Generating Vesicular Stomatitis Virus Pseudotype Bearing the Severe Acute Respiratory Syndrome Coronavirus Spike Envelope Glycoprotein for Rapid and Safe Neutralization Test or Cell-Entry Assay

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ABSTRACT: We generated a recombinant vesicular stomatitis virus (VSV) pseudotype (VSV Δ G*SG) by replacing the envelope G gene with the GFP gene and complementing with spike glycoprotein (S) of SARS-CoV *in trans*. The neutralization and infection blocking tests showed that the VSV Δ G*SG and SARS-CoV reacted similarly to SARS-CoV specific antiserum, suggesting the VSVΔ G*SG can be a safe replacement of the live SARS-CoV for neutralization test and cell-entry assay.

KEYWORDS: SARS coronavirus; spike protein; VSV; pseudotype

INTRODUCTION

The handling of severe acute respiratory syndrome coronavirus (SARS CoV) is strictly restricted in bio-safety level 3 plus laboratory facility, which limits the relative research and vaccine development.[1] The spike glycoprotein (S) mediates SARS CoV to attach the receptor, enter the host cells by membrane fusion, and induce neutralization antibodies.[2–4] In this study, the vesicular

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stomatitis virus (VSV) pseudotype bearing the S of SARS CoV was generated to replace the live virus to establish a novel, rapid, and safe neutralization test or cell-entry assay.

METHODS

The leader sequence of CD5 and residues 12 to 1,255 of the codon optimized S gene of SARS CoV which contains 17 residues of the cytoplasmic tail, and attaches 9 amino acids within the cytoplasmic tail mostly close to the transmembrane domain of VSV G protein, was cloned into pCAGGS.[2,4,5] The resulting plasmid pCAGGS-SG was used to transfect cells and the surface expression of S was confirmed by indirect fluorescence assay and Western blot. To generate pseudotype recombinant VSV with S of SARS CoV (VSVΔG*SG), 293T cells were transfected with pCAGGS-SG, and then infected with a recombinant VSV (VSVΔG*G), in which the open reading frame of G was replaced with the green fluorescent protein (GFP) gene and complemented with G *in trans*.[5,6] Three hundred $TCID_{50}$ of SARS CoV or 300 infectious units (IU) of VSVΔG*-SG was added to twofold diluted SARS CoV specific chicken antisera. After incubation for 1 hour, the mixtures containing 100 $TCID_{50}$ of virus or 100 IU VSVΔG*SG were added to the rinsed Vero E6 cells in triplicate wells of 96-well plates. Serum from specific pathogen free (SPF) chickens was included as negative controls. The cells infected with SARS CoV were monitored daily for the cytopathic effect for 5 days. Neutralizing titers were expressed as the reciprocal of the highest serum dilution that fully inhibited the virus replication in cells infected with SARS CoV or fully inhibited GFP expression in cells infected with VSVΔG*-SG. The GFP-positive cells were counted at 16 hours post infection under a fluorescence microscope.

RESULTS AND DISCUSSION

The GFP expression in the infected Vero E6 cells confirmed the infectious ability of the VSVΔG*-SG and also indicated that the virus titers ranged from 10^5 to 10^6 IU/mL in the 293T cells supernatant. A polyclone serum against SARS CoV blocks the infectivity of the VSVΔG*SGG in Vero E6 virus, and the blocking titer is similar to the titer blocking the live SARS CoV. These results indicated that the VSVΔG*SG will be a safe and useful replacement of the live SARS CoV for SARS neutralization test, cell-entry assay, and novel vaccine or antiviral drugs development.

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REFERENCES

1. Rota, P.A., M.S. Oberste, S.S. Monroe, *et al*. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science **300:** 1394–1399.
2. Li, W., M.J. Moore, N. Vasilieva, *et al*. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature **426:** 450–454.
3. Yang, Z.Y., W.P. Kong, Y. Huang, *et al*. 2004. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature **428:** 561–564.
4. Moore, M.J., T. Dorfman, W. Li, *et al*. 2004. Retroviruses pseudotyped with the severe acute respiratory syndrome coronavirus spike protein efficiently infect cells expressing angiotensin-converting enzyme 2. J. Virol. **78:** 10628–10635.
5. Takada, A., C. Robison, H. Goto, *et al*. 1997. A system for functional analysis of ebola virus glycoprotein. Proc. Natl. Acad. Sci. USA **94:** 14764–14769.
6. Ogino, M., H. Ebihara, B.H. Lee, *et al*. Use of vesicular stomatitis virus pseudotypes bearing hantaan or seoul virus envelope proteins in a rapid and safe neutralization test. Clin. Diagn. Lab. Immunol. **10:** 154–160.