Inefficiency of Sera from Mice Treated with Pseudotyped SARS-CoV to Neutralize 2019-nCoV Infection

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Dear Editor,

An outbreak of unusual pneumonia in Wuhan, China recently was caused by infection of a novel type of coronavirus. The virus and disease were denoted as 2019-nCoV and COVID-19, respectively, by the World Health Organization (WHO). Most recently, 2019-nCoV was renamed SARS-CoV-2 by Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) (Gorbalenya et al. 2020), or HCoV-19, as a common name for the consistency with COVID-19, by a group of virologists in China (Jiang et al. 2020a, b). As of 7 March 2020, a total of 80,651 confirmed cases, including 3070 deaths, were reported in China (China CDC 2020). Global spread is undeniable with serious implications for public health, thus calling for rapid development of effective therapeutics and prophylactics (Jiang et al. 2020a, b).

Very recently, it was found that horse anti-SARS-CoV and convalescent SARS patient sera had neutralizing effects against 2019-nCoV at a low dilution of 1:80 and 1:25, respectively (Zhou et al. 2020; Hoffmann et al. 2020). Although several SARS-CoV receptor-binding domain (RBD)-specific human monoclonal antibodies (mAbs), such as S230, m396 and 80R, exhibited no cross-reactivity with 2019-nCoV RBD (Wrapp et al. 2020). CR3022, a SARS-CoV RBD-specific human mAb, was reported to bind with the RBD of spike (S) protein in 2019-nCoV with potential cross-neutralizing activity (Tian et al. 2020). These studies suggest that the anti-SARS-CoV antibodies might be useful for the treatment of 2019-nCoV infection and people with history of SARS-CoV infection many years ago might be resistant to 2019-nCoV infection.

The S protein in coronavirus plays an essential role in virus entry, and it is also the main target of neutralizing antibodies (Du et al. 2009). Sequence alignment indicated a 75.9% amino acid sequence identity between the S protein of SARS-CoV and that of 2019-nCoV (Supplementary Figure S1, Supplementary Table S1). SARS-related coronavirus (SARSr-CoV) WIV1 and Rs3367 have 92.3% amino acid sequence identity to SARS-CoV in S protein, while those between SARS-CoV and MERS-CoV is 28.7% (Supplementary Figure S1, Supplementary Table S1). It is worth noting that the RBDs of SARS-CoV and 2019-nCoV exhibited a significant difference, although they bind the same receptor (angiotensin-converting enzyme 2, ACE2) during virus infection (Zhou et al. 2020). Therefore, despite sequence and receptor similarities, it is unclear whether the anti-SARS-CoV serum produced by immunization with S protein-based vaccine could cross-neutralize 2019-nCoV infection.

We previously developed SARS pseudovirus (SARS-PsV) and MERS pseudovirus (MERS-PsV), which were prepared by cotransfecting the plasmids of pNL4-3.luc.RE and pcDNA3.1-SARS, or MERS-CoV-S protein, to HEK293T cells (Fig. 1A) (Xia et al. 2019). These pseudoviruses (PsVs) bear the S protein and a defective HIV-1 genome, including a luciferase reporter gene, and could simulate the virus to infect target cells, thus having native conformations (Fig. 1A) (Xia et al. 2019). Consequently, we believed these PsVs to be potential immunogens to produce neutralizing antibodies. Female Balb/C mice (n = 5) were subcutaneously injected with SARS-PsV, MERS-PsV or PBS as a control using Freund’s complete adjuvant for the prime immunization and vaccinated at
28 days after the first immunization using Freund’s incomplete adjuvant for the boost immunization (Fig. 1B). Sera samples were collected from day 7 after the final vaccination.

We first tested day-35 sera for neutralization of SARS-PsV infection using ACE2-expressing 293T cells. As shown in Fig. 1C, compared with the PBS-treated group, significant neutralization (P < 0.0001) was observed in the sera from SARS-PsV-treated mice at dilution of 1:100, 1:200, 1:400 and 1:800. The percent of inhibition of SARS-PsV infection in sera from the SARS-PsV-vaccinated group could reach ~97%, even at a dilution of 1:800. Then, the NT50 value of mouse sera against SARS-PsV, MERS-PsV, WIV1-PsV, Rs3367-PsV and 2019-nCoV-PsV was detected as 5945, 92% and 1:800 (Fig. 1E). In spite of the high neutralization antibody titers of sera from SARS-PsV-treated mice, even at a dilution of 1:100, compared with the sera from PBS-treated control (Fig. 1H and 1I), demonstrating that sera from mice treated with SARS-CoV S protein could potentially cross-neutralize infection by SARS-CoV and SARSr-CoVs, but weakly for 2019-nCoV infection, although its S protein sequence is highly similar to that of SARS-CoV spike protein (Supplementary Figure S1, Supplementary Table S1).

Taken together, our results indicated that the sera of mice treated with pseudotyped SARS-CoV exhibited low titer of 2019-nCoV neutralization activity (1:100), implying that it may not be practical to treat 2019-nCoV infection with anti-SARS-CoV antibodies and that people with history of SARS-CoV infection many years ago may not be resistant to 2019-nCoV infection. However, we cannot exclude the possibility that the low titer of the cross-neutralizing antibody is due to the low density of S protein on the surface of pseudovirus particles. Furthermore, modifying or reconstructing the SARS-CoV S protein may enhance the exposure of conformational neutralizing epitopes in RBD, in order to increase its immunogenicity for eliciting higher cross-neutralizing antibody responses against 2019-nCoV and emerging/reemerging coronaviruses in the future.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights The mice related experiments were carried in strict accordance with institutional regulations (approval number 20190221-070, approval date 21 February 2019).
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