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Original article

Construction and infection of a new simian/human immunodeficiency chimeric virus (SHIV) containing the integrase gene of the human immunodeficiency virus type 1 genome and analysis of its adaptation to monkey cells

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Abstract

Expanding the HIV-1-derived regions in the SHIV genome may help to clarify the viral restriction factors determining the host range. In this study, we constructed a new SHIV having the reverse transcriptase and integrase-encoding regions of HIV-1 in addition to the 3′ half genomic region of HIV-1. This SHIV, termed SHIVrti/3rn, could replicate in a monkey $CD4^+$T cell line, HSC-F, although its replication in monkey PBMCs was very weak. After SHIVrti/3rn was passaged in HSC-F cells for 26 weeks, it gradually began to replicate in monkey PBMCs. This monkey-cell-adapted virus, termed SHIVrti/3rnP, could replicate in rhesus macaques. The whole genome of SHIVrti/3rnP was sequenced and was found to differ from SHIVrti/3rn at eleven positions. We constructed a series of mutants having some or all of these mutations and investigated their replication kinetics. The mutational analysis revealed that all of the mutations, but mainly the mutations in *env*, were responsible for the adaptation in HSC-F cells and were enough to replicate in rhesus PBMCs. Of all the SHIVs reported so far that can infect rhesus monkeys in vivo, SHIVrti/3rnP is the one that is genetically the closest to HIV-1.

Keywords: SHIV; Chimeric virus; Integrase; Animal model; Monkey; Adaptation

1. Introduction

An obstacle to research on human immunodeficiency virus type 1 (HIV-1) is that the only susceptible animal to HIV-1 infection is chimpanzee (*Pan troglodytes*), which is an endangered species. Thus, infection of simian immunodeficiency virus (SIV) in macaque monkeys has been used as an alternative animal model for HIV-1 infection in humans. However, differences between HIV-1 and SIV have limited the utility of SIV/monkey models to test the functions of HIV-1 gene products in vivo. To overcome this limitation, simian/human immunodeficiency chimeric viruses (SHIVs) containing *env* and its adjacent accessory genes of HIV-1 have been constructed and shown to infect monkeys [1,2]. Because SHIVs have the Env proteins of HIV-1, they could be used as challenge viruses to evaluate vaccines targeting Env in monkeys [3]. The prototype SHIVs were non-pathogenic, but other groups developed pathogenic SHIVs by passaging initially avirulent strains of SHIVs in animals [4,5]. These pathogenic SHIVs have proved to be powerful tools for investigating the pathogenic nature of HIV-1. Nowadays, SHIV/monkey systems are widely

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used to understand the biological properties of HIV-1 and to develop preventive measures against AIDS.

SHIVs have not only helped to establish animal models for HIV-1 infection in humans, but they also have provided important clues about why HIV-1 cannot replicate in monkey cells. The ability of SHIVs to replicate in monkey cells suggested that replaceable genes were not the determinants for the replication defect of HIV-1 in monkey cells. In this respect, *rt*, *vpr*, *vpu*, *tat*, *rev*, *env* and *nef* seemed not to be involved in the species-specific tropism of HIV-1 replication [6]. In addition, the capsid of HIV-1 has been reported to be one of the targets of a restriction factor in monkey cells [7], and a cellular protein TRIM5α was shown to block HIV-1 infection in Old World monkey cells [8]. In other studies, Vif was reported to overcome another species-specific intracellular restriction factor in primate cells [9], and APOBEC3 family proteins as host cell factors were found to be deeply involved in the function of Vif [10]. Besides, a novel restriction factor was identified as Lv2 and the determinants for Lv2 were mapped to *gag* and *env* [11]. Thus, unknown restriction mechanisms may still exist in primate cells.

Our strategy has been to construct SHIVs by expanding the HIV-1-derived regions in the SHIV genome, which may help to clarify the viral restriction factors determining the host range and to elucidate the mechanisms of innate defense against the HIV-1 infection in monkey cells. It was previously shown that the *rt* region of *pol* of SIV can be replaced by that of HIV-1 [12]. Recently, we constructed a new SHIV termed SHIVrt/3rn, which had the *rt* region of *pol* in addition to the 3′ half of the HIV-1 genome [13]. SHIVrt/3rn could infect and replicate not only in monkey peripheral blood mononuclear cells (PBMCs) but also in macaque monkeys. Among the monkey-infecting SHIVs constructed at that time, SHIVrt/3rn had the broadest region of the HIV-1 genome.

In this study, we report the construction of a new SHIV containing the integrase (IN)-encoding region (*int* region) of *pol* of HIV-1 in addition to *rt* and the 3′ half of the HIV-1 genome on the background of SIVmac. The new SHIV, termed SHIVrti/3rn, could replicate not only in monkey PBMCs but also in rhesus monkeys in vivo after adapting to monkey cells. Having *rt*, *int*, *vpu*, *vpr*, *tat*, *rev*, *env* and *nef* of HIV-1, SHIVrti/3rn is much closer to HIV-1 than any other rhesus monkey-infecting SHIVs. Moreover, we constructed a series of mutants containing some or all of the mutations identified in the monkey-cell-adapted strain of SHIVrti/3rn and investigated the effect of the respective mutations on the viral replication. Recently, two HIV-1 derivatives, termed stHIV-1 [14] and NL-DT5 [15] having only the intact *vif* and CA or a part of it of SIV respectively, were found to replicate in rhesus PBMCs after passaging several times in human and/or monkey cell lines. To the best of our knowledge, however, there is no evidence that these derivatives were able to replicate in rhesus monkeys, although the replication of a derivative virus of NL-DT5 (termed NL-DT5R) in pig-tailed macaques was reported very recently [16]. Therefore, we can still say that SHIVrti/3rn is the closest to HIV-1 among the rhesus monkey-infecting SHIVs reported so far.

2. Materials and methods

2.1. DNA constructs

Infectious molecular clones of HIV-1 (pNL432) [17] and SIVmac (pMA239) [18] were used as parental provirus DNAs. In addition, two infectious molecular clones, pSHIV-rti (Ido et al., ms. in preparation) and pNM-3rn [8] were used. The former plasmid contains *rt* and *int* of HIV-1 on an SIVmac background. Briefly, the junction of *pr* of SIVmac and *rt* of HIV-1 was created as described previously [13] and the junction of *int* of HIV-1 and *vif* of SIVmac was created by inserting the PpuMI − PpuMI fragment (nt 5492 and nt 5370 in pMA239) containing the full *vif* of SIVmac between the two AvrII sites in *int* of HIV-1 (nt 5431 and nt 5661 in pNL432) after blunt-ligation. The latter plasmid possesses *env* and adjacent accessory genes such as *vpr*, *vpu*, *tat*, *rev* and *nef* of HIV-1 on an SIVmac background.

A fragment of pSHIV-rti spanning from SpeI (nt 2026 in pMA239, *gag*) to NspV (nt 6131 in pMA239, *vif*) was inserted into the corresponding position of pNM-3rn. The newly generated full-genome plasmid was termed pSHIVrti/3rn (Fig. 1).

Substitution mutant plasmids of pSHIVrti/3rn were created by site-directed mutagenesis by PCR with appropriately modified primers based upon the sequencing analysis. As for the T-to-C mutation in the primer binding site (PBS), we replaced a fragment of pSHIV-rti/3rn spanning from NarI (nt 1079 in pMA239, PBS) to DraIII (nt 1627 in pMA239, *gag*) with the corresponding region of pSHIV-C2/1 KS661. SHIV-C2/1 KS661 is a molecular clone derived from a pathogenic SHIV-C2/1 strain that has the same T-to-C substitution in the PBS [19].

2.2. Cell cultures

M8166, a $CD4^+$ human T cell line, is a subclone of C8166 cells [20]. HSC-F is a cynomolgus monkey $CD4^+$T cell line from a fetal splenocyte that was immortalized by infection with *Herpesvirus saimiri* subtype C [21]. M8166 cells and HSC-F cells were maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS). 293T cells were maintained in Dulbecco's modified Eagle's medium containing 10% FBS. PBMCs from healthy rhesus monkeys (*Macaca mulatta*) were cultured as described previously [8]. For the depletion of $CD8^+$ cells, rhesus PBMCs were treated with mouse anti-human CD8 monoclonal antibody (NU-Ts/c; Nichirei, Japan) and sheep anti-mouse IgG magnetic beads (Dynabeads M-450; Dynal A. S, Oslo, Norway).

2.3. Transfection and infection

To generate infectious virus particles, 5 μg of pSHIVrti/3rn was introduced into 1.5×10^6 M 8166 cells by the DEAE-dextran method [18]. The culture medium was changed every 3 days and the supernatant was filtered (0.45 μm pore size) and stored at −80 °C. Then, virion-associated RT activity was measured as described previously [22]. The supernatant