Received: 23 December 2021 | Revised: 17 January 2022 | Accepted: 23 January 2022

DOI: 10.1111/all.15247

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Autoimmunity and Clinical Immunology

Interdependencies of cellular and humoral immune responses in heterologous and homologous SARS-CoV-2 vaccination

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Abstract

Background: Homologous and heterologous SARS-CoV-2 vaccinations yield different spike protein-directed humoral and cellular immune responses. This study aimed to explore their currently unknown interdependencies.

Methods: COV-ADAPT is a prospective, observational cohort study of 417 healthcare workers who received vaccination with homologous ChAdOx1 nCoV-19, homologous BNT162b2 or with heterologous ChAdOx1 nCoV-19/BNT162b2. We assessed humoral (anti-spike-RBD-IgG, neutralizing antibodies, and avidity) and cellular (spike-induced T-cell interferon-γ release) immune responses in blood samples up to 2 weeks before (T1) and 2–12 weeks following secondary immunization (T2).

Results: Initial vaccination with ChAdOx1 nCoV-19 resulted in lower anti-spike-RBD-IgG compared with BNT162b2 (70 ± 114 vs. 226 ± 279 BAU/ml, $p < .01$) at T1. Booster vaccination with BNT162b2 proved superior to ChAdOx1 nCoV-19 at T2 (anti-spike-RBD-IgG: ChAdOx1 nCoV-19/BNT162b2 2387 ± 1627 and homologous BNT162b2 3202 ± 2184 vs. homologous ChAdOx1 nCoV-19 413 ± 461 BAU/ml, both $p < .001$; spike-induced T-cell interferon-γ release: ChAdOx1 nCoV-19/BNT162b2 5069 ± 6733 and homologous BNT162b2 4880 ± 7570 vs. homologous ChAdOx1 nCoV-19 1152 ± 2243 mIU/ml, both $p < .001$). No significant differences were detected between BNT162b2-boostered groups at T2. For ChAdOx1 nCoV-19, no booster effect on T-cell activation could be observed. We found associations between anti-spike-RBD-IgG levels (ChAdOx1 nCoV-19/BNT162b2 and homologous BNT162b2) and T-cell responses (homologous ChAdOx1 nCoV-19 and ChAdOx1 nCoV-19/BNT162b2) from T1 to T2. Additionally, anti-spike-RBD-IgG and T-cell

Abbreviations: COVID-19, coronavirus disease 2019; IFN, interferon; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; T, time point.

Moritz Schnelle and Luise Erpenbeck should be considered joint senior author.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), ER723/3-1 (to L.E.).

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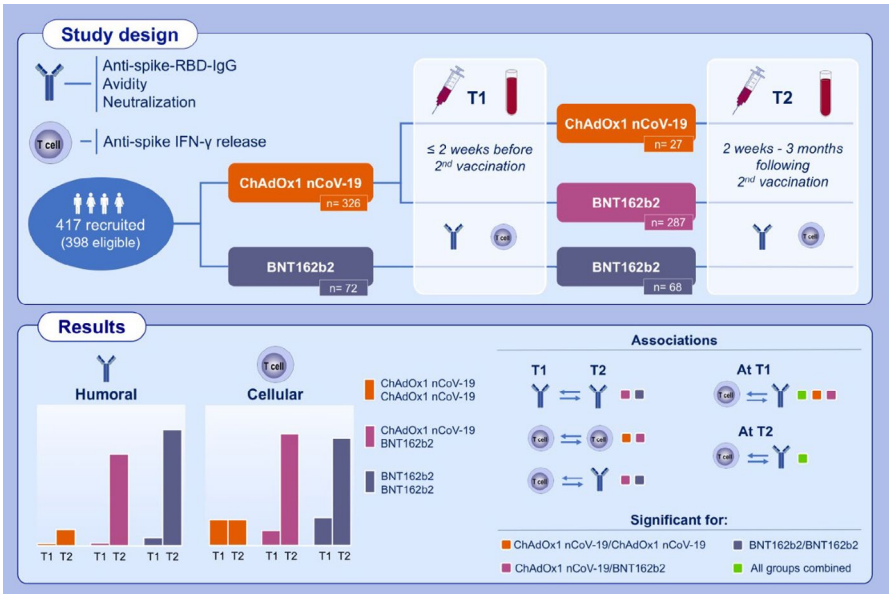
Funding information
Deutsche Forschungsgemeinschaft

response were linked at both time points (all groups combined). All regimes yielded neutralizing antibodies and increased antibody avidity at T2.

Conclusions: Interdependencies between humoral and cellular immune responses differ between common SARS-CoV-2 vaccination regimes. T-cell activation is unlikely to compensate for poor humoral responses.

KEYWORDS
BNT162b2, ChAdOx1 nCoV-19, immune response, SARS-CoV-2, vaccination



GRAPHICAL ABSTRACT
Booster with BNT162b2 elicits strong humoral and cellular immune responses independent of the prime vaccination, whereas ChAdOx1 nCoV-19 booster does not further enhance the cellular response. Levels of humoral and cellular immune responses following COVID-19 vaccinations are related and interdependencies between them differ amongst vaccination regimes. Poor humoral immune responses are unlikely to be compensated by strong cellular activation.

1 | INTRODUCTION

COVID-19 caused by SARS-CoV-2 was declared a pandemic disease by the WHO in March 2020 and has since resulted in more than five million casualties worldwide.[1–4] SARS-CoV-2 enters macrophages, type II pneumocytes, pericytes, and muscle cells by utilizing the angiotensin-converting enzyme 2.[5] As a reaction, innate and acquired immune responses are mounted, including the production of antibodies and specific T-cells.[6–9]

While SARS-CoV-2 expresses four major structural proteins (spike, membrane, envelope, and nucleocapsid proteins), vaccine production has focused on the spike protein because of its immunogenicity and its importance for the induction of neutralizing antibodies.[10–12] Current EMA (European Medicines Agency)-authorized vaccines have all been shown to induce significant levels of antibodies against the spike protein and to provide efficient protection against the virus.[13–17]

Overall, levels of anti-spike-RBD (receptor-binding domain)-IgG and neutralizing antibodies (which correlate strongly) appear to be good measures of vaccine efficacy.[18–21] Recent publications report a vaccine efficiency of 80% against infections with the Alpha variant (B.1.1.7), with anti-spike-RBD-IgG titers of 506 BAU/ml or 90% efficacy for titers above 775 BAU/ml.[20,22] Similar studies for the Delta (B.1.617.2) or Omicron (B.1.1.529) variants are expected to be published very soon.[23] It must be assumed that higher antibody titers are necessary to prevent infections. Additionally, recent research has shown declining antibody titers and waning vaccine efficacy over time.[17,24,25] T-cells may also contribute considerably to protective immunity against SARS-CoV-2,[26] although little is known about the relative importance of T-cells in preventing SARS-CoV-2 infections.

Due to cases of cerebral venous thrombosis following ChAdOx1 nCoV-19 (AstraZeneca) vaccinations particularly in younger individuals, immunizations with ChAdOx1 nCoV-19 were discontinued in younger people in many European countries. As a consequence, heterologous ChAdOx1 nCoV-19/BNT162b2 (Comirnaty, BioNTech) vaccination was proposed. At that time, there was a lack of data on immunogenicity and safety of this combination.[27–29]

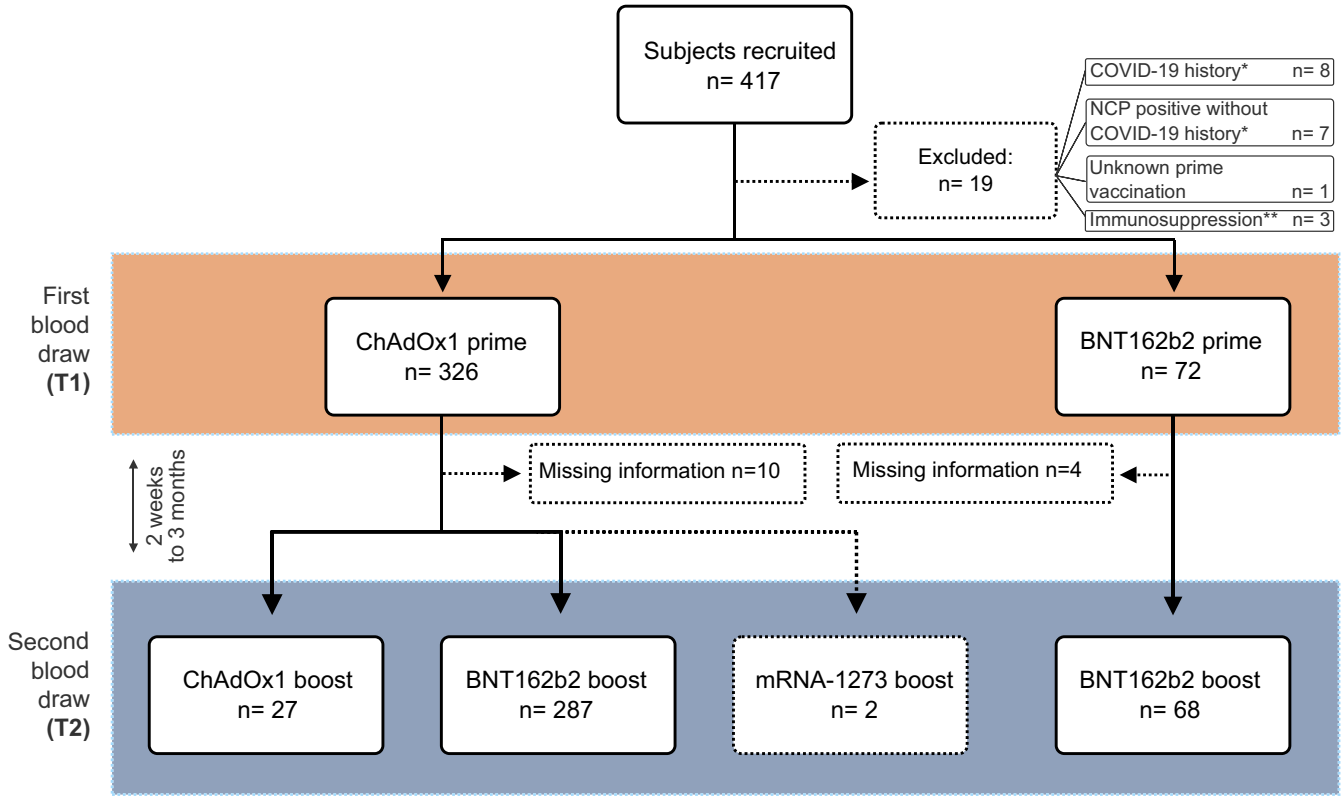


FIGURE 1 Diagram of participant recruitment and study procedure. Dashed lines indicate excluded groups. *The term "COVID-19 history" refers to PCR-confirmed SARS-CoV-2 infection. **Immunosuppression: adalimumab ($n = 1$), ropeginterferon alfa-2b ($n = 1$), and apremilast ($n = 1$). ChAdOx1=ChAdOx1 nCoV-19

In the meantime, research has shown that heterologous immunization with ChAdOx1 nCoV-19/BNT162b2 is safe and at least equally effective to the homologous BNT162b2 regimen.[30–35] Nevertheless, cellular immune responses (e.g., spike protein-directed T-cell responses) and individual titer developments (e.g., IgG titers against the spike protein) and particularly the relationship between these two branches have not been assessed in larger cohorts.

We are still in the process of determining optimal dosing intervals and combinations of the currently available vaccines. The goal of this study was to determine and correlate humoral and cellular immune responses against the spike protein following heterologous ChAdOx1 nCoV-19/BNT162b2 vaccination and compare them with homologous ChAdOx1 nCoV-19 or homologous BNT162b2 regimens, respectively. These findings will help to develop optimized protocols for population-based vaccination programs.

2 | MATERIALS AND METHODS

2.1 | Cohort

The prospective, observational COV-ADAPT cohort study was conducted at the University Medical Center Göttingen, Germany (UMG). Employees and affiliates of the UMG between 18 and 75 years who received routine first (prime) and second (booster) vaccination against COVID-19 at the UMG vaccination center were eligible for inclusion in the study unless they were currently afflicted with COVID-19 or were in domestic quarantine. The study was approved by the UMG ethics committee (21/5/21). Study design and study implementation were performed in accordance with the guidelines of Good Clinical Practice (ICH 1996) and the Declaration of Helsinki. The study was registered with the German Clinical Trials Register (DRKS00026029).

Participants received boosters with EMA-authorized vaccines between 14 May, 2021, and 14 July, 2021. The UMG vaccination center distributed vaccines in accordance with the recommendations of the German standing committee on vaccinations (STIKO) and depending on the availability of the vaccines. Prior to the study inclusion, participants had either had received an initial dose of BNT162b2 (Comirnaty, BioNTech) or ChAdOx1 nCoV-19 (Vaxzevria, AstraZeneca), or had had COVID-19.

After written informed consent was obtained, blood samples were collected. We assessed vaccination regime, age, sex, previous COVID-19, medications, and comorbid diseases by questionnaire. Study subjects were labelled "post COVID-19," if they had had a PCR-confirmed SARS-CoV-2 infection. All other indications of a prior SARS-CoV-2 infection were summarized as "COVID-19 contact".

We registered and excluded all cases with immunosuppressive medication as well as those whose medication frequently has

immunologically relevant side effects (Figure 1). We did not exclude subjects whose concomitant diseases were expected to have no or at most moderate influence on the outcome of vaccination such as well-controlled diabetes mellitus, cardiovascular disease, asthma, or allergies. No study participant reported comorbid diseases, which would be expected to strongly influence the immune system. Two subjects that had received a ChAdOx1 nCoV-19/mRNA-1273 (Moderna) combination were excluded from further analysis as this group was too small.

2.2 | Measurement of humoral and cellular immune responses

Blood samples were collected at the UMG up to 2 weeks before (T1) and 2 weeks to 3 months following booster immunization (T2).

The following analyses were performed:

1. IgG antibodies directed against the receptor binding domain of the spike protein (anti-spike-RBD-IgG) via the SARS-CoV-2-IgG-II-Quant assay on the Architect i2000SR (Abbott Laboratories).
2. Anti-nucleocapsid-IgGs (NCP) to detect previous SARS-CoV-2 infection using the anti-SARS-CoV-2-NCP-ELISA (IgG) (Euroimmun) on the DSX Automated ELISA System (Thermo Labsystems).
3. Neutralizing antibodies (nABs) against SARS-CoV-2 through the DIA-SARS-CoV-2-nAb assay (DiaProph, Kiev, Ukraine; distributed by AlphaScience GmbH) on the DSX Automated ELISA System (Thermo Labsystems).
4. Antibody avidity via the DIA-SARS-CoV-2-S-IgG-av avidity assay (DiaProph) on the DSX Automated ELISA System (Thermo Labsystems).
5. The cellular immune response using the SARS-CoV-2-spike-specific-IFN-γ-release assay (IGRA) (Euroimmun) on the DSX Automated ELISA System (Thermo Labsystems).

2.3 | Statistics

Statistical analyses were performed by the Scientific Core Facility for medical biometry and statistical bioinformatics (MBSB) of the UMG using the statistics software R. The significance levels were set to alpha = 5% for all statistical tests.

For further details, please see the Appendix S1.

3 | RESULTS

3.1 | COV-ADAPT Study Design

From 14 May, 2021, to 14 July, 2021, we recruited 417 participants between the ages of 18 and 65 years for a first blood sampling (T1) up to 2 weeks prior to their receiving a routine COVID-19 booster vaccination at the UMG vaccination center (Figure 1). Participant characteristics are summarized in Table 1. Of the 398 eligible study participants, 326 had received ChAdOx1 nCoV-19 as their prime vaccination and 72 BNT162b2.

A second blood sample was taken 2 weeks to 3 months after booster vaccination (T2). At T2, 382 of the 398 eligible study participants could be allocated to groups according to their vaccination regimes: homologous ChAdOx1 nCoV-19 ($n = 27$), ChAdOx1 nCoV-19/BNT162b2 ($n = 287$), and homologous BNT162b2 ($n = 68$), the others were lost to follow-up (Figure 1, Table S1). Subjects with homologous ChAdOx1 nCoV-19 vaccinations were older on average than subjects with a BNT162b2 booster (see Table 1). We accounted for inhomogeneity by controlling for age and sex.

3.2 | Strong primary immune response with mRNA vaccination (BNT162b2) and high anti-spike-RBD-IgG titers after booster with BNT162b2, independent of prime vaccination

We analyzed titers of IgG against the spike-RBD at T1 (Figure 2A) and found that individuals who had received BNT162b2 as their prime vaccination had achieved significantly higher titers, with an average of 226 ± 279 BAU/ml (mean ± standard deviation [SD]), as compared with ChAdOx1 nCoV-19 (70 ± 114 BAU/ml). Samples at T2 showed that booster vaccinations significantly increased the levels of anti-spike-RBD-IgG for all included vaccine combinations (Figure 2A). However, we observed a superior anti-spike-RBD-IgG response for subjects with a BNT162b2 booster regardless of the initial vaccine, as compared with homologous ChAdOx1 nCoV-19 vaccination (Figure 2A, blue triangles). Among those with BNT162b2 booster (i.e., ChAdOx1 nCoV-19/BNT162b2 and homologous BNT162b2 groups), no significant difference in anti-spike-RBD-IgG could be found at T2. Of note, anti-spike-RBD-IgG titers in individuals with a BNT162b2 booster vaccination exceeded the geometric mean of the homologous ChAdOx1 nCoV-19 vaccinated subjects in all participants but one (Figure 2B).

Regression analysis, corrected for age, sex, and elapsed time between the 2nd vaccination and T2 (Figure 2C), showed a strong positive association between anti-spike-RBD-IgG titers at T1 and T2 in the ChAdOx1 nCoV-19/BNT162b2 group (b = 0.38, CI = [0.31;0.45], $p < .001$) and a weaker association in the homologous BNT162b2 group (b = 0.11, CI = [0.01;0.21], $p = .039$). Subjects with higher titers after prime vaccination achieved higher titers following booster vaccination. For the homologous ChAdOx1 nCoV-19 group, a similar tendency was found but did not reach statistical significance (b = 0.42, CI = [−0.06;0.90], $p = .085$).

We further analyzed whether age (Figure S1A), time between booster and T2 (Figure S1C), or sex (Figure S2) impacted on the humoral response. We found that anti-spike-RBD-IgG titers showed a trend toward lower titers at higher ages only in the groups with a BNT162b2 booster (Figure S1A), but not in the

TABLE 1 Patient characteristics and immune responses following different SARS-CoV-2 vaccination regimes

Parameter	Total	ChAdOx1 ChAdOx1	ChAdOx1 BNT162b2	BNT162b2 BNT162b2
n	399	27	287	68
Age [years]				
Mean ± SD	35 ± 13	57 ± 8	34 ± 13	30 ± 9
Sex				
Male	100 (25.1%)	4 (14.8%)	68 (23.7%)	19 (27.9%)
Female	297 (74.4%)	23 (85.2%)	218 (76.0%)	48 (70.6%)
Unknown	2 (0.5%)	0 (0.0%)	1 (0.3%)	1 (1.5%)
IgG T1 [BAU/ml]				
Mean ± SD	94 ± 140	55 ± 52	72 ± 119	201 ± 194
TC T1 [mIU/ml]				
Mean ± SD	791 ± 1803	1152 ± 2243	669 ± 1578	1240 ± 2509
IgG T2 [BAU/ml]				
Mean ± SD	2370 ± 1787	413 ± 461	2387 ± 1627	3202 ± 2184
Missing	32	0	8	10
TC T2 [mIU/ml]				
Mean ± SD	4747 ± 6677	1680 ± 1854	5069 ± 6733	4880 ± 7570
Missing	35	0	11	10
Avidity T1				
Negative	45 (11.3%)	7 (25.9%)	26 (9.1%)	11 (16.2%)
Low	184 (46.2%)	12 (44.4%)	118 (41.3%)	48 (70.6%)
High	169 (42.5%)	8 (29.6%)	142 (49.7%)	9 (13.2%)
Missing	1	0	1	0
Avidity T2				
High	366 (100.0%)	27 (100.0%)	276 (100.0%)	60 (100.0%)
Missing	33	0	11	8
Neutralization T1				
Negative	41 (10.3%)	5 (18.5%)	25 (8.7%)	8 (11.8%)
Positive	357 (89.7%)	22 (81.5%)	261 (91.3%)	60 (88.2%)
Missing	1	0	1	0
Neutralization T2				
Negative	37 (10.1%)	0 (0.0%)	31 (11.2%)	6 (10.0%)
Positive	329 (89.9%)	27 (100.0%)	245 (88.8%)	54 (90.0%)
Missing	33	0	11	8

Note: Patient characteristics and measurements of immune responses up to 2 weeks before (T1) and 2 weeks to 3 months following booster vaccination (T2) according to vaccination regimes. "Total" refers to 417 recruited patients minus 8 subjects with previously proven COVID-19, 7 subjects with detectable positive or border-line anti-NCP IgG antibody titers despite anamnestic negative COVID-19-history and 3 subjects with immunosuppressive drug intake.

Abbreviations: ChAdOx1, ChAdOx1 nCoV-19; IgG, anti-spike-RBD-IgG; TC, spike-directed IFN-γ T-cell release.

homologous ChAdOx1 nCoV-19 group. Sex did not have a significant influence (Figure S2). Furthermore, the magnitude of anti-spike-RBD-IgG titers differed between the booster vaccination and T2 (Figure S1C), while the response tended to increase with elapsed time in the homologous ChAdOx1 nCoV-19 group (albeit not significantly), titers declined in both groups that had received the BNT162b2 booster (significant in the ChAdOx1 nCoV-19/BNT162b2 group).

3.3 | Robust spike-directed T-cell response following mRNA (BNT162b2) booster but not following homologous ChAdOx1 nCoV-19 vaccination

Next, we measured the spike-directed IFN-γ T cell response in our study groups. There was no significant difference between individuals whose prime vaccination was with either ChAdOx1 nCoV-19 or BNT162b2 at T1, with averages of 707 ± 1631 mIU/ml and

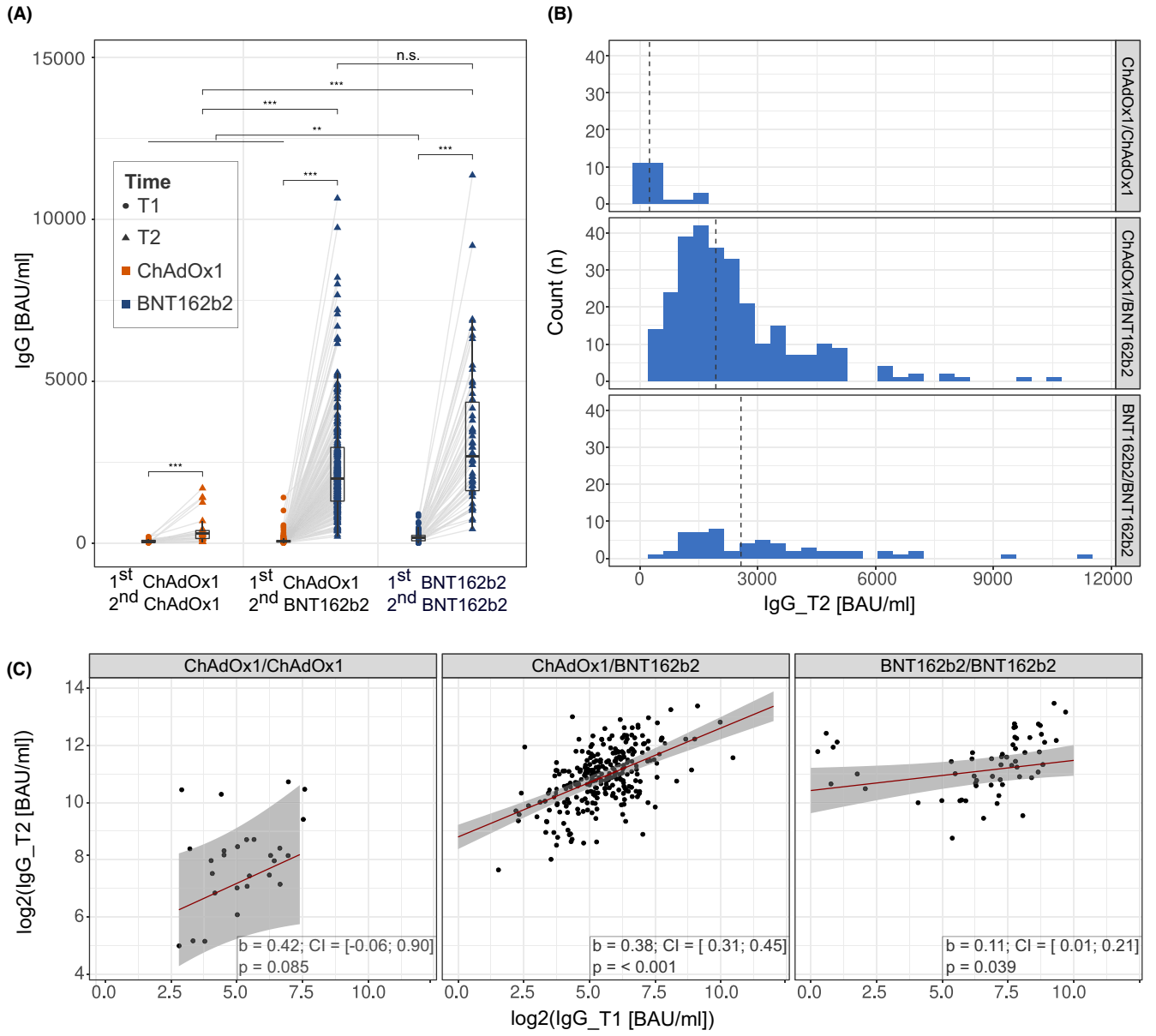


FIGURE 2 Anti-spike-RBD-IgG titers up to 2 weeks before (T1) and 2 weeks to 3 months following booster vaccination (T2) according to vaccination regimes. (A) Anti-spike-RBD-IgG (IgG) [BAU/ml] at both time points by vaccination regime. Significance asterisks indicate results from contrast tests within a linear mixed effect model for log(IgG) with vaccination regime and time and their interaction as predictors, adjusted for age and sex. The *p* values are adjusted for multiple testing using Holm's procedure. ***$p < .001$; **$p < .01$; *$p < .05$. (B) Distribution (as histograms) of anti-spike-RBD-IgG [BAU/ml] measured at T2 in the different vaccination regimes (facets). The dashed lines show the geometric group means. (C) Regression (log_2) of IgG at T2 on IgG at T1 controlling for age, sex, and time between second vaccination and T2. B values, *p* values and confidence intervals (CI) are displayed in the figures. ChAdOx1, ChAdOx1 nCoV-19

1277 ± 2514 mIU/ml (Figure 3A, dots). In contrast to anti-spike-RBD-IgG titers, the distribution was not strikingly different as medians partially overlapped (Figure 3B). Remarkably, there was also no statistically significant booster effect between T1 and T2 in subjects homologously vaccinated with ChAdOx1 nCoV-19. In contrast, booster with BNT162b2 yielded a significantly increased IFN-γ T-cell response at T2, regardless of the prime vaccination (Figure 3A, blue triangles). Similar to the findings of the humoral response, there was no significant difference in T-cell responses between the ChAdOx1 nCoV-19/BNT162b2 and the homologous BNT162b2 group.

Regression analysis between T-cell responses at T1 and T2 (Figure 3C) showed significant linear associations particularly for the homologous ChAdOx1 nCoV-19 group (b = 0.78, CI = [0.40;1.15], $p < .001$). For the ChAdOx1 nCoV-19/BNT162b2 group, there was also a positive association (b = 0.47, CI = [0.34;0.61], $p < .001$), whereas for the homologous BNT162b2 group, no significant association could be detected (b = 0.14, CI = [−0.05;0.32], $p = .139$).

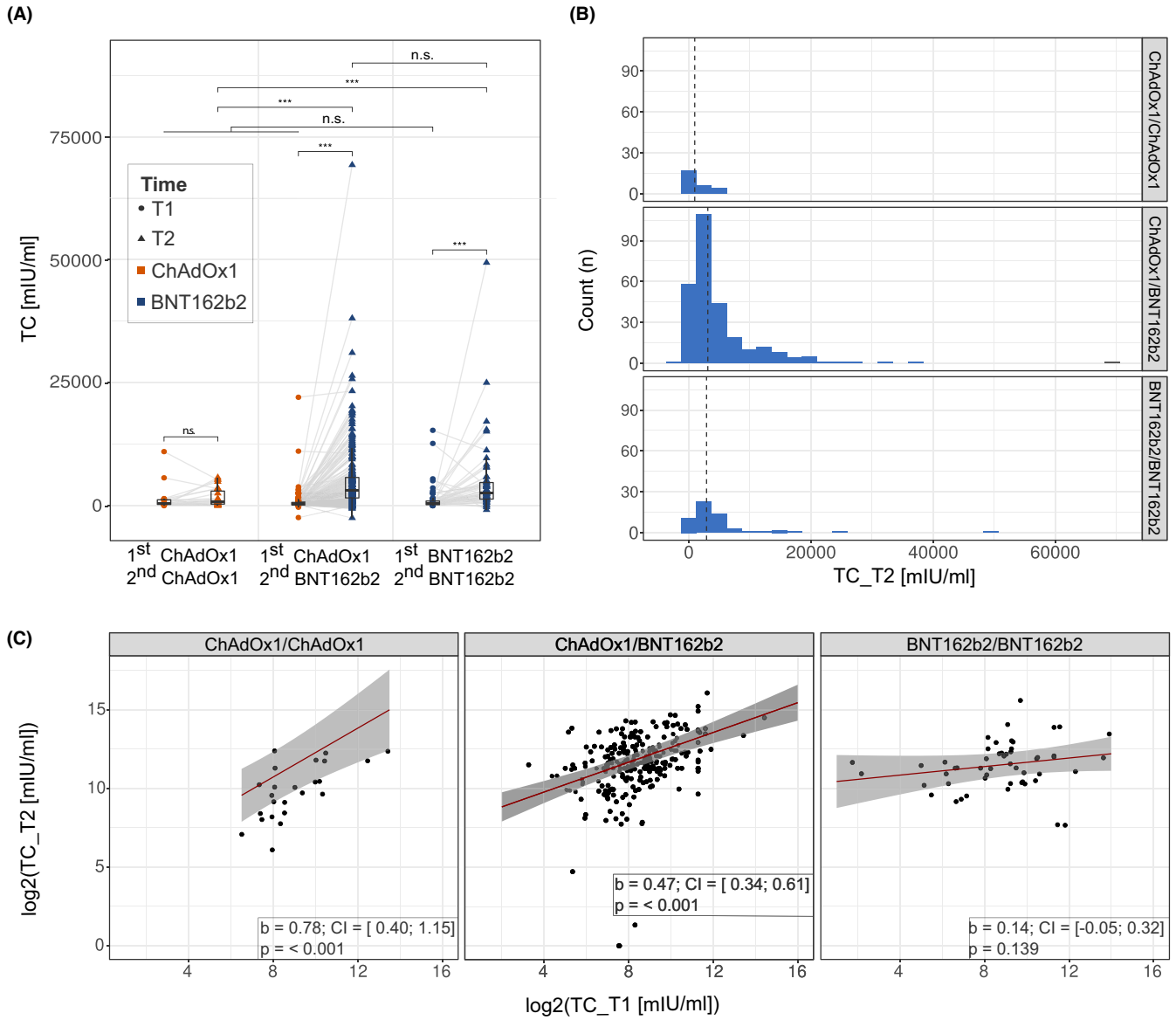


FIGURE 3 T-cell responses up to 2 weeks before (T1) and 2 weeks to 3 months following booster vaccination (T2) according to vaccination regimes. (A) Spike-directed IFN-γ T-cell responses (TC) [mIU/ml] at both time points by vaccination regime. Significance asterisks indicate results from contrast tests within a linear mixed effect model for log(TC) with vaccination regime and time and their interaction as predictors and additionally adjusted for age and sex. The *p* values are adjusted for multiple testing using Holm's procedure. ***$p < .001$; **$p < .01$; *$p < .05$. (B) Distribution (as histograms) of spike-directed IFN-γ T-cell responses [mIU/ml] measured at T2 in the different vaccination regimes (facets). The dashed lines show the geometric group means. (C) Regression (log_2) of TC at T2 on TC at T1 controlling for age, sex, and time between second vaccination and T2. B values, *p* values and confidence intervals (CI) are displayed in the figures. ChAdOx1, ChAdOx1 nCoV-19

When examining the parameters such as age (Figure S1B), time between booster and T2 (Figure S1D), and sex (Figure S2) with respect to IFN-γ T-cell response, it was weakly (not statistically significantly) increased with age only in the homologous ChAdOx1 nCoV-19 group. Our data point toward a slight reduction in spike-directed IFN-γ T-cell activity depending on the elapsed time between booster vaccination and T2 for the ChAdOx1 nCoV-19/BNT162b2 group. This effect could not be observed for homologous BNT162b2 or ChAdOx1 nCoV-19 vaccines (Figure S1D).

3.4 | Associations between humoral and cellular immune responses in primary and booster vaccination

To assess the association between anti-spike-RBD-IgG titers and spike-directed IFN-γ T-cell responses (Figure 4), separate regression analyses for T1 (Figure 4A,B) and T2 (Figure 4C,D) were performed. The analysis was conducted for all the study participants combined (Figure 4A,C) as well as for the different vaccination regimes (Figure 4B,D). For the study group as a whole, there was a

clear positive association between anti-spike-RBD-IgG titers and spike-directed IFN-γ T-cell responses for both T1 and T2 (b = 0.21, CI = [0.10;0.32], $p < .001$ for T1 and b = 0.35, CI = [0.17;0.53], $p < .001$ for T2). However, when considering vaccination groups separately, significant correlations were only found at T1 in the homologous ChAdOx1 nCoV-19 and the ChAdOx1 nCoV-19/BNT162b2 groups, whereas no association was seen in the homologous BNT162b2 group at T1. At T2, none of the vaccine regimes yielded a significant correlation, although in the ChAdOx1 nCoV-19/BNT162b2 group, a trend was detectable (b = 0.26, CI = [0.00;0.53], $p = .054$).

An additional analysis investigated the correlation between the spike-directed T-cell IFN-γ responses at T1 and anti-spike-RBD-IgG

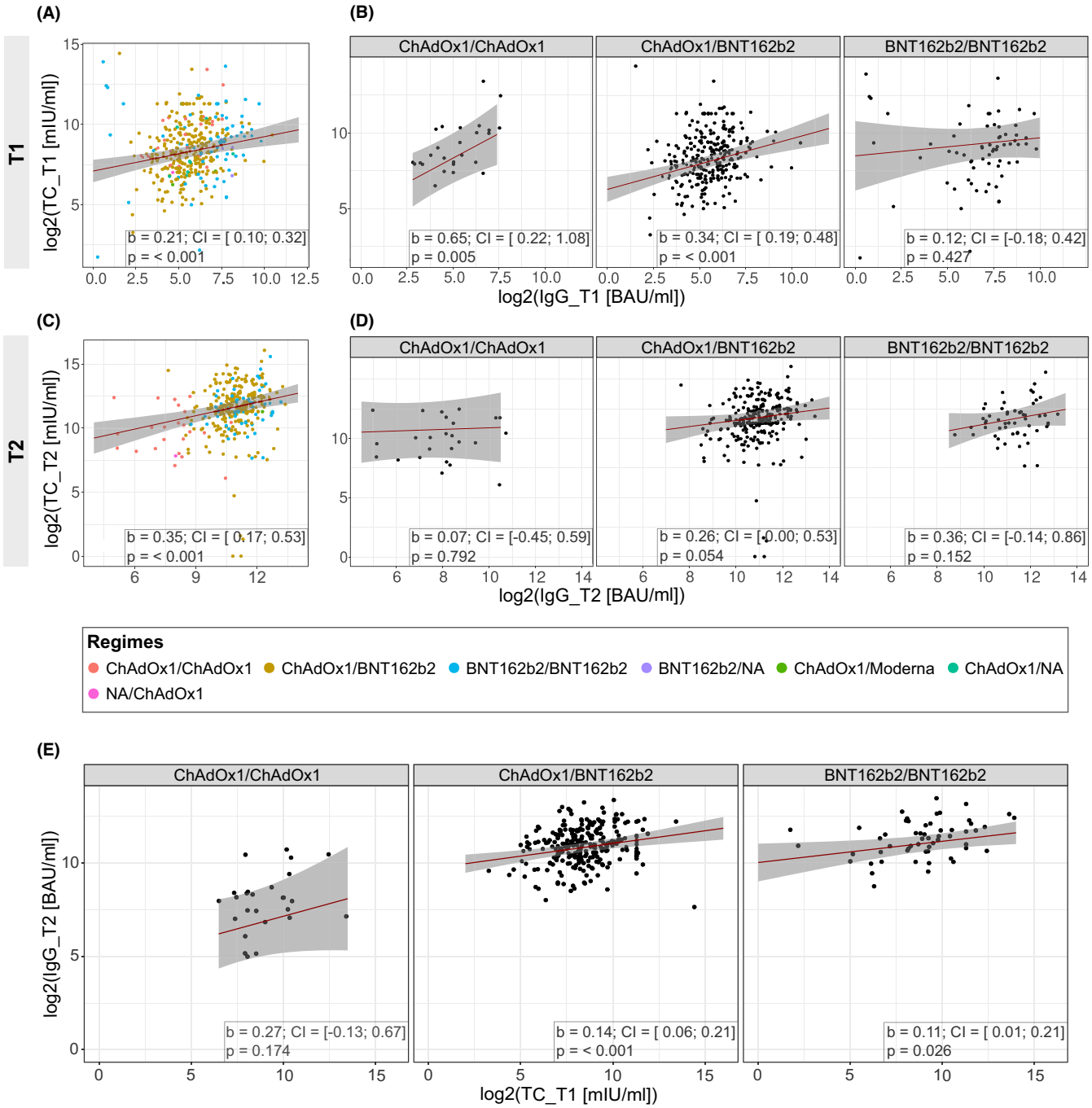


FIGURE 4 Regression of the spike-directed IFN-γ T-cell responses (TC) and anti-spike-RBD-IgG (IgG) up to 2 weeks before (T1) and 2 weeks to 3 months following booster vaccination (T2). (A) Regression (log_2) at T1 controlling for age, sex, and time between first vaccination and T1 for all study participants and (B) for the respective subgroups. (C) Regression (log_2) at T2 controlling for age, sex, and time between second vaccination and T2 in all study participants and (D) for the respective subgroups. (E) Regression (log_2) of TC at T1 and IgG at T2, controlling for age, sex, and time between second vaccination and T2 for the respective subgroups. B values, *p* values and confidence intervals (CI) are displayed in the figures. ChAdOx1, ChAdOx1 nCoV-19

at T2 (Figure 4E). Significant correlations were found for the ChAdOx1 nCoV-19/BNT162b2 and homologous BNT162b2 groups, that is, good T-cell responses after T1 were associated with a good antibody response following booster vaccination at T2 (b = 0.14, CI = [0.06;0.21], $p < .001$ for ChAdOx1 nCoV-19/BNT162b2 and b = 0.11, CI = [0.01;0.21], $p = .026$ for homologous BNT162b2).

3.5 | Neutralizing antibodies and increasing antibody avidity in all three regimens

The neutralization index (NI) as a parameter for the development of neutralizing antibodies against SARS-CoV-2 (Figure 5A) and the relative avidity index (RAI) (Figure 5B) was also determined for T1 and T2. Both qualitative parameters complement the quantitative determination of the antibody titers.

In all three groups, there were measurable titers of neutralizing antibodies already at T1. We found a significant increase in the NI from T1 to T2, most pronounced in both the homologous ChAdOx1 nCoV-19 and the ChAdOx1 nCoV-19/BNT162b2 groups (Figure 5A, Table S2). The more moderate increase in the homologous BNT162b2 group can be attributed to the already high level of neutralization indices at T1.

Interestingly, in both BNT162b2-boostered groups, a small number of participants (31/276 for the ChAdOx1 nCoV-19/BNT162b2 group and 6/60 for the homologous BNT162b2 group) presented with no neutralizing antibodies at T2, even though the majority of these participants had measurable neutralizing antibodies at T1. Only three subjects showed neither neutralizing antibodies at T1 nor at T2, all of whom had received homologous BNT162b2. Of note, the lack of neutralizing antibodies did not correspond to a lack of anti-spike-RBD-IgG, as these participants did have above average anti-spike-RBD-IgG titers when compared with their respective groups (Figure S3).

In our study, individuals who were prime vaccinated with ChAdOx1 nCoV-19 reached a significantly higher avidity index at T1 when compared with BNT162b2 (Figure 5B, Table S3). All groups showed a significant increase in avidity at T2. Interestingly, the homologous BNT162b2 group also reached the lowest avidity at T2, which was significantly lower than for both groups with prime ChAdOx1 nCoV-19 vaccination. We thus found the ratio between antibody titers and avidity to be different between the vaccination schemes. The reason for this could either be that (i) ChAdOx1 nCoV-19 yields fewer but more avid antibodies or (ii) our avidity assay is insufficiently quantitative.

4 | DISCUSSION

COV-ADAPT is an observational cohort study providing real-world data on heterologous vaccination with ChAdOx1 nCoV-19/BNT162b2 compared with homologous ChAdOx1 nCoV-19 and BNT162b2-vaccinations in a large cohort of 417 healthcare workers. As the focus of this study was the evolution of the immune response after prime and booster vaccination, baseline values of participants before both vaccines were not included. We correlated immune responses on an individual level after prime vaccination with the responses after secondary immunization (booster) to evaluate the predictability of the quality of the immune response. We were able to demonstrate that humoral and cellular immune responses correlate with one another, suggesting that a poor humoral immune response is unlikely to be ameliorated by a strong cellular response.

In our study, we found a superior effectiveness in regard to immune stimulation for BNT162b2, either in a homologous vaccine regime or as a heterologous combination with ChAdOx1 nCoV-19. This superior effectiveness was observed both in terms of the humoral immune response (anti-spike-RBD-IgG titers) and the cellular component, that is, the spike-directed IFN-γ T-cell responses. Our findings corroborate the previous findings of comparability of the IgG response against the spike protein in heterologous ChAdOx1 nCoV-19/BNT162b2 regimes to homologous BNT162b2 and superiority to homologous ChAdOx1 nCoV-19.[31–35] Furthermore, these studies showed a tolerable and manageable reactogenicity after heterologous immunization with ChAdOx1 nCoV-19/BNT162b2 compared with homologous ChAdOx1 nCoV-19 and BNT162b2.[30–33,35]

The combination of vector- and mRNA-based vaccines was initially thought to provide the benefits of both vaccination techniques. The hope was that this combination would yield the strong IgG responses known from mRNA vaccines as well as enhanced T-cell responses.[36] Both responses appear to play an important role in vaccine-induced protection against SARS-CoV-2 infection, particularly in the early phase after vaccination.[12,26] Specifically, $CD8^+$ cell responses were expected to be increased through heterologous vaccination with ChAdOx1 nCoV-19/BNT162b2, as based on animal models and pathophysiological considerations.[36,37] Several studies in smaller subpopulations appeared to support this hypothesis.[31,32,38] However, in our large dataset, we were not able to confirm a general superiority of the ChAdOx1 nCoV-19/BNT162b2 regime compared with homologous BNT162b2 vaccination in terms of T-cell stimulation. At T2, booster vaccination with BNT162b2 significantly increased spike-directed IFN-γ release of T-cells in individuals from the ChAdOx1 nCoV-19/BNT162b2 and the homologous BNT162b2 groups, with no statistically relevant difference.

Booster vaccination with ChAdOx1 nCoV-19 had no discernible influence on IFN-γ release by T-cells, suggesting that it is ineffective for increasing T-cell-mediated immunity. This is in line with recent findings by Hillus et al.[35] Of note, Schmidt et al.[32] showed that all three vaccination regimes (homologous ChAdOx1 nCoV-19, heterologous ChAdOx1 nCoV-19/BNT162b2, homologous BNT162b2) induced polyfunctional T-cells, which slightly differed in the induced $CD4^+$ and $CD8^+$ T-cell subpopulations. We did not account for different T-cell subpopulations and can hence not exclude subtle functional advantages of a heterologous vaccination.

We found that the humoral (anti-spike-RBD-IgG) responses at T1 and T2 were positively associated for both BNT162b2-boostered groups, with a more pronounced effect in the ChAdOx1 nCoV-19/BNT162b2 group. Thus, a strong or weak initial humoral response

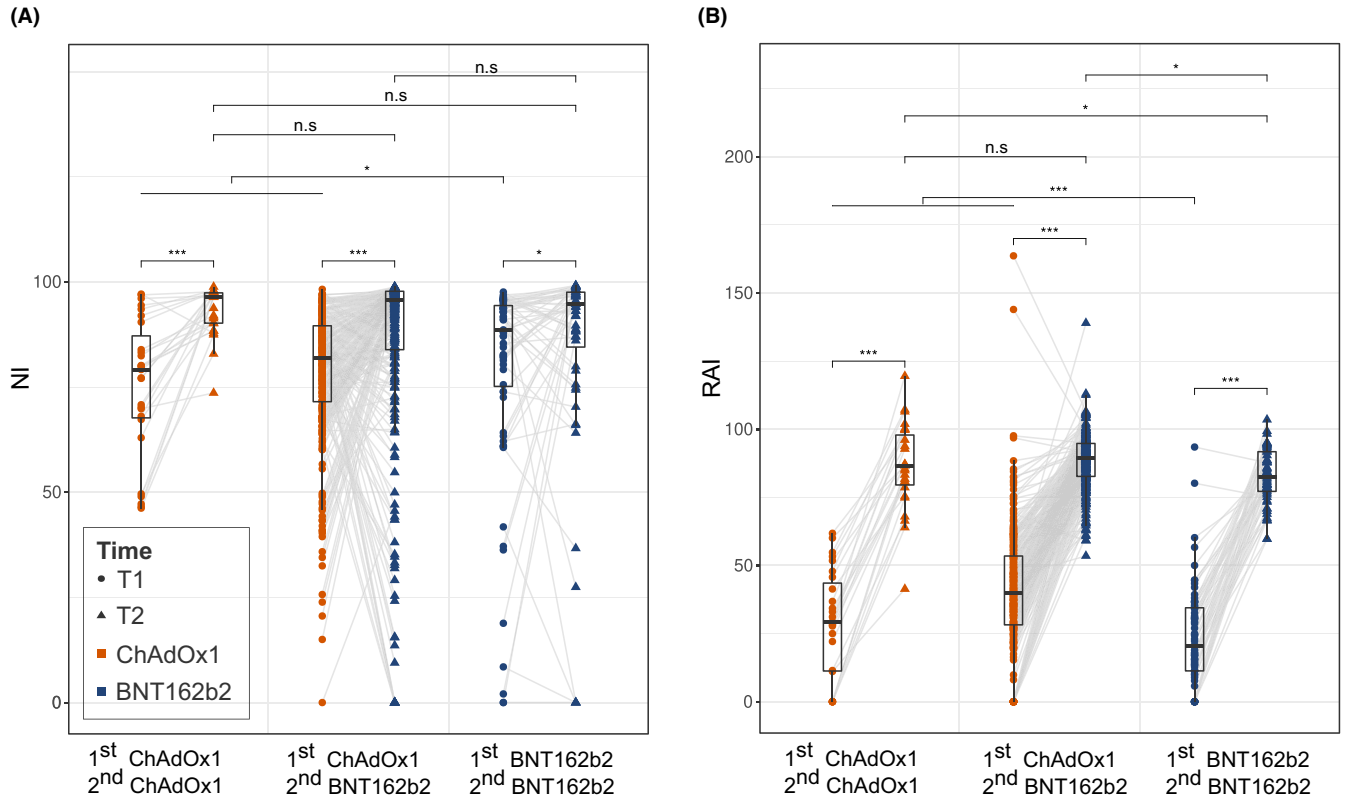


FIGURE 5 Neutralization index (NI) and relative avidity index (RAI) up to 2 weeks before (T1) and 2 weeks to 3 months following booster vaccination (T2) according to vaccination regimens. (A) NI, (B) RAI. Significance asterisks indicate results from contrast tests within a linear mixed effect model with vaccination regime and time and their interaction as predictors and additionally adjusted for age and sex. The *p* values are adjusted for multiple testing using Holm's procedure. ***$p < .001$; **$p < .01$; *$p < .05$. ChAdOx1, ChAdOx1 nCoV-19

appears to be predictive for the development of high or low titers of protective anti-spike-RBD-IgG at T2, respectively. The lack of statistical significance in the ChAdOx1 nCoV-19 group may be attributed to the small group size ($n = 27$).

Additionally, we found a strong and significant positive association between IFN-γ release by T-cells at T1 and T2 for the homologous ChAdOx1 nCoV-19 and ChAdOx1 nCoV-19/BNT162b2 groups. Thus, participants in these two groups who started off with a strong response also showed strong responses at T2, while weak responses remained weak at T2. The positive association in the homologous ChAdOx1 nCoV-19 group was not accompanied by an increased T-cell response from T1 to T2. T-cell activity therefore depended heavily on the reaction to the prime vaccine and could not be further increased by the ChAdOx1 nCoV-19 booster. In contrast, T-cell responses generally increased from T1 to T2 in the ChAdOx1 nCoV-19/BNT162b2 and homologous BNT162b2 groups, indicating a BNT162b2-induced booster effect.

On an aggregate level, humoral and cellular responses were found to be associated with each other at T1 and T2, respectively. When looking at the particular vaccine regimes separately, a significant association between these responses could only be detected for homologous ChAdOx1 nCoV-19 and ChAdOx1 nCoV-19/BNT162b2 groups at T1. The loss of significance for all vaccination regimes at T2 could be attributed to a stronger augmentation of anti-spike-RBD-IgG titers compared to the T-cell responses. One may speculate that antibody titers have a higher capacity to increase as compared to the cellular compartment of the immune system, resulting in a loss of linear association.

A significant association between IFN-γ responses at T1 and anti-spike-RBD-IgG at T2 was found for the ChAdOx1 nCoV-19/BNT162b2 and homologous BNT162b2 groups, suggesting that good initial T cell responses correspond with good humoral responses at T2. This is in line with the notion that functional T-helper cells and memory T-cells are fundamental for launching a successful immune response.[39] Thus, an effective spike-directed T-cell activation at T1 leads to increased anti-spike-RBD titers at re-exposure to the antigen.

While quantitative immune responses did not differ between males and females, subjects of higher age and male sex showed lower avidity after prime vaccination. Avidity, a measure for the binding strength of a multivalent bond between antigen and antibody,[40] is often used as a parameter of the quality of an antibody response. Such general sex-dependent differences in immune responses might contribute to increased COVID-19 severity in men and older individuals, as has been observed in previous studies.[41] However, due to the semi-quantitative nature of the test used here, all subjects reached the maximum test category "high avidity" at T2. We therefore did not find associations between vaccination regimes and/or specific participant characteristics.

Most individuals who received BNT162b2 as a booster developed significant immune responses, which were, by all quantitative parameters measured in this study, highly superior to immunity induced by homologous ChAdOx1 nCoV-19. Unexpectedly, there were no significant differences between the three vaccination regimens in regard to the neutralization indices at both time points. A possible explanation is that the relative neutralization capacity had already been high (90%) after prime vaccination. However, in contrast to the homologous ChAdOx1 nCoV-19 group, some individuals presented with a lack of neutralizing antibodies at T2 following booster vaccination with BNT162b2.

Our findings revealed an association between humoral and cellular immune responses following vaccination, and that a poor humoral immune response is unlikely to be compensated by a strong cellular immune response. The distinct differences between vaccination regimes, as demonstrated in this study, should be taken into account for population-based vaccine programs.

Future studies could evaluate the persistence of the observed interdependencies with increasing time after prime-boost vaccination. Such studies are needed to determine the stability of the observed immune responses over time and may contribute to guidelines for the combination of different vaccines. Furthermore, it will be important to follow-up on the immune response of our participants after their 3rd (booster) and possibly 4th vaccination to assess the evolvement of the immune response after repetitive COVID-19 vaccine application. Indeed, many countries are currently administering booster shots only months after completion of prime-boost immunizations. It will be important to assess the interdependencies between humoral and cellular immune responses among different vaccine combinations to overcome waning immune responses and decreasing vaccine efficacy.

ACKNOWLEDGEMENTS

We would like to thank Prof. Carsten Watzl for his scientific input and thorough review of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (DFG), ER723/3-1 (to L.E.). Open access funding enabled and organized by ProjektDEAL.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

LE, MS, MMH, and AZ designed the study with input from MPS, UG, HSB, and JS. LE, MMH, MS, LM, AB, TMH, AA, AE, JSH, KZ, MS, and AS recruited patients and performed experiments. MMH and LM collected data. MMH and AL extracted and compiled data. All authors discussed data. MMH, LE, and MS drafted the manuscript. AF significantly contributed during the paper revision process. All authors jointly discussed, reviewed, and amended the manuscript. Data verification was performed by MMH and LM.

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REFERENCES

1. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Bio Medica Atenei Parm*. 2020;91(1):157-160. doi:10.23750/abm.v91i1.9397
2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273. doi:10.1038/s41586-020-2012-7
3. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017
4. The Johns Hopkins Coronavirus Resource Center (CRC). COVID-19 map. https://coronavirus.jhu.edu/map.html. Accessed December 10, 2021.
5. Chu H, Chan JFW, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clin Infect Dis*. 2020;71(6):1400-1409. doi:10.1093/cid/ciaa410
6. Grabbe S, Beissert S, Enk A. Systemic immunosuppression in times of COVID-19: do we need to rethink our standards? *JDDG J Dtsch Dermatol Ges*. 2020;18(8):810-813. doi:10.1111/ddg.14194
7. Schön MP, Berking C, Biedermann T, et al. COVID-19 and immunological regulations - from basic and translational aspects to clinical implications. *JDDG J Dtsch Dermatol Ges*. 2020;18(8):795-807. doi:10.1111/ddg.14169
8. Azkur AK, Akdis M, Azkur D, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy*. 2020;75(7):1564-1581. doi:10.1111/all.14364
9. Gattinger P, Niespodziana K, Stiasny K, et al. Neutralization of SARS-CoV-2 requires antibodies against conformational receptor-binding domain epitopes. *Allergy*. 2022;77(1):230-242. doi:10.1111/all.15066
10. Buchholz UJ, Bukreyev A, Yang L, et al. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. *Proc Natl Acad Sci*. 2004;101(26):9804-9809. doi:10.1073/pnas.0403492101
11. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nat Rev Immunol*. 2020;20(10):615-632. doi:10.1038/s41577-020-00434-6
12. Sadarangani M, Marchant A, Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. *Nat Rev Immunol*. 2021;21:475-484. doi:10.1038/s41577-021-00578-z
13. Walsh EE, Frenck RW, Falsey AR, et al. Safety and immunogenicity of two RNA-based covid-19 vaccine candidates. *N Engl J Med*. 2020;383(25):2439-2450. doi:10.1056/NEJMoa2027906
14. Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet*. 2020;396(10249):467-478. doi:10.1016/S0140-6736(20)31604-4
15. Sadoff J, Le Gars M, Shukarev G, et al. Interim results of a phase 1–2a trial of Ad26.COV2.S covid-19 vaccine. *N Engl J Med*. 2021;384(19):1824-1835. doi:10.1056/NEJMoa2034201
16. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 — Preliminary report. *N Engl J Med*. 2020;383(20):1920-1931. doi:10.1056/NEJMoa2022483
17. Shrotri M, Navaratnam AMD, Nguyen V, et al. Spike-antibody waning after second dose of BNT162b2 or ChAdOx1. *Lancet*. 2021;398(10298):385-387. doi:10.1016/S0140-6736(21)01642-1
18. Earle KA, Ambrosino DM, Fiore-Gartland A, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine*. 2021;39(32):4423-4428. doi:10.1016/j.vaccine.2021.05.063

19. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(7):1205-1211. doi:10.1038/s41591-021-01377-8
20. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*. 2021;375(6576):43-50. doi:10.1126/science.abm3425
21. Blain H, Tuaillon E, Gamon L, et al. Receptor binding domain-IgG levels correlate with protection in residents facing SARS-CoV-2 B.1.1.7 outbreaks. *Allergy*. 2021:all.15142. doi:10.1111/all.15142. Online ahead of print.
22. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med*. 2021;27(11):2032-2040. doi:10.1038/s41591-021-01540-1
23. Aldridge RW, Yavlinsky A, Nguyen V, et al. Waning of SARS-CoV-2 antibodies targeting the Spike protein in individuals post second dose of ChAdOx1 and BNT162b2 COVID-19 vaccines and risk of breakthrough infections: analysis of the Virus Watch community cohort [preprint]. *medRxiv*. 2021. doi:10.1101/2021.11.05.21265968
24. Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *Lancet*. 2021;398(10309):1407-1416. doi:10.1016/S0140-6736(21)02183-8
25. Choi A, Koch M, Wu K, et al. Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in healthy adults: an interim analysis. *Nat Med*. 2021;27(11):2025-2031. doi:10.1038/s41591-021-01527-y
26. Kalimuddin S, Tham CYL, Qui M, et al. Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. *Med*. 2021;2(6):682-688.e4. doi:10.1016/j.medj.2021.04.003
27. Deming ME, Lyke KE. A 'mix and match' approach to SARS-CoV-2 vaccination. *Nat Med*. 2021;27:1510-1511. doi:10.1038/s41591-021-01463-x
28. Wise J. Covid-19: European countries suspend use of Oxford-AstraZeneca vaccine after reports of blood clots. *BMJ*. 2021;372:n699. doi:10.1136/bmj.n699
29. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. *N Engl J Med*. 2021;384(22):2092-2101. doi:10.1056/NEJMoa2104840
30. Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet*. 2021;398(10295):121-130. doi:10.1016/S0140-6736(21)01420-3
31. Barros-Martins J, Hammerschmidt SI, Cossmann A, et al. Immune responses against SARS-CoV-2 variants after heterologous and homologous ChAdOx1 nCoV-19/BNT162b2 vaccination. *Nat Med*. 2021;27(9):1525-1529. doi:10.1038/s41591-021-01449-9
32. Schmidt T, Klemis V, Schub D, et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. *Nat Med*. 2021;27:1530-1535. doi:10.1038/s41591-021-01464-w
33. Liu X, Shaw RH, Stuart ASV, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. *Lancet*. 2021;398(10303):856-869. doi:10.1016/S0140-6736(21)01694-9
34. Pozzetto B, Legros V, Djebali S, et al. Immunogenicity and efficacy of heterologous ChadOx1/BNT162b2 vaccination. *Nature*. 2021;600:701-706. doi:10.1038/s41586-021-04120-y
35. Hillus D, Schwarz T, Tober-Lau P, et al. Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1 nCoV-19 and BNT162b2: a prospective cohort study. *Lancet Respir Med*. 2021;9(11):1255-1265. doi:10.1016/S2213-2600(21)00357-X
36. Ledford H. Could mixing COVID vaccines boost immune response? *Nature*. 2021;590(7846):375-376. doi:10.1038/d41586-021-00315-5
37. Spencer AJ, McKay PF, Belij-Rammerstorfer S, et al. Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. *Nat Commun*. 2021;12(1):2893. doi:10.1038/s41467-021-23173-1
38. Chiu NC, Chi H, Tu YK, et al. To mix or not to mix? A rapid systematic review of heterologous prime–boost covid-19 vaccination. *Expert Rev Vaccines*. 2021;20(10):1211-1220. doi:10.1080/14760584.2021.1971522
39. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020;181(7):1489-1501.e15. doi:10.1016/j.cell.2020.05.015
40. Aalberse RC, Kleine Budde I, Stapel SO, van Ree R. Structural aspects of cross-reactivity and its relation to antibody affinity. *Allergy*. 2001;56(s67):27-29. doi:10.1034/j.1398-9995.2001.00909.x
41. Brodin P. Immune determinants of COVID-19 disease presentation and severity. *Nat Med*. 2021;27(1):28-33. doi:10.1038/s41591-020-01202-8

SUPPORTING INFORMATION

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How to cite this article: Hollstein MM, Münsterkötter L, Schön MP, et al. Interdependencies of cellular and humoral immune responses in heterologous and homologous SARS-CoV-2 vaccination. *Allergy*. 2022;00:1–12. doi:10.1111/all.15247