SARS-CoV-2-specific memory B cell responses are maintained after recovery from natural infection and post vaccination

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Abstract

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), has resulted in major worldwide disruption and loss of life over the last 2 years. Many research studies have shown waning serological SARS-CoV-2-specific IgG antibody titres over time, yet it is unclear whether these changes are reflected in the potential functional reactivation of SARS-CoV-2 antigen-specific MBC populations. This is especially true in the contexts of differing COVID-19 disease severity and after vaccination regimens. This study aimed to investigate these by polyclonal in vitro reactivation of MBC populations followed by analysis using SAR-CoV-2 antigen-specific B cell ELISpots and IgG antibody ELISAs. Natural disease-associated differences were investigated in 52 donors who have recovered from COVID-19 with varying disease severity, from asymptomatic to severe COVID-19 disease, accompanied by a longitudinal evaluation in a subset of donors. Overall, these data showed limited disease severity-associated differences between donor groups but did show that COVID-19 serologically positive donors had strong antigen-specific MBC-associated responses. MBC responses were better maintained 6 months after recovery from infection when compared to serological antigen-specific IgG antibody titres. A similar investigation after vaccination using 14 donors showed robust serological antigen-specific antibody responses against spike protein that waned over time. MBC-associated responses against spike protein were also observed but showed less waning over time, indicating maintenance of a protective response 6 months after vaccination. Further research is required to evaluate these putatively functional SARS-CoV-2-specific responses in the context of long-term protection mediated by vaccination against this pathogen.

Introduction

COVID-19 (Coronavirus disease 2019), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), has been associated with over 450 million cases and 6 million deaths (according to the WHO up to March 2022). Symptoms of COVID-19 vary between infected individuals; ranging from no discernible signs of infection to mild symptoms such as cough, fever and loss of taste and smell to more severe symptoms including severe difficulty breathing, organ dysfunction and in some cases death [13, 18, 21]. Immunity to SARS-CoV-2 infection can result from natural exposure or vaccination regimens via classical adaptive immunity consistent with observations in other pathogenic viruses [16, 22, 24], where the production of neutralizing antibodies by B cells is involved in the resolution of infection, as well as protection against subsequent re-exposure to pathogens [1, 10, 20]. This long-term protection is considered immunological memory. In terms of B cells, this can be further broken down into two forms; serological memory (the continued production of antibodies by long-lived plasma cells) and cellular memory (maintained by the production of "classical" memory B lymphocytes that can be reactivated upon reinfection) [15, 35].

When considering antigen-specific B cell responses to SARS-CoV-2, these are predominantly mounted against regions within the Nucleocapsid (N) and Spike (S) protein antigens [3, 25]. Many studies have investigated B cell-associated responses to these SARS-CoV-2 antigens after the resolution of natural infection. The evaluation of serological memory in numerous studies has shown both disease severity-associated differences in circulating antigen-specific antibody titres, as well as waning of N and S protein-specific antibodies over time after both disease resolution [4, 7, 14, 27, 28, 30, 33] and vaccination [17, 23]. However, contrary to these observations, other

studies looking at N and S-specific memory B cells (MBC) have shown the maintenance or an increase in these populations after either the resolution of infection or after vaccination with the BNT162b2 mRNA COVID-19 vaccine [2, 4, 6, 8, 12, 26, 29, 30, 36, 38]. Although the potential reactivation and production of N and S-specific antibodies from these MBC populations in vitro has been investigated, studies are limited and further investigation is required [2, 11, 19, 31, 34].

Methods

Sample acquisition & donor stratification

Donor blood from 52 individuals was acquired from health care professionals at high risk of SARS-CoV-2 exposure from Guy's and St Thomas' NHS Foundation Trust (GSTT) and Valentine Health Partnership as well as from patients within GSTT (United Kingdom). Baseline 1 month post convalescence samples were obtained during the first wave of SARS-CoV-2 infection in the UK (May-August 2020), and 12 donors were followed-up at 6 months. Donor stratification into disease severity-associated groups was based upon self-reported symptoms at the time of sampling and SARS-CoV-2 N and S protein-specific IgG serology. Donors were assigned to one of the following groups: Healthy Controls (n=8, no reported COVID-19 symptoms and negative serology); Asymptomatic (n=6, no reported symptoms but with positive serology to at least one antigen); Mild/Moderate symptoms with Negative serology (n=11, reported symptoms consistent with COVID-19 disease including new persistent cough, fever, muscle fatigue and shortness of breath developed in the period following March 2020, and negative serology); Mild/Moderate symptoms with Positive serology (n=17, reported symptoms consistent with COVID-19 disease that did not require hospital admission and positive serology to at least one antigen), and Severe (n=10, requiring mechanical ventilation on ICU due to COVID-19 disease confirmed by positive PCR test). For the vaccine study, 14 individuals with no known prior SARS-CoV-2 infection were recruited. These donors were sampled prior to vaccination and/or at 1 and 6 months post vaccination with a second dose of BNT162b2. Blood was collected by venipuncture in CPT Tubes (BD Biosciences) and processed within 2 hours of collection.

Sample processing

Donor blood underwent density gradient centrifugation separation of plasma and PBMCs. Plasma was stored at -80°C prior to serological analysis for the stratification of donors. PBMCs were cryopreserved (Full growth media 10% DMSO) and stored in liquid nitrogen until required.

Cell culture & antigen-specific B cell ELISpot assay

Total PBMCs were thawed, washed and counted before being resuspended at $1x10^6$ cells/mL. IL-2 (10ng/mL, Peprotech) and Resiquimod (R848, 2.5µg/mL, Sigma-Aldrich) were added for the polyclonal activation of B cell populations. Furthermore, anti-CD40 antibody (0.5µg/mL, InVivoMAb), Insulin (5µg/mL, Sigma-Aldrich) and Transferrin (35µg/mL, Sigma-Aldrich) were added. Cells were cultured for 5 days in a humidified incubator (37°C, 5% CO_2) after which supernatants were used ELISA assays and remaining cells were used for flow cytometry and B cell ELISpot assays. ELISpot assay was performed in duplicate following the manufacturer's instructions (U-CyTech B cell ELISpot Kit). SARS-CoV-2 N (MyBioSource) and S (Generated in-house as per [33]) proteins were coated at 2µg/mL in 50µL of PBS and cells incubated for 6 hours on the ELISpot plate prior to standard development procedures.

Flow Cytometry

Flow cytometry was performed on a selection of samples from different stratified groups before and after stimulatory PBMC cultures. Cells were washed and underwent FcR blocking (Miltenyi Biotec) prior to staining with Zombie Aqua™ Fixable Viability Dye (BioLegend), anti-CD19 (BioLegend), anti-CD27 (Miltenyi Biotec), anti-CD38 (BioLegend) and anti-CD138 antibodies

(BioLegend). Samples were analysed on the Attune NxT flow cytometer (ThermoFisher Scientific).

Antigen-specific antibody ELISAs

Anti-IgG ELISA were performed in duplicate as per previously reported protocols [33] using both donor plasma (10-fold dilution) and cell culture supernatants (run neat) for all donors in this study. ELISA plates were coated with 2μg/mL N and S proteins (described previously) overnight at 4°C, and plates were read at 405nm absorbance using an ELISA plate reader (SpectraMax Plus).

Data analyses

B cell ELISpots were analysed using Fiji ImageJ with the cell-counter plugin [32]. Absolute spots were counted, from which the relative proportion of antigen-specific IgG-producing cells could be evaluated in the total IgG-producing B cell compartment. Flow cytometry data was analysed using FlowJo^{TM} software [5]. In this study, gating was performed on live single cells for the evaluation of CD19 and CD138 expression. CD27 and CD38 expression was evaluated within the CD19 positive population. Raw absorbance values in the ELISA assay were normalised against negative media-only controls on each ELISA plate and plotted as relative absorbance. The visual representation of data, using box and whisker plots with bars representing minimum and maximum values, was prepared using GraphPad Prism [9]. Statistical evaluation was performed using non-parametric one-way ANOVA (Kruskal-Wallis test) or paired t-test (Wilcoxon signed-rank test). Significant differences are represented on each graph (* $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$).

Results

Self-reported symptoms and antigen-specific serology split donors into five distinct groups

For the naturally infected disease severity-associated study, 52 donors had their plasma and PBMCs isolated 1 month post convalescence (or their self-reported end of symptoms), of which 12 who showed antibody positivity (one healthy control donor later developed COVID-19 disease, referred to as HC:Positive in figures) were followed up at approximately 6 months post convalescence. Initial sample grouping (table 1) was performed as described in the methods. The number of donors in each group varied from 6 to 17. There were no significant differences between these groups based on age. Due to the sizes of the groups, evaluation of the significance of gender was not possible, but was skewed towards females.

In vitro polyclonal activation caused B cell differentiation and was required to observe SARS-CoV-2 antigen-specific IgG-producing responses

Initial assays were performed to establish whether in vitro polyclonal activation of donor PBMCs was successful and responsible for any measured SARS-CoV-2 antigen-specific IgG-producing responses. Flow cytometry was performed on a selection of samples from different groups before culture and after 5 days of culture in order to investigate the differentiation state of B cells and antibody secreting cells (ASC) using surface expression of CD27, CD38 and CD138. Flow cytometry data (Figure 1) showed a consistent increase in CD19+ B cells after 5 days of culture (Figure 1B) irrespective of COVID-19 disease-severity. After 5 days of culture there were also consistent decreases in CD27- CD38- mostly naïve B cells (Figure 1C) as well as CD27+ CD38- MBCs (Figure 1D); with concurrent increases in CD27-/+ CD38+ GC-like B cells (Figure 1E) and CD27+ $CD38^{hi}$ plasmablast-like B cells (Figure 1F). There were no significant differences

between stratified donor groups when looking either before culture or after 5 days of culture. Only within the positive group were significant differences between before and after culture seen, likely due to the larger group size evaluated by flow cytometry. Importantly, there were no measurable CD138+ plasma cells at baseline (or after culture) in any group (Figure 1G). The requirement for polyclonal stimulation to initiate a response was further supported by data showing that uncultured SARS-CoV-2 positive donor PBMCs did not result in antigen-specific responses upon reactivation when measured by subsequent ELISpot assay, where the same samples did show SARS-CoV-2 antigen-specific responses after culture (Figure 1H-J).

Evaluation of N and S protein serological IgG levels indicated severity-associated differences in serological memory as well as consistent waning over time

Comparison between groups of serological N and S protein-specific IgG antibodies in donor plasma at 1 month of convalescence (Figure 2) showed significantly higher N protein-specific IgG in both the severe group relative to both healthy control and negative groups ($P<0.0001$ in both comparisons) and the positive group relative to both healthy control and negative groups ($P=0.0005$ and 0.0007 respectively). These same significant differences were also seen in the S-specific IgG ($P=0.0002$ between severe and healthy control groups; $P=0.0001$ between severe and negative groups; $P=0.0036$ between positive and healthy control groups; and $P=0.0025$ between positive and negative groups). Investigation of antigen-specific IgG levels over time showed a general, but not significant, waning of S protein-specific IgG between 1 and 6 months of convalescence irrespective of donor grouping. However, there was shown to be a significant decrease in N protein-specific IgG in the positive group at 6 months post convalescence relative to 1 month ($P=0.0156$).

SARS-CoV-2 antigen-specific IgG-producing B cell responses associated with MBC reactivation were detected in positive donors with limited association to disease severity

Following polyclonal activation of donor PBMCs, the evaluation of N and S antigen-specific IgG-secreting B cell clones (associated with the reactivation of MBCs) was investigated by ELISpot (Figure 3), and the secretion of antigen-specific IgG was investigated by ELISA. For both N and S protein-specific responses after activation of MBCs, there were few differences between reporting these data as absolute numbers of ASCs or as a percentage of IgG-producing cells. When looking at absolute numbers of ASCs, there were significantly greater N protein-specific ASCs observed in severe donors relative to both healthy control and negative groups (P=0.004 and 0.0016 respectively). Furthermore, there were significantly greater responses in the positive group relative to both healthy control and negative groups (P=0.0266 and 0.0113 respective). S protein-specific responses showed similar statistically relevant significant differences (P=0.0003 between severe and healthy control groups; P=0.0027 between severe and negative groups; and P=0.0089 between positive and healthy control groups). When comparing these responses over time, no significant differences were seen between 1 and 6 months post convalescence, independent of antigen and sample group. However, contrary to the waning of serological memory over time, this appeared to be more variable with no trend towards a reduction in response.

Evaluation of these same memory responses upon reactivation was also possible using the cell culture supernatants and performing N and S protein-specific IgG ELISAs (Figure 4). N-specific IgG was mostly under the limit of detection in these cell culture supernatants. However, S-specific IgG showed the same statistically significant differences as seen in the reactivation of MBC-associated responses (P=0.0016 between severe and healthy control groups; P=0.0001 between

severe and negative groups; P=0.0295 between positive and healthy control groups; and P=0.0027 between positive and negative groups).

Serological antibody levels and MBC-associated responses post vaccination showed similar patterns to those observed in the naturally infected disease-severity associated study

The same assays were performed on a group of donors before and after vaccination where possible. As expected, serological N-specific IgG antibody levels showed no significant difference between the healthy control and either 1 month or 6 month post vaccination groups (Figure 5). However, the naturally infected severe disease severity cohort, used in this study to indicate magnitude of vaccine-associated responses relative to those observed by natural infection, showed significantly higher N-specific IgG levels than the healthy control, 1 month and 6 month post vaccination groups ($P<0.0001$, $P=0.002$ and $P=0.0279$ respectively). S-specific serological IgG antibody levels in both the 1 and 6 months post vaccination groups were significantly higher than the healthy control group ($P<0.0001$ and $P=0.0048$ respectively). The 1 month post vaccination group was significantly higher than the severe group ($P=0.0022$), but not significantly higher than the 6 month group when looking at all donors evaluated. Longitudinal evaluation where possible showed no change in N-specific IgG antibody levels before and after vaccination, but did show significant increase in S-specific IgG antibody levels 1 month after vaccination compared to before vaccination ($P<0.0001$), as well as a significant waning at 6 months after vaccination compared to 1 month ($P=0.0285$).

The same comparisons were made for the reactivation of potentially functional antigen-specific MBC responses using the ELISpot assay (Figure 6). When looking at N-specific MBC responses

231 in terms of absolute numbers, there were significantly higher responses in the severe group relative
232 to the healthy control group and both the 1 month and 6 months post vaccination groups (P=0.0026,
233 P<0.0001 and P=0.0407 respectively). S-specific MBC responses in terms of absolute numbers
234 showed that the 1 month and 6 month post vaccination groups as well as the severe group were
235 significantly higher than the healthy control group (P=0.0002, P=0.0242 and P=0.0004
236 respectively). In terms of the longitudinal evaluation of S-specific responses, there was a
237 significant increase 1 month post vaccination relative to prior to vaccination (P=0.0003), and
238 although there may be some waning, there was no significant decrease at 6 months post vaccination
239 (P=0.8695), which remained significantly higher than prior to vaccination (P=0.0119). Similar to
240 before, the secretion of N and S-specific IgG antibodies from these cultures were evaluated by
241 ELISA to establish if these supported the ELISpot data (Figure 7). The S-specific results appeared
242 to align to those observed in the S-specific ELISpot assay.

Discussion

This study first aimed to investigate MBC responses in a cohort of 52 individuals with differing COVID-19 disease severity to understand whether disease severity-associated differences exist in the potential protection against subsequent reinfection with SARS-CoV-2. The stratification of donors in this study was performed based on self-reported symptomology, in line with other SARS-CoV-2 studies [37, 39]. The existence a negative donor group was due to the presence of self-reported symptoms consistent with COVID-19 in these individuals despite a negative serological antibody result. We did not wish to assume that negative serology data in these individuals was proof that they had not had COVID-19 disease. The work from this study largely supports existing studies that have shown some disease-severity associated differences in SARS-CoV-2 antigen-specific IgG titres, particularly in the early periods of convalescence [4, 7, 14, 27, 28, 30, 33]. They have also shown consistent waning of specific IgG titres. Despite this waning of serological antibodies, studies investigating SARS-CoV-2-specific MBCs have shown persistent and sometimes increasing antigen-specific MBC populations over the observed periods of convalescence [4, 6, 12, 26, 29, 30, 36, 38]. In the few studies that have investigated the reactivation of these MBC-associated responses, these have shown significantly higher antigen-specific MBC-associated responses in recovered patients than healthy controls but no significant differences between asymptomatic and symptomatic groups [11, 19, 31, 34]. Investigation of these MBC-associated responses within this study showed that although significant differences were observed between either the severe and the positive donor groups (containing mild-moderate COVID-19 disease individuals) and the negative and healthy control donor groups, there were no significant differences in terms of the MBC-associated responses between the asymptomatic, positive and severe donor groups. Furthermore, there were no disease severity-associated

differences in the MBC-associated responses in the 13 donors evaluated over time with no clear waning in any COVID-19 infected donor group. These findings are consistent with other studies investigating the reactivation of MBC responses, where although these responses appear robust when compared to serological antigen-specific antibody levels, there appears to be little association of these MBC-dependent responses to the severity of acute COVID-19 disease [11, 19, 31, 34].

This study also aimed to investigate the longevity of S protein-specific MBC-associated activation in response to ongoing vaccination regimens in 14 donors up to 6 months after the second dose with BNT162b2. In this study the naturally infected severe donor group was used as a comparison and the Nucleocapsid protein as a control. Studies after SARS-CoV-2 vaccination have shown similar waning of S-specific serological IgG titres [17, 23], and maintenance of antigen-specific MBC populations [2, 8] as described before. When considering the reactivation of MBC-associated responses in the context of vaccination, robust S-specific responses have been observed by ELISpot up to 6 months after vaccination [2]. This study showed significantly higher levels of serological S-specific IgG after both 1 and 6 months post vaccination compared to the healthy control group as well as waning between 1 and 6 months post vaccination where longitudinally evaluated. Investigation of the reactivation of MBC-associated responses in these same donors showed the same significant increases in S-specific producing B cells, but with no significant waning between 1 and 6 months post vaccination. Despite this lack of waning, there did appear to be a trend towards the reduction in these S-specific MBC reactivation-associated responses that could potentially indicate poor longer-term protection.

In summary, this study has shown that MBC-associated responses in an in vitro reactivation model do not always reflect serological antibody levels of antigen-specific IgG after both natural infection with and vaccination against SARS-CoV-2. These potentially functional antigen-specific reactivation responses appear to be robust with little to no waning over time. This highlights that individuals may maintain protection against SARS-CoV-2, but this needs to be evaluated in the longer term to inform future vaccination regimens as well as the putative risk of emerging novel viral variants.

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Data Availability

Further information and requests for resources should be directed to and will the Lead Contact, David Fear (David.fear@kcl.ac.uk)

Conflict of interests

Declaration of interests: The authors declare no conflict of interests.

Ethics statement

Informed consent was given prior to the collection of all samples used in this study, observing all guidelines at the host institutions. The sample collection protocols used for this project was reviewed and approved by the London Bridge Research ethics committee (14/LO/1699 & 19/SC/0232).

Author contributions

All experimental work and analysis was carried out by TM and RH. DF and MS-H conceived of the original project. KD supplied information, reagents and support for experimental approaches. This article was written by TM and DF and edited and approved by all authors listed.

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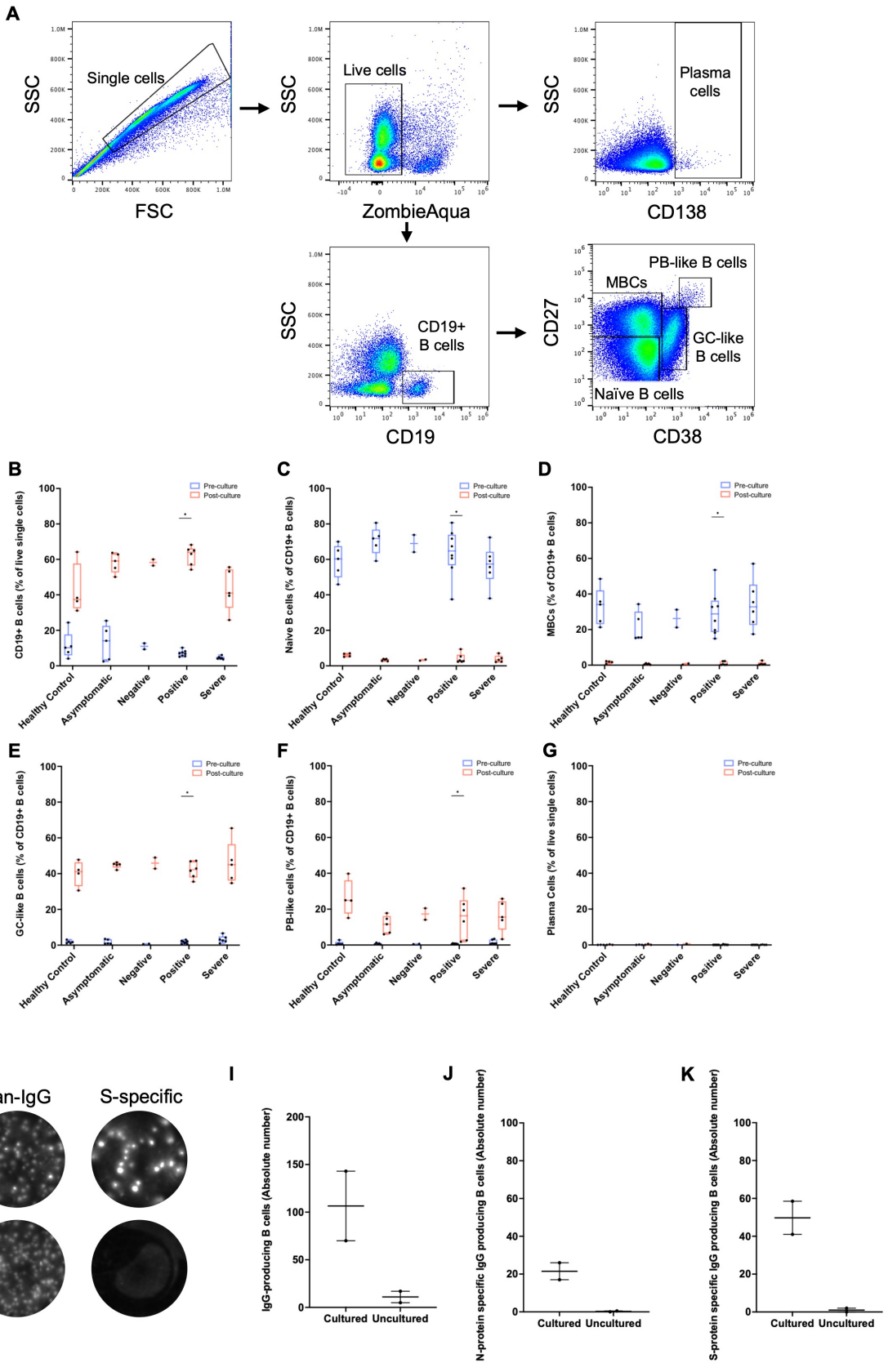
Tables & legends

Table 1. Demographic data & IgG serology results for samples based on disease-severity associated groups. This table shows the number of unique donors in each disease severity group; the number of donors followed up in each group; the average age of each group with statistical comparison (non-parametric one-way ANOVA) to the healthy control group; the sex distribution within each group; and the number and frequency of the N and S protein antigen-specific IgG seropositive donors within each group.

					IgG Seropositive (Percentage)	
Group	**Unique donors**	**Number followed-up**	**Average age (P value)**	**Male/Female (Unknown)**	**N protein**	**S protein**
Healthy Control	8	1	45.1 (N/A)	4/4 (0)	0 (0%)	0 (0%)
Asymptomatic	6	4	36.0 (0.5299)	1/4 (1)	6 (100%)	4 (66.6%)
Negative	11	0	38.5 (>0.9999)	3/8 (0)	0 (0%)	0 (0%)
Positive	17	8	41.8 (>0.9999)	6/11 (0)	15 (88.2%)	16 (94.1%)
Severe	10	0	54.8 (>0.9999)	5/5 (0)	10 (100%)	10 (100%)
Overall	52	13	43.4	17/31 (1)	31 (59.6%)	30 (57.7%)


A
Single cells
SSC
FSC
Live cells
ZombieAqua
Plasma cells
CD138
CD19+
B cells
CD19
PB-like B cells
MBCs
GC-like
B cells
Naïve B cells
CD27
CD38
B
C
D
E
F
G
Pre-culture
Post-culture
CD19+ B cells (% of live single cells)
Naive B cells (% of CD19+ B cells)
MBCs (% of CD19+ B cells)
GC-like B cells (% of CD19+ B cells)
PB-like cells (% of CD19+ B cells)
Plasma Cells (% of live single cells)
Healthy Control
Asymptomatic
Negative
Positive
Severe
H
Pan-IgG
S-specific
+ve
-ve
I
IgG-producing B cells (Absolute number)
J
N-protein specific IgG producing B cells (Absolute number)
K
S-protein specific IgG producing B cells (Absolute number)
Cultured
Uncultured

429

Figure 1. Polyclonal stimulating cultures result in B cell activation and are required to observe antigen-specific B cell responses. These data show the flow cytometry gating strategy (A), the relative frequency of CD19+ B cells in the live single cell population both before (blue) and after (red) culture (B), the relative frequency of CD27- CD38- Naïve B cells within the CD19+ B cell population (C), the relative frequency of CD27+ MBCs (D), the relative frequency of CD27-/+ CD38+ GC-like B cells (E), the relative frequency of CD27+ CD38hi PB-like B cells (F), and the relative frequency of CD138+ Plasma cells within the live single cell population (G). Furthermore, ELISpots were performed on cultures and uncultured donors. These data show example images of ELISpot assay wells for pan-IgG secreting and S-specific antibody secreting cells in positive and negative donors (H), the absolute number of IgG-producing B cells (I), the absolute number of N protein-specific IgG secreting B cells (J), and the absolute number of S protein-specific IgG secreting B cells (K). Data are plotted as box and whisker plots with bars representing minimum and maximum values. Statistical evaluation (B-G) was performed using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test (Wilcoxon signed-rank test).

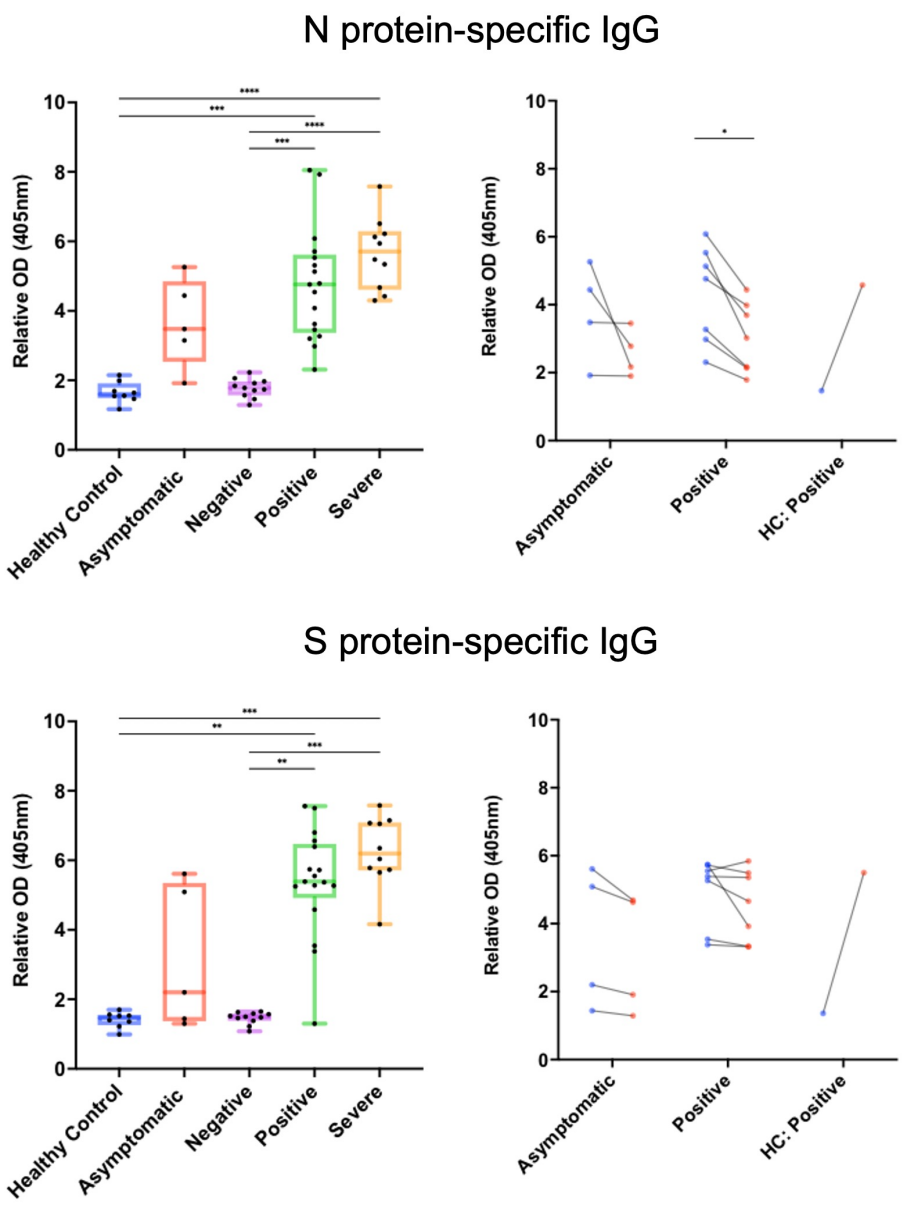


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Figure 2. SARS-CoV-2 N and S protein-specific serological IgG showed some disease-severity group-associated differences and waning of antibodies after 6 months where evaluated. These data for all groups are shown for both N (top) and S proteins (bottom). On the left, the relative absorbance to negative controls is reported for each donor based on their stratified group. On the right, initial 1 month (blue) and subsequent 6 month (red) timepoints are shown in the donors where these were evaluated. Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).

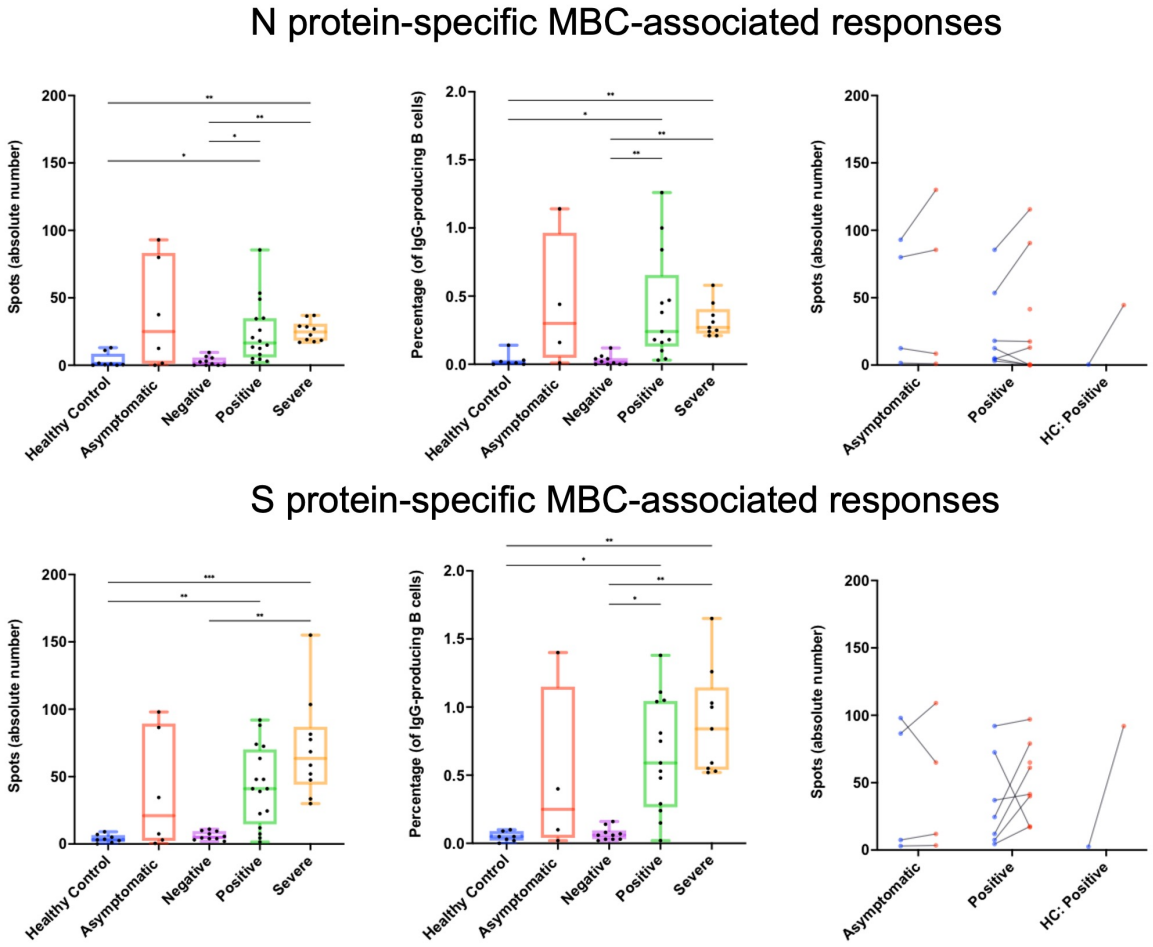
N protein-specific MBC-associated responses
Spots (absolute number)
Percentage (of IgG-producing B cells)
Spots (absolute number)
Healthy Control
Asymptomatic
Negative
Positive
Severe
Asymptomatic
Positive
HC: Positive
S protein-specific MBC-associated responses
Spots (absolute number)
Percentage (of IgG-producing B cells)
Spots (absolute number)
Healthy Control
Asymptomatic
Negative
Positive
Severe
Asymptomatic
Positive
HC: Positive

Figure 3. SARS-CoV-2 N and S protein-specific MBC-associated reactivation showed limited group-associated differences and no waning at later timepoints. These data are shown for both N (top) and S (bottom) protein-specific responses upon polyclonal cell culture stimulation. Data for all groups are represented as both absolute number of visualised spots (left) and as a percentage of IgG producing B cells (centre). Furthermore, data are also shown for initial (blue) and subsequent (red) timepoint analysis where possible in the donors evaluated (right). Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).

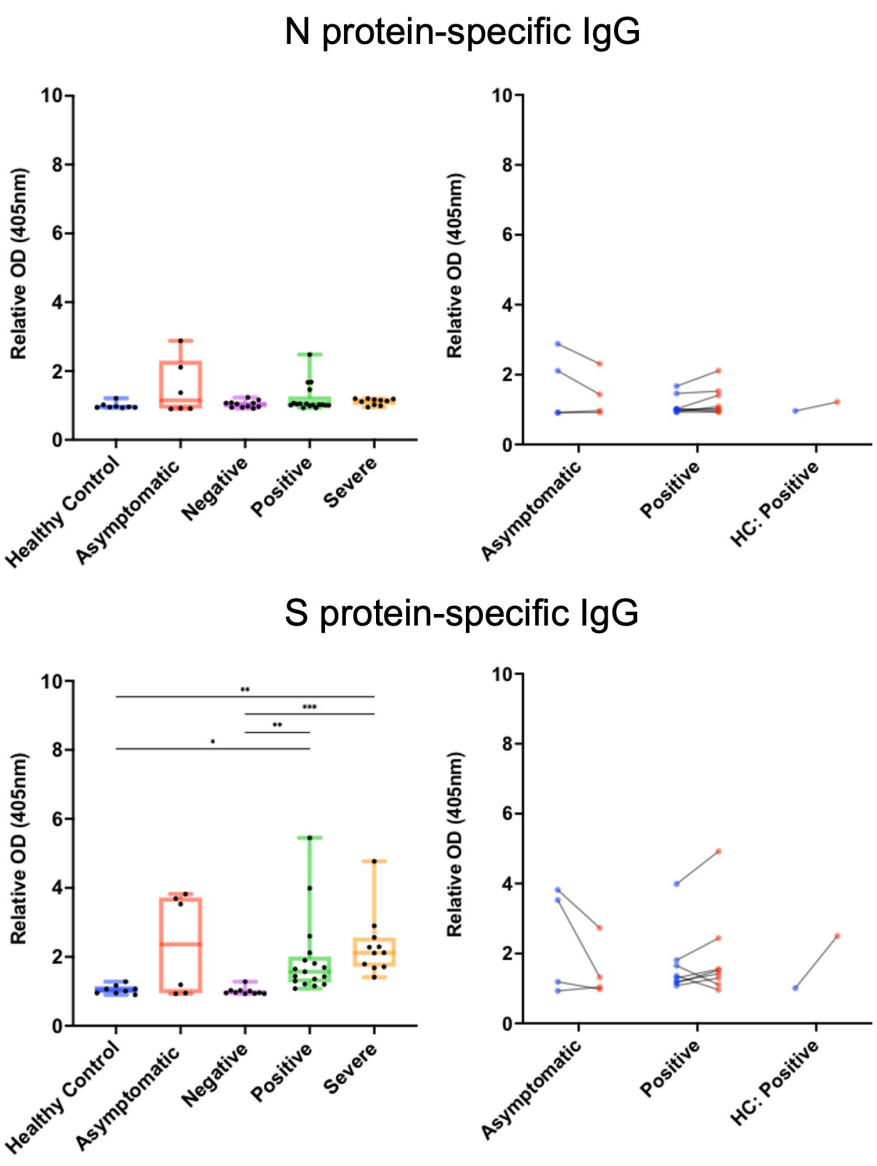
N protein-specific IgG
Relative OD (405nm)
Healthy Control
Asymptomatic
Negative
Positive
Severe
Asymptomatic
Positive
HC: Positive
S protein-specific IgG
Relative OD (405nm)
Healthy Control
Asymptomatic
Negative
Positive
Severe
Asymptomatic
Positive
HC: Positive

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Figure 4. SARS-CoV-2 N and S protein-specific IgG in the cell culture supernatants showed limited group-associated differences and inconsistent responses at later timepoints. These data for all groups are shown for both N (top) and S protein (bottom) in 1 month post convalescence cultured samples (left) and followed-up 6 month samples (red) where possible (right). Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).

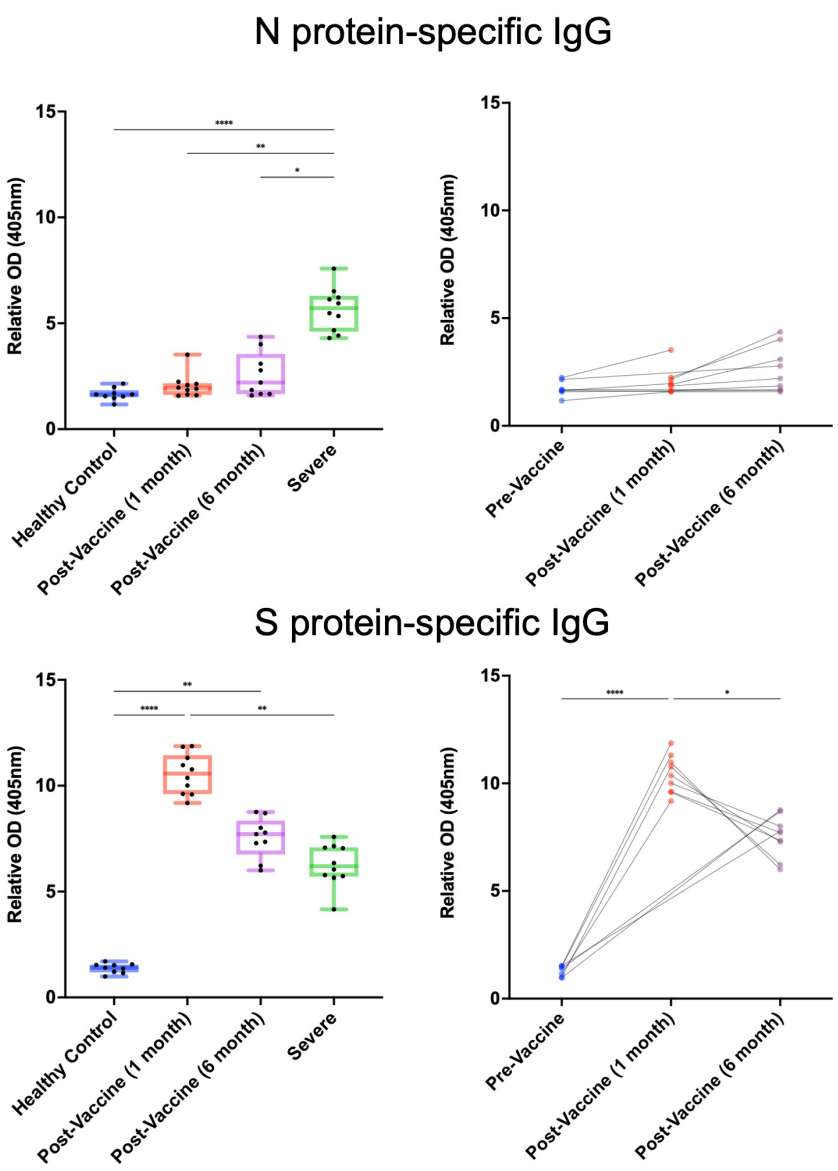

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Figure 5. SARS-CoV-2 N and S protein-specific serological IgG levels post vaccination indicated robust S-specific responses and waning over time. These data are shown for both N (top) and S proteins (bottom). On the left, the relative absorbance to negative controls is reported for each donor based on their stratified group. On the right, initial 1 month (blue) and subsequent 6 month (red for 1 month; purple for 6 months) timepoints are shown where evaluated. Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).

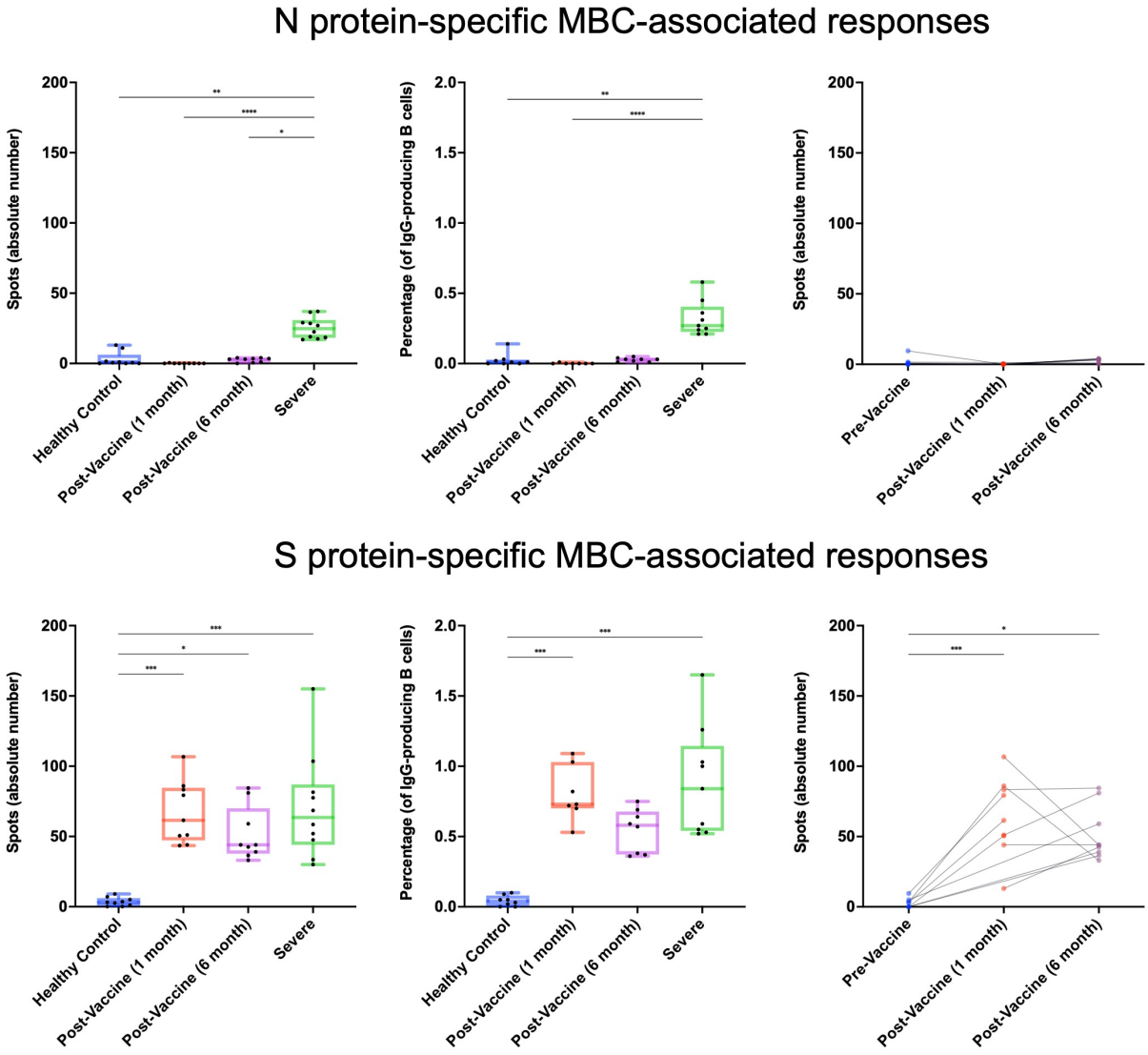
N protein-specific MBC-associated responses
Spots (absolute number)
Percentage (of IgG-producing B cells)
Spots (absolute number)
Healthy Control
Post-Vaccine (1 month)
Post-Vaccine (6 month)
Severe
Pre-Vaccine
Post-Vaccine (1 month)
Post-Vaccine (6 month)
S protein-specific MBC-associated responses
Spots (absolute number)
Percentage (of IgG-producing B cells)
Spots (absolute number)
Healthy Control
Post-Vaccine (1 month)
Post-Vaccine (6 month)
Severe
Pre-Vaccine
Post-Vaccine (1 month)
Post-Vaccine (6 month)

Figure 6. SARS-CoV-2 N and S protein-specific MBC-associated reactivation showed robust S-specific responses due to vaccination. These data for all groups are shown for both N (top) and S (bottom) protein-specific responses upon polyclonal cell culture stimulation. Data are represented as both absolute number of visualised spots (left) and as a percentage of IgG producing B cells (centre). Furthermore, data are also shown for initial (blue) and subsequent (red for 1 month; purple for 6 months) timepoint analysis where possible (right). Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on the box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).

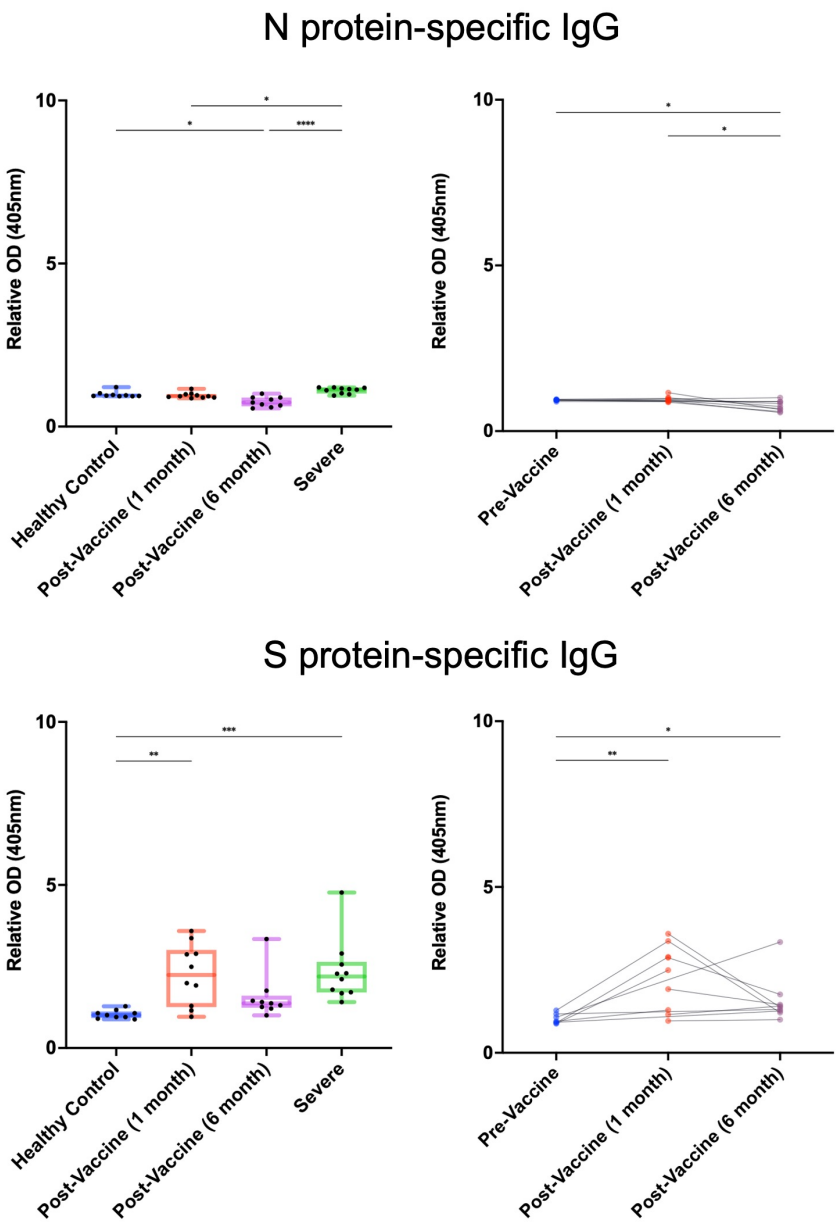

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Figure 7. SARS-CoV-2 N and S protein-specific IgG in the cell culture supernatants showed significantly higher S-specific responses after vaccination with limited waning over time. These data are shown for both N (top) and S protein (bottom) in post vaccination groups (left) and longitudinally followed-up (red for 1 month; purple for 6 months) donors (right) where possible. Data are plotted as box and whisker plots with bars representing minimum and maximum values or as line graphs between two points. Statistical evaluation was performed on the box and whisker plots using non-parametric one-way ANOVA (Kruskal-Wallis test) and paired t-test for the line graphs (Wilcoxon signed-rank test).