Research Article

Natural killer cell-mediated ADCC in SARS-CoV-2-infected individuals and vaccine recipients

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COVID-19, caused by SARS-CoV-2, has emerged as a global pandemic. While immune responses of the adaptive immune system have been in the focus of research, the role of NK cells in COVID-19 remains less well understood. Here, we characterized NK cell-mediated SARS-CoV-2 antibody-dependent cellular cytotoxicity (ADCC) against SARS-CoV-2 spike-1 (S1) and nucleocapsid (NC) protein. Serum samples from SARS-CoV-2 resolvers induced significant CD107a-expression by NK cells in response to S1 and NC, while serum samples from SARS-CoV-2-negative individuals did not. Furthermore, serum samples from individuals that received the BNT162b2 vaccine induced strong CD107a expression by NK cells that increased with the second vaccination and was significantly higher than observed in infected individuals. As expected, vaccine-induced responses were only directed against S1 and not against NC protein. S1-specific CD107a responses by NK cells were significantly correlated to NK cell-mediated killing of S1-expressing cells. Interestingly, screening of serum samples collected prior to the COVID-19 pandemic identified two individuals with cross-reactive antibodies against SARS-CoV-2 S1, which also induced degranulation of NK cells. Taken together, these data demonstrate that antibodies induced by SARS-CoV-2 infection and anti-SARS-CoV-2 vaccines can trigger significant NK cell-mediated ADCC activity, and identify some cross-reactive ADCC-activity against SARS-CoV-2 by endemic coronavirus-specific antibodies.

Keywords: ADCC · COVID-19 · Innate immunity · NK cells · Vaccine

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

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible human betacoronavirus and the cause of the global coronavirus disease 2019 (COVID-19) pandemic. While some individuals infected with SARS-CoV-2 develop severe COVID-19, including acute respiratory distress syndrome and multi-organ failure, most individuals experience only mild-to-moderate disease or asymptomatic infection [1]. The precise mechanisms by which innate and adaptive immune responses mediate this heterogeneous outcome of SARS-CoV-2 infection are not sufficiently understood. Increasing data suggest that the rapid development of antiviral antibody and CD8+ T-cell responses is associated with better COVID-19 outcome [2–5], and that SARS-CoV-2 spike 1 (S1) protein-specific antibodies and T-cell responses are responsible for the efficient protection from disease mediated by SARS-CoV-2 vaccines [6, 7]. Neutralizing antibodies against SARS-CoV-2, especially directed at the receptor binding domain (RBD) of S1, have been implicated in protective immunity following vaccination [8]. However, many individuals recovering from COVID-19 only develop relatively low titers of neutralizing SARS-CoV-2 antibody responses [9, 10], and studies have shown that the strongest antibody responses are observed in individuals with severe COVID-19 [11]. These data indicate that neutralizing antibodies may be more critical for protection against SARS-CoV-2 infection than for the resolution of disease [12]. In addition to their ability of neutralizing viruses, virus-specific antibodies can also provide functional antiviral activity through the binding to Fc receptors expressed on immune cells [12], including antibody-dependent cellular phagocytosis and antibody-dependent cellular cytotoxicity (ADCC).

During ADCC, virus-specific antibodies bind to viral antigens present on the surface of infected cells, and recruit cytotoxic effector cells, in particular NK cells, through their CD16 receptor (FcγIII-Receptor) [13]. CD16-mediated activation of NK cells results in degranulation with the release of cytotoxic molecules such as perforin and granzyme [14, 15]. Antiviral activity mediated by ADCC has been described for several viral infections, including HIV-1, influenza, and Ebola [16–18]. Furthermore, studies using the rhesus macaque model for SIV infection have shown that antibody-mediated protection is reduced when the Fc-fraction of neutralizing antibodies is cleaved [19], suggesting that Fc-mediated antiviral activity is an important additional effector function of antibodies. While the presence of antibodies that can mediate ADCC has been described in convalescent plasma of patients with COVID-19 [20, 21], less is known about the induction of functional antibodies that can mediate ADCC by currently used vaccines against SARS-CoV-2. Furthermore, several papers have described cross-reactivity of antibodies induced by common endemic coronaviruses against SARS-CoV-2 [3, 22, 23], and also suggested that these pre-existing cross-reactive antibodies might be associated with less severe COVID-19 [24]. However, the ability of these antibodies to mediate antiviral function against SARS-CoV-2 remains unknown. Here, we describe the presence of functional SARS-CoV-2-specific antibodies that can mediate ADCC in the convalescent serum of COVID-19 patients and in some longitudinal serum samples collected prior to the COVID-19 pandemic. We furthermore show induction of strong S1 protein-specific ADCC activity of NK cells in response to serum collected from individuals vaccinated with the BNT162b2 SARS-CoV-2 vaccine, which exceeded the ADCC activity of convalescent antibodies even at high dilutions. Taken together, these data provide novel insights into NK cell-mediated ADCC in COVID-19 and the induction of functional antibody responses by SARS-CoV-2 vaccination.

Results

Antibodies induced by COVID-19 can trigger CD16-mediated NK cell responses

NK cells can lyse virus-infected cells by ADCC mediated by the FcγIII-Receptor, also known as CD16, upon binding to the Fc-part of IgG antibodies [13]. Here, we established a degranulation assay that uses CD107a, a sensitive marker of NK cell degranulation [25], to measure CD16-mediated NK-cell activation. Serum samples of PCR-confirmed SARS-CoV-2 resolvers were screened for specific antibodies against the SARS-CoV-2 S1/S2 spike ($n = 15$) and nucleocapsid (NC) protein (14 out of 15 tested), and as expected, significantly higher antibody titers were detected in these COVID-19 resolvers compared to healthy control individuals ($n = 15$) (Fig. 1A). To quantify ADCC activity mediated by SARS-CoV-2-specific antibodies, 96-well plates were coated with either the SARS-CoV-2 spike 1 (S1) or NC protein and subsequently incubated with serum samples derived from COVID-19 resolvers or healthy control individuals to identify serum antibodies binding to S1 and NC. Fresh NK cells isolated from healthy donor PBMC were added and NK cells were analyzed for CD107a expression following a 4-h incubation period. CD107a expression by NK cells was significantly higher following incubation with serum of SARS-CoV-2 resolvers compared to SARS-CoV-2-negative control individuals (Fig. 1B, Supporting Information Fig. S1), and SARS-CoV-2 S1-specific ADCC activity was significantly higher than NC-specific ADCC activity in SARS-CoV-2 resolvers. SARS-CoV-2 S1- and NC-specific ADCC activity was also detected in COVID-19 resolvers with low SARS-CoV-2-specific antibody titers. Furthermore, statistical analysis revealed a strong negative correlation between CD107a expression and CD16 downregulation on NK cells (Spearman's rho = –0.97, *p* value = 2.6×10^{-20}, Supporting Information Fig. S2A). Serum samples of healthy control individuals did not induce CD107a expression of NK cells in response to S1, but samples from some individuals triggered low levels of CD107a expression of NK cells against NC. It has previously been described that the SARS-CoV-2 NC protein is more conserved than the S1 protein, and that it has some homologies to other endemic occurring coronaviruses [26, 27], potentially explaining these low-level responses against NC in SARS-CoV-2-negative individuals. Taken together, these data demonstrate that antibodies induced by
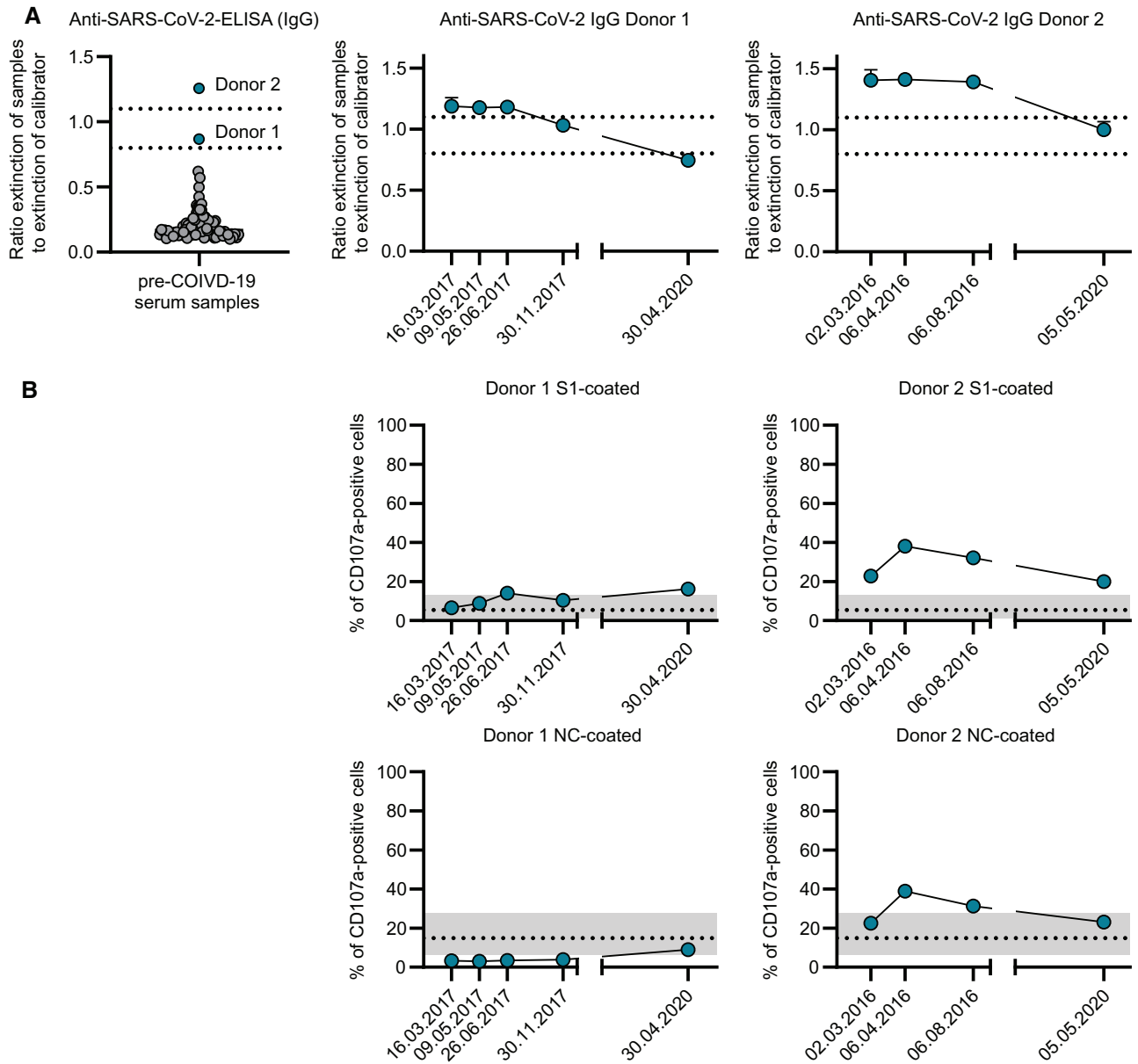
Figure 1. Antibodies against the SARS-CoV-2 spike 1 (S1) and nucleocapsid (NC) protein induce NK cell degranulation. (A) Spike and NC antibody titers were determined by Ig sandwich assays. Results in confirmed COVID-19 resolvers were compared to serum samples of healthy individuals. Squared data points represent results outside the assay detection limit (3.8 and 400 AU/mL). Cut off for positive results was set to 15 AU/mL and 1 COI. (B) Statistical comparison of CD107a expression by NK cells in response to SARS-CoV-2 S1 and NC protein-directed serum antibodies between confirmed SARS-CoV-2 resolvers ($n = 15$) and SARS-CoV-2-negative ($n = 15$) individuals. The experimental setup contained duplicates and triplicates and median values including the IRQ are shown. For resolver and healthy individuals, replicates were combined to single data points. Statistical analysis was performed using nonparametric Mann–Whitney test (****$p < 0.0001$).

COVID-19 can induce significant ADCC responses mediated by NK cells.

Antibodies from some pre-COVID-19 samples can induce NK cell activation against SARS-CoV-2 S1

Previous studies have described the existence of cross-reactive antibodies against SARS-CoV-2 in pre-COVID-19 sera [3, 22, 23], and the above data indicated some cross-reactivity of sera from COVID-19 negative individuals with the NC protein of SARS-CoV-2. To determine whether serum samples collected prior to the COVID-19 pandemic can induce NK cell-mediated ADCC activity against SARS-CoV-2 S1, we screened 85 serum and plasma samples that were collected before the first description of the SARS-CoV-2 outbreak, using the EUROIMMUN anti-SARS-CoV-2 S1 IgG ELISA. These studies identified two individuals (Donors 1 and

2) with detectable cross-reactive responses against SARS-CoV-2 (Fig. 2A). Donor 2 exhibited responses above the cut-off provided by EUROIMMUN for a positive result, while Donor 1 exhibited responses within the range of marginal positive responses (Fig. 2A). Subsequent analysis using the EUROIMMUN assay of longitudinal samples collected between 2013 and 2018 from both donors revealed antibodies reacting against SARS-CoV-2 S1, and demonstrated stable responses over several months that subsequently started to decline to levels below the cut-off for positive responses in 2020 (Fig. 2A). In addition, both donors were screened using the validated *recom*Line assay for antibodies against endemic human coronaviruses to provide information about previous seasonal infections. Both donors had significant antibody titers indicating a resolved betacoronavirus infection in Donor 1 and alphacoronavirus infection in Donor 2 at the time point of sample collection (Supporting Information Fig. S3). Antibody titers declined over time, in line with the observed

Figure 2. Anti-SARS-CoV-2 cross-reactive antibodies found in pre-COVID-19 serum are capable of inducing NK cell degranulation. (A) Anti-SARS-CoV-2 Spike 1 ELISA confirmed cross-reactive IgG antibodies in two pre-COVID-19 collected serum samples, with decreasing antibody titers over time. Dotted lines mark the distributer's range for negative (0.8) and positive (1.1) results (*n* = 85). (B) Pre-COVID-19 cross-reactive antibodies stimulated CD107a expression by NK cells. Dotted lines mark the mean value and the gray area represents the range of results observed in SARS-CoV-2-negative serum samples. Experiments were performed using triplicates and replicates were combined into single data points.

anti-SARS-CoV-2 IgG levels. In order to determine whether these antibodies detected in the EUROIMMUNE assay were able to mediate activation of NK cells in response to S1 and NC protein, we used these samples in the NK cell degranulation assay described above. While the low-level antibodies detected in Donor 1 did not induce NK cell degranulation in response to S1 and NC protein, serum samples of Donor 2, who had stronger antibody responses against SARS-CoV-2 S1 in the ELISA, also induced strong CD107a expression of NK cells in S1-coated plates (Fig. 2B). Samples were run in the same assay as those described

in Fig. 1, allowing comparison of responses between samples from pre-COVID-19 and COVID-19 resolvers. While CD107a expression of NK cells in Donor 2 was lower than those observed in COVID-19 resolvers, responses were considerably higher compared to healthy control individuals in response to S1. Results observed in Donor 1 were comparable to those seen in SARS-CoV-2-negative individuals. Overall, these results provide evidence for the presence of SARS-CoV-2 cross-reactive antibodies that can mediate functional NK cell responses in samples collected prior to the COVID-19 pandemic in some rare individuals.
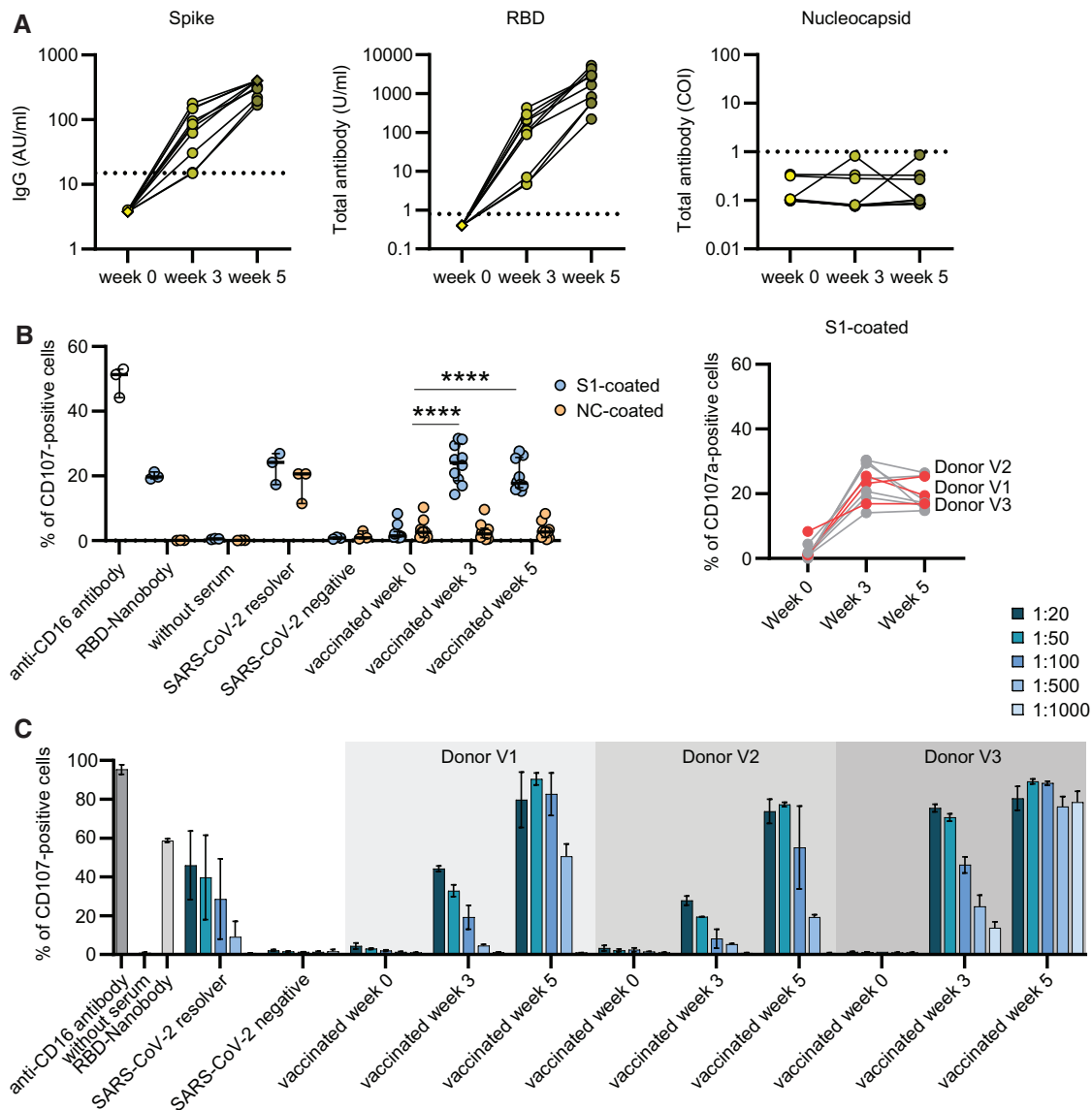
Figure 3. BNT162b2 vaccine-induced SARS-CoV-2 spike 1-directed serum antibodies trigger NK cell degranulation. (A) Longitudinal antibody titers against spike, RBD and NC were determined by Ig sandwich assays in BNT162b2-vaccinated health-care workers ($n = 10$). (B) CD107a expression by NK cells in response to SARS-CoV-2 S1 and NC protein-directed serum antibodies in vaccinated individuals compared to SARS-CoV-2 resolvers ($n = 3$) and healthy control individuals ($n = 3$). Samples of vaccinated individuals were each collected on the day of the first vaccination (week 0), the day of the second vaccination (week 3), and 2 weeks after the second vaccination (week 5). As CD107a levels remain constant between weeks 3 and 5, a selection of donors ($n = 3$, marked in red) was titrated in a follow-up CD107a degranulation assay. The experimental setup contained triplicates and median values and IRQ are shown. For resolver, healthy, and vaccinated individuals, replicates were combined into single data points. Statistical analysis was performed using nonparametric Mann–Whitney test (****$p < 0.0001$). (C) CD107a expression by NK cells after serial dilution (1:20, 1:50, 1:100, 1:500, 1:1000) of vaccinated ($n = 3$), SARS-CoV-2 resolver ($n = 2$), and SARS-CoV-2-negative ($n = 1$) serum samples.

BNT162b2 vaccination induces highly functional antibodies that mediated strong NK cell activation

Since December 2020, a number of SARS-CoV-2 vaccines have been approved in Germany, among which the BNT162b2 vaccine by Pfizer/BioNTech represented the first [28]. Using serum samples longitudinally collected from health-care workers receiving the BNT162b2 vaccine at the University Medical Center Hamburg-Eppendorf, we investigated the ability of vaccine-

induced antibodies to trigger NK cell-mediated ADCC using the CD107a degranulation assay. Serum samples were collected from BNT162b2-vaccinated individuals ($n = 10$) on the day of the first vaccination (week 0), the day of the second vaccination (week 3) and 2 weeks after the second vaccination (week 5). Vaccine-induced antibody production was confirmed using SARS-CoV-2 specific spike-, RBD-, and NC-Ig sandwich assays (Fig. 3A), revealing antibodies exclusively directed toward the spike protein, including the RBD. Serum samples of COVID-19

resolvers and healthy SARS-CoV-2-negative individuals were used as controls. CD107a expression levels of NK cells induced by serum samples diluted 1:10 on day 0 were comparable to those of SARS-CoV-2-negative individuals, while a significant increase in serum-mediated CD107a-expression was observed for samples collected at weeks 3 and 5, reaching values similar to those observed in COVID-19 resolvers (Fig. 3B, Supporting Information Fig. S4). As expected, NK cell degranulation was only observed in response to S1 protein, while NC protein-directed ADCC responses remained low. Interestingly, while S1-specific antibody titers induced by vaccination continued to increase from weeks 3 to 5 (Fig. 3A), serum-mediated CD107a expression on NK cells remained stable (Fig. 3B). To assess whether this plateauing of NK cell-mediated ADCC responses was due to a saturation effect in the CD107a degranulation assay or even a result of the prozone effect, we performed additional NK cell degranulation assays using serial serum antibody dilutions, including serum samples diluted at 1:20, 1:50, 1:100, 1:500, and 1:1000 (Fig. 3C). These additional experiments demonstrated that serum samples from BNT162b2-vaccinated individuals diluted up to 1:500 and for one donor even up to 1:1000 continued to trigger strong NK cell degranulation at week 5. These BNT162b2-induced ADCC responses exceeded those observed in individuals that had been infected with SARS-CoV-2, in which CD107a expression levels by NK cells started to decrease with increasing serum sample dilution. Overall, CD107a expression levels by NK cells using 1:100 diluted serums were significantly correlated to S1 antibody titers (Spearman's rho = 0,95, p value = 0.0002, Supporting Information Fig. S2B). Taken together, these data demonstrate that the BNT162b2 vaccine can induce highly functional antibodies against the SARS-CoV-2 S1 protein that can mediate strong NK cell activation even at dilutions at which reduction of NK cell activation is observed for antibodies induced by natural SARS-CoV-2 infection.

Specific RBD-directed serum antibodies induce ADCC-mediated target cell killing by NK cells

Antibody-induced degranulation of NK cells results not only in the release of cytokines, but also of perforin and granzyme, mediating target cell killing [13]. To determine whether antibody-mediated NK cell degranulation also resulted in killing of target cells expressing SARS-CoV-2 antigen, we used the Burkitt lymphoma cell line Raji, which is known for its resistance to NK cell-mediated killing under normal conditions [29]. We modified Raji cells by inducing surface expression of the SARS-CoV-2 S1 receptor-binding domain (RBD) (Fig. 4A). As a result, S1 RBD-directed antibodies induced by SARS-CoV-2 infection bound to RBD-expressing Raji cells, while no binding was observed using serum from SARS-CoV-2-negative control individuals (Fig. 4A). Furthermore, serum from SARS-CoV-2 resolvers and from BNT162b2-vaccinated individuals triggered strong CD107a-expression by NK cells in response to RBD-expressing Raji cells and killing of RBD-expressing Raji cells, while serum from SARS-

CoV-2-negative control individuals did not (Fig. 4B and C). As described for the plate-degranulation assay, CD16 downregulation was in strong negative correlation with CD107a expression (Spearman's rho = –0.83, p value = 7.52×10^{-5}, Supporting Information Fig. S2C). Furthermore, comparing the results from the degranulation assay with the killing assay, a strong correlation was observed as well (Spearman's rho = 0.85, p value = 0.0016, Supporting Information Fig. S2D). For both, the plate-bound degranulation assay and Raji cell ADCC killing assay, CD56^{dim} CD16^{bright} NK cells were the major population contribution to the detected NK cell degranulation (Supporting Information Fig. S5). Overall, CD107a expression by NK cells was significantly associated with NK cell-mediated killing of Raji cells (Fig. 4D, Spearman's r = 0.86, p value = 1.82×10^{-6}), demonstrating that SARS-CoV-2 antibody-dependent activation of NK cells resulted in ADCC.

Discussion

While the induction of significant virus-specific antibodies has been described in COVID-19, the functional activities of these antibodies, including their ability to induce NK cell-mediated ADCC, are insufficiently understood. Here, we show strong ADCC activity of antibodies induced by natural SARS-CoV-2 infection that was even stronger for antibodies induced by the BNT162b2 vaccine. In addition, we observed in some instances anti-SARS-CoV-2 ADCC activity mediated by cross-reactive antibodies from serum samples collected prior to the COVID-19 pandemic, supporting a potential beneficial role of cross-reactive antibodies in COVID-19 outcome, as recently suggested [24]. NK cell activation by SARS-CoV-2-directed antibodies resulted in NK cell-mediated lysis of antigen-expressing cells. Taken together, these data demonstrate that antibodies directed against SARS-CoV-2 can induce NK cell-mediated ADCC activity, and that antibodies induced by the BNT162b2 vaccine mediate significantly stronger functional activity of NK cells.

In accordance with other studies, we observed strong induction of SARS-CoV-2-specific antibodies after both natural SARS-CoV-2 infection and vaccination [30, 31]. Interestingly, serum samples of vaccinated individuals exceeded results detected in resolvers at higher dilution levels, indicating that previous studies might have underestimated antibody-mediated functions. A possible explanation is the prozone effect, a phenomenon also known as hook effect, in which high antibody titers interfere with proper antigen–antibody interactions and thus lead to false negative or inaccurately low results in immunoassays [32–34]. However, the precise functional correlates by which these antibodies mediate their antiviral functions are insufficiently understood. COVID-19 recovered patients and vaccinated individuals have both shown the presence of RBD-specific memory B cells and anti-SARS-CoV-2 spike protein- and RBD-binding neutralizing antibodies [35–37]. While virus neutralization represents an important correlate of protective antiviral immunity, other nonneutralizing Fc effector functions mediated by antibodies, including ADCC or
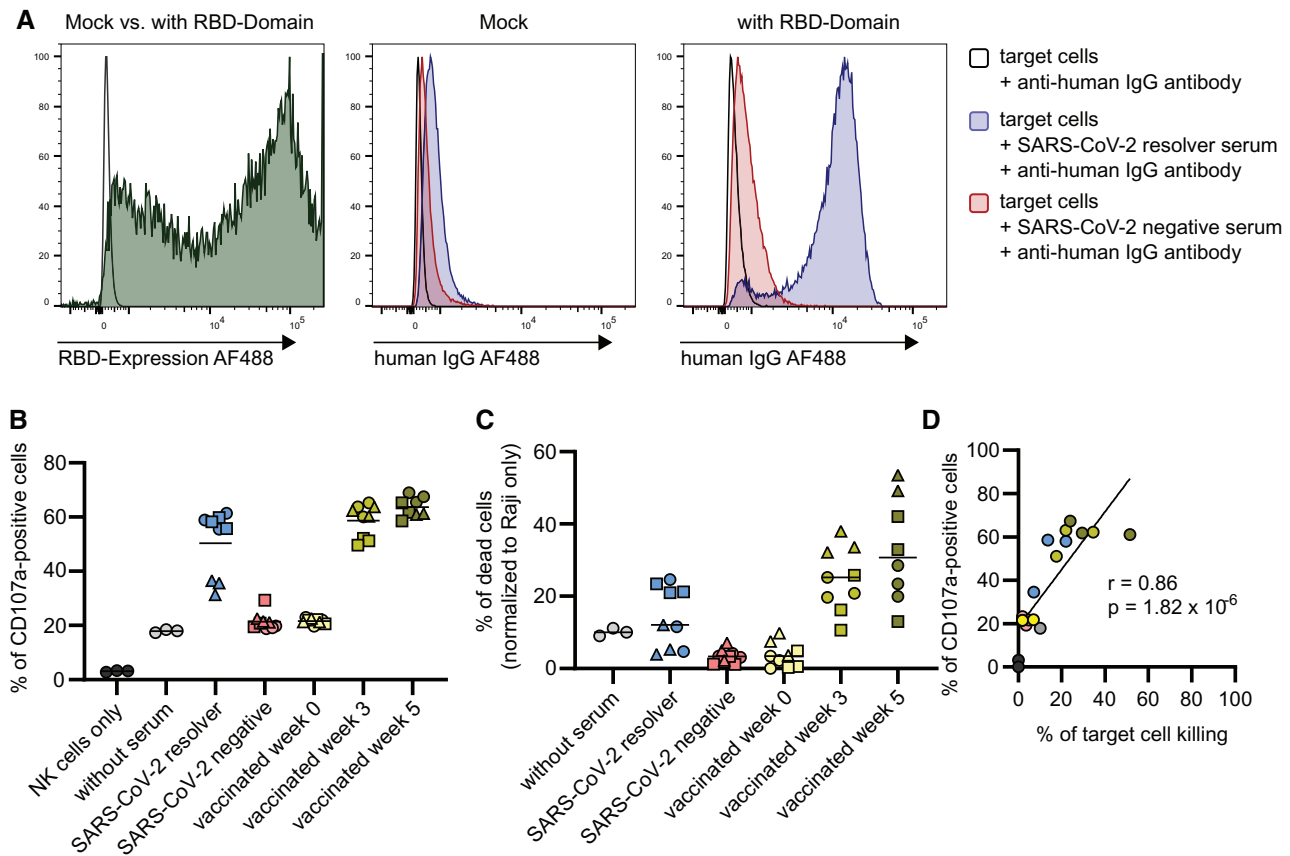
Figure 4. SARS-CoV-2-RBD-directed antibodies induce ADCC-mediated target cell killing by NK cells. (A) RBD expression was determined in mock- and RBD-nucleofected Raji cells. In addition, binding capability of SARS-CoV-2-directed antibodies toward the RBD expressed on nucleofected Raji cells was analyzed in serum samples of SARS-CoV-2 resolver and healthy individuals. (B) RBD-mediated antibodies induce CD107a expression in SARS-CoV-2 resolvers ($n = 3$) and vaccinated individuals ($n = 3$) at 3 and 5 weeks after their initial vaccination. Raji cells alone induce low CD107a-expression levels comparable to those seen in healthy individuals ($n = 3$) and on the day of the first vaccination (week 0, $n = 3$). Replicates of the same serum donor are indicated by shape and color. (C) Killing of target cells increased after incubation with serum of SARS-CoV-2 resolver and vaccinated (weeks 3 and 5) individuals, while it remains low in healthy individuals. Replicates of the same serum donor are indicated by shape and color. (D) CD107a expression by NK cells shows a positive correlation to target cell killing. Mean value of replicates is shown. A linear regression was calculated and statistical analysis was performed using nonparametric Mann–Whitney test.

phagocytosis, can also contribute to viral control and clearance, and the contribution of these different effector functions mediated by antibodies to COVID-19 outcome is not entirely understood. Some recent studies have linked the humoral immune response against SARS-CoV-2 infection to phagocytosis mediated by neutrophils and monocytes [30]. Here, we show that NK cell-mediated lysis of SARS-CoV-2 RBD-expressing target cells represents another important function of antibodies. NK cell-mediated ADCC activity was significantly correlated to antibody titers quantified by SARS-CoV-2 specific Roche Sandwich ELISA. Remarkably, vaccine-induced antibodies mediated significantly stronger ADCC activity at low concentrations compared to antibodies induced by natural SARS-CoV-2 infection, providing an additional correlate for the strong protective effect observed by SARS-CoV-2 vaccines in vivo. While this study focuses on ADCC mediated by RBD-specific antibodies, it has been shown that COVID-19 resolver can also develop high titers of NC-specific antibodies. However, their functional relevance is still controversially discussed, as there is

a lack of knowledge regarding host interactions with NC itself. A recent study reported that NC-specific antibodies play an important role in inhibiting complement hyperactivation, resulting in better disease outcome [38]. In contrast, NC-specific antibodies might also be involved in a more severe disease progression as a result of antibody-dependent enhancement [39]. Here, we observed NK cell activation by NC-specific antibodies, and further studies will be necessary to gain insight into their clinical relevance.

The existence of SARS-CoV-2 cross-reactive antibodies induced by endemic coronaviruses has been described by previous studies [3, 22, 23]. Sequence homologies between different coronavirus strains are especially observed for the NC protein [26, 27], but also exist for the spike protein. This is in line with our data showing mild CD107a expression induced by NC protein-directed antibodies in healthy individuals, in which no S1-specific antibodies were detected. While most diagnostic SARS-CoV-2 antibody tests only use small parts of these viral antigens to ensure high

Table 1. Characteristics of study participants

Group	N	Median age in years	Interquartile range (IQR) age in years	Gender
COVID-19 resolver	15	51	30.5	Male: 9 Female: 6
Healthy controls	15	40	33	Male: 7 Female: 8
BNT162b2-vaccinated	10	50	26.25	Male: 4 Female: 6

specificity for SARS-CoV-2, we performed our experiments using full-length proteins of the initial Wuhan-Hu-1 strain. This allowed us to more broadly determine not only SARS-CoV-2-induced antibodies, but also to analyze the impact of cross-reactive antibodies from seasonal coronavirus infections. Screening of 85 serum samples collected prior to the first documented SARS-CoV-2 case not only confirmed the existence of cross-reactive antibodies as shown by others [24], but also showed that that these cross-reactive antibodies can mediate functional ADCC responses by NK cells. Previous studies have described that endemic coronavirus-induced cross-reactive antibodies can be associated with less severe COVID-19 outcomes [40], and our data on cross-reactive ADCgC activity indicate that the beneficial impact of cross-reactive antibodies in SARS-CoV-2 infection might not only be mediated by T and B cells, but also by NK cells. In conclusion, we demonstrate that antibodies induced by COVID-19 and anti-SARS-CoV-2 vaccines can trigger significant NK cell-mediated ADCC activity, and identify some cross-reactive NK cell activity against SARS-CoV-2 by endemic coronavirus-specific antibodies.

Materials and methods

Serum samples

Serum samples were collected from COVID-19 resolver who had SARS-CoV-2 PCR-confirmed disease, and from BNT162b2-vaccinated individuals by the Center for Diagnostics at the University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany (Table 1). Healthy individuals with no history of COVID-19 were used as controls. Pre-COVID-19 serum samples were derived from cryopreserved samples collected between 2013 and 2018 at the Leibniz Institute for Experimental Virology.

Antibody titer

Serum samples were analyzed using the qualitative anti-NC Ig assay (Elecsys Anti-SARS-CoV-2, Roche), the qualitative anti-Spike IgG (Liaison SARS-CoV-2 S1/S2 IgG, DiaSorin, Saluggia, Italy), and the quantitative anti-Spike RBD Ig assays (Elecsys Anti-SARS-CoV-2 Spike, Roche, Mannheim, Germany). CLIAs were performed using the immune-analyzer (cobas e411, Roche; and

Liaison XL, Diasorin) according to the manufacture's recommendations.

recomLine assay

Serum samples were analyzed for IgG antibodies induced by endemic human coronaviruses using the qualitative MIKROGEN DIAGNOSTIK *recom*Line SARS-CoV-2 IgG immunoassay according to the manufacture's recommendations.

ELISA

Pre-COVID-19 serum samples were screened for cross-reactive antibody responses against SARS-CoV-2 using an anti-SARS-CoV-2 IgG ELISA (EUROIMMUN, EI 2606–9601 G). EUROIMMUN recommends interpreting ratios below 0.8 as negative, above 1.1 as positive, and in between as marginal.

Peripheral blood sample acquisition, processing, and enrichment of NK cells

Citrate-treated peripheral blood samples were obtained from healthy blood donors recruited at the Institute for Transfusion Medicine of the UKE and the Healthy Blood Donor Cohort at the Leibniz Institute for Experimental Virology in Hamburg, Germany. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of healthy donors by density-gradient centrifugation, washed, and resuspended in complete medium (RPMI-1640 medium [Sigma] supplemented with 10 % [v/v] fetal bovine serum, Sigma). Primary NK cells were isolated and enriched from freshly isolated PBMC through negative-selection strategy using the EasySep™ Human NK cell Enrichment Kit (StemCell Technologies) according to the manufacturer's protocol. NK cells were cultivated in complete media at 2×10^6 cells/ml and stimulated with human recombinant IL-15 (5 ng/ml, hrIL-15; R&D Systems).

Assessment of NK cell degranulation

Nontissue culture-treated flat bottom 96-well plates (Falcon®; VWR) were coated with 1 μg of COVID-19 Spike Glycoprotein-S1 (Abeomics) in PBS for 48 h at 4°C. As a positive control for CD107a expression, control wells were coated with a human

anti-CD16 antibody (0.5 μg). After blocking for 2 h with blocking buffer, serum (diluted in 1x PBS) was added and incubated for 1 h at 4℃. NK cells were added in complete medium supplemented with IL-15 and anti-human CD107a antibody (BioLegend) in a 1:100 dilution. BD GolgiStop™-Protein Transport Inhibitor (4 μl/6 ml; BD Bioscience) was added 1 h later followed by additional 3 h of incubation. NK cells were stained for CD3 (BD Bioscience), CD14 (BioLegend), CD16 (BioLegend), CD19 (BioLegend), CD56 (BioLegend), a live/dead-marker (NIR, Invitrogen), and fixed in 1× BD Cellfix (BD Bioscience). CD107a expression levels were analyzed by flow cytometry. Each degranulation assay was performed with NK cells from a single donor. Between assays, different NK cell donors were used to ensure reproducibility. Replicates for serum samples are combined in single data points. A summarized visualization for all controls (anti-CD16 antibody, RBD-Nanobody, and without serum) is presented in Supporting Information Fig. S6.

Cell lines and plasmids used

Raji cells were used to assess NK cell-mediated target cell killing. Cells were cultured in complete medium (RPMI-1640 medium (Sigma)) supplemented with 10 % (v/v) fetal bovine serum (Sigma). Plasmids containing the information for a GPI-anchored RBD of the initial Wuhan-Hu-1 strain were produced and provided by the Institute of Immunology of the UKE.

Nucleofection of NK cell resistant cell lines

Raji cells were nucleofected using the Amaxa™ SG-Cell Line 4D-Nucleofector™ X Kit (Lonza), as suggested by the distributer's user manual. Nucleofection efficiency was assessed using RBD-directed Nanobody-Fc Fusion constructs [41] and anti-IgG antibodies. The Nanobody VHH72 fused to either a rabbit or human Fc construct was provided by the Institute of Immunology, UKE.

NK cell killing assay

RBD-nucleofected Raji cells served as target cells for NK cells isolated from whole blood. Target cells were incubated with serum samples of SARS-CoV-2 resolver and healthy individuals, as well as with serum of vaccinated individuals (weeks 0, 3, and 5) in a 1:20 dilution. NK cells were added in a 5:1 effector-to-target ratio and incubated for 5 h at 37℃. CD107a antibody and BD GolgiStop were added, as described above. In the end, cells were stained with a live/dead marker and for CD3, CD16, CD19, and CD56 expression and fixed with 1× BD Cellfix. Before analyzing the samples using flow cytometry, Precision Count Beads™ (BioLegend) were added to obtain absolute cell counts. The assay was performed with NK cells from a single NK cell donor but with serum samples of multiple individuals in duplicates and triplicates.

Antibodies and flow cytometry

Multiparameter flow cytometry was used to assess CD107a expression and target cell killing/ADCC by NK cells. Different antibodies and reagents were used to determine cell characteristics such as phenotype, viability, purity, and functional capacity. A detailed list of all antibodies is provided in Supporting Information Table S1, and gating strategies are displayed in Supporting Information Figs. 7–9. Acquisition of flow cytometry data was performed using a BD LSR Fortessa (BD Biosciences) and BD FACS Diva software (BD Biosciences) in the core facility Fluorescence Cytometry at the Leibniz Institute for Experimental Virology. Data were further analyzed using FlowJo software v10.1 (BD Biosciences) and is presented according to the guidelines of the journal [42].

Graphical display and statistical analysis

Graphpad Prism 9.0.1 (GraphPad Software) was used for statistical analysis and graphical display of the data. Statistical analysis was performed using the nonparametric Mann–Whitney test and the nonparametric Spearman's rho correlation including simple linear regression. If not indicated otherwise, median values with interquartile range are shown for each group.

Conflict of Interest: The authors have declared no financial or commercial conflict of interest.

Ethics statement: All samples were collected under protocols approved by the ethics committee of the Ärztekammer Hamburg, and all study participants provided written informed consent.

Author contributions: M.A. and M.B. initiated the study. M.A., K.H., and J.J. designed the experiments. H.H. performed the ELISA of pre-COVID-19 samples. K.H. performed NK cell experiments and data analysis. F.K.-N. and S.M. provided plasmids and nanobody Fc-fusion constructs. B.F and K.R. provided transduced cell lines and participated in flow analysis. J.SzW., F.H., T.T.B., and M.L. organized studies design to collect serum samples and provided us with serum and antibody titers. M.L. provided us with serum titers of endemic coronavirus antibodies. S.P. organized

buffy coats of healthy blood donors for NK cell isolation. M.M.A. and M.L. provided ethical statements of study participants. K.H and M.A. wrote the manuscript and all authors contributed with proofreading and feedback.

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://publons.com/publon/10.1002/eji.202149470.

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Abbreviations: **ADCC:** antibody-dependent cellular cytotoxicity · **S1:** Spike 1 · **NC:** nucleocapsid · **SARS-CoV-2:** severe acute respiratory syndrome coronavirus 2 · **COVID-19:** coronavirus induced disease 2019

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