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BRIEF REPORT

Natural Killer Cell-Mediated Antibody-Dependent Cellular Cytotoxicity Against SARS-CoV-2 After Natural Infection Is More Potent Than After Vaccination

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We compared the ability of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike-specific antibodies to induce natural killer cell-mediated antibody-dependent cellular cytotoxicity (ADCC) in patients with natural infection and vaccinated persons. Analyzing plasma samples from 39 coronavirus disease 2019 (COVID-19) patients and 11 vaccinated individuals, significant induction of ADCC could be observed over a period of more than 3 months in both vaccinated and recovered individuals. Although plasma antibody concentrations were lower in recovered patients, we found antibodies elicited by natural infection induced a significantly stronger ADCC response compared to those induced by vaccination, which may affect protection conferred by vaccination.

Keywords. ADCC; COVID-19; NK cell; mRNA vaccine.

In response to the coronavirus disease 2019 (COVID-19) pandemic, extensive research efforts have led to the rapid development of several safe and effective vaccines. It is not yet clear to what extent and for how long a previous infection or vaccination protects against (re-)infection or disease. However, increasing data indicate that immunity declines over time after both natural infection [1] and vaccination [2]. In a recently published study, natural immunity was shown to confer longer-lasting and stronger protection against infection and symptomatic disease than immunity induced by 2 doses of the BioNTech/Pfizer mRNA BNT162b2 vaccine [3]. The mechanisms underlying these differences remained unclear but it is tempting to speculate that a more pronounced immune response triggered by natural infection compared to vaccination-induced immune activation may play a role here.

In both postinfection patients and vaccinated individuals, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific antibodies are important in mediating immunity. In particular, neutralizing antibodies that can directly block infection appear to be associated with protective immunity [4]. However, the development and persistence of neutralizing antibodies seems to vary according to the degree of disease severity [5]. Beyond neutralization, antibodies can also provide antiviral protection by recruiting complement and/or Fc receptors present on all immune cells. These extraneutralizing functions include the ability to activate antibody-dependent cellular cytotoxicity (ADCC) [6]. Natural killer (NK) cells, a heterogeneous lymphocyte subset, are a main mediator of ADCC. Here, binding of NK cell-expressed FcγRIII (CD16) to the Fc part of an antibody bound to an antigen on a target cell induces NK-cell activation and subsequent killing of the target cell. NK-cell–mediated ADCC has been shown to contribute to both natural and vaccine-induced antiviral immunity [7] and recent studies suggest ADCC also plays a role in natural SARS-CoV-2 infection [8]. The extent to which mRNA-based COVID-19 vaccination can induce NK-cell–mediated ADCC and whether this differs from natural infection is currently unclear and was therefore investigated in the present study.

METHODS

Study Design

A total of 39 patients with natural SARS-CoV-2 infection (female sex, 17/39 [44%]; mean age, 57 years [range, 25–83 years]) and 11 healthcare workers vaccinated with mRNA-1273 (ModernaTX) (female sex, 5/11 [45%]; mean age, 39 years [range, 27–60 years]) were enrolled into this study after written informed consent was obtained. All vaccinated donors had tested negative for SARS-CoV-2 infection multiple times prior to study inclusion. In addition, all plasma samples from this group were tested negative for nucleocapsid protein antibodies, further ruling out a history of SARS-CoV-2 infection. COVID-19 patients were stratified according to the World Health Organization Ordinal Scale for Clinical Improvement, with grade 2 to 4 being considered moderate disease and 5 to 8 severe disease.

In COVID-19 patients, blood samples were obtained between day 3 and 244 after onset of symptoms; 12 of these patients were sampled 2 times, once during active infection and once at the post-COVID unit, making it a total of 51 samples.

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In the vaccine group, blood samples were analyzed at 4 different time points: day 1–7, day 21–28, day 48–70, and day 105–127 after the first dose. All individuals received the second dose on day 28 after the first vaccination. As a control, historic samples collected before December 2019 were used.

This study was approved by the Institutional Review board of the University Hospital Bonn (073/19 and 134/20).

Plasma and NK-Cell Isolation

Plasma was isolated from blood collected in LiHep tubes (Sarstedt) by centrifugation. Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood using Ficoll-Paque. Then, NK cells were isolated from total PBMC using the MACS NK Cell Isolation Kit (Miltenyi) according to the protocol. NK cells were isolated from a single unvaccinated, uninfected individual.

ADCC Assay

SARS-CoV-2 spike protein or SARS-CoV-2 nucleocapsid protein (SinoBiological) were coated onto 96-well flat-bottom plates (Greiner Bio-One). After incubation at 4°C overnight, blocking with fetal calf serum for 1 hour, and washing with phosphate-buffered saline, plates were incubated with plasma at different concentrations at 4°C overnight. Following further washing steps, purified NK cells (50 000 per well) and anti-CD107a–fluorescein isothiocyanate (BD Biosciences) were added. After 1 hour, Golgi-Stop (BD Biosciences) and Brefeldin-A (Enzo Life Sciences) were added to each well. Extra- and intracellular staining was performed before/after fixation and permeabilization with the Cytofix/Cytoperm Kit (BD Biosciences). Cells were stained using anti-CD56–allophycocyanin (APC), anti-CD3–APCfire, anti-CD16–peridinin chlorophyll, and ZombieAqua (all obtained from Biolegend) and analyzed on a BD FACSCanto II.

PMA/Ionomycin (Cell Signaling Technology) stimulated cells were used as positive controls. NK cells cultured without plasma or antigen and NK cells alone served as negative controls.

Statistical Analyses

All statistics were performed using GraphPad Prism 9.1.2. Appropriate tests were selected to compare normal or nonnormal distributed values, paired or unpaired samples. Differences between time points were analyzed by Kruskal-Wallis test corrected for multiple comparisons by controlling false discovery rate (Benjamini, Krieger, Yekutieli). $P < .05$ was considered significant.

Antibody Quantification and Neutralization Assay

Plasma samples were analyzed using the V-Plex COVID-19 Serology Assay Kit and the V-Plex COVID-19 ACE2 Neutralization Assay Kit (Meso Scale Discovery) according to the manufacturer's protocol.

RESULTS

First, we analyzed plasma antibody levels in vaccinated persons at 4 different time points. Significant anti–SARS-CoV-2 spike immunoglobulin G (IgG) levels were detectable 3 to 4 weeks after the first vaccination, peaked around day 60 (after the second dose was administered), and then decreased again (Figure 1A). Similar observations were made regarding the concentrations of neutralizing anti–SARS-CoV-2 spike IgG (Figure 1A) and the neutralizing capacity of these antibodies (Figure 1B).

Next, we sought to examine the antibodies' ability to mediate NK-cell activation via ADCC. To this end, different dilutions of patient plasma were incubated with plate-bound SARS-CoV-2 spike protein. After washing, purified circulating NK cells from a healthy control were added and induction of CD107a as a marker for NK-cell degranulation as well as interferon-γ (IFN-γ) production were measured. Using this approach, we found plasma from COVID-19 patients to trigger NK-cell cytotoxicity as well as IFN-γ production in a dose-dependent manner, with induction of significant NK-cell CD107a expression at a 1:100 dilution in all tested samples. In contrast, control plasma sampled before 2019 did not affect degranulation, excluding an unspecific effect (Supplementary Figure 1A–1D). Based on these findings, a dilution of 1:100 was used in all further experiments.

In vaccinated individuals, robust induction of ADCC and IFN-γ production was observed 3 to 4 weeks after the first dose of vaccination and remained stable for more than 100 days (Figure 1C). Both plasma-induced NK-cell cytotoxicity and IFN-γ production correlated positively with SARS-CoV-2 spike-specific antibody concentrations (Figure 1D).

In COVID-19 patients, highest plasma concentrations of anti–SARS-CoV-2 spike levels were detected between weeks 2 and 4 after symptom onset. Over time, there was a gradual decrease in antibody levels, but significant antibody concentrations were still detectable after more than 100 days (Figure 2A).

COVID-19 plasma samples obtained during the first few days after onset of symptoms did not trigger ADCC. Beginning after week 1, however, a significant induction of NK-cell cytotoxicity and IFN-γ production was found, which reached its peak at around 10–20 days after first symptoms occurred. Over time, the capacity of COVID-19 plasma to induce NK-cell–mediated ADCC waned but was still visible in the majority of patients beyond day 100 (Figure 2A). Both antibody levels and functional NK-cell responses tended to be higher in patients with severe disease compared to those with moderate COVID-19 in the first weeks after symptom onset. However, this was not statistically significant. No such differences were found in the samples analyzed at later time points (Figure 2B).

Of particular interest, however, was the comparison between the 2 groups. Here, we observed that plasma from recovered patients had lower antibody levels on average but was more effective in inducing NK-cell–mediated ADCC and

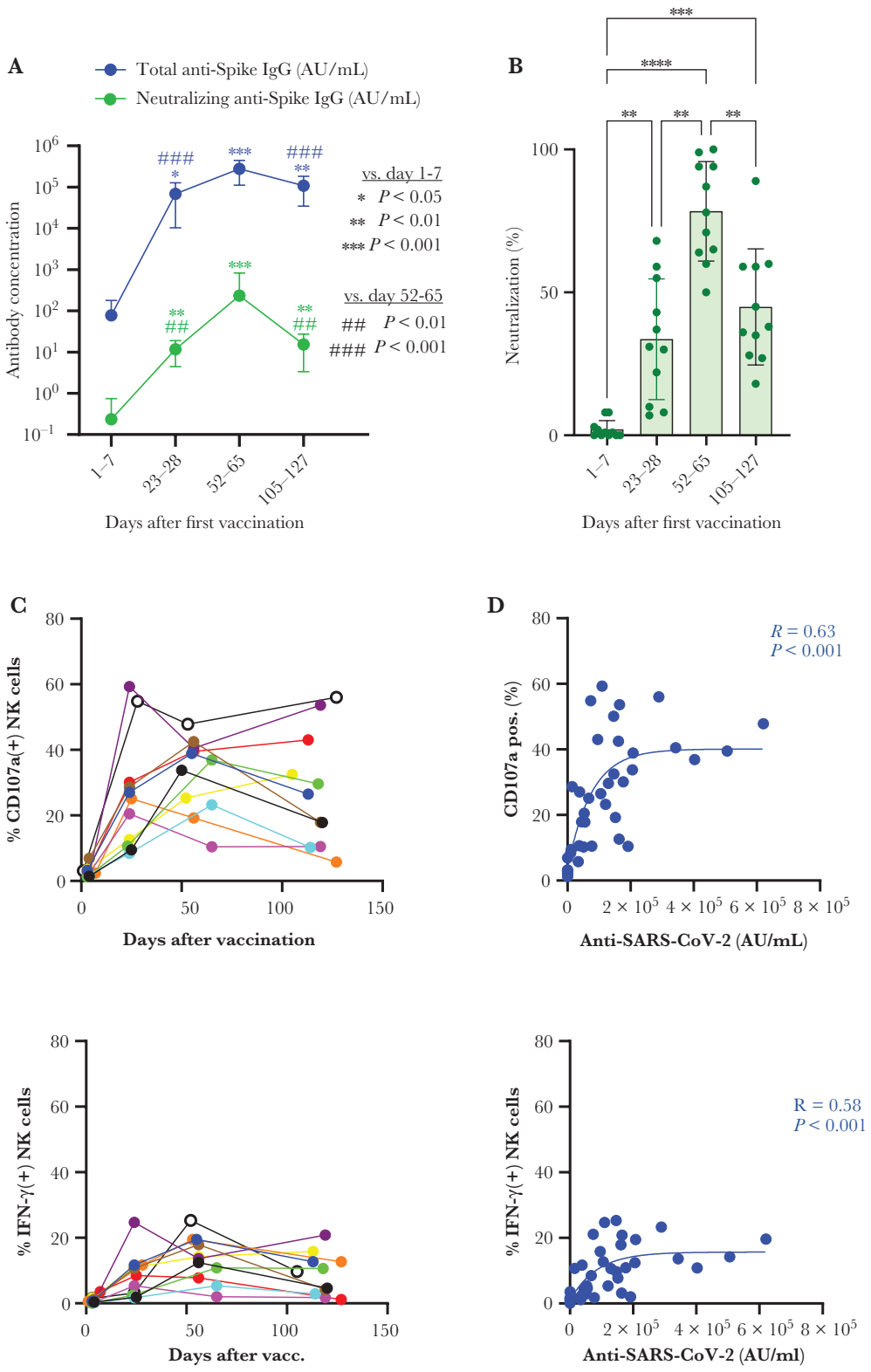

Figure 1. Antibody concentrations and induction of NK-cell–mediated ADCC in vaccinated individuals. Plasma concentrations of total and neutralizing anti-spike IgG (*A*) as well as neutralization capacity of antibodies (*B*) were analyzed at 4 different time points in 11 vaccinated persons. Columns show mean with SD. *C*, Anti-spike–induced NK-cell degranulation and IFN-γ production were analyzed as a surrogate of ADCC using plasma from vaccinated donors at a 1:100 dilution. Each color represents a single individual (n = 11). *D*, Correlation between antibody-induced NK-cell degranulation or IFN-γ production and total anti-spike concentration in vaccinated individuals (n = 11). * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; IFN-γ, interferon-γ; IgG, immunoglobulin G; NK, natural killer; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

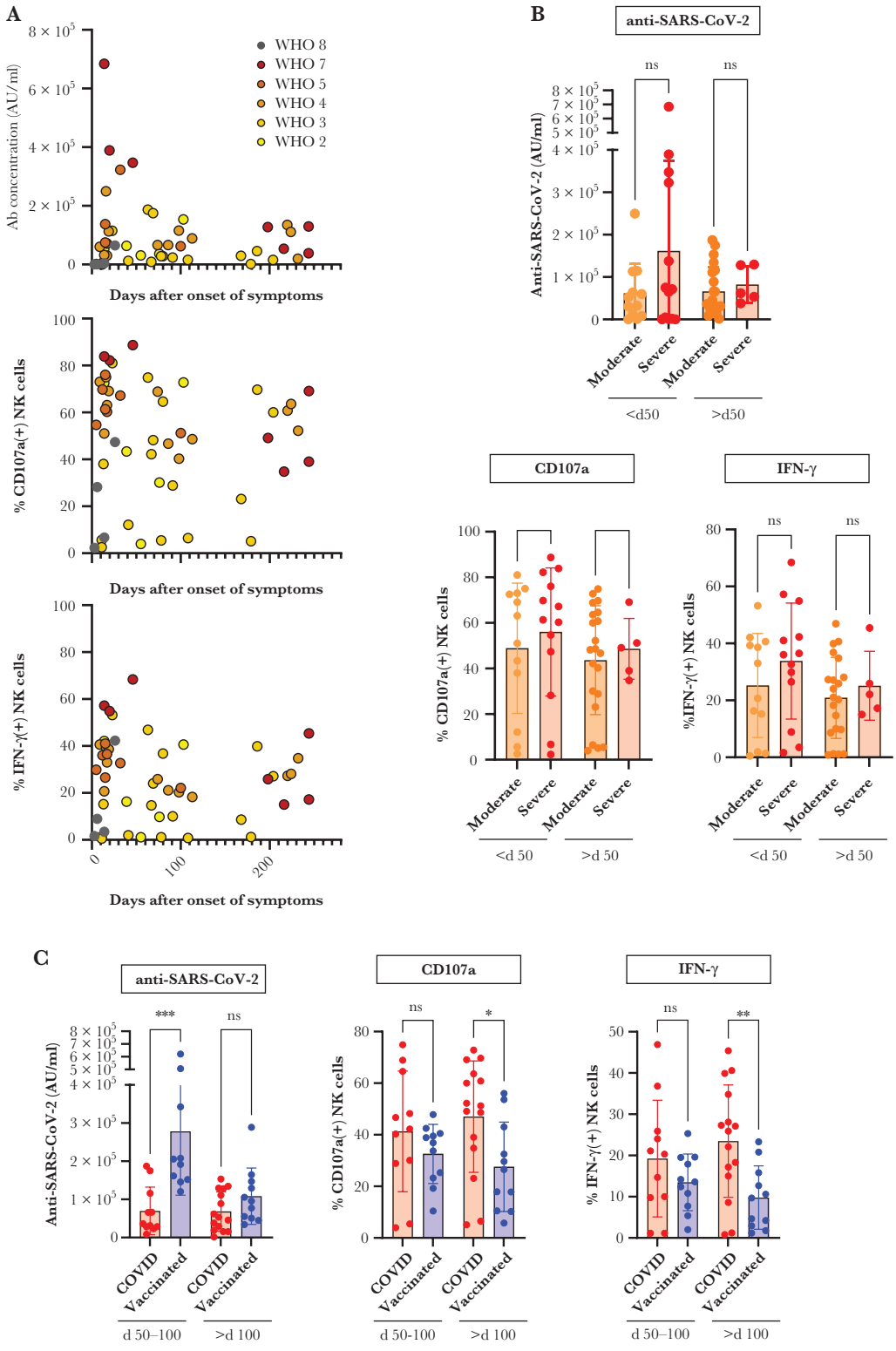

Figure 2. Anti–SARS-CoV-2 spike plasma concentrations and induction of NK-cell–mediated ADCC in natural infection. *A*, Plasma anti-spike concentrations in COVID-19 patients' plasma samples and NK-cell activation assessed as CD107a or IFN-γ expression (n = 51) at a 1:100 dilution, over time. The color of the dots indicates the maximum score on the WHO ordinal scale of the respective patient. *B*, Comparison of anti–SARS-CoV-2 spike plasma concentrations and induction of NK-cell–mediated ADCC measured by CD107a or IFN-γ expression in patients with moderate and severe COVID-19 before (moderate, n = 12; severe, n = 13) or beyond day 50 (moderate, n = 21; severe, n = 5) after onset of symptoms. *C*, Plasma concentrations of total anti-spike and the ability of spike-specific antibodies to induce NK-cell–mediated ADCC and IFN-γ production were analyzed in recovered COVID-19 patients (n = 26) and vaccinated individuals (n = 11) between day 50 and 100 and beyond day 100 after onset of symptoms or first vaccination, respectively. Columns show mean with SD. * $P < .05$, ** $P < .01$, *** $P < .001$. Abbreviations: Ab, antibody; ADCC, antibody-dependent cellular cytotoxicity; COVID-19, coronavirus disease 2019; IFN-γ, interferon-γ; NK, natural killer; ns, not significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

IFN-γ production than plasma from vaccinated individuals (Figure 2C). This difference was particularly prominent in samples collected more than 100 days after onset of symptoms or first vaccination.

Taken together, our data indicate a stronger and longer lasting induction of NK-cell–mediated ADCC after natural SARS-CoV-2 infection than after vaccination.

DISCUSSION

In the present study, we investigated the extent and duration of NK-cell–mediated ADCC induced by mRNA-based COVID-19 vaccination compared to natural infection. Our data show that antibodies capable of inducing NK-cell-mediated ADCC are elicited in recovered patients as well as after vaccination, and that this can still be detected more than 3 months after the onset of disease or vaccination. Of particular interest was the observation that, despite having similar antibody levels, these antibodies were less effective in inducing ADCC in vaccinated compared to recovered individuals. The underlying mechanisms are still unclear at the moment. However, it seems plausible that specific characteristics of the induced antibodies play an important role.

The effector function of antibodies is modulated by *N*-linked glycosylation in the Fc region of the antibody. In particular, the absence of nuclear fucose on the Fc-N glycan increases the binding affinity of IgG1-Fc to the FcγRIIIa on NK cells and results in enhanced ADCC activity [9, 10]. Thus, differences in fucosylation of vaccine-induced versus infection-induced antibodies would be a possible explanation here. Accordingly, it has been shown that high titers of antibodies with low fucosylation can be found, especially in severe COVID-19 [8, 11]. Further studies are warranted to address this issue.

In the present study, plasma samples obtained from patients with severe disease in the first weeks after symptom onset tended to induce a stronger ADCC response than those from individuals with moderate COVID-19. Although these differences did not reach statistical significance, they are similar to results from a previous study that also found increased ADCC activity in severe disease [12]. The authors, therefore, discussed that the ADCC response might contribute to immunopathogenesis in COVID-19. However, a significant difference between patients with moderate and severe COVID-19 was only found between day 21 and 30 after symptom onset. In addition, Yu et al [12] showed that patients who survived severe disease had higher ADCC activity than those who died, which might indicate a beneficial effect of ADCC. Indeed, several studies have shown that ADCC is important for an effective antiviral immune response [7]. Therefore, our finding of reduced triggering of ADCC following vaccination may contribute to the recent observation that vaccine-induced immunity provides less-effective protection against infection and symptomatic disease than immunity induced by natural infection. However, it is likely that multiple mechanisms are relevant here. In contrast to vaccination, the immune response after infection is not limited to the spike protein. For example, in recovered patients we also found ADCC induction by nucleocapsid protein-binding antibodies, whereas this was not the case in vaccinated individuals (Supplementary Figure 2).

In our study, we compared the ability of antibodies induced by vaccination or infection to trigger NK-cell–mediated ADCC. Therefore, we used NK cells from a healthy, nonvaccinated donor as effector cells to exclude a possible effect of qualitative or quantitative differences of the NK-cell pool.

Recent work, however, indicates that alterations in lymphocyte functions may persist in convalescent COVID-19 patients [13]. We recently demonstrated severe COVID-19 to be associated with marked impairment of NK-cell activity, which was still detectable more than 3 weeks after symptom onset [14]. Whether such an NK-cell dysfunction persists in patients that recovered from COVID-19 and how this may affect the ability of NK cells to mediate ADCC is currently unclear and warrants further research.

In our study, we used antibody binding to plate-bound spike protein as a surrogate assay. Therefore, the question may arise whether our findings reflect the in vivo situation, because here recognition of the antigen at the surface of the infected cells is required. However, in a recent study, Ding et al demonstrated antibody binding to spike protein expressed at the surface of infected cells [15].

In summary, our data demonstrate that both after natural infection as well as after vaccination SARS-CoV-2–specific antibodies capable of inducing NK-cell–mediated ADCC can be detected, but suggest that antibodies elicited in infected patients are more effective in triggering ADCC.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. G. R. received travel expenses and honoraria from Gilead. S. S. received travel expenses and congress fees from Abbvie, Gilead, and Johnson & Johnson not related to the topic of the manuscript; and holds shares from BionTech (Fonds-based). E. L. is a cofounder and consultant to IFM Therapeutics and Odyssey Therapeutics; and is cofounder and chairman of the board of Dioscure Therapeutics. C. B. has received honoraria for lectures and/or consultancies from AbbVie, Gilead, Janssen, MSD, and ViiV. J. K. R. has received honoraria for consulting or speaking at educational events from Abivax, Galapagos, Gilead, Janssen, MSD, NPO Petrovax Pharm LLC, Theratechnologies, and ViiV.

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.

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