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• LETTER TO THE EDITOR •

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Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses

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Dear Editor,

The 2002–2003 global pandemic caused by severe acute respiratory syndrome coronavirus (SARS-CoV) infected around 8,000 people with 10% mortality (http://www.who.int/csr/sars/en/). The virus has a positive-stranded RNA genome that encodes a large polyprotein (1a and 1ab), four structural proteins, including spike (S), small envelop (E), membrane (M), and nucleocapsid (N), as well as several accessory proteins (Hu et al., 2015). The S protein plays a key role in cellular entry and is functionally divided into two subunits: S1 at the N-terminal end responsible for cell attachment and S2 at the C-terminal end responsible for membrane fusion (Jiang et al., 2005). Angiotensin I converting enzyme 2 (ACE2) was identified as a cellular receptor of SARS-CoV (Li et al., 2003). A fragment (residues 318-520) of the SARS-CoV S1 subunit was identified as the receptor-binding domain (RBD) that plays a key role in binding ACE2 and, hence, mediating virus entry (Wong et al., 2004). Therefore, RBD in the S1 subunit of S protein contains the major neutralizing epitopes for inducing neutralizing antibodies, thus serving as an important target for developing immunotherapeutics and vaccines (Jiang et al., 2005).

SARS-CoV is considered to be an emerging zoonotic pathogen crossing species barriers to infect humans (Hu et al., 2015). Since the first outbreak, genetically diverse SARS-like coronaviruses (SL-CoVs) have been discovered in horseshoe bats worldwide (Hu et al., 2015). These bat viruses are highly similar to SARS-CoV in most gene encoding regions, but they display wide diversity in S proteins, particularly at their RBDs. Based on the alignment of the RBD sequences of SL-CoVs with those of SARS-CoV, SL-CoVs can be classified into two clades (Figure S1 in Supporting Information). Clade one includes WIV1 and SHC014, which are identical in size and share the same receptor as SARS-CoV, and clade two, as represented by Rp3, has deletions in the RBD and does not use ACE2 (Hu et al., 2015; Zhou et al., 2013). WIV1 and SHC014 both use the ACE2 receptor, but their respective RBDs are highly variable in that the RBD of W1V1 is very similar to that of SARS-CoV, while the SHC014 RBD is much more variable, suggesting that their neutralizing epitopes are different. Therefore, in this study, we tested the cross-neutralization activity of SARS-CoV RBD-specific antibodies after *in vitro* infection by these two bat SL-CoV strains.

The SL-CoV strains were propagated in Vero E6 cells

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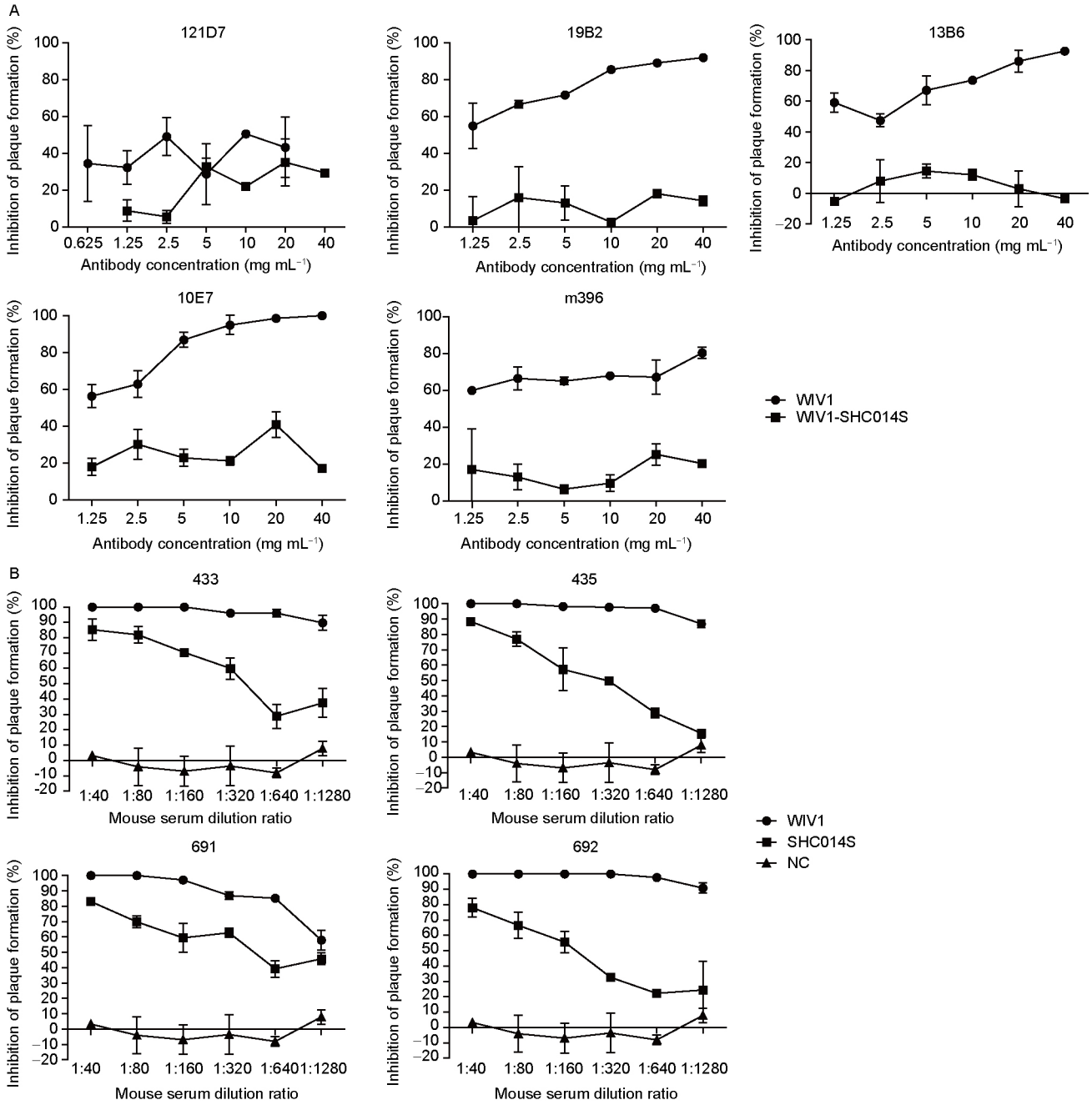

Figure 1 Cross-neutralization activity of anti-SARS-CoV RBD antibodies against infection of bat SL-CoV strain WIV1 and SHC014. The mAbs (A) and mouse sera (pAbs) (B) were diluted as indicated in the figure. The neutralization activities were measured by plaque reduction neutralization assay. NC, negative control.

(ATCC). Four mouse monoclonal antibodies (mAbs) (ID number: 19B2, 10E7, 13B6, and 121D7) and four mouse polyclonal antibodies (pAbs) (ID number: 433, 435, 691, and 692) against SARS-CoV RBD were produced in the Viral Immunology Laboratory, Lindsley F. Kimball Research Institute, New York Blood Center. The mAbs were generated by immunization of BALB/c mice with recombinant SARS-CoV RBD protein containing a C-terminal Fc (RBD-Fc), and purified from culture supernatants of stable hybridoma cell lines using protein A-Sepharose 4 Fast Flow (He et al., 2005). The pAbs were generated by immunization of BALB/c mice with SARS-CoV RBD-Fc (for 433 and 435) or RBD protein containing a C-terminal His_6 (RBD-His) (for 691 and 692), and collection of respective sera at 10 days after the 3rd dose. These mouse mAbs and pAbs demonstrated potent neutralization activity against infection of pseudotyped SARS-CoV that expresses the S protein of SARS-CoV (Figure S2A and B in Supporting Information). A human mAb, m396, previously isolated from a large antibody Fab library and shown to potently

cross-neutralize SARS-CoV isolates (Zhu et al., 2007), was kindly provided by Dr. Dimiter S. Dimitrov (Cancer and Inflammation Program, NCI, NIH, USA).

Since SHC014 could not be successfully isolated, a recombinant virus (rWIV1-SHC014S) was constructed based on the WIV1 backbone with the replacement of *SHC014S* gene, as described previously (Zeng et al., 2016). The S sequence of SHC014 was amplified with primer pair (F-SHC014-*Bsa* I, 5′-AGTGGTCTCAACGAA-CATGAAATTGTTAGTTTTAGTTTTTGCTAC-3′ and R-SHC014-*Bsa* I, 5′-TCAGGTCTCAGTTCGTTTATGTG-TAATGTAATTTGACACCCTTG-3′), digested with *Bsa* I, and inserted into an artificial bacterial chromosome along with the other viral cDNA fragments.

Vero E6 cells were plated at 1.5×10^5 cells well^{-1} in 24-well tissue culture plates and grown overnight. The aforementioned mAbs and mouse pAbs (antisera) were diluted in DMEM starting at a ratio of 1:20. Serial 2-fold dilutions were mixed with an equal volume of 50 plaque-forming units (PFU) of SL-CoV WIV1 or rWIV1-SHC014S, and incubated at 37°C for 1 h. Serum from a C57BL/6 naïve mouse was used as negative control. The mixture was added to monolayers of Vero E6 cells, incubated at 37°C for 1 h, and then changed to a semi-solid medium (DMEM containing 1% methylcellulose and 2% FBS). Plaques were counted at 4–5 days post-infection. The neutralization titers of the antibodies were calculated.

With the exception of mAb 121D7, which had only marginal neutralization activity against WIV1 infection, all tested mAbs, including 19B2, 10E7, 13B6, and m396, had NT_{50} (50% neutralization titer) less than 1.25 μg mL^{-1} against WIV1 infection. However, none of these mAbs showed sufficiently high neutralization activity against rWIV1-SHC014S infection, with only ~20% of rWIV1-SHC014S virus neutralized at most antibody concentrations (Figure 1A). While all four mouse antisera (pAbs 433, 435, 691, and 692) had only low neutralization activity against rWIV1-SHC014 infection, they all demonstrated neutralization >90% of WIV1 virus at a dilution of 1:320 (Figure 1B and Figure S3 in Supporting Information).

In summary, our results have demonstrated that most SARS-CoV RBD-specific antibodies tested in this study could cross-neutralize SL-CoV strain WIV1, but not SHC014. While SARS-CoV and WIV1 have comparable RBD, the RBD of SHC014 is much more variable, as previously noted (Figure S1 in Supporting Information). More specifically, the RBD of SHC014 has a difference of 24 amino acids (aa) compared to that of SARS-CoV, while the RBD of WIV1 only has a difference of 8 aa. This may explain why SHC014 could not be cross-neutralized effectively by most antibodies (mAbs or pAbs) targeting SARS-CoV RBD. The fact that SHC014 retains its ability to infect human cells implies that the available antibodies and vaccines based on SARS-CoV RBD will not protect the next SARS-like disease caused by bat SL-CoV SHC014 strain. Thus, further development of effective vaccines and treatments for potential infection by the SL-CoV strains, as represented by SHC014, is urgently needed.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Alignment of the RBDs of SARS-CoV Tor2 strain and bat SL-CoV strains WIV1, SHC014, and Rp3.

Figure S2 Neutralization activity of anti-SARS-CoV RBD antibodies against infection of pseudotyped SARS-CoV.

Figure S3 Plaque neutralization of SL-CoV WIV1 and SHC014 strains by anti-SARS-CoV RBD sera.

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