Infection of a Chimeric Simian and Human Immunodeficiency Virus with CCR5-Specific HIV-1 Envelope to Rhesus Macaques

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ABSTRACT. Human immunodeficiency virus (HIV) infects lymphocytes and macrophages via CD4 and chemokine receptors. In this study, the infectivity of a chimeric simian and human immunodeficiency virus (SHIV) having a CCR5-specific HIV-1 envelope gene was examined. A SHIV strain termed SHIV-JRFL could enter cells via CD4 with a chemokine receptor CCR5, not CXCR4, and the viral replication was suppressed by recombinant human RANTES, one of β-chemokines. The intravenous inoculation of SHIV-JRFL into two rhesus macaques resulted in a systemic infection, though it was rather weak. During the early infection, the production of RANTES from Con A-stimulated PBMCs of the infected monkeys increased. These results suggested that β-chemokine has the potential to limit the infectivity of an R5-type SHIV.

KEY WORDS: CCR5, RANTES, SHIV.

Table 1. Coreceptor usage of SHIV-JRFL and SHIV-NM-3rN

<table>
<thead>
<tr>
<th>Coreceptor</th>
<th>SHIV-JRFL</th>
<th>SHIV-NM-3rN</th>
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<tr>
<td>CXCR4</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>CCR5</td>
<td>+</td>
<td>–</td>
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Coreceptor specific GFP expression was detected by flow cytometry. The detected value was represented as <1% (−), 2–5% (+) and >5% (++).
PBMC expressed CD4 and both chemokine receptors. Although rhesus macaque macrophages also expressed CD4 and CCR5, the restriction of SHIV-JRFL to rhesus macaque macrophages might be ascribed to other cellular reasons, such as low expression of CD4, rather than viral factors [2, 22].

To assess the role of the HIV co-receptor CCR5 in SHIV-JRFL replication in macaque T cells, we next determined whether RANTES, one of the ligands of CCR5, could block the entry of SHIV-JRFL into rhesus macaque PBMCs. One day before virus infection, recombinant human RANTES (Peprotech, Inc., Rockey Hill, N.J.) was added to the culture of Con A-stimulated rhesus macaque PBMCs at concentrations of 0, 10, 20, and 100 ng/ml. Virus stock of SHIV-JRFL or SHIV-NM-3rN was added to each well at a moi of 0.01. The culture supernatants were collected every three days and viral replication was monitored by reverse transcriptase (RT) activity, as described previously [11]. The viral inhibition by RANTES was compared at 16 days post infection, at the peak value of each viral replication in rhesus macaque PBMCs. The inhibitory effect of RANTES was expressed as R/Ro, where R and Ro are the viral replication in the presence and absence of RANTES, respectively. We found that RANTES clearly inhibited SHIV-JRFL in rhesus macaque PBMC culture in a dose-dependent manner, while it did not inhibit SHIV-NM-3rN (Fig. 1). RANTES was able to control replication of the R5 virus, but not the X4 virus, in rhesus macaque PBMC culture, indicating that SHIV-JRFL is an R5 type virus.

To examine the infectivity of SHIV-JRFL in vivo, two rhesus macaques (Macaca Mulatta) (MM226 and MM227), about four kilograms in weight, were anesthetized by intramuscular injection of ketamine chloride and intravenously inoculated with $1 \times 10^4$ TCID$_{50}$ of SHIV-JRFL. Throughout the experimental period, the monkeys were housed in accordance with regulations approved by the Institutional Animal Care and Use Committee of the Institute for Virus Research, Kyoto University. The blood was periodically collected from all monkeys. PBMCs were separated from heparinized blood by Percoll gradient and plasma samples were frozen at $-80^\circ$C until analysis. The viral infection was assessed by virus recovery from PBMCs, detection of proviral DNAs in PBMCs and viral RNA loads in plasma. Infectious viruses were recovered by coculture of $1 \times 10^6$ cells of CD8-depleted PBMCs with $1 \times 10^6$ cells of HSC-F for at least one month. Depletion of CD8$^+$ cells was performed as previously described [11]. Virus recovery was confirmed by the appearance of syncytium formation and RT activity in the culture supernatants. Proviral DNAs were detected by nested PCR for the amplification of HIV-1 V3 region (359 bp) as previously described [17]. Cellular DNAs were extracted from PBMCs of the inoculated monkeys with a DNaseq Tissue Kit (QIAGEN, Germany) and added to a mixture including AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT). Table 2 summarizes the results of virological and immunological status of SHIV-JRFL-inoculated monkeys. The proviral DNAs in PBMCs were first detected at 1 week post inoculation (wpi) in MM207 and at 2 wpi in MM206, and persistently detected in both monkeys.

| Table 2. Virological and immunological status of SHIV-JRFL-inoculated monkeys |
|-------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Monkey | 0 | 1 | 2 | 3 | 4 | 8 | 12 | 20 | 26 | 40 | 52 |
| MM226 | Proviral DNA | – | – | + | + | + | + | + | – | + | (+) |
| | Virus isolation$^a$ | – | – | + | – | – | – | – | – | – | – |
| | Antibody response | – | – | – | – | 512 | 1024 | 2048 | 4096 | 2048 | 2048 |
| MM227 | Proviral DNA | – | – | – | + | + | + | + | – | + | (+) |
| | Virus isolation$^a$ | – | – | – | – | – | – | – | – | – | – |
| | Antibody response | – | – | – | 128 | 2048 | 4096 | 16384 | 16384 | 16384 | 16384 |

$^a$ Virus isolation was performed by coculture with monkey T cell lines, HSC-F, and CD8$^+$ cells-depleted PBMCs.

$^b$ The inguinal LNs were collected from each monkey at 4 and 52 weeks post inoculation by biopsy. The detection of proviral DNAs in LNs was represented in parentheses.
till 52 wpi. The infectious viruses were isolated from PBMCs early after infection or from the inguinal lymph nodes obtained at 4 wpi by biopsy. HIV-1 Env-specific antibodies gradually increased in the plasma of both monkeys and were detected at serial dilutions of 4096 or 16384 by the particle agglutination method (Serodia HIV-1/2, Fujirebio Inc., Tokyo, Japan). Thus, it is obvious that the infection of SHIV-JRFL to rhesus macaques was achieved.

Viral RNA loads in plasma were also determined by quantitative RT-PCR as previously described [28]. The viral RNA of SHIV-JRFL was detected in the plasma of both monkeys and the viral RNA loads transiently increased in the early phase of infection (Fig. 2). However, the peak value of viral RNA load of SHIV-JRFL in the plasma at 2 wpi was far lower approximately 0.1% than that of SHIV-NM-3rN [28].

To examine the reason why the infectivity of SHIV-JRFL to rhesus macaque monkeys were weak, we analyzed the RANTES production in PBMCs of the infected monkeys early after infection. The PBMCs (2 × 10^5 cells) were stimulated in RPMI medium containing 1 µg/ml of Con A and cultured in RPMI medium containing recombinant human IL-2 for three days. The concentration of RANTES in the culture supernatants was measured using a human RANTES ELISA kit (R & D Systems, Minneapolis, Minn), which is known to cross-react with rhesus chemokine [1, 13].

As shown in Fig. 3, RANTES was detected in the culture supernatants in Con A-stimulated PBMCs of both monkeys after SHIV-JRFL infection although it was not detected in unstimulated PBMCs (data not shown). In the PBMCs of both monkeys, RANTES production gradually increased and peaked at two or three wpi. Thus, SHIV-JRFL transiently induced the production of RANTES early after infection, which might block viral infection, especially an R5 type-virus infection. These results confirmed previous reports that infection of macaques with a live -attenuated SIV induced the production of β-chemokines [1, 13].

Although SHIV utilizing CXCR4 or both CXCR4 and CCR5 were examined well about the disease progression and as challenge virus against vaccine candidate, little is examined about R5 type-SHIV. Recently, more pathogenic R5 type-SHIV was reported to infect into rhesus macaques by both intravenous and mucosal routes and induce simian AIDS [5, 15, 16]. SHIV SF162P3 was generated from non-pathogenic R5 type-SHIV, SHIVSF162 molecular clone, by sequential blood-bone marrow transfusions into rhesus macaques [15, 16]. Importantly, in vivo adaptation to a pathogenic variant of the parental virus was required to obtain the pathogenicities of SHIV. To generate more pathogenic SHIV-JRFL variant, we should attempt in vivo adaptation of the virus enough to overcome host defense against the virus infection in rhesus macaques.

HIV-1 infects various cells, expressing not only CXCR4 but also CCR5, of infected individuals. Therefore, both X4 type-SHIV and R5 type-SHIV should be developed and characterized to mimic natural HIV-1 infection using macaque model. Further studies are necessary to understand the pathogenicity of each SHIV in vivo.
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