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1 The E484K mutation in the SARS-CoV-2 spike protein reduces but does not abolish neutralizing
2 activity of human convalescent and post-vaccination sera.

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18 **Abstract**

19 One year in the coronavirus disease 2019 (COVID-19) pandemic, the first vaccines are being
20 rolled out under emergency use authorizations. It is of great concern that newly emerging
21 variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can escape antibody-
22 mediated protection induced by previous infection or vaccination through mutations in the
23 spike protein. The glutamate (E) to Lysine (K) substitution at position 484 (E484K) in the
24 receptor binding domain (RBD) of the spike protein is present in the rapidly spreading variants
25 of concern belonging to the B.1.351 and P.1 lineages. We performed *in vitro*
26 microneutralization assays with both the USA-WA1/2020 virus and a recombinant (r)SARS-CoV-
27 2 virus that is identical to USA-WA1/2020 except for the E484K mutation introduced in the
28 spike RBD. We selected 34 sera from study participants based on their SARS-CoV-2 spike ELISA
29 antibody titer (negative [N=4] versus weak [N=8], moderate [N=11] or strong positive [N=11]).
30 In addition, we included sera from five individuals who received two doses of the Pfizer SARS-
31 CoV-2 vaccine BNT162b2. Serum neutralization efficiency was lower against the E484K rSARS-
32 CoV-2 (vaccination samples: 3.4 fold; convalescent low IgG: 2.4 fold, moderate IgG: 4.2 fold and
33 high IgG: 2.6 fold) compared to USA-WA1/2020. For some of the convalescent donor sera with
34 low or moderate IgG against the SARS-CoV-2 spike, the drop in neutralization efficiency resulted
35 in neutralization ID_{50} values similar to negative control samples, with low or even absence of
36 neutralization of the E484K rSARS-CoV-2. However, human sera with high neutralization titers
37 against the USA-WA1/2020 strain were still able to neutralize the E484K rSARS-CoV-2.
38 Therefore, it is important to aim for the highest titers possible induced by vaccination to
39 enhance protection against newly emerging SARS-CoV-2 variants. Two vaccine doses may be
40 needed for induction of high antibody titers against SARS-CoV-2. Postponing the second
41 vaccination is suggested by some public health authorities in order to provide more individuals
42 with a primer vaccination. Our data suggests that this may leave vaccinees less protected
43 against newly emerging variants.

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44 **Introduction**

45 One year in the coronavirus disease 2019 (COVID-19) pandemic, the first vaccines are being rolled
46 out under emergency use authorizations. Recently, rapidly spreading variants of severe acute
47 respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, have been
48 reported. It is of great concern that these newly emerging variants can escape neutralizing
49 antibodies induced by previous infection and/or vaccination through mutations in the spike (S)
50 protein, including the receptor binding domain (RBD), a target for neutralizing antibodies. We
51 and others have previously reported that the asparagine (N) to tyrosine (Y) substitution at
52 position 501 (N501Y), present in variants of concern belonging to the B.1.1.7, B.1.351 and P.1
53 lineages, does not seem to affect *in vitro* neutralization of SARS-CoV-2 viruses by human sera
54 from convalescent or vaccinated human donors. However, there remains concern about
55 additional substitutions like E484K present in B.1.351 and P.1 lineages allowing escape from
56 neutralizing antibodies (1–4), thereby potentially rendering vaccine-induced immunity less
57 protective.

58 In order to investigate the impact of the E484K mutation in the neutralizing activity of SARS-CoV-
59 2 specific antisera, we performed *in vitro* microneutralization assays with both the USA-
60 WA1/2020 virus and a recombinant (r)SARS-CoV-2 virus that is identical to USA-WA1/2020 except
61 for the E484K mutation introduced in the spike RBD.

62 The E484K mutant rSARS-CoV-2 was generated using previously described reverse genetics based
63 on the use of a bacterial artificial chromosome (BAC) (5–7). The USA-WA1/2020 reflects SARS-
64 CoV-2 strains that circulated in the early phase of the COVID-19 pandemic. A total of 34 sera were
65 selected from study participants based on their SARS-CoV-2 S enzyme linked immunosorbent

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66 assay (ELISA) antibody titer (negative [N=4] versus weak [N=8], moderate [N=11] or strong
67 positive [N=11]). In addition, we included sera from five individuals who received two doses of
68 the Pfizer SARS-CoV-2 vaccine BNT162b2 (V1-V5). Demographics and available metadata for each
69 participant is summarized in Supplementary Table 1. We performed all experiments in a blinded
70 manner. The same sera have been tested for neutralization studies with a N501Y SARS-CoV-2
71 variant in our recent report (8).

72 **Results**

73 Sera from vaccinated donors gave high neutralization titers, similar to convalescent samples with
74 the highest neutralization titers. Serum neutralization efficiency was lower against the E484K
75 rSARS-CoV-2 (vaccination samples: 3.4 fold; convalescent low IgG: 2.4 fold, moderate IgG: 4.2
76 fold and high IgG: 2.6 fold based on geometric means) which was significantly different for the
77 convalescent sera (see Figure 1), suggesting that the single E484K mutation in the RBD affects
78 binding by serum polyclonal neutralizing antibodies from both convalescent and vaccinated
79 donors. In the case of convalescent donor sera with low or moderate IgG against SARS-CoV-2 S
80 protein, the drop in neutralization efficiency could result in neutralization ID_{50} values similar to
81 negative control samples, resulting in low or even absence of neutralization of the E484K
82 recombinant virus by those sera.

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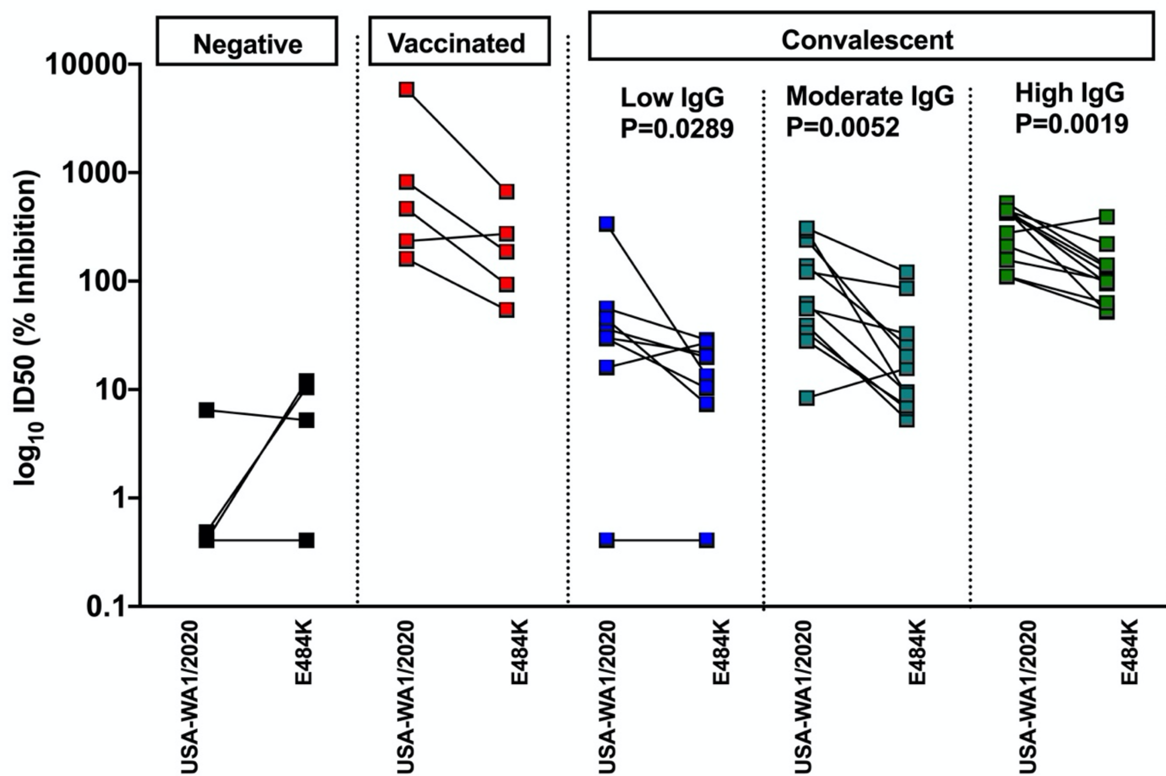

83

84 Figure 1. Human convalescent and post-vaccination sera neutralize E484K rSARS-CoV-2 less

85 efficient than USA-WA1/2020 in an *in vitro* microneutralization assay. Convalescent sera are

86 subdivided in low, moderate and high IgG classes based on anti-spike ELISA titers. Two-sided

87 Mann Whitney-U tests were performed to calculate statistical differences.

88 **Conclusions**

89 These data indicate that the E484K mutation present in circulating SARS-CoV-2 strains that

90 belong to the B.1.351 and P.1 lineages reduces the neutralizing activity of human polyclonal sera

91 induced in convalescent (infected with previous strains) and vaccinated individuals. The

92 significant impact of a single point mutation in the neutralizing activity of polyclonal sera

93 highlights the need for the rapid characterization of SARS-CoV-2 variants. However, human sera

94 with high neutralization titers against the USA-WA1/2020 strain were still able to neutralize the

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95 E484K rSARS-CoV-2. Therefore, it is important to aim for the highest titers possible induced by
96 vaccination, as this should enhance the chances for protection even in the case of antigenic drift
97 of circulating SARS-CoV-2 strains. Currently deployed SARS-CoV-2 vaccines are recommended as
98 a prime-boost regimen. Because of vaccine shortage and relatively strong seroconversion being
99 observed after a single dose, some public health authorities recommended to postpone the
100 second booster vaccination in order to be able to provide more individuals with a first primer
101 vaccination. This will result in lower neutralizing antibody titers. Our data show that this may be
102 problematic in the context of newly emerging SARS-CoV-2 variants, as it may leave some
103 vaccinees unprotected. It is currently unknown which neutralization titer correlates with (full)
104 protection, and to what extent immune mechanisms beyond direct virus neutralization
105 contribute to protection, especially for specific target groups with comorbidities that are
106 currently being prioritized for vaccination.
107 Viruses that belong to the B.1.351 and P.1 lineages have originally been described in the Republic
108 of South Africa and Brazil, but are now reported on multiple continents already. Therefore, while
109 it is premature to update vaccines based on these lineages, it is important that the worldwide
110 vaccination effort will aim at fully vaccinating as many people as possible using vaccination
111 strategies that result in induction of high neutralizing antibody titers.
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161 **Conflicts of interest**

162 The García-Sastre Laboratory has received research support from Pfizer, Senhwa Biosciences
163 and 7Hills Pharma. Adolfo García-Sastre has consulting agreements for the following companies
164 involving cash and/or stock: Vivaldi Biosciences, Contrafect, 7Hills Pharma, Avimex, Vaxalto,
165 Accurius and Esperovax. The Icahn School of Medicine at Mount Sinai has filed patent

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166 applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines
167 which list Florian Krammer as co-inventor. Daniel Stadlbauer and Viviana Simon are also listed
168 on the serological assay patent application as co-inventors. Florian Krammer has consulted for
169 Merck and Pfizer (before 2020), and is currently consulting for Seqirus and Avimex. The
170 Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

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171 **Supplementary Table 1: Description of serum samples obtained from human**
172 **subjects**
173

	SERUM				
	Seropositive, vaccine	Spike IgG response	Sex	Age group (yrs)	Days post 1 vaccine dose (Pfizer)
	V1	Strong positive	F	>60	68
	V2	Strong positive	M	30-40	47
	V3	Strong positive	F	50-60	47
	V4	Strong positive	F	40-50	49
	V5	Strong positive	F	30-40	48
	Seropositive, infection	Spike IgG response	Sex	Age group (yrs)	Days post onset of symptoms
	P1	Weak positive	M	20-29	260
	P2	Weak positive	M	50-59	NA
	P3	Weak positive	F	30-39	111
	P4	Weak positive	F	30-39	221
	P5	Weak positive	F	30-39	254
	P6	Weak positive	F	20-29	247
	P7	Weak positive	M	30-39	220
	P8	Weak positive	F	20-29	Asymptomatic
	P9	Moderate positive	M	30-39	NA
	P10	Moderate positive	F	30-39	197
	P11	Moderate positive	F	50-59	Asymptomatic
	P12	Moderate positive	F	30-39	Asymptomatic
	P13	Moderate positive	M	30-39	234
	P14	Moderate positive	F	20-29	273
	P15	Moderate positive	M	30-39	Asymptomatic
	P16	Moderate positive	F	20-29	258
	P17	Moderate positive	F	20-29	246
	P18	Moderate positive	M	20-29	Asymptomatic
	P19	Moderate positive	F	50-59	204
	P20	Strong positive	F	50-59	NA
	P21	Strong positive	F	30-39	245
	P22	Strong positive	M	NA	170
	P23	Strong positive	F	>60	Asymptomatic
	P24	Strong positive	F	40-49	NA
	P25	Strong positive	F	50-59	191
	P26	Strong positive	F	30-39	NA
	P27	Strong positive	F	50-59	113
	P28	Strong positive	M	>60	Asymptomatic
	P29	Strong positive	M	18-19	218

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	P30	Strong positive	M	50-59	219
	Seronegative, post pandemic	Spike IgG response	Sex	Age group (yrs)	Days from last negative serology
	N1	Negative	F	40-50	23
	N2	Negative	F	20-29	24
	N3	Negative	F	20-29	23
	N4	Negative	F	30-35	22

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175 ## Supplementary Methods section:

176 **50% tissue culture infective dose ($TCID_{50}$) calculation and *in vitro* microneutralization assay:**

177 To estimate the neutralizing efficiency of human sera, *in vitro* microneutralization assays were
178 performed. Human sera were inactivated at 56°C for 30 min. Serum samples were serially diluted
179 3-fold starting from 1:30 dilution in Vero-E6-infection medium (DMEM+ 2% FBS+ 1% non-
180 essential amino acids). The samples were incubated with 450 tissue culture infective dose 50
181 ($TCID_{50}$) of either USA-WA1/2020 or rSARS-CoV-2 E484K for 1 hour in an incubator at 37°C, 5%
182 CO_2 followed by incubation with pre-seeded Vero-E6 at 37°C for 48 hours. The plates were fixed
183 in 4% formaldehyde at 4°C overnight. For $TCID_{50}$ calculation, the virus stock was serially diluted
184 10-fold starting with 1:10 dilution and incubated on Vero-E6 cells for 48 hours followed by
185 fixation in 4% Formaldehyde. The cells were washed with 1xPBS and permeabilized with 0.1%
186 Triton X-100 in 1XPBS. The cells were washed again and blocked in 5% non-fat milk in 1xPBS+
187 0.1% Tween-20 for 1 hour at room temperature. After blocking, the cells were incubated with
188 anti-SARS-CoV-2 NP and anti-spike monoclonal antibodies, mixed in 1:1 ratio, for 1.5 hours at
189 room temperature. The cells were washed in 1xPBS and incubated with 1:5000 diluted HRP-
190 conjugated anti-mouse IgG secondary antibody for 1 hour at RT followed by a brief PBS wash.
191 Finally, 100µl tetramethyl benzidine (TMB) substrate was added and incubated at RT until blue
192 color appeared, and the reaction was terminated with 50µl 1M H_2SO_4. Absorbance was recorded
193 at 450nm and 650nm and percentage reduction in infection was calculated as compared to
194 negative control.

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197 **Serum samples from human subjects:**

198 The study protocols for the collection of clinical specimens from individuals with and without
199 SARS-CoV-2 infection by the Personalized Virology Initiative were reviewed and approved by the
200 Mount Sinai Hospital Institutional Review Board (IRB-16-00791; IRB-20-03374). All participants
201 provided informed consent prior to collection of specimen and clinical information. All specimens
202 were coded prior to processing.

203
204