Generation and Characterization of SARS-CoV-2 Spike Protein-Pseudotyped Lentivirus Particles for Use in Neutralizing Antibody Titer Testing of Human Serum

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Background

- SARS-CoV-2 (SARS-2) is a newly emerged pandemic coronavirus which causes COVID-19.
- The extent to which those that recover from infection or are vaccinated are protected from subsequent infection by their adaptive immunity is not well understood. This protection comes from neutralizing antibodies which bind to spike proteins (S-protein) on the outside of virions, preventing its interaction with ACE-2 proteins on target cells. The virus is neutralized because the antibody prevents the virus from infecting the host cell, and this function can be measured in convalescent patient serum.
- To date, it is not known how long these neutralizing antibodies remain in circulation at high enough titers to protect against infection months or years after infection or vaccination. Current COVID-19 serology tests are only able to determine if the individual tested has antibodies against the virus or not, but they do not reveal whether any of the antibodies are neutralizing antibodies **(6)**.
- Pseudotyped viruses are often generated for use when using the wildtype infectious virus is considered too risky and when only one round of infection is required.
- A pseudotyped virus involves generating chimeric virus particles in which the envelope protein of a virus (i.e., SARS-2 spike) is utilized on another viral backbone that is virtually harmless, because it is missing the gene for its own envelope protein. It is for this reason that pseudotyping has been utilized with viruses such as HIV **(3)**, SARS-CoV-1 **(5)**, and Ebola virus **(1)**.

Purpose

The objective of this study is to generate SARS-2 pseudotyped retrovirus particles expressing green fluorescent protein (GFP) when they infect target cells. The pseudotyped particles will enable us to measure anti-S-protein neutralizing antibodies in human serum without the risks inherent in using infectious SARS-2 for these assays.

Methods

Generation of envelope and backbone constructs:

- SARS-2 envelope expression construct, VSV-G envelope construct, and envelope gene-deleted lentivirus backbone constructs generated from plasmid DNA expression vectors, amplified in transformed DH5α E. coli, isolated and purified on columns.

Production and characterization of SARS-2 pseudovirus:

- Refer to **Figure 1**.

Production and characterization of control pseudovirus:

- Control pseudovirus particles generated by using the same co-transfection protocol but with a vesicular stomatitis virus (VSV) envelope G protein (VSV-G)-expressing vector instead of the SARS-2 S-protein-expressing vector.

Confirmation of ACE2 receptor on HEK cell lines:

- Refer to **Figure 2**.

Figure 1

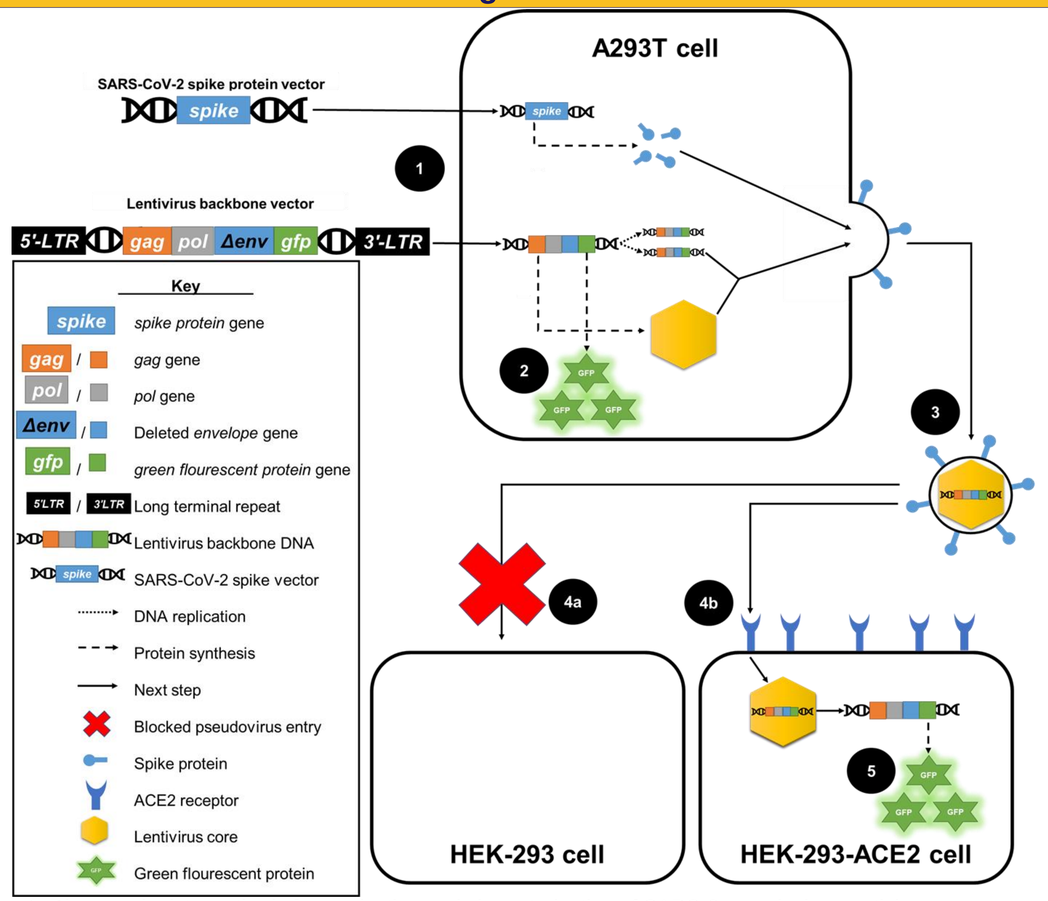

Figure 1. Diagram depicts methods for production and characterization of SARS-2 pseudovirus particles.

1) Co-transfection of A293T cells with a GFP-expressing, envelope gene-deleted lentivirus backbone vector and with an epitope-tagged SARS-2 S-protein expressing vector **(2)(4)**.
2) GFP expression of transfected A293T cells measured by fluorescence microscopy.
3) Pseudotyped particles harvested from co-transfection culture supernatant (48 hours), concentrated through 100kD centrifugal filter tubes and stored in small volume aliquots at -80C.
4) Infection of HEK-293 cell lines with pseudotyped particles.
   a) No SARS-2 pseudovirus infection in HEK-293 cell line.
   b) SARS-2 pseudovirus infection expected in ACE2 expressing HEK-293 cell line.
5) GFP expression of infected HEK-293-ACE2 cells measured by fluorescence microscopy.

Figure 2

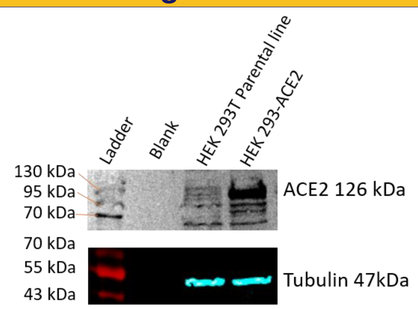

Figure 2. Image depicts Western Blot identifying the presence and absence of ACE2 receptor (126 kDa) in the HEK 293-ACE2 and HEK 293T parental cell lines respectively. Tubulin (47 kDa) used as a loading control.

Figure 3

Figure 3. Image depicts GFP expression in HEK-293-ACE2 cells infected with SARS-2 pseudotyped particles at a 100-fold dilution.

Results

- Co-transduction of A293T cells with a 1:1 or 1:2 ratio of SARS-2 S-protein expression vector to lentivirus backbone vector at 2.5ug total plasmid DNA yielded 34.8% and 34.3% transduction efficiencies, respectively. Following the same protocol, co-transduction with VSV-G envelope construct instead of SARS-2 S-protein expression vector resulted in 36.9% and 34.2% transduction efficiencies at 1:1 and 1:2 ratios, respectively (**Figure 1, step 2**). There is no significant statistical difference between the transduction efficiencies between the SARS-2 and VSV-G pseudotyping protocol.
- In huACE-2 non-expressing HEK-293 cells incubated with the generated pseudotyped particles, GFP expression was observed in those infected with the VSV-G pseudotyped particles but not with the SARS-2 pseudotyped particles (**Figure 1, step 4a**).
- A total of 6 GFP-expressing cells identified between 3 of 9 HEK-293-ACE2 cultures infected with a 100-fold dilution of SARS-2 pseudotyped virus aliquots (**Figure 1, step 5**)(**Figure 3**). At the same dilution of VSV-G pseudotyped virus aliquots, thousands of GFP expressing cells identified within 2 of 2 cultures.
- Western blot analysis of HEK 293-ACE2 cells confirms the presence of huACE2 receptor **(Figure 2)**. This assay rules out the possibility that the absence of GFP expression in the HEK 293-ACE2 cells infected with the SARS-2 spike-pseudotyped virions was due to the absence of huACE2 receptors.

Discussion / Conclusion

- Further research needs to be performed to identify solutions to successfully infect huACE2 positive cells with SARS-2 pseudotyped particles.
- Completion of this research will permit investigators to analyze antibody-positive serum samples for neutralizing antibodies within BSL2 conditions. It may also assist future research that further investigates the level of neutralizing antibody protection induced by initial SARS-2 infections, how well these individuals may be protected against SARS-2 reinfection, and how long this protection may or may not last over time in subsequent serological studies. These are important questions that are currently being asked and have not been studied yet.

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- ACE2-expressing HEK-293 cells and SARS-2 envelope expression construct obtained from the BEI reagent program at the National Institute of Health (NIH).

References

1. Akihito Yonezawa, Marielle Cavrois, and Warner C. Greene. Studies of Ebola Virus Glycoprotein-Mediated Entry and Fusion by Using Pseudotyped Human Immunodeficiency Virus Type 1 Virions: Involvement of Cytoskeletal Proteins and Enhancement by Tumor Necrosis Factor Alpha. J. Virology 79:918-926, 2005.
2. Binshan Shi, Hamayun J. Sharifi, Sara DiGrigoli, Michaela Kinnetz, Katie Mellon, Wenwei Hu and Carlos M. C. de Noronha. Inhibition of HIV early replication by the p53 and its downstream gene p21. Virol. J. 15:53, 2018.
3. Christopher Aiken. Pseudotyping human immunodeficiency virus type 1 (HIV-1) by the glycoprotein of vesicular stomatitis virus targets HIV-1 entry to an endocytic pathway and suppresses both the requirement for Nef and the sensitivity to cyclosporin A. J. Virology 71:5871-5877, 1997.
4. Kristen A. Porter, Lauren N. Kelley, Michael D. Nekorchuk, James H. Jones, Amy B. Hahn, Carlos M. C. de Noronha, Jonathan A. Harton, and Karen M. Duus. CIITA enhances HIV-1 attachment to CD4+ T cells leading to enhanced infection and cell depletion. J. Immunology 185:6480-6488, 2010.
5. Yuchun Nie, Peigang Wang, Xuanling Shi, Guangwen Wang, Jian Chen, Aihua Zheng, Wei Wang, Zai Wang, Xiuxia Qu, Min Luo, Lei Tan, Xijun Song, Xiaolei Yin, Jianguo Chen, Mingxiao Ding, Hongkui Deng. Highly infectious SARS-CoV pseudotyped virus reveals the cell tropism and its correlation with receptor expression. Biochemical and Biophysical Research Communications 321:994-1000, 2004.
6. Yu H-Qiong, Sun B-Qing, Fang Z-Fu. Distinct features of SARSCoV-2-specific IgA response in COVID-19 patients. Eur Respir J, 2020; in press (https://doi.org/10.1183/13993003.01526-2020)