Humoral and cellular immune responses against SARS-CoV-2 variants and human coronaviruses after single BNT162b2 vaccination

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Summary: We assessed single vaccine shot induced B and T cell responses against SARS-CoV-2, variants of concern, and human coronaviruses to determine protection against COVID-19. Our results provide important information about surrogates of protection, test result interpretation and clinical decision-making.

Abstract

Background: Vaccine-induced neutralizing antibodies are key in combating the COVID-19 pandemic. However, delays of boost immunization due to limited availability of vaccines may leave individuals vulnerable to infection and prolonged or severe disease courses. The emergence of SARS-CoV-2 variants of concern (VOC), B.1.1.7 (United Kingdom), B.1.351 (South Africa), and P.1 (Brazil), may exacerbate this issue, as the latter two are able to evade control by antibodies.

Methods: We assessed humoral and T cell responses against SARS-CoV-2 WT, VOC and endemic human coronaviruses (hCoV) that were induced after single and double vaccination with BNT162b2.

Results: Despite readily detectable IgG against the receptor-binding domain (RBD) of the SARS-CoV-2 S protein at day 14 after a single vaccination, inhibition of SARS-CoV-2 S-driven host cell entry was weak and particularly low for the B.1.351 variant. Frequencies of SARS-CoV-2 WT and VOC specific T cells were low in many vaccinees after application of a single dose and influenced by immunity against endemic hCoV. The second vaccination significantly boosted T cell frequencies reactive for WT, B.1.1.7 and B.1.351 variants.

Conclusion: These results call into question whether neutralizing antibodies significantly contribute to protection against COVID-19 upon single vaccination and suggest that cellular immunity is central for the early defenses against COVID-19.

Key words: SARS-CoV-2, vaccination, antibodies, T cells

Introduction

Several vaccines encoding the viral spike (S) protein have been approved to combat the coronavirus disease 2019 (COVID-19) pandemic [1-3]. The recent emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOC) B.1.1.7 in the UK, B.1.351 in South Africa, and P.1 in Brazil may threaten measures to control the COVID-19 pandemic due to their ease of transmission [4, 5] and, in case of variants B.1.351 and P.1, resistance to neutralization by monoclonal antibodies (mAbs) and partial resistance to neutralization by antibodies induced upon infection and vaccination [6-10].

Results from clinical trials and real-world data suggest that vaccine protection against COVID-19 begins around two weeks after the first vaccine dose [1, 2, 11, 12]. However, only modest neutralization activity of sera was observed shortly before the second vaccine administration, and robust increase in neutralizing antibody titers required a second boosting dose [13, 14]. Due to the accelerating pandemic and the associated need to provide at least partial protection at the population level, the U.K. Joint Committee on Vaccines and Immunization has proposed extending the time to the second vaccine dose to enable first vaccination of more individuals within a short time period [15].

However, delaying time until second vaccination may lead to a sizable population of vaccinees with incomplete or short-lived anti-SARS-CoV-2 immunity and this approach may favor the emergence of escape variants. In order to address this question, we analyzed cellular and humoral immune responses induced by a single dose vaccination of the mRNA vaccine BNT162b2. We also determined the impact of preexisting immunity against human coronavirus (hCoV) on the vaccine response.

Results

Anti-SARS-CoV-2 S IgG and IgA levels were determined in individuals early (mean 8.7 days, range 2 to 14 days) and late (mean 20.6 days, range 17-27 days) after immunization with a single 30 µg dose of BNT162b2 (n=124). In addition, samples obtained at mean 21 days (range 6-36 days) after a second 30 µg dose (n=69) were analyzed. Antibodies of the IgG subtype directed against the S1 subunit of SARS-CoV-2 S became detectable around day 14 after the first shot. Almost all participants had measurable IgG levels 17 days after the first BNT162b2 dose and higher IgG levels after the second shot as compared to convalescent COVID-19 patients after mild disease (Fig. 1 A-B). Anti-SARS-CoV-2 IgA was detectable in most individuals at a mean of 20.2 days (range 19-25 days) after the first vaccination with further increase after the booster (Fig. 1C). The magnitude of the anti-SARS-CoV-2 S IgG antibody response was significantly higher 21 days after the second BNT162b2 dose and higher than convalescent COVID-19 patients after mild disease (Fig. 1B). Restricting the analysis on intra-individual responses over time gave similar results (Fig. 1D+E).

When testing plasma samples in a surrogate virus neutralization test (sVNT) for inhibition of RBD binding to plate-bound ACE2 receptor, a similar picture emerged (Fig. 1F, Suppl. Fig. S1). Most plasma samples from day 2 to 14 after the first BNT162b2 vaccination remained below the cut-off (30%) of the assay. In contrast, almost all participants had anti-SARS-CoV-2 S1 RBD inhibitory antibodies detectable after day 17 post first BNT162b2 vaccination. The second vaccination significantly increased inhibitory activity in this assay to levels above those in convalescent COVID-19 patients. To further assess the inhibitory activity of plasma samples after the first BNT162b2, we diluted plasma with >50 % inhibition in the sVNT and compared the results to those from convalescent COVID-19 patients or individuals 21 days after the second BNT162b2 vaccination. Plasma samples with inhibitory activity less than 90% at the highest plasma concentration (1:20) showed a rapid and linear decline by dilution. Only samples with baseline inhibition > 90% maintained > 50% inhibition in the sVNT upon further dilution (Fig. 1G) indicating low antibody concentrations in most plasma samples. Our data support the finding that anti-SARS-CoV-2

antibodies need little affinity maturation [16, 17], and become detectable in the plasma at 10-14 days post first vaccination.

The sVNT showed a highly statistically significant correlation with anti-SARS-CoV-2 S IgG concentrations (Fig. 1H) and, interestingly, convalescent COVID-19 patients exhibited higher SARS-CoV-2 inhibiting activity despite having lower IgG levels as compared to single vaccinated individuals. Finally, sVNT correlated closely to inhibition of SARS-CoV-2 S-driven host cell entry in a vesicular stomatitis virus (VSV)-pseudotype-based assay for detection of neutralizing antibody responses (Fig. 1I).

Next, we determined whether antibodies induced by a single BNT162b2 vaccination inhibited host cell entry driven by WT S protein (harboring D614G) and the S proteins of variants B.1.1.7, B.1.351 and P.1. For this, we used a VSV-based vector pseudotyped with respective S proteins, as previously described [8]. Plasma collected from patients with severe, current COVID-19 WT was included as a control. These plasma samples reduced entry driven by WT S and the S protein of variant B.1.1.7 with similar efficiency (Fig. 2A). In contrast, blockade of entry driven by the S protein of P.1 and particularly the B.1.351 variant was less efficient (Fig. 2A), which is consistent with our published data [8]. Similarly, and again in line with our previous results [8], plasma collected from vaccinees 21 days after the second BNT162b2 dose efficiently neutralized entry driven by the WT S protein and inhibition of entry driven by the S protein of B.1.1.7 was only marginally reduced (Fig. 2C, Suppl. Fig. S2B). In contrast, inhibition of entry driven by the S proteins of variants P.1 and particularly B.1.351 was less efficient (Fig. 2C, Suppl. Fig. S2B). Finally, plasma samples from the same donors obtained 21 days after the first dose exerted no (n = 8) or low (n = 5) inhibitory activity and reduced inhibition of entry driven by the S protein of B.1.351 was observed (Fig. 2B, Suppl. Fig. S2A). The overall summary of the inhibition analysis of n=19 vaccinees after a single dose is depicted in Fig. 2D and suggests that a single vaccination may frequently fail to induce a measurable neutralizing antibody response. Moreover, if such a response is induced, it may fail to protect against infection with the B.1.351 variant.

Besides neutralizing antibodies, the S protein also harbors T-cell epitopes which are central in COVID-19 immunity [18, 19]. To assess T cell immunity post vaccination, we analyzed the frequencies of T cells producing interferon-gamma (IFNγ) upon stimulation with peptide pools derived from the S protein of SARS-CoV-2, hCoV-OC43 and hCoV-299E, and cytomegalovirus (CMV) pp65 (as control) by enzyme-linked immunospot assay (EliSpot). T cells reactive to peptide stimulation from SARS-CoV-2 WT, B.1.1.7, and B.1.351 were undetectable in more than 40% of vaccinees after a single BNT162b2 shot (Fig. 3A+B, Suppl. Fig. S3) but increased significantly following boosting (Fig. 3A-C). Using an alternative *in vitro* SARS-CoV-2 specific cytokine release assay analogous to the tuberculosis IFNγ release assay [20, 21], we observed significantly increased IFNγ production by PBMCs after the first and second BNT162b2 vaccination as compared to controls, but responses remained low in a sizable proportion of individuals after only one vaccination (Fig. 4A). Interestingly, when we analyzed the IFNγ release assay results in single vaccinated individuals with low or absent spw in the EliSpot assay (Fig. 4A, green open circles), we found no clear correlation. In other words, in some vaccinees, low T cell frequencies were accompanied by strong IFNγ release, illustrating that both the quantity of specific T cells as well as qualitative cytokine production per cell strongly impact the overall antiviral response against SARS-CoV.2. This notion is further supported by the observation that T cells from some vacinees in our study released increased levels TNFα and IL-2 (Fig. 4A), while no other cytokine or chemokines where significantly increased (Suppl. Fig. S4).

Prompted by weak antibody neutralization activity in almost all individuals and low or even undetectable T cell frequencies after the first vaccination, we performed experiments to expand vaccine-induced T cells. For this, we stimulated PBMCs with SARS-CoV-2 S1 and S2 peptide pools from WT and VOC for seven days, which led to expansion and detection of responding T cells even in those individuals with initially low or no T cell response (Fig. 4B). This expansion was absent in healthy non-vaccinated controls with no anti-SARS-CoV-2 S IgG. In combination with our T cell

analysis, we conclude that a single BNT162b2 dose is able to generate an effective antiviral T cell response, likely contributing to the clinical efficiency observed two weeks after immunization.

SARS-CoV-2 is a member of the coronavirus family that includes hCoV-OC43, hCoV-HKU1, hCoV-229E, and hCoV-NL63. For the two hCoV variants tested in our work, we observed a significant expansion of hCoV-OC43 reactive T cells and an increase in hCoV-229E responsive T cells in the EliSpot (Fig.3 A+B), suggesting a strong overlap of hCoV with SARS-CoV-2 immunity upon vaccination. This overlap in response was further demonstrated by the significant positive correlation of SARS-CoV-2 WT and variants B.1.1.7 responsive T cell frequencies with those against hCoVs OC43 and 229E after the first and second BNT162b2 vaccination (Suppl. Fig. 5A), while no correlation between SARS-CoV-2 T cell responses and those towards the unrelated virus CMV occurred (Suppl. Fig. 5A). Importantly, T cell frequencies against SARS-CoV-2 WT also correlated closely and increasingly after the second vaccination with those observed for SARS-CoV-2 VOC (Suppl. Fig. 5B).

Discussion

Our comprehensive immunological analysis of B and T cell responses in a large number of individuals after a single BNT162b2 vaccination reveals important findings for the understanding of potential surrogates for protection against SARS-CoV2 WT, VOC, and preexisting cross-reactive immune responses against endemic hCoV. The overall summary of the inhibition analysis of vaccinees suggests that a single vaccination dose may frequently fail to induce a measurable neutralizing antibody response. Moreover, if such a response is induced, it may fail to protect against infection with the B.1.351 variant.

Clinical trials and real-world data from UK and Israel have shown protection against COVID-19 caused by SARS-CoV-2 WT or the B.1.1.7 variant [22] at around 14 days after the first vaccination against SARS-CoV-2 [1, 2][22]. Even before the second dose, BNT162b2 was highly effective, with a vaccine efficacy of 92.6%, a finding similar to the first-dose efficacy of 92.1% reported for the mRNA-1273 vaccine [23]. A retrospective study from Israel reported adjusted rate reductions of COVID-19

of 47% (95% CI 17–66) and 85% (71–92) for days 1–14 and days 15–28 after the first dose, respectively [12]. Similar declines after the first vaccination were reported by others [22]. A large prospective cohort study in Scotland revealed that the first dose of the BNT162b2 mRNA vaccine was associated with a vaccine effect of 91% for reduced COVID-19 hospital admission 28–34 days post-vaccination, although some of the observed effects may have been influenced by other factors [11]. These studies convincingly confirm the clinical efficacy of single and full BNT162b2 vaccination. However, they provide no information of surrogates for protection, some find presumably indirect vaccine program-associated effects at immediately after vaccination before immunologic mechanisms could play a role [11, 12], and none determined single vaccine dose efficacy after the surge of SARS-CoV-2 VOC.

Before the second BNT162b2 shot, we did not observe high titer neutralizing antibodies even against SARS-CoV-2 WT, in line with a pre-print report by Angyal A et al., in which neutralizing antibodies to B.1.351 were not detectable in infection-naive individuals following a single BNT162b2 dose [24]. One might speculate that high titer neutralizing antibodies may be a more important surrogate for outcomes after SARS-CoV-2 infection or after treatment with convalescent plasma [25], but less for protection from SARS-CoV-2 infection or COVID-19 after vaccination with BNT162b2. On the other hand, fully vaccinated individuals had only slightly reduced but overall largely preserved neutralizing titers against the B.1.1.7 lineage, indicating that the B.1.1.7 variant will not escape BNT162b2-mediated protection [26].

Our data on vaccine-induced immune responses are in line with our previous analyses in convalescent COVID-19 patients [17] and shows that the magnitude of B and T cell responses against SARS-CoV-2 upon vaccination is wide-ranging and differs for distinct virus variants. Particularly, the magnitude of SARS-CoV-2-specific T cell responses shows great heterogeneity and is not readily detectable after a single shot. This data suggests efficient T memory cell generation or booster of natural immunity against coronavirus variants after single vaccination, which was reactive after long-term *in vitro* stimulation with WT and mutant SARS-CoV-2 S peptide variants. These results provide

evidence for potentially effective, albeit weak, T cell immune responses against SARS-CoV-2 WT and VOC in a relevant proportion of individuals vaccinated with only the initial dose. This is in line with a study by Angyal and colleagues, in which one dose of vaccine elicited a significant but modest increase in T-cell responses in SARS-CoV-2-naive individuals, which was much more pronounced in individuals with prior infection. T-cell responses after two doses in naïve individuals were comparable to those elicited by a single dose in previously infected participants. The authors concluded that a single dose of vaccine generates comparable antibody and T-cell levels to those detected weeks or months after natural infection, which are highly likely to confer similar levels of protection against infection/re-infection [24]. In a study in health care workers without prior infection, a single BNT162b2 shot resulted in inferior immunity against VOC as compared to a boost vaccination in individuals with prior COVID-19. In addition, peptide pools containing B.1.1.7 and B.1.351 spike mutations led to increased, abrogated or unchanged T cell responses depending on human leukocyte antigen polymorphisms [27]. Studies in convalescent COVID-19 patients have described that efficient SARS-CoV-2-specific T cell responses are associated with milder disease [17, 28], suggesting that T cell responses may be central to control of SARS-CoV-2 infection. However, our study does not allow us to estimate whether these exclusively S-protein specific T cell responses significantly add to protection against COVID-19. Specific correlates of protection can only be established by studies observing a significant number of re-infections over time [17]. We suggest in-depth T cell analysis for post-vaccine responses in individuals with incomplete antibody responses due to e.g. immune deficiency in order to determine whether T cell responses are measurable in these patients and provide a potential replacement for antibody mediated protection. Importantly, undetectable T cell responses in standard T cell stimulation assays should not be interpreted as absence of T cells responsive to SARS-CoV-2 S protein after vaccination.

Our findings on strongly related intraindividual hCoV and SARS-CoV-2 immune responses are in line with our analyses in convalescent COVID-19 patients [29] and previously described associations described overlapping B cell responses against α and β-hCoVs [30]. Cross-reactivity

against SARS-CoV-2 and endemic hCoVs are mediated primarily by memory CD4+ T cell responses directed against conserved epitopes and have been reported in up to 50% of individuals [17, 18, 31-33]. Predictably, T cell frequencies against SARS-CoV-2 WT also correlated closely and increasingly after the second vaccination with those observed for SARS-CoV-2 VOC. Whether such cross-reactivity also occurs through COVID-19 vaccination and whether individuals with cross-reactive T cells may respond differently to vaccines than those without such memory cannot be concluded from our data, since we did not assess pre-vaccination responses.[34-36].

Our study is limited by the fact that we were unable to assess T cell responses before vaccination and that we only investigated one mRNA based vaccine. Secondly, the analyzed cellular responses would benefit from further identification of T cell subsets and viral epitopes involved [24]. Thirdly, our study only considers systemic responses and studies of airway compartments or tissue-resident T cells may be important to gain additional insights into protective immunity after vaccination against COVID-19.

In summary, our data demonstrate suboptimal neutralizing antibody activity against SARS-CoV-2 WT and VOC after a single BNT162b2 vaccination, consistent with previous studies[24, 35]. T cells, which responded equally to spike-derived peptides from SARS-CoV-2 WT, B.1.1.7 and B.1.351 were detectable with a broad inter-individual range and influenced by cross-reactive T cells against hCoV. We propose that non-neutralizing antibody function and/or cellular immunity constitute an important outcome after vaccination and may be part of the early defense against SARS-CoV-2 infection. We conclude that studies confidently assessing COVID-19 protection and sterile immunity against SARS-CoV-2 have yet to be completed and until then, variations in effective immunization programs cannot be confidently recommended [35, 37].

Methods

The study was approved by the Internal Review Board of Hannover Medical School (MHH, approval number 3639_2017, 8973_BO-K_2020, 9226_BO_K_2020, 9255_BO_K_2020, 9459_BO_K_2020) and University Medicine Göttingen (approval number SeptImmun Study 25/4/19 Ü). Following written informed consent, peripheral blood samples were obtained by venipuncture. Vaccinees for this analysis were health care professionals enrolled into the CoCo Study in 2020 before vaccination (See Table 1) for detecting silent seroconversions against SARS-CoV-2 infection [38]. Individuals with previous PCR confirmed SARS-CoV-2 infection or SARS-CoV-2 seroconversion before vaccinations were excluded from this analysis. Blood samples from individuals vaccinated with the BioNTech/Pfizer vaccine BNT162b2 were obtained mean 17.6 day (range 2 to 27 days) after the first and mean 21 days (range 6 to 36 days) after the second dose. Characteristic for convalescent COVID-19 patients with reverse transcriptase polymerase chain reaction (RT-PCR) test confirmed SARS-CoV-2 infection before the occurrence of VOC in Germany are summarized in Table 1. After blood collection, we obtained plasma from EDTA or lithium heparin blood (S-Monovette, Sarstedt) and stored it at minus 80°C until use. PBMCs were isolated from whole blood samples by Ficoll gradient centrifugation and stored in liquid nitrogen until use. For virus neutralization assays, we incubated plasma samples at 56°C for 30 min to inactivate putative infectious virus.

Serology

SARS-CoV-2 IgG serology was performed by quantitative ELISA (anti-SARS-COV-2 S1 spike protein domain/receptor binding domain IgG SARS-CoV-2-QuantiVac, Euroimmun, Lübeck, Germany) in all individuals according to the manufacturer's instructions (dilution 1:400). Antibody levels are expressed as RU/mL assessed from a calibration curve with values above 10 RU/mL defined as positive. Anti-SARS-COV-2 S1 spike protein domain IgA; Euroimmun, Lübeck, Germany) was done according to the manufacturer's instructions. Antibody amounts are expressed as IgA ratio (optical density divided by calibrator). The cPass Neutralization Antibody Detection kit (GeneScript) was used

to detect 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. For additional information of viral entry inhibition assays and detection of IFNγ release of SARS-CoV-2 S responsive T cells, see Supplementary Data.

Statistics

Data are presented as single results where possible with median of groups depicted as lines. Alternatively, data are depicted as mean ± standard deviation (SD). Comparison between groups were performed by two-sided unpaired or paired student's test, ANOVA with Bonferroni's post hoc analysis, Kruskal-Wallis test, or Fisher's exact test, where appropriate. Statistical analysis was performed by GraphPad Prism 5.01, which was also used for data illustration. A p-value of <0.05 was considered significant.

NOTES

Acknowledgments

We thank all participants of the CoCo study. We would like to thank Marion Hitzigrath, Sabine Buyny, Melanie Ignacio, Annika Heidemann, Luis Manthey, Till Redeker, Elisa Armbrecht, Annkathrin Anton, Christian Sturm, Julia Wahlen, Anna Zeisler, Oliver Keil, Mathäus Vetter, Andreas Bode, Birgit Heinisch, Gudrun Mielke, Nele Stein Daniel Gussarow, Juliane Ebersold, Nicole Neumann, Dörthe Rokitta, and Sophie Meyer for technical and logistical support. We thank the entire CoCo study team for help and Marcus Wortmann from the IT department for providing support during the vaccination rollout.

Funding

This work was supported by unrestricted grants from Novartis, Gilead, Kinderherz Hannover e.V., and Pari and in part by grants from the state of Lower Saxony [14 - 76103-184 CORONA-12/20] and the Federal Ministry of Health [ZMVI1-2520COR804]. GMNB is supported by German Center for Infection Research, MSW received unrestricted funding from SARTORIUS lung research.

Potential conflicts of interest: GMNB, CH, GMR, RB, and AD-J received unrestricted funding to their institution from Norvatis, Gilead, Kinderherz Hannover e.V., Pari for the CoCo Study. BE-V receiced funding paid to their institution from the State of Lower Saxony and SP received funding from the State of Lower Saxony (paid to their institution), the German Research Foundation, and the Federal Ministry of Education and Research. SP reports support paid to their institution by DFG and BMBF, during the conduct of the study. RB has the patent Method for identifying T-cell stimulating protein fragments" (PCT/DE99/00175) issued; has received royalties for granting use of patent "Method for identifying T-cell stimulating protein fragments" (PCT/DE99/00175) and "Method for antigen-specific stimulation of T lymphocytes with synthetic peptide libraries" (US 7,994,096 B1). MSW received unrestricted personal funding support from Sartorius; received personal payment, 1-time, for consulting for Amomed, outside the submitted work. MMH received personal payment or honoraria for consulting or lectures from Acceleron, Actelion, Bayer, GSK, Janssen, MSD, Pfizer. AB reports payment to their institution by State of Lower Saxony, during the conduct of the study. All other authors have declared no potential conflicts of interest.

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Table 1: Characteristics of convalescent COVID-19 patients and BNT162b2 vaccinees.

Convalescent COVID-19 patients							
				COVID-19 Severity			
N =	**Age in years (range)**	**Male/female**	**Weeks since diagnosis (range)**	**outpatient**	**hospitalized**	**ICU**	
37	41 (19-74)	13/24	9.03 (3-38)	33	1	3	Fig. 1
26	37 (19-57)	10/16	8.5 (3-36)	25	1	0	Fig. 3A Suppl. Fig. S3
20	54 (20-84)*	12/8	10.1 (7-14)	4	9	7	Fig. 4A
BNT162b2 vaccinees							
148	40.6 (22-66)	63/85	NA	NA	NA	NA	

* p<0.001, ANOVA with Bonferroni's post hoc analysis) compared the other convalescent COVID-19 patients and the vaccinees. No significant differences for age or sex between the groups. NA=not applicable

Figure legends

Figure 1: Humoral immune response after BNT162b2 vaccination. (A) Time course of anti-SARS-CoV-2 S protein IgG (n=124) after the first BNT162b2 dose. **(B)** Anti-SARS-CoV-2 S IgG responses in relative units per mL (RU/mL) after the first (V1, grey and green dots, n=30 and n=87, respectively) and second (V2, blue dots, n=87) BNT162b2 dose as indicated. Note the overlap to data in Fig. 1A. Recovered COVID-19 patients (RC, n=37) are depicted in purple. **(C)** Anti-SARS-CoV-2 S IgA responses after the first (n=65) and second (n=88) BNT162b2 vaccination as well as in recovered COVID-19 patients (n=37). ELISA results are depicted as sample/calibrator ratio and labeled as in B. **(D, E)** Intra-individual anti-SARS-CoV-2 S IgG and IgA responses as a function of time (grey dots, n=5-10, green dots, n=31-38, and blue dots, n=29-36). **(F)** Inhibition in the sVNT after the first or second BNT162b2 vaccination or in recovered COVID-19 patients (RC) as indicated (grey dots, n=16, green dots, n=84, blue dots, n=51, purple dots n=37). **(G)** Reciprocal plasma dilutions of the sVNT in convalescent COVID-19 patients (purple), after the second BNT162b2 dose (blue) or after the first BNT162b2 dose (green/grey). Note that only samples >50% inhibition at the lowest dilution (1:20) were further titrated. **(H)** Correlation of inhibition (sVNT) and anti-SARS-CoV-2 S IgG (ELISA) after the first or second BNT162b2 vaccination and in recovered COVID-19 patients as indicated for F. **(I)** Correlation of the sVNT inhibition with the NT50 of the pseudotype virus neutralization results (n=23) as indicated. Dotted lines represent the assay cut offs as suggested by the manufacturer. *p<0.05, **p<0.001, ***p<0.0001 by two-tailed paired student's t test (D, E) or ANOVA with Bonferroni's post hoc analysis (B, C, F).

Figure 2: S protein of SARS-CoV-2 WT, B1.1.7, B.1351, and P.1 variants show reduced neutralization sensitivity against plasma from single and twice BNT162b2 vaccinated individuals. Pseudotypes bearing the indicated S proteins were incubated with different dilutions of plasma derived from patients with severe COVID-19 **(A)** or plasma obtained shortly before the second dose

BNT162b2 **(B)** or 21 days after the second dose **(C)** and inoculated onto Vero target cells. Transduction efficiency was quantified by measuring virus-encoded luciferase activity in cell lysates at 16-20 h post transduction. The results are shown as % inhibition. For normalization, S protein-driven entry in the absence of plasma was set as 0%. Presented are the data from a single experiment performed with technical triplicates. Error bars indicate SD. Most results were confirmed in a second biological replicate. For more results and negative control see Suppl. Fig. S2. **(D)** Plasma dilutions that lead to a 50% reduction in S protein-driven transduction (neutralization titer 50, NT50) were calculated for convalescent COVID-19 plasma (purple, n=3) and vaccinee plasma after the first (green, n=19) and second BNT162b2 dose (blue, n=10). The line represents the median NT50 of single vaccinated individuals. WT = wildtype, NP = no plasma.

Figure 3: T cell frequencies against SARS-CoV-2 WT and variants B.1.1.7 and B.1.351, and hCoV OC43 and 229E after the first and second BNT162b2 dose. IFNγ EliSpot data for the SARS-CoV-2 variants and two hCoV from individuals vaccinated once (n=78-88) or twice (n=27-37) are shown. The first samples (grey dots day 2-14, green dots day 17-27 after the first dose) depict data from before the second vaccination and the second sample (blue dots) from 21 days after the second BNT162b2 dose. Purple dots represent results from convalescent patients mean 8.5 weeks (range 3-36 weeks) after mild COVID-19. **(A)** Data are depicted as the number of spots per well (spw)/2.5x10^5 PBMCs. For CMVpp65, values from individuals irrespective if their CMV serostatus are depicted (note the separate axis on the right). Bars represent median. **(B)** Number of individuals with zero (black) or ≥1 (grey) spw in the IFNγ EliSpot after the first (left column) or second (right column) BNT162b2 dose. **(C)** Changes in T cell frequencies as a function of time between the first (green dots) and the second (blue dots) vaccination. *p<0.05, **p<0.01, ***p<0.001, by two-tailed student's t test (A) paired t-test (C) or Fisher's exact test (B), ns=not significant.

Figure 4: SARS-CoV-2 S protein induced cytokine release after a single and double BNT162b2 dose. **(A)** IFNγ release assay results obtained from PBMCs of single (V1, green dots/circles) or twice (V2, blue dots) vaccinated individuals after restimulation with SARS-CoV-2 S protein for 24 h and assessment of IFNγ in the supernatant by ELISA or by multiplex cytokine/chemokine quantification assay for TNFα and IL-2. Green circles indicate individuals with ≤ 1 spw/2.5x10^5 PBMCs in the EliSpot assay after the first vaccination. All twice vaccinated individuals analyzed here had > 1 spw/2.5x10^5 PBMCs. Negative controls (N Ctr, grey dots) are from the same individuals as after the second vaccination (blue dots) but from PBMC collected in 2019 before vaccination. Purple dots represent results from PBMCs of convalescent patients with mild to severe COVID-19 (RC) at a mean 10.1 weeks (range 7-14 weeks) after symptom onset. For additional cytokine/chemokine results see Suppl. Fig. S4. **p<0.01, ***p<0.001, calculated by Kruskal-Wallis test. **(B)** PBMCs from individuals with low or zero spw after a single BNT162b2 dose (n=11) or healthy controls without anti-SARS-CoV-2 S IgG (both green dots) were *in vitro* stimulated with overlapping peptide pools from SARS-CoV-2 S WT protein for seven days and again assessed in the IFNγ EliSpot with S protein-derived peptide pools from SARS-CoV-2 WT or variants B.1.1.7 and B.1.351 as indicated. The black dots depict spw of *in vitro* stimulated cells of the vaccinated individuals, the grey dots depict spw of controls. Groups were compared by two-tailed paired student's t test.

Figure 1

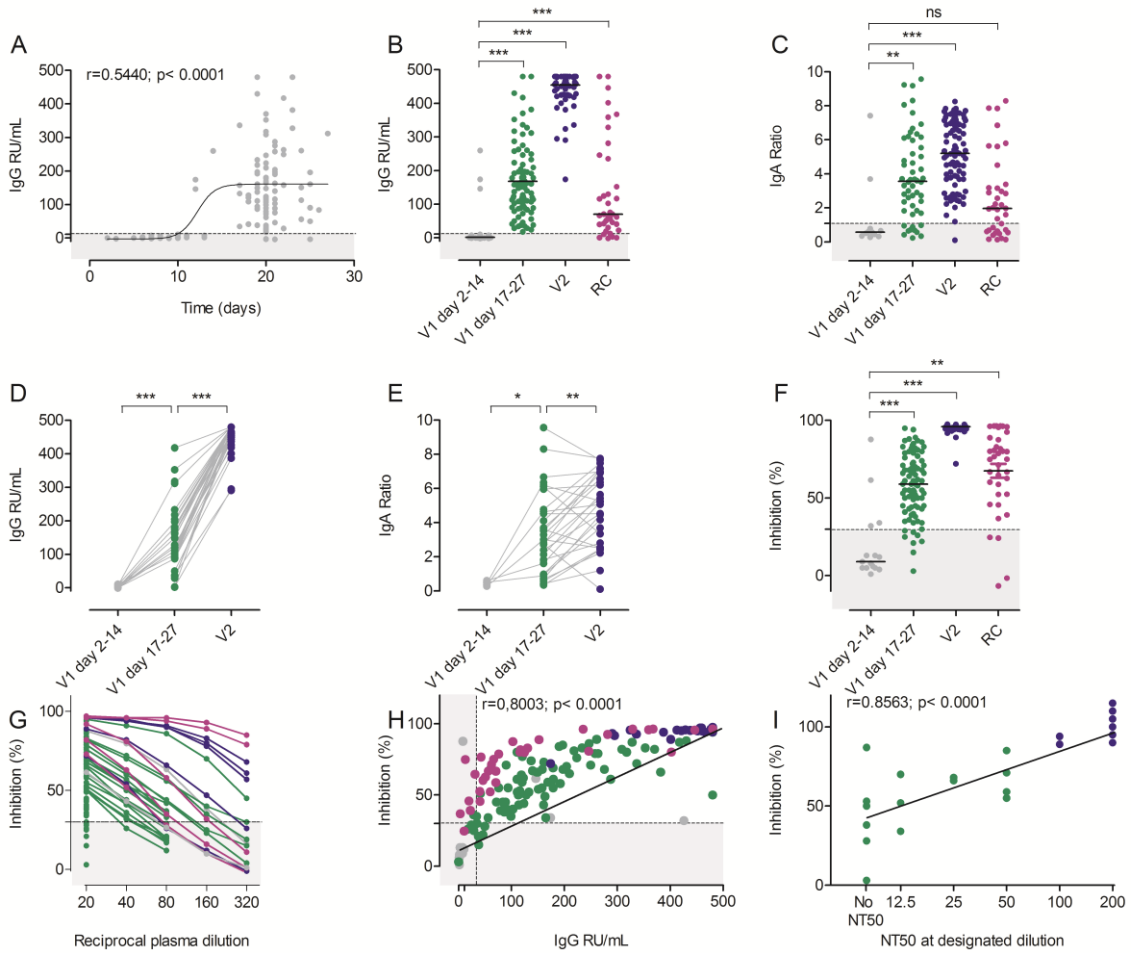

Figure 2

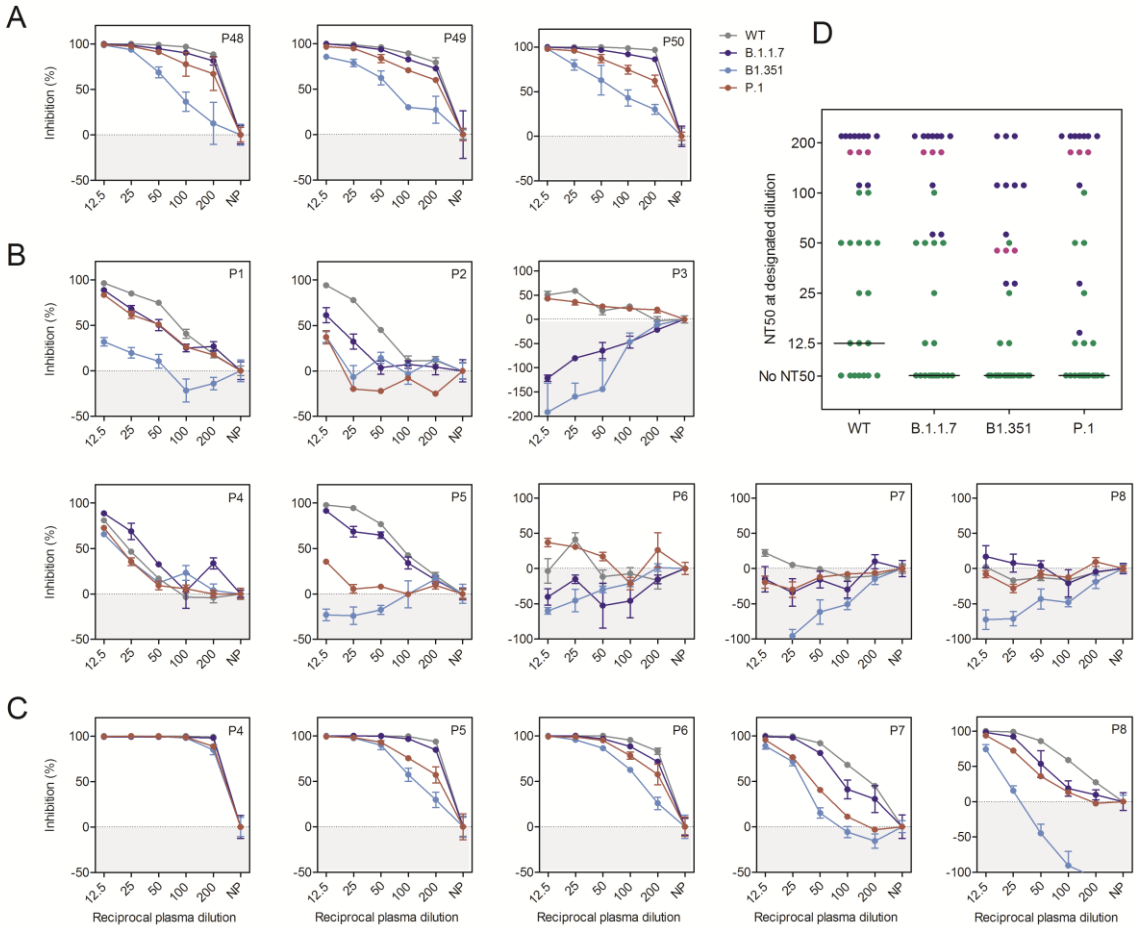

Figure 3

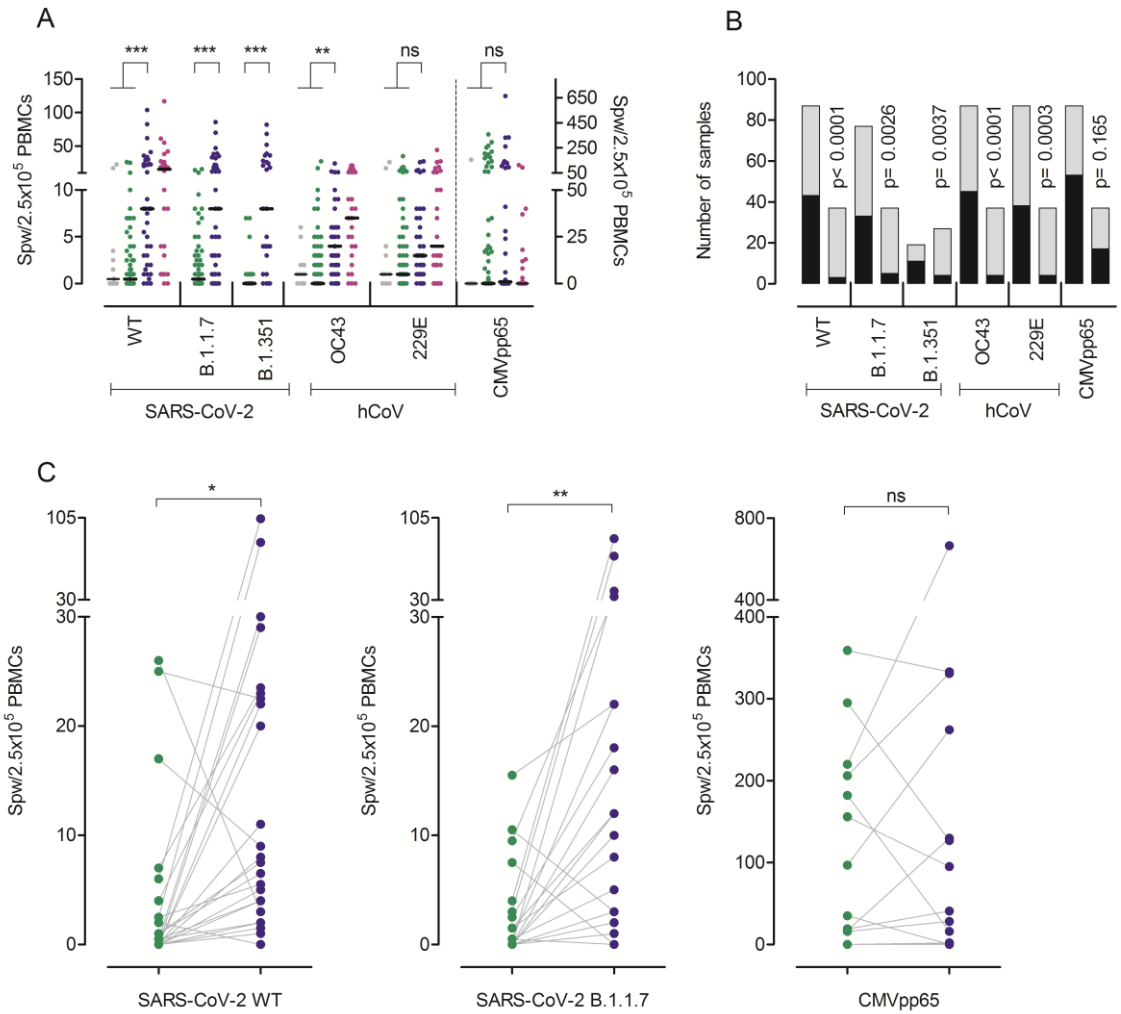

Figure 4