# Development of recombinant rotavirus vaccine carrying herpes simplex virus 2 gene based on reverse genetics technology

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## ABSTRACT

***Introduction.*** Herpes simplex virus type 2 (HSV-2) can cause genital herpes, which reduces the patient's QOL associated with pain. In addition, genital herpes can increase the risk of human immunodeficiency virus infection. Vaccine development has been attempted for many years, but it has not yet been put into practical use. Meanwhile, rotavirus (RV) is one of the major causes of severe gastroenteritis in infants and young children worldwide. Currently, two live attenuated vaccines are widely used to prevent severe RV infection in children. Our laboratory has developed entirely plasmid-based reverse genetics systems of RV, which allowed the generation of recombinant reassortant RV containing a foreign gene. In this study, we sought to develop simian RV (SA11), in which HSV-2 glycoprotein D (gD2) gene was inserted by reverse genetics system, as a herpes vaccine, and evaluated its immunogenicity in mice (**Fig 1**).

***Methods.*** Artificially synthesized gD2 DNA fragment was inserted into T7 expression NSP1 plasmids (pT7/NSP1-gD2) (**Fig 2**). Using the plasmid, recombinant SA11-gD2 virus (rSA11-gD2) was generated (**Fig 3**). gD2 protein expression in infected MA104 cells was verified by Western blotting (**Fig 4**). rSA11-gD2 was orally inoculated into BALB/c suckling mice twice two weeks apart and into eight-week-old mice three times apart four weeks. Diarrhea score was evaluated for seven days after first inoculation in suckling mice. Serum IgG and IgA titers against RV and gD2 were measured by ELISA.

***Results.*** Insertion of the gD2 gene and synthesis of gD2 protein were demonstrated by sequence analysis and Wester blotting analysis, respectively. Diarrhea occurred after the first inoculation of rSA11-gD2 in suckling mice (**Fig 6**). Although IgG and IgA antibodies against RV were induced, gD2 antibody was not detected in convalescent sera in the suckling mice (**Fig 7**). Meanwhile, in the eight-week-old mice inoculated with three times of rSA11-gD2, significant increases in not only IgG and IgA against RV but also IgG against gD2 were demonstrated (**Fig 8**).

***Conclusion.*** Simian RV containing gD2 gene, which was developed by the reverse genetics, induced IgG antibody against gD2 in orally inoculated mice. This strategy can be used to develop genital herpes vaccine.

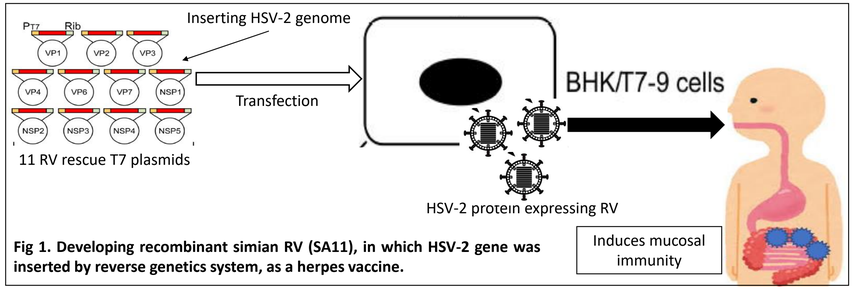


**Fig 1. Developing recombinant simian RV (SA11), in which HSV-2 gene was inserted by reverse genetics system, as a herpes vaccine.**

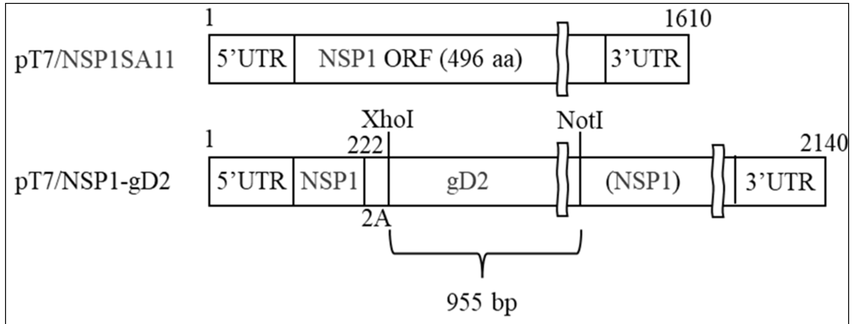


**Fig 2. Schematic presentation of the plasmids carrying the NSP1 genes for rescue of the wild-type, which is rSA11-L2, and rSA11-gD2 viruses (pT7/NSP1SA11 and pT7/NSP1-gD2, respectively).** To generate plasmid pT7/NSP1-gD2, nucleotides 223 to 643 in the NSP1 ORF were replaced with the gD2 gene.

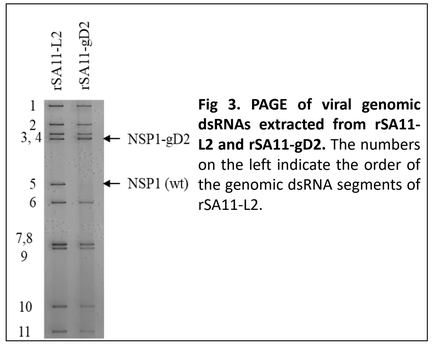


**Fig 3. PAGE of viral genomic dsRNAs extracted from rSA11-L2 and rSA11-gD2.** The numbers on the left indicate the order of the genomic dsRNA segments of rSA11-L2.

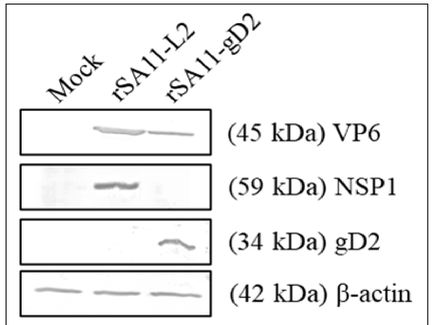


**Fig 4. Expression of RV and HSV-2 proteins in MA104 cells infected with rSA11-L2 and rSA11-gD2**. As a control, uninfected MA104 cells were assigned as mock. Whole-cell lysates of infected cells were analyzed by Western blotting using anti-VP6, anti-NSP1, anti-gD2, and anti-β-actin.

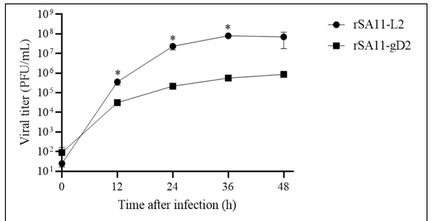


**Fig 5. Multiple-step growth curves for rSA11-L2 and rSA11-gD2.** MA104 cells were infected with rSA11-L2 and rSA11-gD2 at an MOI of 0.01 and then incubated for up to 48 h. Virus titres in the cultures were determined by plaque assays. The data shown are the mean viral titers and SDs for three independent cell cultures. *, P <0.05.

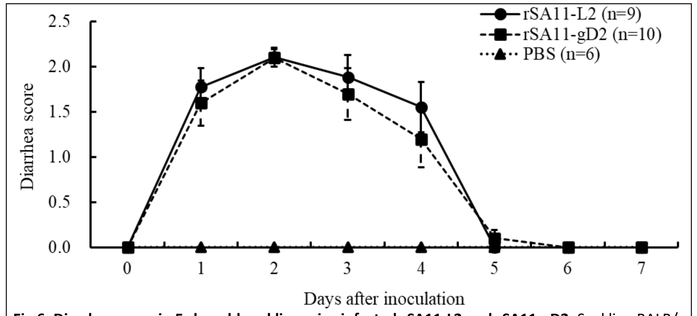


**Fig 6. Diarrhea score in 5-day-old suckling mice infected rSA11-L2 and rSA11-gD2.** Suckling BALB/c mice were orally administered PBS, rSA11-L2, rSA11-gD2 at day 0. Mice were orally inoculated with 100 µL of rSA11-L2 containing 5.5 × $10^7$ pfu/mL, rSA11-gD2 containing 4.8 × $10^6$ pfu/mL, and PBS. Diarrhea score of mice was monitored from day 0 to day 7 every day. Diarrhea score was measured individually on the basis of the following scale: 0: normal; 1: loose stool; 2: moderate diarrhea (defined as orange-yellow colored stool); 3: severe diarrhea (defined as light yellow colored stool).

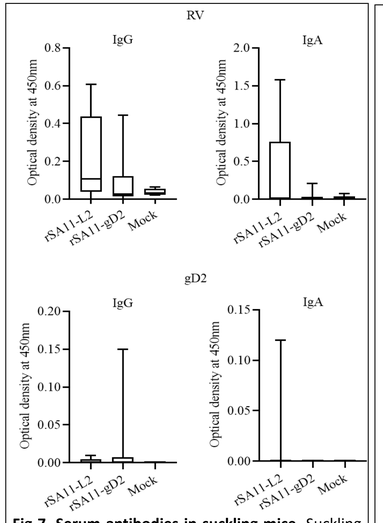


**Fig 7. Serum antibodies in suckling mice.** Suckling 5-day-old BALB/c mice were orally inoculated with rSA11-L2 (5.5×$10^7$ pfu/mL), rSA11-gD2 (4.8×$10^6$ pfu/mL), and PBS twice, 2 weeks apart in a volume of 100 µL at the first dose and 200 µL at the second dose. Sera were obtained 27 days later from the second administration. ELISA results of anti RV or gD2 IgG or IgA at OD 450 nm using horseradish peroxidase labeled as a tracer. The results are expressed as OD.

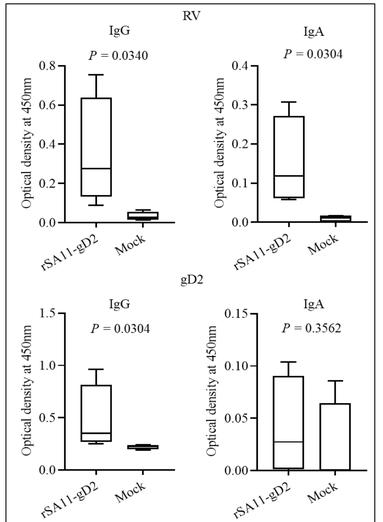


**Fig 8. Serum antibodies in 8-week-old mice**. Eight-week-old BALB/c mice were orally inoculated with rSA11-gD2 (4.8×$10^6$ pfu/mL), and PBS three times, 4 weeks apart, by the oral route in a volume of 400 µL. Sera were obtained 27 days later from the third administration. ELISA results of anti RV or gD2 IgG or IgA at OD 450 nm using horseradish peroxidase labeled as a tracer. The results are expressed as OD.