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Case Report

Boosted production of antibodies that neutralized different SARS-CoV-2 variants in a COVID-19 convalescent following messenger RNA vaccination - a case study

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ABSTRACT

Vaccinated convalescents do not develop severe COVID-19 after infection with new SARS-CoV-2 variants. We questioned how messenger RNA (mRNA) vaccination of convalescents provides protection from emerging virus variants. From the cohort of 71 convalescent plasma donors, we identified a patient who developed immune response to infection with SARS-CoV-2 variant of 20A clade and who subsequently received mRNA vaccine encoding spike (S) protein of strain of 19A clade. We showed that vaccination increased the production of immune cells and anti-S antibodies in the serum. Serum antibodies neutralized not only 19A and 20A, but also 20B, 20H, 21J, and 21K virus variants. One of the serum antibodies (100F8) completely neutralized 20A, 21J, and partially 21K strains. 100F8 was structurally similar to published Ab188 antibody, which recognized non-conserved epitope on the S protein. We proposed that 100F8 and other serum antibodies of the patient which recognized non- and conserved epitopes of the S protein, could have additive or synergistic effects to neutralize various virus variants. Thus, mRNA vaccination could be beneficial for convalescents because it boosts production of neutralizing antibodies with broad-spectrum activity.

Introduction

In 2019, SARS-CoV-2 crossed the species barrier and infected the first person in China. Since then, there have been almost 700 million cases of COVID-19 and almost 7 million related deaths worldwide. Epidemiological studies and sequencing-based surveillance revealed that the virus evolved to adapt to the new host and evade the host immune response developed by previous infections with the virus and vaccinations [1]. The virus acquired mutations in its genome which resulted in substitutions, insertions, and deletions of amino acids in its proteins. Mutations in the viral spike (S) protein were used to define viral clades, lineages, and variants of concern (VoC) [2].

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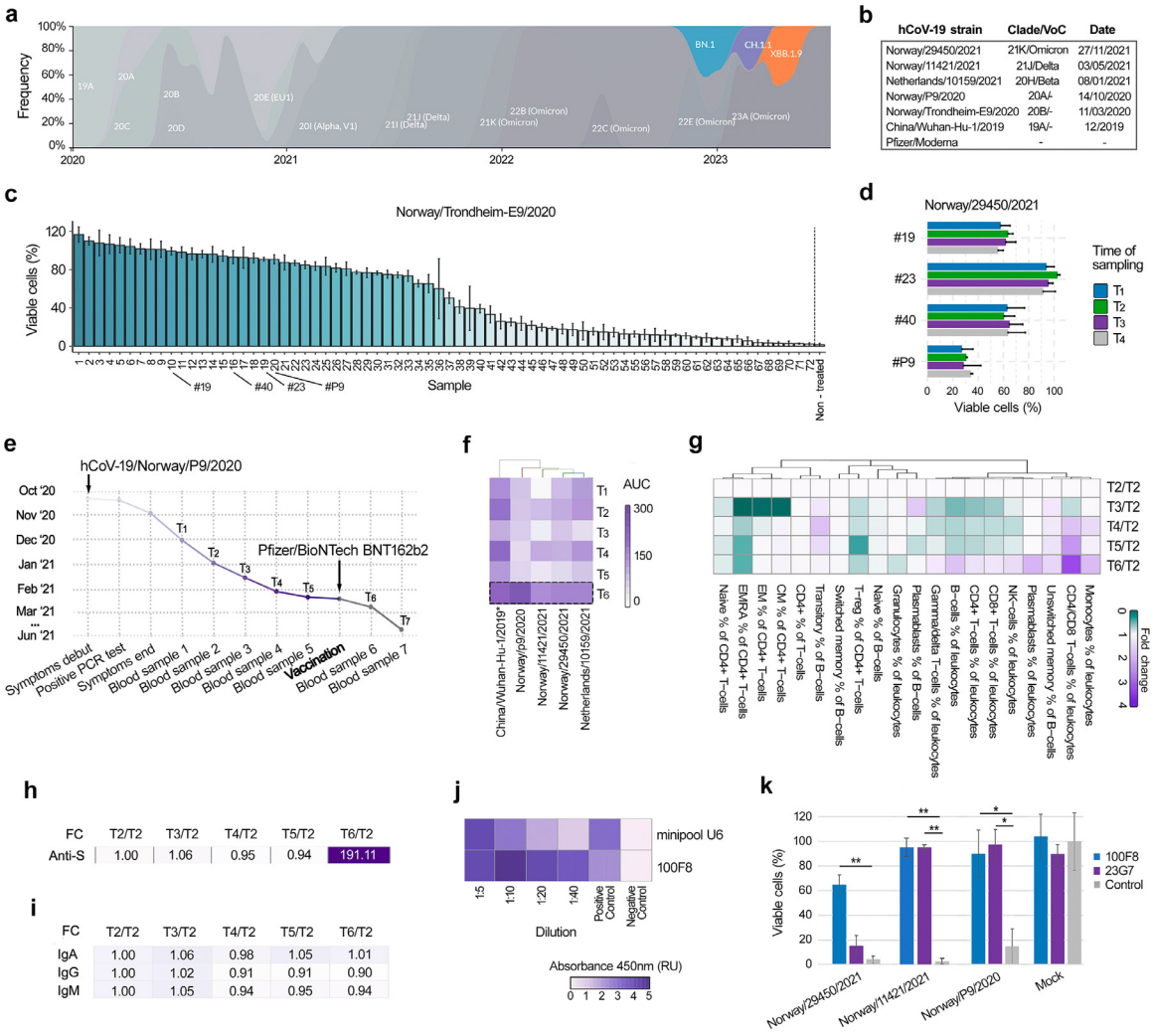

Figure 1. mRNA vaccination of COVID-19 convalescent patient boosted production of immune cells and antibodies neutralizing various SARS-CoV-2 variants. (a) Frequencies of viral clades in Norway based on Nextstrain SARS-CoV-2 resources. Frequencies were colored by clades and normalized to 100% at each time point. (b) SARS-CoV-2 strains used in this study. (c) The hCoV-19/Norway/Trondheim-E9/2020 strain (moi 0.01) was incubated with 1:10 dilution of sera obtained from 72 patients recovered from COVID-19, and one healthy blood donor. The mixtures were added to Vero-E6 cells. Cell viability was measured after 72 hours using CTG assay. Mean; n = 3. (d) The hCoV-19/Norway/29450/2021 strain (clade 21K) (moi 0.01) was incubated with 1:10 dilution of sera obtained from four patients recovered from COVID-19. The mixtures were added to Vero-E6 cells. Cell viability was measured after 72 hours using CTG assay. Mean; n = 3. (e) Graph showing the time of onset of COVID-19 symptoms, positive polymerase chain reaction test, end of symptoms, blood sampling, and vaccination of donor #P9. (f) Indicated strains were incubated with different dilutions of serum samples from patient #P9 patient. The mixtures were added to Vero-E6 cells. Cell viability was measured after 72 h using CTG assay. Mean; n = 3. Heatmap with area under the dose-response curve scores (area under the curve) is shown. (g) Heatmap showing FC of specific immune cell populations of patient peripheral blood mononuclear cells between T2, T3, T4, T5, T6, and T2 points measured by flow cytometry. (h) Heatmap showing FC of serum anti-spike antibody levels between T2, T3, T4, T5, T6, and T2 time points measured by ELISA. (i) Heatmap showing FC of total serum IgA, IgG, and IgM levels between T2, T3, T4, T5, T6, and T2 time points. (j) Heatmap showing binding of different dilutions of antibodies from ELISA-positive antibody minipool U6 and 100F8 antibody to spike protein of 21J strain measured by ELISA. (k) The indicated strains of 20A, 21J, and 21K clades (moi 0.01) were incubated with 1:10 dilution of cell culture supernatants containing 100F8 antibody, control 23G7 antibody (30 ng/ml) or PBS. The mixtures were added to Vero-E6 cells. Cell viability was measured after 72 hours using CTG assay. Mean; n = 3. ** - $P < 0.001$, * - $P < 0.01$ by *t*-test.
CTG, cell titer glo; ELISA, enzyme-linked immunosorbent assay; FC, fold changes; hCoV, human coronavirus; Ig, immunoglobulin; moi, multiplicity of infection; mRNA, messenger RNA.

Considerable variation has been observed in composition and titers of neutralizing antibodies among post-infected individuals [3,4]. The titers negatively correlated with time since infection allowing new virus strains to reinfect after 6-12 months and cause COVID-19 again [5]. Vaccination reduced the risk of COVID-19-related mortality; however, the effectiveness of vaccines has also declined with time and the advent of new VoCs [6].

It has been shown that people who were both infected and received messenger RNA (mRNA) vaccination did not develop severe COVID-19 after infection with new virus variants [7,8]. Here we questioned how mRNA vaccination of convalescents provided protection against severe COVID-19 regardless of virus variant.

Case

In Norway, the first COVID-19 outbreak began in February 2020 with virus of 19A clade followed by outbreaks associated with viruses of 20A-E, 20I (Alpha VoC), 21J (Delta VoC), and 21K, 22B, 22C, 22E, and 23A (Omicron VoC) clades (Figure 1a, Figure S1) [9].

We examined capacity of 72 serum samples collected from convalescent patients in 2020 in Norway to neutralize human coronavirus (hCoV)-19/Norway/Trondheim-E9/2020 strain of 20B clade which is almost identical to parental hCoV-19/Wuhan-Hu-1/2019 strain (19A; Figure 1b, Figure S1) [4]. Approximately half of the samples neutralized the strain and protected Vero-E6 cells from virus-mediated death (Figure 1c).

We noticed that serum samples from patients #19, #23, #40, and #P9 efficiently neutralized hCoV-19/Norway/Trondheim-E9/2020 strain. We collected three follow-up samples from each of the patients with a sampling interval of approximately 1-3 weeks. (Figure 1e, Figure S2). We tested neutralization capacity of these and initial sera against genetically engineered mCherry-expressing hCoV-19/Wuhan-Hu-1/2019 (clade 19B), hCoV-19/Norway/P9/2020 (clade 20A), hCoV-19/Netherlands/NoordHolland-10159/2021 (clade 20H), hCoV-19/Norway/11421/2021 (clade 21J), and hCoV-19/Norway/29450/2021 (clade 21K). The serum samples efficiently neutralized all strains except for the 21K (Omicron variant; Figure 1d, Figure S2). The serum samples from patient #P9 had the lowest neutralizing capacity for 21K strain.

The low neutralization capacity of the serum sample of patient #P9 toward 21K strain could be attributed to the substantial mismatch between sequences of S protein of hCoV-19/Norway/P9/2020, which infected patients in 2020 and hCoV-19/Norway/29450/2021, which emerged in 2021. hCoV-19/Norway/29450/2021 had nine substantial amino acid substitutions (T93I, G140D, N206I, S368L, S370P, S372P, E481A, N498Y, and P678H), VYY deletion at position 141-143, and RE and LLA insertions at positions 206-207 and 238-240, respectively (Figure S1).

Patient #P9 received first dose of Pfizer/BioNTech BNT162b2 mRNA vaccine in February 2021, 4 months after recovery from hCoV-19/Norway/P9/2020 infection (Figure 1e). The differences between patient- and the vaccine-encoded S proteins are K986P and V987P substitution and HV deletion at positions 68-69 (Figure S1). Despite this difference, the vaccination boosted neutralization capacity of serum against 21K variant and other tested SARS-CoV-2 strains several folds according to area under the curve scores (Figure 1f, Figure S3).

We analyzed serum and peripheral blood mononuclear cell samples from patient #P9 collected before (T2-T5) and after vaccination (T6). We observed substantial increase in clusters of differentiation (CD4/CD8) T-cells, B-cells, and plasmablasts after vaccination (Figure 1g). We also observed ~200-fold increase in anti-S antibody production (Figure 1h). We did not detect substantial changes in total immunoglobulin (Ig)A, IgM, IgG, total protein levels (Figure 1i), specific cytokines, and growth factors (Figure S4). Altogether, these results indicate that vaccination after COVID-19 boosted production of immune cells, which produced broadly neutralizing antibodies.

To obtain broadly neutralizing antibodies we collected peripheral blood mononuclear cells from patient #P9 3 months after vaccination (T7; Figure 1e). For this, we enriched B cells expressing antibodies against the S1-domain of S-protein and cloned the antibody-expressing genes using the HybriFree technology (Figure S5) [10]. Antibody library pools and single clones were generated in the hIgG1 framework, expressed in the CHO cell line, and tested for antigen binding by enzyme-linked immunosorbent assay. Antibody 100F8 from minipool U6 efficiently interacted with S1 protein of 21J variant (Figure 1j). The antibody completely neutralized parental 20B, and 21J strains, as well as partially neutralized 21K strains. By contrast, 99S8 antibody from the same patient and control antibody 23G7, which we cloned preciously from 20A-infected patients, did not neutralize 20K variant (Figure 1k; Figure S6) [11]. We sequenced full-length heavy and light chains of antibody 100F8. The Basic Local Alignment Search (BLAST) found regions of local similarity between 100F8 and other SARS-CoV-2 neutralizing antibodies, such as Ab188 (Figure S5) [12]. Ab188 recognized non-conserved region on the receptor-binding domain of S protein (Figure S7) but neutralized Omicron variant [12]. Since other serum antibodies can recognize conserved epitopes on the S protein and can have additive or synergistic effects with 100F8/Ab188, together they could neutralize previous and emerging virus variants. Thus, our results suggest that patient #P9 developed broadly neutralizing antibodies after infection with 20A strain and vaccination with an mRNA vaccine encoding S protein derived from hCoV-19/Wuhan-Hu-1/2019 strain.

Discussion

In this retrospective case study, we showed that mRNA vaccination of convalescent patients boosted production of immune cells and broadly acting antibodies, which could provide protection against various SARS-CoV-2 variants. Clearly, there are limitations to the study. Most obvious that only one convalescent vaccinated individual and one antibody were selected for in-depth analysis. However, similar studies with more volunteers and broad range of antibodies are available to strengthen our conclusions [12].

Our previous epitope-resolved profiling of the SARS-CoV-2 antibody response identified cross-reactivity with endemic human coronaviruses [13]. In particular, it revealed that some antibodies can cross-recognize conserved S2 region of S proteins of endemic and pandemic viruses. Perhaps, vaccination with endemic coronavirus vaccines early in SARS-CoV-2 outbreak could induce production of broadly acting antibodies, and thus could reduce morbidity and mortality, decrease hospitalization time and treatment costs, and prevent viral spread. This indicates the importance of developing vaccines against emerging and re-emerging human viruses to slow down the next epidemic and pandemic.

Conclusion

Corona and other zoonotic viruses re-surface regularly. Vaccination remains the main way to prevent severe viral diseases. Even if a patient recovers from the infection, vaccination can boost the immune response and protect people from reinfection with new variants of the virus. We showed that this is achieved through increased production broadly acting antibodies, which could recognize conserved and variable epitopes of the virus surface proteins.

Declarations of Competing Interest

The authors have no competing interests to declare.

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Author contributions

Conceptualization, D.K., M.H.F. S.A.N.; methodology, A.I., E.R., E.S., W.W., P.J., H.L., T.S., M.U., G.K., M-L.V., K.P., K.W., S.A.N., M.K., K.E., M.H.F., S.A.N., and D.K.; software, A.I.; validation, E.R., and D.K.; data curation, A.I.; writing - original draft preparation, A.I., D.K.; writing-review and editing, all authors.; visualization, A.I.; supervision, M.B., D.K., T.T., M.U., M.B., R.K., E.Z.; project administration, D.K.; funding acquisition, M.H.F., D.K., E.Z., M.B., T.T., M.U. All authors have read and agreed to the published version of the manuscript.

Data availability statement

All data generated or analyzed during this study are included in this manuscript and its supplementary information files.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.10.011.

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