

Emerging SARS-CoV-2 variants reduce neutralization sensitivity to convalescent sera and monoclonal antibodies

Running Title: SARS-CoV-2 variants exhibit neutralization resistant

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19 **ABSTRACT**

20 SARS-CoV-2 Spike-specific antibodies contribute the majority of the
21 neutralizing activity in most convalescent human sera. Two SARS-CoV-2
22 variants, N501Y.V1 (also known as B.1.1.7 lineage or VOC-202012/01) and
23 N501Y.V2 (B.1.351 lineage), reported from the United Kingdom and South
24 Africa, contain several mutations in the receptor binding domain of Spike and
25 are of particular concern. To address the infectivity and neutralization escape
26 phenotypes potentially caused by these mutations, we used SARS-CoV-2
27 pseudovirus system to compare the viral infectivity, as well as the
28 neutralization activities of convalescent sera and monoclonal antibodies
29 (mAbs) against SARS-CoV-2 variants. Our results showed that N501Y Variant
30 1 and Variant 2 increase viral infectivity compared to the reference strain
31 (wild-type, WT) *in vitro*. At 8 months after symptom onset, 17 serum samples
32 of 20 participants (85%) retaining titers of $ID_{50} > 40$ against WT pseudovirus,
33 whereas the NAb titers of 8 samples (40%) and 18 samples (90%) decreased
34 below the threshold against N501Y.V1 and N501Y.V2, respectively. In addition,
35 both N501Y Variant 1 and Variant 2 reduced neutralization sensitivity to most
36 (6/8) mAbs tested, while N501Y.V2 even abrogated neutralizing activity of two
37 mAbs. Taken together the results suggest that N501Y.V1 and N501Y.V2
38 reduce neutralization sensitivity to some convalescent sera and mAbs.

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41 **INTRODUCTION**

42 Coronaviruses are enveloped, positive-stranded RNA viruses that contain the
43 largest known RNA genomes to date. As severe acute respiratory syndrome
44 coronavirus 2 (SARS-CoV-2) continues to circulate in the human population,
45 multiple mutations accumulate over time, which may affect its transmission,
46 virulence and antigenicity. Neutralizing antibodies (NAbs) elicited by natural
47 infection or vaccination are likely to be a key immune correlate for protection
48 against SARS-CoV-2 infection. Decline of antibodies response to
49 SARS-CoV-2 in convalescent individuals and reinfections by different
50 viral-variants have been reported ¹⁻³. It is therefore important to gain insights
51 into infectivity and antigenicity of SARS-CoV-2 variants.

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53 Spike-specific antibodies contribute the majority of the neutralizing activity in
54 most convalescent human sera. Two SARS-CoV-2 variants, N501Y.V1 (also
55 known as B.1.1.7 lineage or VOC-202012/01) and N501Y.V2 (B.1.351 lineage),
56 reported from the United Kingdom (UK) and South Africa, contain several
57 mutations in the receptor binding domain (RBD) of Spike and are of particular
58 concern. To address the infectivity and neutralization escape phenotypes
59 potentially caused by these mutations, we used SARS-CoV-2 pseudovirus
60 system to compare the viral infectivity, as well as the neutralization activities of
61 convalescent sera and monoclonal antibodies (mAbs) against SARS-CoV-2
62 variants.

63 **METHODS**

64 The blood samples ($n = 40$) of 20 patients with COVID-19 obtained in February
65 and October 2020 in Chongqing were previously reported.² Eight RBD-specific
66 mAbs with neutralizing capability against SARS-CoV-2 were obtained from the
67 blood samples of COVID-19 convalescent patients.⁴ DNA sequences encoding
68 reference strain (wild-type, WT) and mutant Spike proteins of SARS-CoV-2
69 were codon-optimized and synthesized by Sino Biological Inc (Beijing, China)
70 and GenScript Inc (Nanjing, China). Using luciferase-expressing lentiviral
71 pseudotype system, we expressed WT, N501Y.V1 (Variant 1) and N501Y.V2
72 (Variant 2) mutant Spike proteins in enveloped virions, respectively. The
73 neutralizing antibodies (NAbs) were measured by pseudovirus-based assays
74 in 293T-ACE2 cells. The inhibitory dose (ID_{50}) was calculated as the titers of
75 NAbs.

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77 **Ethical Approval**

78 The study was approved by the Ethics Commission of Chongqing Medical
79 University (reference number 2020003). Written informed consent was waived
80 by the Ethics Commission of the designated hospital for emerging infectious
81 diseases.

82

83 **RESULTS**

84 First, the infectivity of pseudotyped viral particles were measured by luciferase
85 assay as previously described.⁵ As shown in [Fig. 1a](#), the entry efficiencies of
86 Spike pseudotyped viruses bearing N501Y Variant 1 or Variant 2 mutant were
87 about 3 to 4.4 times higher than that of the WT pseudovirus when viral input
88 was normalized, suggesting that these spike variants promote the infectivity of
89 SARS-CoV-2. Then, we assessed the neutralizing efficacy of 40 convalescent
90 serum samples from 20 individuals at two time points with pseudovirus
91 neutralization assay. At follow-up time point 1, corresponding to a median of 25
92 days (range 5–33 days) post-symptom onset, most sera were significantly less
93 effective in neutralizing the N501Y Variant 1 and Variant 2 compare to WT
94 pseudovirus ([Fig. 1b](#)). The mean nAb titers were 825 for WT, 343 for Variant 1,
95 and 148 for Variant 2. The neutralizing activity of 2 samples against N501Y.V1
96 was reduced by >10-fold. Notably, the NAb titers of 6 samples (30%)
97 decreased below the threshold against Variant 2 ([Fig. 1b](#)). At follow-up time
98 point 2 (about 8 months post-symptom onset), 17 samples of 20 participants
99 (85%) retaining titers of $ID_{50} > 40$ against WT pseudovirus, whereas the NAb
100 titers of 8 samples (40%) and 18 samples (90%) decreased below the
101 threshold against N501Y Variant 1 and Variant 2, respectively ([Fig. 1c](#)). These
102 data indicate that N501Y Variant 1 and Variant 2 escape from neutralizing
103 antibodies in some COVID-19 convalescent sera.

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105 In addition, we assessed the impact of these variants on neutralizing activity of
106 human monoclonal antibodies (mAbs) isolated from COVID-19 convalescent
107 patients. All eight antibodies potently neutralized the WT pseudovirus, while
108 two mAbs (CQ016 and CQ045) are only minimally affected by the variants.
109 However, the neutralization activities of six mAbs were reduced or abolished
110 by either N501Y Variant 1 or Variant 2 (Fig. 1d). Among them, three mAbs
111 were less effective against N501Y.V1 and five against N501Y.V2 by 3-folds or
112 more (Fig. 1d). Notably, two mAbs (CQ026 and CQ038) showed no
113 neutralizing activity to N501Y.V2. Moreover, the Variant 2 reduced the
114 neutralization sensitivity with the most potent mAb CQ046 by 26 folds,
115 compared with that of WT pseudovirus. The IC₅₀ of mAb CQ046 increased
116 from 7.4 ng/ml (WT) to 194 ng/ml (Variant 2) (Fig. 1e). Together, both N501Y
117 Variant 1 and Variant 2 reduced neutralization sensitivity to most mAbs tested,
118 while N501Y.V2 even abrogated neutralizing activity of two mAbs.

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121 **DISCUSSION**

122 Our findings indicated that N501Y Variant 1 and Variant 2 increase viral
123 infectivity compared to the reference strain *in vitro*. Notably, both N501Y
124 Variant 1 and Variant 2 contain the D614G and N501Y mutations in Spike
125 protein. The findings that Variant 1 and Variant 2 enhanced the infectivity of
126 SARS-CoV-2 *in vitro* are highly consistent with previous studies, which
127 demonstrated that D614G and N501Y mutations enhanced the fitness and
128 transmissibility of the virus as evidenced by structure analysis and the
129 increased number of clinical cases.^{6,7} Another key question is whether some
130 mutations may enable immune evasion. It is reported that neutralization
131 escape mutants can be selected by passaging virus in the presence of NAbs.⁸
132 Here, we observed that two naturally occurring SARS-CoV-2 variants, N501Y
133 Variant 1 and Variant 2, were more resistant to neutralization by some mAb
134 and convalescent sera from patients that were infected in mid- to late- January
135 2020 when a ‘first wave’ virus was mainly circulating in China. Consistently,
136 Spike variants with the H60/V70 deletion or E484K mutation have significantly
137 reduced susceptibility to neutralization by the polyclonal serum antibodies of
138 some individuals.^{9,10} Whether these patients were at high risk of reinfection
139 with ‘second wave’ variants should be explored in further studies. It is also
140 urgent to assess the effectiveness of currently authorized vaccines against
141 these variants.

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143 Collectively, this study will be helpful for understanding SARS-CoV-2 infectivity
144 and for the design of vaccines against COVID-19. Given the evolving nature of
145 the SARS-CoV-2 RNA genome, antibody therapeutics and vaccine
146 development require further considerations to accommodate mutations in
147 Spike that may affect the antigenicity of the virus. Limitations of this study
148 include its small sample size and the use of non-replicating pseudovirus
149 system. Therefore, further studies with authentic SARS-CoV-2 viruses are
150 required.

151

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165 and designed the study. J.H. and P.P. performed the experiments. B.L. and L.F.
166 provided the samples. F.L. was responsible for mAb purification. K.W.
167 performed statistical analysis. All authors provided scientific expertise and the
168 interpretation of data for the work. K.W. drafted the manuscript. All authors
169 have approved the final version of the manuscript.

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171 **Conflict of Interest:** The authors declare no conflicts of interest.

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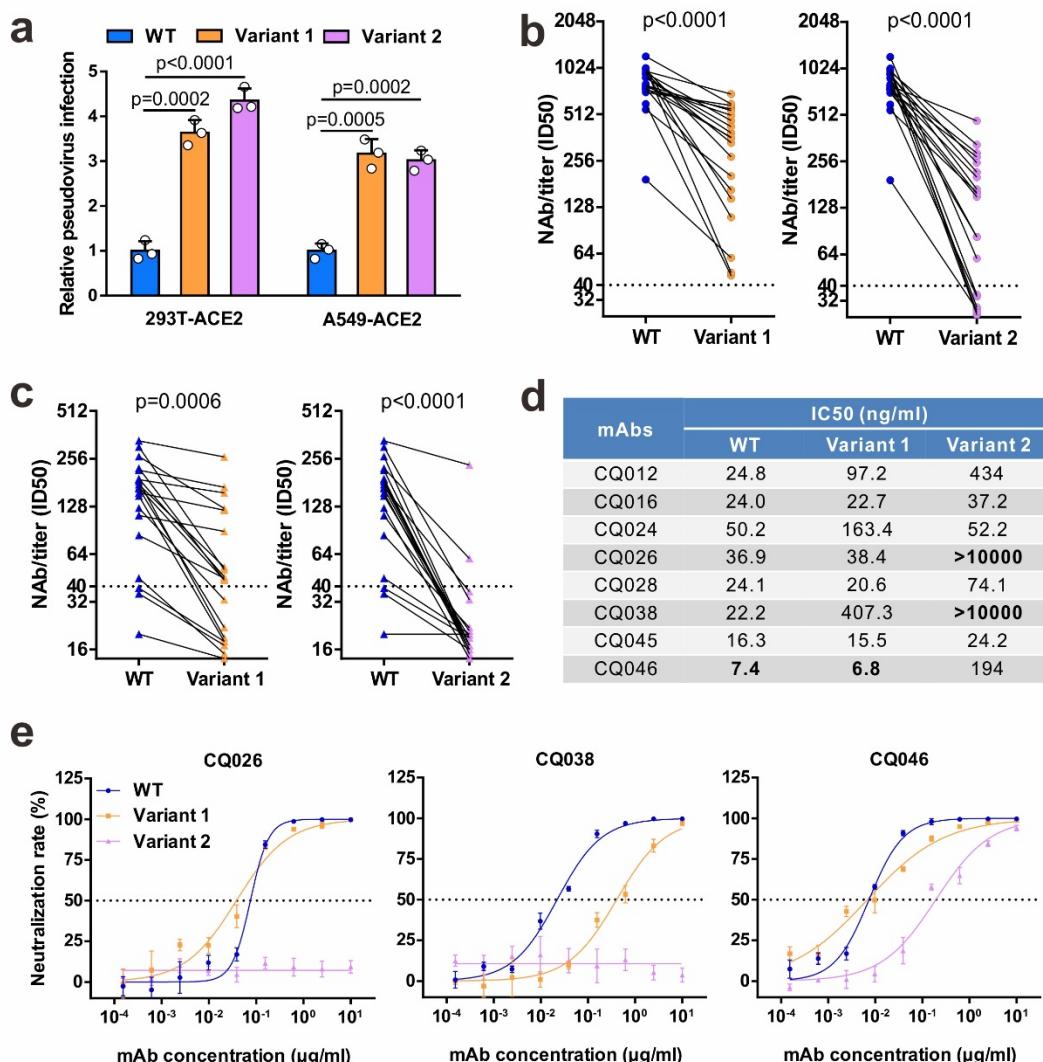
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208 **Figure and Figure Legend**



209

210 **Fig.1 Neutralizing activities of convalescent sera and monoclonal antibodies**
211 against SARS-CoV-2 variants. **a** Infectivity of WT and variant pseudovirus
212 conducted in 293T-ACE2 and A549-ACE2 cells. Cells were inoculated with
213 equivalent doses of each pseudotyped virus. WT, wild-type Spike (GenBank:
214 QHD43416) pseudotyped virus; Variant 1, N501Y.V1 mutant Spike
215 pseudotyped virus (containing H60/V70 deletion, Y144 deletion, N501Y,
216 A570D, D614G, P681H, T716I, S982A, D1118H); Variant 2, N501Y.V2 mutant
217 Spike pseudotyped virus (containing K417N, E484K, N501Y, D614G). **b-c**

218 Neutralization of WT and variant pseudoviruses by convalescent sera.

219 Pseudovirus-based neutralizing assay were performed to detect neutralizing

220 antibody (NAb) titers against SARS-CoV-2. The thresholds of detection were

221 1:40 of ID₅₀. Twenty sera (indicated by circles) were drawn 5 to 33 days

222 post-symptom onset (**b**); 20 sera (indicated by triangles) were drawn ~ 8

223 months post-symptom onset (**c**). **d-e** The half-maximal inhibitory

224 concentrations (IC₅₀) for tested monoclonal antibodies (mAbs) against

225 pseudoviruses (**d**) and representative neutralization curves (**e**). Statistical

226 significance was determined by One-way ANOVA.