

1 **Distinct antigenic properties of the SARS-CoV-2** 2 **Omicron lineages BA.4 and BA.5**

3 Authors: Brian J. Willett^{1#}, Ashwini Kurshan^{2#}, Nazia Thakur^{3,4#}, Joseph Newman^{3#}, Maria
4 Manali¹, Grace Tyson¹, Nicola Logan¹, Pablo R. Murcia¹, Luke B. Snell⁵, Jonathan D.
5 Edgeworth⁵, Jie Zhou⁶, Ksenia Sukhova⁶, Gayatri Amirthalingam⁷, Kevin Brown⁷, Bryan
6 Charleston³, Michael H. Malim², Emma C. Thomson¹, Wendy S. Barclay⁶, Dalan Bailey^{3‡},
7 Katie J. Doores^{2‡} and Thomas P. Peacock^{6‡},

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9 **Affiliations**

10 ¹MRC-University of Glasgow Centre for Virus Research, Glasgow, UK

11 ²Department of Infectious Diseases, School of Immunology & Microbial Sciences, King's College
12 London; London, UK.

13 ³The Pirbright Institute, Woking, Surrey, UK

14 ⁴Nuffield Department of Medicine, The Jenner Institute, Oxford, UK

15 ⁵Centre for Clinical Infection and Diagnostics Research, Department of Infectious Diseases, Guy's and
16 St Thomas' NHS Foundation Trust; London, UK.

17 ⁶Department of Infectious Diseases, Imperial College London, UK

18 ⁷Immunisation and Vaccine Preventable Diseases Division, UKHSA

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20 *Corresponding author: Thomas P. Peacock (thomas.peacock09@imperial.ac.uk)

21 #these authors contributed equally to this work

22 ‡these authors contributed equally to this work

23

24 **Abstract**

25 Over the course of the pandemic variants have arisen at a steady rate. The
26 most recent variants to emerge, BA.4 and BA.5, form part of the Omicron lineage
27 and were first found in Southern Africa where they are driving the current wave
28 of infection.

29 In this report, we perform an in-depth characterisation of the antigenicity
30 of the BA.4/BA.5 Spike protein by comparing sera collected post-vaccination,

31 post-BA.1 or BA.2 infection, or post breakthrough infection of vaccinated
32 individuals with the Omicron variant. In addition, we assess sensitivity to
33 neutralisation by commonly used therapeutic monoclonal antibodies.

34 We find sera collected post-vaccination have a similar ability to neutralise
35 BA.1, BA.2 and BA.4/BA.5. In contrast, in the absence of vaccination, prior
36 infection with BA.2 or, in particular, BA.1 results in an antibody response that
37 neutralises BA.4/BA.5 poorly. Breakthrough infection with Omicron in vaccinees
38 leads to a broad neutralising response against the new variants. The sensitivity
39 of BA.4/BA.5 to neutralisation by therapeutic monoclonal antibodies was similar
40 to that of BA.2.

41 These data suggest BA.4/BA.5 are antigenically distinct from BA.1 and, to
42 a lesser extent, BA.2. The enhanced breadth of neutralisation observed
43 following breakthrough infection with Omicron suggests that vaccination with
44 heterologous or multivalent antigens may represent viable strategies for the
45 development of cross-neutralising antibody responses.

46 **Keywords:** SARS-CoV-2; Pandemic; COVID-19; Omicron; BA.4; BA.5; variant of concern; antigenicity

47

48 **Main Text**

49 In November 2021 a novel SARS-CoV-2 variant of concern, Omicron, was
50 identified in Southern Africa¹. The Omicron complex is now composed of five
51 related lineages – BA.1 to BA.5, with BA.4 and BA.5 recently being described in
52 Southern Africa². The Omicron lineages BA.1 and BA.2 have been described as
53 having a large antigenic distance from previous variants and current vaccine
54 strains³⁻⁵, but a more modest antigenic distance between one another^{6,7}.

55 As of May 2022, BA.4 and BA.5 have become the predominant variants in
56 South Africa and are rising rapidly in several European countries. There are also

57 clear signs BA.4 and BA.5 are driving a 5th wave of cases in South Africa². BA.4
58 and BA.5 encode identical Spike proteins and are most closely related to BA.2,
59 containing several additional mutations – Δ 69-70, L452R and F486V, but both
60 are lacking the Q493R substitution relative to BA.2². As L452R and F486V have
61 been predicted to have an antigenic influence on Spike⁸, we examined the
62 relative antigenic properties of the major Omicron complex family members,
63 BA.1, BA.2, and BA.4 using human and animal vaccine sera, post-infection and
64 vaccine-breakthrough sera, and therapeutic monoclonal antibodies (mAbs) in
65 current use.

66 First we tested sera from triple-vaccinated individuals and found that
67 these sera had a similar drop in neutralising titre for all Omicron lineages (6-15-
68 fold), including an 8- to 10-fold drop against BA.4/BA.5 (Figure 1A). Using an
69 older vaccinee cohort, we saw a similar pattern with similar drops to all Omicron
70 lineages (Figure 1B,C). We used this same cohort to further investigate the effect
71 of the 3rd dose on BA.4/BA.5-specific titres. We found that for both three dose
72 BNT162b2 and two dose ChAdOx1 + BNT162b2 boost vaccine regimes, the
73 booster dose increased BA.4 neutralising titres by \geq 10-fold, similar to that
74 observed for BA.1 and BA.2.

75 We next investigated the cross-reactivity between different variants using
76 human and hamster sera collected post-infection. Using sera from unvaccinated
77 individuals with only a single known exposure to BA.1, we found a 23-fold drop
78 in relative neutralising titres against BA.4/BA.5, and a more modest reduction
79 against BA.2 (7.6-fold, Figure 1D). To validate the observations with human sera,
80 which could potentially contain individuals who had asymptomatic exposure to
81 pre-Omicron SARS-CoV-2 strains, we examined hamster sera collected post-
82 infection (BA.1 or BA.2; Figure 1E,F). Consistent with the human sera, we found

83 post-BA.1 hamster sera displayed a marked drop off in cross-neutralisation of
84 BA.4/BA.5 (70-fold), while a more modest drop off was observed against BA.2
85 (9-fold). Hamster sera collected post-BA.2 infection displayed a less marked
86 drop off in cross-neutralisation of BA.4/BA.5 (6-fold), while neutralisation of
87 BA.1 was reduced by 2.3-fold. A similar pattern of cross-neutralisation was
88 noted for a single human BA.2 infection-only sera (Figure 1F). We detected a
89 similar pattern of cross-neutralisation with sera from unvaccinated people with
90 unspecified Omicron infections. This cohort highlighted the extreme variability
91 in immune responses to Omicron, with some individuals generating very poor
92 post-infection antibody titres at the timepoints tested (Figure 1G). These data
93 suggest that in naïve individuals, BA.4/BA.5 has a distinct antigenic profile, highly
94 distinct to that of BA.1 and closer to that of BA.2.

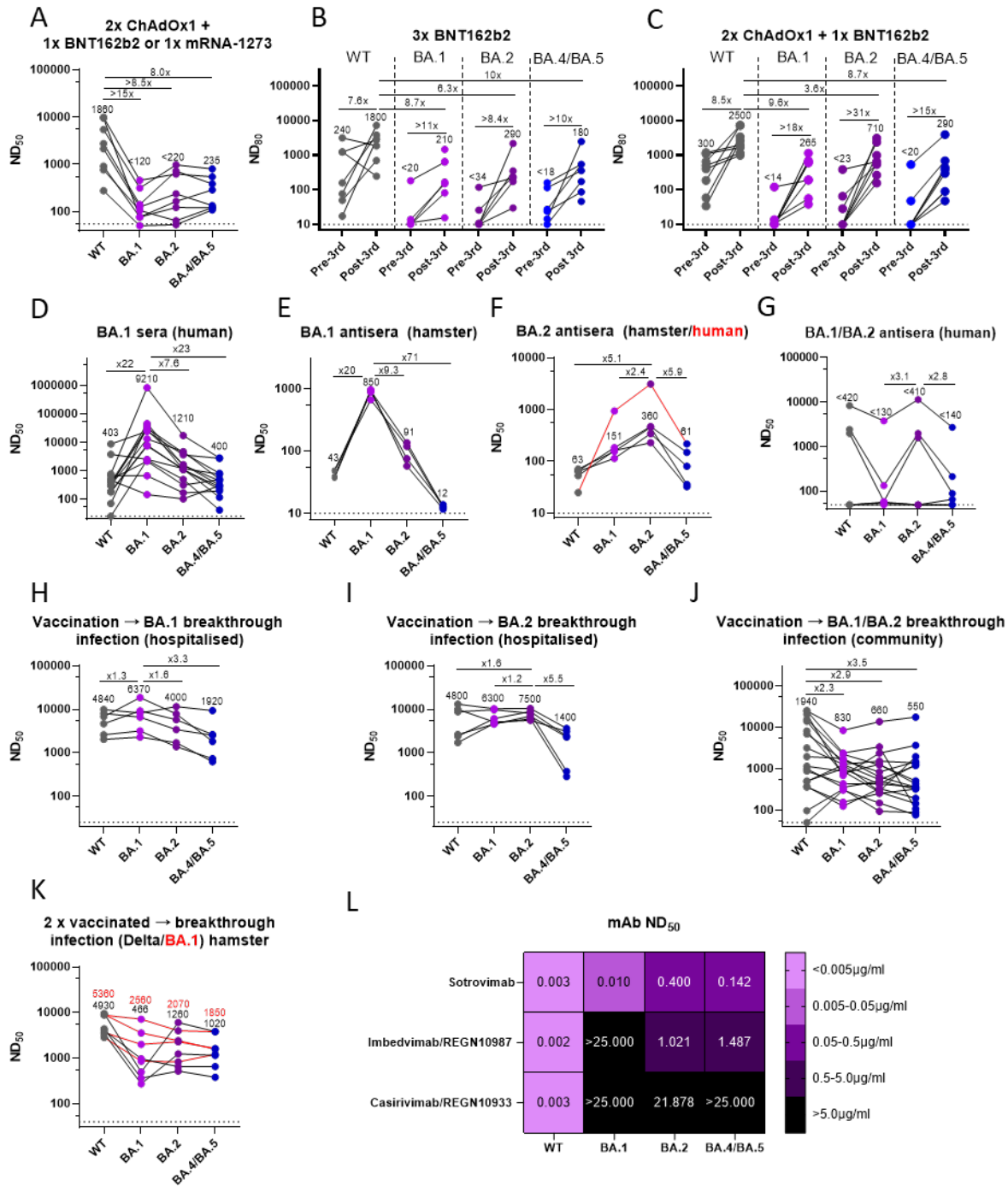
95 We next tested sera from breakthrough infection, specifically from
96 vaccinated individuals with known infections with BA.1 or BA.2, likely the most
97 common exposure history in UK adults. Generally, Omicron breakthrough
98 infections resulted in a robust pan-Omicron neutralising response. We observed
99 reductions in neutralisation of 3.3-fold between BA.4/BA.5 and BA.1 for BA.1
100 breakthrough infection (Figure 1H) consistent with several other reports⁹⁻¹², or
101 5.5-fold between BA.4/BA.5 and BA.2 for BA.2 breakthrough sera (Figure 1I). For
102 community antisera from Omicron breakthrough infections where the lineage
103 was unspecified, there were comparable drops in titre against all Omicron
104 lineages (between 2.3 - 3.5-fold, Figure 1J). Further, we tested the responses of
105 hamsters vaccinated twice with an ancestral vaccine strain and then infected
106 with either Delta or BA.1. Surprisingly, we found Delta breakthrough after
107 vaccination resulted in a cross-neutralising response to BA.2 and BA.4/BA.5 (<2-
108 fold drop), but not BA.1. BA.1 breakthrough in vaccinated hamsters also led to a
109 moderately cross-neutralising response with BA.4/BA.5 being neutralised to a

110 similar degree to BA.1 or BA.2 (<2-fold difference; Figure 1K). Overall, these data
111 suggest that breakthrough infection with BA.1 or BA.2 post-vaccination leads to
112 a cross-neutralising ‘pan-Omicron’ antibody response.

113 Finally, we tested the neutralisation activity of 3 commonly used mAbs –
114 sotrovimab (a monotherapy developed by GSK), casirivimab/REGN10933 and
115 imbedvimab/REGN10987 (which together make up the Regeneron cocktail,
116 REGEN-COV/Ronapreve). We found that BA.4/BA.5 showed a broadly similar
117 pattern of mAb sensitivity to that of BA.2 (Figure 1L), being recognised less well
118 by sotrovimab than BA.1 or WT Spike, with marginally better recognition by
119 imbedvimab than BA.1, as seen by others^{10,12,13}.

120 To conclude, BA.4/BA.5 are antigenically distinct from previous Omicron
121 variants, particularly BA.1, and are still at a substantial distance from the closely
122 related BA.2. Sera elicited by breakthrough infections in vaccinated individuals
123 with Omicron generally showed broad cross-neutralising activity, indicating that
124 priming with an ancestral antigen and boosting with an Omicron-derived antigen
125 (in this case BA.1 or BA.2 infection) elicits a pan-Omicron neutralising response.
126 These data suggest that boost vaccination with Omicron-derived antigens could
127 be an effective approach to inducing cross-protective immunity.

128



129

130 **Figure 1. Antigenic properties of the BA.4/BA.5 Omicron Spike.** A-C) Vaccine antisera from (A) the DOVE cohort
 131 vaccinated with two doses of ChAdOx1 (Oxford/AstraZeneca) with a booster dose of either BNT162b2
 132 (Pfizer/BioNTech) or mRNA-1273 (Moderna) or (B,C) CONSENSUS geriatric cohort, vaccinated with two doses of
 133 ChAdOx1 or BNT162b2 and a booster dose of BNT162b2. Sera collected at the latest timepoint available prior to
 134 3rd vaccine dose and at 4-6 weeks post 3rd dose. D-G) Post-infection sera from (D, F) hospitalised or (G)
 135 community infections. (E, F) Sera from previously infected hamsters. (H-J) Sera from Omicron-breakthrough
 136 infections of vaccinees in either (H, I) hospitalised or (J) the community. (K) Sera taken from breakthrough
 137 infections in hamsters vaccinated with two doses of an ancestral spike containing vaccine. (L) ND₅₀ in µg/ml
 138 values for commonly used monoclonal antibodies against Omicron lineages. Neutralisation assays annotated
 139 with geometric mean titres and fold changes throughout.

140

141 **Methods**

142 **Neutralisation assays**

143 Pseudovirus neutralisation assays were performed as described
144 elsewhere^{4,7,14-16}. Briefly, HEK 293T cells were transfected with lentiviral
145 packaging plasmids and the named Spike construct - D614G(WT), BA.1, BA.2 or
146 BA.4/5 to produce pseudovirus. Heat inactivated antisera was serially diluted
147 and added to and mixed with a set volume of pseudovirus and incubated for 1 h
148 at 37°C. Sera was then combined with target cells expressing human ACE2
149 (HEK293T or HeLa) and incubated for 48-72 hours. After this time cells were
150 lysed and luciferase signals read by platereader. Antibody titre was then
151 estimated by interpolating the point at which infectivity had been reduced to a
152 set value of inhibition relative to the no serum control samples.

153 **Antisera and ethics**

154 Hamster antisera was generated as we have described previously⁷, and
155 was carried out under a United Kingdom Home Office License, P48DAD9B4.

156 For the first cohort of vaccine antisera, sera were collected from healthy
157 volunteers participating in the COVID-19 **Deployed Vaccine Cohort Study**
158 (DOVE), or from the NHS Greater Glasgow and Clyde (NHSGGC) Biorepository.
159 DOVE is a cross-sectional cohort study to determine the immunogenicity of
160 deployed COVID-19 vaccines against evolving SARS-CoV-2 variants; a post-
161 licensing cross-sectional cohort study of individuals vaccinated with deployed
162 vaccines as part of the UK response to the COVID-19 pandemic. All DOVE
163 participants gave informed consent to take part in the study, which was
164 approved by the North-West Liverpool Central Research Ethics Committee (REC
165 reference 21/NW/0073). Community sera were obtained from by NHSGGC

166 Biorepository (ethical approval 550). Random residual biochemistry serum
167 samples from primary (general practice) and secondary (hospital) healthcare
168 settings were collected by the NHSGGC Biorepository from 2020 to 2022.

169 For the older adult vaccine cohort, healthy participants aged 50-90 years
170 were recruited through North London primary care networks as part of the
171 “COVID-19 vaccine responses after extended immunisation schedules”
172 (CONSENSUS) study (UKHSA). Sera samples used in this study were taken from
173 individuals vaccinated with 2 doses of Pfizer/BioNTech BNT162b2 (n=7) or 2
174 doses of Oxford/AstraZeneca ChAdOx1 nCoV-10/AZD1222 (n=8) 8-12 weeks
175 apart, followed by a 3rd dose of Pfizer/BioNTech BNT162b2. Samples were
176 analysed at the latest timepoint available prior to 3rd vaccine dose and at 4-6
177 weeks post 3rd dose. The protocol was approved by Public Health England
178 Research Ethics Governance Group (reference NR0253; 27/01/21). Participants
179 who were unable to provide informed written consent were excluded from the
180 recruitment process.

181 For sequence confirmed BA.1 or BA.2 antisera surplus serum samples was
182 approved by South Central – Hampshire B REC (20/SC/0310). SARS-CoV-2 cases
183 were diagnosed by RT-PCR of respiratory samples at St Thomas’ Hospital,
184 London. Vaccinated and BA.1 BTI – 5/6 had had 3 vaccine doses. Samples
185 collected 18-27 days POS or post positive test. Vaccinated BA.2 BTI - 5/6 had had
186 3 vaccine doses. Samples collected 9-25 days POS or post positive test. SARS-
187 CoV-2 naïve/BA.1 infected – 18-27 days POS or post positive test. BA.1 or BA.2
188 variant infection were confirmed using whole genome sequencing as previously
189 described ¹⁴ or using MT-PCR ¹⁷.

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191 The authors declare funding sources had no role in the design, collection,
192 analysis, interpretation of data, the writing of the report, or in the decision to
193 submit the paper for publication.

194 **Conflict of interest**

195 The authors declare no conflict of interest.

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209 **Author contributions**

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211 J.Z., K.S., G.A., K.B., and T.P. Data analysed by B.W., A.K., N.T., J.N., L.S., J.E.,
212 M.M., G.T., N.L., P.M., J.Z., K.S. and T.P.. Data interpretation by B.W., D.B., K.D.
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217 References

218

- 219 1. Viana R, Moyo S, Amoako DG, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron
220 variant in southern Africa. *Nature* 2022.
- 221 2. Tegally H, Moir M, Everatt J, et al. Continued Emergence and Evolution of Omicron in South
222 Africa: New BA.4 and BA.5 lineages. *medRxiv* 2022: 2022.05.01.22274406.
- 223 3. Cele S, Jackson L, Khoury DS, et al. Omicron extensively but incompletely escapes Pfizer
224 BNT162b2 neutralization. *Nature* 2022; **602**(7898): 654-6.
- 225 4. Newman J, Thakur N, Peacock TP, et al. Neutralising antibody activity against SARS-CoV-2
226 variants, including Omicron, in an elderly cohort vaccinated with BNT162b2. *medRxiv* 2021:
227 2021.12.23.21268293.
- 228 5. Yu J, Collier AY, Rowe M, et al. Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2
229 Variants. *N Engl J Med* 2022; **386**(16): 1579-80.
- 230 6. Mykytyn AZ, Rissmann M, Kok A, et al. Omicron BA.1 and BA.2 are antigenically distinct
231 SARS-CoV-2 variants. *bioRxiv* 2022: 2022.02.23.481644.
- 232 7. Zhou J, Sukhova K, McKay PF, et al. Omicron breakthrough infections in vaccinated or
233 previously infected hamsters. *bioRxiv* 2022: 2022.05.20.492779.
- 234 8. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the
235 SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma
236 antibodies. *Cell Host & Microbe* 2021; **29**(3): 463-76.e6.
- 237 9. Khan K, Karim F, Ganga Y, et al. Omicron sub-lineages BA.4/BA.5 escape BA.1 infection
238 elicited neutralizing immunity. *medRxiv* 2022: 2022.04.29.22274477.
- 239 10. Cao Y, Yisimayi A, Jian F, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by
240 Omicron infection. *bioRxiv* 2022: 2022.04.30.489997.
- 241 11. Hachmann NP, Miller J, Collier A-rY, et al. Neutralization Escape by the SARS-CoV-2 Omicron
242 Variants BA.2.12.1 and BA.4/BA.5. *medRxiv* 2022: 2022.05.16.22275151.
- 243 12. Tuekprakhon A, Huo J, Nutalai R, et al. Further antibody escape by Omicron BA.4 and BA.5
244 from vaccine and BA.1 serum. *bioRxiv* 2022: 2022.05.21.492554.
- 245 13. Yamasoba D, Kosugi Y, Kimura I, et al. Sensitivity of novel SARS-CoV-2 Omicron subvariants,
246 BA.2.11, BA.2.12.1, BA.4 and BA.5 to therapeutic monoclonal antibodies. *bioRxiv* 2022:
247 2022.05.03.490409.
- 248 14. Dupont L, Snell LB, Graham C, et al. Neutralizing antibody activity in convalescent sera from
249 infection in humans with SARS-CoV-2 and variants of concern. *Nat Microbiol* 2021; **6**(11): 1433-42.
- 250 15. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing
251 antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol*
252 2020; **5**(12): 1598-607.
- 253 16. Willett BJ, Grove J, MacLean OA, et al. SARS-CoV-2 Omicron is an immune escape variant
254 with an altered cell entry pathway. *Nature Microbiology* 2022; **In press**: 2022.01.03.21268111.
- 255 17. Hale R, Crowley P, Dervisevic S, et al. Development of a Multiplex Tandem PCR (MT-PCR)
256 Assay for the Detection of Emerging SARS-CoV-2 Variants. *Viruses* 2021; **13**(10).

257