Antibody course and memory B-cell response in the first year after SARS-CoV-2 infection

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Summary of main point: During the first year after infection the half-life of SARS CoV-2 spike antibodies increased from three months to two years. Most infected individuals had robust virus-specific memory B-cell responses 12 months after infection.

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Abstract

Background: The possibility of repeat infections with SARS-CoV-2 raises questions regarding quality and longevity of the virus-induced immune response.

Methods: The antibody course and memory B-cell (MBC) response against SARS-CoV-2 proteins, influenza virus nucleoprotein (NP) and tetanus toxin (Ttx) were examined in adults with mild to moderate SARS-CoV-2 infection in the first year after infection.

Results: The concentration of SARS-CoV-2 RBD-specific antibodies was low compared with the concentration of influenza virus NP-specific antibodies. The SARS-CoV-2 RBD antibody half-life increased from 95 days in the first six months to 781 days after 9-12 months. The SARS-CoV-2 NP antibody half-life increased from 88 to 248 days. Two thirds of the subjects had SARS CoV-2-specific MBC responses 12 months after infection. SARS-CoV-2 antibody levels correlated with the MBC frequency at 12 months.

Conclusions: The low concentration of SARS-CoV-2 spike protein antibodies indicates that re-exposure to the virus or vaccination are required to use the B-cell immunity to full capacity. The existence of a robust SARS CoV-2 MBC response at 12 months in most subjects and the substantially increasing antibody half-life provide evidence that the immune response is developing into long-term immunity. The early antibody reaction and the ensuing MBC response are interdependent.

Keywords: SARS-CoV-2, COVID-19, antibody course, memory B-cells, antibody half-life

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Introduction

Previous infection with SARS-CoV-2 protects approximately 80% of infected individuals from repeat infection for at least several months. The level of protection decreases with age and was less than 50% among individuals 65 years and older [1]. This argues for a need to further explore the magnitude and course of the virus-specific immune response. Previous studies have shown that SARS-CoV-2 infection induces a virus-specific IgG antibody response that peaks at 20-24 days after infection and subsequently declines [2–4]. It was also reported that in the first 9 months after infection the average half-lives of IgG antibodies against the viral nucleoprotein (NP) and spike (S) protein were 36-85 and 36-344 days, respectively [2,3,5–11]. At later time points the antibodies decayed more slowly indicating different phases of antibody decline [2,9,10,12,13].

SARS-CoV-2 infection also induces virus-specific memory B-cells (MBCs). The magnitude of the MBC response increases with time from infection and reached a maximum 4-5 months after symptom onset [4,7,10,12,14,15]. At 8-9 months after infection, SARS-CoV-2-specific MBCs were found in 69.2 to 100% of recovered subjects suggesting that the infection induces robust memory B-cell responses [4,16].

To examine the development of the SARS-CoV-2 antibody half-life and to test if the antibody and MBC response to SARS-CoV-2 differ from the immune response against more frequently encountered natural and vaccine antigens, we measured the concentration of antibodies against SARS-CoV-2 RBD and NP, influenza virus NP and tetanus toxin (Ttx) at different time points. We calculated the antibody half-life for different time intervals, studied the magnitude of the MBC responses a year after infection and determined the relationship of these responses to each other.

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Material and methods

Participants of the study

Participants (n = 55) with SARS-CoV-2 infection and uninfected control subjects (n = 15) were recruited for the study. In infected individuals, the days post symptom onset (PSO) were counted from the first day of symptoms reported or, in the case of asymptomatic infection, the day of the first positive RT-PCR. Serum samples were collected 4 or 5 times during the first year PSO. Heparinized blood samples were taken at 12 months PSO. Blood samples were obtained after informed consent. Sera were stored at -20°C. Heparinized blood samples were examined the same day or the day following blood drawing. The study was approved by the Ethics Commission of the Medical Faculty at the University of Leipzig (ethical vote 147/20-ek).

Measurement of antibody concentrations

The concentration of antibodies against the SARS-CoV-2 receptor binding domain (RBD) in sera was determined with the Abbott SARS-CoV-2 IgG II Quant assay using the ARCHITECT i2000SR system (Abbott, Chicago, U. S. A.). This led to antibody concentrations in arbitrary units (AU)/ml. Sera below 50 AU/ml were regarded as negative. To convert the AU values into WHO binding antibody units (BAU), AU values were divided by 7 according to information from the manufacturer. To determine the SARS-CoV-2 RBD antibody concentration in µg/ml, the human anti-SARS-CoV-2 spike S1 RBD monoclonal antibody (mAb) CR3022 (Antibodies-online.com) was examined with the Abbott SARS-CoV-2 IgG II Quant assay. The measurement showed that 520 Abbott AU were equivalent to 1 µg anti-SARS-CoV-2 RBD mAb CR3022. Therefore, the RBD antibody concentrations in AU/ml were converted into µg/ml by multiplying 1 AU with $1.92x10^{-3}$ µg.

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IgG antibodies against the SARS-CoV-2 and the influenza virus nucleoproteins (NP) were measured by in-house ELISAs as previously described [17,18]. Serial dilutions of the National Institute of Biological Standards and Controls (NIBSC) Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (code 20/162) and a recombinant mAb against influenza virus NP [19] were used as concentration standards. Sera were diluted 1:100 (SARS-CoV-2 NP ELISA) or 1:5000 (influenza virus NP ELISA). Standard and serum were incubated for 60 minutes. Plates were washed and HRP-conjugated anti-human IgG antibody (P0214, Dako, Agilent Technologies, Inc. or no. 109-036-098, Jackson Immunoresearch Laboratories, Inc.) was added and incubated for another hour. TMB substrate (SeramunBlau® slow2 85) was added for 15 minutes and the reaction was stopped with 1 N H_2SO_4. The optical density (OD) was measured with a Tecan Sunrise photometer at 450 nm wavelength (reference wave length 570 nm). Measurements were performed in duplicates. Mean OD values were calculated and the OD of the blank was subtracted. Standard curves were created using a four parameters logistic (4-PL) regression (SARS-CoV-2 NP) or a polynomial function (influenza virus NP). Sera below 34 AU/ml anti-SARS-CoV-2 NP antibody and below 0.78 µg/ml anti-influenza NP antibody were regarded as negative. Ttx-specific IgG concentrations were measured in international units (IU)/ml with the SERION ELISA classic Tetanus IgG (Virion\Serion GmbH) ELISA kit.

Examination of antigen-specific MBCs

PBMCs were isolated from heparinized blood (15 ml) by ficoll density gradient centrifugation and resuspended in RPMI-1640 medium containing 20 % fetal calf serum (FCS), penicillin, streptomycin, sodium pyruvate, non-essential amino acids, 1 µg/ml R848 (Resiquimod, Sigma-Aldrich, Merck KGaA) and 0.11 µg/ml interleukin-2 (Proleukin, Novartis AG). Cells were cultured for 5 days at 3 $x10^6$ PBMC in 2 ml medium in a 24 well plate at 37°C and 5% CO_2. MBCs were examined by ELISpot using 96-well Multiscreen-IP filter plates (Millipore, Merck KGaA). The plates were washed for 15 seconds with 35% ethanol and with PBS and coated with 50 µl SARS-CoV NP-maltose binding

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protein (MBP) fusion protein (2 µg/well), SARS-CoV-2 RBD (1 µg/well [17]), influenza virus NP-MBP fusion protein (2 µg/well) or Ttx (5 µg/well, lot 317490, GSK Vaccines). As controls, wells were coated with PBS or MBP (1 µg/well). Total numbers of IgG-secreting cells were determined with wells coated with mouse anti-human IgG mAb (clone MT91/145, Mabtech AB). The plates were incubated overnight at 4°C or for 2 hours at 37°C, washed and blocked for an hour with medium containing 20 % FCS. Stimulated cells were added to the antigen-coated wells (300,000 cells) and to anti-IgG coated wells (5,000 cells). Plates were incubated at 37°C for 20 hours.

The next day, plates were washed, alkaline phosphatase (AP)-conjugated goat anti-human IgG (no. 109-055-098, Jackson Immunoresearch Laboratories, Inc., diluted 1:5000) was added and incubated for 2 hours at 37°C. Plates were washed and NBT/BCIP substrate (AP conjugate substrate kit, Bio-Rad Laboratories, Inc.) was added for 5 minutes. Plates were washed with water, dried overnight and read with the AID EliSpot/FluoroSpot reader. Uncoated wells were used as negative control for SARS-CoV-2 RBD and Ttx. Wells coated with MBP were used as negative control for SARS-CoV-2 NP and influenza NP, because the antigens contain MBP as fusion protein [18]. Positive MBC results were defined as showing at least 10 spots per well and at least 3 times the spots in negative control wells [20]. MBCs were measured in duplicates. Mean values were calculated and the percentage of antigen-specific MBCs was calculated by the following equation:

$$\text{MBC (\%)} = \frac{(No.spots\ Ag{-}coated\ wells - No.spots\ negative\ control\ wells)/60}{No.\ IgG\ spots - No.of\ spots\ negative\ control\ wells} . 100$$

Calculations and statistical methods

Antibody concentrations were compared with the paired one-sided Wilcoxon signed-rank test. To calculate the antibody half-lives, the data were censored in the following way: Individuals with antibody concentrations rising by more than 25 % (RBD: n = 3), antibody negative sera and 12 month values of vaccinated subjects (n = 4) were excluded. For the anti-Ttx antibody half-life, an

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unvaccinated subject and 15 individuals who were vaccinated in the preceding 3 years or during the study were excluded. For influenza NP-specific antibody half-life calculations, 19 individuals with influenza vaccination in autumn/winter 2020/21 were excluded after vaccination.

The antibody course was described by an exponential decay model. Antibody concentrations were log transformed based on the natural logarithm. A linear mixed model was applied for the half-life of the whole time period PSO. Correlated random intercept and slope were allowed. The half-life between two consecutive observation points were calculated with linear regression analysis. Both regression models follow equation (1).

$$\ln(y) = \beta_0 + \beta_1 T \quad (1)$$

where y represents the antibody concentration, β_0 the intercept, β_1 the slope of the curve and T the days after symptom onset [21]. In the linear regression model, the mean of the individual slopes was taken as the model slope. The half-life was calculated by dividing ln(0.5) by the slopes according to equation (2).

$$T_{1/2} = \ln(0.5)/\beta_1 \quad (2)$$

95% confidence intervals (CI) of the half-lives were calculated by applying the 95% confidence intervals of the slopes in equation (2).

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The percentage of subjects with MBCs and the frequency of MBCs were determined. Participants who were not vaccinated against tetanus were excluded from the analysis of tetanus-specific MBCs. Comparison of the positivity rate of MBCs in paired samples was performed with the mid-p McNemar test. The positivity rates of the SARS-CoV-2 recovered and naïve cohort were compared with the Fisher's exact test. Modified Wald 95% confidence intervals were calculated for ELISpot positivity rates. The frequency of MBCs was determined in samples with detectable B-cell responses. Paired samples were compared with the Wilcoxon signed-rank test, unpaired samples were compared with the Mann-Whitney U test. The relationship between variables was investigated using Spearman's rank correlation. The level of significance for the statistical analyses was 0.05.

R version 3.6.3 and R Studio version 1.4.1106, LibreOffice Calc and Microsoft Excel were used for statistical analysis and graphs.

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Results

Participants

The subjects (n = 55) had been infected with SARS-CoV-2 between March and May 2020. Fifty-four participants had a positive SARS-CoV-2 RT-PCR at time of diagnosis. One participant was enrolled 7 months after clinical diagnosis (Cov-046). The median age of the participants at diagnosis was 46 years (range: 21-72 years), 58.2% of the participants were female. The participants had asymptomatic infection, mild or moderate disease (see Suppl. Table 1). Blood samples were collected 2-10 weeks (mean 1.5 months), 6 (n = 53) or 7 (n = 2), 9 and 12 months PSO. Four participants that were vaccinated against COVID-19 during the study were excluded from subsequent analyses. Uninfected male and female individuals with negative SARS-CoV-2 RBD antibody test (n = 15) were enclosed as control for the MBC response.

IgG antibody concentrations

The concentration of SARS-CoV-2 antibodies varied greatly in the participants. Two to ten weeks PSO, the RBD-specific antibody concentration ranged from 0.13 to 26.8 µg/ml. The median SARS-CoV-2 RBD antibody concentration 2-10 weeks after infection was 1.54 µg/ml and the mean 3.6 µg/ml. During the study period, the median RBD antibody titer declined to 0.61 µg/ml. The concentration of influenza virus NP-specific antibodies at the first time point was between 2.9 and 47.1 µg/ml (median 17.2, mean 19.5 µg/ml) and declined during the study to a median of 14.1 µg/ml (mean 13.8 µg/ml). The difference between the concentration of the SARS-CoV-2 RBD and influenza virus NP-specific antibodies at 0.5-2.6 months PSO was statistically significant ($p < 0.0001$) (Tab. 1). The severity of disease correlated weakly with the SARS CoV-2 RBD-specific antibody concentration in the first 9 months. On the average, individuals with systemic symptoms had higher SARS CoV-2 RBD antibody concentrations than patients with local disease (Tab. 2). The decline of SARS-CoV-2 RBD and NP antibodies between examinations was statistically significant during the whole study period (Suppl. Fig. 1). The RBD- and NP-specific antibody concentrations correlated at all time points. Thus, participants with high antibody responses to SARS-CoV-2 RBD tended to have high antibody responses to NP as well **(**Table 2, Suppl. Fig. 2).

Antibody half-lives

Between the time point of maximum antibody concentration and the measurement at 12 months PSO, the SARS-CoV-2 RBD-specific antibodies declined with a half-life ($T_{1/2}$) of 158 days (95% CI: 141-181 days). Similarly, the SARS-CoV-2 NP-specific antibodies declined with a $T_{1/2}$ of 119 days (95% CI: 103-141 days). On closer inspection, the antibody half-lives increased during this period. The $T_{1/2}$ of the RBD-specific antibody response was 95 days (95% CI: 83-111 days) from 2-10 weeks to

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6-7 months PSO, 213 days (95% CI: 180-262 days) from 6-7 to 9 months PSO and 781 days (95% CI: 452-2881 days) from 9 to 12 months PSO. Similarly, the half-life for SARS-CoV-2 NP IgG antibodies increased from 88 days (95% CI: 76-104 days) through 150 days (95% CI: 114-221 days) to 248 days (95% CI: 180-399 days). The half-life of antibodies against influenza virus NP for the whole observation period was 1367 days (95% CI: 873-3100 days) and the $T_{1/2}$ of Ttx-specific antibodies was 11043 days (95% CI: 1790 days - ∞) (Fig. 1).

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Memory B-cell responses

Twelve months PSO, SARS-CoV-2 RBD and NP-specific MBCs were detected in 68.6% (95% CI: 54.9-79.7%) of the individuals. Influenza virus NP and Ttx-specific MBCs were found in 76.5% (95% CI: 63.0-86.1%) and 78.0% (95% CI: 64.5-87.3%) of the subjects, respectively. No SARS-CoV-2-specific MBCs were found in the uninfected control group. Influenza virus NP-specific MBCs were found in 73.3% (95% CI: 47.5-89.3%) and Ttx-specific B-cells in 92.3% (95% CI: 64.2-100.0%) samples from uninfected control subjects. In pairwise comparisons, the differences in the frequency of MBCs for different antigens were statistically insignificant (p = 0.27-1). Thus, MBCs to SARS-CoV-2 proteins were found as frequently as MBCs specific for influenza virus NP and tetanus toxin (Table 2, Suppl. Fig. 3). In individuals with measurable MBCs, the median frequency of SARS-CoV-2 RBD-specific MBCs was 0.46% and the median of SARS-CoV-2 NP-specific B cells was 0.49% among all activated, antibody-secreting cells. The difference was insignificant (p = 0.28). The median frequency of influenza virus NP and Ttx-specific MBCs was 0.33% and 0.41 %, respectively. The RBD-specific MBC response was slightly more vigorous than the influenza virus NP-specific response (p = 0.02). In the group of SARS-CoV-2 naïve individuals, the median frequency of influenza virus NP-specific MBCs was 0.54% and the median frequency of Ttx-specific MBCs was 0.385%. The differences to the recovered cohort were insignificant (p = 0.21 and 0.85) (Fig. 2).

SARS-CoV-2 RBD and NP specific MBC frequencies correlated moderately with each other (rho = 0.40, p = 0.03), but did not correlate with the influenza NP or Ttx-specific MBC response (rho = -0.13 to 0.13, p = 0.49-0.99) (Table 2 and Suppl. Fig. 4**)**.

Correlation of MBC and antibody responses

The maximum SARS-CoV-2 RBD and NP-specific antibody concentrations and the concentrations at 6-7 months PSO correlated with the magnitude of the MBC response 12 months PSO. Thus, higher SARS-CoV-2 antibody concentrations early in the infection indicated a more vigorous MBC response at 12 months after infection. The RBD-specific antibody responses at 9 and 12 months also correlated with the MBC response. Antibody concentrations and MBC responses to influenza NP and tetanus toxin did no correlate (Table 2, Suppl. Fig. 5).

Discussion

The goal of the study was to measure the SARS-2 RBD and NP-specific antibody concentration at several time points in the first year after infection and the memory B-cell response a year PSO, to determine the antibody half-lives and to compare the results among each other and with the immune responses to influenza virus NP and Ttx. For the comparison, we determined the concentration of SARS-CoV-2 RBD-specific IgG antibodies in μg/ml using the monoclonal RBD-specific antibody CR3022 as a concentration standard. The individual concentrations of the serum antibodies varied by more than a hundredfold. The mean concentration of SARS-CoV-2 RBD antibodies in the first 2-10 weeks (3.6 μg/ml) was similar to that previously found by Ibarrondo et al. (3.48 $\log_{10}$ ng/ml or 3.02 μg/ml) [5].

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The influenza virus NP-specific IgG antibody concentration was more than 10 times greater than the concentration of RBD-specific IgG indicating that the SARS-CoV-2 antibody response is relatively weak compared with the antibody response against another respiratory virus. It suggests that in principle the immune system is capable to generate markedly higher antibody concentrations after appropriate stimulation. The finding also indicates that the participants have considerably more influenza NP-specific than SARS-CoV-2 RBD-specific plasma cells in the body. This finding is in line with the observation that the frequency of bone marrow residing influenza virus haemagglutinin-specific IgG-secreting plasma cells outnumbered the frequency of SARS-CoV-2 S-specific bone marrow plasma cells in subjects who recovered from SARS-CoV-2 infection [22].

Between the first few weeks and 12 months after infection, the SARS-CoV-2 RBD and NP antibody concentrations declined significantly. In the first six months, the antibody half-lives averaged approximately 3 months. Similar observations have been made by other research groups [2,3,5–12,22–26]. During the observation, the antibody decline slowed down. Between 9 and 12 months PSO, the RBD antibody half-life increased to more than 2 years and the NP antibody half-life to 7 months. The half-life values are similar to those that have been reported by Gallais et al. [12]. The increasing half-life indicates that predictions about the course of the antibodies must consider the time interval between infection and antibody testing. For instance, when the concentration of SARS-CoV-2 spike IgG is being measured during the first few weeks after infection, half of these antibodies decay in approximately three months. In contrast, when antibody concentrations are being determined 9 months after infection, the average antibody titer remains above 50% of that concentration for the following 2 years.

Even at 9-12 months PSO, the SARS-CoV-2 RBD-specific antibody half-life was shorter than the half-lives of influenza virus NP and Ttx-specific antibodies. As the half-life of antibodies to other viral infections and vaccine antigens increases over 2-3 years, it is likely that the half-life of SARS-CoV-2 antibodies will further extend [27].

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The difference in the half-life of SARS-CoV-2 spike and NP-specific antibodies is striking and has previously been reported by other research groups [8,10–12]. If IgG antibodies against NP and RBD are being catabolized at the same rate - what likely is the case - the longer serum half-life of RBD-specific antibodies indicates that the immune system constantly replaces a higher fraction of RBD- than NP-specific antibodies.

The concentrations of SARS-CoV-2 RBD and NP-specific antibodies in the subjects correlated at early and later time points after infection. Thus, in principle the magnitude of the antibody response to one of the proteins allows prediction of the level of antibodies against the other protein.

Previous studies that examined the SARS-CoV-2-specific MBC response observed that the MBC frequency increased during 4-5 months after infection and the frequency of RBD-specific MBCs decreased between 6.2 and 12 months after infection [4,7,14,28]. We found SARS-CoV-2-specific MBCs in 68.6% of the participants 12 months PSO. The percentage of subjects with detectable SARS-CoV-2 RBD- and NP-specific MBCs was identical and similar to the percentage of subjects with a measurable influenza NP and Ttx-specific MBC response.

In the subjects with detectable MBC responses the frequency of SARS-CoV-2 RBD, NP, influenza virus NP and Ttx-specific MBCs was comparable. Thus, at 12 months after infection the MBC response generated by the SARS-CoV-2 was as robust as the MBC response induced by repeated exposure with influenza virus or Ttx. In a related study, Turner et al. reported comparable frequencies of SARS-CoV-2 spike protein and influenza virus haemagglutinin-specific MBCs 7 months after SARS-CoV-2 infection [22]. The observation that the SARS CoV-2-specific MBC frequency is of similar magnitude as the influenza NP and Ttx-specifc MBC response suggests that the MBC frequency has reached a physiological optimum. This is different to the antibody response after SARS CoV-2 infection that was relatively weak. In vitro, the MBCs rapidly differentiated into antibody-secreting plasma cells upon activation. This suggests that in persons with MBCs re-exposure to SARS-CoV-2 or a vaccine will probably induce a swift rise of antibodies.

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In the study, the SARS-CoV-2 RBD- and NP-specific MBC frequencies correlated closely. Thus, individuals with a higher RBD-specific MBC frequency had more circulating SARS-CoV-2 NP-specific MBCs and participants with fewer RBD-specific MBCs had less SARS-CoV-2 NP-specific MBCs.

The determination of the MBC response allowed a comparison between the antibody concentration and the extent of the MBC response at 12 months PSO. The maximum SARS-CoV-2 RBD and NP antibody concentration and the antibody concentration at 6-7 months correlated with the magnitude of the MBC responses. This indicates that the extent of the early antibody response is predictive of the MBC response at 12 months. It also suggests that the SARS-CoV-2-specific antibody and MBC levels are being regulated by a common immunologic mechanism. The strong correlation of the SARS CoV_2 MBC frequency and serum antibody concentration is remarkable, because MBC frequency and antibody concentration correlate uncommonly. For instance, in our study, the influenza NP and Ttx-specific antibody and MBC concentrations did not correlate. Likewise, Amanna et al. previously reported that the immune responses to only three of eight viral and vaccine antigens correlated significantly [29].

The low antibody concentration but vigorous MBC response after SARS CoV-2 infection indicate that repeated antigen exposure may preferentially boost the antibody response and the generation of long-lived plasma cells.

It has been shown that SARS-CoV-2 vaccination markedly boosts the antibody response in infected individuals [28]. This study describes the immunologic situation in which this effect occurs. A year after infection the antibody level is comparably low, the spike-protein antibody half-life has increased to two years and most SARS-CoV-2 infected individuals have developed a robust virus-specific MBC response.

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Figure legends

Fig. 1. Decay curves and half-life of antibody concentrations. Course and half-life of serum antibodies for the whole time period (max-M12) and separate time intervals from maximum values to month 6-7 (max-M6), month 6-7 to 9 (M6-M9) and month 9-12 (M9-M12). **A:** Antibodies against SARS-CoV-2 RBD and NP for the whole study period. **B:** SARS-CoV-2 RBD antibodies from maximum values to 6-7 months, 6-8 to 9 months and 9-12 months; **C:** SARS-CoV-2 NP antibodies from maximum values to 6-7 months, 6-8 to 9 months and 9-12 months; **D:** Course and half-lives of influenza virus NP and Ttx-specific antibodies in the study period. The red lines indicate the limit of detection of the antibody tests.

Fig. 2. Frequency of memory B-cells. Frequency of MBCs specific for SARS-CoV-2, influenza virus NP and Ttx antigens among all activated, antibody-secreting cells in recovered and naïve participants.

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Tables

Table 1: Median and range of SARS-CoV-2 RBD and influenza NP antibody concentrations at different time points after infection

	Antibody concentration (µg/ml)	
Months PSO[1]	**Median (25-75 IQR)[3]**	**Range[3]**
SARS-CoV-2 RBD		
0.5-2.6[2]	1.54 (0.61-4.45)	0.13-26.8
6-7	0.75 (0.30-1.53)	<0.1-5.53
9	0.56 (0.19-1.14)	<0.1-4.08
12	0.61 (0.2–1.18)	<0.1-4.47
Influenza virus NP		
0.5-2.6[2]	17.2 (9.8-27.8)	2.9-47.1
6-7	15.3 (9.7-22.9)	2.7-49.3
9	12.0 (7.2-19.9)	1.7-45.8
12	14.1 (6.2-17.8)	0.7-40.3

[1]PSO: post symptom onset; [2]equivalent to 2-10 weeks; maximum values if two blood specimens from this period were examined; [3]cut-off SARS-CoV-2 RBD antibodies: 0.1 µg/ml

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Table 2: Overview of IgG and memory B-cell responses and antibody half-lives

Correlation of disease severity and antibody concentrations (rho)				
	Max	**M6**	**M9**	**M12**
SARS-CoV-2 RBD	0.30*	0.32*	0.29*	0.20
SARS-CoV-2 NP	-0.01	0.17	-0.09	-0.03
Correlation of the concentration of SARS CoV-2 RBD and N antibodies (rho)				
	2-10 weeks	**M6**	**M9**	**M12**
SARS-CoV-2 RBD/NP	0.65***	0.61***	0.54***	0.50***
Percentage of individuals with antigen-specific memory B-cells				
% positive individuals	**RBD**	**SARS CoV-2 NP**	**Influenza NP**	**Tetanus toxin**
Infected	68.6	68.6	76.5	78.0
Uninfected	none	none	73.3	92.3
Correlation of memory B-cell responses (rho)				
	SARS-CoV-2 NP	**Influenza NP**	**Tetanus toxin**	
SARS-CoV-2 RBD	0.40*	-0.002	0.04	
SARS-CoV-2 NP	-	-0.13	0.13	
Influenza NP	-	-	0.13	
Correlation of antibody and memory B-cell responses (rho)				
	Max/first value[1]	**M6**	**M9**	**M12**
SARS-CoV-2 RBD	0.60***	0.62***	0.56***	0.51**
SARS-CoV-2 NP	0.53**	0.40*	0.004	-0.02
Influenza NP	0.21	0.15	0.14	0.29
Tetanus toxin	0.10	0.06	0.07	0.06

Max: maximum values; M6, M9, M12: measurement at month 6-7, 9, and 12 post symptom onset.

Rho: Spearman rank coefficient of correlation. Significance level * p < 0.05, ** p < 0.01, *** p < 0.001. [1] Maximum antibody levels were used for SARS-CoV-2 RBD and NP; the first measured antibody values were used for Influenza NP and Ttx.

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Figure 1

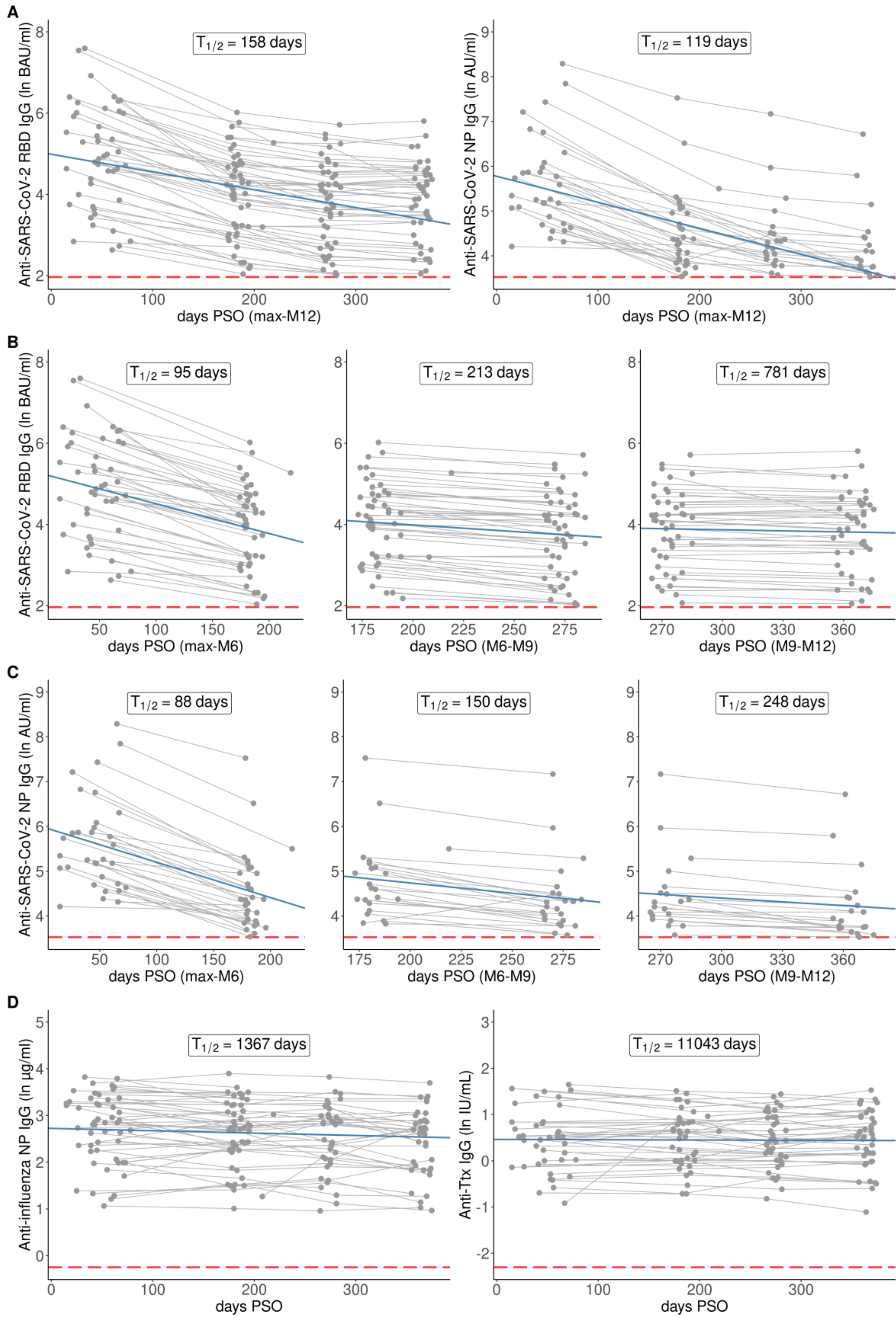
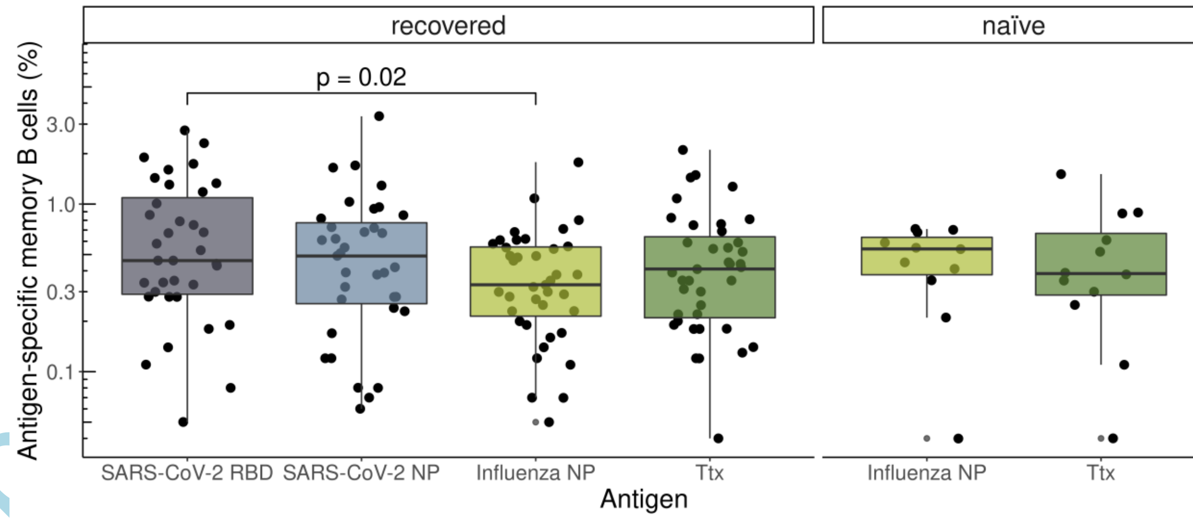


Figure 2



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