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

Yoshimi ENOSE¹⁾, Ariko MIYAKE¹⁾, Eiji IDO¹⁾ and Masanori HAYAMI¹⁾

¹⁾*Institute for Virus Research, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan*

(Received 21 June 2002/Accepted 11 November 2002)

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.

J. Vet. Med. Sci. 65(2): 283–286, 2003

Human immunodeficiency virus (HIV) infects lymphocytes and macrophages and is biologically classified into two main groups, macrophage-tropic (M-tropic) and T cell-tropic (T-tropic) strains. The entry of HIV-1 into target cells is mediated by the interaction between the viral envelope glycoprotein and a cellular receptor complex consisting of CD4 and one or more members of the CC or CXC chemokine receptor family [6, 8–10, 12]. Berger *et al.* [3] have proposed defining the phenotype of HIV-1 isolates based on the usage of co-receptors. The biological phenotype of a virus can change during disease progression in HIV-infected patients. M-tropic viruses, which are known to use CCR5 as a co-receptor, predominate in early infection, while T-tropic viruses that use CXCR4 appear as the disease progress [7]. Therefore, delineating the whole infection process of a CCR5-type virus (R5-virus) is important for understanding viral transmission and progression of the disease to AIDS.

Chimeric simian and human immunodeficiency viruses (SHIVs) which have an HIV-1 envelope gene (*env*) inserted into the SIV genome were constructed in several laboratories, and used to infect macaque monkeys [14, 18, 20, 25, 26]. SHIV generally showed the phenotype and the usage of co-receptors similar to those of the parental HIV-1 *env* that was inserted to the SHIV [5, 14–16, 21]. Therefore, the co-receptor usage and the biological property of the HIV-1 Env can be analyzed *in vivo* by using the SHIV. In this study, to examine the infectivity of an R5-type SHIV in rhesus macaques, we inoculated such a strain termed SHIV-JRFL to rhesus macaques and analyzed its viral infectivity.

SHIV-JRFL was constructed by insertion of a *Stu*I - *Bgl*II fragment of the HIV-1 JRFL *env* (nucleotides 6139–6910 in HIV-1 strain JRFL, GenBank accession number: U63632) in SHIV-NM-3rN [4]. This chimeric virus could efficiently replicate in human PBMCs and macrophages, and has the same cell tropism characteristics as HIV-1 JRFL, which can also replicate in human PBMCs and macrophages [19, 24].

However, SHIV-JRFL could not replicate in rhesus macaque macrophages, but could replicate in rhesus PBMCs [4]. In this study, we first determined the co-receptor usage of SHIV-JRFL and the parental T-tropic virus, SHIV-NM-3rN, by using GHOST cells transfected with either CXCR4 or CCR5 and a reporter construct encoding enhanced green fluorescent protein (GFP). These cell lines were obtained through the NIAID/NIH AIDS Research and Reference Reagent Program and were contributed by Vincent N. KewalRamani and Dan R. Littman [23]. The viral stock was prepared from the culture supernatant of a cynomolgus macaque T cell line, HSC-F, infected with SHIV-JRFL or SHIV-NM-3rN, and the 50% tissue culture infectious dose (TCID₅₀) of the viral stock was determined in HSC-F. After infection to GHOST cell lines with undiluted virus stocks, GFP expression was detected by flow cytometry at 3 days post infection. As the results, SHIV-JRFL used CD4 and CCR5, but not CXCR4, to enter the cells, while SHIV-NM-3rN used CD4 and CXCR4, but not CCR5 (Table 1). These co-receptor usages of SHIV-JRFL and SHIV-NM-3rN were the same as those of HIV-1 JRFL and HIV-1 NL432, respectively [27], showing that HIV-1 Env region inserted into SHIV determined the co-receptor usage of the virus strain. Thus, SHIV-JRFL could infect the PBMC using CD4 and CCR5, because rhesus macaque

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

Virus	GHOST cell	
	CXCR4	CCR5
SHIV-JRFL	–	+
SHIV-NM-3rN	++	–

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., Rocky Hill, N.J.) was added to the culture of Con A-stimulated rhesus macaque PBMCs at concentration 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/R₀, where R and R₀ 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 1×10^4 TCID₅₀ 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°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 1×10^6 cells of CD8-

Fig. 1. Inhibition of replication of SHIV-JRFL and SHIV-NM-3rN by recombinant human RANTES in rhesus macaque PBMCs. Virus replication was measured by reverse transcriptase activity of the culture supernatant 16 days post infection. Values are expressed as the percentages of the viral replication compared with the value in the absence of recombinant RANTES.

depleted PBMCs with 1×10^6 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 DNeasy 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 ^{b)}	8	12	20	26	40	52 ^{b)}
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.

Fig. 2. Plasma viral RNA loads in two SHIV-JRFL-infected monkeys (MM226 and MM227). Total RNAs were prepared from plasma of each monkey and RT-PCR was performed using SIV gag specific primers. The amplified PCR products were quantified in real time using SIV gag specific labeled probe [28].

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 $\mu\text{g}/\text{ml}$ 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

Fig. 3. Production of RANTES by PBMCs obtained from SHIV-JRFL-infected monkeys (MM226 and MM227). RANTES was assayed in the supernatants from Con A-stimulated PBMCs by ELISA. Data show the concentrations of RANTES in each culture supernatant.

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, SHIV_{SF162} 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*.

ACKNOWLEDGMENTS. This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Education and Science, Japan. Y. E. is recipient of a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

REFERENCES

- Ahmed, R. K., Nilsson, C., Wang, Y., Lehner, T., Biberfeld, G. and Thorstensson, R. 1999. *J. Gen. Virol.* **80**: 1569–1574.
- Bannert, N., Schenten, D., Craig, S. and Sodroski, J. 2000. *J. Virol.* **74**: 10984–10993.
- Berger, E. A., Doms, R. W., Fenyo, E. M., Korber, B. T., Littman, D. R., Moore, J. P., Sattentau, Q. J., Schuitemaker, H., Sodroski, J. and Weiss, R. A. 1998. *Nature (Lond.)* **391**: 240.
- Chen, J., Ido, E., Jin, M., Kuwata, T., Igarashi, T., Mizuno, A., Koyanagi, Y. and Hayami, M. 1998. *J. Gen. Virol.* **79**: 741–745.
- Chen, Z., Huang, Y., Zhao, X., Skulsky, E., Lin, D., Ip, J., Gettie, A. and Ho, D. D. 2000. *J. Virol.* **74**: 6501–6510.
- Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. and Sodroski, J. 1996. *Cell* **85**: 1135–1148.
- Connor, R. I., Sheridan, K. E., Ceradini, D., Choe, S. and Landau, N. R. 1997. *J. Exp. Med.* **185**: 621–628.
- Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhardt, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R. and Landau, N. R. 1996. *Nature (Lond.)* **381**: 661–666.
- Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G. and Doms, R. W. 1996. *Cell* **85**: 1149–1158.
- Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P. and Paxton, W. A. 1996. *Nature (Lond.)* **381**: 667–673.
- Enose, Y., Ibuki, K., Tamaru, K., Ui, M., Kuwata, T., Shimada, T. and Hayami, M. 1999. *J. Gen. Virol.* **80**: 847–855.
- Feng, Y., Broder, C. C., Kennedy, P. E. and Berger, E. A. 1996. *Science* **272**: 872–877.
- Gauduin, M. C., Glickman, R. L., Ahmad, S., Yilma, T. and Johnson, R. P. 1999. *Proc. Natl. Acad. Sci. U. S. A.* **96**: 14031–14036.
- Harouse, J. M., Tan, R. C., Gettie, A., Dailey, P., Marx, P. A., Luciw, P. A. and Cheng-Mayer, C. 1998. *Virology* **248**: 95–107.
- Harouse, J. M., Gettie, A., Tan, R. C., Blanchard, J. and Cheng-Mayer, C. 1999. *Science* **284**: 816–819.
- Harouse, J. M., Gettie, A., Eshetu, T., Tan, R. C., Bohm, R., Blanchard, J., Baskin, G. and Cheng-Mayer, C. 2001. *J. Virol.* **75**: 1990–1995.
- Igarashi, T., Kuwata, T., Takehisa, J., Ibuki, K., Shibata, R., Mukai, R., Komatsu, T., Adachi, A., Ido, E. and Hayami, M. 1996. *J. Gen. Virol.* **77**: 1649–1658.
- Joag, S. V., Li, Z., Foresman, L., Stephens, E. B., Zhao, L. J., Adany, I., Pinson, D. M., McClure, H. M. and Narayan, O. 1996. *J. Virol.* **70**: 3189–3197.
- Koyanagi, Y., Miles, S., Mitsuyasu, R. T., Merrill, J. E., Vinters, H. V. and Chen, I. S. Y. 1987. *Science* **236**: 819–822.
- Kuwata, T., Igarashi, T., Ido, E., Jin, M., Mizuno, A., Chen, J. and Hayami, M. 1995. *J. Gen. Virol.* **76**: 2181–2191.
- Luciw, P. A., Pratt, L. E., Shaw, K. E., Levy, J. A. and Cheng-Mayer, C. 1995. *Proc. Natl. Acad. Sci. U. S. A.* **92**: 7490–7494.
- Mori, K., Rosenzweig, M. and Desrosiers, R. C. 2000. *J. Virol.* **74**: 10852–10859.
- Morner, A., Bjorndal, A., Albert, J., Kewalramani, V. N., Littman, D. R., Inoue, R., Thorstensson, R., Fenyo, E. M. and Bjorling, E. 1999. *J. Virol.* **73**: 2343–2349.
- O'Brien, W. A., Koyanagi, Y., Namazie, A., Zhao, J. Q., Diagne, A., Idler, K., Zack, J. A. and Chen, I. S. Y. 1990. *Nature (Lond.)* **348**: 69–73.
- Reimann, K. A., Li, J. T., Veazey, R., Halloran, M., Park, I. W., Karlsson, G. B., Sodroski, J. and Letvin, N. L. 1996. *J. Virol.* **70**: 6922–6928.
- Shibata, R., Maldarelli, F., Siemon, C., Matano, T., Parta, M., Miller, G., Fredrickson, T. and Martin, M. A. 1997. *J. Infect. Dis.* **176**: 362–373.
- Smyth, R. J., Yi, Y., Singh, A. and Collman, R. G. 1998. *J. Virol.* **72**: 4478–4484.
- Ui, M., Kuwata, T., Igarashi, T., Miyazaki, Y., Tamaru, K., Shimada, T., Nakamura, M., Uesaka, H., Yamamoto, H. and Hayami, M. 1999. *J. Med. Primatol.* **28**: 242–248.