Replication and cytopathogenicity of human immunodeficiency virus type 1 (HIV-1)/simian immunodeficiency virus agm3 chimeric viruses in human and monkey cells: the 5' half of the HIV-1 genome is responsible for virus cytopathogenicity

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Two chimeric viruses were constructed between human immunodeficiency virus type 1 (HIV-1) and an apathogenic simian immunodeficiency virus (SIVagm3mc) from African green monkeys. One of the chimeras, HE-A391, expressed the HIV-1-derived env, vpu, tat and rev genes and the SIVagm3mc-derived LTR and the gag, pol and vif genes. The other chimera, SE-H13, contained the SIVagm3mc-derived env, tat and rev genes and the HIV-1-derived LTR and the gag, pol, vif and nef genes. Both constructs yielded infectious viruses and their phenotypes (growth-competence and cell-killing capacity) were examined in various CD4⁺ cells including human and monkey PBMCs. The results indicated that the replicative properties of the chimeras were mainly dependent on the 5'-genomic half of the parental viruses, and the determinant for viral cytopathogenicity was located within the 5' half of the HIV-1 genome.

Human immunodeficiency virus type 1 (HIV-1) is the aetiological agent of AIDS, and depletion of CD4⁺ T lymphocytes is recognized as the principal event leading to the severe immune dysfunction observed in HIV-infected individuals (Fauci, 1988). This depletion may be caused, at least in part, by the cytopathogenicity of HIV-1. Despite innumerable research efforts, however, it is still not known which gene(s) of the viral genome is responsible for the depletion of CD4⁺ cells and the eventual destruction of the immune system. Appropriate animal models for AIDS using HIV-1 or related viruses are therefore needed for our better understanding of the nature of viral pathogenicity.

Various simian immunodeficiency viruses (SIV), which are genetically closely related to HIV, have been isolated from several species of nonhuman primates (Hayami et al., 1994). SIVmac, isolated from macaque monkeys (Macaca mulatta), can induce an AIDS-like disease in macaque monkeys (Letvin et al., 1985). In contrast SIVagm, from African green monkeys (AGM; Cercopithecus aethiops), is apathogenic in its natural host (Ohta et al., 1988; Cichutek & Norley, 1993). Further, no AIDS-like symptoms are observed in AGMs and cynomolgus monkeys (Macaca fascicularis) experimentally inoculated with the virus (Honjo et al., 1990).

SIVagm is growth-competent but not cytopathogenic in human PBMC, whereas most HIV-1 strains are cytopathogenic in such cells. In this study, we constructed two HIV-1/SIVagm chimeric viruses in an attempt to generate chimeras which can replicate in monkey PBMC. A newly obtained proviral DNA (pSIVagm3) for SIVagm (Baier et al., 1989; Dittmar et al., 1995) and pNL432 (a cloned provirus of HIV-1) (Adachi et al., 1986) were used. The molecularly cloned SIVagm, termed SIVagm3mc, was shown to be biologically active both in vivo and in vitro (Baier et al., 1989). The growth-competence and cytopathogenic phenotypes (syncytium formation and capacity to induce cell death) of the chimeric viruses were characterized and compared with those of the parental viruses.

Chimeric proviral plasmids were constructed by inserting an appropriate fragment excised from one parental provirus DNA into the corresponding position of the other proviral plasmid utilizing appropriate restriction sites (Fig. 1A). In brief, one chimera designated pHE-A391 was constructed by inserting an EcoRI-XhoI fragment of pNL432 into the SspI and StuI sites of pSIVagm3 with the aid of 8 bp EcoRI and XhoI linkers (Takara Shuzo, Co., Japan) respectively. The other chimera, designated pSE-H13, was constructed by inserting a SacI-DraI fragment of pSIVagm3 into the SalI and BamHI sites of pNL432 with the aid of a SalI–SacI fragment (SalI–EcoRI (33 bp) from the polylinker site of plasmid pBluescript KS(+) plus EcoRI–SacI (6 bp) from pUC18) and blunting the BamHI site. Thus, pHE-A391 carried the HIV-1-derived env, vpu, tat genes and the SIVagm3mc-derived LTR and the gag, pol, vif and nef genes. The other chimera, SE-H13, contained the HIV-1-derived LTR and the SIVagm3mc-derived env, vpu, tat and rev genes and the HIV-1-derived LTR and the gag, pol, vif and nef genes. Both constructs yielded infectious viruses and their phenotypes (growth-competence and cell-killing capacity) were examined in various CD4⁺ cells including human and monkey PBMCs. The results indicated that the replicative properties of the chimeras were mainly dependent on the 5'-genomic half of the parental viruses, and the determinant for viral cytopathogenicity was located within the 5' half of the HIV-1 genome.

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Fig. 1. Generation of HIV-1/SIVagm3 chimeric viruses. (a) Structures of parental and recombinant chimeric viruses. The genes derived from pNL432 (HIV-1) and pSIVagm3 are represented by white and black bars respectively. The positions of restriction sites used for construction of the chimeras are also indicated. Functionally inactive genes due to intragene ligations are represented by grey bars. The nucleotide numbers were taken from the Los Alamos database for pNL432 and from Baier et al. (1990) for pSIVagm3. (b) Expression of viral proteins in infected M8166 cells. M8166 cells were infected with supernatant from SW480 cells transfected with each proviral plasmid. The cell lysates were prepared when syncytium formation was observed throughout the majority of the cells, resolved on 11% polyacrylamide gels, and Western immunoblotted with either anti-NL432 serum (panel i) or anti-SIVagm serum (panel ii). The former serum was taken from a cynomolgus monkey infected with the HIV-1/SIVmac chimeric virus NM-3 (Sakuragi et al., 1992) and the latter serum was taken from a naturally SIVagm-infected AGM. Size markers are shown on the left. The mobility of HIV-1 capsid proteins (p24) is slightly faster than that of the SIVagm capsid proteins (p26).

First, we examined whether or not the chimeric constructs were able to produce infectious viruses. SW480 cells, a human colon carcinoma cell line, were transfected with each proviral plasmid as described (Adachi et al., 1986). The culture
Fig. 2. Biological properties of HIV-1/SIVagm3 chimeric viruses. (a) Growth kinetics of HIV-1/SIVagm3 chimeras and their parental viruses in human PBMC (panel i) and AGM PBMC (panel ii). The RT activity in the culture supernatants was measured as described by Willy et al. (1988). (b) Effects on cell viability of HIV-1/SIVagm3 chimeras and their parental viruses in CD8⁺-cell depleted human PBMC (panel i) and CD8⁺-depleted AGM PBMC (panel ii). Depletion of CD8⁺-cells was done with the anti-human CD8 monoclonal antibody NV-Ts/c (Nichirei Co., Japan), and magnetic beads coated with a sheep anti-mouse IgG antibody (Dynabeads M-450, Dynal) according to the procedures recommended by the manufacturers. Live cells were counted in a haemocytometer after dilution with 0.1% trypan blue in PBS. ○, HIV-1; ○, SIVagm3mc; ▲, HE-A391; △, SE-H13; □, mock-infected. The results of representative experiments are shown.

The growth kinetics of chimeric and parental viruses were examined in human and AGM PBMCs (Fig. 2a). Human and AGM PBMCs were separated from heparinized whole blood and maintained in RPMI 1640 medium containing 10% FCS and human interleukin-2 (IL-2) after concanavalin A stimulation.
as described (Ohta et al., 1988). To infect human or AGM PBMC, appropriate volumes of the virus stock (containing 2 × 10⁶ RT units, which is approximately equivalent to 1 × 10⁶ TCID₅₀) prepared in M8166 cells were added. The PBMCs (approximately 1 × 10⁶ cells per well in a 96-well plate) were incubated for 4 h or overnight at 37 °C, and resuspended in fresh RPMI 1640 medium containing 10% FCS and IL-2. As shown in Fig. 2(a), panel (i), HIV-1 NL432 and SE-H13 were highly replication-competent in human PBMC, whereas SIVagm3mc and HE-A391 replicated poorly. Although the rise in RT values for the latter two was inconspicuous, virus production was confirmed by rescue of infectious virus by cocultivation with uninfected M8166 cells. As for cytopathogenicity, the former two viruses induced typical syncytium formation (ballooning) in human PBMC whereas the latter two did not. In AGM PBMC (Fig. 2a, panel ii), the chimeras HE-A391 and SE-H13 and the parental virus SIVagm3mc replicated, although replication of the chimeras was weak. Nonetheless, HIV-1 replicated only transiently in AGM PBMC. It is noteworthy that considerable cell death was observed under the microscope even in the absence of syncytium formation throughout the course of infection with SE-H13 and with HIV-1 around 4 to 6 days p.i.

In order to quantify the effect of virus on cell viability, CD8⁺ cell-depleted human and monkey PBMCs were infected with the viruses and viable cells were counted at 2 day intervals (Fig. 2b). We noticed that HIV-1 and SE-H13 caused significant cell death with human PBMC (panel i). Cell death started around 4 days p.i., and as a consequence the viable cell number did not rise above a certain value. In contrast, no significant cell death was observed on infection with either SIVagm3mc or HE-A391. Cell numbers increased at a rate similar to that of mock-infected controls during the course of infection, although a slight suppression was seen in the case of HE-A391. These results indicated that not only the replication-competence but also the cytopathogenic (cell-killing) properties of HIV-1 and SE-H13 were similar.

The severe cell-killing effect of SE-H13 was also clearly manifested in AGM PBMC (Fig. 2b, panel ii). When infected with SE-H13, the rise in viable cell number was limited because of the cytopathogenicity of this chimeric virus. In contrast, neither HE-A391 nor SIVagm3mc seemed to be cytopathogenic in monkey PBMC. The fact that the increase in cell number after HIV-1 infection was almost to that in mock-infected controls indicates that the AGM cells must have quickly resumed their normal proliferation rate after the poor transient replication of HIV-1. It is emphasized that SE-H13 (expressing the 5’ half of HIV-1) was cytopathogenic whereas HE-A391 (expressing the 5’ half of SIVagm3mc) did not cause any noticeable cell death, despite having the same replicative capacity as SE-H13. It should also be emphasized that no detectable cytopathogenic effect was observed with SIVagm3mc, despite its high level of replication in monkey PBMC.

We previously reported the generation of a series of chimeric viruses between pNL432 and pSA212 (a cloned provirus of SIVagm strain TYO-1 (Shibata et al., 1990a). The chimeras generated were able to infect human CD4⁺ cell lines (Shibata et al., 1990b), but neither the virus produced from the parental clone (pSA212) nor the derived chimeras were replication-competent in monkey PBMC. The use of pSIVagm3 in place of pSA212 allowed us to extend our limited knowledge of the biological properties of the virus because the two HIV-1/SIVagm3 chimeric viruses constructed in the present study were replication-competent in both human and AGM PBMCs.

The replicative properties of SE-H13 were very similar to those of HIV-1 in human cells as shown in Fig. 2(a, panel i, and b, panel i). On the other hand, HE-A391 was similar to SIVagm3mc. A similar relationship between these two virus pairs was also observed in monkey cells (Fig. 2a, panel ii, and b, panel ii), although this similarity was less clear because of the poor replication of HIV-1 in AGM PBMC. As already mentioned, SE-H13 contains the LTR and gag to vif genes and HE-A391 possesses the LTR and gag to vif genes of SIVagm3mc. Therefore, we conclude that the replicative properties of the chimeras were mainly dependent on the 5’ half of the parental virus genomes with respect to species-tropism. This conclusion is consistent with that of our previous report (Shibata et al., 1991), in which we suggested that the important genetic region for macaque tropism lies within the 5’ half of the SIVmac genome. It is generally believed that the env gene is critical for the virus phenotype including cell-tropism (Kuiken et al., 1992; Shioda et al., 1991), but the species-tropism appears to be decided in a different manner.

A summary of the cytopathogenic properties of the chimeras and their parental viruses is given in Table 1. The cytopathogenic properties (syncytium formation and cell death) of both HIV-1 and SE-H13 were clearly demonstrated in human PBMC. The ability of SE-H13 to kill AGM PBMC was significant. It should be noted that induction of cell death did not always correlate with formation of syncytia in AGM PBMC.

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* Whether or not HIV-1 can induce cell death is unclear because it only transiently replicates in AGM PBMC.
PBMC infected with SE-H13, no apparent syncytium formation was observed. Nevertheless, the virus caused severe cell death despite its poor replication. In contrast, SIVagm3mc and HE-A391 did not induce any detectable cytopathogenic effect (neither syncytium formation nor cell death), although they were able to replicate well in these cells. We therefore conclude that the 5’ half of the viral genome is also important for viral cytopathogenicity. Corroborating our previous findings, the determinant(s) for viral cytopathogenicity (especially cell-killing capacity) was located within the 5’-half of the genomic region of HIV-1. Further efforts are required to identify the determinant(s) at the molecular level and clarify the nature of viral pathogenicity in vivo.

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