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Construction and *in vivo* infection of a new simian/human immunodeficiency virus chimera containing the reverse transcriptase gene and the 3′ half of the genomic region of human immunodeficiency virus type 1

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A new simian/human immunodeficiency virus (SHIV) chimera with the reverse transcriptase (RT)-encoding region of *pol*, in addition to the 3′ region encoding *vpr*, *vpu*, *tat*, *rev*, *env* and *nef* of HIV-1, on an SIV_{mac} (SIV from a macaque monkey) background was constructed. This new SHIV chimera, named SHIVrt/3rn, could replicate in monkey peripheral blood mononuclear cells (PBMCs) as well as in the human and monkey $CD4^+$ T-cell lines M8166 and HSC-F. Since SHIVrt/3rn contains the RT gene of HIV-1, replication of the virus in M8166 cells was inhibited by an HIV-1-specific non-nucleoside RT inhibitor, MKC-442, with a sensitivity similar to that of HIV-1. To investigate the replication competence of SHIVrt/3rn *in vivo*, two rhesus monkeys were inoculated intravenously with the virus. At 2 to 4 weeks post-inoculation (p.i.), plasma viral RNA loads of both monkeys showed a peak value of more than 10^4 copies ml^{-1}. Infectious virus was isolated from the PBMCs of one monkey at 2 and 3 weeks p.i. and from the other at 4 weeks p.i. Moreover, proviral DNA was detected constantly throughout the observation period, starting from 3 weeks p.i. An antibody response, detected first at 3 weeks p.i., was maintained at high titres. These results indicate that SHIVrt/3rn can infect and replicate *in vivo*. SHIVrt/3rn, having part of HIV-1 *pol* in addition to the 3′ part of the HIV-1 genome is genetically more close to HIV-1 than any of the other monkey-infecting SHIVs reported previously.

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INTRODUCTION

To clarify the pathogenesis of AIDS and to develop therapeutic and preventive measures, it is necessary to establish an animal model for the infection of human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS. However, there is a crucial obstacle: HIV-1 can infect only a limited range of primates other than humans, such as chimpanzees (Fultz *et al.*, 1986; Gajdusek *et al.*, 1985). The HIV-1/chimpanzee system has provided the closest model for HIV-1 infection in humans. However, such animals are not suitable for experimental use. One of the reasons for this is that HIV-1 infection hardly induces AIDS-like symptoms in infected chimpanzees (Novembre *et al.*, 1997). Besides, the chimpanzee is an endangered species and is expensive to acquire and care for. As an alternative, simian immunodeficiency virus (SIV) and macaque monkeys have been used as an animal model for AIDS. SIV_{mac} isolated from rhesus monkeys (*Macaca mulatta*) can infect Asian macaque monkeys persistently and induce a fatal disease very similar to human AIDS (Letvin *et al.*, 1985). SIV_{mac239} (Naidu *et al.*, 1988), a pathogenic molecular clone of SIV_{mac}, made this model useful for analysing not only the infectivity and immunogenicity but also the pathogenicity of HIV-related viruses. For instance, studies with this model demonstrated the importance of the SIV *nef* gene for its pathogenesis *in vivo* (Kestler *et al.*, 1991) and that gene-deleted live-attenuated SIV vaccines provided the most effective protection against challenge infection with pathogenic SIVs (Daniel *et al.*, 1992). Moreover, Veazey *et al.* (1998) showed the importance of the gastrointestinal tract as a target organ using the SIV/monkey model.

SIV is genetically similar, but not identical, to HIV-1. The findings obtained from the SIV/monkey system cannot be applied directly to the case of HIV-1 infection in humans. Therefore, to develop a better animal model, we previously generated simian/human immunodeficiency chimeric viruses (SHIVs) that can infect monkeys. These SHIVs possessed the 3′ half of several HIV-1-derived genes, including the *env* gene, using the SIV genome as a backbone

(Kuwata *et al.*, 1995; Li *et al.*, 1992; Shibata *et al.*, 1991). The SHIVs containing the *env* gene of HIV-1 were shown to be useful for evaluating the efficacy of anti-HIV-1 vaccine candidates targeting Env proteins by using them as challenge viruses for vaccinated monkeys (Ui *et al.*, 1999). Although these SHIVs were shown to be non-pathogenic (Hayami *et al.*, 1999), pathogenic SHIVs were also developed by animal passage, starting from initially non-pathogenic SHIVs (Joag *et al.*, 1996, 1997; Reimann *et al.*, 1996). Using the SHIV/monkey system, Harouse *et al.* (1999) demonstrated the role of co-receptor usage of HIV-1 Env for pathogenesis. Thus, SHIV/monkey systems have been valuable tools for understanding, at least, in part, the biological properties of HIV-1 and for developing HIV vaccines.

Most of the SHIVs reported to date, including the SHIVs generated by our group, had the 3′ half of the HIV-1 genome on an SIV backbone. For instance, one of the SHIVs generated by us, which was termed NM-3rn, possessed *vpr*, *vpu*, *tat*, *rev*, *env* and *nef* of HIV-1 (Kuwata *et al.*, 1995). These findings naturally lead us to the following question: to what extent can we replace SIV genes with those of HIV-1 without losing their infectivity to monkeys? The answer to this question may help to develop a better animal model for AIDS, one that ultimately mimics HIV-1 infection in humans.

We have been trying to construct a new SHIV chimera that has a broader HIV-1-derived region by the addition of various regions of the 5′ part of HIV-1, including *pol*, to the previously reported SHIV containing the 3′ half of HIV-1. Here, we report that we succeeded in constructing a new SHIV chimera, one which contains the reverse transcriptase (RT)-encoding region (*rt* region) of *pol*, in addition to the 3′ half of the HIV-1 genome, and that this virus was able to infect and replicate in monkey peripheral blood mononuclear cells (PBMCs) and in macaque monkeys *in vivo*. This newly constructed SHIV has more of the HIV-1 genome, such as *rt*, *vpr*, *vpu*, *tat*, *rev*, *env* and *nef*, than any of the SHIVs reported so far.

METHODS

DNA constructs. Infectious molecular clones of HIV-1 (pNL432) (Adachi *et al.*, 1986) and SIV_{mac239} (pMA239) (Naidu *et al.*, 1988; Shibata *et al.*, 1991) were used as parent proviral DNAs. To generate chimeric junctions at the N- and C-terminal ends of the *rt* gene, we utilized PCR-based site-directed mutagenesis. We first introduced a *Dra*I site near the C-terminal end of the protease gene of pMA239 (SIV_{mac}); pNL432 (HIV-1) already possesses this site at the corresponding position (nt 2540). The following oligonucleotide primer was used: MAPR-R, 5′-CTTTAGCTATGGGAAAA**TTTAAA**GACAT CCCCAGAGCTG-3′; this primer was designed to create a *Dra*I site (shown in boldface letters), without alteration to the amino acid sequence (letters underlined represent the mutated nucleotide).

Next, we introduced an *Xba*I site near the N-terminal end of the integrase gene for both HIV-1 and SIV_{mac} by PCR-based site-directed mutagenesis. For HIV-1, the following oligonucleotide primer was used: NLRT-R, 5′-TTCCA**TCTAGA**AATAGTACTTTCCTGATTCCAGC-3′. For SIV_{mac}, the oligonuceotide primer MAIN-F, 5′-CTCTT**TCTAGA** AAAGATAGAGCCAGCACAAGAAG-3′, was used; these primers were designed to create an *Xba*I site into the respective sequences (nt 4232 for HIV-1 and nt 4786 for SIV_{mac}) without alteration to the amino acid sequences (letters in boldface represent the *Xba*I site and letters underlined represent the mutated nucleotides).

With the restriction sites at the junctions of the protease/RT and RT/integrase genes, two retrovirus sequences, SIV_{mac} and HIV-1, were reassembled by conventional molecular recombinant techniques to produce a chimeric *pol* gene. The *Spe*I (nt 2026 in pMA239, *gag*) to *Nsp*V (nt 6131 in pMA239, *vif*) fragment of this chimeric gene was then inserted into the corresponding position of an HIV-1 *env*-possessing SHIV plasmid, pNM-3rn (Kuwata *et al.*, 1995), to generate a novel full-genome plasmid, termed pSHIVrt/3rn.

Cell cultures. M8166 is a subclone of C8166 (Clapham *et al.*, 1987), a $CD4^+$ human T-cell line. HSC-F is a cynomolgus monkey $CD4^+$ T-cell line from a foetal splenocyte that was immortalized by infection with herpesvirus saimiri subtype C (Akari *et al.*, 1996). M8166 and HSC-F cells were maintained in RPMI 1640 medium containing 10 % heat-inactivated foetal bovine serum (FBS). PBMCs of healthy rhesus monkeys were separated from heparinized whole blood by Percoll density-gradient centrifugation, stimulated with 25 μg concanavalin A ml^{-1} for 24 h and maintained in RPMI 1640 medium containing 10 % FBS and 400 units recombinant IL-2 ml^{-1}, as described previously (Kuwata *et al.*, 1995).

Transfection and infection. For generating infectious virus particles from a full-genome plasmid DNA, 5 μg pSHIVrt/3rn was introduced into $1{\cdot}5 \times 10^6$ M8166 cells using the DEAE–dextran method (Naidu *et al.*, 1988). Culture medium was changed every 3 days and the supernatant was filtered (0·45 μm pore size) and stored at −80 °C. Virion-associated RT activity was measured as described previously (Willey *et al.*, 1988). The supernatant with the highest RT activity was used as virus stock. To determine the virus infectivity of the stocks, the $TCID_{50}$ was calculated using M8166 cells, as described previously (Igarashi *et al.*, 1994). The virus inoculum used for *in vitro* infection was adjusted to contain a certain amount of RT units (typically $3–4 \times 10^3$ RT units) by adding the appropriate volume of the medium to the virus stock. M8166 cells, HSC-F cells or monkey PBMCs (1×10^6 cells per well) were infected with a virus and cultured in a 96-well plate. The culture supernatant was harvested every 3 days and its RT activity was monitored.

Effect of an RT inhibitor on virus replication. MKC-442 is a non-nucleoside-type RT inhibitor (Yuasa *et al.*, 1993) that inhibits the RT activity of HIV-1 specifically, but not that of SIV. The ability of MKC-442 to block virus replication was examined. MKC-442 was diluted from a 2 mM stock solution with the culture medium and used at 200 and 20 nM.

Inoculation of monkeys. All animals were housed in a P3-level monkey storage facility and were treated in accordance with regulations approved by the Committee for Experimental Use of Nonhuman Primates in the Institute for Virus Research, Kyoto University, Japan. To investigate the replication competence of SHIVrt/3rn *in vivo*, two female adult rhesus monkeys (*Macaca mulatta*) were inoculated intravenously with the virus stock of SHIVrt/3rn containing 1×10^5 $TCID_{50}$ per monkey. After inoculation, blood samples were collected periodically from the inoculated monkeys and were separated into plasma and PBMCs. Then, plasma viral RNA loads, proviral DNA and $CD4^+$ cells were analysed. Virus isolation was attempted as described below.

Virus isolation. We attempted to isolate infectious virus from the PBMCs of inoculated monkeys as follows. Serially threefold diluted PBMCs were co-cultured with $2{\cdot}5 \times 10^5$ M8166 cells for 4 weeks in RPMI 1640 containing 10 % of FBS in a 48-well plate. Virus recovery was judged by the cytopathic effect (CPE) of syncytia formation and a rise in RT activity of the culture supernatants.

Detection of proviral DNA in PBMCs. Proviral DNA in PBMCs of inoculated monkeys was detected with nested DNA PCR, as described previously (Igarashi *et al.*, 1996). The primers used in this study were designed to amplify the V3 region of HIV-1 (NL432) *env* specifically, which is present in the SHIVrt/3rn genome.

Determination of plasma viral RNA loads. Plasma viral RNA loads after inoculation were determined by quantitative RT-PCR, as described previously (Kozyrev *et al.*, 2002).

Titration of antibody. Antibody titres of the monkey plasma after inoculation with SHIVrt/3rn were measured by particle agglutination according to the instructions of the manufacturer (Serodia HIV-1/2, Fujirebio).

CD4$^+$ cell kinetics. After two rhesus monkeys were inoculated with SHIVrt/3rn, PBMCs were isolated from the animals and CD4$^+$ cell numbers in the PBMCs were calculated, as described previously (Ui *et al.*, 1999).

RESULTS

Construction of SHIV

Most of the SHIVs constructed to date have only the *env* gene and the adjacent accessory genes of HIV-1. In this study, a new type of SHIV containing not only *env* but also a part of *pol* of HIV-1 was constructed. The parental molecular clone of the SHIV was NM-3rn (Kuwata *et al.*, 1995), which contains the genes from *vpr* to *nef* from HIV-1; the rest of the genes were from SIV$_{mac}$. We replaced the *rt* region of *pol* of SIV$_{mac}$ with that of HIV-1. The newly constructed SHIV was designated SHIVrt/3rn (Fig. 1).

Replication of SHIVrt/3rn *in vitro*

To obtain infectious virions, we transfected M8166 cells, a CD4$^+$ human T-cell line, with the full-genome plasmid of SHIVrt/3rn and measured virion-associated RT activity released in culture supernatants. To confirm whether the supernatant fractions with RT activity contain infectious virions, we inoculated the supernatants of the transfected cell culture onto M8166 cells. As a control, we used NM-3rN, which possesses HIV-1-derived genes from *vpr* to *env* on an SIV$_{mac}$ background. It should be noted that *nef* of NM-3rN is from SIV$_{mac}$, while *nef* of NM-3rn is from HIV-1. In the present study, we compared the replication of SHIVrt/3rn with that of NM-3rN because this virus has been used in our laboratory and because the replication profiles of NM-3rN and NM-3rn do not differ from each other *in vitro* (Kuwata *et al.*, 1995). After infection, SHIVrt/3rn, as well as NM-3rN, induced CPE in the infected M8166 cells. An increase in RT activity in the culture supernatant was also apparent (data not shown). Together, these data indicate that pSHIVrt/3rn was capable of producing infectious virions.

To investigate the virus growth kinetics of SHIVrt/3rn, we infected M8166 cells, a monkey CD4$^+$ T-cell line (HSC-F), and monkey PBMCs with the virus and monitored RT activity in culture supernatants (Fig. 2). In M8166 cells, the

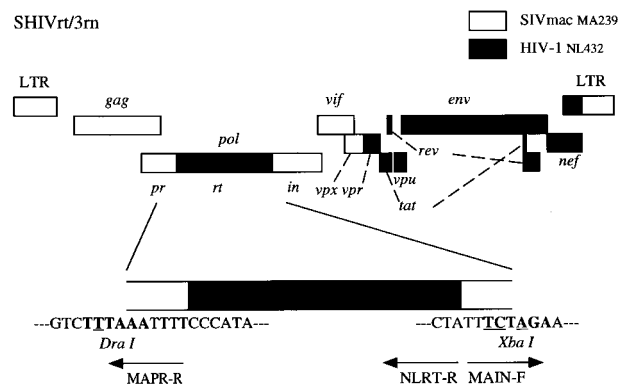

Fig. 1. Genomic structures of the newly constructed SHIVrt/3rn. Solid and open boxes represent sequences derived from HIV-1 (NL432) and SIV$_{mac}$ (MA239), respectively. Boldface letters represent the introduced *Dra*I and *Xba*I sites and underlined letters are the mutated nucleotides. Arrows indicate the directions of the primers used.

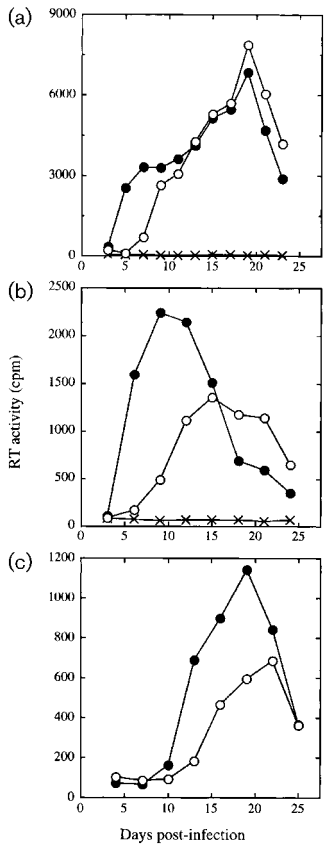

Fig. 2. Replication of chimeric viruses in (a) a human CD4$^+$ cell line M8166, (b) a monkey CD4$^+$ cell line HSC-F and (c) PBMCs from a rhesus monkey. Cell-free virus stocks from M8166 cells transfected with the respective chimeric DNA clones were infected and virion-associated RT activity in the culture supernatants was monitored every 2 or 3 days. ○, SHIVrt/3rn; ●, NM-3rN; ×, mock infection.

replication of SHIVrt/3rn reached a peak at about 19 days post-infection (p.i.) (Fig. 2a). This profile of kinetics was similar to that of NM-3rN, except that there was a slight delay in the initial rise in RT activity, indicating that SHIVrt/3rn could replicate well in this human $CD4^+$ cell line with almost the same replication competence as NM-3rN. In HSC-F cells, the replication of SHIVrt/3rn was delayed compared with that of NM-3rN and reached a peak at about 15 days p.i. (Fig. 2b). In monkey PBMCs, replication of SHIVrt/3rn reached a peak at about 22 days p.i. (Fig. 2c). Judging from the RT values, SHIVrt/3rn replicated to somewhat lower titres than NM-3rN. Nevertheless, it was clearly able to replicate in monkey PBMCs.

Effect of MKC-442, an HIV-1-specific non-nucleoside RT inhibitor, on virus growth

MKC-442 did not inhibit the replication of NM-3rN (which has the RT of SIV_{mac}) at any concentration examined, but it completely inhibited the replication of SHIVrt/3rn (which has the RT of HIV-1) at 200 nM (Fig. 3). In the presence of 20 nM MKC-442, growth of SHIVrt/3rn was initially blocked but, eventually, slow growth was observed approximately 10 days later compared with that at the start (in the absence of MKC-442) (Fig. 3b).

Replication of SHIVrt/3rn *in vivo*

To investigate the competence of SHIVrt/3rn to infect and replicate *in vivo*, we inoculated intravenously two female rhesus monkeys (MM251 and MM257) with the virus stock containing 1×10^5 $TCID_{50}$ per each monkey and performed virus isolation, DNA PCR, quantitative RT-PCR and FACScan analysis. Here we show the results obtained up to 32 weeks p.i.

Firstly, we examined whether or not there were infected PBMCs that could generate infectious virus by co-culturing with M8166 cells (Table 1). From the PBMCs of MM251, infectious virus was isolated at 2 and 3 weeks p.i., and at 4 weeks p.i. from the PBMCs of MM257. To detect proviral DNA in the isolated PBMCs, we performed DNA PCR using the extracted chromosomal DNA from the PBMCs (Table 1). In the PBMCs from both MM251 and MM257, proviral DNA was detected constantly throughout the observation period, starting from 3 weeks p.i. until 32 weeks p.i. To determine plasma viral RNA loads, we extracted RNA from the isolated plasma samples and performed quantitative RT-PCR (Fig. 4a). The plasma viral RNA loads of MM251 exhibited a peak during 2 to 3 weeks p.i., and those of MM257 did so during 3–4 weeks p.i. The number of RNA copies at the peak period was $2{\cdot}6 \times 10^4$ and $1{\cdot}2 \times 10^4$ copies ml^{-1}, respectively. As for MM251, viral RNA was detected in the plasma samples up to 10 weeks p.i. On the other hand, no viral RNA was detected in the plasma samples of MM257 after 6 weeks p.i. To detect and titrate antibodies against HIV-1, we measured particle agglutination antibody titres using the Serodia HIV-1/2 kit (Fujirebio) (Table 1). Antibodies were detected in both

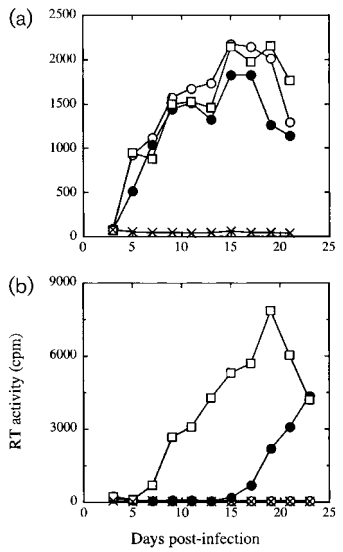

Fig. 3. Inhibition of the growth of NM-3rN (a) and SHIVrt/3rn (b) by MKC-442 in M8166 cells. Cell-free virus stocks from M8166 cells transfected with the respective chimeric DNA clones were infected in the presence of 20 nM (●) or 200 nM (○) MKC-442 and virion-associated RT activity in the culture supernatants was monitored every 2 days. □, Medium; ×, mock infection.

Table 1. Virological and immunological status of SHIVrt/3rn-inoculated monkeys

	DNA PCR*		Virus isolation†		Antibody titre‡	
Week	MM251	MM257	MM251	MM257	MM251	MM257
0	−	−	ND	ND	ND	ND
1	−	−	−	−	ND	ND
2	−	−	+	−	<32	<32
3	+	+	+	−	128	128
4	+	+	−	+	512	128
6	+	+	−	−	2048	256
8	+	+	−	−	2048	512
10	+	+	−	−	2048	512
12	+	+	−	−	2048	512
16	+	+	−	−	2048	4096
20	+	+	−	−	4096	4096
26	+	+	−	−	4096	4096
32	+	+	−	−	4096	4096

*The presence of viral DNA in PBMCs was determined by PCR using primer pairs for amplification of the V3 region of HIV-1 *env*. +, Viral DNA detected; −, no viral DNA detected.

†Virus isolation was performed by co-culture of collected PBMCs and M8166 cells. +, Virus isolated; −, no virus isolated.

‡Antibody titres were measured by particle agglutination (Serodia HIV-1/2, Fujirebio).

ND, Not done.

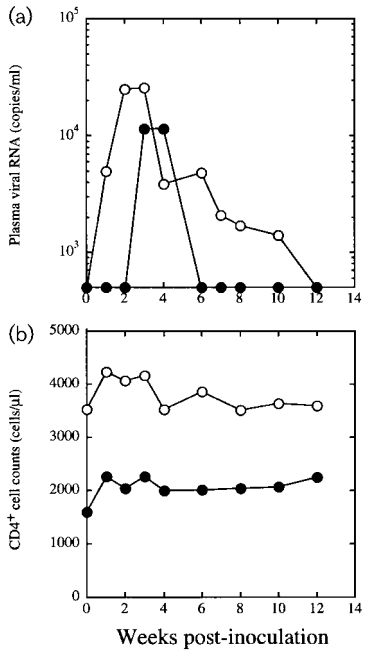

Fig. 4. (a) Plasma viral RNA loads of the SHIVrt/3rn-infected monkeys. Plasma viral RNA loads were measured by quantitative RT-PCR, as described in Methods. (b) Changes in the number of circulating $CD4^+$ cells with time. ○, MM251; ●, MM257.

monkeys first at 3 weeks p.i. and were maintained with high titres (4096) up to 32 weeks p.i. In addition, we analysed the number of $CD4^+$ cells in these rhesus monkeys after the inoculation. Both monkeys showed no significant decrease in the number of $CD4^+$ cells up to 32 weeks p.i. (Fig. 4b), suggesting that SHIVrt/3rn is non-pathogenic at this stage.

All of the above results indicate that SHIVrt/3rn could efficiently infect and replicate in rhesus monkeys and induce a humoral response against HIV-1 without causing any apparent symptoms of disease.

DISCUSSION

Most of the SHIVs constructed so far possess the 3′ half of the HIV-1 genome, including the *env* gene; the rest of the genome is from SIV. In this study, we constructed a new SHIV chimera, named SHIVrt/3rn, which has a broader HIV-1-derived region than any of the SHIVs reported previously. Specifically, SHIVrt/3rn differs from the previous SHIVs in that it carries part of the *pol* gene of HIV-1. SHIVrt/3rn replicated well, not only in established $CD4^+$ T-cell lines, such as M8166 and HSC-F, but also in monkey PBMCs (Fig. 2). Moreover, when it was inoculated into rhesus monkeys, it exhibited evidence of virus replication *in vivo* (Fig. 4 and Table 1). This virus has the *rt* region in addition to the 3′ part of the HIV-1genome, that is to say, *vpr*, *vpu*, *tat*, *rev*, *env* and *nef*. Therefore, it is genetically more close to HIV-1 than any of the other monkey-infecting SHIVs reported previously.

Since the newly constructed SHIVrt/3rn has the RT of HIV-1, we confirmed the inhibition of the replication of SHIVrt/3rn by an HIV-1-specific non-nucleoside-type RT inhibitor, MKC-442, *in vitro*. The 90 % effective concentration (EC_{90}) and the 50 % effective concentration (EC_{50}) of MKC-442 against HIV-1 were reported to be 98 and 15 nM, respectively (Baba *et al.*, 1994). EC_{90} and EC_{50} values were defined as the concentrations at which 90 and 50 % of HIV-1 induced CPE in MT-4 cells were protected (Pauwels *et al.*, 1988). In this study, the replication of SHIVrt/3rn was inhibited completely by MKC-442 at 200 nM. In addition, the initial rise in RT activity of SHIVrt/3rn was delayed by 20 nM MKC-442, which is considered to be a consequence of incomplete inhibition. (Fig. 3b). These results indicate that the sensitivity to MKC-442 of SHIVrt/3rn is similar to that of HIV-1, although it should be noted that different cells were employed for this inhibition assay. Today, highly active antiretroviral therapy, in which combinations of RT inhibitors and protease inhibitors are used, has shown satisfactory clinical benefits. Moreover, new anti-HIV drugs targeting other virus components, such as Env, are being developed. Since monotherapy resulted, in most cases, in failure, such entry blockers should be prescribed in combination with other drugs such as RT inhibitors. Having the RT and Env of HIV-1 and having a sensitivity to an RT inhibitor similar to that of HIV-1, SHIVrt/3rn can be used for the *in vivo* evaluation of a new combination therapy of HIV-1-specific RT inhibitors and entry blockers, such as CXCR4 antagonists, in monkeys.

Two rhesus monkeys were inoculated with 1×10^5 $TCID_{50}$ SHIVrt/3rn and both monkeys were infected consequently. The *in vivo* replication of NM-3rn, the parental molecular clone of SHIVrt/3rn, was reported previously (Bogers *et al.*, 1997). According to that report, eight rhesus monkeys were inoculated with NM-3rn at six different virus titres, ranging from $6{\cdot}3 \times 10^3$ to $6{\cdot}3 \times 10^{-1}$ $TCID_{50}$. Seven of the eight monkeys were infected with NM-3rn. Viruses were isolated continuously from 2 to 12 weeks p.i. from five of the seven infected monkeys. In this study, we also performed virus isolation. However, we could isolate viruses only at 2 and 3 weeks p.i. from one of the two monkeys and at 4 weeks p.i. from the other (Table 1). In the case of NM-3rn infection, the results of DNA PCR showed the presence of proviral DNA from all seven monkeys infected with NM-3rn at 2 and 4 weeks p.i. and from all three monkeys analysed out of the seven at 8 weeks p.i. In this study, we also performed DNA PCR and could detect proviral DNA constantly, starting at 3 weeks p.i., from both of the monkeys, although we could not detect it at 2 weeks p.i. (Table 1). These results suggest that SHIVrt/3rn possesses a slightly lower replication competence *in vivo* than NM-3rn. This weak replication competence was also observed *in vitro*, as shown by the growth kinetics in monkey PBMCs (Fig. 2c). The replacement of the *rt* region of SIV with that of HIV-1 might have affected the replication potential of the virus.

Überla *et al.* (1995) constructed a SHIV having the *rt* region

of HIV-1 and the rest of the genome from SIV_{mac} (RT-SHIV). Two rhesus monkeys inoculated with RT-SHIV exhibited a systemic infection and one of them developed an AIDS-like symptom at approximately 6 months p.i. This result suggested that RT-SHIV possessed a higher replication competence *in vivo* than SHIVrt/3rn. This difference may be because SHIVrt/3rn and RT-SHIV use different strains of HIV-1 for the *rt* region, or because SHIVrt/3rn has a broader HIV-1-derived region, one that covers the region from *vpr* to *nef*. Although the reason for the lower replication competence of SHIVrt/3rn is not clear at this stage, we expect that it can be improved by serial animal passages and/or by using different strains of HIV-1.

We are now attempting to make new SHIVs in which the HIV-1-derived region is as broad as possible, without losing the infectivity of the virus to monkeys. We hope this will allow us to establish a novel animal model that ultimately mimics HIV-1 infection in humans and to identify the virus determinants of the species tropism of HIV-1, namely, the virus factors that restrict HIV-1 replication in monkey cells.

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