Induction of robust cellular and humoral immunity against SARS-CoV-2 after a third dose of BNT162b2 vaccine in previously unresponsive elderly

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Brief Communication

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Abstract

Herein, we compared SARS-CoV-2-specific antibody and T-cell responses to two doses of BNT162b2 mRNA in 51 vaccinees > 80 years old and 46 (20–53 years old) controls. The responses of the elderly were much lower and 10% non-responders were identified. Importantly, in four of them, a third vaccination raised the immune response to levels seen in responders after two vaccinations, thus implying that non-response is not fateful even in the elderly.

Brief Communication

Vaccination protects against fatal courses of SARS-CoV-2 infection, also in the elderly[1,2]. Induction of neutralizing serum antibodies was observed after two intramuscular applications of the BNT162b2 mRNA COVID-19 vaccine in people > 80 years of age[3]. However, recent outbreaks among elderly vaccinees[4], and antibody responses inferior to those observed in younger vaccinees[3], prompt discussion on the necessity of a third vaccination.

Herein, we compared vaccine-induced humoral and cellular immune responses to SARS-CoV-2 in 51 individuals aged > 80 years (elderly) and in 46 control individuals (young) (45 aged 20–44 years, plus one 53 year old woman, Table S1). All participants were vaccinated twice, at day 0 and day 21 with BNT162b2 in a vaccination center (elderly) or a doctor's practice (young) in Marburg, Germany, March-May 2021. Analysis of spike-specific IgG and CD40L + IFNγ + CD4 T cells in peripheral blood revealed strong induction of humoral and cellular immunity in response to vaccination (Fig. 1A,B). This finding confirms previous data on antibodies[3]. As for spike-specific CD4 T cells, our data vary from this report[3], as young and elderly groups demonstrated a further increase (10-fold average), between first and second dose (Fig. 1B, day 21 versus day 35). This was previously not noted[3], potentially due to the different method used for their quantification (FluroSpot), as compared to the herein applied multi-parameter flow cytometry (Fig. S1).

Several important differences were noted between young and old vaccinees' immune responses. First, the overall antibody and CD4 T cell response was lower in elderly vaccinees at a high level of significance (Fig. 1A,B, day 35). Secondly, while responses were comparable across young donors, substantial heterogeneity was observed in elderly donors, both in antibody and CD4 T cell responses, whereby several elderly showed scarce or even no reaction. Thirdly, some elderly had high frequencies of responding CD4 T cells before vaccination (Fig. 1B, day 0), likely reflecting cross-reactive activities gained during previous encounters with other corona viruses, as demonstrated before[5,6].

By combining results for antibodies and CD4 T cells for each vaccinee (Fig. 2A,B), we identified five elderly individuals retaining very low levels of specific serum IgG together with almost absence of spike-reactive CD4 T cells (Fig. 2B, red triangles). Remarkably, these elderly were potentially not protected by the previous two doses of the vaccine. Among them, donor #31 received methotrexate for rheumatoid arthritis, while no history of immune modulating medication or disease was evident in the remaining four.

No similar non-responder was found within the young cohort (Fig. 2A). Notably, our local authorities (Regional Counsil of Giessen, Hesse, Germany) informed us about breakthrough infections in several retirement homes. These infections occurred between one and three months after second vaccination with BNT162b2 and 5 out of 45 infected inhabitants > 80 years succumbed to infection. Thus, the lethality of these breakthrough infections is remarkably similar to the frequency of non-responders in our elderly study cohort. No COVID-19 infections were recorded in our cohorts until August 2021.

Aiming to enhance SARS-CoV-2 immunity, all five elderly non-responders received a third dose of the BNT162b2 mRNA vaccine during week 16 after the first dose. At that day, blood analyses demonstrated absence of specific immunity to SARS-CoV-2. The third vaccination was well tolerated. Most importantly, two weeks later four out out of five vaccinees, including #31, demonstrated robust spike-specific T cell and antibody responses, comparable to that detectable in responders after two-dose vaccination (Fig. 2C,D). In donor #54, a healthy man without obvious morbidities, specific serum IgG and T cell frequency also increased, however only to low levels. He was meanwhile vaccinated a fourth time, again with BNT162b2 and without obvious side effects.

Our data shows that the elderly initially hardly responding to two-dose vaccination can mount a virus-specific adaptive immune response after a third BNT162b2 dose. While the reason for primary unresponsiveness in our elderly cohort remains unclear, BNT162b2 unresponsiveness in the elderly is not fateful, and can be overcome by repeated vaccination. To confirm overall intact adaptive immune competence in initial non-responders, we tested for antibody and CD4 T cell reactivity towards control pathogens unrelated to SARS-CoV-2. Measles virus (MV)- or varicella-zoster virus (VZV)-specific IgG did not differ between non-responsive donors and all other aged donors at baseline (day 0, Fig. 2E). Additionally, the T cell responsiveness towards staphylococcal enterotoxin B (SEB) was comparable to that seen in the other elderly vaccinees throughout the observation period (Fig. 2F, Fig. S1). A similar response was observed in the young cohort (exemplified by their data on day 0), or in elderly (> 80 years) having recovered from COVID-19 infection several months before (Fig. S1). These results demonstrate that kinetics of T cell activation in our assay conditions are similar throughout participants and evaluated time points. Furthermore, initial unresponsiveness to vaccination is not indicative of an overall lack of immune competence; consequently, elderly who previously did not respond to vaccination, would likely benefit from a third dose of BNT162b2. Accordingly, in very recent studies of patients after allogeneic HSCT or on hemodialysis, only a subgroup of vaccinees reacted by a rise of antibody levels after a third vaccination, while T cell reactivity was not analyzed[7,8]. A third vaccination was recently shown to increase protection against COVID-19 in people > 60 years (Bar-On et al., 10.1101/2021.08.27.21262679). Likely, this effect combines overcoming of the herein described primary non-responsiveness, and a booster effect in primary responders, whose antibody titers may have gradually declined.

Overall, we show lower immune responses against SARS-CoV-2 in aged versus young vaccines, a finding which in preliminary analyses is also reflected in the antibody neutralization capacity against the SARS-CoV-2 delta variant (data not shown). Nevertheless, 90% of individuals aged > 80 years established adaptive SARS-CoV-2 specific immunity after receiving two doses of the BNT162b2 mRNA vaccine.

However, non-responders can be identified. Therefore, our data are suggestive of routine screening for spike-specific immunity in this population at risk, to assess the extent of immunity after two doses of BNT162b2. Screening should be unbiased and not limited to conditions of immunodeficiency or targeted immunosuppression. Should such tests reveal lack of specific immunity, re-vaccination should be considered.

Declarations

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Methods

Study participants

Blood samples were obtained from elderly aged >80 years by venipuncture before and at additional time points indicated in Figure 1 after primary and secondary vaccination by injection of Tozinameran (BNT162b2 vaccine, Comirnaty®) in the deltoid muscle, at a vaccination centre in Marburg, Germany (Table S1). Based on the lack of appropriate response to routine vaccination, the vaccination center decided to vaccinate five individuals a third time with BNT162b2 vaccine 16 weeks after day 0, and blood samples were again obtained immediately before and 2 weeks after the third vaccination.

Analyses were performed between March and May 2021 and in August 2021 for third vaccination candidates. In May 2021 the study also obtained samples from unvaccinated elderlies >80 years of age living in a retirement home, having recovered from COVID-19 after an outbreak with SARS-CoV2 variant B.1.221 in January 2021. All donors provided informed consent to participate in the study. Few donors were later excluded from the study for reasons given in Table S1.

The study of patients with COVID-19 and vaccinations against COVID-19 was approved by the ethics committee of the medical faculty of the Philipps-University Marburg (study number 40/21-12032021).

Sample processing and clinical lab

Blood serum was isolated from Serum Separator Clot Activator tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) according to the manufacturer's instructions, and stored at -80 °C until analysis.

PBMC were isolated from fresh heparinized whole blood by density gradient centrifugation over Pancoll human (Pan Biotech, Aidenbach, Germany) after dilution with an equal volume of PBS at room temperature. PBMC were washed twice (500 x g, 10 min, 4 °C) in cold PBS supplemented with 0.2% BSA, counted manually, and resuspended in RPMI 1640 media (Gibco, Life Technologies, Carlsbad, CA) supplemented with penicillin, streptomycin, and 10% human AB serum (all Sigma, St. Louis, MO) at 5 x 10^6 cells / mL.

Assessment of antigen-specific T cells

Antigen-reactive T cell responses were analyzed using a protocol based on previous work[9]. A detailed protocol is given in the supplementary section. Briefly, 5 x 10^6 PBMC were stimulated with either SARS-CoV-2 spike protein peptide mix (wildtype, Miltenyi Biotec), SEB (0.7 µg / mL, kindly provided by Prof. Bernhard Fleischer, Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany), or with an equal volume of water as a control, in the presence of anti-CD28 (5 µg / mL) and monensin (1 µg / mL) for 12 hours. Brefeldin A (1 µg / mL) was added 2 hours after the start of the stimulation. The stimulation was stopped by adding 2 nM EDTA. Dead cell labeling was performed by resuspending the cell pellet in 500 µL PBS supplemented with 1:1000 amine reactive Zombie Aqua™ Fixable Viability dye (Biolegend), and PBMC were fixed for 20 minutes using 2% formaldehyde solution (Thermo Scientific, Germany). Thereafter, cells were stained with acocktail of antibodies as detailed in the supplementary section. Acquisition was performed on a MACSQuant 16 flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany).

FlowJo version 10 (BD, Ashland, OR) and OMIQ.ai (Santa Clara, CA) were used for analyzing flow cytometry data. Flow cytometry standard (FCS) files underwent quality control and, where applicable, anomaly removal by FlowAI[10].

Quantification of SARS-CoV-2-specific antibodies

Serum antibodies against the recombinantly expressed S1 subunit of the SARS-CoV-2 spike protein were quantified using the Anti-SARS-CoV-2-QuantiVac-ELISA run on the automated EuroLab Workstation (Euroimmun, Lübeck, Germany), following the manufacturer's protocol. Sera exceeding the detection range of the assay were pre-diluted 1:10 or 1:100 and measured again. Results obtained in relative units per mL were converted into binding antibody units (BAU) / mL by multiplication with the factor 3.2, according to the manufacturer's instructions. Results in BAU / mL are calibrated against the "First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136)". The lower cutoff for this assay is at 35.2 BAU / mL.

Virus-specific antibodies

IgG antibodies against measles and varicella-zoster (VZV) virus were quantified using the commercial Siemens Enzygnost® IgG ELISA kit that was run on an automated Siemens BEPIII® system. Values were

quantified using the alpha method, according to the manufacturer's instructions. The cut-off for VZV-specific IgG was 50 mIU / mL, and 150 mIU / mL for measles-specific IgG.

Statistical analysis details

Prism version 9 (GraphPad software, San Diego, CA) was used to display data, and perform descriptive statistics and significance testing.

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Figures

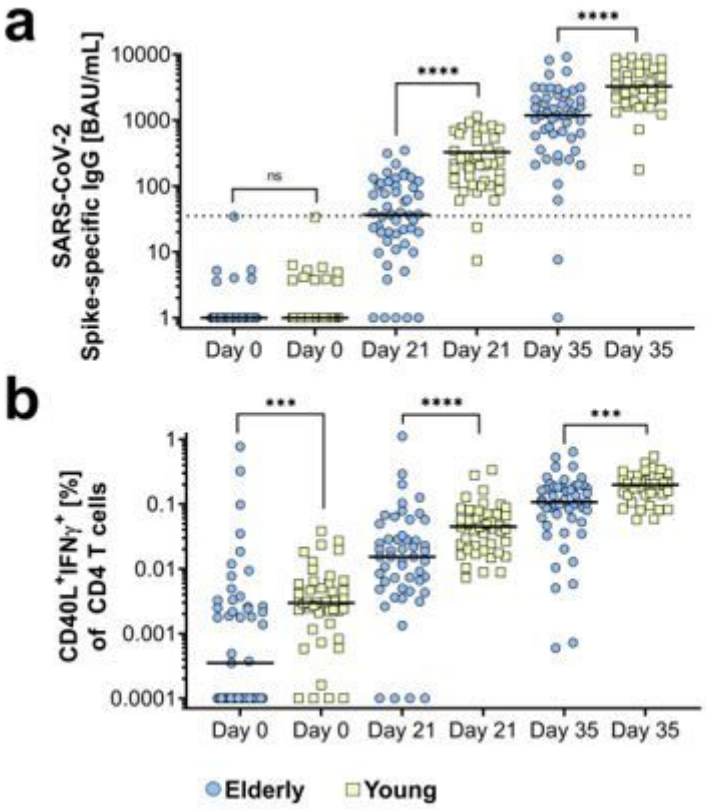

Figure 1

Humoral and cellular SARS-CoV-2 immunity in >80 and 20-53 year-old study participants vaccinated with the BNT162b2 vaccine. SARS-CoV-2 spike-specific serum IgG antibody titers (A) and percentages of SARS-CoV-2 spike-specific CD4 T cells (B) were analyzed in 51 donors aged >80 years (blue symbols) and 46 donors aged 20-53 years (yellow symbols) before (day 0), 21 days after the first and 14 days after the second BNT162b2 (Pfizer-Biontech) vaccination (day 35). Each symbol represents one donor. Dashed lines indicate the cutoff for antibody positivity at 35.2 BAU / mL. *** and **** indicate P value lower than 0.001 and 0.0001 respectively, determined by two-tailed Mann-Whitney test.

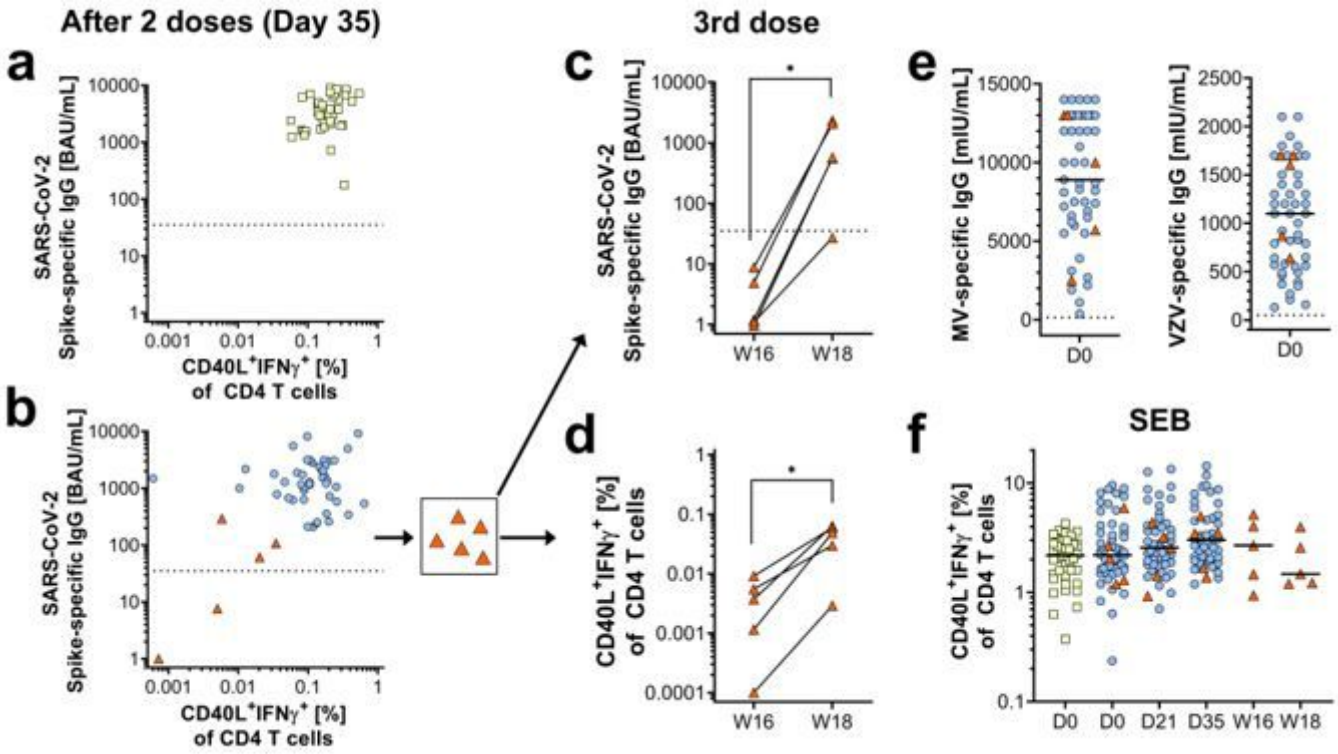

Figure 2

Humoral and cellular SARS-CoV-2 immunity in >80 year-old initial non-responders is rescued after a third dose of the BNT162b2 mRNA vaccine. Combined presentation per person of the SARS-CoV-2 spike-specific serum IgG antibody and percentages of SARS-CoV-2 spike-specific CD4 T cells at day 35 in young (A) and aged donors (B). Five individuals (red triangles) mounted only low specific antibody and T cell responses (B), and were vaccinated a third time in week 16. Antibody and T cell responses to the third BNT162b2 vaccination, measured in week 18, are shown in panels (C) and (D). (E) Antibody titers towards measles virus in young and aged individuals at day 0. (F) T cell response to SEB of the elderly participants measured at the indicated days or weeks, compared to the young cohort on day 0. (A-F) Each dot, square, and triangle represents one donor. Blue dots indicate aged responders, red triangles initial non-responders to the SARS-CoV-2-specific spike glycoprotein, yellow squares young donors. Horizontal lines indicate medians, dashed lines indicate the cutoff for antibody positivity at 35.2 BAU / mL for SARS-CoV-2 specific IgG (A-C) and 150 mIU / mL for anti-measles IgG (E). * and **** indicate P values lower than 0.05 and 0.0001 respectively, determined by one-tailed Wilcoxon matched-pairs signed rank test (C, D) and two-tailed Mann-Whitney test (E).

Supplementary Files

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