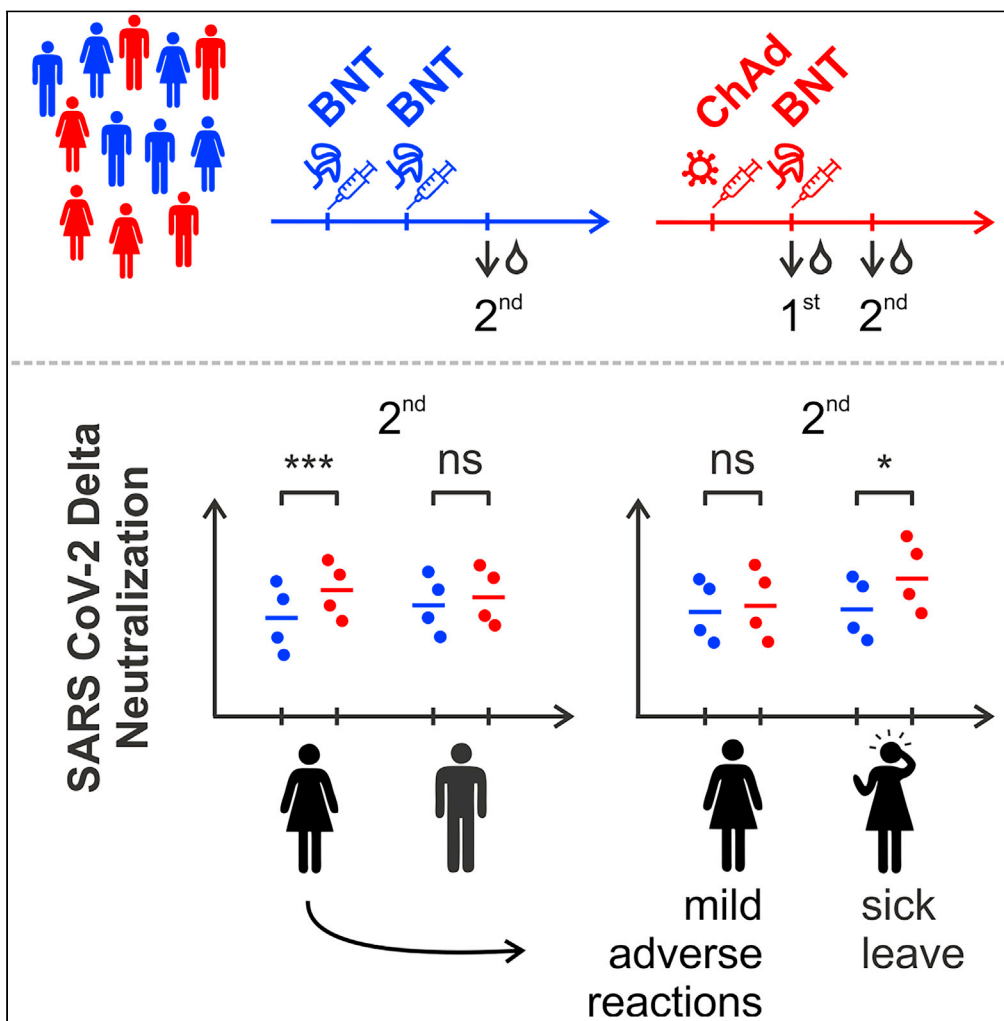# iScience

Article

Increased neutralization of SARS-CoV-2 Delta variant after heterologous ChAdOx1 nCoV-19/ BNT162b2 versus homologous BNT162b2 vaccination

Markus Bauswein, David Peterhoff, Annelie Plentz, ..., Bernd Salzberger, Barbara Schmidt, Stilla Bauernfeind

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Highlights

Heterologous ChAd/BNT vaccination is highly immunogenic

Delta VoC neutralization is increased after heterologous ChAd/BNT vaccination

This effect is attributable to women with sick leave after second vaccination

IgA levels are overall low, but higher after BNT/BNT vaccination

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Article

Increased neutralization of SARS-CoV-2 Delta variant after heterologous ChAdOx1 nCoV-19/ BNT162b2 versus homologous BNT162b2 vaccination

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SUMMARY

Heterologous SARS-CoV-2 vaccine approaches with a second mRNA-based vaccine have been favored in the recommendations of many countries over homologous vector-based ChAdOx1 nCoV-19 vaccination after reports of thromboembolic events and lower efficacy of this regimen. In the middle of 2021, the SARS-CoV-2 Delta variant of concern (VoC) has become predominant in many countries worldwide. Data addressing the neutralization capacity of a heterologous ChAdOx1 nCoV-19/mRNA-based vaccination approach against the Delta VoC in comparison to the widely used homologous mRNA-based vaccine regimen are limited. Here, we compare serological immune responses of a cohort of ChAdOx1 nCoV-19/ BNT162b2-vaccinated participants with those of BNT162b2/BNT162b2 vaccinated ones and show that neutralization capacity against the Delta VoC is significantly increased in sera of ChAdOx1 nCoV-19/BNT162b2-vaccinated participants. This overall effect can be attributed to ChAdOx1 nCoV-19/BNT162b2-vaccinated women, especially those with more severe adverse effects leading to sick leave following second immunization.

INTRODUCTION

Heterologous prime-boost vaccination approaches were first described in the context of AIDS vaccine development (Lu, 2009). Since then, heterologous regimens have been shown to protect successfully against different viral diseases and to be more immunogenic than homologous schemes in many cases (Kardani et al., 2016). With the emergence of vaccine-induced thrombosis and immune thrombotic thrombocytopenia syndrome (VITT) following vaccination with the adenoviral vector vaccine ChAdOx1 nCoV-19 (ChAd), a temporary suspension of vaccinations with ChAd was suggested in many European countries in March 2021. After re-evaluation by the European Medicines Agency (EMA), German authorities recommended to use ChAd only in vaccinees 60 years and older. Therefore, a second vaccination with an mRNA vaccine was recommended to younger ChAd-primed vaccinees. At the time of this recommendation, only some animal data suggested that heterologous COVID-19 vaccines worked well (He et al., 2021; Spencer et al., 2021). Meanwhile, several studies showed the increased immunogenicity of a heterologous immunization with a second mRNA-based vaccine compared to a homologous ChAd/ChAd vaccine regimen, including data on neutralization of Alpha, Beta and Gamma variants of concern (VoC) (Barros-Martins et al., 2021; Hillus et al., 2021; Normark et al., 2021; Tenbusch et al., 2021). In the middle of 2021, the SARS-CoV-2 Delta VoC (B.1.617.2) became predominant in many countries worldwide. Data addressing the neutralization capacity of the Delta VoC after heterologous ChAd/BNT162b2 vaccination in comparison to the widely used BNT162b2/BNT162b2 (BNT/BNT) regimen are limited.

In the present study, we compare safety and immunogenicity of the heterologous ChAd/BNT and the homologous BNT/BNT vaccination scheme and provide data on live virus Delta VoC neutralization capacity of both regimens.

RESULTS

Study cohorts and reactogenicity of heterologous and homologous vaccination

To compare the heterologous ChAd/BNT with the homologous BNT/BNT vaccine approach, two different study cohorts were used (baseline characteristics of study participants are provided in Table 1). 109

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Table 1. Descriptive characteristics of vaccinees

	All vaccines			Vaccinees included in neutralization assay		
	Chad/BNT n = 103	BNT/BNT n = 88	p value	Chad/BNT n = 59	BNT/BNT n = 60	pvalue
Male sex - no. (%)	30 (29.1)	44 (50.0)	**0.0050**	28 (47.5)	30 (50.0)	0.9252
Median age (range, IQR)	43 (20–64, 22)	42 (23–64, 24.5)	0.9827	42 (21–64, 21)	43.5 (23–63, 23)	0.1902
Median BMI (range, IQR)	23.3 (18.0–40.4, 5.0)	24.5 (17.6–35.9, 5.6)	0.4624	23.5 (18.6–40.2, 4.5)	24.5 (17.6–35.9, 4.6)	0.9476
Smoker - no. (%)	8 (7.8)	7 (8.0)	1.0000	2 (3.4)	5 (8.3)	0.4529
Immunosuppression	2 (2.0) [a]	3 (3.4)	0.8609	1 (1.7)	0	0.9916
Any comorbidity	29 (28.4) [a]	22 (25.0)	0.7143	15 (25.9) [d]	16 (26.7)	1.0000
Adverse reactions after 1st vaccination						
- No/mild	15 (14.9) [b]	59 (67.1)	**<0.0001**	11 (18.6)	44 (73.3) [b]	**<0.0001**
- Moderate	19 (18.8) [b]	9 (10.2)	0.1609	11 (18.6)	2 (3.3)	**0.0145**
- Severe	67 (66.3) [b]	20 (22.7)	**<0.0001**	37 (62.7)	14 (23.3)	**<0.0001**
Adverse reactions after 2ND vaccination						
- No/mild	40 (39.6) [b]	45 (51.1)	0.1189	27 (45.8)	30 (50.0)	0.7803
- Moderate	29 (28.7) [b]	0	**<0.0001**	11 (18.6)	0	**0.0005**
- Severe	32 (31.7) [b]	43 (48.9)	0.0238	21 (35.6)	30 (50.0)	0.1604
Prophylactic medication before 1st vaccination	11 (10.9) [b]	3 (3.4)	0.0876	5 (8.8) [e]	3 (5.0)	0.6600
Prophylactic medication before 2nd vaccination	17 (17.0) [c]	5 (5.7)	0.0262	12 (20.7) [d]	4 (6.7)	0.0483
Median time interval between 1st and 2nd vaccination - days (range, IQR)	66 (65–86, 8)	33 (21–38, 12.5)	**<0.0001**	66 (65–86, 8)	33 (21–38, 12)	**<0.0001**
Median time interval between 2nd vaccination and blood sample collection - days (range, IQR)	28 (28–42, 0)	49 (35–58, 7)	**<0.0001**	28 (28–42, 0)	49 (35–58, 6)	**<0.0001**

BMI = body mass index; IQR = interquartile range.
[a] n = 102.
[b] n = 101.
[c] n = 100.
[d] n = 58.
[e] n = 57.

vaccinees were enrolled in a prospective observational study assessing the heterologous ChAd/BNT vaccination schedule. No randomization of vaccinees took place due to the study protocol as all participants having received ChAd as prime immunization were offered BNT as second vaccination as recommended by the Germans authorities. Five vaccinees were lost to follow-up and excluded from the analysis. One was excluded because of a history of SARS-CoV-2 infection. 103 vaccinees were finally included in the analysis. Median time interval between prime and second vaccination was 66 days (range 65–86). Blood samples were collected immediately before second immunization and in median 28 days afterward (range 28–42). Thirty participants were male (29.1%). Median age was 43 years. Smoking (8; 7.8%) and immunosuppression (2; 2.0%) were rare. Twenty-nine vaccinees (28.4%) reported to have any comorbidity. Severe adverse reactions defined as any symptom(s) resulting in sick leave were more frequent after prime vaccination with ChAd (67 versus 32 vaccinees). More vaccinees took prophylactic antipyretic medication before second vaccination with BNT than before prime vaccination (17 versus 11). Ninety vaccinees were enrolled in a study assessing the homologous BNT/BNT vaccination schedule (Bauernfeind et al., 2021). Two vaccinees were excluded from the analysis because of a history of SARS-CoV-2 infection. Participants were recruited from a cohort of 735 vaccinees according to adverse reactions after prime and second vaccination in order to compare those with no adverse reactions to those with most severe adverse reactions resulting in sick leave. Median time interval between prime and second vaccination was 33 days (range 21–38), between second immunization and blood sample collection 49 days (range 35–58). Males and females were equally represented. Median age was 42 years. The BNT/BNT did not differ from the ChAd/BNT cohort with regard
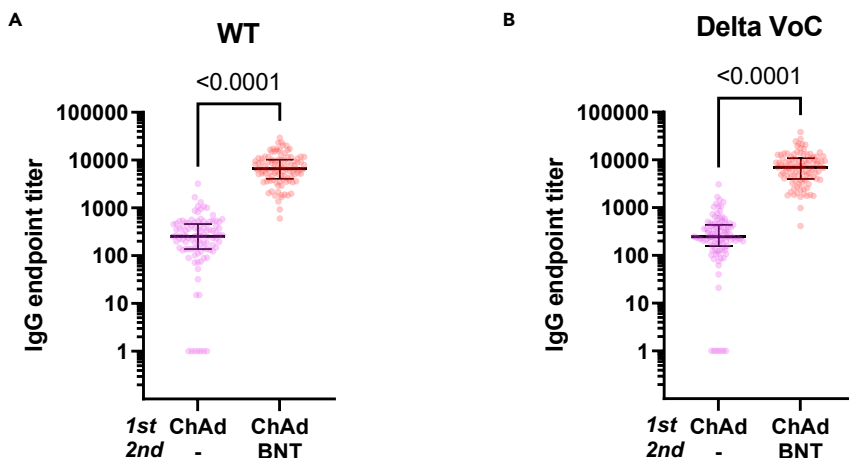
Figure 1. Increased IgG endpoint titers by second immunization with BNT after ChAd as prime vaccination
Blood samples were collected immediately before second immunization and in median 28 days afterward. For 102 participants, blood samples after prime and second immunization were available. Individual endpoint IgG titers and median with interquartile range (IQR) as measured by WT RBD (A) and Delta VoC RBD (B) ELISA. For statistical analysis, Wilcoxon tests for paired values were performed and p values are given.

to age, body mass index (BMI), smoking, immunosuppression, and prevalence of any comorbidity. Sick leave after prime vaccination was significantly more frequent among ChAd/BNT vaccinees ($p < 0.001$), whereas more BNT/BNT vaccinees had to stay at home after second vaccination ($p = 0.0238$). Prophylactic antipyretic medication was rarely taken by BNT/BNT vaccinees.

Second heterologous immunization increases IgG titers against SARS-CoV-2 wild type and Delta variant

After ChAd prime, second immunization by BNT led to a 25.9-fold and a 29.0-fold increase in median IgG endpoint titers as measured by a wild type (WT; Wuhan Hu-1) S protein receptor-binding domain (RBD) ELISA (Figure 1A) and a Delta VoC RBD ELISA (Figure 1B), respectively (n = 102; prime serum not available for 1 participant).

Increased neutralization of Delta variant after heterologous vaccination

Evaluating serum samples after second immunization, ChAd/BNT-vaccinated participants (n = 103) showed a significant 1.4-fold higher median IgG endpoint titer as measured in the Delta VoC RBD ELISA compared to BNT/BNT vaccinees (n = 88) (Figure 2A). With regard to WT RBD ELISA, the median difference in endpoint titers was only 1.2-fold (Figure 2B).

To further investigate whether the difference in IgG levels directed against the Delta VoC RBD also affected the neutralization capacity of sera between vaccine regimens, sera of 59 ChAd/BNT-vaccinated partici-pants were tested for neutralization activity against the SARS-CoV-2 Delta VoC and compared to sera of 60 BNT/BNT-immunized vaccinees. Sera for neutralization tests were selected in a manner to balance age, sex, and mild vs. moderate to more severe (sick leave) vaccine-related reactions after second immu-nization (characteristics see Table 1). Male participants with more severe reactions resulting in sick leave, however, were overrepresented in the homologous vaccine group. Neutralization tests against the Delta VoC were performed as live virus neutralization assay using a SARS-CoV-2 Delta VoC strain (lineage AY.33/B.1.617.2.33) isolated from a patient's respiratory specimen. Overall, tested ChAd/BNT vaccinees (n = 59) showed a significantly higher capacity to neutralize the Delta VoC compared to tested BNT/ BNT-vaccinated participants (n = 60) (Figure 2C). This effect in neutralization capacity is not biased by se-lection of sera for neutralization assay as the effect of endpoint titers between vaccine regimens as measured in the Delta VoC RBD ELISA for the selected subgroup corresponded to the 1.4-fold increase observed in the entire group (Figure 2D). Subgroup analyses revealed that the higher neutralization capac-ity of ChAd/BNT vaccination against the Delta VoC in comparison to BNT/BNT immunization was only detectable in female participants (Figure 3A). This was not due to higher neutralization capacity of female vaccinees within the heterologous vaccine group as there was no significant difference between female and
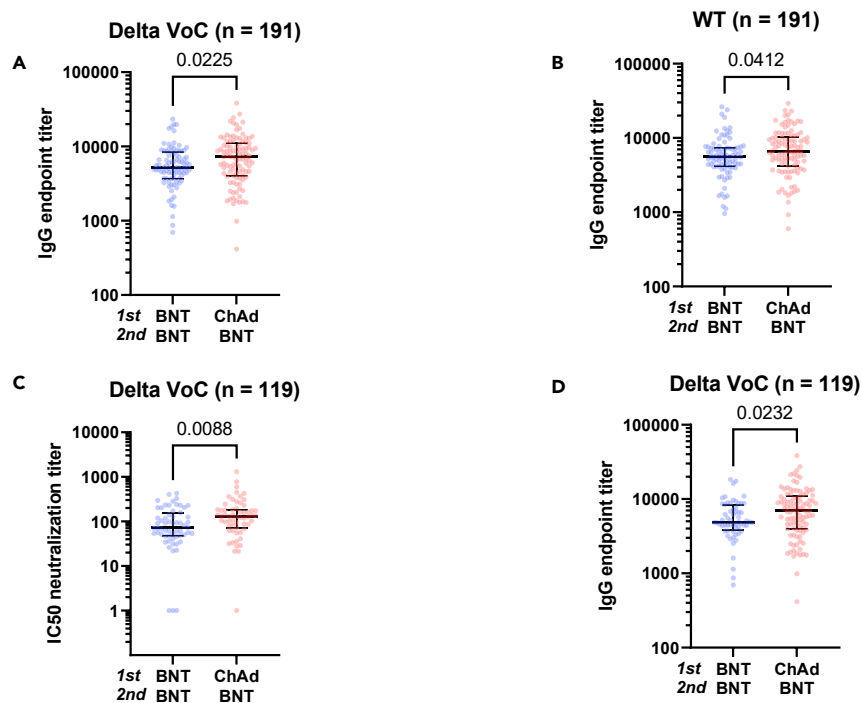
Figure 2. Improved neutralization of SARS-CoV-2 Delta VoC after heterologous ChAd/BNT versus homologous BNT/BNT vaccination

(A and B) Delta VoC RBD ELISA and WT RBD ELISA IgG endpoint titers for ChAd/BNT-vaccinated participants (n = 103) in comparison to BNT/BNT vaccinees (n = 88) (A + B).

(C) Neutralization of live Delta VoC for sera of BNT/BNT-vaccinated participants (n = 60) in comparison to sera of ChAd/BNT vaccinees (n = 59). Neutralization activity is expressed as inverse dilution (titer) at half-maximal inhibition of virus replication (IC_{50} for log10-transformed RNA levels). Samples without neutralizing activity at a 1:20 dilution were assigned to a titer of 1.

(D) Delta VoC RBD ELISA IgG endpoint titers for the subgroup of participants whose sera were included in neutralization assay. Shown are individual values and median (with IQR). For statistical analysis, Mann-Whitney tests were performed and *p* values are given.

male participants, but because of significantly lower neutralization capacity of women with BNT/BNT vaccination. With regard to neutralization capacity of Delta VoC, only women benefited from a ChAd prime, adjusting their Delta VoC neutralization capacity to those of BNT/BNT- and ChAd/BNT-vaccinated men. Further, a significantly higher neutralization capacity against the Delta VoC between corresponding subgroups of BNT/BNT and ChAd/BNT vaccinees was only found in the subgroup of female participants with severe adverse reactions after second vaccination leading to sick leave, but not in the subgroup of women without this characteristic even if the difference in medians pointed to a similar effect (Figure 3B).

In each vaccine group, the titers of IgG antibodies directed against Delta VoC RBD correlated with Delta VoC neutralization capacity (Figures 4A+4B). Of note, all 4 participants who failed to neutralize the Delta VoC at a serum dilution of 1:20 (IC_{50} neutralization titer assigned to 1) had comparatively lower IgG-antibody endpoint titers against Delta VoC RBD in the range of approximately 1:1,000. Delta VoC RBD ELISA endpoint titers (Figures 4A+4B) showed a better correlation with Delta VoC neutralization than WT RBD ELISA endpoint titers (Figures 4C+4D).

IgA levels are increased after homologous vaccination

In contrast to elevated IgG levels against WT as well as Delta variant RBD and improved Delta variant neutralization, heterologous ChAd/BNT vaccination led to significantly decreased SARS-CoV-2-specific IgA levels as measured by a WT RBD ELISA compared to homologous BNT/BNT immunization (Figure 5). However, as the median IgA level even for BNT/BNT vaccination was below the cutoff of the used test
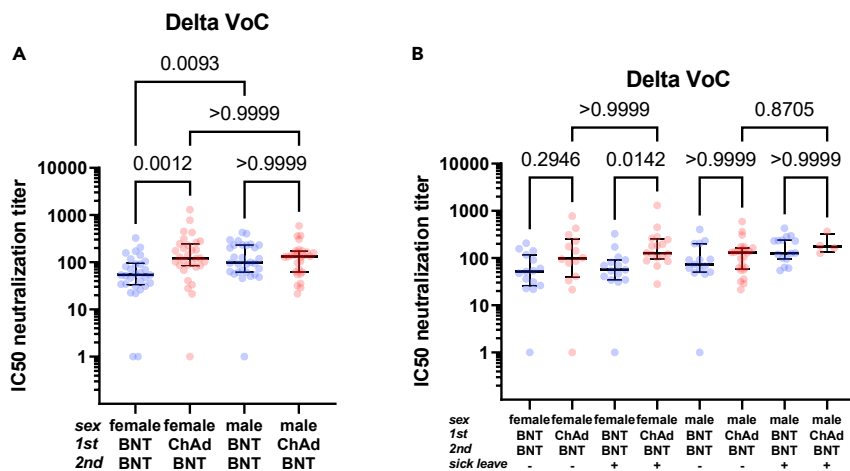
Figure 3. Sera of female ChAd/BNT vaccinees neutralize the Delta VoC more efficiently than BNT/BNT-vaccinated participants

Sera of BNT/BNT-vaccinated women (n = 30) were compared to sera of female ChAd/BNT vaccinees (n = 31) (A). Neutralization activity against the Delta VoC in relation to sex and more severe reactions (defined as sick leave) after second immunization (B). Individual values and median (with IQR). Statistical analysis: Kruskal-Wallis test and Dunn's multiple comparisons tests as post-tests. p values are given.

system and approximately 10 times lower than the median IgA level of patients with COVID-19 more than 10 days after a positive qRT-PCR test as measured in the identical ELISA system (Peterhoff et al., 2021), it remains to be elusive if this difference between vaccine regimens is able to contribute to altered mucosal or even sterile immunity.

DISCUSSION

Our finding of improved neutralization of the Delta VoC for heterologous ChAd/BNT vaccine regimen compared to homologous BNT/BNT immunization is in line with recently published results of neutralization capacity against other variants of concern. In a pseudovirus neutralization test, Hillus et al. showed increased neutralization capacity against the Alpha and Beta VoC for heterologous vaccine regimen, while data on Delta variant neutralization are not included in their study (Hillus et al., 2021). They can attribute this effect of improved neutralization to an increase in anti-S1-IgG avidity after heterologous vaccination. As in our study, they already see a slight increase in anti-RBD-IgG (WT) in their data (in median 1.04-fold), but in contrast to our data this effect is smaller and not significant. Different characteristics as prime-second-vaccination interval and interval between second immunization and sample collection might contribute to this difference. Another study demonstrated an increased Delta VoC neutralization for heterologous ChAd/BNT vaccine regimen in comparison to homologous ChAd/ChAd immunization, while data on BNT/BNT vaccine regimen were not included (Behrens et al., 2021). In contrast to Alpha, Beta and Gamma VoC neutralization and in contrast to our data on Delta VoC neutralization, Hammerschmidt et al. recently showed increased neutralization titers against the Delta VoC after BNT/BNT vaccination in comparison to ChAd/BNT immunization (Hammerschmidt et al., 2021). While we tested Delta VoC neutralization by live virus neutralization, they used a surrogate virus neutralization test (sVNT). Our neutralization assay data are supported by increased IgG against Delta VoCRBD as measured in our cohort. It remains speculative whether the different approach to measure neutralization capacity or possible differences in study cohort characteristics contribute to the different findings compared to Hammerschmidt et al..

Sex plays an important role in immune response to vaccines. Women often have more side effects and higher antibody responses to bacterial and viral vaccines (Klein and Flanagan, 2016). Correspondingly, the increased neutralization capacity of Delta VoC after ChAd/BNT vaccination in our data is mainly attributable to female vaccinees. This effect, however, is not due to higher neutralization capacity of women in comparison to men within the ChAd/BNT vaccination group, but because of comparatively lower neutralization capacity of BNT/BNT-vaccinated women. Whether there is an association between adverse reactions and immune response after vaccination, is incompletely understood. In a previous study, we showed
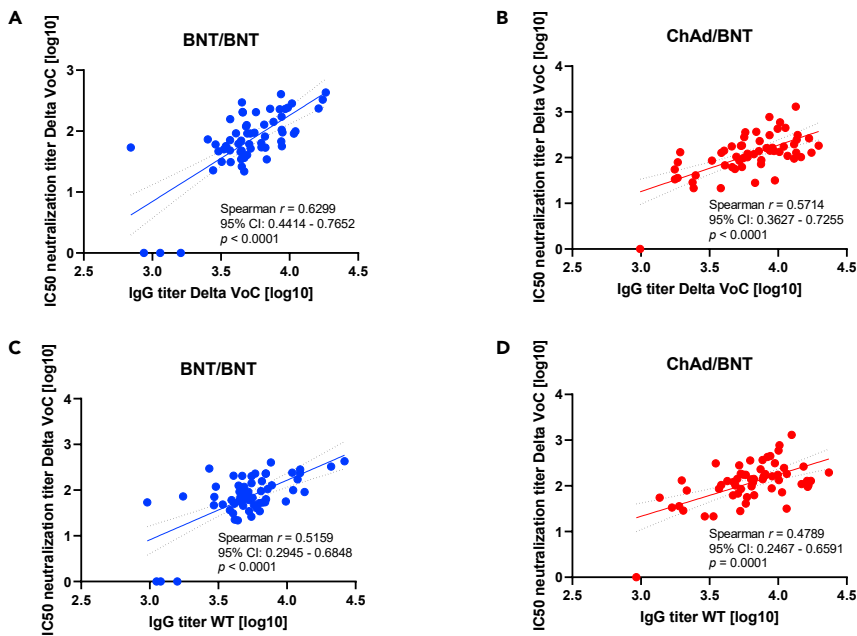
Figure 4. Correlation between SARS-CoV-2-RBD-specific IgG endpoint titers (ELISA) and neutralization activity against the Delta VoC

All values are log10-transformed. X axis and Y axis represent inverse IgG endpoint titers and inverse dilutions (titers) at half-maximal replication inhibition (IC_{50} for log10-transformed RNA copies), respectively. Samples without neutralizing activity at a 1:20 dilution were assigned to a value of 1 (0 after log10 transformation). A Spearman rank correlation test was performed. Lines represent linear regression with 95% confidence interval. **(A + B)** Delta VoC ELISA; **(C + D)** WT ELISA.

for the homologous BNT/BNT regimen that men profited from severe adverse reactions leading to sick leave in respect of both higher SARS-CoV-2 RBDIgG levels and improved neutralization capacity (Bauern-feind et al., 2021). The SARS-CoV-2 vaccine type does not only influence immune responses but also adverse reactions which are again partially sex-dependent. Whereas women are more prone to immune thrombotic thrombocytopenia syndrome after ChAd/ChAd, men are more affected by myocarditis after
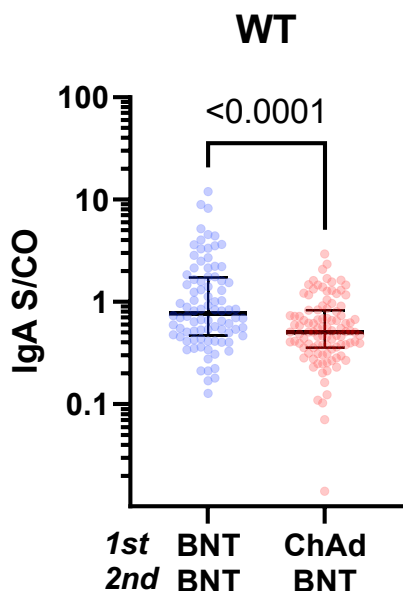

Figure 5. SARS-CoV-2-specific IgA (WT, RBD) is increased in BNT/BNT vaccinees compared to ChAd/BNT immunization

Samples were collected after second immunization; n = 88 (BNT/BNT), n = 103 (ChAd/BNT). Individual values and median (with IQR). Statistical analysis: Mann-Whitney test. *p* values are given.

mRNA vaccination (Abu Mouch et al., 2021; Greinacher et al., 2021). More studies are needed to describe the influence of sex and adverse reactions on vaccination-induced immune response.

Our data support earlier findings of high immunogenicity of a heterologous vaccine regimen and, for the subgroup of female vaccinees with more severe side effects, offer a significantly enhanced immunogenicity with regard to neutralization of the Delta VoC compared to homologous BNT immunization.

Limitations of the study

Our study on heterologous ChAd/BNT vaccination was designed as a prospective observational study without randomization of vaccinees in different regimens and without blinding. Further, time intervals between second immunization and blood sampling between ChAd/BNT vaccine group (median 28 days) and BNT/BNT vaccine group (median 49 days) differed. Owing to the study design of the BNT/BNT study, side effects in the homologous vaccine group might not be distributed as in a non-collected cohort for this characteristic. Because of the recruitment of vaccinees from the working population, the age range was limited (20–64 years) and results may not be attributable to other age groups. Meanwhile, the SARS-CoV-2 Delta VoC is underlying a rapid evolution and currently consists of more than hundred sublineages (O'Toole et al., 2021). For the neutralization assay, a live Delta VoC virus of lineage AY.33/B1.617.2.33 was used. Neutralization capacities of vaccinees' sera might change with respect to other sublineages.

STAR★METHODS

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AUTHOR CONTRIBUTIONS

Conceptualization, S.B. and A.P.; Methodology, D.P., B.Sc., M.B., S.B., and A.H.; Investigation, S.B., A.P., M.B., D.P., and B.Sc.; Writing – Original Draft, M.B., S.B., and D.P.; Writing – Review & Editing, B.Sc.; Resources, A.G., R.W., B.Sc., and B.Sa.; Supervision, A.G., R.W., B.Sa., and B.Sc.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Polyclonal rabbit anti-human-IgG, HRP-conjugated	Agilent	Cat# P021402-2; RRID: AB_2893418
Polyclonal rabbit anti-human-IgA, HRP-conjugated	Agilent	Cat# P021602-2; RRID: AB_2893419
Bacterial and virus strains		
SARS-CoV-2 Delta VoC strain (lineage AY.33/B.1.617.2.33)	Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	GenBank: OK_149285
Biological samples		
Human serum samples The studies were conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethics Committee of the University of Regensburg (*cross*: reference number: 21-2374-101; *immunoreakt*: reference number: 21-2332-101). Informed consent was obtained from all subjects involved in the studies.	Department of Infection Prevention and Infectious Diseases, University Hospital Regensburg, Regensburg, Germany; Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	N/A
Chemicals, peptides, and recombinant proteins		
RBD WT	Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	N/A
RBD Delta	Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	N/A
ExpiFectamine™ 293 Transfection Kit	Thermo Fisher Scientific	Cat# A14524
Expi293™ Expression Medium	Thermo Fisher Scientific	Cat# A1435101
Opti-MEM™ I Reduced Serum Medium	Thermo Fisher Scientific	Cat# 31985062
HisTrapExcel 5 ml	Cytiva	Cat# 17371206
Imidazole	Sigma	Cat# 56749
PD-10 Desalting Columns (Sephadex G-25)	Cytiva	Cat# 17085101
Amicon Ultra-15, 10 kDa	Merck Millipore	Cat# UFC901024
Dulbecco's Phosphate Buffered Saline	gibco	Cat# 14190-094
Tween 20	Caelo	Cat# 3472
Fat free milk powder	Heirler	www.heirler.de
Mikrogen TMB Substrate Solution	Mikrogen	Cat# 12003
Mikrogen Stopp (24.9% H_3PO_4)	Mikrogen	Cat# 12004
DMEM	gibco	Cat# 41966-029
FBS	PanBiotech	Cat# P30-3306
Penicillin+Streptamicin	PanBiotech	Cat# P06-07100
L-Glutamine	PanBiotech	Cat# P04-80100
0.1 M NaCl	VWR International	Cat# 32038-1EA
0.1 M Tris	Affymetrix	Cat# 75825
IGEPAL CA-630	VWR International	Cat# J61055.AP
RNAse Inhibitor	Applied Biosystems	Cat# N8080119

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Ion Torrent™ NGS Reverse Transcription Kit	Thermo Fisher Scientific	Cat# A45003
Ion AmpliSeq SARS-CoV-2 Insight Research Assay	Thermo Fisher Scientific	Cat# A51305
Deposited data		
Dataset uploaded to Mendeley	This study	Mendeley Data: https://doi.org/10.17632/tzncbsfgkk.1
Experimental models: Cell lines		
Caco-2 cells	CLS Cell Lines Service	Cat# 300137
Oligonucleotides		
SARS-CoV-2 RTqPCR ●E_Sarbeco_F (ACAGGTACGTTAATAGT TAATAGCGT) ●E_Sarbeco_R (ATATTGCAGCAGTACG CACACA) ●E_Sarbeco_P1 (FAM-ACACTAGCCA TCCTTACTGCGCTTCG-BBQ)	TIB MOLBIOL (Corman et al., 2020)	N/A
Recombinant DNA		
pcDNA5/FRT/TO_SARS-CoV-2_RBD_WT	Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	N/A
pcDNA5/FRT/TO_SARS-CoV-2_RBD_Delta	Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Germany	N/A
Software and algorithms		
GraphPadPrism 9.2.0	GraphPad Software	www.graphpad.com
Stata 17	Stata Corporation	www.stata.com
CorelDRAW2018	Corel Corporation	www.coreldraw.com
Torrent Suite 5.12.2	Thermo Fisher Scientific	www.thermofisher.com
Other		
NuncMaxisorp Plates	Thermo Fisher Scientific	Cat# 446469
Cell culture plate, 96 well, flat bottom	Sarstedt	Cat# 83.3924

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Markus Bauswein (markus.bauswein@ukr.de).

Materials availability

Materials are available on personal request, but we may require a completed materials transfer agreement if there is potential for commercial application.

Data and code availability

- Datasets generated in this study have been uploaded to Mendeley Data: https://doi.org/10.17632/tzncbsfgkk.1.https://data.mendeley.com . Sequence of SARS-CoV-2 Delta VoC used for neutralization assay is available under GenBank: OK_149285. .

- This paper does not report original code.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

At our University Hospital Center vaccination clinic COVID-19 vaccinees - mostly health care workers and some university employees - were enrolled in two studies, *cross* (registration number DRKS00025271) and *immunoreakt* (registration number DRKS00025316) (Bauernfeind et al., 2021).

The *cross* study assessed immunogenicity and reactogenicity of the heterologous ChAd/BNT vaccination schedule. The enrolment took place from May 19th to June 2nd, 2021. 109 vaccinees primed with ChAd were included, median age was 43 years (range 20-64), 32 were male (29.4%). At enrolment, a blood sample was taken and the second vaccination with BNT was administered. The second blood sample was taken 28 days (range 28-42) afterward and a questionnaire was distributed.

The *immunoreakt* study assessed immunogenicity according to reactogenicity of the homologous BNT vaccination scheme. 735 vaccinees indicated adverse reactions after first and second vaccination as follows:

a) no or only minor symptoms on the injection side

b) moderate - not further classified

c) severe - classified as (any) symptom(s) resulting in sick leave or would have resulted in sick leave when the vaccination was followed by day(s) off

The study was designed to compare vaccinees with the most severe adverse reactions to those with no or only minor injection side symptoms. The enrolment took place from June 7th to 18th, 2021. 90 vaccinees were enrolled, median age was 42 years (range 23-64), 45 were male (50.0%). 45 participants had side effects resulting in sick leave after first and/or second vaccination. 45 participants had no or only minor symptoms on the injection side after first and second vaccination. One blood sample was taken 49 days (range 35 - 58) after second vaccination with BNT, participants were asked to fill in a questionnaire identical to that of the *cross* study.

Ethical issues

The studies were conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethics Committee of the University of Regensburg (*cross*: reference number: 21-2374-101; *immunoreakt*: reference number: 21-2332-101). Informed consent was obtained from all subjects involved in the studies.

METHOD DETAILS

Generation of SARS-CoV-2 RBD WT and RBD variants

The SARS-CoV-2 RBD WT (Wuhan Hu-1) encoding sequence was codon usage-optimized and synthesized by GeneArt AG (part of Thermo Fisher Scientific Inc.). The RBD was subsequently cloned into a modified pcDNA5/FRT/TO encoding an N-terminal tPA signal peptide and a C-terminal avi-hexahistidine tag (sequence: GGSGLNDIFEAQKIEWHEGSHHHHHH). The plasmid encoding the RBD of SARS-CoV-2 Delta V1 (B.1.617.2) was generated by introducing the corresponding mutations L452R and T478K via overlap extension PCR (Higuchi R et al., 1988) in the WT sequence and by re-cloning into the original pcDNA5/FRT/TO-derivate.

For expression and purification of the antigens, Expi293F™ cells (Thermo Fisher Scientific; A14527) were transfected with plasmids encoding the SARS-CoV-2 RBD variants according to the manufacturer's recommendations. After 5 days of protein expression, supernatants were harvested by centrifugation. Supernatants were loaded onto an immobilized metal chelate affinity chromatography column (HisTrap Excel, Cytiva), washed with Dulbecco's Phosphate Buffered Saline (PBS, Sigma) containing 10 mM imidazole (Sigma) and eluted over a linear gradient of 10–500 mM imidazole in PBS. The protein was buffer exchanged to PBS and concentrated to approximately 1–2 mg/ml by ultrafiltration.

SARS-CoV-2 binding antibodies

SARS-CoV-2 binding antibodies were detected by an in-house ELISA (Peterhoff et al., 2021). NuncMaxiSorp plates (Thermo Fisher Scientific) were coated with 50 μl RBD at 2 μg/ml in PBS (Sigma) at 4°C over night. After blocking for 1 h at room temperature (RT) with 200 μl 5% fat free milk powder (Heirler) in PBS

containing 0.1% Tween 20 (Caelo), plates were washed three times with 200 μl PBS containing 0.1% Tween 20 (PBS-T). Next, 50 μl of serum dilutions in 1% fat free milk powder in PBS-T were added and the plates were incubated for 1 h at RT. After ten 200 μl PBS-T washing steps, secondary antibody horseradish peroxidase conjugate was added in 50 μl PBS-T (polyclonal rabbit anti-human IgG/HRP conjugate (Agilent, Dako) at 1:5,000 dilution or polyclonal rabbit anti-human IgA/HRP conjugate (Agilent, Dako) at 1:4,000 dilution). After 1 h incubation at RT, the plates were washed ten times with 200 μl PBS-T. 50 μl TMB substrate solution (Mikrogen) was added to each well, incubated for 4 min at RT, and stopped with 25 μl of 1.0 N sulfuric acid. Optical density was determined by measuring at 450 nm (OD450) and 630 nm (OD630) in three technical replicates. For evaluation, OD630 values were subtracted from OD450 values as background.

ELISA antigens were the receptor-binding domain (RBD) of SARS-CoV-2 WT and SARS-CoV-2 Delta strain. For determination of the serum titers, sera were measured in eight two-fold serial dilutions starting at 1:200. Cut-off values were determined as described before by measuring pre-pandemic SARS-CoV-2 naïve sera (Peterhoff et al., 2021). Endpoint titers were calculated from a 4-parameter logistic function (4PL, GraphPad Prism version 9.2.0) of the titration data in combination with the previously determined cut-off values.

Delta variant neutralization test (NT)

SARS-CoV-2 Delta variant was isolated from a patient's respiratory specimen using Caco-2 cells (CLS Cell Lines Service GmbH, Eppelheim, Germany). Sequence is available under GenBank: OK_149285.

Caco-2 cells were plated in 96-well flat bottom plates at 15,000 cells/well and infected using a multiplicity of infection (MOI) of 0.05. Prior to infection, the virus inoculum was incubated with serial dilutions of serum samples (1:20; 1:120; 1:720; 1:4,320; 1:25,920) for one hour and then added to the cells. To remove the input virus, media were exchanged 12 hours post plating and dilutions of serum samples were replenished. 2.5 days post infection, viral loads were determined in cell culture supernatants using qRT-PCR. In brief, RNA was extracted from 50 μl of cell culture supernatants with an equimolar volume of DL buffer (0.1 M NaCl, 0.01 M Tris, 0.5% IGEPAL CA-630 in DEPCH20, pH 7.4) with added RNAse inhibitor for 30 min (Shatzkes et al., 2014) and then subjected to reverse transcription and amplification using the TaqPath[TM] 1-Step qRT-PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) with published probe and primers targeting the E-gene of SARS-CoV-2 (Corman et al., 2020) on a StepOnePlus Real-Time PCR System (Thermo Fisher). For absolute quantification, an *in vitro* transcribed RNA standard was used, as previously described for a different system (Wenzel et al., 2009). The analytical sensitivity of the assay was ≤ 300 RNA copies/mL. Half maximal inhibitory concentrations (IC_{50}) for log10-transformed SARS-CoV-2 RNA copies were calculated by non-linear 4PL regression (constraints: bottom = 3.5; top = 6.5; $IC_{50} > 0$) using GraphPad Prism version 9.2.0. Samples without neutralizing activity at a 1:20 dilution were assigned to a value of 1 (0 after log10 transformation).

Genomic sequencing

SARS-CoV-2 whole genome sequencing was performed from the original nasopharyngeal aspirate. Here, the Ion Torrent™ NGS Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, USA) was used for synthesis of cDNA starting from 800 genome equivalents. Quantification of viral copies was determined by qRT-PCR as described for cell-culture supernatants above. Targeted amplification of SARS-CoV-2 genomes was conducted using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay covering >99% of the viral genome (~30kb) along with 5 human expression controls. Automated preparation of sequencing libraries was conducted on an IonChef™ instrument followed by high-throughput sequencing on an Ion-Torrent™ GeneStudio S5 Plus sequencer (Thermo Fisher Scientific, Waltham, USA). Sequences were analyzed with the Torrent Suite 5.12.2 resulting into a total of 974,910 reads and a 6,653-fold mean coverage of the full genome. The GenerateConsensus plugin version 5.16.0.10 was used for assembling of sequencing reads and consensus sequence generation.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 9.2.0 and Stata 17. For paired values, Wilcoxon tests were performed. For unpaired values, Mann-Whitney tests or Kruskal-Wallis tests followed by Dunn's multiple comparisons test as post-test were performed. $P < 0.05$ was considered significant. Data are presented as median (with IQR).