Infection of macaque monkeys with a chimeric human and simian immunodeficiency virus

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Two macaque monkeys were inoculated with a chimeric human and simian immunodeficiency virus carrying the tat, rev, vpu and env genes of human immunodeficiency virus type 1. Infectious virus was recovered from one of the monkeys at 2 and 6 weeks post-infection. The hybrid nature of the isolated viruses was verified by Southern and Western blotting analyses. Both of the monkeys infected with the chimera elicited a humoral antibody response against the virus.

The host range of human immunodeficiency virus type 1 (HIV-1), the major causative virus of human AIDS, is narrow and other animal species susceptible to HIV-1 infection are few. However, a number of systems for the study of animal models of HIV-1 infection have been reported. These include transgenic mice (Leonard et al., 1988), higher primates (Alter et al., 1984; Fultz et al., 1986; Gajdusek et al., 1985; Lusso et al., 1988; Nara et al., 1987), rabbits (Filice et al., 1988; Kulaga et al., 1989) and SCID-hu mice (Namikawa et al., 1988). Although the experimental systems described are of value for certain aspects of HIV-1 research, a new approach to investi-gate extensively the biology and molecular biology of HIV-1 in vivo, and to facilitate a practical study should be undertaken. The similarities between HIV-1 and simian immunodeficiency virus (SIV) in genome sequence and organization, and in their biological properties, have suggested that SIV systems would provide useful animal models for the study of AIDS (Desrosiers & Ringler, 1989). In fact, macaque monkeys experimentally infected with molecularly cloned SIVs develop the disease (Dewhurst et al., 1990; Kestler et al., 1990).

The reason for the narrow host range of HIV-1 is presently unknown. However, by using chimeric clones generated between macaque monkey-tropic SIV and HIV-1, and SIV mutants, we have recently demonstrated that the vpx, vpr, tat, rev, env and nef genes of SIV are not essential for macaque cell tropism (Shibata et al., 1991). We have also found that one of the chimeric viruses, which carries HIV-1 tat, rev, vpu and env genes, can productively infect macaque cells in vitro (Shibata et al., 1991). In this communication, we report the inoculation of the infectious chimeric virus into macaque monkeys.

The chimeric construct NM-3 used in this study (Fig. 1a), which is a hybrid clone between HIV-1 NL432 (Adachi et al., 1986) and SIV MAC239 (Naidu et al., 1988), is infectious to peripheral blood mononuclear cell (PBMC) cultures from cynomolgus monkeys (Macaca fascicularis) (Shibata et al., 1991). To determine whether various PBMC cultures differ in their susceptibility to the virus, NM-3 virus obtained from transfected SW480 cells (Adachi et al., 1986) was inoculated into PBMC preparations from four cynomolgus monkeys. For controls, PBMC cultures were also infected with the parental viruses of NM-3 (NL432 and MAC239) and monitored for their replication. HIV-1 NL432 did not grow in any of the cynomolgus monkey PBMC cultures (Fig. 1b) as reported for other HIV-1 strains (McClure et al., 1987). In contrast, the NM-3 virus replicated well in all four PBMC cultures to an extent similar to that of the cynomolgus monkey PBMC cultures (Fig. 1b) as reported for other HIV-1 strains (McClure et al., 1987).
Short communication

(a) Structure of NM-3 genome. Restriction enzyme sites used to construct NM-3 are indicated with nucleotide positions. (GenBank accession numbers: M19921 for HIV-1 NL432 and M33262 for SIV MAC239.) Speckled boxes represent truncated genes due to recombination.

(b) Kinetics of progeny virus production (RT production; Willey et al., 1988) in parallel cultures of PBMCs from four cynomolgus monkeys (A to D) at different times post-inoculation with the chimera (●), SIV MAC239 (○), HIV-1 NL432 (●) or no virus (○). To generate virus stocks for infection, SW480 cells (Adachi et al., 1986) were transfected with molecular clones pNL432 (Adachi et al., 1986), pMA239 (Shibata et al., 1991) or procNM-3 (Shibata et al., 1991), and cell-free supernatants were collected on day 2. PBMCs, separated and prepared from monkeys by the standard method (Ohta et al., 1988), were infected with the same amounts of virus (RT units) as described by Folks et al. (1985).

[approximately 10^4 TCID_{50} in M8166 cells (Clapham et al., 1987) per monkey]. The monkeys were examined for the presence of proviral DNA copies at 2 and 12 weeks post-inoculation. Genomic DNAs were prepared by the standard method (Folks et al., 1985) from PBMC cultures of the two cynomolgus monkeys and analysed by the polymerase chain reaction (PCR) (Saiki et al., 1988). Proviral DNAs were detected in the PBMC DNAs from both monkeys (Fig. 2). However, copy numbers of viral DNA appeared to decrease during the course of infection (especially in monkey no. 2). The two monkeys were also checked at intervals (2, 6, 8, 10 and 12 weeks post-inoculation) for the presence of infectious virus by cocultivation of PBMCs from monkeys infected with NM-3 virus and M8166 cells, which are highly susceptible to infection by various isolates of HIV and SIV. Virus production was monitored for 1 month by extensive microscopic observation of cytopathic effects and reverse transcriptase (RT) assay. Viable virus was recovered only from monkey no. 1 at 2 and 6 weeks post-inoculation. Virus was not isolated by the cocultivation of PBMCs from the infected monkeys (8 and 10 weeks post-inoculation) and PBMCs from uninfected monkeys.

The general structure of the re-isolated virus was determined by Southern and Western blotting analyses of virus-producing M8166 cells (Fig. 3). Four DNA probes were used for Southern hybridization. HIV-1 sequences could be detected by probes N1 (4.3 and 1.2 kb

Fig. 1. Growth of HIV-1/SIV chimeric virus NM-3 in macaque PBMCs. (a) Structure of NM-3 genome. Restriction enzyme sites used to construct NM-3 are indicated with nucleotide positions. (GenBank accession numbers: M19921 for HIV-1 NL432 and M33262 for SIV MAC239.) Speckled boxes represent truncated genes due to recombination. (b) Kinetics of progeny virus production (RT production; Willey et al., 1988) in parallel cultures of PBMCs from four cynomolgus monkeys (A to D) at different times post-inoculation with the chimera (●), SIV MAC239 (○), HIV-1 NL432 (●) or no virus (○). To generate virus stocks for infection, SW480 cells (Adachi et al., 1986) were transfected with molecular clones pNL432 (Adachi et al., 1986), pMA239 (Shibata et al., 1991) or procNM-3 (Shibata et al., 1991), and cell-free supernatants were collected on day 2. PBMCs, separated and prepared from monkeys by the standard method (Ohta et al., 1988), were infected with the same amounts of virus (RT units) as described by Folks et al. (1985).

Fig. 2. Presence of proviral DNA in macaque monkeys inoculated with NM-3 virus. PBMCs for PCR analysis (Saiki et al., 1988) were prepared from two cynomolgus monkeys (nos 1 and 2) infected with the NM-3 virus at 2 and 12 weeks post-inoculation. Samples (1 μg genomic DNA) were subjected to 35 cycles of PCR. The location (arrowheads) and sequence of the primer pair used to amplify the HIV-1 NL432 env gene are indicated in (a). (b) PCR products were run through a 3% agarose gel and subjected to Southern blotting analysis (Folks et al., 1985). A BgII-BglII DNA fragment (a) was labelled with 32P using the random primer DNA labelling kit (Takara Shuzo) and used as a probe. The size of the specific product was predicted to be 660 bp. PBMC genomic DNA was from: monkey no. 1 at 2 weeks post-inoculation (lane 1), 12 weeks post-inoculation (lane 3) and before infection (lane 5); and monkey no. 2 at 2 weeks post-inoculation (lane 2), 12 weeks post-inoculation (lane 4) and before infection (lane 6).
bands (fragments A and C, respectively, in Fig. 3) by \textit{HindIII} digestion] and N2 [4-3 and 2-1 kb (fragments A and B, respectively)] (lanes h). Probes M1 and M2 recognize 6-2 kb (fragment a) and 3-5 kb (fragment b) \textit{HindIII} cleavage products of SIV MAC239 DNA, respectively (lanes s). The Southern hybridization profile of NM-3 virus, obtained by cocultivation of PBMCs and M8166 cells, was exactly as predicted from its initial structure (Fig. 3b). HIV-1 2-1 kb and SIV 6-2 kb bands were detected by probes N2 and M1, respectively (lanes 3). In addition, no specific signals were observed with probes N1 and M2 (lanes 3). The hybrid nature of the NM-3 virus isolated from monkey no. 1 was further confirmed by Western blotting (Fig. 3c). The human antiserum used here (Fig. 3c, upper panel) can react with HIV-1 env-gp120, gag-p24 (lane h) and SIV gag-p26 (lane s). The monkey serum (Fig. 3c, lower panel) recognizes SIV env-gp41, env-gp130 and gag-p26 (lane s). As demonstrated in Fig. 3(b), NM-3 virus had SIV gag and HIV-1 env in agreement with the chimeric structure (lanes 3).

To investigate the humoral antibody response to NM-3 virus infection, sera were sampled from the two monkeys at 2, 4, 6, 8, 10 and 12 weeks post-inoculation,
and monitored for reactivity with virus antigens by Western blotting (Fig. 4). In sera of two infected monkeys, antibodies appeared 6 to 8 weeks post-inoculation and persisted during the observation period. Consistent with the structure of NM-3 virus, only anti-HIV-1 env and anti-SIV gag antibodies were detected. However, the extent and the appearance of these antibodies during the course of infection varied considerably between the monkeys. Anti-HIV-1 env gp120 activity in monkey no. 2 was clearly detected at 6 weeks post-inoculation and thereafter. In contrast, a relatively low level of activity was detected at 12 weeks post-inoculation in monkey no. 1. Although no recognizable anti-SIV gag antibody was present in the sera of monkey no. 2, a high titre was detected in the sera of monkey no. 1. Of note is the failure to recover virus from monkey no. 2 despite the presence of viral DNA.

In this study, two cynomolgus monkeys were inoculated with the HIV-1/SIV chimeric NM-3 virus, and virus recovery and host response were monitored. Both monkeys demonstrated high antibody responses to the virus following inoculation. High titres of the antibodies were still detected in the sera of the two monkeys at 28 weeks post-inoculation, though the titres declined thereafter (data not shown). The HIV-1 sequence was detected by the PCR method in DNAs prepared from PBMC cultures of the monkeys. Although virus was recovered from the inoculated monkeys only on some occasions, these data strongly suggest that the NM-3 virus is replication-competent in vivo in cynomolgus monkeys.

In addition to enabling basic studies, this macaque system with a chimeric virus may facilitate practical approaches to, for instance, HIV-1 env vaccine research. Apart from small changes that might occur, the chimeric structure of NM-3 virus was maintained during the course of infection in vivo.

The infection process of NM-3 virus in vivo appeared less efficient than in vitro. The infected monkeys were apparently healthy 1 year post-inoculation. Under similar experimental conditions, progeny virus was easily isolated from monkeys inoculated with the parental monkey cell-tropic MAC239 virus (Kestler et al., 1991; S. Sakuragi et al., unpublished observation). The basis for the difference between the in vitro and in vivo activities of NM-3 virus is presently unknown. It is worth mentioning that the nef gene of SIV MAC239 is reportedly required for the maintenance of high virus loads and for the development of AIDS in monkeys (Kestler et al., 1991). The NM-3 virus in this study lacks an intact nef gene. It is also possible that the HIV-1 sequence in the chimeric virus affects the in vivo activity of the virus. It is clear that more chimeric clones should be constructed and analysed.

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References


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