Regimen of Coronavirus Disease 2019 Vaccination Influences Extent and Kinetics of Antibody Avidity

Annika Rüssler,1,a Janine Kimpel,1,a Verena Fleischer,2 Silke Huber,1,a Dorothee von Laer,1,a Wegene Borena,1,a and Reinhard Würzner,1,a on behalf of the HEVACC Study Group

We investigated antibody titers and avidity after heterologous versus homologous coronavirus disease 2019 vaccination over 6 months after the second dose. We found a significantly higher avidity in regimens including at least 1 dose of the adenoviral vector vaccine ChAdOx1-S compared with 2 doses of the mRNA vaccine BNT162b2.

Keywords. antibody kinetics; avidity; heterologous vaccination; SARS-CoV-2.

Since the onset of the coronavirus disease 2019 (COVID-19) pandemic in December 2019, antibody responses have been shown to play a crucial role in protective immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [1, 2]. Reflecting the quality of antibody responses, avidity is a measure of cumulative binding strength of antibodies to the target antigen [3]. We and others previously demonstrated that, regardless of age, the avidity of antibodies increases over time after infection, whereas titers of binding and neutralizing antibodies wane [4–6]. Comparable findings have been published after vaccination [1, 7]. However, little is known about dynamics of antibody avidity after vaccination with respect to different vaccination regimens. Although first avidity data from individuals immunized with messenger ribonucleic acid (mRNA)-based vaccine are available, differences to widely used heterologous vaccination schedules (combination of an adenovirus vaccine with an mRNA vaccine) have not yet been investigated in a large study cohort over time [1, 8]. In this study, we compared the dynamics of antibody avidity over a 6-month period after the second vaccination in a large and partially randomized clinical trial, comprising homologous ChAdOx1-S (AstraZeneca [AZ]) and BNT162b2 (PFizer/BioNTech [BNT]) vaccinees, as well as heterologous AZ/BNT-vaccinated individuals.

METHODS

Study Population

For the present avidity study, we analyzed plasma samples from the HEVACC (heterologous vaccination) clinical trial (ClinicalTrials.gov Identifier NCT04907331) [7]. The local ethics committee (EC) of the Medical University of Innsbruck has approved the HEVACC study with EC number EC 1191/2021. The HEVACC study is a 3-arm, partially randomized, single-blinded, multicenter clinical trial, in which individuals received either 2 homologous AZ doses, 2 homologous BNT doses, or a heterologous AZ/BNT regimen. Participants with a history of prior SARS-CoV-2 infection, indicated by antibodies against the nucleoprotein, or severe immune defects were excluded [7]. Individuals who had been immunized with a first AZ dose were randomized (matched for sex and study center) to receive a second vaccination with either AZ (referred to as AZ/AZ, homologous) or BNT (referred to as AZ/BNT, heterologous). In addition, plasma samples from an observational study cohort of 2-dose BNT-vaccinated individuals (referred to as BNT/BNT) were included. Number and age of the included study participants are shown in Supplementary Table 1.

Plasma Samples

Plasma samples from study participants were collected 30 days (±3 days), 90 days (±3 days), and 180 days (±3 days) after the second vaccination. Plasma samples deviating from collection time points were excluded from the study. Participants who missed a blood collection were reinvited for the next time point of blood collection. Participants with a breakthrough infection (confirmed by PCR or anti-N seroconversion) or those who received a booster vaccination were excluded from further study visits. In total, we analyzed 241 and 74 plasma samples from homologous AZ/AZ or BNT/BNT study cohorts, respectively, as well as further 329 plasma samples from the heterologous AZ/BNT group. Details on numbers of plasma samples collected at each time point are shown in Supplementary Table 2.

Serological Testing

Plasma samples were analyzed for binding antibodies and antibody avidity using an anti-SARS-CoV-2 enzyme-linked...
In the avidity analysis, which was performed as previously described [4], antibodies were assessed using the fully automated 4-plate benchtop instrument Immunomat (Virion/Serion, Würzburg, Germany) and given as binding antibody units (BAU)/mL with an assay lower limit of quantification of 3.2 BAU/mL. Thus, only samples with titers >3.2 BAU/mL were included in the avidity analysis, which was performed as previously described [4]. In brief, after centrifugation for 5 minutes at 8000 rpm, clarified plasma supernatant was diluted 1:401 in sample buffer and transferred in duplicate to a microtiter plate, precoated with the S1-domain of the SARS-CoV-2 ancestral spike protein. One well remained untreated whereas a duplicate was incubated with urea (5.5 M for 10 minutes). Plates were analyzed using a Tecan Sunrise absorbance plate reader (Tecan Austria GmbH, Groedig, Austria) at 620 nm (reference) and 450 nm (sample). Samples below (extinction <0.3) or exceeding the linear range (extinction >3.0) were diluted less (1:101) or more (1:1001), respectively. Antibody avidity was calculated as ratio of the absorbance of a sample in presence and absence of urea and was expressed as relative avidity index (RAI) in percentages.

**Statistical Analysis**

Statistical differences were calculated with a non-parametric one-way analysis of variance (ANOVA) followed by Kruskal-Wallis test with Dunn’s multiple comparison test using GraphPad Prism 9.0.1 (GraphPad Software, Inc., La Jolla, CA). To account for the effect of major confounders on the level of avidity, we performed hierarchical multivariable linear regression analysis adjusting for antibody concentration, age, and sex (SPSS, Version 25.0.; IBM Corp., Armonk, NY).

**RESULTS**

In all 3 study cohorts (Figure 1), in homologous AZ/AZ or BNT/BNT, as well as in heterologous AZ/BNT-vaccinated individuals, titers of anti-S-specific IgG antibodies declined over a period of 6 months after second vaccination (Figure 1A). Levels of binding antibodies significantly waned from a median of 193.6 BAU/mL to 59.3 BAU/mL in the AZ/AZ group, from 985.6 to 157.8 BAU/mL in the AZ/BNT group, and from 1430 to 163.7 BAU/mL in the BNT/BNT group, between Day 30 and Day 180. However, we found significantly higher titers in the heterologous AZ/BNT and the homologous BNT/BNT group compared to the homologous AZ/AZ group at almost all time points of plasma sampling. As an indicator of binding strength of antibodies and antibody functionality, we additionally investigated the avidity in collected samples (Figure 1B). We observed that, irrespective of sex and age of vaccinees (see Supplementary Figures 1 and 2), avidity significantly increased over time in all study cohorts to a median RAI of 74.48% (95% confidence interval [CI], 72.86–76.6) in the heterologous AZ/BNT, 72.7% (median; 95% CI, 70.40–74.28) in homologous AZ/AZ, and 65.57% (median; 95% CI, 62.35–69.40) in the homologous BNT/BNT group, 180 days after the second vaccination. It is notable that, when performing this analysis, we discovered remarkable differences in the dynamics of avidity of the antibodies among the 3 study cohorts. Study participants prevaccinated with 1 dose of AZ possessed significantly higher avidity as early as 30 days after their BNT boost vaccination, compared with individuals that received 2 doses of AZ or BNT (Figure 1B). Moreover, although individuals belonging to the homologous AZ/AZ or the heterologous AZ/BNT cohort appeared to have already reached the plateau of maximal avidity 90 days after their second vaccination (median = 69.26% and 95% CI = 66.77–71.25 or median = 73.15% and 95% CI = 70.99–74.68, respectively), this was significantly lower in the homologous BNT/BNT group. Maximum avidity in the homologous BNT/BNT study population was not detected until 6 months after the boost vaccination (median = 65.57%; 95% CI, 62.35–69.40). The observed association between vaccine regimen and avidity persisted even when we controlled for the effects of antibody concentration, age, and sex (Supplementary Table 3).

**DISCUSSION**

In this study, we investigated antibody responses after heterologous AZ/BNT vaccination compared with homologous AZ/AZ or BNT/BNT vaccination regimens over a 6-month period after the second vaccine dose. In line with other studies examining immune responses after SARS-CoV-2 infection or vaccination, we found declining levels of IgG antibodies against SARS-CoV-2 and increasing antibody avidities in all examined study cohorts over time [1, 4–7, 9]. In particular, we and others demonstrated that a second dose vaccination with an mRNA-based vaccine induces significantly higher titers of binding antibodies compared to a second dose with an adenoviral vector vaccine, irrespective of whether study participants had received primary immunization with AZ or BNT. However, a higher antibody avidity seems to correlate with the initial inclusion of an adenoviral vector vaccine, in this study AZ, within the vaccination regimen. After receiving their second vaccination with AZ or BNT, we found a remarkably higher avidity in study cohorts prevaccinated with AZ, irrespective of age and sex of study participants. By combining the advantages of early rise in avidity of antibodies after immunization with an adenoviral vector vaccine and higher levels of binding antibodies induced by at least 1 dose of an mRNA vaccine, a heterologous vaccination regimen therefore elicits a significant advantage to vaccinees over their homologous counterparts. We acknowledge that the effect size of the association between vaccine regimen and avidity was rather moderate.
as we adjusted for the effect of antibody concentration. However, the persistence of the statistical significance is worthy of note and needs further elaboration. Both elevated levels of binding antibodies as well as antibody avidity have recently been shown to correlate with virus neutralization [1, 7, 10]. It is noteworthy that antibody avidity after vaccination was associated with even broader recognition of SARS-CoV-2 epitopes and thus with an improved capacity to neutralize emerged SARS-CoV-2 variants of concern [1]. Our study has the limitation of a small sample size in the homologous BNT/BNT group, and sample size decreased in all study cohorts over time, because some study participants had already received their third vaccination outside the study or had a breakthrough infection and therefore had to be excluded. We recommend that future larger studies—also targeting risks of breakthrough infections across vaccine regimen—elaborate further on this finding. However, the observed statistical significance despite the small sample size delivered a strong insight into a potential impact of vaccination schedules on antibody avidity.

**CONCLUSIONS**

Hence, our study demonstrates the benefit of including at least 1 dose of an adenoviral vector in initial COVID-19 vaccination schemes to achieve a level of antibodies with significantly higher avidity. However, further studies investigating the avidity differences after a third shot and other heterologous vaccination schedules would be of value.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copy-edited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

Acknowledgments. We thank Albert Falch, Bianca Neurauter, Eva Hochmuth, Evelyn Peer, Lisa-Maria Raschbichler, Luiza...
Hoch, and Maria Huber for excellent technical support and organizational help. We also thank Sabine Embacher and Kathrin Becker from the Clinical Trial Center of the Medical University of Innsbruck for supporting the coordination of the study.

Financial support. This study was funded by the Medical University of Innsbruck, Austria, and the Austrian Science Fund (HOROS W-1253).

Potential conflicts of interest. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serological assays and Newcastle disease virus-based SARS-CoV-2 vaccines, which list Florian Krammer as coinventor. Mount Sinai has launched a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020) and is currently consulting for Pfizer, Seqirus, 3rd Rock Ventures, and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. R. W. received regular support from Pfizer to organize the annual Tyrolean Vaccination Day. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

HEVACC Study Group

David Bante, Barbara Falkensammer, and Helena Schäfer (Institute of Virology, Department of Hygiene, Microbiology and Public Health, Medical University of Innsbruck, Innsbruck, Austria); Florian Krammer (Department of Microbiology and Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY); Peter Pichler and Ursula Wiedermann (Institute of Specific Prophylaxis and Tropical Medicine, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria); Daniel Rainer (Hospital Schwaz, Schwaz, Austria); Tobias Trips and August Zabernigg (Hospital Kufstein, Kufstein, Austria).

References

1. Wratil PR, Stern M, Priller A, et al. Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern. Nat Med 2022; 28:496–503.
2. Seekircher L, Bánki Z, Kimpel J, et al. Immune response to 2-dose BNT162b2 vaccination and risk of SARS-CoV-2 breakthrough infection: the Shieldvacc-2 study. medRxiv [preprint] 2022.04.19.22273872. https://doi.org/10.1101/2022.04.19.22273872.
3. Bauer G. The potential significance of high avidity immunoglobulin G (IgG) for protective immunity towards SARS-CoV-2. Int J Infect Dis 2021; 106:61–4.
4. Pichler D, Baumgartner M, Kimpel J, et al. Marked increase in avidity of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibodies 7–8 months after infection is not diminished in old age. J Infect Dis 2021; 224:764–70.
5. Tauzin A, Gendron-Lepage G, Nayrac M, et al. Evolution of anti-RBD IgG avidity following SARS-CoV-2 infection. Viruses 2022; 14:532.
6. Borowa N, Bánki Z, Bates K, et al. Persistence of immunity to SARS-CoV-2 over time in the ski resort Ischgl. EBioMedicine 2021; 10:3534.
7. Bánki Z, Mateus J, Rössler A, et al. Heterologous ChAdOx1/BNT162b2 vaccination induces stronger immune response than homologous ChAdOx1 vaccination: the pragmatic, multi-center, three-arm, partially randomized HEVACC trial. eBioMedicine 2022; 80:104073.
8. Rose R, Neumann F, Grobe O, Lorentz T, Fickenscher H, Krummbholz A. Humoral immune response after different SARS-CoV-2 vaccination regimens. BMC Med 2022; 20:31.
9. Liu X, Shaw RH, Stuart ASV, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenviral vectored and mRNA COVID-19 vaccine (com-COV): a single-blind, randomised, non-inferiority trial. Lancet 2021; 398:856–69.
10. Pozzetto B, Legros V, Djebali S, et al. Immunogenicity and efficacy of heterologous ChAdOx1–BNT162b2 vaccination. Nature 2021; 600:701–6.