Generation of a Chimeric Human and Simian Immunodeficiency Virus Infectious to Monkey Peripheral Blood Mononuclear Cells

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We constructed five chimeric clones between human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency viruses (SIVs) and four SIV MAC mutations by recombinant DNA techniques. Three chimeric clones and all mutants with an alteration in either the vif, vpx, vpr, or nef gene were infectious to human CD4-positive cell lines. The susceptibility of macaque monkey peripheral blood mononuclear cells (PBMC) to infection by these mutants and chimeras was examined in vitro. Macaque PBMC supported the replication of wild-type and vpx, vpr, and nef mutant SIV MAC strains. A chimera carrying the long terminal repeats (LTRs), gag, pol, vif, and vpx of SIV MAC and tat, rev, vpu, and env of HIV-1 was also replication competent in PBMC. In contrast, HIV-1, the vif mutant of SIV MAC, a chimera containing rev and env of SIV MAC, and a chimera containing vpx, vpr, tat, rev, and env of SIV MAC did not grow in PBMC. Western immunoblotting analysis of the replicating chimera in PBMC confirmed the hybrid nature of the virus. These data strongly suggested that the sequence important for macaque cell tropism lies within the LTR, gag, pol, and/or vif sequences of the SIV MAC genome.

AIDS is caused by two related but distinct groups of human lentiviruses, human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2) (5, 11, 31, 41). HIV-1 is associated with the AIDS pandemic throughout the world, while HIV-2 infection is less widespread, found mainly in West Africa. HIV-1 and HIV-2-related viruses (simian immunodeficiency viruses [SIVs], including SIV MAC isolated from rhesus monkeys [Macaca mulatta], SIV Mne from pigtailed macaques [M. nemestrina], and SIV AMN from sooty mangabeys [Cercocebus atys]) can infect macaque monkeys persistently, and some strains cause a fatal disease like human AIDS (6, 13, 15, 19, 26, 30, 35). Persistent infection of macaque or African green monkeys (Cercopithecus aethiops) with SIV AGM (from African green monkeys) has also been reported (4, 24). In contrast, HIV-1 can infect only higher primates, such as chimpanzees (Pan troglodytes) (2, 20, 21, 37) and gibbons (Hylobates lar) (33). Infection of rabbits (17, 29) and severe combined immunodeficient mice reconstituted with human peripheral blood leukocytes (36) with HIV-1 has also been described.

The reason for the narrow host range of HIV-1 is not known. Comparative studies of primate lentiviruses have shown the close relationship between HIV-1 and SIVs. The genome organization of HIV-1 (including SIV cpz from chimpanzees) and SIVs (including HIV-2) is similar except for the vpu and vpx genes, which are unique to HIV-1 and SIVs, respectively (14). Mutational analyses and complementation studies by us and others showed a functional similarity and exchangeability between some genes of HIV-1 and SIVs (3, 16, 22, 25, 32, 34, 42, 43, 45, 46, 49). Furthermore, we have recently described replication-competent chimeras between HIV-1 and SIV AGM (47). In this study, we focused on the host range difference between viruses derived from well-characterized molecular clones of HIV-1 (HIV-1NL432) and SIV MAC (SIV MAC239). HIV-1NL432 replicates efficiently in human peripheral blood mononuclear cells (PBMC) and various human T-cell lines (1, 38, 39), but does not productively infect PBMC of rhesus monkeys, cynomolgus monkeys (Macaca fascicularis), or African green monkeys (unpublished data). In contrast, SIV MAC239 can replicate in macaque PBMC, human PBMC, and human cell lines (27, 35). This clone can induce AIDS in rhesus monkeys (26). We assumed that the determinant(s) for the dual tropism must reside in the genome of SIV MAC239 and performed genetic mapping of the determinant(s) by construction of SIV MAC239 mutants and chimeric clones between HIV-1NL432 and SIV MAC239. We demonstrate here that the vpx, vpr, tat, rev, env, and nef genes of SIV MAC239 are not essential for the dual tropism and that a chimeric virus containing tat, rev, vpu, and env derived from HIV-1NL432 can productively infect macaque PBMC.

MATERIALS AND METHODS

Cells. A human colon carcinoma cell line, SW480 (1), was maintained in Dulbecco’s modified Eagle’s medium containing 10% heat-inactivated fetal calf serum (FCS). CD4+ human leukemia cell lines MT-4 (23), CEMx174 (44), and Molt6 (a subclone of C8166 [10]) were maintained in RPMI 1640 medium containing 10% FCS. PBMC from cynomolgus monkeys (M. fascicularis) were separated from heparinized whole blood, stimulated with 10 μg of concanavalin A (ConA) per ml for 24 h, and maintained in RPMI 1640 medium containing 10% FCS and human interleukin-2 as described previously (40).

Transfection. Uncleaved plasmid DNA was introduced into SW480 cells by the calcium phosphate coprecipitation method (1).

Infection. Culture supernatants of virus-producing cells were filtered (0.45-μm pore size), and appropriate volumes were added to leukemia cell lines (18) or macaque PBMC 3 days after ConA stimulation as described previously (40).

RT assays. Virion-associated reverse transcriptase (RT) activity was measured as described previously (50).

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Western immunoblotting. Lysates of transfected or infected cells were prepared as described before (50), and proteins were resolved on 10 or 12% sodium dodecyl sulfate-polyacrylamide gels, followed by electrophoretic transfer to nitrocellulose membranes. The membranes were incubated at room temperature with sera from individuals infected with HIV-1 or SIVmac overnight and with 125I-protein A for 3 h, washed, and visualized by autoradiography (50).

DNA constructs. The infectious molecular clone pNL432 (HIV-1) has been described (1). A SIVmac infectious clone, pMA239, was constructed by inserting the HindIII (41 nucleotides [nt] upstream of the beginning of the 5' long terminal repeat [LTR])-SacI (1,153 nt downstream of the end of the 3' LTR) fragment of the lambda phage clone of SIVmac239 (35) into the plasmid vector pUC18. All mutants of SIVmac239 and chimeric clones were constructed from pMA239 and pNL432 by standard recombinant DNA techniques (Fig. 1 and 2). Mutants pMA-Sc (mutation in vif), pMA-ET (vpx), pMA-N2 (vpr), and pMA-Nc ( nef) were generated by digesting appropriate subclones of pMA239 with restriction enzymes, end-modified by T4 DNA polymerase, ligating the blunt ends with T4 DNA ligase, and recloning the mutated DNAs into pMA239. To construct pMA-Nc, an XhoI linker (8 bp) was inserted into the blunt-ended NcoI site. Thus, pMA-Sc contains a 4-bp deletion at SacI (nt 5755); pMA-ET, a 4-bp insertion at EcoT22I (nt 6052); pMA-N2, a 4-bp insertion at NcoI (nt 6193); and pMA-Nc, a 12-bp insertion at NcoI (nt 9281). Chimeric clones were constructed by inserting an appropriate fragment cut out from one of the infectious clones into another proviral clone or a subclone derived from it. prcNM-1 was constructed by inserting a SacI (blunt-ended, nt 5755)-XhoI (nt 9281) fragment of pMA-Nc between the EcoRI (blunt-ended, nt 5743) and XhoI (nt 8887) sites of pNL432. prcNM-2 was constructed by inserting an XbaI (blunt-ended, nt 6439)-XhoI (nt 9281) fragment of pMA-Nc between the Clal (blunt-ended, nt 6153) and XhoI (nt 8887) sites of pNL-Ss (39) identical to pNL432 except for Clal linker insertion at SspI. prcNM-3 was made by inserting an EcoRI (blunt-ended, nt 5743)-XhoI (nt 8887) fragment of pNL432 between the Eco47III (nt 6351) and XhoI (nt 9281) sites of pMA-Nc. prcNM-4 was made by inserting an SpeI (nt 1770)-Eco47III (nt 6351) fragment between the SpeI (nt 1507) and EcoRI (blunt-ended, nt 5743) sites of pNL432. prcNM-5 was made by inserting an NarI (nt 823)-PstI (nt 1684) fragment of pMA239 between the NarI (nt 637) and PstI (nt 1415) sites of pNL432.

RESULTS

Construction of mutants of SIVmac239 and chimeric clones between HIV-1NL432 and SIVmac239. The genome organizations of the parental molecular clones of HIV-1 (pNL432) and SIVmac (pMA239) are similar except for the vpu and vpx genes (Fig. 1). Numerous studies on various HIV and SIV isolates indicated that three structural genes (gag, pol, and env) and two regulatory genes (tat and rev) are essential for virus infectivity, whereas other genes (vif, vpx, vpr, vpu, and nef) are dispensable (for a review, see reference 8). In order to determine whether the accessory genes of SIVmac239 are essential for infectivity to macaque cells, we generated four SIVmac239 mutants (Fig. 2). Frame-shift mutations were introduced to inactivate vif, vpr, and vpu. pMA-Sc was expected to produce an aberrant vif protein (149 amino acids [aa]), whose N-terminal aa were identical to those of native vif (214 aa). Similarly, the
C-terminal 30 aa of the vpx protein (112 aa) and 76 aa of the vif protein (91 aa) would not be translated from pMA-ET and pMA-N2, respectively. The original pMA239 contains an in-frame stop codon in the nef open reading frame (ORF) at 93 aa, which truncates an otherwise 263-aa protein (26) (Fig. 1). We constructed pMA-Nc, which contains three irrelevant aa insertions at aa 70 in nef (Fig. 2).

In addition to these proviral mutants of SIVMAC239, several chimeric recombinant clones were made to determine the sequence important for macaque cell tropism (Fig. 2). Since mutations in the gag, pol, env, tat, and rev genes would deprive the virus of its infectivity in both human and macaque cells, chimeric clones were constructed so as to replace these essential genes with their counterparts from HIV-1 (Fig. 2). Chimeric viruses rcnM-1 and -2 were considered to represent HIV-1 containing SIVMAC env. There were some structural differences between the two in the central region. rcnM-1 contained many SIVMAC genes located in that region. Conversely, rcnM-3 was SIVMAC containing HIV-1 env. The other chimeras were designed to contain a shorter SIVMAC sequence than rcnM-3. rcnM-4 carried the 3′ half of gag (the C-terminal half of the capsid protein and the nucleic acid-binding protein), pol, vif, and vpx of SIVMAC. rcnM-5 contained only the 5′ half of gag (the matrix protein and the N-terminal half of the capsid protein) derived from SIVMAC. While rcnM-1, -2, and -5 have HIV-1 LTRs, rcnM-3 and -4 have SIVMAC LTRs.

Immunoblot analysis of transfected SW480 cells was performed to examine the viral proteins produced by these chimeric viruses. As shown in Fig. 3, HIV-1NL432 products p24 (gag, the capsid protein), p35 (gag, the precursor), and gp120 (env, the extracellular glycoprotein) were detected by the human antiserum (lanes h in Fig. 3A). The SIVMAC proteins p26 (gag, the capsid protein), p35 (gag, the precursor), gp41 (env, the transmembrane protein), and gp130 (env, the extracellular glycoprotein) were detected by the macaque antiserum (lanes m in Fig. 3B). The macaque antiserum was weakly reactive with gag proteins of HIV-1NL432 (lanes h in Fig. 3B). The chimeras rcnM-1 and 2 produced SIVMAC env (lanes 1 and 2 in Fig. 3B) and HIV-1NL432 gag proteins (lanes 1 and 2 in Fig. 3A), as expected from their structures. The protein profile of rcnM-3 was the opposite (lanes 3). rcnM-4 produced amounts of HIV-1NL432 env similar to those of the wild-type clone (lane 4 in Fig. 3A). However, the mature capsid protein, which was expected to be chimeric, could hardly be detected by either antiserum, whereas unprocessed p55gag was observed (lanes 4). This clone gave poor RT activity in the culture fluids after transfection (data not shown). The construct prCNM-5 produced HIV-1NL432 env (lane 5 in Fig. 3A) and chimeric gag proteins which were processed efficiently (lanes 5).

**Growth of mutants and chimeras in human and macaque cells.** Growth potentials of the mutants and the chimeras in three human CD4+ cell lines (MT-4 [23], M8166 [10], and CEMx174 [44]) which were susceptible to infection with HIV-1NL432 and SIVMAC were determined (summarized in Fig. 2). For infection, equal amounts of RT units of the virus samples (culture supernatants of transfected SW480...
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transfection, resolved anti-SIVMAC Western glycoproteins p55), pNL432; s, weeks. When there RT production were within 2 cells) were used. The infected cultures were monitored for RT production and cytopathic effect (CPE) for at least 3 weeks. When there was no sign of productive infection, observation periods were extended to 4 weeks. In each infection experiment, a high input dose was used. Under these conditions, both wild-type viruses killed most of the cells within 2 to 3 weeks. Infectivity of rcNM-4 and -5, carrying chimeric capsid proteins, could not be detected in any of the three human CD4+ cell lines (Fig. 2). The other three chimeras and all mutants of SIVMAC239 were infectious and cytopathic, although rcNM-1 and -2 and the vif mutant exhibited restricted host ranges (Fig. 2). All these replication-competent viruses could be serially passaged in the permissive cell lines. The three chimeric clones could grow in MT-4 cells, but less efficiently than the two parental viruses (Fig. 4). The vif mutation resulted in severe replication defects (Fig. 5). The vif mutant showed delayed growth kinetics in M8166 cells relative to the wild-type virus. MT-4 and CEMx174 cells did not support replication of the vif mutant.

The viable mutants and chimeric clones were examined for their ability to replicate in macaque PBMC. Figure 6 shows the growth kinetics in two PBMC preparations obtained from different cynomolgus monkeys. RT activity in

![Figure 3](https://example.com/figure3.png)  
**FIG. 3.** Expression of viral proteins in SW480 cells transfected with the chimeric clones. Cell lysates were prepared 48 h after transfection, resolved on 12% polyacrylamide gels, and analyzed by Western blotting with either an anti-HIV-1 human serum (A) or anti-SIVMAC macaque serum (B). The same amount of cell lysate was applied to the corresponding lanes in both panels. env extracellular glycoproteins (EG) of HIV-1 (gp120) and SIVMAC (gp130), env transmembrane glycoprotein (TM, gp41) of SIVMAC, gag precursors (pre-GAG, p55), and gag capsid proteins (CA) of HIV-1 (p24) and SIVMAC (p26) are indicated. Lanes: c, mock transfection; h, pNL432; s, pMA239; 1 to 5, prcNM-1 to -5, respectively.

![Figure 4](https://example.com/figure4.png)  
**FIG. 4.** Replication of chimeric viruses in the human CD4+ cell line MT-4. The results of two independent experiments are shown. MT-4 cells were infected with the same amounts (RT units) of virus obtained from SW480 cells transfected with pNL432 (h), pMA239 (s), and the chimeric clones prcNM-1 to -3 (1 to 3, respectively). RT production was monitored at intervals. c, negative control.

![Figure 5](https://example.com/figure5.png)  
**FIG. 5.** Growth kinetics of the vif mutant in human CD4+ cell lines MT-4, M8166, and CEMx174. Cells were infected with cell-free virus samples obtained from SW480 cells transfected with pUC18 (as a negative control) (c), pMA239 (s), and pMA-Sc (f). Input viral RT units were equivalent between the wild type (s) and the vif mutant (f). RT production in the infected cells was monitored at intervals.
the supernatant of macaque PBMC infected with HIV-1NL432 disappeared by day 9, while PBMC infected with SIVMAC239 produced RT activity continuously for 3 to 4 weeks. Among the mutants of SIVMAC239, only the vif mutant did not productively infect macaque PBMC. There were no significant differences in the kinetics of virus replication among the wild type, vpx, vpr, and nef mutants of SIVMAC239. Two of three chimeric viruses were replication defective in macaque PBMC. Chimeras rcNM-1 and -2, which contained the env gene derived from SIVMAC239, did not grow in the PBMC in two independent experiments (data not shown). In contrast, rcNM-3, which carried LTR, gag, pol, vif, and vpx of SIVMAC239, productively infected macaque cells (Fig. 6). RT production in two cell preparations infected with the chimera persisted during the observation period. In addition, the progeny viruses retained the ability to grow in other macaque PBMC preparations (data not shown). The culture fluids of infected PBMC on day 21 (panel 2 in Fig. 6) could induce CPE when inoculated into M8166 cells. The 21-day culture fluids of PBMC infected with the wild type or vpx, vpr, or nef mutant of SIVMAC239 also contained infectious virions, but those infected with the vif mutant and HIV-1NL432 did not.

We confirmed the protein profile expressed by rcNM-3 (Fig. 7). The M8166 cells, exhibiting severe CPE after inoculation of the 21-day culture fluids of PBMC infected with SIVMAC239 or rcNM-3, were analyzed by Western blotting with both human antiserum and macaque antiserum (Fig. 7, lanes s and 3). Since the 21-day culture fluids of PBMC infected with HIV-1NL432 induced no CPE or viral protein synthesis (lanes h), M8166 cells productively infected with HIV-1NL432 (a cell-free supernatant of SW480 cells transfected with pNL432) were also analyzed as a positive control (lanes h'). The cell lysates were resolved on 12% (A) or 10% (B) polyacrylamide gels and analyzed by Western blotting with either an anti-HIV-1 human serum (A) or anti-SIVmac macaque serum (B). The same amount of cell lysates was applied to the corresponding lanes in both panels. env extracellular glycoproteins of HIV-1 (EG120) and SIVMAC (EG130), env transmembrane protein (TM41) of SIVMAC, gag precursors of HIV-1 (pre-GAG55a) and SIVMAC (pre-GAG55a), gag capsid proteins of HIV-1 (CA24) and SIVMAC (CA26), and gag matrix protein of HIV-1 (MA17) are indicated.

**DISCUSSION**

Our results clearly indicated that rcNM-3, the chimeric virus carrying the SIVMAC239-derived LTR, gag, pol, vif, and vpx, productively infected both human cell lines and macaque PBMC, like wild-type SIVMAC239. The other two chimeras (rcNM-1 and -2), whose structures were almost
opposites of that of rcNM-3, could not replicate in macaque cells. It is unlikely that vpx, which is a gene unique to HIV-2 and SIVs, is the determinant for macaque cell tropism, because the vpx mutant can grow in macaque PBMC and rcNM-1 cannot. The vif mutant clearly showed a restricted host range in human cell lines (Fig. 5), suggesting that vif may play a role in the cell type specificity of virus infection. However, whether vif also affects species specificity between human and macaque is unclear. It was somewhat surprising that the envelope proteins were not the determinants for the dual tropism, because some studies have suggested that the env region is important for virus host range, such as restricted T-cell-line tropism (28, 51), macrophage tropism (9, 12, 38), and macaque PBMC tropism (27). However, our previous study on chimeric viruses between HIV-1 and SIV_AGM suggested that env is not the determinant for the replication defect of SIV_AGM in MT-4 cells (47). The fact that a viral gene other than env determines host range has been observed with Fv-1 restriction of murine leukemia viruses (the determinant was mapped within gag [48]). In sum, we conclude that the determinant(s) for the dual tropism of SIVMAC239 is LTR, gag, pol, and/or vif.

At present, the precise sequence responsible for macaque cell tropism is not clear. rcNM-4 and -5 could not replicate even in human CD4+ cell lines (Fig. 2). An additional eight chimeric clones generated by recombination at common restriction sites located in gag and pol (PstI at nt 1415, SpeI at nt 1507, PstI at nt 2839, and KpnI at nt 4154 [pNL432] were used for recombination with the corresponding sites of pMA239) were also noninfectious, and some of them showed inefficient gag processing or poor RT production in transfection experiments (data not shown). These results suggest that the chimeric proteins (capsid proteins and RT) between the two distinct viruses could not function even when recombinations were performed at conserved restriction sites. Further study is required to elucidate the determinant(s) of dual tropism.

Productive infection of macaque PBMC with rcNM-3, the chimeric virus carrying HIV-1NL4-32-derived tat, rev, vpu, and env, also raises the possibility of animal experiments with HIV-SIV chimeras. If this chimera can also infect macaque monkeys in vivo, we would obtain an animal model especially useful for developing subunit vaccines consisting of the virus envelope glycoproteins. It was reported that two of two chimpanzees immunized with the recombinant gp120 were protected from HIV-1 infection (7). The macaque system with the chimeric virus may greatly facilitate such vaccine research.

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