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LETTER TO THE EDITOR

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Unremarkable antibody responses against various infectious agents after inoculation with the BNT162b2 COVID-19 vaccine

Coronavirus disease 2019 (COVID-19) messenger ribonucleic acid (mRNA) vaccines are highly effective in preventing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and disease onset.[1] The mRNA contained in vaccines can activate innate immune responses and is also translated into protein antigens in the cytoplasm, which can induce strong humoral and cell-mediated immune responses.[2] Since mRNA vaccines can be rapidly designed and developed after identifying the sequence of an emerging infectious agent, this vaccine platform is ideal for producing vaccines against emerging infectious diseases. Therefore, mRNA vaccines might become commonly used to prevent a variety of infectious diseases, especially emerging infectious diseases, in the future. Thus, it is important to elucidate the full spectrum of immune responses elicited by mRNA vaccines.

Some microbial molecules and microorganisms can nonspecifically induce the proliferation and differentiation of antibody-secreting cells, in a process called polyclonal B cell activation. This phenomenon can increase antibody titers against various infectious agents and induce auto-antibodies.[3] Since mRNA vaccines mimic common viral infections and can induce extremely strong immune responses, it is important to determine whether a COVID-19 mRNA vaccine can induce polyclonal B cell activation.

Twenty-four healthy adult health care workers (15 males and 9 females) aged between 28 and 60 years (median age: 46.5 years) who received two doses of the BNT162b2 vaccine were enrolled in this study (Approval No. HM20-089). All subjects did not have previous SARS-CoV-2 infection. Serum samples were collected at 3 points: before vaccination (Pre), 2 weeks after the first dose of the BNT162b2 vaccine (Post 1), and 2 weeks after the second dose of the BNT162b2 vaccine (Post 2). Immunoglobulin G (IgG) antibodies against the spike glycoprotein (S) and nucleoprotein (N) of SARS-CoV-2 were measured using a prototype indirect enzyme immunoassay (DK20-COV4E) provided by Denka. Antibody titers were calculated in BAU/ml with calibrators assigned to the first World Health Organization international standard (NIBSC code 20/136).[4] To measure IgG levels for antibodies against the other 9 microorganisms, we used commercial Enzyme Immunosorbent Assay kits (Antibodies- enzyme immunoassay "SEIKEN" provided by Denka).

Changes in serum SARS-CoV-2 N-IgG and S-IgG antibody titers before and after the two doses of the BNT162b2 vaccination are shown in Figure 1. SARS-CoV-2 N-IgG antibodies were not detected in any of the serum samples. SARS-CoV-2 S-IgG antibodies were not detected in any participants before vaccination, but a significant increase in antibody titers was observed in 21 (87.5%) of 24 participants after the first dose. Furthermore, a significant booster effect was observed in all participants. Changes in serum antibody titers for the 9 microorganisms are shown in Figure 2. Seropositivity for 9 microorganisms (11 antibodies) in prevaccination serum samples were as follows: 100% for rubella virus, varicella-zoster virus, and Epstein-Barr virus (EBV) (EBNA); 96% for measles virus and cytomegalovirus; 92% for EBV (VCA) and human parvovirus B19; 71% for *Bordetella pertussis* (FHA); 58% for mumps virus and herpes simplex virus; and 54% for *Bordetella pertussis* (PT). Two doses of the BNT162b2 vaccine did not change antibody titers against the 9 microorganisms (11 antibodies) at all.

Extremely strong immune response induced by COVID-19 mRNA vaccines raises concern about immune-mediated adverse reactions. Several autoimmune adverse reactions, such as Graves' disease,[5] autoimmune hepatitis,[6,7] and acute autoimmune transverse myelitis,[8] have already been reported in individuals after COVID-19 mRNA vaccination. It is considered that molecular mimicry can play an important role in causing vaccine-induced cross-reactive immunity against host tissue antigens. SARS-CoV-2 S protein, N protein, and membrane protein all cross-react with thyroid peroxidase.[9] In addition, polyclonal B cell activation[10] and bystander activation enhance cytokine production and expansion of autoreactive B and T cells. For example, the relationship between systemic lupus erythematosus and EBV, which can cause polyclonal B cell activation, has been intensively investigated.[11,12] Although the number of participants was limited, current data suggest that the BNT162b2 vaccine can elicit a strong immune response against the SARS-CoV-2 S protein in naïve healthy adults, but polyclonal B cell activation seems to be less common in these participants. However, since the small sample size is a study limitation, future studies will need to analyze a large number of participants to confirm this hypothesis.

In addition to certain viral infections, genetic factors are considered to be involved in the production of autoreactive antibodies. The possibility of polyclonal B cell activation should be examined in patients with autoimmune diseases after receiving the COVID-19 vaccine.

Abbreviations: BAU, binding antibody unit; COVID-19, coronavirus disease 2019; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; EU, enzyme-linked immunosorbent assay unit; FHA, filamentous hemagglutinin; IgG, immunoglobulin G; mRNA, messenger ribonucleic acid; PT, pertussis toxin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

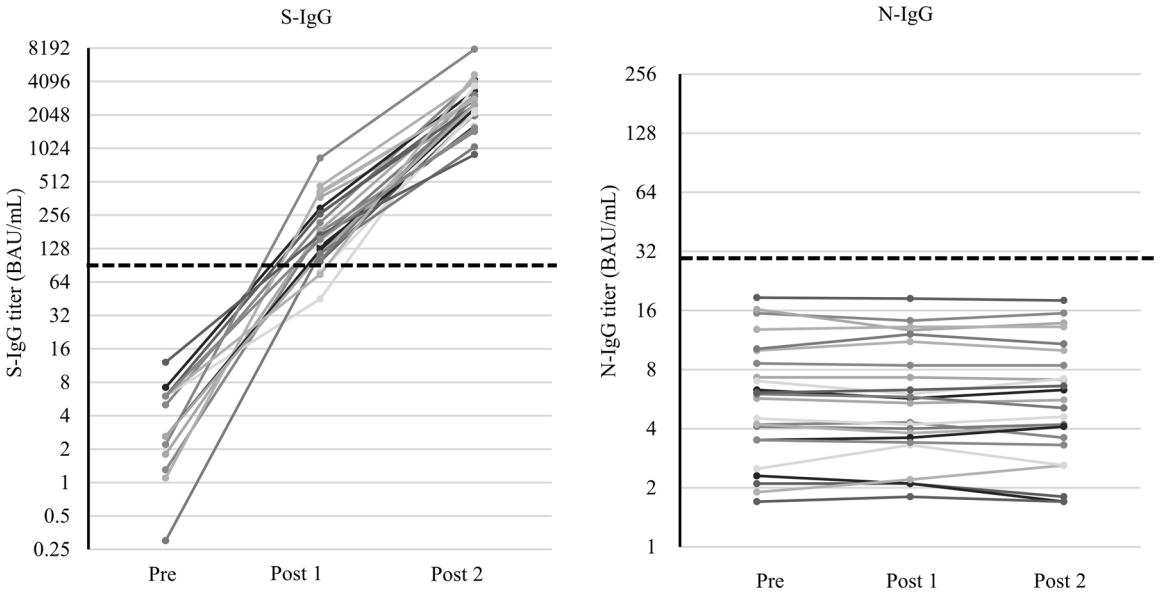

FIGURE 1 Changes in serum SARS-CoV-2 N-IgG and S-IgG antibody titers before and after two doses of the BNT162b2 vaccine measured with ELISA. Dotted lines indicate cutoff values. IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

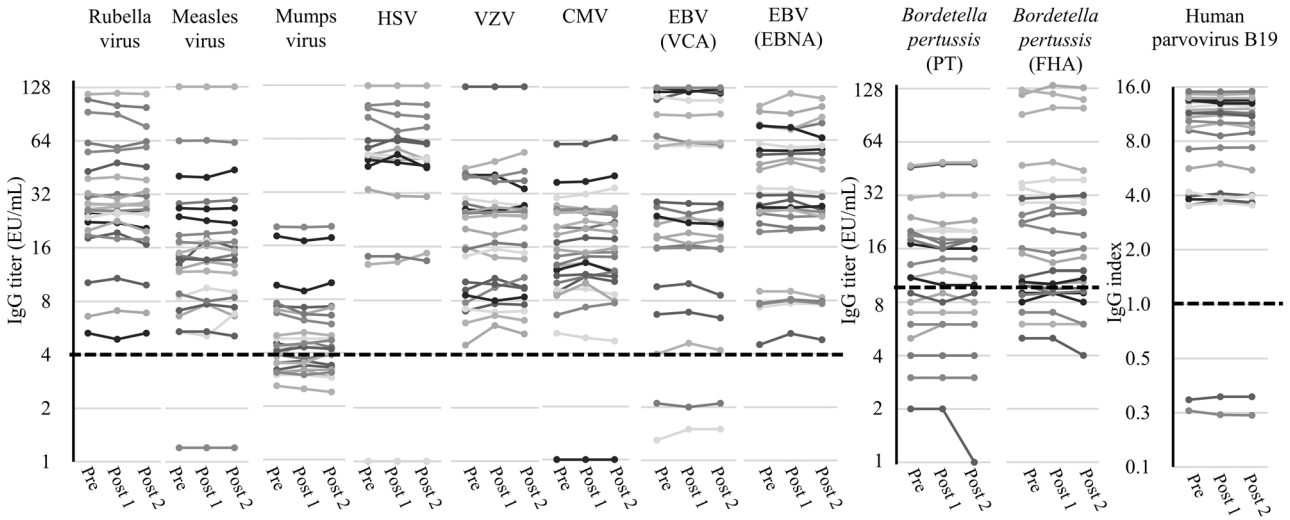

FIGURE 2 Changes in serum antibody titers against 9 other microorganisms (11 antibodies) before and after two doses of the BNT162b2 vaccine. Dotted lines indicate cutoff values. CMV, cytomegalovirus; EBNA, EB-nuclear antigen; EBV, Epstein-Barr virus; FHA, filamentous hemagglutinin; HSV, herpes simplex virus; PT, pertussis toxin; VCA, viral capsid antigen; VZV, varicella-zoster virus

AUTHOR CONTRIBUTIONS

Kei Kozawa: carried out conceptualization, data collection, data analysis, and literature research, and wrote and edited the manuscript. **Hiroki Miura, Yoshiki Kawamura, Yuki Higashimoto, and Masaru Ihira**: carried out data analysis and edited the manuscript. **Tetsushi Yoshikawa**: contributed conceptualization, discussion, editing, and supervision.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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