Neutralizing antibody responses 300 days after SARS-CoV-2 infection and induction of high antibody titers after vaccination

Doris Urlaub1, Natalie Wolfsdorff1, Jan-Erik Hoffmann2, Stefanie Dorok2, Markus Hoffmann3,4, Moritz Anft5, Naomi Piers1, Patrick Günther6, Bernhard Schaaf7,8, Uwe Cassens9, Peter Bröde1, Maren Claus1, Lea K. Picard1, Sabine Wingert1, Simone Backes10, Deniz Durak11, Nina Babel5, Stefan Pöhlmann3,4, Frank Renken6 and Carsten Watzl1

1 Department for Immunology, Leibniz Research Centre for Working Environment and Human Factors (IfADo) at TU Dortmund, Dortmund, Germany
2 Protein Chemistry Facility, Max Planck Institute of Molecular Physiology, Dortmund, Germany
3 Infection Biology Unit, German Primate Center - Leibniz Institute for Primate Research, Göttingen, Germany
4 Faculty of Biology and Psychology, Georg-August-University Göttingen, Göttingen, Germany
5 Center for Translational Medicine and Immune Diagnostics Laboratory, Marien Hospital Herne University Hospital of the Ruhr-University Bochum, Herne, Germany
6 Department of Structural Biochemistry, Max Planck Institute of Molecular Physiology, Dortmund, Germany
7 Department of Respiratory Medicine and Infectious Diseases, Klinikum Dortmund, Dortmund, Germany
8 Faculty of Health, University Witten/Herdecke, Herdecke, Germany
9 Institute for Transfusion Medicine, Laboratory Medicine and Medical Microbiology, Medical Center Dortmund, Dortmund, Germany
10 Institute for Virology and Immunobiology, University of Wuerzburg, Wuerzburg, Germany
11 Dortmund Health Department, Dortmund, Germany

Neutralizing antibodies against SARS-CoV-2 are important to protect against infection and/or disease. Using an assay to detect antibodies directed against the receptor binding domain (RBD) of SARS-CoV-2 Spike, we identified individuals with SARS-CoV-2 infection after an outbreak at a local health institution. All but one COVID-19 patient developed detectable anti-RBD antibodies and 77% had virus neutralizing antibody titers of >1:25. Antibody levels declined slightly over time. However, we still detected virus neutralizing antibody titers in 64% of the COVID-19 patients at >300 days after infection, demonstrating durability of neutralizing antibody levels after infection. Importantly, full COVID-19 vaccination of these individuals resulted in higher antibody titers compared to fully vaccinated individuals in the absence of prior infection. These data demonstrate long-lived antibody-mediated immunity after SARS-CoV-2 infection, and a clear benefit of two vaccine doses for recovered individuals.

Keywords: SARS-CoV-2 · COVID-19 · Vaccines · antibody titers · waning immunity

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Introduction

Most people develop antibodies against SARS-CoV-2 during infection, which not only provide immunity to reinfection but can be used to identify previously infected individuals [1]. For virus neutralization, antibodies directed against the SARS-CoV-2 spike protein and especially antibodies against the receptor binding domain (RBD) are important [2]. Therefore, most COVID-19 vaccines use the spike protein as an antigen and there is a good correlation between vaccine efficacy and the levels of neutralizing antibodies [3, 4], suggesting that antibody titers are important for immunity against SARS-CoV-2. Recently, the waning of antibody titers within 6 months after full vaccination has gained much attention, resulting in a reduced protection from symptomatic infection [5–9]. Therefore, booster vaccinations have been recommended in many countries. However, longitudinal data about the durability of antibody titers after SARS-CoV-2 infection and the effects of full vaccination of recovered individuals are scarce.

Here, we use an ELISA to detect anti-RBD-specific antibodies [10]. We used this assay to detect previously infected individuals in a local outbreak and to follow the stability of anti-RBD antibodies and their virus neutralizing activity up to 300 days after infection. Finally, our data show that full vaccination of recovered individuals at ≥300 days after SARS-CoV-2 infection results in higher antibody titers compared to fully vaccinated individuals without prior infection.

Results and discussion

The presence of SARS-CoV-2-spike-specific antibodies is a clear indication of a prior infection in the absence of vaccination. Therefore, we used an ELISA with the RBD of the spike protein of SARS-CoV-2 as an antigen [10], to test volunteers in a public health institution with several documented cases of SARS-CoV-2 infection early during the pandemic (March 2020). We defined three clusters: People in cluster one (n = 20) did have a PCR-confirmed SARS-CoV-2 infection (Supporting information Table S1) 48–93 days before sampling (mean 59 days). People in cluster two (n = 55) did not test positive by PCR but did have contact with individuals from cluster one. Finally, cluster three (n = 73) defined people without positive PCR and without contact to individuals in cluster one. All but one individual from cluster one tested positive for anti-RBD antibodies (Fig. 1A) and we found two individuals in each of the other clusters who had clearly detectable antibodies, strongly suggesting a prior infection that was not diagnosed by PCR. We tested the PCR-positive sample that gave a negative test result in our antibody assay with two different commercial anti-SARS-CoV-2 ELISA and also obtained a negative result. However, we could detect SARS-CoV-2-specific T-cell responses in a blood sample from this individual (data not shown), suggesting that he/she was indeed infected with SARS-CoV-2, but failed to produce detectable anti-RBD antibody levels. This is in line with other reports showing that a small percentage of SARS-CoV-2 infected individuals do not produce detectable antibodies [1]. These data

Figure 1. Detecting and examining the durability of anti-RBD antibody levels. (A) ELISA to detect SARS-CoV-2-specific antibodies using the RBD of the spike protein as antigen. Dotted lines indicate the thresholds for positive (>1) and negative (<0.5) signals. Twenty people from cluster 1 were tested positive by PCR, 55 people in cluster 2 had known interactions with cluster 1, 73 people in cluster 3 were not knowingly in contact with infected people. Dots are single values combined from a total of four assays. (B) Twenty-two of the individuals who were tested positive for antibodies during the first round of sampling (sample A) were tested again at about 150 days (sample B) and 300 days (sample C) after the infection. The anti-RBD antibody level calibrated to the WHO standard is displayed over time, the dotted line at 30.3 BAU/ml indicates the threshold for positive values on this scale. Eighteen PCR positive people from cluster 1 are shown in black (the date of the positive PCR test was set as timepoint 0) and four people without PCR confirmed infection in are shown in grey (mean timepoint of the outbreak was set to 0). (C) A simple linear regression was used to calculate the slopes that indicate the changes of signal intensity between the timepoints A to C. Changes are shown as individual values and the median as horizontal bar. (A) Measurements for cluster 1 and positive samples from cluster 2+3 are representative of three independent experiments. (B–C) Antibody titers were determined by measuring four different dilutions of each sample.
declined over time and in samples from 300 days post infection of the individuals (Fig. 2A). Also, virus neutralization activity was reduced in the samples 59 days after infection, we found full virus neutralization (
>90%) at 1:25 serum dilution in 77% (17 of 22) of the individuals (Fig. 2A). Also, virus neutralization activity declined over time and in samples from 300 days post infection we found >90% virus neutralization in 64% (14 of 22) of the individuals. When we compared anti-RBD antibody levels to the virus neutralization activity 300 days after infection, we observed a clear correlation. Anti-RBD antibody titers of >100 BAU/mL correlated with >90% virus neutralization activity at 1:25 serum dilution (Fig. 2B). However, anti-RBD antibody titers of below 100 BAU/mL did not show full virus neutralization, and the extent of virus neutralization was quite variable between individuals. This demonstrates that not all anti-RBD antibodies have neutralizing activity, but above a threshold of 100 BAU/mL our ELISA result could reliably predict virus neutralization. Neutralization assays were performed with the pseudotyped viruses carrying the spike protein of the original Wuhan strain of SARS-CoV-2, as the infections occurred before the emergence of variants of concern. While the data show a relative stability of neutralizing antibodies, neutralization of variants of concern, especially Omicron, would be much reduced.

As recommended, most of the previously infected individuals got vaccinated >300 days after their infection using different COVID-19 vaccines. In all cases, the vaccination resulted in a strong increase in anti-RBD titers (Fig. 3A–C), even in the individuals who failed to develop detectable antibodies after the infection (Fig. 3C). However, the titers differed depending on the vaccines used. Vaccination with AZD1222 resulted in a GMT of 895 BAU/mL (95% CI: 362–2215), heterologous vaccination with AZD1222 followed by BNT162b resulted in GMT 3929 BAU/mL (95% CI: 2235–6906), and vaccination with BNT162b2 resulted in GMT 4581 BAU/mL (95% CI 1999–10496). As most previously infected individuals received two vaccine doses, we were interested to compare the anti-RBD titers to fully vaccinated individuals without prior infection. Full vaccination of individuals without prior infection with AZD1222 resulted in a GMT of 238 BAU/mL (95% CI: 185–305), heterologous vaccination with AZD1222 followed by BNT162b resulted in GMT 1180 BAU/mL (95% CI: 972–1432), and vaccination with BNT162b2 resulted in GMT 1151 BAU/mL (95% CI 828–1600). Therefore, antibody titers were significantly higher in previously infected individuals after full vaccination compared to fully vaccinated individuals without any documented prior infection (Fig. 3D–F). While it may not be surprising that three antigen exposures induce stronger antibody responses [10], this contrasts with other studies that showed that a second vaccination of previously infected individuals did not result in higher antibody titers [16]. However, the long time (>300 days) between infection and vaccination may have contributed to higher antibody titers in our cohort [17].

Concluding remarks

Comparing the antibody titers after infection (Fig. 1) or vaccination (Fig. 3) clearly shows that COVID-19 vaccines can induce higher antibody titers compared to recovered individuals. As our ELISA shows a high correlation with neutralizing antibodies, this may suggest a higher protection from vaccination compared to infection. However, the decline of these antibody titers appears to be faster after vaccination [14], suggesting that recovered individuals retain their immunity for a longer period of time, which is in line with a recent report [18]. More importantly, immunizing recovered individuals with two doses of a COVID-19 vaccine about 300 days after the infection induces significantly higher antibody titers compared to vaccinated noninfected individuals. A recent report suggested that the decline of antibodies in
Immunity to infection

AzD inf. + AzD 10 100 1000 10000 100000 anti-RBD BAU/ml AzD+ BNT inf. + AzD+ BNT 10 100 1000 10000 100000 anti-RBD BAU/ml BNT inf. + BNT 10 100 1000 10000 100000 anti-RBD BAU/ml pre vac. 2x AzD 0.1 1 10 100 1000 10000 100000 anti-RBD BAU/ml pre vac. AZD+ BNT 0.1 1 10 100 1000 10000 100000 anti-RBD BAU/ml pre vac. 2x BNT

Figure 3. Comparison of titers after SARS-CoV-2 infection and/or vaccination. Previously infected individuals are shown before and after vaccination with two doses of the indicated vaccine combination (A, B, C). Comparison of titers after infection and vaccination versus vaccination only (D, E, F). Shown are values from 6 (A, D), 11 (B, E), 4 (C, F) infected individuals and 59 (D), 106 (E), and 29 (F) uninfected individuals after two doses of the indicated vaccines. Groups were compared using Wilcoxon test (A, B, C) or Mann-Whitney test (D, E, F). Horizontal lines in D, E, and F indicate the geometric mean. (A–F) Antibody titers were determined by measuring four different dilutions of each sample.

Vaccinated recovered individuals continue to be slower compared to vaccinated noninfected individuals [17], which would translate in a lasting immunity that could be stable for several years.

Materials and methods

Cell lines

Cells were incubated in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% (Hek 293T) or 5% (Vero) fetal bovine serum and penicillin/streptomycin (all from Gibco) at 37°C and 5% CO₂ in a humidified incubator and subcultured three times per week.

Serum samples

Samples were taken from venous blood or capillary blood from the fingertip into appropriate tubes (Monovettes or Microvottes with serum gel, Sarstedt). Serum was frozen at −80°C until use.

ELISA

The RBD sequence (spike glycoprotein amino acids 319–541) of the Wuhan strain of SARS-CoV-2 with a C-terminal HIS-tag was expressed in HEK 293-F cells and purified on a HisTrap Excel column using a ÄKTApure purification system. Ninety-six well flat bottom plates (maxisorp; Nunc) were coated with 3 μg/mL of SARS-CoV-2 spike RBD overnight at 4°C. Plates were washed, blocked with Biolegend ELISA diluent, and then incubated with serum samples diluted in blocking buffer. The S Antibody (humanized anti-Spike antibody by Dianova/Cusabio, Stock concentration: 0.3 mg/mL) was used as positive control (1:5000 final dilution) and calibrator (1:40 000 final dilution). As a secondary antibody, HRP conjugated anti-human IgG (Dianova), was used and signals were detected with 1 Step Ultra TMB (Pierce). The relative absorbance was calculated using the formula: (sample-negative control)/(calibrator-negative control). Values > 1 are positive, values < 1 but > 0.5 are borderline and should be repeated, and values < 0.5 are negative at a 1:100 dilution of the sample. To calculate antibody titers in BAU/mL, the samples were diluted from 1:100 to 1:12 500 and the dilution was calculated for which the relative absorbance would be 1. To do so, hyperbolic curves with the formula \( Y = B_{\text{max}} \times X / (K_d + X) \) were used.
Neutralization assay

VSV$^\Delta G$-fLuc were pseudotyped with SARS-CoV-2 spike using either the full-length Spike or a truncated spike ($\Delta 21AA$ C terminal) according to published methods [15, 19, 20]. Hek 293T cells were transfected with the pCG1-SARS-2-S or pCG1-SARS-2-S-trunc vector using Lipofectamine 2000 (Thermo Fisher Scientific). The next day, these cells were inoculated with a replication-deficient VSV$^\Delta G$-fLuc that contains expression cassettes for eGFP and firefly luciferase instead of the VSV-G open reading frame (kindly provided by Gert Zimmer, Institute of Virology and Immunology, Mittelhäusern, Switzerland). After 1 h at 37°C, the cells were washed and fresh medium containing an anti-VSV-G antibody (11, mouse hybridoma supernatant from CRL-2700; ATCC) was added to neutralize the VSV$^\Delta G$-fLuc input virus. SARS-CoV2 spike pseudotyped virus particles were harvested the next day, clarified by centrifugation, and frozen at −80°C until use.

Vero cells were seeded in 96-well plates with white walls and clear bottom to be about 70% confluent the next day. First, the pseudotyped virus with full length or truncated spike was preincubated for 30 min at 37°C with serum diluted at 1:25. Then medium was removed from Vero cells and 40 μL of pretreated virus was added per well. After 1 h at 37°C, 60 μL fresh medium was added and cells were incubated overnight. Neutralization was quantified by measuring firefly luciferase activity using a commercial substrate (Beetle-Juice, PJK), signal intensity from a sample with virus but without serum was interpreted as 0% and signal without virus as 100% neutralization.

Acknowledgments: We thank the rest of the Protein Chemistry Facility team for assistance during protein production, as well as Raphael Gasper and Petra Janning from MPI Dortmund for protein analysis. This work was supported in part by a grant from the Volkswagen Foundation (Grant number 98 579) to CW. Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest: The authors declare no conflict of interest.

Ethics approval statement for human studies: This study was approved by the ethics committee of IfADo (#178) and all participants gave informed consent.

Author contributions: D.U., N.W., N.P., M.C., L.P., S.W. planned and carried out the experiments; D.U., P.B., C.W. analyzed the data; M.A., U.C., N.B. provided T cell and additional antibody data; J.-E.H., S.D., P.G. produced and purified recombinant RBD; M.H., B.S., B.B., D.D, S.P, F.R. provided reagents and expertise; S.R. and C.W. supervised the project; and D.U., C.W. wrote the manuscript.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer review: The peer review history for this article is available at https://pubons.com/publon/10.1002/eji.202149758

References

1 Vanshylla, K., Di Cristanziano, V., Kleipass, F., Dewald, F., Schommers, P., Gieselmann, L., Gruell, H., et al., Kinetics and correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. Cell Host Microbe. 2021. 29: 917–929.

2 Amanat, F., Thapa, M., Lei, T., Ahmed, S. M. S., Adelsberg, D. C., Carreno, J. M., Strohmeier, S., et al., SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S. Cell 2021. 184: 3936–3948.

3 Khoury, D. S., Cromer, D., Reynaldi, A., Schlub, T. E., Wheatley, A. K., Juno, J. A., Subbarao, K., et al., Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat. Med. 2021. 27: 1205–1211.

4 Earle, K. A., Ambrosino, D. M., Fiore-Gartland, A., Goldblatt, D., Gilbert, P. B., Siber, G. R., Dull, P. and Plotkin, S. A., Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine 2021. 39: 4423–4428.

5 Tartof, S. Y., Slezak, J. M., Fischer, H., Hong, V., Ackerson, B. K., Ranasinghe, O. N., Frankland, T. B., 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: 1407–1416.

6 Fowlkes, A., Gagliani, M., Groover, K., Thiese, M. S., Tyner, H., Ellingson, K. and Cohorts, H.-R., Effectiveness of COVID-19 vaccines in preventing SARS-CoV-2 infection among frontline workers before and during B.1.617.2 (Delta) variant predominance—eight U.S. locations, December 2020–August 2021. MMWR Morb. Mortal. Wkly. Rep. 2021. 70: 1167–1169.

7 Self, W. H., Tenforde, M. W., Rhoads, J. P., Gagliani, M., Ginde, A. A., Douin, D. J., Olson, S. M., et al., Comparative effectiveness of moderna, pfizer-BionTech, and Janssen (Johnson & Johnson) vaccines in preventing COVID-19 hospitalizations among adults without immunocompromising conditions—United States, March-August 2021. MMWR Morb. Mortal. Wkly. Rep. 2021. 70: 1337–1343.

8 Chemaitelly, H., Tang, P., Hasan, M. R., AlMukdad, S., Yassine, H. M., Benslimane, F. M., Al Khathib, H. A., et al., Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. N. Engl. J. Med. 2021. 385: e83.

9 Goldberg, Y., Mandel, M., Bar-On, Y. M., Bodenheimer, O., Freedman, L., Haas, E. J., Milo, R., et al., Waning immunity after the BNT162b2 Vaccine in Israel. N. Engl. J. Med. 2021. 385: e85.

10 Urlaub, D., Wolfsdorff, N., Durak, D., Renken, F. and Watzl, C., SARS-CoV-2 infection shortly after BNT162b2 vaccination results in high anti-spike antibody levels in nursing home residents and staff. Immun. Inflamm. Dis. 2021. 9: 1702–1706.

11 Dan, J. M., Mateus, J., Kato, Y., Hastie, K. M., Yu, E. D., Faliti, C. E., Grifoni, A., et al., Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021. 371:eabf6063.
Immunity to infection

12 Wei, J., Matthews, P. C., Stoesser, N., Maddox, T., Lorenzi, L., Studley, R., Bell, J. I., et al., Anti-spike antibody response to natural SARS-CoV-2 infection in the general population. Nat. Commun. 2021. 12: 6250.

13 Wajnberg, A., Amanat, F., Firpo, A., Altman, D. R., Bailey, M. J., Mansour, M., McMahon, M., et al., Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 2020. 370: 1227–1230.

14 Tober-Lau, P., Schwarz, T., Vanshylla, K., Hillus, D., Gruell, H., Group, E. C. S., Suttrop, N., et al., Long-term immunogenicity of BNT162b2 vaccination in older people and younger health-care workers. Lancet Respir. Med. 2021. 9: e104–e105.

15 Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, T. S., et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020. 181: 271–280.

16 Krammer, F., Srivastava, K., Alshammary, H., Amoako, A. A., Awawda, M. H., Beach, K. F., Bermudez-Gonzalez, M. C., et al., Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N. Engl. J. Med. 2021. 384: 1372–1374.

17 Zhong, D., Xiao, S., Debes, A. K., Egbert, E. R., Caturegli, P., Colantuoni, E., and Milstone, A. M., Durability of antibody levels after vaccination with mRNA SARS-CoV-2 vaccine in individuals with or without prior infection. JAMA 2021. 326: 2524–2526.

18 Israel, A., Shenhar, Y., Green, I., Merzon, E., Golan-Cohen, A., Schaffer, A. A., Ruppin, E., et al., Large-scale study of antibody titer decay following BNT162b2 mRNA vaccine or SARS-CoV-2 infection. Vaccines (Basel) 2021. 10: 64.

19 Berger Rentsch, M. and Zimmer, G., A vesicular stomatitis virus replicon-based bioassay for the rapid and sensitive determination of multi-species type I interferon. PLoS One 2011. 6: e25858.

20 Whitt, M. A., Generation of VSV pseudotypes using recombinant DeltaG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. J. Virol. Methods 2010. 169: 365–374.

Abbreviations: BAU: binding antibody units · CI: confidence interval · GMT: geometric mean titer · RBD: receptor binding domain

Full correspondence: Prof. Carsten Watzl, Leibniz Research Centre for Working Environment and Human Factors (IfADo) at TU Dortmund, Ardeystrasse 67, 44139 Dortmund, Germany.
e-mail: wattl@ifado.de

Received: 7/12/2021
Revised: 2/7/2022
Accepted: 2/3/2022
Accepted article online: 5/3/2022