Characterization of antibody and T-cell response after second booster vaccination

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Abstract

The recent surge of infections with SARS-CoV-2 Omicron subvariants of prompted countries, such as Israel and Germany, to call for an accelerated booster vaccination program for health care workers and vulnerable groups in order to limit disease and transmission. However, detailed studies analyzing the correlates of protection over time after second booster vaccination are still lacking. Here, we examined the production of Spike receptor binding domain (RBD) -specific antibodies as well as neutralizing antibodies from subjects before, two, and seven weeks after the second booster vaccination against the D614G harboring B.1 variant as well as the variants of concern (VOC) Alpha, Beta, Delta in addition to Omicron BA.1 and BA.2. The second booster vaccination resulted in an increase in anti-RBD IgG antibodies and neutralizing antibodies against B.1 in all individuals tested, then remained nearly constant over the observed period. In addition, a 2nd booster resulted in an increase in neutralizing antibodies against VOCs Alpha, Beta, Delta, and Omicron subvariants BA.1 and BA.2. However, compared to B.1 the neutralizing capacity of both Omicron subvariants remained low. Neutralization of Omicron BA.1 and BA.2 was limited even after the 2nd booster vaccination indicating that an antibody-mediated protection against infection with this VOC is unlikely, as evidenced by the fact that three of the quadruple vaccinated individuals became infected with BA.1 during the course of the study. Moreover, T cell activation measured by interferon gamma release was detected in all subjects after the 2nd booster vaccination. This may offer protection suggesting protection against severe disease. T-cell activation was independent of the age of the subjects, but correlated with the amount of Spike-specific antibodies. Interestingly, in subjects with Omicron BA.1 breakthrough infection, a significant increase in neutralizing antibodies to all tested VOCs studied was observed after the 2nd booster vaccination. Taken together, our data suggest inferior protection from breakthrough infection with the Omicron subvariant BA.1 when compared to other VOCs after four vaccine doses.

Introduction

For two years counting, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has been affecting public health systems worldwide and has led to 6.02 million deaths so far (as of March10th, 2022 by the WHO) [1,2].

Since the end of 2020, different COVID-19 vaccines have been licensed, produced and distributed to the population in Germany as the main measure of protection against severe disease as well as containment of the pandemic [3]. Vaccination as well as natural infection induce a humoral and cellular immune response [4]. Diagnostic assays measuring these responses serve as valuable tools to evaluate protection efficacy. Particularly, the induction of antibodies that neutralize the virus are critical for the protective humoral immune response and level of those neutralizing antibodies have been reported to correlate with the severity of COVID-19 [5]. Those antibodies are substantially directed against the viral surface protein Spike [6] and decreasing titers over time lead to reduced protection (“immune waning”) [7–10].

During the ongoing pandemic, various SARS-CoV-2 lineages and variants have emerged of which the WHO classified some as variants of concern (VOC). Criteria for a VOC include increased transmissibility, virulence /clinical disease or decrease of effectiveness of therapeutic measurements such as immune escape from current vaccines-induced antibodies [11]. While the first VOC Alpha (B.1.1.7) [12] has been defined mainly by a highly increased transmissibility, others such as Beta (B.1.351) [13,14], Delta (B.1.617) [15,16] and Gamma (P.1) [17] revealed dominant immune escape capacities [18]. At the end of 2021, the variant Omicron (B.1.1.529) [19 20] was identified and has since spread rapidly around the world. Omicron is characterized by a multitude of mutations particularly in the Spike sequence and up to now at least three circulating sub-lineages (BA.1, BA.2 [21] and BA.3 [22]) have been identified. Studies support the notion that Omicron results in milder infections and less severe disease outcome [23], however health systems face consequences due to high patient numbers resulting from high transmissibility and immune escape leading to infections in vaccinated and recovered patients. While biopharmaceutical companies are working on current strain-specific vaccines [5,24,25], due to development, licensing and production processes, all currently approved and administered vaccines are based on ancestral virus strains [26].

As a consequence of immune escape and waning, Germany as well as other countries recently have recommended booster vaccinations with messenger RNA (mRNA) -based vaccines following a completed primary vaccination series [8,9,27–29]. However, in anticipation, some health care providers have started to administer their employees with 1st booster vaccinations, at a time when many citizens had not yet completed their primary vaccination series. As this group consists of health care workers with high risk of exposure and transmission, 2nd booster vaccinations were recently provided to this group during the Delta wave in winter 2021, immediately preceding the Omicron wave. Some countries, including Israel have administered 2nd booster vaccinations on a larger scale to its inhabitants [30]. A report from an ongoing clinical trial in Israel was recently published endorsed by an official recommendation for citizens > 60 years, vulnerable individuals and health care workers in January 2022, which the German institutions subsequently followed [31].

In this study, we evaluated a cohort of non-immunosuppressed individuals consisting of mainly health care workers before and after 2nd booster vaccination over time and analyzed the dynamics of serum antibody production and the activity of neutralizing antibodies against different variants of concern. Furthermore, these data were evaluated in parallel with findings of immunocompetent, otherwise healthy subjects who recently recovered from a SARS-CoV-2 Omicron BA.1 infection.

Results

Characterization of study cohort after 2nd booster vaccination

We investigated a cohort of 26 healthy subjects with a mean age of 49.6 years who were immunized against SARS-CoV-2 with a 2nd booster 4+/-0.5 months after the first booster. Vaccination schedules were not uniform among individuals, and a detailed summary of vaccination regimens is provided in Suppl. Table 1. Blood was collected before (N=15), at 2.4 +/-1 weeks (N=26), and at 7.3 +/-1.4 weeks (N=15) after the 2nd booster dose and antibody responses were subsequently analyzed (Fig 1A, Suppl. Table 1). During the study period, three individuals contracted an infection with the Omicron variant BA.1 (Suppl. Table 1).

In addition, convalescent sera were examined from 17 subjects who had had a documented infection with the Omicron variant BA.1 at a period comparable to the interval to 2nd booster vaccination in the other subjects (7.3+/-1.4 weeks versus 6.2+/-1.6 weeks) (Fig. 1B, Suppl. Table 2). All SARS-CoV-2-positive subjects enrolled in this study had symptomatic infections (WHO score 1-2).

Anti-Spike IgG antibody levels after 2nd booster vaccination

Anti-SARS-CoV-2 Spike RGB IgG antibodies determined by means of chemiluminescence microparticle-immunoassays were analyzed in all individuals and plotted comparing the different time points (Fig. 2A, Suppl. Table 1). As shown in Figure 2A, the 2nd booster dose of SARS-CoV-2 vaccine resulted in a significant increase in SARS-CoV-2 specific anti-RBD-antibodies two weeks after vaccination compared to t0 (Fig. 1A, 5.7 fold (range 3 – 27, $p<0.0005$). The antibody level did not correlate with the vaccination schedule (Fig. 2C) or with the age of the subjects (Fig. 2D). A slight decrease of 20.5% in IgG levels was detected 7.3 +/-1.4 weeks after the 2nd booster. The progression of antibody levels was the same in 8 individually recorded subjects (Fig. 2B). However, IgG levels in individuals who experienced a SARS-CoV-2 infection after the 2nd booster vaccination (red lines) continued to increase during the course (Fig. 2B). Thus, natural SARS-CoV-2 infection after 2nd booster vaccination can induce high IgG levels (Fig. 2A, B).

The primary routes of entry of SARS-CoV-2 are the oral and nasal cavities, thus mucosal immunity is critical to protect against infection [32]. In order to estimate whether IgG levels in saliva correspond to the level of IgGs in serum, we collected saliva of 10 specimens of subjects from our cohort on t2 after 2nd booster vaccination. Suppl. Fig. 1 demonstrates a correlation between serum and salivary IgG levels in our subjects and both COVID-19 recovered subjects (red dots) appear within the highest range.

Finally, we examined the level of SARS-CoV-2-specific antibodies in 17 time-matched subjects who had SARS-CoV-2 infection 2.2+/-0.8 months after the recommended 1st booster vaccination and who had

blood drawn 6.2+/-1.6 weeks after diagnosis, a period similar to t2 (7.3 +/-1.4 weeks, Fig. 1B, supplemental Table 2). Fig. 2A shows that Omicron infection after the 1st booster vaccination led to a larger increase in anti-RBD IgGs in our subjects compared with a 2nd booster vaccination (2.5 fold, p<0.0323).

Booster vaccination-elicited neutralizing antibodies against SARS-CoV-2 variants

Neutralizing antibodies are the most commonly measured indicator of protection against SARS-CoV-2 infection. Thus, a sufficiently high level of neutralizing antibodies is required for protection against infection. However, antibodies directed against SARS-CoV-2 are produced in varying quality and quantity after vaccination or recovery, and these levels decrease over time [33]. Thus, we evaluated the activity of vaccine-elicited sera after the 2nd booster vaccination to neutralize *in vitro* SARS-CoV-2 infection using authentic virus isolates and characterized protective capacities among the SARS-CoV-2 VOCs Alpha, Beta, Delta, and Omicron BA.1 and BA.2. As shown in Fig. 3A, neutralizing antibodies against the parental strain B1 harboring the D614G mutation were detected in all but one individual 4 months after the 1st booster vaccination (t0). After the 2nd booster (t1), neutralizing antibodies against B.1 increased 7 fold (t1; geometric mean (GMT) 40 vs 287) and remained at a comparable level after 7.2 weeks (t2 GMT 287 vs 307) (Fig. 3A). The Alpha variant remained sensitive to neutralization as previously described in vaccinated as well as convalescent sera [14], but did not exhibit a further increase after 2nd booster (Fig. 3B). Three months after the 1st booster (t0), only 60-20% of sera showed neutralization activity against the immune escape associated VOCs Beta, Delta, and Omicron subvariants BA.1 and BA.2, which increased after the 2nd booster vaccination (Fig. 3C - F). However, in a direct comparison of VOCs, neutralization efficiency of Beta, BA.1, and BA.2 was poorest 3 months after the 1st booster, but was increased after the 2nd booster and was then comparable to neutralization of of Alpha (Fig. 3G). However, all VOCs remained significantly below the activity against B.1 (Fig. 3G).

Overall, we observed an increase in neutralizing antibodies after the 2nd booster, but only low titers were achieved, especially for Beta, BA.1 and BA.2, which do not reliably protect against infection with this variant *in vitro*.

Neutralizing antibodies against diverse variants of SARS-CoV-2 after Omicron BA.1 infection

During the Omicron waves around the globe, breakthrough infections in vaccinated as well as boostered individuals were frequently reported. Thus, we furthermore evaluated individuals who contracted an

infection either after the first or the second booster and assessed their level of neutralizing antibodies against different SARS-CoV-2 variants. As shown in Fig. 3, infection with BA.1 resulted in significant enhancement of neutralizing antibodies 6.2 weeks after infection against Alpha, Beta, Delta, BA.1 and BA.2 (Fig. 3 B-F). Overall, sera from subjects after booster vaccinations and consecutive Omicron infection exhibited the most efficient neutralization activity against a broad range of variants that is significantly increased compared to a 2nd booster alone (Fig. 3 G, H). Only neutralisation of the parental variant B.1 were not further increased.

Evaluation of Interferon Gamma Release after second booster vaccination

Memory T cell responses have been described to be essential for protection of severe outcomes of SARS-CoV-2 infection [34]. To evaluate antigen-specific T cell activation, we assessed 16 subjects using an interferon-y release assay (IGRA) 7.3 weeks after the 2nd booster vaccination. Overall, we detected activated T cells against SARS-CoV-2 in all quadruple-vaccinated subjects, and activation weakly correlated with the number of anti-Spike antibodies in serum (Fig. 4A) but not with the age of the subjects (Fig. 4B). The subject with a recent infection after the 2nd booster vaccination (shown as a red dot) had the highest score in the IGRA test.

Discussion

We examined the immune response of vaccinated subjects at two time points (2 weeks and 7 weeks) after a 2nd booster dose. Limitations of our study include the small number of subjects with 10 subjects covering all three time points (Suppl. Table 1). Using this cohort, our data show that the 2nd booster vaccination led to i) an intermediate increase in anti-Spike-RBD antibodies peaking two weeks after vaccination that remained at a similar level after 7 weeks, and ii) resulted in an activated T-cell response in the subjects in our study. Furthermore, the 2nd booster vaccination did not result in a substantial increase of neutralizing antibodies to the VOC Alpha, Beta, Delta and Omicron BA.1 and BA.2.

Various studies demonstrated that waning of IgG levels after COVID-19 immunization is universal and has been shown after two-dose prime vaccination basic and booster vaccinations[27,35–37], which prompted recommendations for additional booster vaccinations. In line with an ongoing clinical trial evaluating vaccine safety and efficacy of mRNA vaccines in health care workers in Israel [31], we found that IgG levels after the 2nd booster vaccination increased ~ 6–10 fold two weeks after administration, respectively [27,35–37]. However, this increase is considered moderate due to high baseline levels after 4–5 months after the 1st booster compared to IgG levels after two-dose prime immunization or first booster (app. 7 month after basic vaccination, > 30 fold depending on the study) [31]. While independent studies have limited comparability, it becomes clear that long term investigations regarding immune waning after different immunization schemes in healthy subjects are important to judge long-term vaccination schemes after the pandemic phase.

A further challenge of the pandemic is the emergence of novel immune escape variants of SARS-CoV-2 such as the Omicron lineage first identified in November 2021 [31,38]. We observed that sera from subjects who contracted SARS-CoV-2 Omicron BA.1 after the first, but also after the 2nd booster exhibited significantly elevated IgG levels against SARS-CoV-2 Spike-RBD compared to individuals that had been vaccinated 4 times. Increased levels of anti-Spike-RBD IgG after breakthrough infections preceded after basic immunization or booster vaccinations have been described earlier [13,20], thereby indicating different and/or additive mechanisms of immune responses after infection compared to vaccination [25]. However, detailed information about this phenomenon is lacking.

Three of our subjects of the study had a known symptomatic infection with BA.1 during the study one to six weeks after the 2nd booster vaccination as also reported in [6,39,40]. Also these samples were further examined for the activity of neutralizing antibodies against authentic variants after 2nd booster vaccination including the currently circulating Omicron variants BA.1 and BA.2. We demonstrated that the level of neutralizing antibodies against the Omicron subvariants was severely reduced compared to the parental B.1 variant at all time points despite high levels of anti-Spike IgGs after 2nd booster. This is in agreement with recently published studies assessing the activity of VOC such as Omicron after 1st booster vaccination [31]. Reduced neutralization of the Omicron subvariants was also described for convalescent sera of individuals infected with other SARS-CoV-2 variants [10,41–44]. In this report, we did not detect a significant difference between the Omicron sublineages BA.1 and BA.2 in sera after 2nd booster or subjects who recovered from Omicron BA.1 as observed in reports assessing sera after 1st booster vaccination [27,45]. In contrast, neutralizing antibodies against all variants including Delta and Alpha were detected in individuals after the 1st booster followed by infection with the SARS-CoV-2 variant Omicron BA.1. Intriguingly, Omicron BA.1 infection strongly enhances neutralizing antibodies against the Beta variant compared to 2nd booster vaccination alone (Fig. 3). Both variants as well as BA.2 share a mutation on position N501Y in the RBD of the Spike protein that has been assigned to strong immune escape characteristics [46]. These results suggest protection against infection with related variants such as BA.2 and possibly Beta after exposure to Omicron BA.1 infection in vaccinated or convalescent individuals [46], but reinfections with BA.2 after BA.1 infection have been extremely rarely reported [47].

Our data support the notion that a fourth dose of vaccination restores levels of anti-Spike antibodies and therefore counteracts immune waning [48]. However, the protection against *in vitro* infections, as measured with neutralization assays, is limited against immune escape associated VOCs such as Omicron BA.1 and BA.2. In consequence, the finding that booster vaccination led to increased, but low levels of neutralizing antibodies [10,49] could be interpreted that at-risk individuals such as immunocompromised patients or the elderly who have prominent immune waning [50] and high risk for severe disease could profit from vaccine doses every three months, particularly during times of high SARS-CoV-2 incidence. The positive effect of regular vaccination of the young and healthy population might be restricted, as currently the infection rate with Omicron remains high and the burden of disease remains low. Furthermore, in light of unequal vaccine distribution worldwide, the benefit from vaccinations with

periods < 6 months might be negligible for the healthy population particularly during low incidence periods.

A different approach lies in the development and rollout of vaccines against immune escape variants such as Omicron as recently announced by biopharmaceutical companies [32]. However, this variant-specific vaccine development appears to be hampered by the rapid evolution of SARS-CoV-2 and the risk of future variants of concern. Omicron BA.1 has been suggested to be emerge due to evolutionary pressure in a recovered and vaccinated population forcing the virus to develop immune escape variants [20], a phenomenon that will likely persists and might be further enhanced as the endemic state approaches and the non-therapeutic measures disappear.

Materials And Methods

Ethics statement

This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the Ethics Committee of the Faculty of Medicine at Goethe University Frankfurt (No. 2022-640).

Collection of clinical serum samples

Peripheral blood was drawn prior, 2 weeks or 7.5 weeks after the second booster vaccination and prepared for serological assays accordingly. For sera isolation, samples were centrifuged at 2000 x g, room temperature for 10 min. Prior neutralization assay, samples were inactivated at 56°C for 30 min.

Anti-Spike-IgG assay

Serum anti-Spike receptor binding domain (RBD) IgG antibody concentration was determined using the SARS-CoV-2 IgG II Quant assay and the Alinity I device (Abbott Diagnostics, Wiesbaden, Germany) with an analytical measurement range from 2.98–5680 binding antibody units per mL (BAU/mL).

Anti-Spike-IgG assays in saliva

Saliva specimens were collected and centrifuged in salivette tubes (Sarstedt) as instructed by the manufacturer. The samples were frozen (-20°C) and thawed prior analysis. For IgG testing, samples were diluted (1:2) in buffer plus (Euroimmun). The ELISA (Euroimmun) was performed using the manufacturers controls and protocols optimizied for salivary testing.

Interferon gamma release assay (IGRA)

The Interferon (IFN)-gamma release assay (IGRA) was applied to quantitate IFN-γ release by SARS-CoV-2-specific T cells and to assess hereby the cellular immunity to SARS-CoV-2 using Quan-T-Cell ELISA, Euroimmun, Germany. The assay was carried out according to the manufacturer's instructions. In brief, in the first step a specific T-cell stimulation was performed with antigens based on the S1 domain of the SARS-CoV-2-Spike-protein. Therefore, 500 µl of heparinized blood was added into stimulation tubes containing these specific SARS-CoV-2 antigens and incubated for 22-24 hours at 37 °C. The anti IFN-gamma ELISA assay was measured the next day using the Quan-T-ELISA (Euroimmun).

SARS-CoV-2 variants

The following SARS-CoV-2 virus variants were used in this study: B.1 (FFM7/2020), MT358643 [51]; Alpha (B.1.1.7) (FFM-UK7931/2021), MZ427280 [14]; Beta (B.1.351) (FFM-ZAF1/2021), MW822592[14]; Delta (B.1.167.2) (FFM-IND8424/2021), MZ31514 [16]; Omicron BA.1 (B.1.1.529a (EPI_ISL_6959868); Omicron BA.2 (B.1.1.529b) (EPI_ISL_6959871)[10].

Cell culture, virus propagation and virus strains

All reagents for cell culture were purchased from Sigma (St. Louis, MO, USA). The establishment of the A549-AT cell line stably co-expressing ACE2 and TMPRSS2 is described in [52]. Cells were cultivated in Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS), 4 mM L-glutamine, 100 IU/mL of penicillin, and 100 µg/mL of streptomycin at 37°C and 5% CO_2.

SARS-CoV-2 isolates were propagated and collected as described in [53]. In brief, cell culture supernatant was collected after Caco2 cells show cytopathic effects and subsequently aliquoted and stored at -80°C. Median tissue culture infective dose ($TCID_{50}$) was applied to determine the titer as described by Spearman [54] and Kaerber [55]. All handling with SARS-CoV-2 was performed under biosafety level 3 (BSL-3) conditions at the Institute for Medical Virology, Frankfurt.

Neutralisation assay

Heat inactivated sera were serially diluted (1:2) in MEM supplemented with 1%FCS, 4 mM L-glutamine, 100 IU/mL of penicillin, and 100 µg/mL of streptomycin. All specimens were tested in duplicates. Sera dilutions were incubated with 4000 $TCID_{50}$/mL of SARS-CoV-2 for 1 h at 37°C. Antibody/virus mix was then added to A549 A+T cells and CPE formation was monitored after 72 h using a light microscope. For each experiment, all virus strains were tested in parallel. Values below ≤ 10 are considered borderline neutralization.

Statistics

Graph Pad Prism (version 9.3.1, GraphPad Software, LLC) was used for statistical analysis. Statistical differences between groups was calculated using t-tests. The used test was indicated in the figure legend. Adobe Illustrator (Adobe) was used for figure design.

Declarations

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Figures

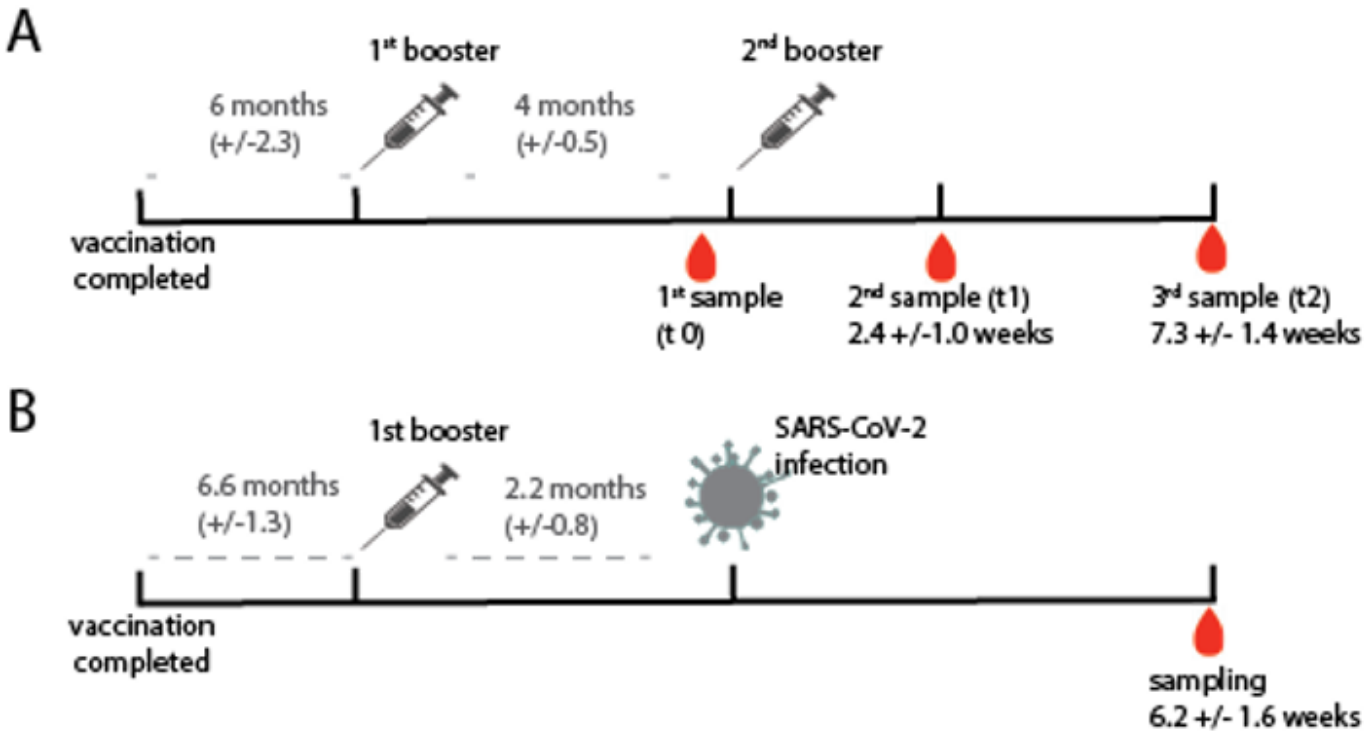

Figure 1

Study characteristics

Overview of vaccination scheme and sampling time points in **A** cohort after 2nd booster and **B** SARS-CoV-2 Omicron BA.1 infection after 1st booster.

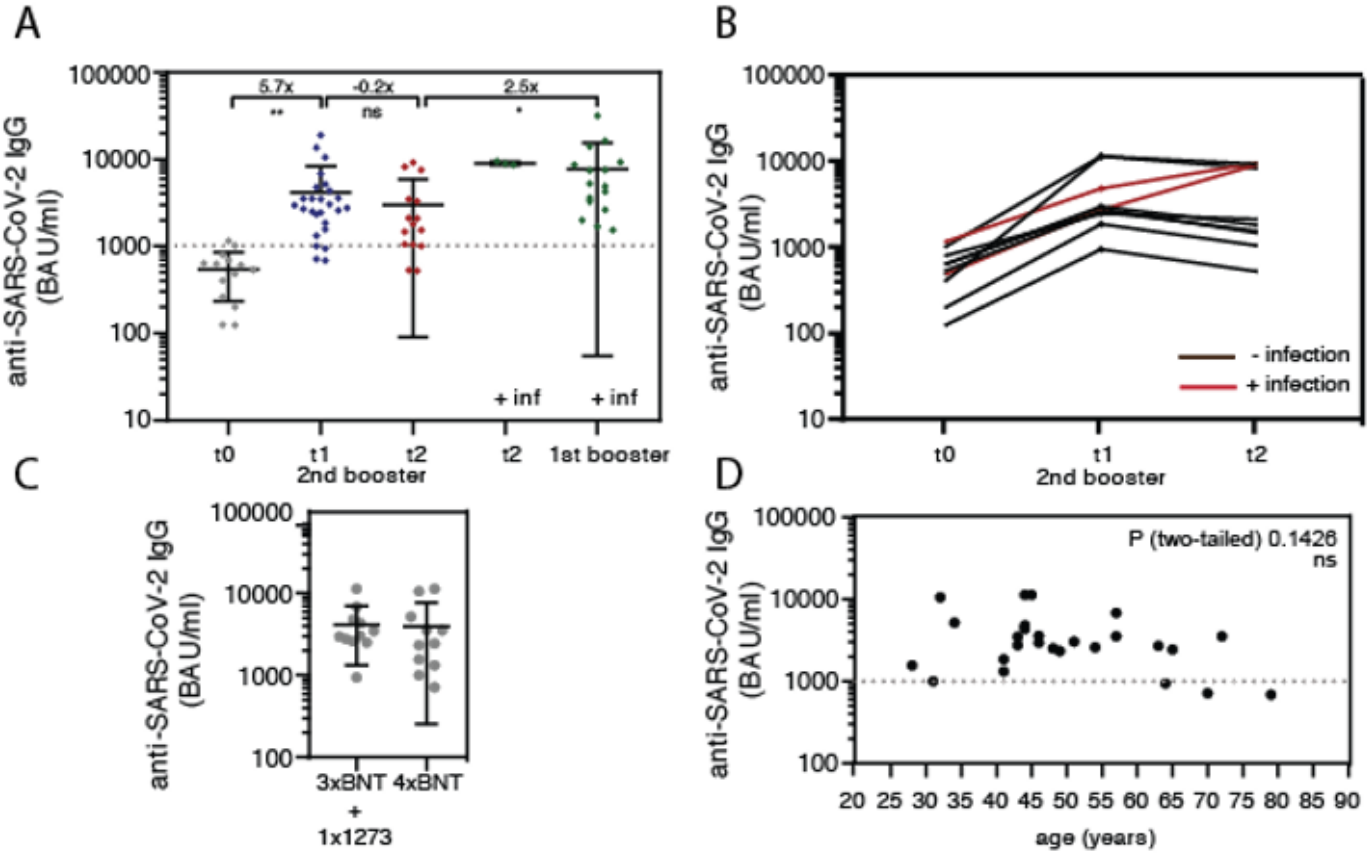

Figure 2

Characterization of anti-Spike IgG antibody response after 2nd booster vaccination or SARS-CoV-2 infection after first or second booster vaccination

(A) Anti-SARS-CoV-2 IgG levels were determined and indicated as binding antibody units per milliliter (BAU/mL) in samples before 2nd booster (t0), 2 weeks (t1), 7.3 weeks (t2), or infection after 1st or 2nd booster. (t-test, Mann-Whitney) **(B)** Individual responses of 8 individuals after 2nd booster vaccination and 2 after 2nd booster followed by a SARS-CoV-2 infection are plotted. **(C)** anti-SARS-CoV-2 IgG levels dissected between the most common immunization schemes in Germany in 2021 (3x BNT162b2 and 1X mRNA-1273 and 4x BNT162b2). (t-test, Mann-Whitney) **(D)** Correlation between age and IgG levels do not show any significance (linear regression – not significant). Abbreviations: BNT = BNT162b2; 1273=mRNA-1273.

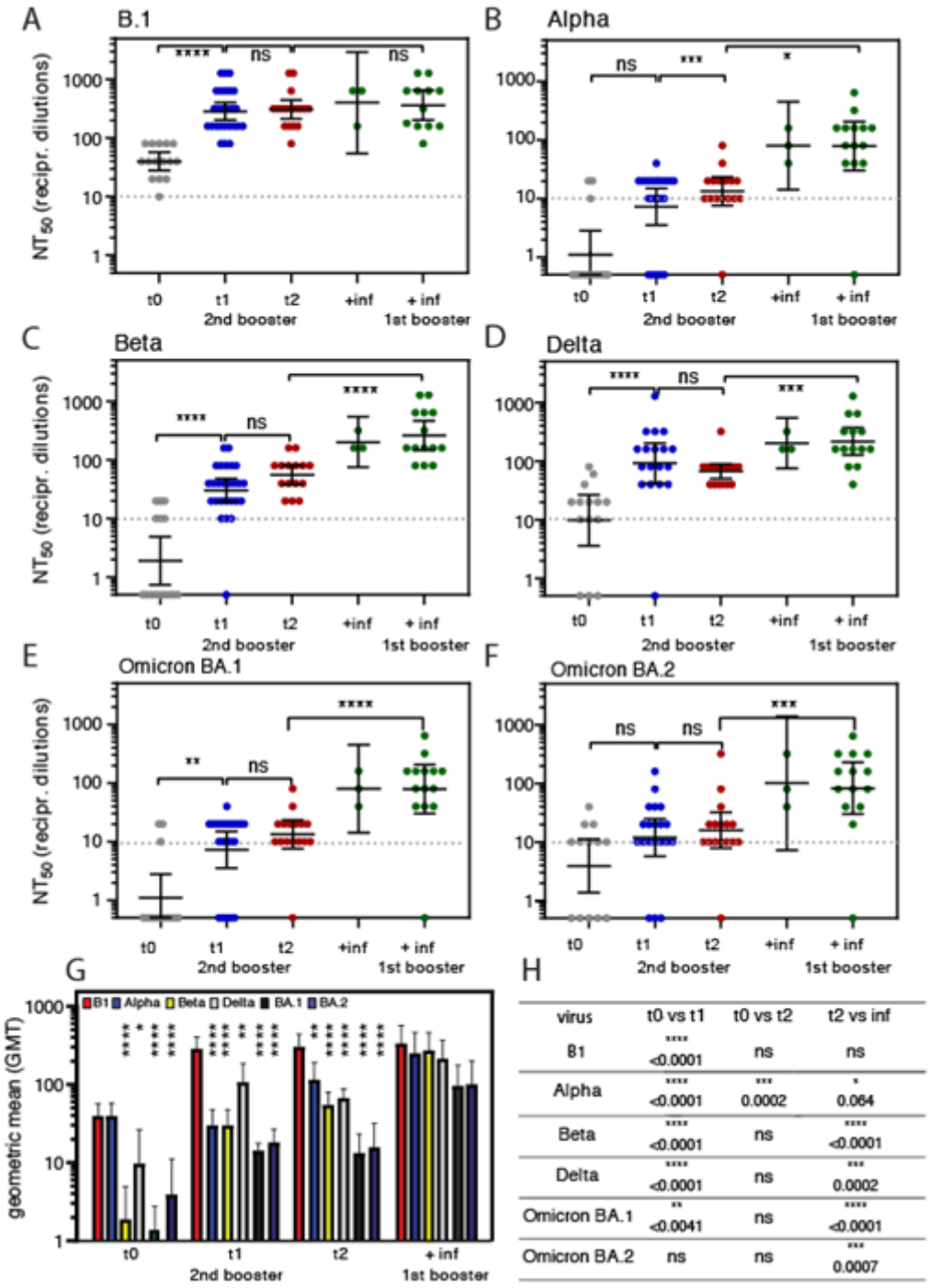

Figure 3

Characterization of neutralization titers of sera after 2nd booster and reconvalescent sera against VOC.

Neutralization assays were conducted using the authentic SARS-CoV-2 strains B.1 **(A)**, Alpha **(B)**, Beta **(C)**, Delta **(D)**, Omicron BA.1 **(E)** and Omicron BA.2 **(F)** using sera from recipients after 2nd booster vaccinations at t0 (N=15), t1 (N=26) and t2 (N=15). The analysis also includes sera from individuals after breakthrough infections after 2nd booster (N=3) and 1st booster (N=17). (t-test; Mann-Whitney, error bars = geometric mean with 95% CI). Statistics are summarized in (H). **(G)** Graph depicting the geometric means

of tested groups as indicated by the color code. (t-test, Mann-Whitney) **(H)** Summary of statistical analysis (t-test, Mann-Whitney).

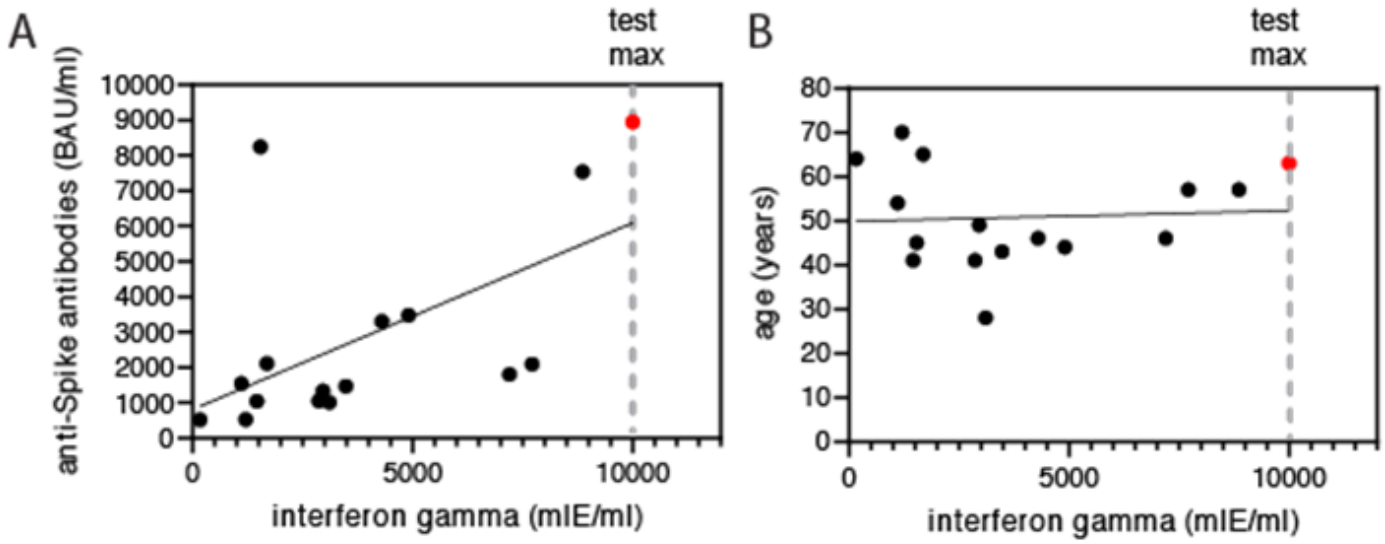

Figure 4

Activation of T cells after 2nd booster vaccination

Interferon gamma release assays were performed with 16 subjects 7.3 weeks after 2nd booster vaccination (suppl. Table 1). Interferon gamma concentration is measured in mIE/ml. Results were plotted against (**A)** the anti-Spike IgGs (BAU/ml) (linear regression – R = 0.3) or **(B)** age (years) (liner regression – not significant).

Supplementary Files

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- Supplement.docx