Preserved T-cell Response in anti-CD20 Treated Multiple Sclerosis Patients Following SARS-CoV-2 Vaccination

Simon Faissner (simon.faissner@rub.de)
Ruhr-University Bochum https://orcid.org/0000-0002-3412-762X
Neele Heitmann
Ruhr-University Bochum
Ricarda Rohling
Ruhr-University Bochum
Ulas Ceylan
Ruhr-University Bochum
Marielena Bongert
Ruhr-University Bochum
Corinna Marheinecke
Ruhr-University Bochum
Xiomara Pedreiturria
Katholisches Klinikum Bochum https://orcid.org/0000-0002-3024-3826
Ilya Ayzenberg
Ruhr-University Bochum
Kerstin Hellwig
Ruhr-University Bochum
Ingo Schmitz
Ruhr University Bochum https://orcid.org/0000-0002-5360-0419
Stephanie Pfänder
Department of Molecular and Medical Virology
Ralf Gold
Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Germany.

Article

Keywords: SARS-CoV-2 vaccination, multiple sclerosis, anti-CD20 therapy, T cell response

Posted Date: December 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1109886/v1

Abstract

The SARS-CoV-2 pandemic has tremendous implications for the management of patients with autoimmune conditions such as multiple sclerosis (MS) under immune therapies targeting $CD20^+$ B cells (aCD20). We here investigated humoral and cellular immune responses, including neutralization against SARS-CoV-2 WT and delta variant and T cell responses of aCD20-treated MS patients following SARS-CoV-2 vaccination compared to healthy controls. aCD20-treated MS patients had lower anti-SARS-CoV-2-Spike titers, which correlated with B-cell repopulation. Sera of aCD20 treated patients had reduced capacity to neutralize WT and delta pseudoviruses *in vitro*. On the contrary, aCD20 treated patients elicited higher frequencies of $CD3^+$ T cells, Th1 cells, Th2 cells, Tc1 cells and $CD8^+IFN\text{-}\gamma^+IL\text{-}2^+$ cells. In summary, aCD20 treated patients have a reduced humoral immune response, depending on B cell repopulation, in accordance with a shift of cellular immune response to a stronger Th1, Th2 and Tc1 phenotype, suggesting strong cellular protection against SARS-CoV-2.

Introduction

Coronavirus-disease-2019 (COVID-19) induced by SARS-CoV-2 is a potentially life-threatening disease, having led to a global pandemic since early 2020. This raised concerns especially for vulnerable patient groups with impaired immunity, namely patients with chronic autoimmune conditions under highly active immunotherapy such as multiple sclerosis (MS). During the last 20 years, scientific progress led to the development of nearly 20 medications for MS therapy, including monoclonal antibodies targeting B cells via anti-CD20 (aCD20) such as rituximab or ocrelizumab[1]. aCD20 treated patients are at higher risk for hospitalization and a more severe course of COVID19[2]. Since the end of 2020, the first vaccine candidates received emergency use authorization, showing high efficacy against SARS-CoV-2 infection[3]. Since B cells are crucial for the development of a humoral response, investigating the immune response following SARS-CoV-2 vaccination is of huge importance. First reports in 2021 showed reduced humoral response in aCD20 treated MS patients[4]. Most research so far focused on humoral immunity regarding the presence of neutralizing antibody titers following vaccination. However, T cells can exert long lasting and robust immune responses, provide more longevity compared to neutralizing antibodies and are additionally less prone for escape mechanisms[5]. Since knowledge about cellular immune response in aCD20 treated patients is sparse, we here analyzed humoral and T cell responses of MS patients under aCD20 therapy 4-8 weeks following a second vaccination against SARS-CoV-2. Data were corroborated by the analysis of the neutralization capacity of respective sera against the wildtype and the currently circulating delta variant of concern of SARS-CoV-2 *in vitro*. Those data will help to better stratify aCD20 treated patients regarding risk management and vaccination strategy.

Methods

Study design

The study was authorized by the local ethics committee of the Ruhr-University Bochum (20-6953-bio). Patients were recruited at the Department of Neurology, Ruhr-University Bochum, St. Josef-Hospital. We included n=10 healthy age-matched controls and n=36 B-cell depleted MS patients (n=20 ocrelizumab, n=16 off-label rituximab). All patients provided written informed consent. The demographics of the study participants are presented in Table 1. Samples were collected within 4-8 weeks following the second vaccination against SARS-CoV-2.

Table 1
Demographic and clinical characteristics of recruited MS patients and healthy controls. Age, disease duration and EDSS are presented as mean. SD: standard deviation, SEM: standard error of the mean.

	MS	**HC**
Number	36	10
Male (%)	8 (22%)	5 (50%)
Female (%)	28 (78%)	5 (50%)
Age ± SD (range)	40,8 ± 10,6 (20 to 58)	38,7 ± 15,4 (22 to 60)
Disease duration ± SD (range)	10,2 ± 7,5 (1 to 29)	NA
EDSS ± SD (range)	2,25 ± 1,4 (0 to 6,5)	NA
EDSS median	2	NA
EDSS ± SEM	0,24	NA
Treatment	**MS total 36**	**HC total 10**
Ocrelizumab	20 (56%)	-
Rituximab	16 (44%)	-
no therapy	-	10 (100%)
Vaccination	**MS total 36**	**HC total 10**
Comirnaty (Pfizer/Biontech)	31 (86%)	5 (50%)
Vaxzevria (Astrazeneca)	3 (8%)	-
Spikevax (Moderna)	1 (3%)	-
Mixed (Vaxzevria and Comirnaty)	1 (3%)	5 (50%)

Anti-SARS-CoV-2 spike antibody titer

Titers were evaluated using the Elecsys anti-SARS-CoV-2 S assay (range 0.4-2,500 U/ml; Roche).

Cell isolation and cryopreservation

Blood collection for peripheral blood mononuclear cells (PBMC) isolation was conducted using four 7.5 ml KABEVETTE® G EDTA tubes per patient. Blood residues in the EDTA tubes were transferred to the collecting tube by washing each tube with 7.5 ml PBS. The 30 ml blood sample was slowly placed on top of 15 ml ROTI®Sep 1077 human density gradient (Carl Roth) in a 50 ml centrifuge tube. Density gradient centrifugation was performed at 800 g for 30 min. without break. The interface containing the PBMCs was withdrawn from the gradient and washed twice with PBS at 1200 rpm for 10 minutes. The cell pellet was resuspended in 10 ml PBS and cell number was determined using trypan blue in an Improved Neubauer chamber. 10-20x10^6 cells were cryopreserved in 1 ml CTL-Cryo-ABC Freezing media Kit (ImmunoSpot®) according to the manufacturer instructions. The cryotubes were cooled down overnight in a MrFrosty (Sigma) at 80°C and stored at 80°C.

PBMC stimulation using SARS-CoV-2 peptide pool

PBMCs were thawed in a 37°C water bath and diluted in 10 ml RPMI medium 1640 (Gibco) with GlutaMAX ™ and 1% Penicillin/Streptomycin (Pen/Strep) (both Thermofisher) and centrifuged at 1200 rpm for 10 min. The pellet was washed once with 10 ml PBS and resuspended in 1 ml OpT®mizer ™ CTS ™ (Thermofisher). Cell number was determined using trypan blue in an Improved Neubauer chamber. 80 µl per sample were seeded in duplicates in a round bottom 96 well plate (Sarstedt) at a concentration of 2.5x10^6 cells per ml. Cells were stimulated with 2 µg/ml PepMix SARS-CoV-2 (JPT) for 16-18 hours together with Brefeldin A solution (BioLegend). The peptide mix consisted of a pool of 315 (158+157) peptides derived from a peptide scan (15mers with 11 aa overlap) through Spike glycoprotein (Swiss-Prot ID: P0DTC2) of SARS-CoV-2. Additional wells in duplicates were seeded for unstained control, dead cell positive control and unstimulated control without PepMix SARS-CoV-2 for each treatment group.

Flow cytometry

After peptide stimulation for 16-18 hours the cells were stained for flow cytometry against extracellular markers (BioLegend, anti-CD3 clone: SK7 RRID: AB_2616890, anti-CD4 clone: SK3 RRID: AB_1937227, anti-CD8a clone: RPA-T8 RRID: AB_2629694) and intracellular markers (BioLegend, anti-IFN-γ clone: 4S.B3 RRID: AB_961357, anti-IL-2 clone: MQ1-17H12 RRID: AB_315096, anti-IL-4 clone: MP4-25D2 RRID: AB_315127). Antibody dilutions are presented in Supplementary Table 1. Extracellular staining was conducted together with viability stain Zombie Aqua™ Dye (BioLegend) for 30 min. at 4°C. Fixation and permeabilization were performed with Fixation Buffer (BioLegend) and intracellular staining perm wash buffer (BioLegend) before intracellular staining according to the manufacturer's instructions. Subsequently, for intracellular staining cells were incubated for 30 min. at room temperature. Flow cytometry was performed using a FACSCelesta™ Flow cytometer (BD) with High Throughput Sampler and FACSDiva™ Software (BD FACS Diva Software, Version 9.0). The gating strategy is shown in Fig. S1. Flow cytometry data were analyzed with FlowJo™ (BD, version 10.7.1).

SARS-CoV-2 neutralization assay

SARS-CoV-2 pseudoviruses were prepared as described previously[6]. Briefly, sera were incubated for 30 min at 56°C in order to inactivate complement factors. Single cycle VSV∗ΔG(FLuc) pseudoviruses

bearing the SARS-CoV-2 spike (D614G) protein[7] or SARS-CoV-2 B.1.617.2 (Delta) (EPI_ISL_1921353) spike in the envelope were incubated with quadruplicates of twofold serial dilutions from 1:20 to 1:2560 of immune sera in 96-well plates prior to infection of Vero E6 cells ($1x10^4$ cells/well) in DMEM with 10% FBS (Life Technologies). At 18 hours post infection, firefly luciferase (FLuc) reporter activity was determined as previously described[8] using a CentroXS LB960 (Berthold) and the reciprocal antibody dilution causing 50% inhibition of the luciferase reporter was calculated as pseudovirus neutralization dose 50% (PVND50). Detection range is defined to be between 1:20 of above 1:2560.

Statistics

GraphPad Prism (version 9.2.0) was used for statistical analysis. Data were tested for Gaussian distribution using Kolmogorov-Smirnov test. Data were analyzed with Mann-Whitney U test, correlations were calculated using Spearman correlation.

Data Availability

All data are available from the corresponding author SF upon reasonable request.

Results

Baseline characteristics

We included 36 MS-Patients with a mean age of 40.8 years and a disease duration of 10.2 ± 7.5 years (mean ± SD; Table 1) and n=10 age-controlled controls (age 38.7 ± 15.4, HC). MS-patients had a mean EDSS of 2.25 ± 1.4 (range 0 to 6.5). 56% of aCD-20 treated patients were under therapy with ocrelizumab, 44% received off-label rituximab. The majority of MS-patients had been vaccinated with an mRNA-vaccine (86% Comirnaty, Pfizer/Biontech).

Anti-SARS-CoV-2-Spike antibody spike titer correlates with repopulating B cells

Anti-SARS-CoV-2-Spike antibodies were significantly lower in aCD20 treated MS patients compared to healthy controls (HC 2337 U/ml ± 147.3 (mean ± SEM); aCD20 243.3 U/ml ± 103.7; $p<0.0001$; Fig. 1a). HC showed no correlation of $CD19^+$ B cells with the titer since 80% of the patients had a titer of 2500 U/ml at the maximum range (Fig. 1b). On the contrary, in aCD20 treated patients the anti-SARS-CoV-2-Spike titer correlated with the frequency of $CD19^+$ B cells ($r=0.6844$, $p<0.0001$; Fig. 1c). Age did not correlate with anti-SARS-CoV-2-spike antibodies (Fig. 1d, e).

aCD20-treated patients developed fewer neutralizing antibodies against the WT and delta variant of SARS-CoV-2

The participants' sera were tested for their capacity to reduce the SARS-CoV-2-virus variants' infection rate *in vitro* using a neutralization assay against wild type (WT) and delta variant of SARS-CoV-2. The capacity of respective sera to reduce the infectivity of mammalian cells using WT and Delta

pseudoviruses was calculated as 50% inhibition (ND_{50}) of a luciferase reporter virus. Sera of anti-CD20 treated MS patients had significantly less neutralizing effect on WT ($p<0.01$) and Delta variants ($p<0.0001$) of SARS-CoV-2 compared to HCs (Fig. 2a,b). We also analyzed whether there was an association of anti-SARS-CoV-2 spike titers and neutralization capacity of respective sera. In HC, there was a significant correlation of anti-SARS-CoV-2 spike and WT neutralization ($r=0.7006$, $p=0.0222$), but not for delta, whereas no correlation could be observed in aCD20 treated patients (Fig. 2c, d).

Higher frequency of $CD3^+$ T cells following SARS-COV peptide stimulation in aCD20 treated MS patients

Since aCD20 treated MS patients revealed a restricted humoral immune response, we proceeded to evaluate cellular immune response following stimulation with a peptide pool of SARS-CoV-2 (pool of 315 (158+157) peptides derived from Spike glycoprotein (Swiss-Prot ID: P0DTC2)). aCD20 treated MS patients revealed a higher frequency of overall $CD3^+$ T cells than HC (HC 56.78% ± 2.358; aCD20 72.29% ± 1.908 (mean ± SEM); $p<0.0001$) (Fig. 3a). Frequencies of $CD4^+$ T helper cells were also higher in aCD20 treated MS patients compared to HC (HC 30.02% ± 1.411; aCD20 38.43 ± 3.171; $p<0.05$). $CD8^+$ cytotoxic T cells did not differ from HC (Fig. 3c). The frequency of T cells, T helper cells (Th cells) and cytotoxic T cells was neither in HC nor in aCD20 dependent on the anti-SARS-CoV-2-Spike titer (Fig. S2). In line with this finding, cellular immune response of T cells, T helper cells and cytotoxic T cells did not correlate with neutralization capacity of respective sera except for delta neutralization and correlation with activation of $CD4^+$ T helper cells (HC $r=-0.7619$, $p=0.0368$; aCD20 $r=0.3492$, $p=0.0368$; Fig. S3d).

To evaluate the effect of aging on cellular immune response, we correlated overall $CD3^+$ T cells with age. In aCD20 treated patients, the percentage of T cells in the blood was dependent on the age with a younger age accounting for a higher percentage of T cells ($r=-0.3532$, $p=0.0374$; Fig. S4a). In HC, we observed the same trend, which however lacked significance.

Th1 and Th2 cell populations were significantly increased following SARS-COV peptide stimulation in MS patients under antiCD20 therapy

We further analyzed subpopulations of $CD4^+$ Th cells between respective groups. Th1 cells expressing both CD4 and IFN-γ were significantly more abundant in aCD20 treated MS patients than in HC (HC 3.616% ± 0.3461; aCD20 18.69% ± 1.770 (mean ± SEM); $p<0.0001$) (Fig. 4a, c). Memory $CD4^+$ T helper cells, characterized by the expression of IL-2, did not differ between both groups (Fig. 4b). In contrast, IL-4 expressing $CD4^+$ T helper cells were significantly more abundant in PBMCs of aCD20 treated MS patients than in HC (Fig. 4c). IFN-γ and IL-2 double positive $CD4^+$ Th cells did not significantly differ between both groups, although there was a trend towards higher frequency in aCD20 treated MS patients (Fig. 4d). T helper cell subpopulations did not correlate with anti-SARS-CoV-2-spike titer (Fig. S5).

Higher frequencies of subpopulations of cytotoxic T cells expressing IFN-γ or both IFN-γ and IL-2 following SARS-COV peptide stimulation in aCD20 treated MS patients

We further analyzed subpopulations of $CD8^+$ cytotoxic T cells regarding the expression of IFN-γ and IL-2. $CD8^+$ T cells expressing IFN-γ were abundant with a higher frequency in aCD20 treated MS patients than in HC (HC 1.399% ± 0.3599; aCD20 8.135% ± 0.8551 (mean ± SEM); p<0.0001) (Fig. 5a). $CD8^+$ T cells expressing IL-2 did not differ (Fig. 5b). On the contrary, $CD8^+$ T cells expressing both IFN-γ and IL-2 were found with higher frequency in aCD20 treated MS patients (HC 4.399% ± 0.6898; aCD20 17.63% ± 2.314 (mean ± SEM); p<0.0001) (Fig. 5c). In aCD20 treated MS patients, anti-SARS-CoV-2-Spike titers correlated with Tc1 cell percentages (r=0.3904; p=0.0204; Fig. S6). In HCs, this correlation was not present. Anti-SARS-CoV-2-Spike titers did not correlate with cytotoxic T cell subsets expressing IL-2 or IFN-γ together with IL-2 (Fig. S6).

Discussion

The SARS-CoV-2 pandemic has profound implications for the management of patients with chronic disorders, especially patients with chronic immune diseases in need of a permanent immunomodulatory or immunosuppressive therapy such as patients with multiple sclerosis, rheumatoid arthritis or transplant patients. The treatment of MS patients has seen huge progress during the last twenty years, offering a broad armamentarium of medications for different severities and MS courses[1,9]. Early during the pandemic there was a debate regarding the risk of MS patients to develop a severe course of COVID-19. We[10] and others showed that MS per se is not associated with a higher risk for severe COVID-19. There are, however, subgroups of patients at higher risk. Data from 657 suspected and 1683 confirmed COVID-19 MS patients under disease modifying therapy showed that older age, progressive MS phenotype and higher disability are associated with worse COVID-19 outcomes[2]. Moreover, treatment with aCD20 therapy with ocrelizumab and rituximab was associated with higher rates of hospitalization (aOR 1.75, 95% CI 1.29-2.38; aOR 2.76, 95% CI 1.87-4.07) and ICU admission (aOR 2.55, 95% CI 1.49-4.36; aOR 4.32, 95% CI 2.27-8.23) and, for rituximab, with artificial ventilation (aOR 6.15, 95% CI 3.09-12.27)[2].

The development of vaccines against SARS-CoV-2 raised the question whether especially patients under B cell depletion might be at risk to develop only a reduced humoral and cellular immune response due to the lack of B cells, important for co-stimulation. While there is evidence, that B cell depleted MS-patients are at higher risk for a severe course of COVID-19[2], there are only anecdotal reports of patients under B cell depleting therapy vaccinated against SARS-CoV-2 with breakthrough infection[11]; hence, it remains unclear how vaccination against SARS-CoV-2 might influence this risk.

We here demonstrate that patients under B cell depletion show a reduced humoral immune response by measuring anti-SARS-CoV-2 spike antibodies, in line with a reduced capacity to neutralize WT and delta pseudovirus *in vitro*. Development of anti-SARS-CoV-2 spike antibodies correlated strongly with repopulation of B cells. On the contrary, aCD20 treated patients exhibited a stronger cellular immune response following SARS-COV peptide stimulation compared to HC. This was mainly characterized by higher frequencies of overall $CD3^+$ T cells, IFN-γ expressing $CD4^+$ T helper (Th1) cells, IL-4 expressing $CD4^+$ T helper cells (Th2), IFN-γ expressing $CD8^+$ cytotoxic T cells (Tc1) cells and IFN-γ IL-2 expressing

$CD8^+$ T cells, suggesting an enhanced cellular immune response of Th1, Th2 and Tc1 in aCD20 treated MS patients.

The first report raising questions regarding the development of a proper immune response in aCD20 treated patients was shown in early 2021 in MS patients treated in Israel[4]. Those data showed that patients under B cell depleting therapy indeed develop only in 22.7% of the cases a humoral immune response against SARS-CoV-2 spike 29.5-55 days after the second vaccine dose[4]. Apart from MS, there are numerous reports having shown impaired humoral immune response in immunosuppressed patients such as in rheumatoid arthritis, systemic lupus erythematosus and vasculitis under different immunosuppressive therapies including rituximab[12]. Using the IFN-γ enzyme linked immunosorbent spot (ELISpot) assay, preserved cellular immune response in B cell depleted patients with impaired humoral response was demonstrated in a group of rituximab treated rheumatology patients[13], however without analyzing distinct immune subpopulations. Apostolidis et al. showed a reduced spike-specific and receptor-binding domain (RBD)-specific antibody and memory B cell responses in most patients in a group of n=20 MS patients under aCD20 therapy[14]. Moreover, the group showed that CD8 T cell induction was augmented with preserved type 1 helper T (Th1) cell priming[14]. Those data are in line with our findings, showing that aCD20 treated patients present a strong response of both Th1 and Tc1 cells.

Th1 cells producing IFN-γ and IL-2 are considered as a subpopulation of cells with superior protective cellular capacity[15]. Hence, it might be speculated that patients under aCD20 therapy might at least be in part protected against SARS-CoV-2 infection due to a skewed cytotoxic T cell response. The cytotoxic T cell response is fast, potent, and detectable 10-12 days after a prime vaccination[3]. On the contrary, a prime vaccination only leads to a limited presence of class-switched B cells that could produce S1-specific IgG, while the second booster vaccination mobilizes antigen-specific memory B cells to the periphery after boost[16]. In addition, the T cell response is broader, targeting multiple SARS-CoV-2 variants of concern following BNT162b2 mRNA-vaccination due to conservation of T cell epitopes on SARS-CoV-2 variants, whereas some variants can partially escape humoral immunity[17]. A stable and fully functional $CD8^+$ T cell response is already mobilized one week after prime vaccination with the mRNA vaccine BNT162b2, when circulating $CD4^+$ T cells and neutralizing antibodies are still weakly detectable[16]. The longevity of this protective T cell immunity induced by mRNA vaccination remains unclear. Patients who recovered from SARS following infection during the outbreak in 2003 possess indeed long-lasting memory T cells that are reactive to the N protein of SARS-CoV 17 years later[18]. Moreover, these T cells displayed robust cross-reactivity to the N protein of SARS-CoV-2, suggesting that beta-coronaviruses induce multi-specific and long-lasting T cell immunity against the structural N protein[18]. Memory B-cells on the contrary are not durable in coronavirus infection and disappear within less than three years following infection[5].

In summary, our study provides evidence that patients under B cell depleting therapy have a reduced humoral response and that the development of an anti-SARS-CoV-2 titer depends on the reconstitution of B cells, in line with reduced capacity to neutralize WT and delta variant of SARS-CoV-2. Moreover, we

show that B cell depletion leads to a shift of cellular immune response, characterized by strong Th1, Th2 and Tc1 response, suggesting strong cellular immunity against SARS-CoV-2 in aCD20 treated patients. Those data altogether support that, if possible, aCD20 treated patients should be vaccinated or boostered during the reconstitution of B cells. Long-term studies are required to address the question whether vaccination in aCD20 treated patients might be protective against the development of COVID19 in real-world cohorts and to understand dynamics of T cell response over time.

Abbreviations

aCD20
anti-CD20 treated
COVID-19
Coronavirus disease 2019
MS
Multiple sclerosis
PBMCs
peripheral blood mononuclear cells
PVND50
Pseudovirus Neutralizing dose 50
SARS-CoV-2
Severe acute respiratory syndrome coronavirus 2
SD
Standard deviation

Declarations

Acknowledgement We thank Gert Zimmer, Institute for Virology und Immunology, Switzerland and Department of Infectious Diseases and Pathobiology (DIP), Vetsuisse Faculty, University of Bern, Switzerland and Stefan Pöhlmann and Markus Hoffmann, Infection Biology Unit, German Primate Center - Leibniz Institute for Primate Research, Göttingen, Germany, Faculty of Biology and Psychology, Georg-August-University Göttingen, Göttingen, Germany, for providing the WT und Delta plasmid. Funding This research was supported by a grant from the Rose foundation T298/28150/2016. Authors' contributions SF, UC, RR, MB, KH and IA acquired patients´ samples. NH, UC, RR, CM and XP performed the experiments. SF, NH, UC, RR, MB and SP analyzed data. SF, NH and RR made the figures. SF, NH and UC wrote the first draft of the manuscript. RR, MB, CM, XP, IA, KH, IS, SP and RG corrected the manuscript. SF and RG designed and supervised the study. RG acquired funding. All authors read and approved the final manuscript. Competing interests The authors declare no competing interests relevant to the content of this manuscript. Materials & Correspondence All data are available from the corresponding author SF upon reasonable request.

References

1. Faissner, S. & Gold, R. Efficacy and Safety of the Newer Multiple Sclerosis Drugs Approved Since 2010. *CNS drugs* **32**, 269–287, doi:10.1007/s40263-018-0488-6 (2018).
2. Simpson-Yap, S. *et al.* Associations of Disease-Modifying Therapies With COVID-19 Severity in Multiple Sclerosis. *Neurology* **97**, e1870-e1885, doi:10.1212/wnl.0000000000012753 (2021).
3. Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* **383**, 2603–2615, doi:10.1056/NEJMoa2034577 (2020).
4. Achiron, A. *et al.* Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. *Ther Adv Neurol Disord* **14**, 17562864211012835, doi:10.1177/17562864211012835 (2021).
5. Hellerstein, M. What are the roles of antibodies versus a durable, high quality T-cell response in protective immunity against SARS-CoV-2? *Vaccine X* **6**, 100076, doi:10.1016/j.jvacx.2020.100076 (2020).
6. Zettl, F. *et al.* Rapid Quantification of SARS-CoV-2-Neutralizing Antibodies Using Propagation-Defective Vesicular Stomatitis Virus Pseudotypes. *Vaccines (Basel)* **8**, doi:10.3390/vaccines8030386 (2020).
7. Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271-280.e278, doi:10.1016/j.cell.2020.02.052 (2020).
8. Kinast, V. *et al.* C19orf66 is an interferon-induced inhibitor of HCV replication that restricts formation of the viral replication organelle. *J Hepatol* **73**, 549–558, doi:10.1016/j.jhep.2020.03.047 (2020).
9. Faissner, S., Plemel, J. R., Gold, R. & Yong, V. W. Progressive multiple sclerosis: from pathophysiology to therapeutic strategies. *Nature reviews. Drug discovery*, doi:10.1038/s41573-019-0035-2 (2019).
10. Richter, D. *et al.* Multiple sclerosis is not associated with an increased risk for severe COVID-19: a nationwide retrospective cross-sectional study from Germany. *Neurol Res Pract* **3**, 42, doi:10.1186/s42466-021-00143-y (2021).
11. Peeters, M. *et al.* Reduced humoral immune response after BNT162b2 coronavirus disease 2019 messenger RNA vaccination in cancer patients under antineoplastic treatment. *ESMO Open* **6**, 100274, doi:10.1016/j.esmoop.2021.100274 (2021).
12. Ferri, C. *et al.* Impaired immunogenicity to COVID-19 vaccines in autoimmune systemic diseases. High prevalence of non-response in different patients' subgroups. *Journal of autoimmunity* **125**, 102744, doi:10.1016/j.jaut.2021.102744 (2021).
13. Mrak, D. *et al.* SARS-CoV-2 vaccination in rituximab-treated patients: B cells promote humoral immune responses in the presence of T-cell-mediated immunity. *Annals of the rheumatic diseases* **80**, 1345–1350, doi:10.1136/annrheumdis-2021-220781 (2021).
14. Apostolidis, S. A. *et al.* Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nature medicine*, doi:10.1038/s41591-021-01507-2 (2021).

15. McKinstry, K. K. *et al.* Memory CD4 T cell-derived IL-2 synergizes with viral infection to exacerbate lung inflammation. *PLoS pathogens* **15**, e1007989, doi:10.1371/journal.ppat.1007989 (2019).
16. Oberhardt, V. *et al.* Rapid and stable mobilization of CD8(+) T cells by SARS-CoV-2 mRNA vaccine. *Nature* **597**, 268–273, doi:10.1038/s41586-021-03841-4 (2021).
17. Geers, D. *et al.* SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. *Sci Immunol* **6**, doi:10.1126/sciimmunol.abj1750 (2021).
18. Le Bert, N. *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* **584**, 457–462, doi:10.1038/s41586-020-2550-z (2020).

Figures

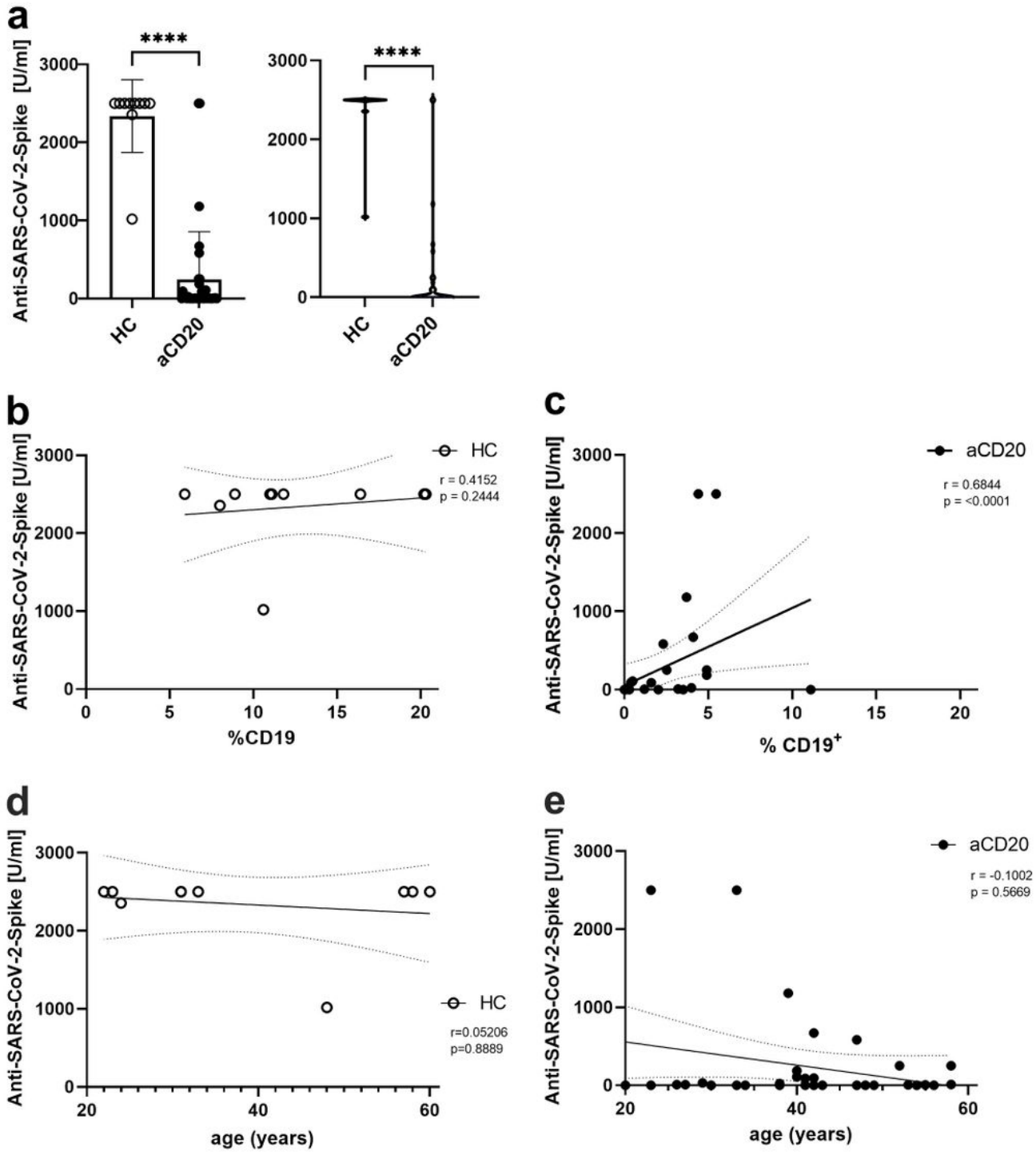

Figure 1

Anti-SARS-CoV-2-spike titer correlates with B-cell repopulation. a) anti-SARS-CoV-2-spike titers were lower in anti-CD20 (aCD20) treated MS patients compared to healthy controls (HC). b, c) Correlation of anti-SARS-CoV-2-Spike titers with CD19+ B cell frequencies, showing strong correlation in aCD20 treated MS patients. d, e) The age of anti-CD20 treated MS patients and HC did not correlate with anti-SARS-CoV-2-

Spike titer. Data were analyzed with non-parametric, two-tailed Mann-Whitney test (a) or non-parametric, two-tailed, Spearman-correlation (b-d). n=36 aCD20, n=10 HC. **** p<0.0001.

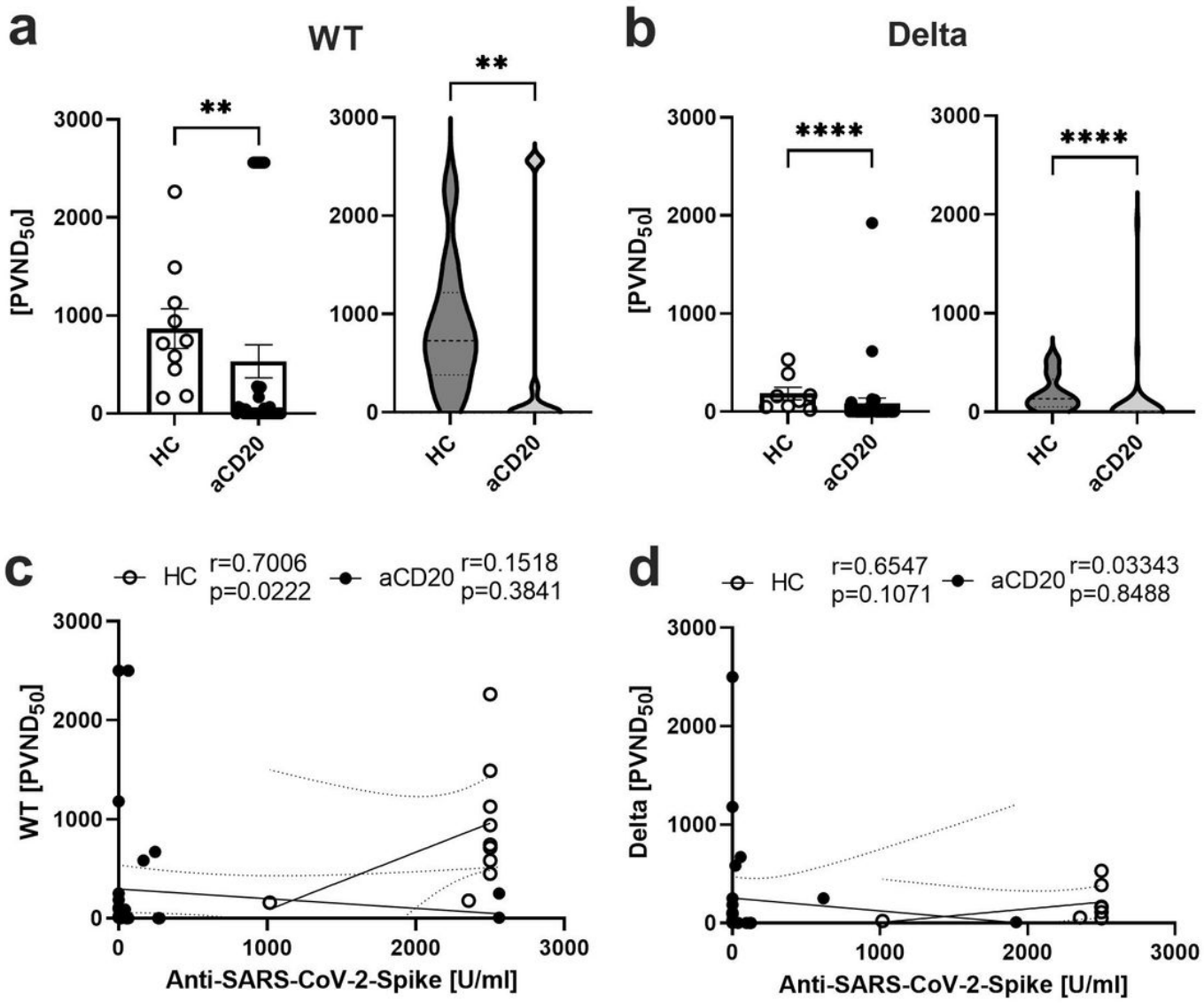

Figure 2

Neutralization of WT and Delta variant in vitro. a) Neutralization of SARS-CoV-2 pseudoviruses WT and b) Delta following 18 h of incubation. Shown is luciferase reporter activity, indicating 50% inhibition (PVND50). c, d) Correlation of anti-SARS-CoV-2 spike and neutralization capacity. Data were analyzed with non-parametric, two-tailed Mann-Whitney test (a, b) or non-parametric, two-tailed Spearman-correlation (c, d). n=36 aCD20, n=10 HC (a), n=8 HC (b). **P < 0.01; ****P < 0.0001.

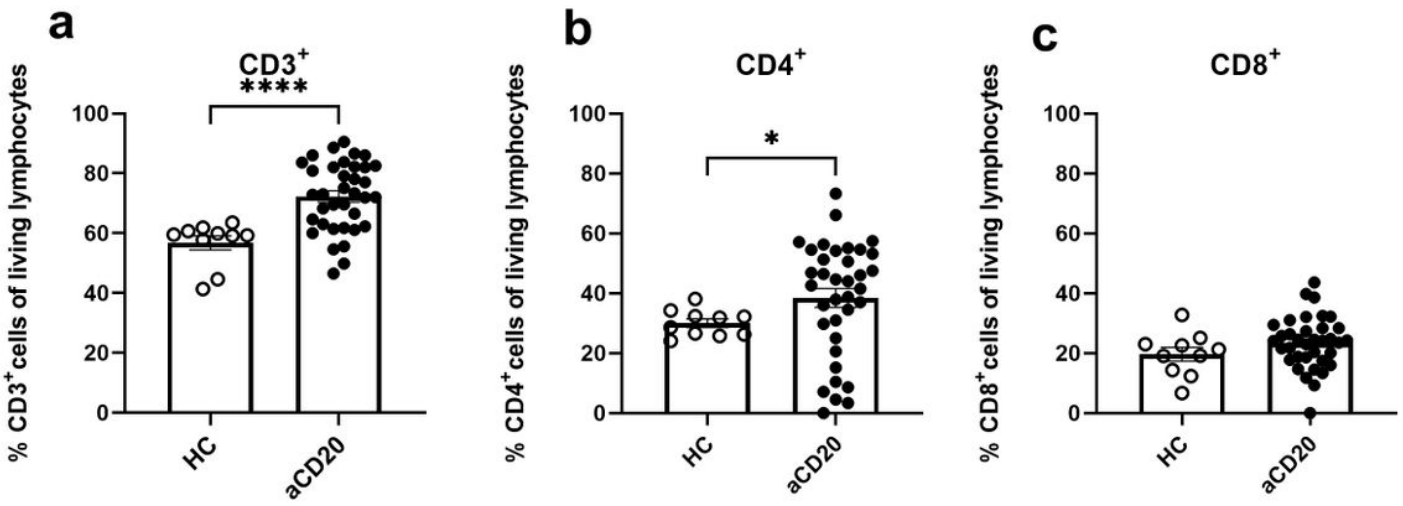

Figure 3

Flow cytometry analysis of T cell populations. PBMCs were stimulated with SARS-CoV-2 peptide pool. a) Higher frequencies of CD3+ T cells and b) CD4+ T helper cells in aCD20 treated MS patients compared to HC. c) CD8+ T cells did not differ. Data were analyzed with non-parametric, two-tailed Mann-Whitney test. n=36 aCD20, n=10 HC. ****P < 0.0001.

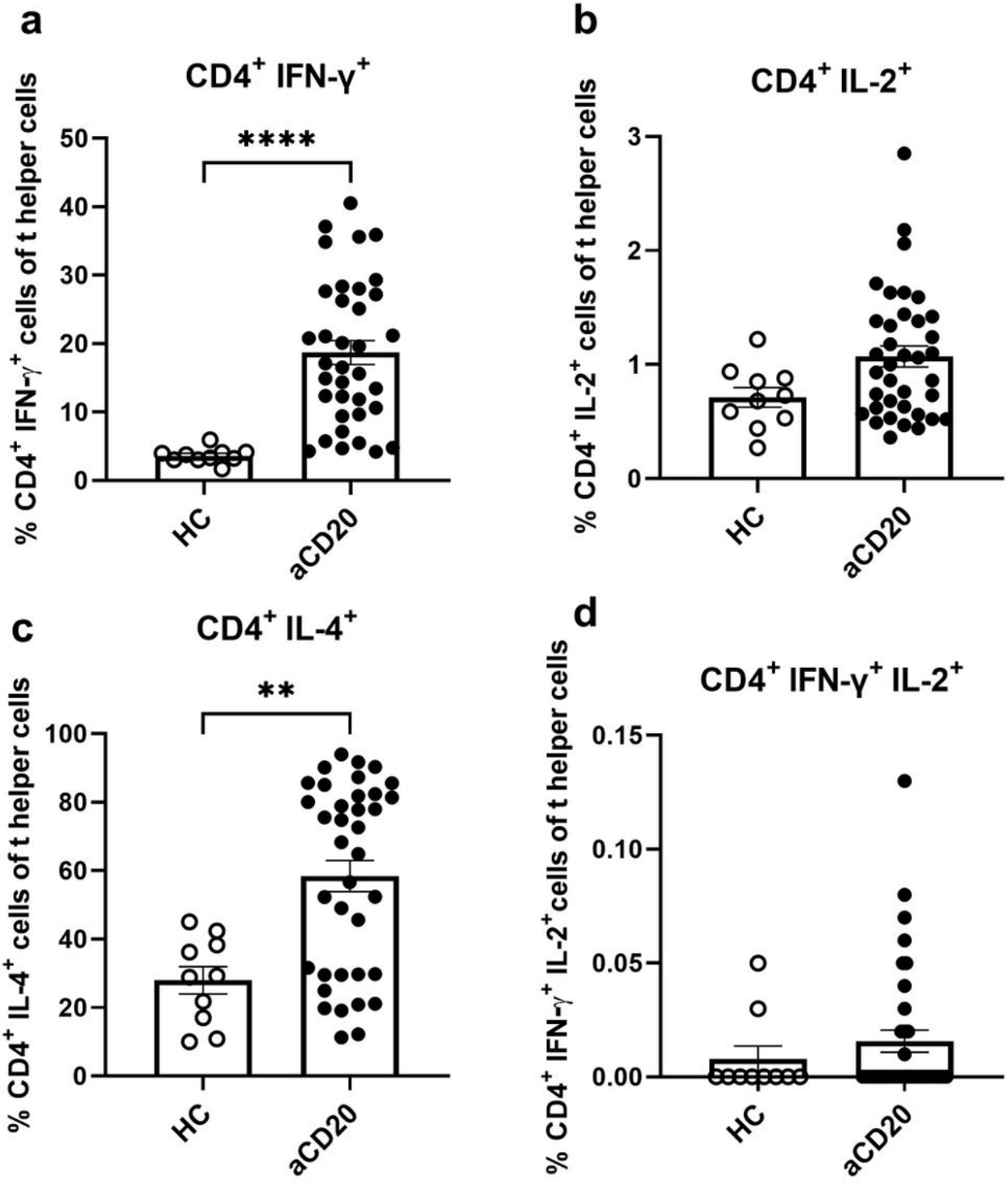

Figure 4

aCD20 treated patients express stronger response of CD4+IFN-γ+ (Th1) and CD4+IL-4+ (Th2) T helper cells. a) Frequencies of CD4+ T cells expressing IFN-γ were significantly higher in aCD20 treated patients than in HC. b) The expression of IL-2 or IFN-γ and IL-2 in aCD20 treated patients did not deviate from those of HC. c) Higher frequencies of IL-4 expressing CD4+ T helper cells in aCD20 compared to HC. Data

were analyzed with non-parametric, two-tailed Mann-Whitney test. n=36 aCD20, n=10 HC. **P < 0.01; ****P < 0.0001.

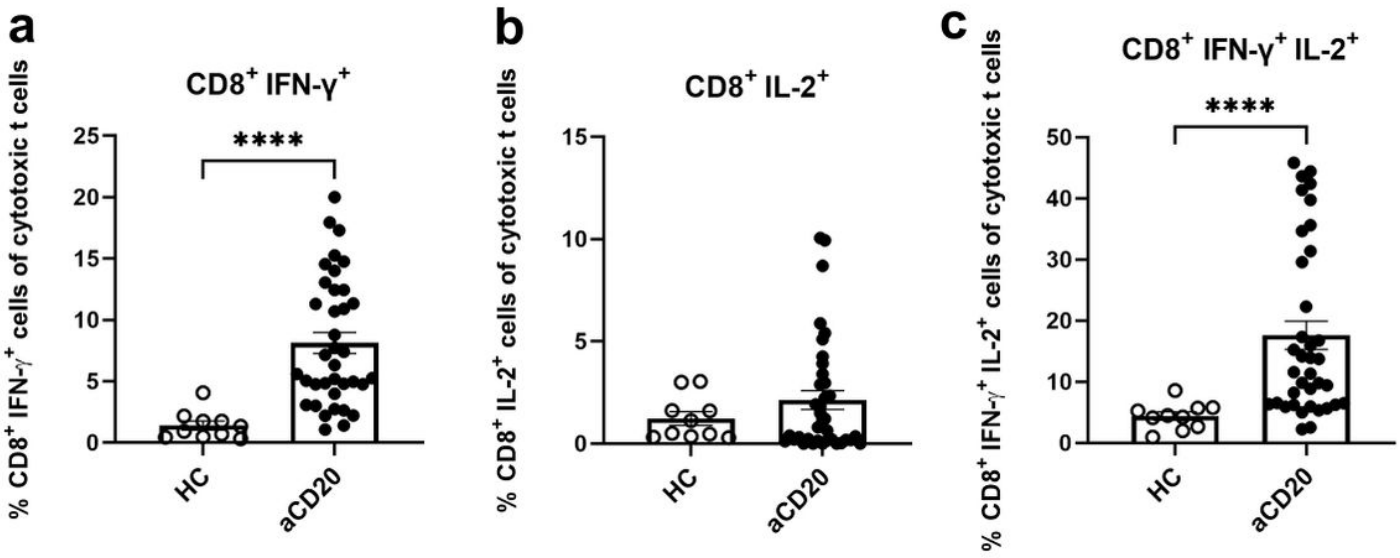

Figure 5

aCD20 treated patients express stronger response of CD8+IFN-γ+ and CD8+IFN-γ+IL-2+ cytotoxic T cells. a) aCD20 treated patients show significantly higher IFN-γ expression in cytotoxic t cell population than HC. b) Frequencies of IL-2 expressing cytotoxic T cells in aCD20 treated MS patients did not differ from HC. c) IFN-γ and IL-2 double positive cytotoxic T cells showed a significantly higher abundance in aCD20 treated MS patients. Data were analyzed with non-parametric, two-tailed Mann-Whitney test. n=36 aCD20, n=10 HC. ****P < 0.0001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- VaccinationaCD20MSSupplementsubmission.pdf