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T cell responses against SARS-CoV-2 and its Omicron variant in a patient with B cell lymphoma after multiple doses of a COVID-19 mRNA vaccine

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ABSTRACT

Anti-SARS-CoV-2 antibodies are crucial for protection from future COVID-19 infections, limiting disease severity, and control of viral transmission. While patients with the most common type of hematologic malignancy, B cell lymphoma, often develop insufficient antibody responses to messenger RNA (mRNA) vaccines, vaccine-induced T cells would have the potential to 'rescue' protective immunity in patients with B cell lymphoma. Here we report the case of a patient with B cell lymphoma with profound B cell depletion after initial chemoimmunotherapy who received a total of six doses of a COVID-19 mRNA vaccine. The patient developed vaccine-induced anti-SARS-CoV-2 antibodies only after the fifth and sixth doses of the vaccine once his B cells had started to recover. Remarkably, even in the context of severe treatment-induced suppression of the humoral immune system, the patient was able to mount virus-specific $CD4^+$ and $CD8^+$ responses that were much stronger than what would be expected in healthy subjects after two to three doses of a COVID-19 mRNA vaccine and which were even able to target the Omicron 'immune escape' variant of the SARS-CoV-2 virus. These findings not only have important implications for anti-COVID-19 vaccination strategies but also for future antitumor vaccines in patients with cancer with profound treatment-induced immunosuppression.

COVID-19 is caused by SARS-CoV-2, which contains the spike (S) and nucleocapsid (N) proteins.[1 2] The S protein has S1 and S2 domains and the virus uses the receptor-binding domain (RBD) within S1 to bind to ACE-2 receptor[3] and enter normal cells such as the pneumocytes in the lungs.[1 4] Unfortunately, patients with hematologic malignancies and COVID-19 show dramatically increased mortality rate,[5 6] which correlates with the intensity of prior antilymphoma treatments.[5–7]

Disease-induced or vaccine-induced anti-SARS-CoV-2 antibodies are crucial for protection from future COVID-19 infections, limiting disease severity, and control of viral transmission.[8 9] Unfortunately, patients with the most common type of hematologic malignancy, namely B cell lymphoma, often develop insufficient antibody responses to messenger RNA (mRNA) vaccines due to the immunosuppression caused by their anti-B cell treatments.[10] In addition to antibody responses, antiviral T cells have been shown to improve survival in patients with COVID-19,[11] including patients with hematologic cancers,[12] and vaccine-induced T cells have the potential to 'rescue' protective immunity in patients with B cell lymphoma. However, it is not entirely clear whether patients with B cell lymphoma are capable of mounting a vaccine-induced T cell response in the framework of treatment-induced immunosuppression and whether such T cells would be able to recognize and target immune escape variants such as Omicron.

In this study we performed a comprehensive monitoring of anti-SARS-CoV-2 antibody and T cell immunity in a patient with B cell lymphoma with profound immunosuppression receiving multiple doses of a COVID-19 mRNA vaccine (For methods used please see online supplemental methods and online supplemental tables 1–3). The patient is a man in his early 70s with diffuse large B cell lymphoma involving the left cervical chain (stage 1) who received four cycles of R-CHOP (rituximab/cyclophosphamide/doxorubicin/vincristine/prednisone) followed by two cycles of rituximab alone. He achieved complete remission which was sustained. While the patient was under treatment with the final two doses of rituximab (figure 1A and online supplemental figure 1), he simultaneously received the first two doses of the BNT162b2 COVID-19 mRNA vaccine (online

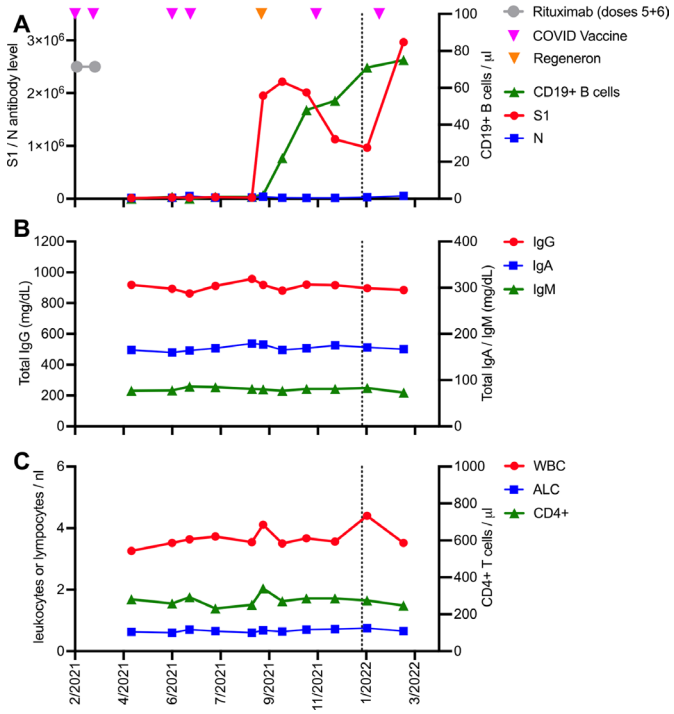

Figure 1 Time course of immune parameters including anti-SARS-CoV-2 antibodies in a patient with lymphoma receiving multiple COVID-19 vaccinations. (A) Absolute numbers of peripheral blood B cells and levels of antibodies directed against SARS-CoV proteins S1 and N after one dose of Regeneron's antibody cocktail REGN-COV2 and multiple doses of the BNT162b2 COVID-19 vaccine, respectively. (B) Absolute serum concentrations of IgG, IgA, and IgM immunoglobulins over time. (C) Absolute white blood cell count (WBC), absolute lymphocyte count (ALC), and number of peripheral blood CD4$^+$ T cells over time. The dotted line indicates the timepoint when comprehensive immunomonitoring was performed.

supplemental figure 1). At that time, he did not have any B cells in his peripheral blood (figure 1A), and accordingly he did not develop antibodies against the S protein of SARS-CoV-2 (figure 1A). Two more doses of the same vaccine did not lead to the development of endogenous antiviral antibodies, and as a consequence he received Regeneron's antibody cocktail REGN-COV2 off-label as an alternative prophylactic measure (figure 1A). Shortly thereafter, anti-S1 antibodies became detectable, presumably due to the exogenous antibodies persisting in his blood (figure 1A). In October 2021 the patient's B cell counts finally started to recover from anti-CD20 treatment (figure 1A). Off-label he received a fifth dose of the COVID-19 vaccine, with normal B cells detectable but still low, which led to a stabilization of total anti-S antibody levels (figure 1A) without any additional doses of the REGN-COV2 antibody cocktail, presumably representing early signs of an initial endogenous humoral immune response to the fifth dose of the vaccine. A sixth dose of the same mRNA COVID-19 vaccine given after normalization of B cell numbers led to a substantial increase in anti-S1 antibody levels. We performed a comprehensive analysis of vaccine-induced T cell and B cell responses between administration of the fifth and sixth doses of the vaccine (figure 1A).

When we asked whether the lack of a vaccine-induced anti-SARS-CoV-2 antibody response to the first four doses was due to an unspecific and global treatment-induced and/or disease-induced immunosuppression, we found that the patient indeed showed lower levels of total IgG, IgM, and IgA immunoglobulins compared with a group of healthy controls (online supplemental figure 2A). However, there was no decline in immunoglobulins over time (figure 1B), and even more importantly the patient with B cell-depleted lymphoma maintained normal levels of IgG antibodies against recall antigens such as influenza A, tetanus toxoid, and Epstein-Barr virus even after six cycles of anti-B cell lymphoma treatment (online supplemental figure 2B).

Taking a closer look at the anti-SARS-CoV-2 immunity our patient had developed after five doses of the vaccine, we detected, in agreement with the routine laboratory assay (figure 1A), IgG antibodies directed against the S1, RBD (online supplemental figure 2C), and S2 proteins, with no detectable anti-N antibodies (online supplemental figure 2E). The antibody titers were lower compared with those of seven healthy controls at 4 weeks after the second dose of a COVID-19 mRNA vaccine (online supplemental figure 2C); however, they still led to an almost 100% antiviral neutralizing activity (online supplemental figure 2D). Consistent with our longitudinal analyses of peripheral B cell numbers in this patient who initially showed therapy-induced B cell depletion (figure 1A), we were able to detect a significant number of CD19$^+$/CD20$^+$ B cells (online supplemental figure 2F), including CD19$^+$ B cells secreting IgG antibodies against the S protein (online supplemental figure 2G), after five doses of the vaccine. Unfortunately, the comparably low antibody titers in our patient were further reduced when binding to the Omicron variant instead of the ancestral S1 and RBD proteins (online supplemental figure 2C). The reduced binding resulted in a dramatically diminished anti-Omicron neutralizing activity of the polyclonal antibodies (online supplemental figure 2D).

We next asked whether our patient evidenced anti-SARS-CoV-2 T cells despite an only very slowly developing humoral antiviral immune component, which was still suboptimal after the fifth dose of the vaccine. We found that the patient actually showed vaccine-induced CD4$^+$ and CD8$^+$ T cell responses in their blood targeting the S protein of the SARS-CoV-2 virus that were much stronger than what we had observed in a group of healthy individuals who had received two doses of the mRNA vaccine (figure 2). Unfortunately, we were not able to measure T cell responses in our patient after only two doses of the vaccine; however, after five doses, the number of SARS-CoV-2-specific CD8$^+$ T cells was almost 100 times higher

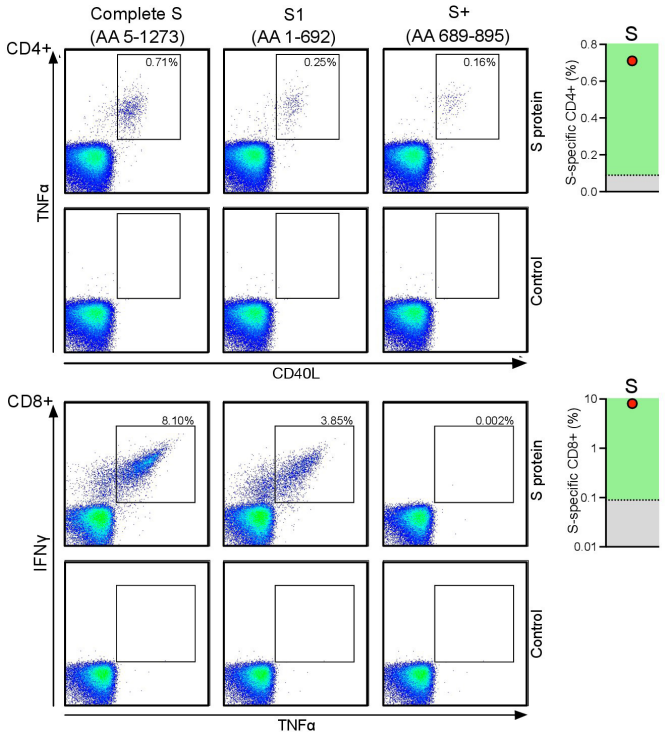

Figure 2 Vaccine-induced SARS-CoV-2-specific T cells in a patient with B cell lymphoma after multiple doses of a COVID-19 mRNA vaccine. After the patient had received five doses of the COVID-19 mRNA vaccine, T cells specific for the S protein of the SARS-CoV-2 were identified ex vivo after short-term stimulation of the total peripheral blood mononuclear cells (PBMC) using libraries of overlapping peptides covering the complete sequence of the protein. Intracellular staining of cytokines followed by flow cytometry served as the read-out assay. SARS-CoV-2-specific $CD4^+$ T cells (upper panel) were defined as tumor necrosis factor (TNF) α/CD40L (CD154) double-positive $CD3^+CD4^+$ T cells, and SARS-CoV-2-specific $CD8^+$ T cells (lower panel) were defined as interferon (IFN) γ/TNFα double-positive $CD3^+CD8^+$ T cells. The number of vaccine-induced $CD4^+$ and $CD8^+$ T cells specific for the complete sequence of the S fusion protein was compared with the number of T cells from the same individual recognizing the N-terminal S1 protein or the N-terminal portion (amino acids 689–895) of the S2 protein ('S+'). Background levels were typically <0.01% of all $CD4^+$ or $CD8^+$ T cells. Plots on the right show the patient's S-specific $CD4^+$ and $CD8^+$ T cells (red dot) in relation to the median number (dotted line) of the same T cells from a group of six healthy control subjects, where the results were available from 4 weeks after the second dose of a COVID-19 mRNA vaccine. mRNA, messenger RNA.

than what we had observed in healthy vaccinated individuals after the two initial doses (figure 2). For both $CD4^+$ and $CD8^+$ T cells targeting the S protein, most of the immunodominant epitopes of the anti-SARS-CoV-2 $CD4^+$ T cells were within the S1 component of the fusion protein (figure 2).

It has previously been shown that SARS-CoV-2 vaccination is capable of inducing T cells with the potential to cross-recognize the Omicron variant[13 14]; however, to the best of our knowledge, the same phenomenon has not been demonstrated in patients with B cell lymphoma. When we examined cross-recognition by our patient's T cells, we found that, while there was a certain decrease in T cell reactivity when exposed to the Omicron variant of the S protein, most of the patient's polyclonal vaccine-induced $CD4^+$ and $CD8^+$ T cells also recognized this immune escape variant of the ancestral SARS-CoV-2 virus (online supplemental figure 3). While this certainly cannot serve as evidence of a protective function of the vaccine-induced T cells, it has indeed previously been shown that postinfection and vaccine-induced anti-SARS-CoV-2 T cells can play a protective role,[12 15–17] and our patient never developed COVID-19 despite multiple known close exposures to family members with a proven infection.

We have shown here that a patient with B cell lymphoma whose B cells were initially depleted following antilymphoma chemoimmunotherapy was not able to mount an antibody response to four doses of a COVID-19 mRNA vaccine. The patient only developed vaccine-induced anti-SARS-CoV-2 antibodies once his B cells had started to recover. Importantly, even in the context of severe treatment-induced suppression of the humoral immune system, the patient was able to mount SARS-CoV-2-specific $CD4^+$ and $CD8^+$ responses that were much stronger than what would be expected in healthy subjects after two to three rounds of a COVID-19 mRNA vaccine and which were even able to target the Omicron 'immune escape' variant[18] of the virus. These findings not only have important implications for anti-COVID-19 vaccination strategies but also for future antitumor vaccines in patients with cancer with profound treatment-induced immunosuppression.

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Contributors DA designed the study, performed the experiments, analyzed the data, made the figures, and wrote the manuscript. RJK collected patient data and samples, analyzed the data, and wrote the manuscript. DO, TI, and EG processed patient samples and performed the experiments. XF and PDB performed the experiments and wrote the manuscript. NMH, TL, SD, JC, and APR analyzed the data and wrote the manuscript.

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Competing interests None declared.

Patient consent for publication Obtained.

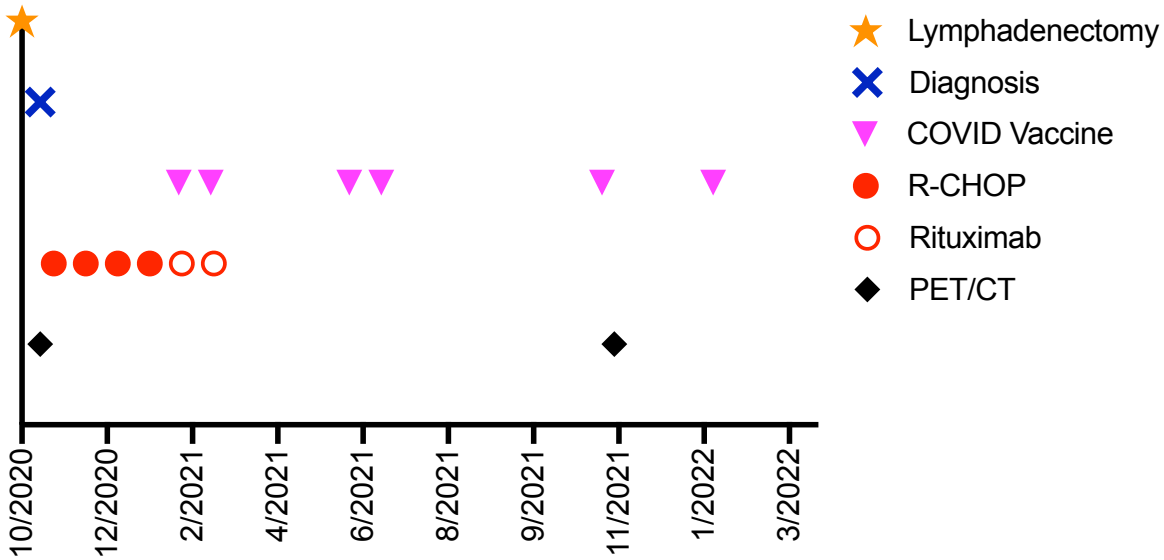
Ethics approval This study involves human participants and was approved by the IRB of the University of Maryland. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

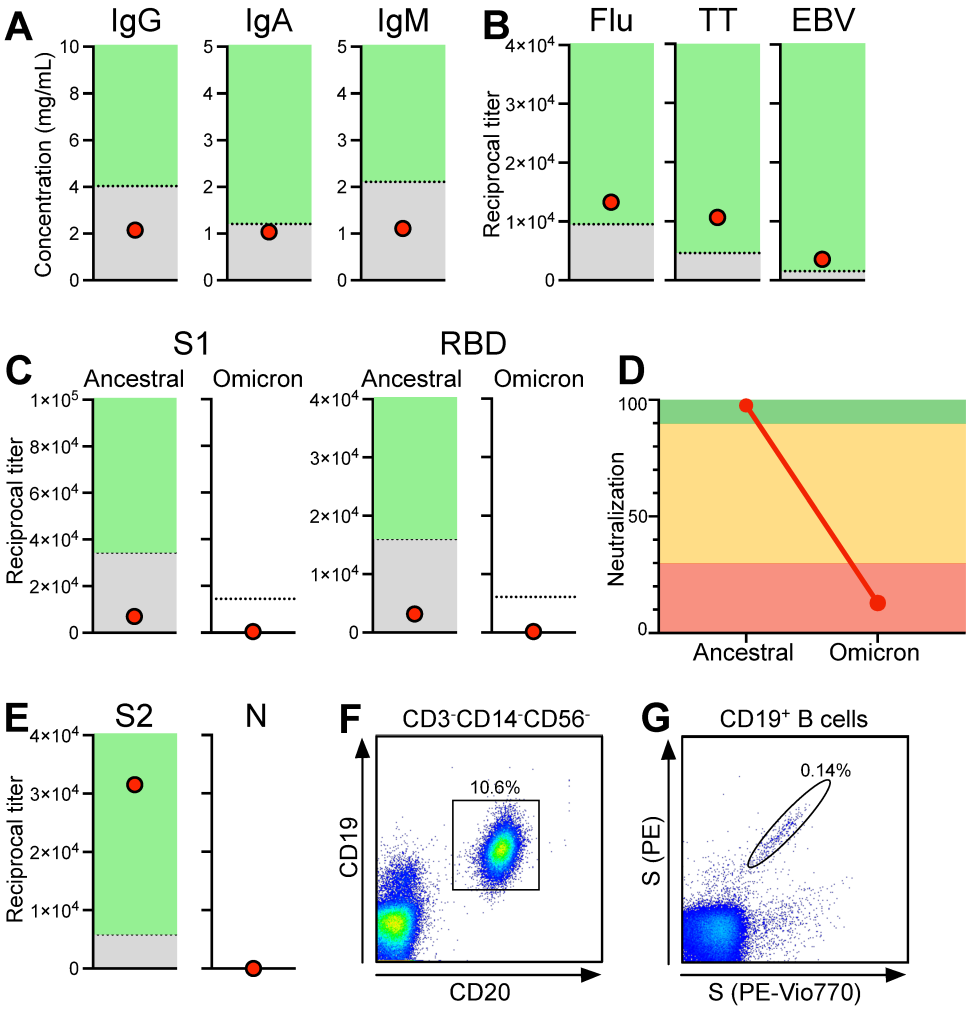
1 Zhou P, Yang X-L, Wang X-G, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270–3.
2 Zhu N, Zhang D, Wang W, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
3 Ou X, Liu Y, Lei X, *et al*. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020;11:1620.
4 Ge X-Y, Li J-L, Yang X-L, *et al*. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* 2013;503:535–8.
5 Lee LYW, Cazier J-B, Starkey T, *et al*. COVID-19 prevalence and mortality in patients with cancer and the effect of primary tumour subtype and patient demographics: a prospective cohort study. *Lancet Oncol* 2020;21:1309–16.
6 Vijenthira A, Gong IY, Fox TA, *et al*. Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. *Blood* 2020;136:2881–92.
7 Busca A*et al*. COVID-19 and CAR-T cells: current challenges and future directions-a report from the EPICOVIDEHA survey by EHA-IDWP. *Blood Adv* 2022.
8 Bergwerk M, Gonen T, Lustig Y, *et al*. Covid-19 breakthrough infections in vaccinated health care workers. *N Engl J Med* 2021;385:1474–84.
9 Lucas C, Klein J, Sundaram ME, *et al*. Delayed production of neutralizing antibodies correlates with fatal COVID-19. *Nat Med* 2021;27:1178–86.
10 Herishanu Y, Avivi I, Aharon A, *et al*. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* 2021;137:3165–73.
11 Sattler A, Angermair S, Stockmann H, *et al*. SARS-CoV-2-specific T cell responses and correlations with COVID-19 patient predisposition. *J Clin Invest* 2020;130:6477–89.
12 Bange EM, Han NA, Wileyto P, *et al*. $CD8^+$ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med* 2021;27:1280–9.
13 Tarke A, Coelho CH, Zhang Z, *et al*. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from alpha to omicron. *Cell* 2022;185:847–59.
14 Liu J, Chandrashekar A, Sellers D, *et al*. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 omicron. *Nature* 2022;603:493–6.
15 Kundu R, Narean JS, Wang L, *et al*. Cross-Reactive memory T cells associate with protection against SARS-CoV-2 infection in COVID-19 contacts. *Nat Commun* 2022;13:80.
16 McMahan K, Yu J, Mercado NB, *et al*. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* 2021;590:630–4.
17 Guo L, Wang G, Wang Y, *et al*. SARS-CoV-2-specific antibody and T-cell responses 1 year after infection in people recovered from COVID-19: a longitudinal cohort study. *Lancet Microbe* 2022;3:e348–56.
18 Liu L, Iketani S, Guo Y, *et al*. Striking antibody evasion manifested by the omicron variant of SARS-CoV-2. *Nature* 2022;602:676–81.

Supplemental Figure 1



Supplemental Figure 1: Time course of clinical events and treatments

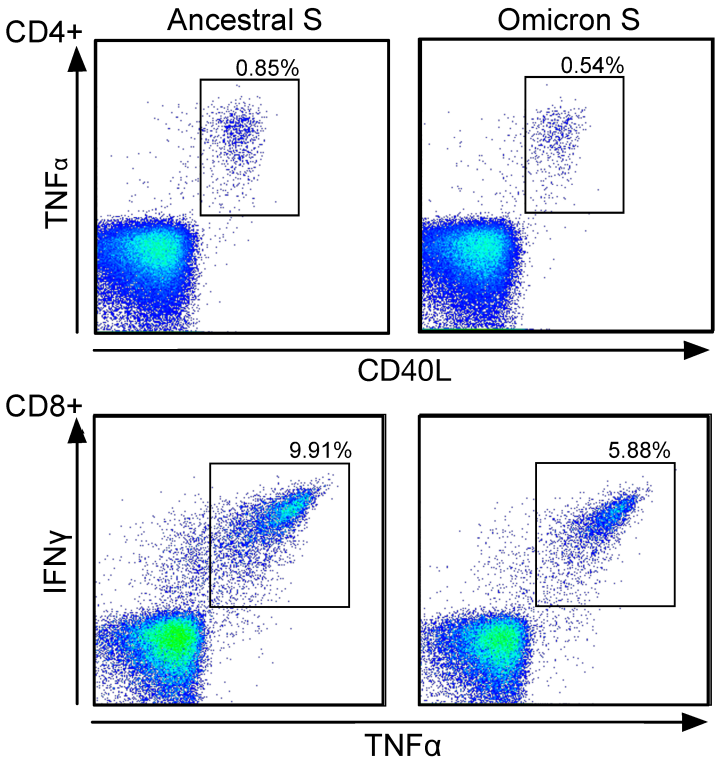
Supplemental Figure 2



Supplemental Figure 2: B cell responses in a patient with B cell lymphoma after multiple rounds of a COVID-19 mRNA vaccine

(A) Absolute levels of IgG, IgA, and IgM antibodies in our patient (red dot) were measured after 5 doses of the COVID-19 mRNA vaccine using a commercially available ELISA. Concentrations are shown in mg/mL and in relation to those of 7 healthy controls at 4 weeks after the second dose of a COVID-19 mRNA vaccine. **(B)** Titers of IgG antibodies against full-length recombinant Influenza A nucleoprotein (Flu), tetanus toxoid (TT), and Epstein-Barr virus (EBV) were measured in an ELISA. **(C)** IgG antibody titers against SARS-CoV-2 proteins S1 and RBD and their Omicron variants. **(D)** Neutralizing activity in the peripheral blood of our B cell lymphoma patient. Green, orange, and red areas indicate different degrees of inhibition (green: >90%, orange: 30-89%, red: <30%). Neutralizing activity is shown for both the original "ancestral" SARS-CoV-2 RBD protein (left) and for its Omicron variant (right). **(E)** The patient's IgG antibody titers against SARS-CoV-2 proteins S2 and N. **(F)** Flow cytometric analysis of B cell subpopulations in the peripheral blood of our B cell lymphoma patient after 5 doses of the vaccine. Dot plots show $CD19^+/CD20^+$ B cells after gating on $CD3^-/CD56^-/CD14^-$ lymphocytes. **(G)** Anti-S antibody-secreting B cells in the B cell lymphoma patient.

Supplemental Figure 3



Supplemental Figure 3: Recognition of ancestral vs. Omicron SARS-CoV-2 S protein by vaccine-induced T cells

The dot plots on the left show our patient's vaccine-induced antiviral $CD4^+$ (upper panel) and $CD8^+$ T cells (lower panel) recognizing peptide pools covering the complete sequence of ancestral vs. Omicron SARS-CoV-2 S protein. Numbers indicate percentages of S protein-specific T cells out of all $CD4^+$ and $CD8^+$ T cells, respectively.

MATERIALS AND METHODS

Study Population and Design

Healthy controls analyzed for this study were enrolled in our prospective, observational cohort study in patients with hematologic malignancies who received care at the University of Maryland Medical Center (UMMC) and had either received or were scheduled to receive any of the two SARS-CoV-2 mRNA vaccines approved by the FDA (IRB HP-00095016). We collected 40ml of heparinized blood from 9 consecutive COVID-19 patients, 21 patients with B cell lymphomas, and 9 healthy controls (Supplemental Table 1). Blood from the lymphoma patient described in this report was collected under a different IRB, HP-00057785. The University of Maryland's Institutional Review Board had approved both studies. Informed consent was obtained, blood samples were collected and plasma was generated from peripheral blood samples after centrifugation at 400g for 10min and frozen immediately at -80C. Peripheral blood mononuclear cells (PBMCs) were isolated using lymphocyte separation density gradient and immediately frozen in liquid nitrogen. Our patients with hematologic malignancies and the healthy controls enrolled received COVID-19 vaccination per the treating physician’s discretion.

Measurement of absolute immunoglobulin levels

Absolute serum concentrations of the different immunoglobulins were measured using Human IgG, IgM, and IgA Enzyme-linked Immunosorbent Assay (ELISA) Kits (Invitrogen, Cat. No. BMS2091, BMS2098, BMS2096) as per the manufacturer’s instructions. Absorbance was read at 450nm with a reference wavelength of 620nm in a microtiter plate reader (Tecan, Morrisville, NC).

Levels of anti- SARS-CoV-2 S1 antibodies

For repeated measurement of anti-SARS-CoV-2 S1 antibody levels at the Center for Cancer Research, quantitative measurements by luciferase immunoprecipitation assay systems (LIPS)

were performed at Dr. Jeffrey I. Cohen's lab at the National Institutes of Health, Bethesda, Maryland as previously described [1].

Analysis of SARS-CoV-2-specific antibodies

For the comprehensive immunomonitoring after 5 doses of the vaccine performed at the University of Maryland, serum antibody responses against recombinant, full-length SARS-CoV-2 proteins (Supplemental Table 2) or viral control proteins (Supplemental Table 1) were determined by ELISA as previously described. Briefly, high-binding ELISA plates (Thermo Fisher, Cat. No. 44-2404-21) were coated with 5µg/mL of the respective proteins in PBS (Gibco, Cat. No. 10010-023) overnight at 4ºC. The next day plates were washed twice with PBS and twice with 0.1% PBS-T (VWR, Cat. No. M147-1L). Plates were then blocked with 5% non-fat dry milk (Santa Cruz, Cat. No. sc2325) in PBS (MPBS) for 1h at room temperature (RT), then washed again as described above. Serum was diluted 1:40 for screening assays and for titration 1:100/1:400/1:1,600/1:6,400 and if necessary 1:25,000 and 1:100,000 in MPBS. Diluted sera were added to plates and incubated for 3H at RT. Plates were washed as described above before incubation with secondary antibodies against pan-human IgG (Southern Biotech, Cat. No. 2040-04) or IgA (Southern Biotech, Cat. No. 2050-04). Secondary antibodies were diluted according to the manufacturers' instructions and plates incubated for 1h at RT. Plates were then washed as described above, PNPP tablets (Southern Biotech, Cat. No. 0201-01) dissolved in diethanolamine (Thermo, Cat. No. 34064) and PNPP substrate solution added to each well for 10min in the dark. 15uL of 3N NaOH (VWR, Cat. No. BDH7472-1) stop solution was added to each well and absorbance was read at 405nm with a reference wavelength of 620nm in a microtiter plate reader (Tecan, Morrisville, NC). Endpoint titers were calculated using serum titration curves for positive samples and pooled sera of 5 healthy donors. For non-SARS-CoV-2 antigens, serum dilutions for anti-GST (glutathione-S-transferase) antibodies (Supplemental Table 1) were used as a negative control.

SARS-CoV-2 neutralization assay

Neutralizing activity of patient sera was assessed using the cPass Neutralization Antibody Detection Kit (GenScript, Cat. No. L00847-A) which is a surrogate test detecting circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein with the ACE2 cell surface receptor. Briefly, samples and controls were diluted with sample dilution buffer and pre-incubated with the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD) or one of its variants listed in Supplemental Table 2 to allow the binding of the circulating neutralization antibodies to HRP-RBD. The mixture was then added to the capture plate, which was pre-coated with the hACE2 protein. The unbound HRP-RBD as well as any HRP-RBD bound to non-neutralizing antibody was captured on the plate, while the circulating neutralization antibodies HRP-RBD complexes remained in the supernatant and were removed during washing. Following a wash cycle, TMB substrate solution was added followed by the Stop Solution. The absorbance of the final solution was read at 450 nm in a microtiter plate reader (Tecan, Morrisville, NC).

Analysis of B cell phenotypes

Approximately 500,000 were stained using the antibody cocktail shown in Supplemental Table 3. B cell subpopulations were analyzed using the BD LSRII flow cytometer.

Analysis of SARS-CoV-2-specific B cells

B cells specific for SARS-CoV-2 were identified using the Spike B Cell Analysis Kit (Miltenyi Biotec, cat no. 130-128-022). Briefly, S protein-tetramers were prepared by incubating SARS-CoV-2 Spike protein (HEK)-Biotin with streptavidin (PE or PE-Vio 770) for 15 minutes at room temperature. For each test 2 µL of each fluorochrome-conjugated antibody (Supplemental Table 4), 5 µL of 7-AAD Staining Solution, 5 µL of spike-tetramer-PE, and 10 µL of spike-tetramer-PE-Vio 770 were used. PBMCs (5-10 × 10^6 were incubated in antibody staining mix for 30 minutes at

4°C. Cells were washed and analyzed by flow cytometry using a MACSQuant® Analyzer 10 (Miltenyi Biotec).

Analysis of SARS-CoV-2-specific T cells

B cells specific for SARS-CoV-2 were identified using different versions of the SARS-CoV-2 T Cell Analysis Kit (Miltenyi Biotec) and pools of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids (aa) overlap, covering the complete sequence of the S protein ("S complete") or sequence domains 1-692 ("S1") or aa 689-895 ("S plus"). For the analysis of T cell responses against the omicron variant of the SARS-CoV-2 Spike Glycoprotein a peptide pool consisting of 315 peptides (15mers with 11 aa overlap) covering the entire sequence of the S protein was used (GenScript, cat. no. RP30121). For the analysis of T cell responses against microbial antigens other than SARS-CoV-2 a pool of 32 MHC class I-specific peptides of 8–12 aa in length derived from cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and influenza virus was used (CEF MHC Class I Plus, Milteny Biotec, cat no. 130-098-426). Briefly, PBMC were thawed, plated cells at a density of 5×10^6/mL in fresh cell culture medium in a 24-well cell culture plate at 37°C and 5% CO_2 overnight. The next morning, cells were resuspended in culture medium at a density of 1×10^7 viable cells per mL and 100 µL of cell suspension per well was plated in a flat-bottom 96-well plate resulting in a total number of 1×10^6 cells per well. For antigen stimulation 2 µL of peptide stock solution was added to the respective wells and mixed by pipetting up and down. As a positive control 2 µL of the CytoStim crosslinking agent were used and as a negative control 2 µL sterile water/10% DMSO solution were added to the respective wells. Cells were incubated at 37 °C and 5% CO_2 for 2 hours, 2 µL of Brefeldin A were added to each well, and cells were incubated at 37 °C and 5% CO_2 for an additional 4 hours. Cells were then resuspended in 100 µL reconstituted Viobility 405/452 Fixable Dye master mix and incubated for 10 minutes at room temperature. Fixation was performed by adding 100 µL Inside Fix to each well followed by incubation for 20 minutes at room temperature. The supernatant was removed

and permeabilization was performed by adding 100 µL Inside Perm to each well. The supernatant was removed and 100 µL of antibody staining mix (rSupplemental Table 4) was added to each well followed by incubation for 10 minutes at room temperature. Cells were washed and analyzed by flow cytometry using a MACSQuant® Analyzer 10 (Miltenyi Biotec).

Statistical analyses

Statistical analyses for serological analyses were performed using GraphPad Prism 9 software (GraphPad Software, San Diego, CA). Groups were compared using the Mann–Whitney U test and paired analyses were performed using the Wilcoxon signed-rank test. For the analysis of clinical characteristics, groups were compared using a student's t-test.

References

1. Burbelo, P.D., et al., *Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients.* medRxiv, 2020: p. 2020.04.20.20071423.

Supplemental Table 1: Control Proteins

Protein	Expression System	Manufacturer	Catalogue No.
Influenza A H1N1 Nucleoprotein (NP)	Baculovirus-Insect Cell	Sino Biological	11675-V08B
Epstein-Barr Virus (EBV) Glycoprotein gp350	Baculovirus-Insect Cell	Sino Biological	40373-V08B
Glutathione S-Transferase Alpha 1 (GSTA1)	HEK293	Sino Biological	15237-H08H
Tetanus Toxoid		Calbiochem	582231

Supplemental Table 2: SARS-CoV-2 Proteins

Protein	Variant	Mutations	Expression System	Conjugate	Manufacturer	Catalogue No.
Spike S1			HEK293		Acro Biosystems	S1N-C52H2
Spike Receptor-Binding Domain (RBD)			HEK293		Acro Biosystems	SPD-C52H2
Spike S2			HEK293		Acro Biosystems	S2N-C52H5
Nucleocapsid (N)			HEK293		Acro Biosystems	NUN-C5221
Spike S1	Omicron (B.1.1.529)	A67V, HV69-70del, T95I, G142D, VYY143-145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H)	HEK293		Acro Biosystems	S1N-C52Ha
Spike Receptor-Binding Domain (RBD)	Omicron (B.1.1.529)	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H	HEK293		Acro Biosystems	SPD-C522e
Receptor-Binding Domain (RBD)	Omicron (B.1.1.529)	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H	HEK293	HRP	GenScript	Z03730

Supplemental Table 3: Monoclonal Antibodies for Flow Cytometry

Specificity	Conjugate	Clone	Isotype	Manufacturer	Test
CD19	APC-Vio 770	LT19,	Mouse IgG1κ	Miltenyi Biotec	Spike B Cell Analysis Kit
CD27	Vio Bright FITC (	M-T271	Mouse IgG1κ	Miltenyi Biotec	
IgG	VioBlue®	IS11-3B2.2.3	Mouse IgG1κ	Miltenyi Biotec	
IgA	VioGreen™	IS11-8E10	Mouse IgG1κ	Miltenyi Biotec	
IgM	APC	PJ2-22H3	Mouse IgG1	Miltenyi Biotec	
CD3	APC	REA613	Recombinant human IgG1	Miltenyi Biotec	SARS-CoV-2 T Cell Analysis Kit
CD4	Vio® Bright B515	REA623	Recombinant human IgG1	Miltenyi Biotec	
CD8	VioGreen™	REA734	Recombinant human IgG1	Miltenyi Biotec	
IFN-γ	PE	REA600	Recombinant human IgG1	Miltenyi Biotec	
TNF-α	PE-Vio® 770	REA656	Recombinant human IgG1	Miltenyi Biotec	
CD14	VioBlue®	REA599	Recombinant human IgG1	Miltenyi Biotec	
CD20	VioBlue®	REA780	Recombinant human IgG1	Miltenyi Biotec	
CD154	APCVio® 770	REA238	Recombinant human IgG1	Miltenyi Biotec	
CD19	Pacific Blue®	LT19	Mouse IgG1	Bio-Rad	Phenotypic B Cell Analysis
CD20	Brilliant Violet 510™	2H7	Mouse IgG2b	BioLegend	
CD24	PE	ML5	Mouse IgG2aκ	BioLegend	
CD27	Alexa Fluor 700	LT27	Mouse IgG2a	Bio-Rad	
CD38	APC/Fire™ 750	HB-7	Mouse IgG1κ	BioLegend	
IgD	FITC	Polyclonal	Goat IgG	Bio-Rad	
CD3	Alexa Fluor® 700	OKT3	Mouse IgG2aκ	Bio-Legend	
CD14	Alexa Fluor® 700	TÜK4	Mouse IgG2aκ	Bio-Rad	
CD56	Alexa Fluor® 700	123C3	Mouse IgG1	Bio-Rad	