Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

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List of Investigators

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Methods

Ethics statement

The ethics committee (EC) of the Medical University of Innsbruck has approved the study with EC numbers: 1330/2020, 1093/2021, 1168/202, 1191/2021, and 1197/2021.

Study participants

Serum from individuals that received two doses of a COVID-19 vaccination were collected either 4-6 months after the second vaccination for mRNA-1273/mRNA-1273 vaccinated individuals or 1 month after the second dose for ChAdOx1-S/ChAdOx1-S, ChAdOx1-S/BNT162b2 and BNT162b2/BNT162b2 vaccinated individuals. The interval between the two vaccine doses was 4-6 weeks for mRNA-1273/mRNA-1273, 11-13 weeks for ChAdOx1-S/ChAdOx1-S, 11-13 weeks for ChAdOx1-S/BNT162b2, and 3-6 weeks for BNT162b2/BNT162b2 vaccinated individuals.

Focus forming neutralization assay

Focus forming neutralization assays were performed as previously described with the following modifications.[1] Briefly, sera were heat inactivated for 30 minutes at 56°C and subsequently centrifuged for 5 minutes at 8,000 rpm in a tabletop centrifuge. Sera were four-fold diluted in duplicate samples in complete medium with 2% fetal calf serum (FCS) starting with a 1:16 dilution. Virus (Alpha (B.1.1.7): isolate C69.1, GISAID ID EPI_ISL_3277382; Beta (B.1.351): isolate C24.1, GISAID ID EPI_ISL_1123262; Delta (B.1.617.2): isolate SARS-CoV-2-hCoV-19/USA/NY-MSHSPSP-PV29995/2021, GISAID ID EPI_ISL_2290769; Omicron (B.1.1.529): isolate E16.1, GISAID ID EPI_ISL_6902053) was added resulting in around 100-200 infected cells in the control wells without serum and mixes were incubated for 1 hour at 37°C. Subsequently, serum/virus mixtures were transferred to confluent Vero cells stably overexpressing TMPRSS2 and ACE2. Two hours after infection, inoculum was removed and fresh medium was added. Ten hours after infection, cells were fixed for 5 minutes with 96% ethanol and subsequently stained using the serum of a convalescent patient and an Alexa Fluor Plus 488-conjugated goat anti-human IgG secondary antibody (Invitrogen, Thermo Fisher Scientific, Vienna, Austria). Plates were analyzed using an ImmunoSpot S6 Ultra-V reader and FluoroSpot software (CTL Europe GmbH, Bonn, Germany). Continuous 50% neutralization titers were calculated in GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA, USA) using a non-linear regression. Titers >1:16 were regarded as positive as seen in Riepler et al. and unpublished data.[1] Titers >1:1024 were set to 1:1024 and titers below 1 to 1.

Statistical analysis

Statistical differences were determined via Kruskal-Wallis test with Dunn's multiple comparisons test using GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA, USA).

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Table S1. Patient characteristics convalescent sera

SAMPLE ID	INFECTION	VACCINATION	AGE [YEARS]	SEX[#]
F620	Alpha	-	63	f
F628	Alpha	-	70	f
F633	Alpha	-	62	f
F635	Alpha	-	32	f
F647	Alpha	-	50	f
F650	Alpha	-	30	m
F658	Alpha	-	56	m
F661	Alpha	-	26	f
F662	Alpha	-	49	f
F667	Alpha	-	53	f
C701	Beta	-	86	m
C709	Beta	-	74	f
C711	Beta	-	68	f
C715	Beta	-	88	f
C770	Beta	-	82	m
C850	Beta	-	91	f
C859	Beta	-	86	m
C860	Beta	-	63	m
G21	Delta	-	30	f
G22	Delta	-	31	m
G23	Delta	-	43	m
G24	Delta	-	31	f
G25	Delta	-	31	m
G26	Delta	-	15	f
G27	Delta	-	10	m

[#] f = female; m = male

Table S2. Patient characteristics vaccinated sera

SAMPLE ID	VACCINATION	MONTHS SINCE 2ND DOSE	AGE [YEARS]	SEX[#]
E916	ChAdOx1-S/ChAdOx1-S	1	25	f
E918	ChAdOx1-S/ChAdOx1-S	1	29	f
E993	ChAdOx1-S/ChAdOx1-S	1	29	m
E995	ChAdOx1-S/ChAdOx1-S	1	37	f
E998	ChAdOx1-S/ChAdOx1-S	1	52	f
E999	ChAdOx1-S/ChAdOx1-S	1	28	f
E1000	ChAdOx1-S/ChAdOx1-S	1	52	f
F5	ChAdOx1-S/ChAdOx1-S	1	38	f
F6	ChAdOx1-S/ChAdOx1-S	1	44	m
F9	ChAdOx1-S/ChAdOx1-S	1	39	f
E919	ChAdOx1-S/BNT162b2	1	29	f
E920	ChAdOx1-S/BNT162b2	1	28	m
E921	ChAdOx1-S/BNT162b2	1	38	f
F41	ChAdOx1-S/BNT162b2	1	34	f
E994	ChAdOx1-S/BNT162b2	1	28	f
E996	ChAdOx1-S/BNT162b2	1	24	m
E997	ChAdOx1-S/BNT162b2	1	28	f
F1	ChAdOx1-S/BNT162b2	1	39	f
F2	ChAdOx1-S/BNT162b2	1	54	f
F3	ChAdOx1-S/BNT162b2	1	26	m
F4	ChAdOx1-S/BNT162b2	1	28	m
F8	ChAdOx1-S/BNT162b2	1	24	f
F12	ChAdOx1-S/BNT162b2	1	23	f
F14	ChAdOx1-S/BNT162b2	1	28	f
F16	ChAdOx1-S/BNT162b2	1	52	f
F17	ChAdOx1-S/BNT162b2	1	53	f
F19	ChAdOx1-S/BNT162b2	1	29	f
F20	ChAdOx1-S/BNT162b2	1	31	m
F43	ChAdOx1-S/BNT162b2	1	25	f
F22	ChAdOx1-S/BNT162b2	1	32	f
F110	BNT162b2/BNT162b2	1	62	f
F120	BNT162b2/BNT162b2	1	42	f
F121	BNT162b2/BNT162b2	1	47	m
F122	BNT162b2/BNT162b2	1	25	f
F124	BNT162b2/BNT162b2	1	29	m
F262	BNT162b2/BNT162b2	1	38	m
F263	BNT162b2/BNT162b2	1	28	f
F269	BNT162b2/BNT162b2	1	29	f
F271	BNT162b2/BNT162b2	1	36	f
F273	BNT162b2/BNT162b2	1	25	f
F289	BNT162b2/BNT162b2	1	36	m
F292	BNT162b2/BNT162b2	1	41	f

F358	BNT162b2/BNT162b2	1	57	f
F359	BNT162b2/BNT162b2	1	26	m
F491	BNT162b2/BNT162b2	1	25	f
F361	BNT162b2/BNT162b2	1	37	m
F353	BNT162b2/BNT162b2	1	30	f
F479	BNT162b2/BNT162b2	1	26	f
F363	BNT162b2/BNT162b2	1	28	m
F552	BNT162b2/BNT162b2	1	30	f
G30	mRNA-1273/mRNA-1273	5	59	m
G33	mRNA-1273/mRNA-1273	5	27	m
G34	mRNA-1273/mRNA-1273	6	28	m
G36	mRNA-1273/mRNA-1273	6	33	f
G37	mRNA-1273/mRNA-1273	4	28	m
G38	mRNA-1273/mRNA-1273	5	37	f
G39	mRNA-1273/mRNA-1273	5	29	f
G40	mRNA-1273/mRNA-1273	5	45	f
G41	mRNA-1273/mRNA-1273	6	32	f
G42	mRNA-1273/mRNA-1273	5	36	f

[#] f = female; m = male

Table S3. Patient characteristics super immune sera

SAMPLE ID	IMMUNE STATUS*	VACCINATION	AGE [YEARS]	SEX#
F653	Con./vacc.	BNT162b2	48	f
F623	Con./vacc.	BNT162b2/BNT162b2	66	m
F652	Con./vacc.	BNT162b2	28	f
F660	Con./vacc.	BNT162b2	59	f
F654	Con./vacc.	BNT162b2	57	m
F678	Vacc./con.	ChAdOx1-S/ChAdOx1-S	56	m
F739	Vacc./con.	BNT162b2/BNT162b2	58	f
F727	Vacc./con.	mRNA-1273/mRNA-1273	49	f
F726	Vacc./con.	mRNA-1273/mRNA-1273	49	m
W1	Vacc./con.	ChAdOx1-S/ChAdOx1-S	46	f

*Vacc./con.: vaccination with two doses mRNA-1273, ChAdOx1-S or BNT162b2 and subsequent infection; Con./vacc.: infection and subsequent vaccination either with one or two doses; # f = female; m = male

Table S4. Statistics

	ALPHA[#]	BETA[#]	DELTA[#]
mRNA-1273/mRNA-1273	***	**	***
ChAdOx1-S/ChAdOx1-S	***	**	***
ChAdOx1-S/BNT162b2	****	**	****
BNT162b2/BNT162b2	****	**	****
Alpha-Convalescent	****	**	ns
Beta-Convalescent	ns	**	ns
Delta-Convalescent	ns	ns	**
Super-immune (Con./vacc.)	n.a.	n.a.	*
Super-immune (Vacc./con.)	n.a.	n.a.	*

Statistical difference to Omicron using Kruskal-Wallis test with Dunn's multiple comparisons test; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns = non-significant; n.a. = not analyzed

References

1. Riepler, L., et al., *Comparison of Four SARS-CoV-2 Neutralization Assays.* Vaccines (Basel), 2020. **9**(1).