived from the two infectious clones, designated pMD121 and pMD122 (Fig. 1), displayed differing degrees of virulence in a CD4-positive T cell line, MolT4 clone 8 (M4-8) (Sakai et al., 1992a). The virus from pMD121 (MD121) grew more rapidly than that from pMD122 (MD122) in M4-8 cells and the viral determinant for this phenotype was the tat gene. In this report, we have examined the sensitivity of monocyte/macrophage cell lines to infection with MD121 and MD122 viruses. We have found that MD122
Fig. 2. Growth kinetics of various recombinant virus clones in T cell lines M4-8 and M8166. To prepare cell-free virus samples, SW480 cells (Adachi et al., 1986) were transfected with 20 ng of DNA clones by the calcium phosphate coprecipitation method (Graham & van der Eb, 1973; Wigler et al., 1979), and 48 h later culture supernatants were collected and filtered (0.45 μm pore size). Cells (10⁶) were infected with 2 × 10⁵ RT units of cell-free virus obtained from transfection as described by Folks et al. (1985), and RT production was monitored at the designated intervals. Virion-associated RT activity was measured as described by Willey et al. (1988). For quantification, spots on DE81 paper were cut out, and the RT activity was determined by scintillation counting. The data presented were obtained in a single experiment. For convenience, the results for recombinant viruses with high tat activity (NcoI-SphI fragment from pSMH111) and low tat activity (NcoI-SphI fragment from pSMH21) (Sakai et al., 1992a) are shown in the upper and lower panels, respectively. The infection experiments were repeated three times, and the results obtained were essentially the same.

Fig. 3. Growth kinetics of various recombinant virus clones in monocytic cell lines HL60 and U937. Methods for infection experiments were the same as described in the legend to Fig. 2. The same amount of the same virus stocks (Fig. 2) was used for infection. The results are presented as described in the legend to Fig. 2. HL60 (JCRB0085) and U937 (CRL1593) cells were obtained from the Japanese Cancer Research Resources Bank.
replicated more rapidly in these cells than MD121, in contrast to their behaviour in T cell lines, and we have shown that this viral property is attributable to the env gene.

To discover whether viruses carrying the MD121 tat gene grow faster than those with the MD122 tat gene in a T cell line other than M4-8, M8166 cells (Shibata et al., 1991) were infected with various viruses (Fig. 1) and viral replication was monitored by reverse transcriptase (RT) assay. The results were similar in both M4-8 and M8166 cell lines (Fig. 2). Human monocytic HL60 cells were then infected with MD121 and MD122, and the viral growth kinetics were determined. Both viruses were able to grow in the cells (Fig. 3). However, the replication kinetics of MD122 were slightly faster than those of MD121. The difference in growth kinetics did not appear to be so small, since the tat gene of MD122 was less active than that of MD121 (Sakai et al., 1992a). The slightly more rapid growth kinetics of MD122 relative to MD121 were also observed in another monocytic cell line, U937 (Fig. 3). Progeny viruses recovered from infected HL60 and U937 cells showed essentially the same growth kinetics as those of their parental viruses in M4-8, M8166, HL60 and U937 cells (data not shown), which indicates that the growth characteristics of both viruses were quite stable.

To define the sequence responsible for the efficient replication in HL60 and U937 cells, growth kinetics in these cells of six recombinant clones from MD121 and MD122 (Fig. 1) were determined. The structural differences between the two parental viruses originated from a Ncol–Apal fragment encompassing the 3' portion of pol, vif, vpr, the first coding exons of tat and rev, and the 5' portion of the env gene (Fig. 1). Highly and poorly replicating viruses were readily distinguished by comparison of the growth kinetics of viruses carrying the same tat gene (within the sequence of the Nhel and Sphl sites in Fig. 1) (see Fig. 3). Among the viruses with the MD121 tat gene, MD121C and MD122B containing the sequence between the Sphl and Apal sites derived from MD122 replicated more rapidly than MD121 and MD121A (Fig. 3, upper part). Essentially the same results were obtained with viruses containing the MD122 tat gene (Fig. 3, lower part). The data shown in Fig. 3 indicate that the Sphl–Apal sequence was a major determinant for the growth properties of the viruses in HL60 and U937 cells, although the tat activity of the viruses appeared to affect their growth rate slightly.

The nucleotide sequences of the Sphl–Apal fragments derived from MD121 and MD122 were then determined and compared (Fig. 4). Seven nucleotide substitutions were identified in the region, which resulted in six amino acid changes in the env-encoded surface (SU) protein (Fig. 4a). Among the substitutions, the change of Asn to Tyr, which results in the loss of the N-glycosylation site of MD121, might affect the three-dimensional structure of the SU protein. The Lys (basic amino acid) to Met (neutral amino acid) substitution could also have affected the conformation of the protein. However, all six substitutions had little effect on the secondary structure and hydrophobicity of the SU protein as analysed by the PROTEUS system of DNASIS (Hitachi Software Engineering) (data not shown). The amino acid
sequence of SIV<sub>MND</sub> env-encoded SU protein was compared with that of the HIV-1 SU protein (Fig. 4b). The six amino acid changes did not appear to be located in functional regions such as the V3 and CD4-binding domains as reported for HIV-1 (Clements et al., 1991; Hattori et al., 1989; Helseth et al., 1991; Lasky et al., 1987; Olshesky et al., 1990).

In this study, we have shown that subtle change(s) in the env-encoded SU protein of SIV<sub>MND</sub> can have a considerable effect on virus replication in monocyte/macrophage cell lines. In the case of HIV-1, the sequence encompassing the V3 loop sequence is responsible for the tropism for primary macrophages (Hwang et al., 1991; Shioda et al., 1991). Because the amino acid substitutions found in the env gene product of SIV<sub>MND</sub> MD122 were probably not within the functional domains already reported for HIV-1, the interaction of the SU protein of the virus with target cells should be investigated in more detail. Another important finding in this report concerns the biological effect of tat activity in T cell and monocytic cell lines. In M4-8 T cells, a small difference in tat activity greatly affected the virus growth rate (Sakai et al., 1992a). In contrast, no such drastic effect was observed in HL60 and U937 cells. This might imply that there is a difference in the requirement for tat activity during virus growth in various cell types.

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References


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