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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 virus (SIV_{MAC}) and four SIV_{MAC} mutants 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-2 and HIV-2-related viruses (simian immunodeficiency viruses [SIVs], including SIV_{MAC} isolated from rhesus monkeys [*Macaca mulatta*], SIV_{Mne} from pig-tailed macaques [*M. nemestrina*], and SIV_{SMM} 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 ($HIV\text{-}1_{NL432}$) and SIV_{MAC} (SIV_{MAC239}). $HIV\text{-}1_{NL432}$ 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 $HIV\text{-}1_{NL432}$ 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 $HIV\text{-}1_{NL432}$ 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 M8166 (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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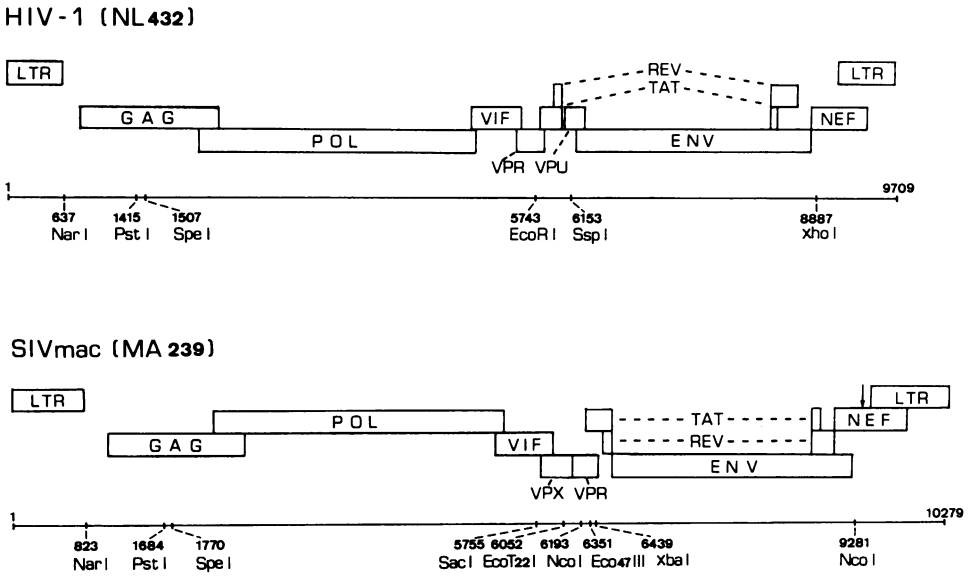


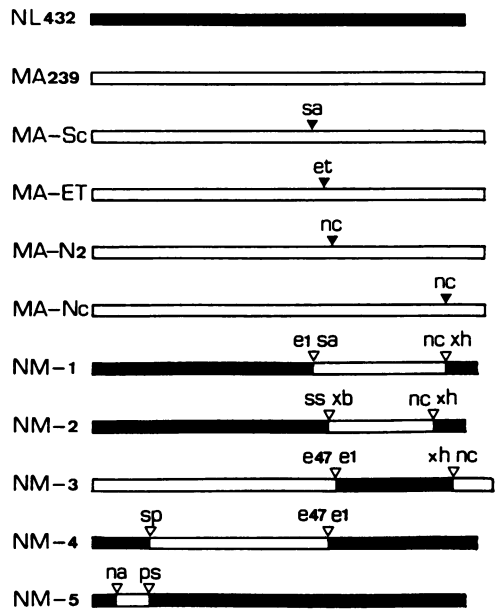
FIG. 1. Schematic representation of infectious DNA clones of HIV-1 (pNL432) and SIV_{MAC} (pMA239) based on the nucleotide sequences filed with the GenBank data base (accession numbers M19921 and M33262). Restriction sites used to make mutants and chimeras are indicated with nucleotide numbers. The boundaries of the LTRs and ORFs of the two clones are as follows. pNL432: 5′ LTR, nt 1 to 634; *gag*, nt 790 to 2292; capsid protein (*gag* p24), nt 1186 to 1878; *pol*, nt 2085 to 5096; *vif*, nt 5041 to 5619; *vpr*, nt 5559 to 5846; *tat* first coding exon, nt 5830 to 6044; *tat* second coding exon, nt 8369 to 8414; *rev* first coding exon, nt 5969 to 6044; *rev* second coding exon, nt 8369 to 8643; *vpu*, nt 6061 to 6306; *env*, nt 6221 to 8785; *nef*, nt 8787 to 9407; 3′ LTR, nt 9076 to 9709. pMA239: 5′ LTR, nt 1 to 818; *gag*, nt 1053 to 2585; capsid protein (*gag* p26), nt 1458 to 2144; *pol*, nt 2228 to 5410; *vif*, nt 5340 to 5984; *vpx*, nt 5812 to 6150; *vpr*, nt 6151 to 6456; *tat* first coding exon, nt 6302 to 6597; *tat* second coding exon, nt 8803 to 8902; *rev* first coding exon, nt 6528 to 6597; *rev* second coding exon, nt 8803 to 9059; *env*, nt 6604 to 9243; *nef*, nt 9077 to 9868; in-frame stop codon in *nef* (indicated by an arrow), nt 9353 to 9355; 3′ LTR, nt 9462 to 10279.

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 SIV_{MAC} overnight and with ^{125}I-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 SIV_{MAC} infectious clone, pMA239, was constructed by inserting the *Hin*dIII (41 nucleotides [nt] upstream of the beginning of the 5′ long terminal repeat [LTR])-*Sac*I (1,153 nt downstream of the end of the 3′ LTR) fragment of the lambda phage clone of SIV_{MAC239} (35) into the plasmid vector pUC18. All mutants of SIV_{MAC239} 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 *Xho*I linker (8 bp) was inserted into the blunt-ended *Nco*I site. Thus, pMA-Sc contains a 4-bp deletion at *Sac*I (nt 5755); pMA-ET, a 4-bp insertion at *Eco*T22I (nt 6052); pMA-N2, a 4-bp insertion at *Nco*I (nt 6193); and pMA-Nc, a 12-bp insertion at *Nco*I (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 *Sac*I (blunt-ended, nt 5755)-*Xho*I (nt 9281) fragment of pMA-Nc between the *Eco*RI (blunt-ended, nt 5743) and *Xho*I (nt 8887) sites of pNL432. prcNM-2 was constructed by inserting an *Xba*I (blunt-ended, nt 6439)-*Xho*I (nt 9281) fragment of pMA-Nc between the *Cla*I (blunt-ended, nt 6153) and *Xho*I (nt 8887) sites of pNL-Ss (39) (identical to pNL432 except for *Cla*I linker insertion at *Ssp*I). prcNM-3 was made by inserting an *Eco*RI (blunt-ended, nt 5743)-*Xho*I (nt 8887) fragment of pNL432 between the *Eco*47III (nt 6351) and *Xho*I (nt 9281) sites of pMA-Nc. prcNM-4 was made by inserting an *Spe*I (nt 1770)-*Eco*47III (nt 6351) fragment between the *Spe*I (nt 1507) and *Eco*RI (blunt-ended, nt 5743) sites of pNL432. prcNM-5 was made by inserting an *Nar*I (nt 823)-*Pst*I (nt 1684) fragment of pMA239 between the *Nar*I (nt 637) and *Pst*I (nt 1415) sites of pNL432.

RESULTS

Construction of mutants of SIV_{MAC239} and chimeric clones between $HIV\text{-}1_{NL432}$ and SIV_{MAC239}. The genome organizations of the parental molecular clones of HIV-1 (pNL432) and SIV_{MAC} (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 SIV_{MAC239} are essential for infectivity to macaque cells, we generated four SIV_{MAC239} mutants (Fig. 2). Frameshift mutations were introduced to inactivate *vif*, *vpx*, and *vpr*. pMA-Sc was expected to produce an aberrant *vif* protein (149 amino acids [aa]), whose N-terminal 138 aa were identical to those of native *vif* (214 aa). Similarly, the



	Origins of LTR and ORFs		Infectivity			
			Human cell lines			Monkey
	HIV-1	SIV_{mac}	MT-4	M8166	CEMx174	PBMC
NL432	all	none	+	+	+	−
MA239	none	all	+	+	+	+
MA-Sc	none	all except vif	−	+	−	−
MA-ET	none	all except vpx	+	+	+	+
MA-N2	none	all except vpr	+	+	+	+
MA-Nc	none	all except nef	+	+	+	+
NM-1	LTR,gag,pol,vif	vpx,vpr,tat,rev,env	+	−	nd	−
NM-2	LTR,gag,pol,vif,vpr,tat	rev,env	+	−	nd	−
NM-3	tat,rev,vpu,env	LTR,gag,pol,vif,vpx	+	+	+	+
NM-4	LTR,5'half of gag,tat, rev,vpu,env,nef	3'half of gag,pol,vif,vpx	−	−	−	nd
NM-5	all except 5'half of gag	5'half of gag	−	−	−	nd

FIG. 2. Structures and infectivities of various viral clones described in this study. Schematic representations of HIV-1_{NL432} (pNL432), SIV_{MAC239} (pMA239), the *vif* mutant of SIV_{MAC239} (pMA-Sc), the *vpx* mutant (pMA-ET), the *vpr* mutant (pMA-N2), the *nef* mutant (pMA-Nc), and the five chimeric viruses (prcNM-1 to -5) are shown on the left. Solid and open boxes represent sequences derived from pNL432 and pMA239, respectively. Solid and open triangles indicate mutated restriction sites and recombined sites, respectively. sa, *Sac*I; et, *Eco*T22I; nc, *Nco*I; e1, *Eco*RI; xh, *Xho*I; ss, *Ssp*I; xb, *Xba*I; e47, *Eco*47III; sp, *Spe*I; na, *Nar*I; ps, *Pst*I. For precise positions of the restriction sites, see Fig. 1. Origins of LTRs and ORFs and growth ability of the viruses in human and monkey cells (see text) are summarized on the right. nd, not done.

C-terminal 30 aa of the *vpx* protein (112 aa) and 76 aa of the *vpr* 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 SIV_{MAC239}, 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 SIV_{MAC} *env*. There were some structural differences between the two in the central region. rcNM-1 contained many SIV_{MAC} genes located in that region. Conversely, rcNM-3 was SIV_{MAC} containing HIV-1 *env*. The other chimeras were designed to contain a shorter SIV_{MAC} 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 SIV_{MAC}. rcNM-5 contained only the 5′ half of *gag* (the matrix protein and the N-terminal half of the capsid protein) derived from SIV_{MAC}. While rcNM-1, -2, and -5 have HIV-1 LTRs, rcNM-3 and -4 have SIV_{MAC} 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-1_{NL432} products p24 (*gag*, the capsid protein), p55 (*gag*, the precursor), and gp120 (*env*, the extracellular glycoprotein) were detected by the human antiserum (lanes h in Fig. 3A). The SIV_{MAC239} proteins p26 (*gag*, the capsid protein), p55 (*gag*, the precursor), gp41 (*env*, the transmembrane protein), and gp130 (*env*, the extracellular glycoprotein) were detected by the macaque antiserum (lanes s in Fig. 3B). The macaque antiserum was weakly reactive with *gag* proteins of HIV-1_{NL432} (lanes h in Fig. 3B). The chimeras rcNM-1 and 2 produced SIV_{MAC239} *env* (lanes 1 and 2 in Fig. 3B) and HIV-1_{NL432} *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-1_{NL432} *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 $p55^{gag}$ 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-1_{NL432} *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-1_{NL432} and SIV_{MAC239} were determined (summarized in Fig. 2). For infection, equal amounts of RT units of the virus samples (culture supernatants of transfected SW480

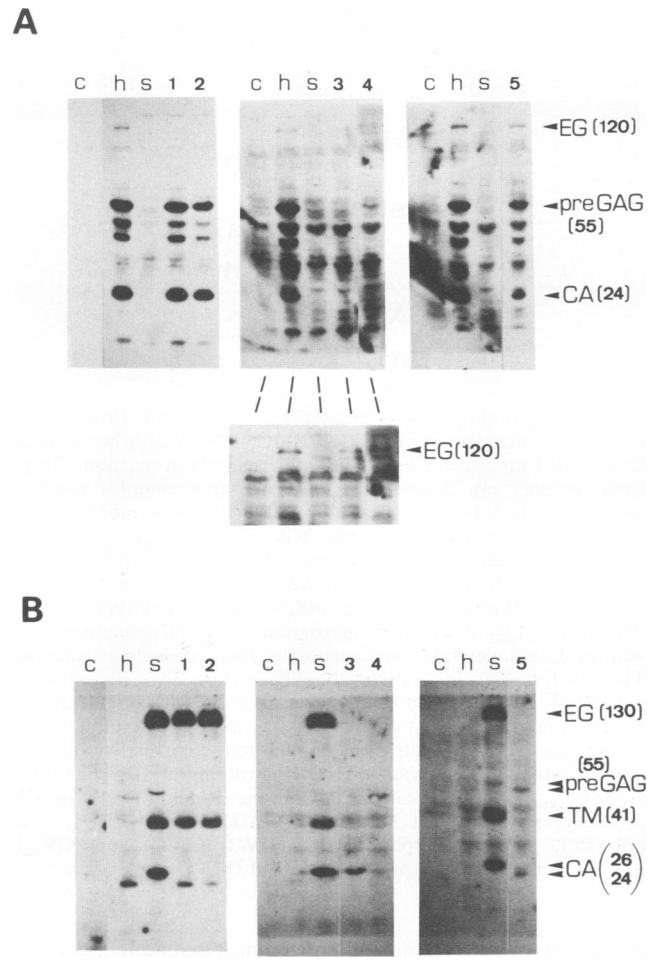


FIG. 3. Expression of viral proteins in SW480 cells transfected with the chimeric clones. Cell lysates were prepared 48 h after transfection, resolved on 12% polyacrylamide gels, and analyzed by Western blotting with either an anti-HIV-1 human serum (A) or anti-SIV_{MAC} macaque serum (B). The same amount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

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 SIV_{MAC239} 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

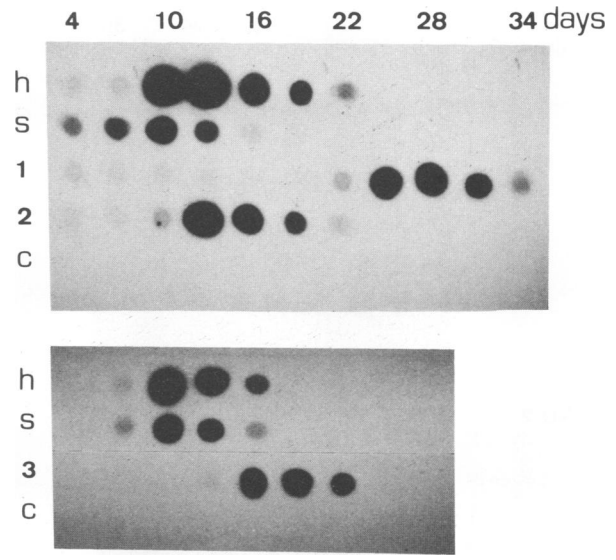


FIG. 4. Replication of chimeric viruses in the human $CD4^+$ cell line MT-4. The results of two independent experiments are shown. MT-4 cells were infected with the same amounts (RT units) of virus obtained from SW480 cells transfected with pNL432 (h), pMA239 (s), and the chimeric clones prcNM-1 to -3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

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

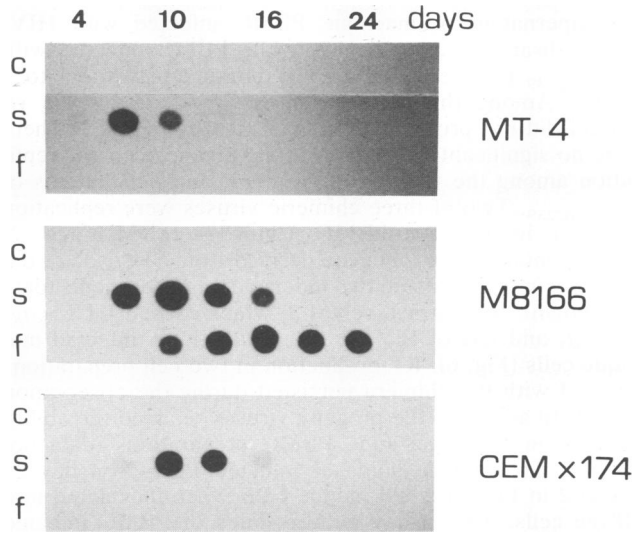


FIG. 5. Growth kinetics of the *vif* mutant in human $CD4^+$ cell lines MT-4, M8166, and CEMx174. Cells were infected with cell-free virus samples obtained from SW480 cells transfected with pUC18 (as a negative control) (c), pMA239 (s), and pMA-Sc (f). Input viral RT units were equivalent between the wild type (s) and the *vif* mutant (f). RT production in the infected cells was monitored at intervals.

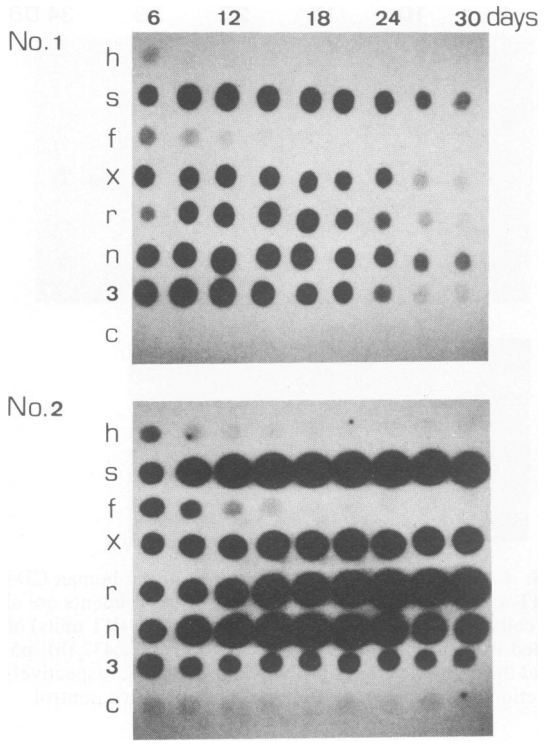


FIG. 6. Growth of chimeric virus and mutants of SIV_{MAC239} in macaque PBMC. The results of two independent experiments with PBMC obtained from two cynomolgus monkeys are shown. ConA-stimulated PBMC were infected with cell-free virus samples obtained from SW480 cells transfected with pNL432 (HIV-1, h), pMA239 (SIV_{MAC}, s), pMA-Sc (the *vif* mutant, f), pMA-ET (the *vpx* mutant, x), pMA-N2 (the *vpr* mutant, r), pMA-Nc (the *nef* mutant, n), and the chimeric clone prcNM-3 (lane 3). RT production was monitored at intervals. c, negative control.

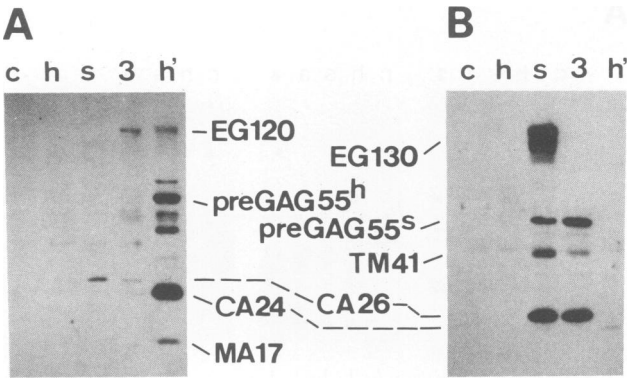


FIG. 7. Protein profile expressed by the chimeric virus rcNM-3 obtained from infected macaque PBMC. M8166 cells were inoculated with 1 ml of cell-free culture supernatants of macaque PBMC from monkey no. 2 (see Fig. 6). The virus samples used for inoculation had been harvested on day 21 from mock-infected PBMC (lanes c) and PBMC infected with HIV-1_{NL432} (lanes h), SIV_{MAC239} (lanes s), or the chimera prcNM-3 (lanes 3). Cell lysates were prepared from M8166 cells 3 weeks (lanes c and h) or 2 weeks (lanes s and 3) after inoculation. M8166 cells productively infected with HIV-1_{NL432} (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-SIV_{MAC} 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 SIV_{MAC} (EG130), *env* transmembrane protein (TM41) of SIV_{MAC}, *gag* precursors of HIV-1 (pre-$GAG55^h$) and SIV_{MAC} (pre-$GAG55^s$), *gag* capsid proteins of HIV-1 (CA24) and SIV_{MAC} (CA26), and *gag* matrix protein of HIV-1 (MA17) are indicated.

the supernatant of macaque PBMC infected with HIV-1_{NL432} disappeared by day 9, while PBMC infected with SIV_{MAC239} produced RT activity continuously for 3 to 4 weeks. Among the mutants of SIV_{MAC239}, 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 SIV_{MAC239}. Two of three chimeric viruses were replication defective in macaque PBMC. Chimeras rcNM-1 and -2, which contained the *env* gene derived from SIV_{MAC239}, 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 SIV_{MAC239}, 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 SIV_{MAC239} also contained infectious virions, but those infected with the *vif* mutant and HIV-1_{NL432} 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 SIV_{MAC239} 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-1_{NL432} induced no CPE or viral protein synthesis (lanes h), M8166 cells productively infected with HIV-1_{NL432} (a cell-free supernatant of the SW480 cells transfected with pNL432) were also analyzed as a positive control (lanes h'). The human antiserum recognized *env* products (gp120) from rcNM-3 (lane 3 in Fig. 7A) and HIV-1_{NL432} (lane h' in Fig. 7A) and numerous *gag*-related proteins from HIV-1_{NL432}. The human antiserum also cross-reacted with the capsid protein (p26) produced by rcNM-3 and SIV_{MAC239} (lanes 3 and s in Fig. 7A). The macaque antiserum clearly detected the *env* protein gp130 of SIV_{MAC239} (lane s in Fig. 7B), which was absent in rcNM-3-infected cells (lane 3 in Fig. 7B). The protein expressed by rcNM-3 (lane 3 in Fig. 7B) comigrating with another *env* protein, gp41, of SIV_{MAC239} probably represented a partial cleavage product, p41, of the SIV_{MAC239} *gag* precursor. The capsid protein derived from SIV_{MAC239} was observed in lanes s and 3 in Fig. 7B, and cross-reacting p24 (the capsid protein of HIV-1_{NL432}) was seen in lane h' in Fig. 7B. These data indicated that the chimera rcNM-3 was replication competent in both human and macaque cells.

DISCUSSION

Our results clearly indicated that rcNM-3, the chimeric virus carrying the SIV_{MAC239}-derived LTR, *gag*, *pol*, *vif*, and *vpx*, productively infected both human cell lines and macaque PBMC, like wild-type SIV_{MAC239}. 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 SIV_{MAC239} 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* (*Pst*I at nt 1415, *Spe*I at nt 1507, *Pst*I at nt 2839, and *Kpn*I 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 transient-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 $HIV\text{-}1_{NL432}$-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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