

1 **SARS-CoV-2 variant exposures elicit antibody responses with differential cross-**
2 **neutralization of established and emerging strains including Delta and Omicron**

3

4 **Running title: Immunity elicited by SARS-2 variants**

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39 **SUMMARY**

40 This study characterizes neutralization of eight different SARS-CoV-2 variants, including Delta
41 and Omicron, with respect to nine different prior exposures, including vaccination, booster, and
42 infections with Delta, Epsilon, and others. Different exposures were found to confer substantially
43 differing neutralization specificity.

44

45 **ABSTRACT**

46 The wide spectrum of SARS-CoV-2 variants with phenotypes impacting transmission and
47 antibody sensitivity necessitates investigation of the immune response to different spike protein
48 versions. Here, we compare the neutralization of variants of concern, including B.1.617.2 (Delta)
49 and B.1.1.529 (Omicron) in sera from individuals exposed to variant infection, vaccination, or
50 both. We demonstrate that neutralizing antibody responses are strongest against variants
51 sharing certain spike mutations with the immunizing exposure. We also observe that exposure
52 to multiple spike variants increases the breadth of variant cross-neutralization. These findings

53 contribute to understanding relationships between exposures and antibody responses and may
54 inform booster vaccination strategies.

55

56 **Keywords:** SARS-CoV-2; COVID-19; neutralization; variant; B.1.617.2 (Delta); B.1.1.529

57 (Omicron); vaccination; natural infection; antibody escape; immune exposure

58 **Background**

59 Genomic surveillance of SARS-CoV-2 continues to identify a diverse spectrum of emerging
60 variants possessing mutations in the spike gene, the main viral determinant of cellular entry and
61 primary target of neutralizing antibodies [1]. Many spike mutations likely result from selective
62 pressure which improves viral fitness through increased transmissibility or evasion of host
63 immunity [2,3]. Studies have demonstrated that sera from vaccinated and naturally infected
64 individuals yield diminished neutralizing activity against certain variants, including the globally
65 dominant Delta variant [4]. Because serum neutralization titer is an important correlate of real-
66 world protective immunity, these findings suggest that antibody responses elicited by exposure
67 to ancestral spike versions (Wuhan or D614G) will be less effective at preventing future infection
68 by certain variants [5]. However, the diversity and prevalence of variants have fluctuated greatly
69 throughout the pandemic, creating a complex population of individuals that may have inherently
70 different capacity to neutralize certain variants depending on the specific genotype of their
71 previous exposures, including vaccination [6].

72

73 In this study, we address the question of variant-elicited immune specificity by determining the
74 breadth of neutralizing activity elicited by exposure to specific SARS-CoV-2 variants, vaccines,
75 or both. To accomplish this, we collected serum from subjects with prior infections by variants
76 B.1 (D614G mutation only), B.1.429 (Epsilon), P.2 (Zeta), B.1.1.519, and B.1.617.2 (Delta),
77 which were identified by viral sequencing. We also collected serum from mRNA vaccine
78 recipients who were infected with the B.1 ancestral spike lineage prior to vaccination, infected
79 with B.1.429 prior to vaccination, or had no prior infection. We measured and compared the
80 neutralization titer of each serum cohort against a panel of pseudoviruses representing each
81 different exposure variant plus the variants of concern B.1.351 (Beta), P.1 (Gamma), B.1.617,
82 B.1.617.2 (Delta), and B.1.1.529 (Omicron), which have one or more spike mutations of interest
83 in common with one of the exposure variants. Our results provide a quantitative comparison of

84 the degree of neutralization specificity produced by different exposures. We also demonstrate
85 the effect of serial exposure to different spike versions in broadening the cross-reactivity of
86 neutralizing antibody responses. Together, these findings describe correlates of protective
87 immunity within the rapidly evolving landscape of SARS-CoV-2 variants and are highly relevant
88 to the design of future vaccination strategies targeting spike antigens.

89

90 **Methods**

91 **Serum collection**

92 Samples for laboratory studies were obtained under informed consent from participants in an
93 ongoing community program “Unidos en Salud”, which provides SARS-CoV-2 testing, genomic
94 surveillance, and vaccination services in San Francisco, California [7]. Subjects with and without
95 symptoms of COVID-19 were screened with the BinaxNOW rapid antigen assay (supplied by
96 California Department of Public Health). Positive rapid tests were followed by immediate
97 disclosure and outreach to household members for testing, supportive community services, and
98 academic partnership for research studies. All samples were sequenced using ARTIC Network
99 V3 primers on an Illumina NovaSeq platform and consensus genomes generated from the
100 resulting raw .fastq files using IDseq [8].

101

102 Convalescent serum donors were selected based on sequence-confirmed infection with the
103 following variants of interest: B.1 (D614G mutation only; n=10 donors), B.1.429 (Epsilon; n=15),
104 B.1.1.519 (n=6), P.2 (Zeta; n=1), B.1.526 (Iota; n=1), B.1.617.2 (Delta; n=3), D614G infection
105 with subsequent BNT162b2 vaccination (n=8), and B.1.429 infection with subsequent
106 BNT162b2 vaccination (n=17). Serum was also collected from healthy recipients of two (n=11)
107 or three (n=7) doses of BNT162b2 or mRNA-1273 vaccines who were confirmed to have no
108 prior SARS-CoV-2 infection by anti-SARS-CoV-2 nucleocapsid IgG assay [9]. All serum was
109 collected from donors an average of 34 days (standard deviation 16.6 days) after exposure to

110 either SARS-CoV-2 or the most recent dose of mRNA vaccine. For pooled serum experiments,
111 samples from the same exposure group were pooled at equal volumes. Serum samples from
112 the closely related exposures P.2 and B.1.526 were pooled together for the “E484K exposure”
113 pool, and samples from BNT162b2 and mRNA-1273 exposures were pooled together for the
114 “vaccine exposure” pool because of the very similar neutralization specificity observed in
115 individual tests of these sera. Serum samples were heat inactivated at 56°C for 30 minutes prior
116 to experimentation. Relevant serum sample metadata and exposure grouping is shown in Table
117 S1A.

118

119 **Pseudovirus production**

120 SARS-CoV-2 pseudoviruses bearing spike proteins of variants of interest were generated using
121 a recombinant vesicular stomatitis virus expressing GFP in place of the VSV glycoprotein
122 (rVSV Δ G-GFP) described previously [10]. The following mutations representative of specific
123 spike variants were cloned in a CMV-driven expression vector and used to produce SARS-CoV-
124 2 spike pseudoviruses: B.1 (D614G), B.1.429/Epsilon (S13I, W152C, L452R, D614G), P.2/Zeta
125 (E484K, D614G), B.1.351/Beta (D80A, D215G, Δ 242-244, K417N, E484K, N501Y, D614G,
126 A701V), P.1/Gamma (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G,
127 H655Y, T1027I, V1176F), B.1.1.519 (T478K, D614G, P681H, T732A), B.1.617 (L452R, E484Q,
128 D614G, P681R), B.1.617.2/Delta (T19R, T95I, G142D, Δ 157-158, L452R, T478K, P681R,
129 D614G, D950N), and B.1.1.529/Omicron (32 spike mutations). All pseudovirus spike mutations
130 are listed in Table S1C. Pseudoviruses were titered on Huh7.5.1 cells overexpressing ACE2
131 and TMPRSS2 (gift of Andreas Puschnik) using GFP expression to measure the concentration
132 of focus forming units (ffu).

133

134 **Pseudovirus neutralization experiments**

135 Huh7.5.1-ACE2-TMPRSS2 cells were seeded in 96-well plates at a density of 7000 cells/well
136 one day prior to pseudovirus inoculation. Serum samples were diluted into complete culture
137 media (DMEM with 10% FBS, 10mM HEPES, 1x Pen-Strep-Glutamine) using the LabCyte Echo
138 525 liquid handler and 1500 ffu of each pseudovirus was added to the diluted serum to reach
139 final dilutions ranging from 1:40-1:5120, including no-serum and no-pseudovirus controls.
140 Serum/pseudovirus mixtures were incubated at 37°C for 1h before being added directly to cells.
141 Cells inoculated with serum/pseudovirus mixtures were incubated at 37°C and 5% CO₂ for 24h,
142 resuspended using 10x TrypLE Select (Gibco), and cells were assessed with the BD Celesta
143 flow cytometer. The WHO International Reference Standard 20/150 was used to validate the
144 pseudovirus assay and compare serum neutralization titers (Table S1B) [11]. All neutralization
145 assays were repeated in a total of three independent experiments with each experiment
146 containing two technical replicates for each condition. Cells were verified to be free of
147 mycoplasma contamination with the MycoAlert Mycoplasma detection kit (Lonza).

148

149 **Data analysis**

150 Pseudovirus flow cytometry data was analyzed with FlowJo to determine the percentage of
151 GFP-positive cells, indicating pseudovirus transduction. Percent neutralization for each
152 condition was calculated by normalizing GFP-positive cell percentage to no-serum control wells.
153 Neutralization titers (NT₅₀ and NT₉₀) were calculated from eight-point response curves
154 generated in GraphPad Prism 7 using four-parameter logistic regression. The fold-change in
155 pseudovirus neutralization titer in each serum group was calculated by normalizing each variant
156 NT₅₀ and NT₉₀ value to D614G pseudovirus NT₅₀ and NT₉₀ values in the same serum group. To
157 compare neutralization titer across a panel of different pseudoviruses and serum groups, the
158 Log₂ fold-change compared to D614G pseudovirus was reported.

159

160 **Results**

161 We compared the 50% and 90% neutralization titers (NT_{50} and NT_{90}) of D614G and B.1.429
162 (Epsilon) pseudoviruses in individual serum samples from subjects exposed to D614G infection,
163 B.1.429 infection, mRNA vaccination, D614G infection with subsequent mRNA vaccination, and
164 B.1.429 infection with subsequent mRNA vaccination (Figure 1). Fold-changes in both NT_{50} and
165 NT_{90} are reported since these values often differ in magnitude due to differences in
166 neutralization curve slope between different variants and sera. In D614G-exposed and vaccine-
167 exposed serum, we observed approximately 2 to 3-fold decreases in average neutralization titer
168 against B.1.429 pseudovirus compared to D614G pseudovirus. As expected, B.1.429-exposed
169 serum neutralized B.1.429 pseudovirus more efficiently than D614G pseudovirus. Of note,
170 previous infection with either D614G or B.1.429 followed by vaccination led to substantially
171 higher neutralization titers against both pseudoviruses. In contrast to other exposure groups,
172 serum from vaccine recipients previously infected by B.1.429 neutralized D614G and B.1.429 at
173 similar titers, with only a 1.3-fold difference in NT_{90} , indicating that exposure to multiple spike
174 variants elicits a potent response with specificity toward the breadth of prior exposures.

175

176 We next investigated how exposure impacts neutralization specificity by crossing a panel of
177 eight different spike variants against serum pools elicited by nine different prior exposures.
178 (Figure 2; Table S1B). A range of reductions in neutralization titer relative to D614G
179 pseudovirus were observed, with B.1.617.2 (Delta), B.1.351 (Beta), and B.1.1.529 (Omicron)
180 exhibiting the greatest resistance to neutralization in serum from vaccinated or D614G-exposed
181 individuals with up to 4-fold, 12-fold, and 65-fold reductions in NT_{90} , respectively. However, for
182 most variants, reductions in neutralization titer were considerably smaller or absent in serum
183 from subjects previously exposed to a variant bearing some or all of the same spike mutations
184 as the variant being tested. Specifically, prior exposure to the E484K mutation in the spike
185 receptor binding domain (RBD) produced the greatest neutralization of four tested variants with
186 mutations at the E484 position: B.1.617, P.1 (Gamma), P.2 (Zeta), and B.1.351 (Beta).

187 Similarly, B.1.617.2 (Delta) was neutralized more effectively by serum elicited by partially
188 homologous exposures B.1.1.519 and B.1.429 and was neutralized most effectively by serum
189 elicited by fully homologous B.1.617.2 exposure. Conversely, in B.1.617.2-exposed serum we
190 observed the least efficient neutralization of the highly divergent spike variants P.1 and B.1.351.
191 Interestingly, although B.1.1.529 (Omicron) substantially escaped neutralization in all
192 convalescent sera and serum from recipients of two vaccine doses, a much more modest 4 to 8-
193 fold reduction in neutralization titer was observed in sera from individuals with previous infection
194 plus vaccination or three vaccine doses.

195

196 **Discussion**

197 In this study, we observe that vaccination and natural SARS-CoV-2 infection elicit neutralizing
198 antibody responses that are most potent against variants that bear spike mutations present in
199 the immunizing exposure. This trend is exemplified by variants with mutations at the spike E484
200 position, which were neutralized more effectively by E484K-exposed serum than other serum
201 types. Importantly, we also show that B.1.617.2 (Delta) is neutralized more effectively by serum
202 elicited by prior exposure to three different variants — B.1.429, B.1.1.519, and B.1.617.2 —
203 which have separate sets of spike mutations partially or fully overlapping with mutations in
204 B.1.617.2. These effects are presumably due to the shared L452R RBD mutation in B.1.429 and
205 B.1.617.2, and the shared T478K RBD mutation and P681 furin cleavage site mutation found in
206 both B.1.1.519 and B.1.617.2. The poor neutralization of P.1 and B.1.351 by Delta-exposed
207 serum further reinforces the notion that cross-neutralization is heavily impacted by antigenic
208 distance between variants [12]. Together, these results demonstrate that serum neutralization
209 specificity is strongest against variants fully homologous to the exposure, but even single
210 shared spike mutations, particularly those in highly antigenic regions such as the RBD, can
211 enhance cross-neutralization as supported in other studies [3,6,13].

212

213 This study also demonstrates the effect of serial exposure to repeated or novel versions of spike
214 on neutralizing antibody response. Infection with B.1.429 (Epsilon) followed by vaccination led
215 to greater cross-neutralization of B.1.429 and B.1.617.2 (Delta) compared to vaccination alone
216 or D614G infection plus vaccination, supporting the notion that exposure to multiple spike
217 variants expands neutralization breadth. Repeated immunizing exposures from infection plus
218 vaccination or booster vaccination led to both an overall increase in neutralization titers and
219 generally broadened neutralization specificity, particularly towards B.1.1.529 (Omicron), which
220 was neutralized most effectively by serum from recipients of three vaccine doses. A limitation of
221 this study is the relatively small number of serum samples, however the shift in neutralization
222 titer between D614G and variant pseudoviruses shows strong consistency between samples.
223

224 These serology data leverage human exposures to an array of naturally occurring spike
225 mutations, including those relevant to the globally dominant B.1.617.2 and recently ascendant
226 B.1.1.529 variants, providing a real-world complement to previous animal studies investigating
227 heterologous boosting or multivalent vaccination strategies [14,15]. Our findings suggest that
228 immunity acquired through natural infection will differ significantly between populations in
229 different regions of the world due to highly variable prevalence of different SARS-CoV-2 variants
230 throughout the course of the ongoing pandemic. These results also reinforce the urgent need for
231 widespread booster vaccination and contribute additional evidence suggesting that
232 heterologous or multivalent boosting strategies may be important and effective measures to
233 address newly emergent variants such as the highly immune evasive B.1.1.529 (Omicron).
234 Future studies investigating immune responses to additional emerging variants in vaccinated
235 and unvaccinated individuals will contribute to identifying spike antigen versions that elicit
236 broadly neutralizing antibody responses.

237

238 **Figure Legends**

239 **Figure 1. Neutralization of D614G and B.1.429 pseudoviruses by serum from individuals**

240 **with different exposures.** Plot of 50% and 90% pseudovirus neutralization titers (NT_{50} and
241 NT_{90}) of serum samples obtained from donors with the indicated infection and/or vaccination
242 exposures. Grey lines connect neutralization titer values for D614G (black dots) and B.1.429
243 (blue dots) pseudoviruses within each individual serum sample. Geometric mean neutralization
244 titers for each serum group are marked with red lines and fold-change in NT_{50} and NT_{90}
245 between D614G and B.1.429 pseudoviruses is shown along with P-value. Dark grey shading
246 marks the interquartile range of titer values in each group and light grey shading marks the 10th-
247 90th percentile of the range. P-values were calculated with a Wilcoxon matched-pairs signed-
248 rank test.

249

250 **Figure 2. Change in variant pseudovirus neutralization titer relative to D614G.** Matrix of

251 normalized neutralization titers for seven different variant pseudoviruses (rows) neutralized by
252 seven different pools of individual sera grouped by exposure (columns). Data is represented as
253 a heat map of the Log₂ fold-change in NT_{50} (top left of each box) and NT_{90} (bottom right of each
254 box) of each variant relative to D614G pseudovirus. All serum samples were collected at least
255 14 days after the date of the subject's positive COVID-19 test or date of most recent vaccine
256 dose. All titer measurements are the mean of at least three independent experiments, each
257 performed with two technical replicates. Positive Log₂ fold-change (blue) indicates an increase
258 in neutralization titer for that variant relative to D614G pseudovirus, while negative Log₂ fold-
259 change (red) indicates a decrease relative to D614G. Statistical significance was determined
260 with unpaired t-tests. All values are statistically significant (P-value < 0.05) except where noted
261 with "ns" to indicate the difference in variant neutralization titer is not significantly different from
262 D614G pseudovirus neutralization titer in that serum pool.

263

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277

278 **Potential conflicts**

279 Dr. DeRisi is a member of the scientific advisory board of The Public Health Company, Inc., and
280 is scientific advisor for Allen & Co. Dr. DeRisi also reports options granted for service on the
281 Scientific Advisory Board of The Public Health Company. None of the other authors have any
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