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Induction of HIV-1-Specific Neutralizing Antibodies in Mice Vaccinated with a Recombinant Sendai Virus Vector

Munehide Kano[1], Tetsuro Matano[1,2]*, Atsushi Kato[3,4], Tatsuo Shioda[4,5] and Yoshiyuki Nagai[1,4,6]

[1]*AIDS Research Center and*
[3]*Department of Viral Diseases and Vaccine Control, National Institute of Infectious Diseases, Gakuen 4-7-1, Musashi-Murayama, Tokyo 208-0011,*
[2]*Department of Microbiology, Graduate School of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033,*
[4]*Department of Viral Infection, Institute of Medical Science, University of Tokyo, Shirokanedai 4-6-1, Minato-ku, Tokyo 108-8539,*
[5]*Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, Yamadaoka 17-13-1, Suita, Osaka 565-0871 and*
[6]*Toyama Institute of Health, Nakataikou-yama 17-1, Kosugi, Imizu, Toyama 939-0363*

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Virus-specific CD8+ T cells and neutralizing antibodies are important effectors against virus infections in the host. We have been studying a recombinant Sendai virus (SeV) vector system as an AIDS vaccine candidate (1-4). We constructed a recombinant SeV vector expressing simian immunodeficiency virus (SIV) Gag protein and showed its potential for efficiently inducing antigen-specific CD8+ T cells in macaques (3,4). In this study, using a mouse model, we examined if the recombinant SeV vector-based vaccine system can induce virus-specific neutralizing antibodies as well.

Two kinds of SeV vectors expressing HIV-1 envelope glycoproteins (Env), SeV/gp160 and SeV/gp140, were recovered as described previously (5). The SeV/gp160 expresses full length HIV-1_{NL4-3} Env, gp160. The SeV/gp140 expresses the extracellular portion of HIV-1_{NL4-3} Env, gp140, consisting of the whole gp120 and the gp41 ectodomains. Balb/c mice were intranasally immunized with 10^7 cell infectious units of SeV/gp160 or SeV/gp140 twice at weeks 0 and 2. The immunized mice were sacrificed 4 weeks after the first immunization, and the sera were prepared from the whole bloods, inactivated at 56°C for 30 min, and subjected to neutralization assay.

The HIV-1_{NL4-3} stock solutions diluted serially fourfold in quadricate were incubated with an equal volume of the inactivated control sera or the sera prepared from the SeV/gp160- or SeV/gp140-immunized mice. After incubation at room temperature for 1 h, the mixtures were used to infect

*Corresponding author: Tel: +81-3-5841-3407, Fax: +81-3-5841-3374, E-mail: matano@m.u-tokyo.ac.jp

Table 1. HIV-1-specific neutralizing activities in immunized mice

Sera	Infectious titer in the presence of the indicated serum TCID50/ml	logarithm		%inhibition
Control Serum				
1st	5.16E+05	5.71		
2nd	2.06E+06	6.31		
Geometric mean (M)	1.03E+06	6.01		
SD		0.43		
M-2SD		5.15		
Serum from SeV/gp140-immunized mice				
#1	7.05E+04	4.85	*	93.2
#2	1.13E+06	6.05		0.0
#3	1.29E+05	5.11	*	87.5
#4	7.76E+04	4.89	*	92.5
Serum from SeV/gp160-immunized mice				
#5	5.20E+06	6.72		0.0
#6	1.13E+06	6.05		0.0
#7	3.28E+06	6.52		0.0
#8	2.06E+06	6.31		0.0
#9	5.16E+05	5.71		49.9
#10	2.38E+06	6.38		0.0

The %inhibition was calculated as (1-[HIV-1 titer in the presence of the serum]/[HIV-1 titer in the presence of the control serum]) × 100 (%). The asterisk indicates significance of neutralizing activities (<M-2SD).

MT-4 cells cultured in 96-well plates at a concentration of 5×10^4 cells per well. After 14 days of culture, the supernatants were harvested and subjected to an enzyme-linked immunosorbent assay (ELISA) in order to detect HIV-1 Gag p24 antigen (Retro-Tek, Cellular Products, Inc., Buffalo, N.Y.), and the infectious virus titer was calculated as described before (6). The obtained virus titers in each condition and their geometric means are shown in Table 1. Three of four sera derived from mice immunized with SeV/gp140 significantly reduced HIV-1 infectivity. However, none of the sera obtained from six SeV/gp160-immunized mice did so. Antibody levels measured by an ELISA using HIV-1_{NL4-3} Env as an antigen were similar for SeV/gp140-immunized and SeV/gp160-immunized groups (data not shown). Therefore, Env antigen expressed as gp140 appeared more effective in inducing neutralizing antibodies than the membrane-bound gp160 Env. However, the obtained level of the neutralizing capacity of the antibodies was low even with SeV/gp140.

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