DISSERTATION

Cellular and humoral immunity towards SARS-CoV-2 infection and vaccination in patients with primary antibody deficiency

Zelluläre und humorale Immunität in Antwort auf eine SARS-CoV-2 Infektion und Impfung bei Patienten mit primären Antikörpermangelerkrankungen

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ABBREVIATIONS

Ab	antibody
Aab	autoantibody
ATM	atypical memory B cells
CHC	convalescent healthy control
CVID	common variable immunodeficiency disorder
COVID-19	coronavirus disease 2019
DMSO	dimethylsulfoxid
dp	double producing
ELISA	enzyme-linked Immunosorbent assay
ELISpot	enzyme linked immuno spot assay
ESID	European Society for Immunodeficiencies
HC	healthy control
HCoV	human endemic coronavirus
IEI	inborn errors of immunity
IFNγ	interferon-γ
IgG	immunoglobulin G
IGRA	interferon-gamma-release-assay
IgRT	immunoglobulin replacement therapy
IL-2	interleukin-2
IMDM	Iscove's modified dulbecco's medium
IQR	interquartile range
IVIG	intravenous immunoglobulin
mAb	monoclonal antibody
MBC	memory B cell
nAb	neutralizing antibodies

NCAP	nuclear capsid protein
NP	nuclear protein
NR	non-responder
OD	optical density
PAD	primary antibody deficiency
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PID	primary immunodeficiency disease
PRNT	plaque reduction neutralization test
PSO	post symptom onset
R	responder
RBD	receptor binding domain
RPMI	Roswell Park Memorial institute medium
RT-PCR	real-time polymerase chain reaction
S	Spike
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SEB	staphylococcus enterotoxin B
sp	single producing
SpO2	blood oxygen saturation level
TNFα	tumor necrosis factor-α
tp	triple producing
VOC	variants of concern
WHO	World Health Organization
XLA	X-linked agammaglobulinemia

ZUSAMMENFASSUNG

Zu Beginn der COVID-19 Pandemie, ausgelöst durch das 2019 in Wuhan, China entdeckte Virus Schweres Akutes Respiratorisches Syndrom-Coronavirus-2 (SARS-CoV-2), wurden Patienten mit angeborenen Immundefekten mit hohem Risiko für schwere Krankheitsverläufe eingestuft. Primäre Antikörperdefizienzen (PAD) stellen die größte Gruppe dieser Immundefekte dar. Zusätzlich zum quantitativen Immunglobulinmangel, tritt eine qualitative Einschränkung der humoralen Immunität auf, gekennzeichnet durch fehlende spezifische Antikörper (Ak)-Antwort nach Infektion oder Impfung. Eine erste klinische Beobachtungstudie bestätigte die erhöhte COVID-19 Morbidität und Mortalität für PAD. Zu Beginn dieser Dissertation waren die Mechanismen der zellulären und humoralen Immunantworten bei SARS-CoV-2-Infektion und Impfung für PAD unbekannt. In den experimentellen Studien wurden polyfunktionale T-Zell-Antworten durchflusszytometrisch analysiert und der Ak-Status, sowie das funktionelle B-Zell-Gedächtnis mittels ELISA und ELISpot-Techniken bestimmt. Studie 1 wies erstmals eine T-Zell-Immunität gegen endemische Coronaviren und deren Potential zur SARS-CoV-2-Kreuzreaktivität bei 7/11 PAD nach. Im Vergleich zu unifizierten und rekonvaleszenten Gesunden war die Erkennungshäufigkeit und Polyfunktionalität bei PAD geringer. Um die Bedeutung der spezifischen COVID-19 Ak-Antwort besser zu verstehen, wurden in Studie 2 T-zelluläre und innate Immunantworten schwerer COVID-19 Verläufe bei PAD ohne Serokonversion untersucht. Obwohl alle untersuchten PAD eine intakte polyfunktionale T-Zell-Immunität aufwiesen und keine gestörte Typ-I-Interferonantwort vorlag, wurden gehäuft SARS-CoV-2 Virämien und persistierende virale Ausscheidungen bis zu 127 Tage beobachtet. Studie 3 untersuchte die COVID-19 Impfantwort bei PAD. Dabei zeigten spezifische Ak-Antworten bei PAD im Vergleich zu Gesunden eine deutlich verminderte Avidität. Zudem konnte die Mehrzahl der serokonvertierten PAD kein funktionelles B-Zell-Gedächtnis aufbauen. Die T-Zell-Antwort war bei 100% der Patienten mit, und bei 83% der Patienten ohne Ak-Antwort intakt. Reaktive follikuläre T-Helferzellen waren bei Ak-positiven PAD erhöht. Zusammenfassend zeigten die Studien normale T-Zell-Antworten bei PAD nach SARS-CoV-2-Infektion oder Impfung. Ungeimpfte PAD ohne spezifische SARS-CoV-2 Ak-Antwort zeigten schwerere Krankheitsverlaufe und prolongierte mukosale Viruspersistenzen. Während klinische Daten den Stellenwert einer prophylaktischen Vakzin-induzierten T-Zell-Immunität untermauern, zeigen qualitative Unterschiede in der

Avidität und funktionellen Gedächtnisformation der humoralen Immunität bei geimpften PAD die Notwendigkeit einer differenzierteren Bewertung jenseits der binären Einteilung in seronegativ und -positiv. Die langfristige Bedeutung therapeutischer Maßnahmen, wie der Einsatz monoklonaler Ak und der Stellenwert der IgG-Ersatztherapie von immunisierten Spendern, ist in dieser Patientengruppe noch nicht hinreichend geklärt.

ABSTRACT

At the onset of the COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), which evoked in Wuhan, China in 2019, patients with inborn errors of immunity were considered as high-risk group prone to more severe disease. Primary antibody deficiencies (PAD) represent the largest group of these immunodeficiencies. In addition to quantitative immunoglobulin deficiency, a qualitative impairment of humoral immunity occurs, that is characterized by a lack of specific antibody (Ab) response after infection or vaccination. An initial observational clinical study during early pandemic stage confirmed the increased incidence of morbidity and mortality due to COVID-19 in PAD. At the beginning of this doctoral thesis, mechanisms of cellular and humoral immune responses to SARS-CoV-2 infection and vaccination in PAD remained elusive. The conducted experimental studies involved the analysis of polyfunctional T cell responses by flow cytometry along with Ab status and functional B cell memory determined by ELISA and ELISpot techniques. Study 1 was the first among literature to demonstrate T cell immunity to human endemic coronaviruses and their potential for SARS-CoV-2 cross-reactivity in 7/11 PAD. However, compared with uninfected and convalescent healthy individuals, detection frequency and polyfunctional capacity were lower in the patient group. In order to better understand the importance of specific COVID-19 Ab response, study 2 examined T cell and innate immune responses of severe COVID-19 PAD cases without seroconversion. Although all PAD investigated had intact polyfunctional T cell immunity and no impaired type I interferon response, frequent SARS-CoV-2 viremia and persistent viral shedding up to 127 days were observed. Study 3 examined COVID-19 vaccination response in PAD patients. Here, specific Ab responses in PAD showed significantly reduced avidity compared with healthy individuals. In addition, the majority of seroconverted PAD failed to from a functional B-cell memory. T cell response was intact in 100% of patients with, and in 83% of patients without SARS-CoV-2 Ab responses. Reactive follicular T helper cells were increased in Ab-positive PAD. In summary, the studies indicate normal T cell responses in PAD subsequent to SARS-CoV-2 infection or vaccination. Unvaccinated PAD without a SARS-CoV-2 Ab response showed more severe disease progression and prolonged mucosal viral persistence. While clinical data support the value of prophylactic vaccine-induced T cell immunity, qualitative differences in the avidity and functional memory formation of humoral immunity in vaccinated PAD highlight the need for a more nuanced assessment

beyond the binary classification of seronegative and –positive patients. The long term value of therapeutic measures, such as the use of monoclonal Ab and the significance of IgG replacement therapy from immunized donors, has not yet been sufficiently clarified in this patient group.

1 INTRODUCTION

1.1 SARS-CoV-2 a novel coronavirus causing a global health crisis

As of November 28th, 2023, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused over 772 million cases with an estimated 6.98 million cumulative deaths leading to a significant global health crisis and bearing a huge socioeconomic burden to the world population (1). SARS-CoV-2, causing coronavirus disease 2019 (COVID-19), first appeared in Wuhan, China in December 2019. This enveloped single-stranded positive-sense RNA virus belongs to the family of human betacoronaviruses comprising OC43, 229E, SARS-CoV-1 and Middle East Respiratory Syndrome (MERS-CoV) amongst others (2).

COVID-19 clinical presentation ranges from asymptomatic, over mild, severe and critical conditions. Infected individuals experience influenza-like symptoms including headache, fatigue, dry cough or a sore throat. Severe and critical cases with bilateral pneumonia could experience acute respiratory distress syndrome (ARDS) where invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO) may be required. Multi-organ involvement like neurological, gastrointestinal and cardiac manifestations are reported (3, 4).

Four essential proteins make up the structure of SARS-CoV-2 including spike (S), envelope (E), membrane (M) and nucleocapsid (NCAP). S and NCAP proteins induce strong immune responses during infection evoking a broad interest of the scientific community to better characterize their role in SARS-CoV-2 driven immunity as well as their potential for diagnostics and as target for active and passive immunization. The homotrimer S protein has two functional subunits. Subunit S1 comprises the N-terminal and receptor binding domain (RBD), allowing attachment to the host cell, whereas subunit S2 (C-terminal domain) catalyzes fusion via the receptor angiotensin-converting enzyme 2 (ACE2). Due to its critical role, the S protein is an attractive target for virus inhibition through neutralizing antibodies (nAb) and vaccine development (5). The highly conserved NCAP inside the virion holds the viral genome. It is critical for viral assembly, replication and regulatory functions in viral life cycle. Detection of NCAP is specific to infected individuals and is therefore a useful marker for distinguishing natural from vaccine acquired SARS-CoV-2 immunity (6). The transmembrane E protein has multiple functions in virulence, viral assembly, budding and activation of different pathways like NOD-, LRR-

and pyrin domain-containing protein 3 inflammasome (7). The M protein dimer presents the most abundant of the four structural proteins and has several pivotal roles in virus maturation and assembly (8).

1.2 Primary antibody deficient patients – a group more prone to severe COVID-19 outcome?

According to the European Society for Immunodeficiencies (ESID) registry, primary antibody deficiencies (PAD) are the largest group of inborn errors of immunity (IEI), affecting up to 56% (9). In this thesis, two PAD patient groups were examined. The first group, patients with Common Variable Immunodeficiency Disorder (CVID), represent the most prevalent IEI. Markedly decreased IgG and a reduction in at least one IgA or IgM isotypes, accompanied by poor vaccine responses are hallmark disease features (10). The second group is Good's Syndrome, an immunodeficiency associated with thymoma, significantly reduced or absent B cells, hypogammaglobulinemia and lack of clinically effective antibodies (Ab) (11). Those rare disorders arise due to impaired B cell function or defects in B cell maturation. Because of low or absent levels of one or more immunoglobulin (Ig) isotypes underpinned by the inability to produce pathogen specific Ab and poor vaccination responses, patients face an increased susceptibility to (severe) infections, of which the majority occur in the respiratory tract (12). Consequently, life-long Ig replacement therapy (IgRT), effective in infection prevention is necessary. IgRT reduces serious infections but patients can still suffer from breakthrough and more frequent infections, because Ig do not contain IgA and IgM (13). Aside from infectious manifestations, approximately 2/3 suffer from non-infectious complications like lymphoproliferations, inflammatory disease, autoimmune disorders and malignancies (14).

The overall impaired humoral immunity makes these patients particularly vulnerable to SARS-CoV-2 infection, which might result in higher prevalence of severe disease and fatal outcome (15). Several prognostic factors with increased risk for mortality were reported including advanced age, ethnicity, type I IFN autoantibodies (Aab) and pre-existing comorbidities like respiratory diseases, diabetes, cardio- or cerebrovascular diseases and malignancy (16, 17). Clinical manifestations resemble those in the general population, but outcomes differ significantly (15). PAD patients endure longer COVID-19 duration often accompanied by prolonged viremia and viral shedding due to impaired

immunity (18). Younger age at infection is observed, which is around 20 years lower compared to the general population (19). Moreover, there is a higher prevalence of comorbidities amongst PAD and increased admission rate to intensive care units with up to 20% (15). Consequently, case fatality rate, before vaccination, was several fold higher with up to 40% (15, 19, 20) compared to cumulative data of the world population with ~1% (1). This increased case fatality rate was associated with significantly younger age among the deceased (19).

1.3 Adaptive immunity to SARS-CoV-2 in the general population and in the presence of humoral immunodeficiency

Adaptive immunity is a key player during viral control and clearance. It is essential for re-recognizing viral antigens and protection from re-infection upon secondary exposure by creating an immunological memory. There are two major components, B and T cells, during cellular and humoral immunity. Regarding SARS-CoV-2 infection and vaccination, it is well described that active immunity in immunocompetent individuals requires the generation of antigen-specific T cells and synthesis of nAb (21-23). Still, there is a lack of information on humoral and T cellular immunity after SARS-CoV-2 infection in PAD patients.

1.3.1 T cellular immunity after SARS-CoV-2 infection

T cell responses towards SARS-CoV-2 in healthy individuals emerge early post-infection and correlate with protection. Current studies reveal a variety of reactions targeting structural and non-structural SARS-CoV-2 proteins with strong responses to S and NCAP proteins (24-27). Data on long-term immunity suggests that T cell response is maintained without loss of functionality up to 10 month (m) post-infection, but seems to be accompanied by a decrease in frequency (28-30). $CD4^+$ response, characterized by polyfunctional cytokine potential, was shown to be present at higher frequency compared to $CD8^+$ T cells (28). Interestingly, in 90% of uninfected healthy individuals there is proof of pre-existing cross-reactive T cells responsive to SARS-CoV-2, originating from previous infection with seasonal endemic human coronaviruses (HCoV), SARS-CoV-1 or MERS. This cross-reactivity likely results from homologous peptide sequences of closely related pathogens, sharing distinct highly conserved T cell epitopes (25, 27, 31-34). The impact of cross-reactive T cells on disease progression and outcome remains uncertain,

but they are thought to enhance immune response during SARS-CoV-2 infection and vaccination (31). Data on T cellular immunity after COVID-19 in PAD is scarce, because most studies solely focus on humoral immunity and clinical outcome, lacking additional qualitative assessments like polyfunctionality/memory phenotype of T cells (15, 20, 35, 36).

1.3.2 Humoral immune response after SARS-CoV-2 infection

Ab provide a first line of defense upon viral encounter, whereas memory B cell (MBC) response is supposed to supplement waning Ab titer and provide long-term infection protection (21). Seroconversion in immunocompetent SARS-CoV-2 infected individuals is reached within the first 2 weeks post symptom onset (PSO) peaking after 2-3 weeks (29, 37). These specific Ab primarily target S and NCAP proteins. While S IgM/A Ab decline relatively fast after 7-10 weeks, S IgG Ab remain stable for about 3 m with modest decrease 5-8 m later (29, 34). Ab against other structural proteins like NCAP wane more rapidly (38).

nAb titers emerge within the first few weeks post-infection and their decline is associated with increased risk of re-infection especially with regard to emerging virus variants of concern (VOC) (39). Notably, 10-15% of healthy convalescents with mild disease lack specific serum Ab (40). S specific class-switched (CS) MBC were detected in most SARS-CoV-2 infected individuals, also in those with loss of specific IgG (28, 41-44). The finding of SARS-CoV-2 long-lived bone marrow plasma cells indicates a long-lived immunity and these cells can respond rapidly upon secondary viral encounter (42, 45).

Ab-mediated immunity is expected to be impaired in PAD patients after SARS-CoV-2 infection, depending on the type of underlying immunodeficiency and frequencies of T and B cell subpopulations. Studies show varying levels in SARS-CoV-2 Ab responses, predominantly assessed in CVID patients, with up to 85% achieving seroconversion, but Ab levels in seroconverted PAD tend to be lower compared to healthy individuals (35, 46-48). Based on flow cytometry markers, generation of SARS-CoV-2 S MBC was proposed in about 1/3 of convalescent CVID patients (46).

1.3.3 COVID-19 vaccine acquired adaptive immunity

Vaccination remains the most effective tool preventing infection in immunocompetent and immunocompromised individuals, although PAD patients share the common risk of poor adaptive immunity and might not benefit from COVID-19 vaccination to the same extent

than healthy individuals (49). Humoral and T cellular responses to mRNA vaccines in PAD were never investigated before the COVID-19 outbreak. However, a diminished or absent humoral response was observed following other type of vaccinations, e.g. against influenza. Generation of T follicular helper (T_{FH}) cells and virus-specific polyfunctional T cells is possible in the majority of PAD patients (50, 51). In patients lacking humoral immunity, T cellular response alone might offer partial protection against severe disease endorsing for COVID-19 vaccination in PAD (49).

Several COVID-19 vaccines were approved by EMA and national authority during the first immunization period in 2021 in Germany, including two mRNA-based vaccines (Pfizer BTN162B2, Moderna mRNA-1273) and one of adenoviral origin (AstraZeneca AZD1222). All three vaccines were proven to be immunogenic and effective generating T cell and serologic responses (52-54).

Studies on COVID-19 vaccine responses in immunocompetent individuals show Ab responses up to 100% and up to 90% for T cell responses (52, 55, 56). There is still uncertainty about the impact of COVID-19 vaccination in individuals with PAD and adaptive immunity after immunization is under ongoing extensive investigation showing varying responses ranging from 29-85% for seroconversion and 29-83% for T cellular responses (57-60). Protective levels of immune responses after vaccination remain unknown and data on longevity and quality of acquired responses are still limited.

1.4. Importance and aims of the doctoral thesis

PAD patients present with moderate to severe quantitative and qualitative Ab deficiency due to impaired B cell function and maturation resulting in the inability to produce clinically effective Ab. Patients are characterized by hampered responses to vaccines, leading to limited humoral protection from vaccination as prophylactic measure and despite ongoing IgRT, patients suffer from infections. This impaired humoral immunity along with higher prevalence of comorbidities puts these patients at higher risk for severe COVID-19. Early in the pandemic, therapeutic approaches and preventive management were scarce and comprehensive analyses on quality and longevity of SARS-CoV-2 immunity in PAD remained limited. Therefore, this thesis aimed to enhance the understanding of SARS-CoV-2 immunity in PAD by characterizing cellular and humoral immune responses. The three studies of this thesis had the following aims:

1. Study 1 aimed to comparatively evaluate reactive T cells in response to peptide pools of human endemic corona virus (HCoV) strains OC43 and 229E as well as SARS-CoV-2 in infection naïve CVID patients with known impaired Ab responses, infection naïve healthy controls (HC) and COVID-19 convalescent HC. The purpose was to characterize T cellular immunity in CVID patients and to find out if SARS-CoV-2-cross-reactive T cells are present.

2. Study 2 followed the observations from study 1 by investigating the impact of SARS-CoV-2 infection on T cellular immunity in severe COVID-19 PAD patients with complete lack of SARS-CoV-2-specific serology. This involved comparative analyses to two control groups of unexposed and convalescent HC. Assessment of type I IFN innate immunity, described as potential risk factor for severe outcomes (17), was performed to better understand immune mechanisms of severe COVID-19 courses in PAD patients. Finally, persistence of viral shedding and RNA viremia in the absence of SARS-CoV-2 Ab were monitored.

3. Study 3 explored COVID-19 vaccine-induced humoral and T cellular immunity. The aim was to elucidate T cellular and MBC responses in SARS-CoV-2 infection naïve, vaccinated seroresponding and non-seroresponding CVID patients compared to infection naïve, vaccinated HC. Quality of SARS-CoV-2 Ab response and functional B cell recall memory was investigated to shed light on humoral immunological memory. SARS-CoV-2-reactive T_{FH} and polyfunctional cytokine T cell responses were analyzed before and after vaccination to examine the impact of vaccination on the development of a T cellular immunity.

2 METHODS

Information on material and methods in this section refer to methodologies in selected publications 1-3 (61-63), accessible as print copies under section 9.

2.1 Study subjects

Blood samples of PAD patients were assembled at the outpatient clinic for immunodeficiencies at the Institute for Medical Immunology, Charité Universitätsmedizin Berlin. All patients were adult and diagnosed based on ESID criteria (64). HC samples were obtained from laboratory staff at the same institute. The studies were approved by the Charité Ethics Committee (EA2/092/20 from June 4th, 2020) and procedures were performed in compliance with the 1964 Declaration of Helsinki and its later amendments. All participants gave written informed consent.

2.2 Isolation of human peripheral blood mononuclear cells (PBMCs)

PBMCs were obtained from heparinized whole blood by density gradient centrifugation using Leucosep tubes (Greiner Bio-One). Blood was diluted 1:2 with phosphate buffered saline (PBS) and layered carefully into prefilled tubes containing Pancoll (Pan Biotech) separation medium. Samples were centrifuged at 800 g for 15 min at room temperature (RT) without deceleration. Enriched cell fraction was harvested by pouring the supernatant into another tube. PBMCs were washed twice with PBS and subsequently centrifuged for 10 min at 300 g. Cell counts were determined using Neubauer chamber and staining cells with an equal volume of Trypan Blue solution (Sigma-Aldrich, St. Louis, U.S.). PBMCs were cryopreserved in freezing medium (50% Iscove's Modified Dulbecco's Medium (IMDM), 40% fetal calve serum (FCS), 10% dimethylsulfoxid (DMSO)) and stored in liquid nitrogen.

2.3 Assessment of SARS-CoV-2 antibodies

SARS-CoV-2 Ab were assessed in cooperation with the group of Victor M. Corman at the Institute of Virology, Charité Universitätsmedizin Berlin. Serum IgG/IgA against the SARS-CoV-2 S1 domain was analyzed using Euroimmun ELISA according to manufacturer’s instructions and fully automated Euroimmun Analyzer I (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). SeraSpot anti-SARS-CoV-2

IgG/IgA immunoassay (Seramun Diagnostica GmbH) was used for NCAP, RBD, S1 and S full detection. Neutralizing SARS-CoV-2 IgGs were analyzed using plaque reduction neutralization test (PRNT) as previously described (65).

2.4. SARS-CoV-2 IgG antibody avidity

Ab avidity was analyzed in cooperation with Victor M. Corman of the Institute of Virology, Charité Universitätsmedizin Berlin. An adjusted SARS-CoV-2 S1 EUROIMMUN ELISA was used. Plates were pre-coated with SARS-CoV-2 S1 and serum samples (1:101 dilution) incubated for 1h at 37°C. After washing, 200µl PBS or urea (5.5M) were applied and incubated for 10min at 37°C. Following washing, conjugate and substrate solution were added according to manufacturer's instructions. OD was measured at 450nm. Avidity index was calculated by dividing the OD of urea by the PBS sample, multiplied by 100.

2.4 Detection of SARS-CoV-2 and HCoV reactive T cells

2.4.1 Cell culture condition for SARS-CoV-2 and HCoV reactive T cells

Cryopreserved PBMCs were thawed at 37°C and rested in IMDM/10% FCS/1% P/S for 24h at 37°C, 5% CO_2 (standard settings). Cells were stimulated with 1µg/ml of SARS-CoV-2 S peptide pool (PM-WCPV-S-1, N- and C-terminal), SARS-CoV-2 NCAP (PM-WCPV-NCAP-1) or HCoV-229E and -OC43 S peptide pools (PM-229E-S-1; PM-OC43-S-1, N- and C-terminal; JPT Peptide Technologies GmbH, Berlin). 3µg/ml of superantigen staphylococcal enterotoxin B (SEB, Sigma-Aldrich, St. Louis, U.S.) was added as positive control and DMSO only as background control. After 2h, 15µg/ml of the secretion inhibitor brefeldin A (Sigma-Aldrich, St. Louis, U.S.) was applied. Stimulation continued for 16h under standard settings.

2.4.2 Flow cytometry analysis of SARS-CoV-2 and HCoV reactive T cells

Following stimulation, cells were washed and extracellular (e.c.) markers (Biolegend, San Diego, U.S. or Thermo Fisher, Waltham, U.S, Table 1) incubated for 30 min at standard settings. After washing, fixation/permeabilization buffer (FoxP3 transcription factor staining buffer set, eBioscience) was added and incubated for 30 min at 4°C. Intracellular (i.c.) staining (Table 1) was done for 30 min at 4°C. Samples were washed and acquired using a CytoflexLX flow cytometer (Beckman Coulter) and analyzed with FlowJo version

10.6.2 (Becton Dickinson, Franklin lakes, U.S.). To exclude unspecific activation, DMSO background signal was subtracted from stimulated samples. SARS-CoV-2 S-reactive T cell responses were defined as $CD4^+CD154^+CD137^+$ or $CD8^+CD137^+$ T cells (later referred to as reactive $CD4^+/CD8^+$ T cells) >0.005% and 20% above background signal within total $CD4^+/CD8^+$ T cells. This threshold aligns with the range in which 95% of all negative samples lie. Boolean combination gating was applied for subset analysis of polyfunctional cytokine-producing T cells.

Table 1: Marker for T cell phenotyping using flow cytometry

Target	Staining	Dilution	Conjugate	Clone
T cell markers study #1 and #2				
Anti-human CD3	i.c.	1:100	BV650	OKT3
Anti-human CD4	i.c.	1:100	PerCp-Cy5.5	SK3
Anti-human CD8	i.c.	1:100	BV510	RPA-T8
Anti-human CD137	i.c.	1:100	PE	4B4-1
Anti-human CD154	i.c.	1:200	BV421	24-31
Anti-human IL-2	i.c.	1:200	APC	MQ1-17H12 (RUO)
Anti-human IFNγ	i.c.	1:20	BV605	4S.B3
Anti-human TNFα	i.c.	1:20	AF700	MAb11
Live/Dead Cell Staining	e.c.	1:100	Fixable Blue	-
Additional T cell markers study #3				
Anti-human CD45-RA	e.c.	1:100	PE-Cy7	HI100
Anti-human CXCR5	e.c.	1:100	PE-Dazzle	J252D4

CXCR5=C-X-C chemokine receptor type 5; e.c.=extracellular, i.c.=intracellular, IFNγ=interferon-γ; IL-2=interleukin 2; TNFα=tumor necrosis factor-α. Own representation: Sophie Steiner

2.5 Detection of SARS-CoV-2 antibody secreting cells by memory B cell ELISpot

2.5.1 Cell culture condition for B cell expansion

Cryopreserved PBMCs were thawed and seeded with $4x10^6$ cells/3ml culture medium (RPMI/10% FCS/1% P/S) in a 6-well plate. Incubation was performed at standard settings for 7d. B cell proliferation was achieved using the protocol from *Crotty et al.* (66) with 6 µg/ml CpG oligodeoxynucleotides 2006 (ODN7909, InvivoGen, San Diego, U.S.), 100 ng/ml of *Pokweed mitogen* (Sigma-Aldrich, St. Louis, U.S.), 1:10,000 *Staphylococcus aureus Cowan I* (SAC) and 50 µM β-Mercaptoethanol (β-Mercaptoethanol, SAC: Merck KGaA, Darmstadt, Germany).

2.5.2 Analysis of B cell subpopulations by flow cytometry

B cell subsets were determined by flow cytometry analysis of PBMCs at day 0 (*ex vivo*) and day 7 after expansion (*in vitro*). After washing, cells were incubated with a LIVE/DEAD marker (Thermo Fisher, Waltham, U.S.) for 30 min at RT. Subsequently, e.c. staining using monoclonal antibodies (mAb) (Biolegend, San Diego, U.S.) was performed (for staining panel refer to selected publication 3, Supplementary Table S4) for 30 min at 4°C. Samples were acquired on a CytoflexLX an analyzed with FlowJo software version 10.6.2..

2.5.3 SARS-CoV-2 specific memory B cell ELISpot

SARS-CoV-2 S antibody secreting cells (ASC) after 7d cell culture were detected using enzyme-linked immuno spot (ELISpot) assay. 96-well MultiScreen Filter Plates (Merck Millipore, Biochrom GmbH, Berlin, Germany) were coated with 1µg/ml trimeric SARS-CoV-2 S protein (D614G mutant, Excellgene, Monthey, Switzerland). As positive control wells were coated with1.2 µg/ml goat anti-human IgG (Jackson ImmunoResearch, West Baltimore Pike, U.S.) and with PBS for exclusion of unspecific Ab binding. Wells were incubated overnight (ON) at 4°C and subsequently washed three times with PBS and blocked with culture medium for 1h at standard settings. During blocking cells of day 7 were washed twice in culture medium. Cells were plated in duplicate onto the plate with $2.5x10^5$/100µl for SARS-CoV-2 S protein and $6.25×10^3$/100 µl for whole IgG and incubated 6h at standard settings. Wells were washed six times with PBS/1% BSA/0.05%Tween. Secondary Ab goat anti-human IgG-HRP (1:500, Invitrogen, Waltham, U.S.) was added and incubated ON at 4°C. After three times washing with PBS, spot development was achieved by adding substrate buffer (0.3 M sodium acetate solution, 0.2 M acetic acid solution, Aqua dest., pH = 5.0, 1:30 3-amino-9-ethyl-carbazole-dimethylformamide solution and 1:100 3% H_2O_2 (Sigma-Aldrich, St. Louis, U.S.)). Spots were analyzed using AID ELISpot reader and 7.0 iSpot software (Autoimmun Diagnostika GmbH, Straßberg, Germany). To prevent duplicate and artificial spot counting, each plate was manually verified.

2.6 SARS-CoV-2 IGRA

Interferon-gamma-release-assay (IGRA, Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) was performed for quantitative IFN-γ determination by SARS-CoV-2-

reactive T cells according to manufacturer's instructions in cooperation with Victor M. Corman at the Institute of Virology, Charité Universitätsmedizin Berlin.

2.7 Analysis of innate immunity to SARS-CoV-2 infection

Experiments on innate immunity were carried out at the clinical diagnostics laboratory, Labor Berlin GmbH.

2.7.1 SIGLEC1 expression on monocytes

EDTA whole blood was used to detect expression of sialic acid binding Ig like lectin 1 (SIGLEC1 or CD169) on monocytes by flow cytometry as previously described (67). Experiments were performed according to an admitted protocol using mouse anti-human SIGLEC1 Ab (clone 7-239), CD14 and CD45 (Beckman Coulter, Pasadena, U.S.).

2.7.2 Autoantibodies against type I IFNs

Analysis of anti-type I IFN Aab was performed as described (68) by the use of an electrochemiluminesence immunoassay-platform (MSD, Rockville, U.S.). Positive cut-off values for light signal counts (LSC) were >1,980 (anti-IFN-α) and >1,961 (anti-IFN-ω).

2.8 Statistics

Kruskal-Wallis-test followed by Dunn's post-test was applied for unpaired comparisons between multiple groups. In order to compare two unpaired groups, two-tailed Mann-Whitney U test was applied. For assessment of paired comparisons within a group, Wilcoxon signed-rank test was performed. Continuous variables are presented as median with interquartile range (IQR). Statistical significance was defined at a p-value of <0.05. Analyses were performed with GraphPad Prism version 9.3.1 (GraphPad Software, Boston. U.S.).

3 RESULTS

Results presented in this section refer to selected publications 1-3 (61-63), accessible as print copies under section 9.

3.1 Study 1: HCoV and SARS-CoV-2 cross-reactive T cells in CVID patients

SARS-CoV-2 reactive T cells against different structural proteins were shown early in the pandemic in healthy individuals, but not in PAD patients. Study 1, initiated in spring 2020, investigated SARS-CoV-2 $CD4^+$ and $CD8^+$ T cell reactivity in infection naïve CVID patients and HC compared to COVID-19 convalescent HC. This was addressed using flow cytometry analyzing reactive T cells towards two common HCoV strains along with pre-existing SARS-CoV-2-reactive T cells and their cytokine profile (IFNγ, TNFα, IL-2) in response to *in vitro* stimulation with the corresponding peptide pools. Seven peptide pools were used, including N- and C-terminal domains for HCoV-OC43, -229E and SARS-CoV-2 S and NCAP. Peptide reactive T cells were characterized by expression of activation markers $CD154^+$ and $CD137^+$ as described in 2.4.2. Gating strategy is shown in selected publication 1, Figure 1 (61).

3.1.1 Study cohort and humoral immune response to SARS-CoV-2

11 infection-naïve CVID patients (median age 51 (29–74)), 12 infection-naïve HCs (median age 35 (25–65)) and 11 post COVID-19 HCs (median age 44 (22–75)) were examined. The CVID cohort was on continuous IgRT for at least 2 years. Post COVID-19 HCs had a previously mild disease course and a median of 73 d (48-95 d) after diagnosis bevor analyzing T cells. Detailed CVID patient and post COVID-19 HC characteristics are described in selected publication 1, Table 1 A-B. COVID-19 convalescent HC had SARS-CoV-2 serum IgG and neutralizing IgG, whereas CVID patients and unexposed HC had no history of COVID-19 being seronegative (selected publication 1, Supplementary Figure 1). CVID patients showed slightly higher $CD3^+$ and $CD8^+$ T cell frequencies, whereas $CD4^+$ T cell frequencies were comparable among the groups (selected publication 1, Supplementary Figure 2) (61).

3.1.2 Unexposed CVID patients and HC show reactive T cells in response to SARS-CoV-2 and HCoV peptide pools

In all groups SARS-CoV-2 and HCoV reactive T cells were detected. Among the 11 CVID

patients, 7 had S-reactive $CD4^+$ T cells against SARS-CoV-2 and 4 of those 7 against HCoV-229E and/or -OC43. SARS-CoV-2 NCAP-reactive $CD4^+$ T cell responses were completely missing and $CD8^+$ NCAP T cell responses were less frequent (selected publication 1, Figure 2 A-B). This was significant for 9 of overall 14 peptide responses with less $CD4^+$ and $CD8^+$ T cell responses to S and NCAP peptide pools of SARS-CoV-2, HCoV-229E and –OC43 in CVID compared to unexposed HC ($p<0.0005$ for one, $p<0.005$ for six, $p<0.05$ for two peptide responses; selected publication 1, Table 2). $CD4^+$ reactive T cell responses to one or more of the SARS-CoV-2 or HCoV S peptide pools were found in unexposed HC (SARS-CoV-2 and HCoVs each 75%) and post COVID-19 HC (SARS-CoV-2: 81%; HCoVs: 63%; selected publication 1, Figure 2 A-B, Table 2 and Supplementary Table 1, 2). No difference was observed in peptide reactive $CD4^+$ or $CD8^+$ T cell frequencies among the three groups. Similar responses to SEB positive control were seen in all groups (selected publication 1, Figure 2 A-D). In uninfected HC, most SARS-CoV-2 S $CD4^+$ and $CD8^+$ T cell responses significantly correlated with responses to S HCoVs (selected publication 1, Table 3 A-B), indicating cross-reactivity. Serum S IgG levels did not correlate with frequencies of T cell responses (data not shown) (61).

3.1.3 SARS-CoV-2 cross-reactive T cells in unexposed HC and CVID patients show polyfunctional cytokine profile

Highest cytokine responses were found for $TNF\alpha^+IL\text{-}2^+$ double producing (dp) and $TNF\alpha^+IFN\gamma^+IL\text{-}2^+$ triple producing (tp) reactive $CD4^+$ and $CD8^+$ T cells (selected publication 1, Figure 3 A-D). There were no differences in cytokine producing T cells between unexposed and post COVID-19 HC. However, peptide stimulation elucidated significantly lower reactive tp $CD4^+$ T cells in CVID patients in response to SARS-CoV-2 S (N-terminal $p=0.002$, C-terminal $p=0.036$) as well as HCoV-OC43 S C-terminal ($p=0.05$) peptide pool (selected publication 1, Figure 3A). NCAP-reactive $CD4^+$ T cells elicited significantly higher tp cytokine frequencies in post COVID-19 compared to unexposed HC ($p=0.0043$, selected publication 1, Figure 3A). This was not observed in CVID patients. In peptide reactive $CD8^+$ T cells no differences were observed between the groups (selected publication 1, Figure 3 B, D). All other cytokine subsets showed no difference (selected publication 1, Supplementary Figure 4). SEB positive control revealed similar results among all groups for reactive $CD4^+$ or $CD8^+$ T cells and their cytokine producing capacity, arguing against impaired T cell function in CVID patients (selected publication 1, Figure 3 E-F) (61).

3.2 Study 2: SARS-CoV-2 T cell response in severe and fatal COVID-19 in primary antibody deficiency patients unable to generate SARS-CoV-2 specific humoral immunity

During study 2, immune status and underlying immunological mechanisms in COVID-19 PAD patients were still poorly understood. PAD patients faced increased risk of severe disease with up to 2/3 requiring hospitalization (15, 69). Therefore, clinical and immunological manifestations in severe COVID-19 PAD patients, unable to build SARS-CoV-2 specific humoral immune responses, were explored (62). We analyzed SARS-CoV-2 Ab, viral load in plasma by RT-PCR and T cellular immunity in response to SARS-CoV-2 S and NCAP peptides by flow cytometry. Innate immune response was assessed via detection of SIGLEC1 on monocytes as marker of type I IFN signature along with anti-type I IFN Aab, which were recently shown to correlate with severe COVID-19 (17).

3.2.1 Study subjects and humoral immune response

The study cohort comprised 5 PAD patients (3 CVID, 2 Good's Syndrome patients, baseline characteristics: selected publication 2, Table 1). Within the PAD group two fatal cases (#1, #5) occurred. Control groups included 6 healthy convalescents (CHC) and 6 infection naïve, unvaccinated HC. SARS-CoV-2 infection in PAD patients was confirmed by SARS-CoV-2 RT-PCR. 4/5 patients presented with extensive bilateral pneumonia accompanied by onset of respiratory insufficiency 8-20 d post symptom onset (PSO). Milder respiratory insufficiency with infiltrates were observed in patient #2 (SpO2: 91% at room air). Detailed clinical case descriptions can be found in selected publication 2, Table 2 and Supplementary Text 1. All PAD patients entirely lacked SARS-CoV-2 Ab responses (selected publication 2, Table 2). Three CHC were positive, two borderline positive and one negative for SARS-CoV-2 S IgG. Five CHC were positive for S IgA, one was negative. None of the infection naïve HCs had SARS-CoV-2 Ab (selected publication 2, Supplementary Figure 1) (62).

3.2.2 Detection of high frequencies of SARS-CoV-2 reactive T cells in COVID-19 PAD patients

T cell responses were analyzed in 4/5 PAD patients, 6 CHC and 6 naïve HC. Patient #5 deceased 30 d PSO and could not be included. Gating strategy is shown in selected publication 2, Supplementary Figure 2. All PAD patients and CHC generated reactive $CD4^+$ T cells in response to SARS-CoV-2 S peptide pools (N-terminal n=4; C-terminal

n=3). Fewer naïve HC showed reactive $CD4^+$ T cells towards N- (n=4) and C-terminal (n=2) domains. Significantly higher frequencies of SARS-CoV-2 S and NCAP-reactive $CD4^+$ T cells were observed in PAD patients compared to CHC (N-terminal: p=0.005, C-terminal: p=0.02, NCAP: p=0.03) and naïve HC (N-terminal: p=0.014; selected publication 2, Figure 1A). Moreover, stimulation with SARS-CoV-2 peptide pools showed higher frequencies of reactive $CD4^+$ T cells in CHC compared to HC (N-terminal: p=0.02, NCAP: p=0.04, selected publication 2, Figure 1A) (62). Study 1 already included patient #1 showing low levels of pre-existing SARS-CoV-2 and HCoV S-reactive $CD4^+$ T cells seven months before his infection (61). In study 2, this patient had high responses to SARS-CoV-2 S and NCAP during acute infection ranging from 1.8–2.2% for $CD4^+$ T cells (selected publication 2, Supplementary Table 1). $CD8^+$ T cell responses were lower compared to CD4+ T cell responses with no differences between all groups (selected publication 2, Supplementary Figure 3A). SEB positive control elucidated similar frequencies of reactive $CD4^+$ and $CD8^+$ T cells among all groups (selected publication 2, Figure 1 B and Supplementary Figure 3B) (62).

3.2.3 SARS-CoV-2 antigen reactive $CD4^+$ T cells of PAD patients and convalescent healthy controls express a polyfunctional cytokine profile

All PAD patients and CHC showed polyfunctional tp reactive $CD4^+$ T cells after SARS-CoV-2 S peptide stimulation (selected publication 2, Figure 2A). PAD patients show significantly elevated levels of SARS-CoV-2 S-reactive $INF\gamma^+TNF\alpha^+IL\text{-}2^+$ tp $CD4^+$ T cells compared to CHC (N-terminal: p=0.02) and naïve HC (N-terminal p=0.014). CHC expressed significantly higher tp SARS-CoV-2-reactive $CD4^+$ T cells than HC (N-terminal: p=0.03, C-terminal: p=0.04, NCAP: p=0.04; selected publication 2, Figure 2A). Highest cytokine responses among SARS-CoV-2-reactive $CD4^+$ T cells were observed for $TNF\alpha^+IL\text{-}2^+$ dp cells with significantly higher responses in PAD patients than in CHC (N-terminal: p=0.014; NCAP: p=0.03) and HC (N-terminal: p=0.005, selected publication, Figure 2B). Other reactive $CD4^+$ and $CD8^+$ T cell cytokine subsets (selected publication 2, Supplementary Figure 4 and 5) did not differ (62).

3.2.4 Detection of viral load in blood, prolonged viral shedding and treatment with specific antibodies in PAD patients

Viral load in blood was detected in cases #1, #4 and #5 with SARS-CoV-2 RNA in blood at $3.2\text{-}8.8x10^6$ copies/ml (selected publication 2, Table 2). Patients #1 and #4 received

convalescent plasma (CP) therapy with nAb of a PRNT≥1:320, leading to a decrease of viral load after 3 d. Patient #5 was treated with 8 g of mAb Casivirimab/Imdevimab (Regeneron Pharmaceuticals), decreasing viral load after 6 d. 14 d later, RT-PCR in plasma was negative and SARS-CoV-2 specific serum IgG (#1, #4, #5) and IgA (#1, #4) was detectable (selected publication 2, Table 3). RT-PCR from nasopharyngeal swab revealed prolonged viral shedding >40 d in all patients with up to 127 d PSO (patient #4) (selected publication 2, Table 2) (62).

3.2.5 Normal innate immune response to SARS-CoV-2 in PAD patients

As marker for innate immunity, SIGLEC1 was measured on monocytes. Acting as downstream IFN signaling molecule, it represents a surrogate marker of type I IFN signature. Elevated SIGLEC1 (CD169) during SARS-CoV-2 infection indicate physiological type I IFN response. 4/5 patients showed this elevated response, apart from patient #4 who had RNAemia and low SIGLEC1 levels (1,423 molecules/monocyte). No IFN-α or IFN-ω Aab in PAD patients were found (selected publication 2, Table 2, Supplementary Table 2) (62).

3.3 Study 3: Impaired B cell recall memory and reduced antibody avidity but robust T cell response in CVID patients after COVID-19 vaccination

COVID-19 vaccine responses in PAD patients were reported with varying results (57-60) regarding Ab and T cell responses and protective levels remained unknown. Given the impaired humoral immunity, the rate of seroresponding PAD patients was unexpected high after COVID-19 vaccination. Therefore, quality and longevity of this response needed to be evaluated. Study 3 investigated humoral and cellular immune response before any (T1) and after the second (T2) COVID-19 vaccination in CVID patients. 8 HC, 10 seroresponding (R) and 6 non-seroresponding (NR) CVID patients were analyzed regarding their SARS-CoV-2 Ab, Ab avidity, functional MBC response and T cellular immunity resulting from COVID-19 vaccination (Figure 1) (63).

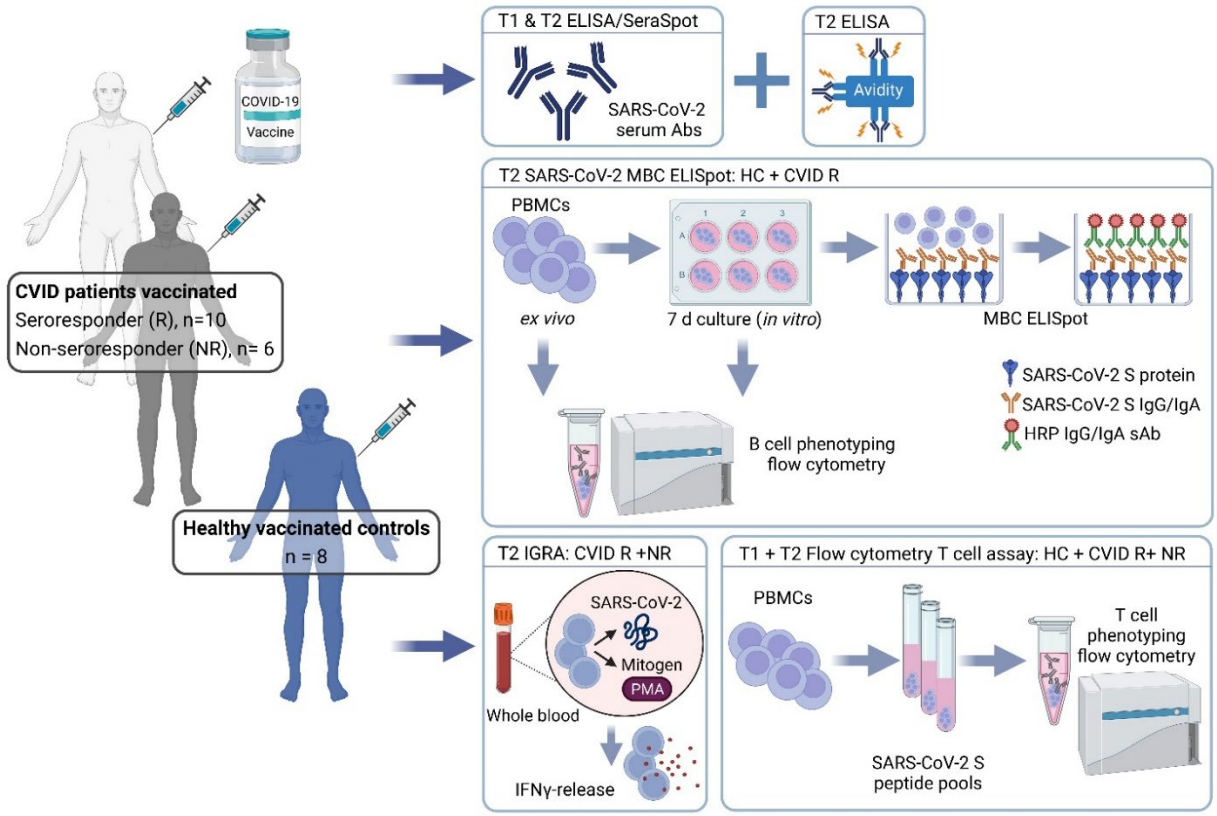

Figure 1: Experimental setup study 3. Humoral and T cellular immunity was investigated at time point 1 before (T1) and 2 (T2) after COVID-19 vaccination in CVID patients and healthy controls (HC). CVID patients with (CVID R: n=10) and without (CVID NR: n=6) seroconversion and HC (n=8) were tested for SARS-CoV-2 serum antibodies (sAb, at T1+T2) and antibody avidity (T2). Functional memory B cell (MBC) recall response was analyzed in HC and CVID R (T2) by ELISpot and flow cytometry B cell phenotyping. T cellular immunity was assessed by Interferon-gamma (IFNγ)-release-assay (IGRA) in CVID R and NR patients (T2) and flow cytometry T cell assay using SARS-CoV-2 spike (S) peptide pools for stimulation (T1+T2) in all groups. Abs = antibodies, HRP = horseradish peroxidase, PBMCs = peripheral mononuclear cell, PMA = Phorbol-12-myristat-13-acetat. Own presentation: Sophie Steiner, created with BioRender.com.

3.3.1 Study cohort characteristics

Cohort characteristics are described in selected publication 3, Supplementary Table S1. SARS-CoV-2 serological parameters were determined by Euroimmun ELISA (S IgG) in all individuals (selected publication 3, Table 1) and confirmed with SeraSpot assay (RBD, S1, S full; nuclear protein (NP)) in CVID R patients (selected publication 3, Supplementary Table S2). No significant differences were observed regarding immunological parameters in CVID patients comprising $CD3^+$, $CD4^+$, $CD8^+$ T cells, $CD19^+$ B cells, B cell subsets, NK cells and IgG, IgA and IgM titers (selected publication 3, Table 2). Non-infectious and infectious manifestations (selected publication 3, Table 3), age (median age 57) and gender were similar in CVID R and NR (63).

3.3.2 Impaired SARS-CoV-2 spike antibody response in CVID patients

An increase of SARS-CoV-2 S IgG after second COVID-19 vaccination was revealed in CVID R ($p=0.002$) and HC ($p=0.008$). Yet, S IgGs were lower in CVID R compared to HC

(p=0.002; selected publication 3, Figure 1A). All individuals were negative for NP (selected publication 3, Supplementary Table S2) as marker for infection naivety (63).

3.3.3 SARS-CoV-2 spike antibody avidity is significantly diminished in seroresponding CVID patients

Despite the presence of SARS-CoV-2 S IgG in CVID R, Ab avidity was significantly reduced compared to HC ($p<0.001$; selected publication 3, Figure 1B). A correlation of S IgG with Ab avidity was found in HC ($p=0.01$; $r=0.833$), but not CVID R patients (selected publication 3, Figure 1C-D) (63).

3.3.4 Formation of B cell memory is impaired in CVID patients despite the presence of circulating antibodies

Due to the presence of SARS-CoV-2 S IgG in CVID R patients, the question arose whether a B cell memory response is formed. SARS-CoV-2 S IgG ELISpot in combination with FACS B cell phenotyping was performed to test for a functional MBC response after second COVID-19 vaccination. Because of alterations in B cell subsets of CVID patients, SARS-CoV-2 S IgG ASCs were calculated per factor 10,000 of plasmablasts (PB) and MBCs used on d 0 of *in vitro* stimulation, to reach comparability between CVID R and HC (63).

3.3.4.1 Memory B cell and plasmablast phenotype in seroresponding CVID patients and HC

Flow cytometry revealed lower levels of class-switched (CS) MBC and CS PB in CVID R compared to HC *ex vivo* (CS MBC: $p<0.0001$; CS PB: $p<0.0001$) and after *in vitro* stimulation (CS MBC: $p=0.003$; CS PB: $p<0.0001$; selected publication 3, Figure 2 A-B). A successful differentiation of MBC into ASCs was shown by a simultaneous decrease of CS MBC and increase of CS PB after *in vitro* stimulation in HC. CVID R MBCs also differentiated into CS PB *in vitro*, although frequencies were much lower compared to HC ($p\leq0.0001$, selected publication 3, Figure 2 B) (63).

3.3.4.2 Deficient memory B cell recall response in seroresponding CVID patients after COVID-19 vaccination

SARS-CoV-2 S IgG ELISpot was performed in 10 CVID R and 8 HC. Results revealed a minor MBC recall response in 3 CVID R patients (30%), while all HC generated SARS-CoV-2 S IgG ASCs after *in vitro* differentiation. After normalizing SARS-CoV-2 S IgG ASC

per 10,000 CS MBC, the count of specific ASC was substantially reduced in CVID R compared to HC (p=0.0007, selected publication 3, Figure 2D). Likewise, lower SARS-CoV-2 S IgG ASC were revealed per 10,000 CS PB for CVID R compared to HC (p=0.001, selected publication 3, Figure 2F). Whole IgG positive control elucidated a response in 7/10 CVID R patients with normalized spots per 10,000 CS MBC and CS PB (selected publication 3, Figure 2E, G) comparable to HC, supporting specificity of the SARS-CoV-2 S IgG ELISpot assay (63).

3.3.5 SARS-CoV-2 T cellular immune response is robustly induced in CVID patients after COVID-19 vaccination

T cell responses before and after COVID-19 vaccination were examined (gating strategy: selected publication 3, Supplementary Figure S2). An additional patient group was introduced comprising CVID patients without seroconversion after two COVID-19 vaccinations (non-seroresponder = CVID NR). T cell reactivity was investigated using IGRA concurrent with a comprehensive flow cytometry T cell assay (63).

3.3.5.1 IFNγ release by SARS-CoV-2-reactive T cells

Quantitative IFNγ release showed a SARS-CoV-2 S response in 6 CVID R and 3 CVID NR. Each group contained one borderline positive CVID patient in response to SARS-CoV-2 S. 3 CVID R and 2 CVID NR were negative. Phorbol-12-myristat-13-acetat (PMA) stimulated positive control samples were positive in 9/10 CVID R and all NR. The one CVID R patient with a negative value for PMA was however positive for SARS-CoV-2 S IFNγ response (selected publication 3, Table 4) (63).

3.3.5.2 SARS-CoV-2 spike reactive polyfunctional CD4+ T cell responses

SARS-CoV-2 S-reactive $CD4^+$ T cells were defined as described in methods section 2.4.2. Stimulation with SARS-CoV-2 S peptide pools elucidated reactive $CD4^+$ T cells in all HC (N-term p=0.016; C-term p=0.008) and CVID R (N-term p=0.004) after vaccination. 5/6 CVID NR showed similar SARS-CoV-2 S-reactive $CD4^+$ T cells compared to the other two groups (selected publication 3, Figure 3A). Positive control SEB showed similar frequencies of reactive $CD4^+$ T cells among all groups pre and post vaccination. Slightly enhanced frequencies of SEB-reactive $CD4^+$ T cells ruled out generally impaired T cell responses in CVID NR (p=0.02, selected publication 3, Figure 3B) (63).

Polyfunctional tp ($IFN\gamma^+TNF\alpha^+IL\text{-}2^+$) reactive $CD4^+$ T cells increased after vaccination within HC (N-term: p=0.008; C-term: p=0.008) and CVID R (N-term: p=0.002; C-term: p=0.04). The 5 CVID NR patients with SARS-CoV-2-reactive $CD4^+$ T cells after vaccination were also characterized by tp cytokine polyfunctionality. Tp cytokine responses were comparable between all groups (selected publication 3, Figure 3C) (63).

3.3.5.3 COVID-19 vaccination induces spike-reactive circulating T_{FH} cells

Peripheral T_{FH} (pT_{FH}) cells were assessed, because of their essential role in providing help for germinal center (GC) B cells to enable specific Ab production upon vaccination. pT_{FH} were defined using the B cell attractant marker C-X-C chemokine receptor type 5 (CXCR5). Greater proportions of $CD4^+CD45RA^-CXCR5^+$ pT_{FH} cells were found in all CVID compared to HC (selected publication 3, Figure 3D). SARS-CoV-2 S-reactive pT_{FH} ($CD4^+CD154^+CD137^+CXCR5^+$) were generated in all groups (selected publication 3, Figure 3E) and significantly increased after stimulation in HC (N-term: p=0.008; C-term: p=0.008) and CVID R (N-terminal: p=0.02). In CVID NR pT_{FH} were also induced but not to a significant extent. Stimulation with SARS-CoV-2 S C-terminal peptide pool resulted in higher S-reactive pT_{FH} post vaccination in HC (p=0.008) and CVID R (p=0.02) compared to CVID NR (selected publication 3, Figure 3E). There was no correlation of S-reactive pT_{FH} with Ab, Ab avidity or reactive polyfunctional tp $CD4^+$ T cells (selected publication 3, Supplementary Figure S3) (63).

4 DISCUSSION

4.1 Study 1: HCoV reactive and SARS-CoV-2 cross-reactive T cells are present in CVID patients prior to COVID-19

In Study 1 (cross)-reactive T cell responses to human endemic and SARS-CoV-2 coronaviruses were examined in CVID patients compared to unexposed and convalescent HC. The results showed HCoV-reactive and SARS-COV-2 cross-reactive T cells in a subset of COVID-19 naïve CVID patients. Nonetheless, fewer SARS-CoV-2 and HCoV-reactive $CD4^+$ and $CD8^+$ T cells were observed in patients, but frequencies in individuals with a detectable T cell response were comparable between all groups. CVID patients exhibited less polyfunctionality in reactive T cells compared to unexposed and convalescent HC suggesting an overall weaker T cell memory response. Additionally, SARS-CoV-2 NCAP-reactive $CD4^+$ T cells were missing in CVID patients (61). Our findings align with prior studies showing that T cell mediated immunity might be affected as alterations in T cell phenotypes, activation status or apoptosis have been described (70). In healthy controls, data during the early phase of the pandemic revealed pre-existing cross-reactive T cells in up to 90% not priorly exposed to SARS-COV-2 (25, 27, 32, 33, 71). We could confirm this observation with 75% of unexposed HC having SARS-CoV-2-reactive T cells in our study. Notably, SARS-CoV-2-reactive T cell level correlated with HCoV reactive T cells in CVID patients and unexposed HC, but not in post COVID-19 HC, indicating cross-reactivity (61).

Our finding of comparable SARS-CoV-2 and HCoV-reactive T cell frequencies in a subset of CVID patients versus naïve and post COVID-19 HC is consistent with previous findings of intact viral T cell responses in CVID patients towards influenza vaccination (50, 51) or Epstein-Barr virus (EBV) and cytomegalovirus infection (72, 73). Generally, CVID patients are under IgRT, which effectively prevents infections (74). During study 1, all CVID patients were under ongoing IgRT. However, SARS-CoV-2 cross-reactive Ab were not detectable in available IgRT products at that moment, despite the fact that approximately 90% of the world population are seropositive for HCoVs (75, 76). In contrast, identified preexisting SARS-CoV-2 cross-reactive T cells likely origin from overlapping HLA viral T cell epitopes with seasonal circulating HCoVs (31-33). A possible explanation why less CVID patients exhibit HCoV and SARS-CoV-2-reactive T cells might be that many patients avoid contact to infected individuals and miss exposure to circulating HCoVs. An

alternative explanation is impaired T cell immunity in CVID (70). Initially, it was unclear whether preexisting SARS-CoV-2 cross-reactive T cell immunity translates into beneficial or detrimental effects. Both possibilities were demonstrated for viruses like EBV, where cross-reactive T cells from influenza infection offered protection (77), but also induced serious symptoms of infectious mononucleosis (78). Advantageous effects were reported in studies focusing on SARS-CoV-1 (79-81) and MERS (82). Recent studies now provide evidence for partial protection from COVID-19 and improved vaccine responses after mRNA and viral vector-based vaccines (31, 53, 83-85). Preexisting cross-reactive T cells exhibit high T cell receptor avidity during heterologous secondary infection (31), able to attenuate virulence by impeding invasive infection or leading to asymptomatic infection with decreased viral transmission (83, 84). Regarding vaccination, cross-reactive T cells augment the effectiveness of COVID-19 vaccines priming the immune system for a more targeted and stronger response (31). Moreover, studies suggest a contribution to *de novo* immune responses by increased T_{FH} responses and magnitude of B cell help (31, 53). Nevertheless, we were not able draw conclusions regarding the role of SARS-CoV-2-cross-reactive T cells in CVID patients due to small sample size and lack of prospective study data.

Another relevant finding was the polyfunctionality ($IFN\gamma^+TNF\alpha^+IL\text{-}2^+$) of SARS-CoV-2 and HCoV-reactive T cells in convalescent and unexposed HC, indicating a memory-like phenotype (61), hence presenting a component of protective immunity (86). In our analyses of CVID patients, a triple cytokine producing phenotype in S-reactive SARS-CoV-2 and HCoV T cells was low or absent and completely missing for SARS-CoV-2 NCAP prior to COVID-19 (61). Previous or less frequent HCoV infections may have contributed to this observation. The finding of similar frequencies of $TNF\alpha^+$ sp and $TNF\alpha^+IL\text{-}2^+$ dp S-reactive T cells in CVID patients and unexposed HC suggests virus contact made a longer time ago, since this type of cytokine producing T cells are associated with long-lasting immunological memory (87). Protective potential of SARS-CoV-2 cross-reactive T cells in PAD patients remains to be further characterized. Clinical and immunological data in the coming years will reveal the value of T cell immunity in PAD patients without specific nAb. This will give further insights on the role of T cell mediated SARS-CoV-2 immunity on overall COVID-19-related morbidity and mortality as well as on the contribution of VOC-related factors.

4.2 Study 2: Intact T cellular and innate immune response in PAD patients without specific antibodies could not prevent severe and fatal COVID-19

A comprehensive immunological analysis of 5 severe COVID-19 PAD patients is presented in study 2. At the time of analyses, all participants had not received SARS-CoV-2 vaccination. Because of COVID-19 pneumonia, all patients suffered from respiratory insufficiency and two had a fatal outcome. Each patient failed to mount SARS-CoV-2 Ab responses. Detailed assessment of SARS-CoV-2 T cellular immune responses towards S and NCAP peptide pools was performed in 4/5 severe COVID-19 PAD patients compared to 6 unexposed HC and 6 convalescent HC (CHC) with mild disease (62). Back then, T cell immunity in severe COVID-19 cases among PAD was unknown (15, 36, 88). All PAD patients, despite undetectable SARS-CoV-2 Ab, mounted a robust polyfunctional $CD4^+$ T cellular response, including NCAP-reactive T cells, indicating a memory-like phenotype. Those responses were higher in PAD patients compared to CHC (62).

Since impaired type I IFN response and presence of type I IFN Aab were associated with increased fatal COVID-19 outcome (17), we examined innate immunity via SIGLEC1 expression on monocytes as surrogate marker for type I IFN response. SIGLEC1 monocyte surface expression increases quickly in response to activation of type I IFN pathway. Consequently, the expression correlates with type I IFN levels (89). In patient #1 and #4, SIGLEC1 on monocytes showed low levels. Therefore, presence of anti-type I IFN-α/-ω Aab were determined in those patients, but were undetectable. Despite intact T cellular and innate immunity, prolonged viral shedding was observed in 5/5 PAD patients with viremia in 3/5 (62).

In line with other studies on SARS-CoV-2 T cellular immunity in healthy COVID-19 convalescents (28, 34, 71), polyfunctional $CD4^+$ T cells were observed in our SARS-CoV-2 seronegative PAD patients. In contrast to study 1 where polyfunctional T cells were low or absent (61), we showed that COVID-19 PAD patients are able to attain polyfunctional SARS-CoV-2-reactive T cells at high levels including NCAP responses (62). In immunocompetent individuals a central memory-like phenotype ($CD45RA^-CCR7^+$) along with a polyfunctional profile >180 d PSO has been shown for SARS-CoV-2-reactive $CD4^+$ T cells arguing for longevity of acquired responses (28). Elevated SARS-CoV-2 T cell responses in PAD patients can be likely attributed to increased disease severity, whereas CHC had an overall mild disease course. Higher $CD4^+$ T cell activation was previously associated with worse clinical outcome (90) possibly reflecting more severe disease on a

cellular level in our patients. Moreover, elevated polyfunctional cytokine-producing $CD4^+$ T cell responses, characterized by a T_{H1} phenotype (IFNγ+TNFα+IL-2+), were observed in severe COVID-19 immunocompetent individuals (91). This aligns with our findings of higher cytokine responses after SARS-CoV-2 infection in PAD patients with a more severe disease compared to mild COVID-19 CHC (62).

The role of cross-reactive T cells in PAD remains unclear. In study 2, we could observe the clinical outcome in a SARS-CoV-2 infected CVID patient with low detectable cross-reactive T cells before infection as seen in study 1 (61). Low preexisting SARS-CoV-2-reactive T cells, in the absence of SARS-CoV-2 humoral immunity, did not avoid severe and consequently fatal disease (62). Thus, a protective function cannot be concluded.

Innate immunity presents a first line of antiviral defense mechanisms, where type I IFN signaling is important. Neutralizing Aab against type I IFNs usually result in hampered viral clearance and are thought to mitigate the capacity to control viral replication, thereby influencing disease severity (17, 92, 93). Recent studies identified anti-cytokine Aab in ~5-10% of patients with life-threatening COVID-19 (17, 92, 94). Regarding IEI patients, type I IFN Aab were described for autoimmune polyendocrine syndrome type 1 (95) and Good's syndrome (96, 97). Nevertheless, a correlation of the high mortality rate in Good's Syndrome with type I IFN Aab was never established. Despite RNAemia along with declining SIGLEC1 expression on monocytes in patient #1 and low SIGLEC1 levels in patient #4, no anti-IFN-α or IFN-ω Aab were observed (62). A possible cause of low monocyte SIGLEC1 could be treatment with corticosteroids impairing innate immunity (98). Taken together; our data show normal innate and T cell immunity in our severe COVID-19 PAD patients. Despite that, all PAD patients suffered from prolonged viral shedding with up to 127 d PSO in patient #4 (Good's syndrome) and from RNAemia in 3/5 cases (62). In severe COVID-19, RNAemia has been observed in the general population, whereas persistent viral shedding was primarily seen in the elderly (99, 100). Both conditions were associated with increased severity and fatal outcome (101-104). Persistent viral shedding increases the already high burden on PAD patients resulting in isolation and fear of recurrent infection. Mucosal viral persistence was moreover associated with intra-host evolution over time reflecting emergence of highly variable, possibly more infectious SARS-CoV-2 variants (105-107). Whether an absent humoral immunity results in increased risk for RNAemia is unknown. However, levels of SARS-CoV-2 RBD-specific Ab responses were shown to correlate with declining RNAemia (108). For XLA patients, with absence of B cells in peripheral blood and complete

agammaglobulinemia, RNAemia was not evaluated and symptoms ranged from asymptomatic to severe (15, 88, 109-111). In our patients with RNAemia, viral clearance and clinical improvement was achieved by CP or mAb treatment (62). In the 7th update on COVID-19 therapeutic recommendations from the WHO, the CDC advises against CP use due to uncertain benefit and potential harms of blood product transfusion-related adverse effects (112). Moreover, mAb are higher concentrated compared to CP and outperform Ab levels acquired after COVID-19 vaccination. Unfortunately, mAb treatment does not trigger immunological memory formation or other immune response pathways, thus it is probably a suitable supplement to vaccination in prophylactic use (113). However, administration of prophylactic measures do not guarantee protection from severe disease in PAD. In summary, extended viral shedding despite normal T cell and innate immunity stresses the significant role of the Ab-mediated immune response in viral clearance.

4.3 Study 3: SARS-CoV-2 immunized CVID patients show impaired B cell recall memory and antibody avidity, but exhibit strong T cell responses

In study 3, a comprehensive analysis of the humoral and cellular immune response to SARS-CoV-2 immunization in CVID patients was performed including Ab avidity, functional MBC recall response and reactive polyfunctional T cell and T_{FH} responses. All CVID patients presented with impaired Ab response to immunization with conjugated pneumococcal vaccine. Despite this, a subset of CVID patients mounted an IgG response upon COVID-19 vaccination. However, Ab levels and avidity were significantly lower in CVID seroresponding patients (CVID R) compared to HC. 7/10 CVID R patients failed to build a functional SARS-CoV-2 MBC response. The 3 patients showing a response had very few specific SARS-CoV-2 S CS MBC and PB derived IgG in ELISpot. Polyfunctional $CD4^+$ T cell responses were intact in the majority of CVID R and non-seroresponding (NR) patients and SARS-CoV-2-reactive T_{FH} were higher in CVID R compared to HC (63). Regarding COVID-19 vaccination there is evidence in immunocompetent individuals for robust humoral and T cellular immunity along with the formation of an immunological memory for at least one year (114). The extent of adaptive immune response and protection from infection or severe disease after SARS-CoV-2 immunization was limited. Immunogenicity in CVID patients was predominantly described for mRNA COVID-19 vaccines (57, 59, 60, 115, 116) and Ab responses differ substantially with a responder

range from 20-93% after two doses (60, 117, 118). This probably occurs due to the complex heterogeneity of immunological impairments, comorbidities and genetic variation amongst CVID patients or different testing methodology. Our observation of a qualitatively different humoral response in CVID R affirm latest articles, where reduced avidity of SARS-CoV-2 Ab after vaccination were observed (119, 120). Sauerwein *et al.* found that even after a third mRNA vaccine booster Ab level and avidity were still lower in CVID compared to HC (120). SARS-CoV-2 Ab responses in PAD were associated with total B cell numbers and frequencies of CS MBC. Patients with low B cells and reduced CS MBCs were less likely to build vaccine-induced Ab (59).

In contrast to other studies on SARS-CoV-2 MBCs in PAD after COVID-19 immunization, only phenotypically assessing S specific MBCs via flow cytometry (46, 60, 121), we evaluated the functional capacity of MBCs using a SARS-CoV-2 S ELISpot assay we established. After *in vitro* stimulation of PBMCs and differentiation of MBCs into ASCs we analyzed the functional ability of specific B cell memory and secretion of SARS-CoV-2 S Ab, revealing a failure of most seroresponding CVID R to develop a robust humoral memory response (63). This is in line with other studies on vaccine-induced B cell responses, where patients developed alternative pathways of B cell immunity by formation of atypical memory B cells (ATM), defined as $CD19^{+}CD24^{-}CD27^{-}CD38^{-}$, with reduced binding capacity to S protein, limited Ab secretion upon stimulation and generation of low affinity Ab (46, 59, 60). ATMs are thought to originate mainly at extra-follicular sites (EF) or to a lesser extent as product of failed GC reactions (60). Contrarily, convalescent CVID patients B cell response was shown to be similar compared to B cell immunity in immunized healthy individuals, characterized by S specific MBCs, undergoing affinity maturation and class-switching in the GC (46). Different from mRNA vaccines, infection is considered to initiate a more stable GC response (122). Several extrinsic components play a role in GC reactions like T_{FH} cells (123). CXCR5+ T_{FH} cells are key players in B cell proliferation, formation of GC and are associated with diversification, affinity maturation and class-switching of Ab (124). We observed a T_{FH} response after stimulation with SARS-CoV-2 S peptide pools in CVID R patients similar to HC at baseline and following immunization. In CVID NR we saw no significant increase of T_{FH} response after vaccination and significantly lower levels after stimulation with the C-terminal peptide pool (63). This is underpinned by pre-pandemic studies showing a link between T_{FH} frequencies and Ab level induced by influenza vaccination (125), conjugated pneumococcal polysaccharide (126) and hepatitis B (127) vaccines. A recent COVID-19

vaccine study in CVID patients observed higher T_{FH} effector $CD4^+$ T cell frequencies compared to immunocompetent individuals before and after vaccination supporting our observation (128). However, Sauerwein *et al.* discovered impaired activation of T_{FH} vaccine responses after restimulation with S peptides (115). Higher T_{FH} frequencies in CVID patients were observed before, particularly in patients with autoimmunity and granulomatous disease, showing an apoptosis, senescence and exhaustion phenotype (129, 130). Regarding these heterogeneous observations for T_{FH}, phenotypic results alone are insufficient to draw conclusions on their functional relevance in immunized CVID patients. Moreover, central memory $CD4^+$ T cells in the periphery express 20-25% of CXCR5 (131). Thus, we cannot rule out that high T_{FH} cells before immunization depict an unspecific activation condition.

Concerning overall vaccine-generated SARS-CoV-2-reactive $CD4^+$ T cells we identified a robust, polyfunctional response (63), similar to the results after infection from study 2 (62). Noticeably, various studies show high prevalence of SARS-CoV-2 T cellular immunity after vaccination in CVID, however in many, T cellular responses are impaired with lower levels or less cytokine secretion (59, 60, 116, 117, 119, 132-135). Impaired responses are predominantly observed in studies relying on assays detecting only IFNγ secretion (60, 117, 134, 135). Hence, testing of T cell responses might benefit from more differentiated flow cytometry assays. Intact T cellular immunity in CVID has been also described for influenza vaccination (50, 51).

Longevity of COVID-19 vaccine-induced immunity is under undergoing investigation. Long-lasting immunological memory of SARS-CoV-2 S specific humoral and cellular responses in CVID was shown up to 15 months (118, 136). However, median S IgG was lower in CVID, but T cell responses remained comparable to immunocompetent HC (118). PAD patients seem to benefit from boosting with a 3^{rd} or 4^{th} vaccination resulting in augmented immunity by increased frequency of seroconverting patients and higher Ab level (113, 133, 136). In summary, study 3 shows a qualitatively different Ab response in seroresponding CVID patients compared to HC expressing reduced avidity and impaired MBC recall response (63). This could hint towards inadequate Ab maturation upon antigen encounter after vaccination. Further studies need to explore MBCs (ATMs) and Ab-binding kinetics in more detail to evaluate if patients have a clinically effective humoral defense response after vaccination.

4.4 Limitations

One critical aspect of all studies is the low number of patients used for the experiments. Another limitation is the higher median age of CVID patients compared to controls, although we did not observe age influencing T cell responses in our cohorts. Due to the variable nature of COVID-19 symptoms and the fact that approximately 10% of HC do not seroconvert after SARS-CoV-2 infection, it is possible that not all unexposed/unvaccinated individuals without history of SARS-CoV-2 infection were infection naïve. Nevertheless, given low infection and high testing rates in Germany during that time the likelihood of overlooked infection is low. Another critical aspect is the heterogeneity of T cell responses among PAD patients reported in the literature, likely resulting from different methodology, sensitivity of assays, time point of analyses and patient heterogeneity regarding immunological and genetic abnormalities. Protective thresholds for Ab and T cell responses still remain unknown. In study 3, different vaccination regimens were administered and time point of sampling after the second COVID-19 vaccination was slightly heterogeneous between patient and control groups. Moreover, results from study 3 are limited to two vaccine doses leaving it undisclosed if a 3rd or 4th vaccination could generate a superior MBC response.

4.5 Conclusion

This doctoral thesis explored humoral and T cellular immune responses to SARS-CoV-2 in PAD patients during the early phase of the COVID-19 pandemic and has implications for patient management to prevent severe disease and fatal outcomes. It provides evidence for preexisting SARS-CoV-2 T cellular immunity in a subset of PAD patients, characterized by lower polyfunctionality (Study 1). Despite the absence of a humoral immune response, PAD patients elicited a robust memory-like polyfunctional T cellular immunity post-infection, similar to immunocompetent individuals (Study 2), which was also observed after vaccination (Study 3). This implicates an overall intact viral T cellular reactivity towards SARS-CoV-2. However, most COVID-19 PAD patients experienced prolonged viral shedding after infection suggesting that T cell response alone is insufficient for viral clearance. This implies a pivotal role of humoral immunity to confer SARS-CoV-2 infection and emphasizes the need for COVID-19 prevention and treatment strategies. Study 3 revealed a high subset of PAD patients able to seroconvert after SARS-CoV-2 vaccination despite the previous inability to generate specific

pneumococcal conjugate vaccine Ab. However, functional MBC response was impaired and Ab at lower levels and poorer quality, as unveiled by reduced avidity. Additionally, vaccinated PAD patients had a SARS-CoV-2 specific T_{FH} response, which was diminished in patients without seroconversion, emphasizing a substantial role for T_{FH} cells throughout vaccine-induced immunity. This challenges the simplified categorization of PAD patients into non-seroresponder and seroresponder, stressing the significance of a comprehensive elaboration of humoral immunity during SARS-CoV-2 infection and vaccination. Taken together, this thesis gives a rationale for SARS-CoV-2 vaccination and monitoring vaccine-induced cellular and humoral responses in PAD patients. Moreover, those findings could influence clinical decisions concerning prophylactic measures like mAb treatment.

4.6 Outlook

Subsequent studies should prioritize assessment of larger patient cohorts, as small sample sizes limit data interpretation. Understanding immunological mechanisms shaping humoral immunity and B cell memory formation remains challenging. It would be valuable to functionally investigate vaccine-induced ATM responses in PAD and to qualitatively explore MBC response e.g. by the use of our SARS-CoV-2 MBC ELISpot assay and functional Ab analyses, such as PRNT. Prospective studies need to focus on the identification of possible underlying B cell differentiation defects associated with the quality of MBC responses. Moreover, comparing different and prolonged vaccination regimens can help to decipher which vaccines are most effective in immunizing patients with the purpose of triggering GC reactions and seroconversion. Recent findings suggest, that more than two doses increase humoral immunity in PAD (113, 133, 136). Thus, repeated vaccination might translate into a functional B cell memory, which we could not observe after two doses. Clarification is necessary if patients would benefit from alternative vaccinations directed against structural proteins like NCAP. Moreover, prophylactic management may benefit from identification of predictive factors influencing weak or strong immune responses to SARS-CoV-2 infection/vaccination.

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6 STATUTORY DECLARATION

I, Sophie Steiner, affirm in lieu of oath by my handwritten signature that I have written the submitted dissertation with the topic: **“Cellular and humoral immunity towards SARS-CoV-2 infection and vaccination in patients with primary antibody deficiency / Zelluläre und humorale Immunität in Antwort auf eine SARS-CoV-2 Infektion und Impfung bei Patienten mit primären Antikörpermangelerkrankungen”** independently and without the undisclosed help of third parties and have not used any sources or aids other than those indicated.

All parts of this thesis that are in letter or in spirit based on publications or lectures of other authors are specified as such in accordance with the citation guidelines. I am responsible for the sections on methodology (in particular practical work, laboratory regulations, statistical processing) and results (in particular figures, charts and tables).

I further affirm that I have correctly marked all the data, data analyses, and conclusions generated in collaboration with other persons and have correctly identified my own contribution as well as the contributions of other persons (see declaration of contribution). I have correctly identified texts or parts of texts that were generated in collaboration with other persons.

My contribution to any publications to this dissertation correspond to those stated in the joint declaration below together with the supervisor. The ICMJE (International Committee of Medical Journal Editors; www.icmje.og) guidelines on authorship have been followed for all publications within the scope of this dissertation. I further declare that I shall comply with the regulations of Charité - Universitätsmedizin Berlin to ensure good scientific practice.

Furthermore, I affirm that I have not submitted this dissertation in identical or similar form to another faculty.

I am aware of the significance of this statutory declaration and the consequences of a false statutory declaration (§§156, 161 of the German Criminal Code)."

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Date	Sophie Steiner

7 DECLARATION OF CONTRIBUTION TO THE PUBLICATIONS

Sophie Steiner contributed the following to the below listed publications:

Publication 1: **Steiner S**, Sotzny F, Bauer S, Na I-K, Schmück-Henneresse M, Corman VM, Schwarz T, Drosten C, Désirée JW, Behrends U, Volk HD, Scheibenbogen C, Hanitsch LG. HCoV- and SARS-CoV-2 Cross-Reactive T Cells in CVID Patients. Frontiers in Immunology. 2020.

Sophie Steiner performed preparation of serum and PBMCs from patient and control blood samples, including cryopreservation, storage and thawing of PBMCs, established the T cell panel setup for flow cytometry and performed T cell assays (cell culture and stimulation of PBMCs, intra- and extracellular antibody staining and acquisition on the flow cytometer). The above mentioned laboratory work was supported during experimental implementation by the technician Sandra Bauer. SARS-CoV-2 Spike IgG ELISA was performed by Sandra Bauer and Sophie Steiner. Neutralizing SARS-CoV-2 IgG (Table 1B, created by Sophie Steiner) was analyzed by Dr. Victor Corman, Dr. Tatjana Schwarz and Prof. Christian Drosten. Sophie Steiner further performed analysis of flow cytometry data and SARS-CoV-2 S IgG ELISA results, did statistical analyses of this data and is responsible for visual data presentation of all figures, all tables as well as all supplementary material and interpreted data. Supplementary Table 2 is based on laboratory routine test results from Labor Berlin GmbH. Dr. Michael Schmück-Henneresse and Dr. Désirée Jaqueline Wendering advised the analysis of reactive SARS-CoV-2 T cells. Dr. Leif G. Hanitsch made CVID patient and convalescent healthy control samples available. Sophie Steiner recruited healthy infection naïve donors. Data was discussed with all co-authors. Prof. Carmen Scheibenbogen, Dr. Leif G. Hanitsch, Prof. Il-Kang Na and Uta Behrends were responsible for the conception of the Study. The manuscript draft was written by Prof. Carmen Scheibenbogen and Dr. Leif G. Hanitsch, further edited by Sophie Steiner and was proof read by all co-authors. Sophie Steiner submitted the manuscript. Sophie Steiner further optimized during the review process and replied to reviewers responses in cooperation with Prof. Carmen Scheibenbogen and Dr. Leif G. Hanitsch based on reviewers comments.

Publication 2: Steiner S, Schwarz T, Corman VM, Gebert L, Kleinschmidt MC, Wald A, Gläser S, Kruse JM, Zickler D, Peric A, Meisel C, Meyer T, Staudacher OL, Wittke K, Kedor C, Bauer S, Al Besher N, Kalus U, Pruß A, Drosten C, Volk HD, Scheibenbogen C, Hanitsch LG. SARS-CoV-2 T Cell Response in Severe and Fatal COVID-19 in Primary Antibody Deficiency Patients Without Specific Humoral Immunity. Frontiers in Immunology. 2022

Sophie Steiner performed preparation of serum and PBMCs from patient and control blood samples, including cryopreservation, storage and thawing of PBMCs and further established, planned and performed all experiments on flow cytometry T cell assays (cell culture and stimulation of PBMCs, intra-and extracellular antibody staining and acquisition on the flow cytometer), analyzed results, is responsible for visual presentation of all figures, all tables, supplementary material and interpretation of data. Sandra Bauer and Laura Gebert supported Sophie Steiner during experimental implementation. Prof. Dr. Victor M. Corman, Dr. Tatjana Schwarz and Prof. Christian Drosten performed ELISA, SeraSpot and plaque reduction neutralization test for SARS-CoV-2 serology as well as SARS-CoV-2 RT-PCR in plasma samples of patients (Table 2, Table 3, created by Sophie Steiner). Supplementary Figure 1 on SARS-CoV-2 antibody levels was created by Sophie Steiner based on the aforementioned data. Prof. Christian Meisel, Dr. Olga Staudacher and Dr. Tim Meyer from Labor Berlin GmbH analyzed SIGLEC1 levels on monocytes and type I IFN autoantibodies in serum (Table 2 and Supplementary Table 2). Dr. Leif G. Hanitsch, Dr. Malte C. Kleinschmidt, Dr. Alexandra Wald, Prof. Sven Gläser, Dr. Jan M. Kruse, Dr. Daniel Zickler, Dr. Kirsten Wittke and Dr. Alexander Peric made patient samples available. Dr. Leif G. Hanitsch recruited convalescent healthy donors and Sophie Steiner recruited healthy infection naïve donors. Nabeel al Besher, Dr. Uwe Kalus and Prof. Axel Pruß made convalescent plasma available. Dr. Leif G. Hanitsch and Prof. Carmen Scheibenbogen were responsible for conception and design of the study. Sophie Steiner, Prof. Carmen Scheibenbogen and Dr. Leif G. Hanitsch interpreted the data and discussed it with all co-authors. The manuscript draft was written by Sophie Steiner and Dr. Leif G. Hanitsch and was proof read by all co-authors. Sophie Steiner submitted the manuscript. Sophie Steiner also optimized during the review process and replied to reviewers responses in cooperation with Dr. Leif G. Hanitsch and Prof. Carmen Scheibenbogen based on reviewers comments.

Publication 3: Steiner S, Schwarz T, Corman VM, Jeworowski LM, Bauer S, Jeworowski LM, Bauer S, Drosten C, Scheibenbogen C, Hanitsch LG. Impaired B Cell Recall Memory and Reduced Antibody Avidity but Robust T Cell Response in CVID Patients After COVID-19 Vaccination. Journal of Clinical Immunology. 2023

Sophie Steiner performed preparation of serum and PBMCs from patient and control blood samples, including cryopreservation, storage and thawing of PBMCs, planned, established and performed all experiments of flow cytometry T cell assays (cell culture, intra-and extracellular antibody staining and acquisition on the flow cytometer), performed stimulation of whole blood samples as well as preparation, freezing and storing of plasma for interferon-gamma release assay, established and performed SARS-CoV-2 memory B cell ELISpot including cell culture and B cell phenotyping, analyzed all results, is responsible for visual presentation of all figures, all tables, supplementary material and interpreted data. Table 2 on immunological parameters is based on laboratory routine test results from Labor Berlin GmbH and was created by Sophie Steiner and Leif G. Hanitsch. Dr. Victor M. Corman, Dr. Tatjana Schwarz, Lara Jeworowksi and Prof. Christian Drosten performed ELISA (Figure 1 A, Table 1, created by Sophie Steiner), SeraSpot (Supplementary Table S2, created by Sophie Steiner) and IgG avidity experiments (Figure 1 B-D and Supplementary Figure S3 A-D, created by Sophie Steiner) for SARS-CoV-2 serology as well as analyses of plasma from interferon-gamma-release-assay with Quan-T-Cell ELISA (Table 4, created by Sophie Steiner). Dr. Leif G. Hanitsch made patient samples available and was responsible for the conception and design of the study. All authors discussed and interpreted the data making substantial intellectual contribution to the work. The manuscript draft was written by Sophie Steiner and Dr. Leif G. Hanitsch and was proof read by all co-authors. Dr. Leif G. Hanitsch submitted the manuscript and Sophie Steiner and Leif G. Hanitsch optimized during the review process and replied based on comments by reviewers.

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Sophie Steiner

8 PRINT COPIES OF THE PUBLICATIONS

8.1 Study 1

frontiers in Immunology

ORIGINAL RESEARCH
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HCoV- and SARS-CoV-2 Cross-Reactive T Cells in CVID Patients

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The inability of patients with CVID to mount specific antibody responses to pathogens has raised concerns on the risk and severity of SARS-CoV-2 infection, but there might be a role for protective T cells in these patients. SARS-CoV-2 reactive T cells have been reported for SARS-CoV-2 unexposed healthy individuals. Until now, there is no data on T cell immunity to SARS-CoV-2 infection in CVID. This study aimed to evaluate reactive T cells to human endemic corona viruses (HCoV) and to study pre-existing SARS-CoV-2 reactive T cells in unexposed CVID patients. We evaluated SARS-CoV-2- and HCoV-229E and –OC43 reactive T cells in response to seven peptide pools, including spike and nucleocapsid (NCAP) proteins, in 11 unexposed CVID, 12 unexposed and 11 post COVID-19 healthy controls (HC). We further characterized reactive T cells by IFNγ, TNFα and IL-2 profiles. SARS-CoV-2 spike-reactive CD4+ T cells were detected in 7 of 11 unexposed CVID patients, albeit with fewer multifunctional (IFNγ/TNFα/IL-2) cells than unexposed HC. CVID patients had no SARS-CoV-2 NCAP reactive CD4+ T cells and less reactive CD8+ cells compared to unexposed HC. We observed a correlation between T cell reactivity against spike of SARS-CoV-2 and HCoVs in unexposed, but not post COVID-19 HC, suggesting cross-reactivity. T cell responses in post COVID-19 HC could be distinguished from unexposed HC by higher frequencies of triple-positive NCAP reactive CD4+ T cells. Taken together, SARS-CoV-2 reactive T cells are detectable in unexposed CVID patients albeit with lower recognition frequencies and polyfunctional potential. Frequencies of triple-functional reactive CD4+ cells might provide a marker to distinguish HCoV cross-reactive from SARS-CoV-2 specific T cell responses. Our data provides evidence, that anti-viral T cell immunity is not relevantly impaired in most CVID patients.

Keywords: common variable immunodeficiency disorder (CVID), coronavirus disease 2019 (COVID-19), T cell response, primary immunodeficiency (PID), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human endemic coronavirus 229E (HCoV-229E), human endemic coronavirus OC-43 (HCoV-OC43)

INTRODUCTION

Clinical presentations of coronavirus disease 2019 (COVID-19) are highly variable, ranging from asymptomatic to severe acute respiratory syndrome (SARS). A number of clinical factors with a more than 2-fold increased risk for mortality have been identified and include advanced age, pre-existing respiratory, cardio- and cerebrovascular diseases, hypertension, diabetes and malignancy (1). Ethnicity has also been described as a risk factor for COVID-19 with increased infection rates and worse clinical outcome in Black, Asian and Minority Ethnic individuals (2). According to European Society for Immunodeficiencies (ESID) criteria, patients with common variable immunodeficiency disorder (CVID) have a relevant IgG and IgA +/- IgM deficiency together with reduced class switched memory B cells and/or an impaired specific antibody response to pathogens or vaccination. Due to the inability to mount specific antibody responses to pathogens, patients with CVID are likely at increased risk for severe COVID-19, however clinical data is still very limited (3, 4).

Standard treatment for CVID is IgG replacement therapy, which is effective in infection prevention (5). Because of the novelty of COVID-19, IgG preparations do not contain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgG yet.

Data on the clinical course of COVID-19 in CVID patients are still very limited. Recently, a fatal outcome was reported and a first report described a moderate to severe course of COVID-19 in 5 CVID patients (4). Authors discussed that CVID patients may be more prone to severe COVID-19 due to preexisting lung inflammatory diseases present in 10% of CVID patients. Data on T cell responses in CVID patients with COVID-19 are currently missing.

Cross-reactivities of T cells against human endemic coronaviruses (HCoV) to SARS-CoV-2 have been proposed and are currently under extensive investigations (6–10). Population studies estimate that approximately 90% express IgG seropositivity to the worldwide circulating endemic HCoV strains, which usually cause milder "common cold" respiratory infections (11). The emerging evidence of pre-existing SARS-CoV-2 reactive T cells shaping the immune response (12), remains to be elucidated in immunodeficient patients. Previous studies provide evidence for normal T cell responses to influenza (vaccine) in CVID patients (13) and to hepatitis B vaccine in X-linked agammaglobulinemia (XLA) patients (14). Here, we aim to characterize the T cell responses to SARS-CoV-2 and two common HCoV strains (229E and OC43) in SARS-CoV-2 unexposed patients with CVID and compare it to T cell responses in unexposed and post-COVID-19 HC.

METHODS

Human Blood Samples

11 patients with confirmed diagnosis of CVID according to ESID criteria were recruited from the outpatient clinic for immunodeficiencies at the Institute for Medical Immunology at the Charité Universitätsmedizin Berlin (**Table 1A**). Recovered healthy controls (HC) with past COVID-19 had been diagnosed by RT-PCR. The clinical course is described in **Table 1B**. HC without a history of COVID-19 were recruited from laboratory staff and had a negative SARS-CoV-2 antibody test. Blood was drawn from patients and HC in June and July 2020. During the time of our study, the weekly incidence rate of SARS-CoV-2 infections in Berlin was at a level of 0.3–2.0/100 000 inhabitants. The study was approved by the Ethics Committee of Charité Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments (EA2/092/20 from June 4th, 2020). All patients and controls gave informed consent.

Quantification of SARS-CoV-2 IgG

Serum IgG against the N-terminal domain of the spike protein including the immunologically relevant receptor binding domain (RBD) of SARS-CoV-2 was determined by ELISA (EUROIMMUN AG). Neutralizing IgG antibodies were determined by plaque reduction similar as described before (15).

Flow Cytometric Analysis of Antigen-Reactive T Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparin blood samples by density gradient centrifugation, frozen at

TABLE 1A | Characteristics of patients and controls.

(A) CVID patients.

ID	age	sex	IgG [g/l](before RT)	IgA[g/l]	IgM[g/l]	CD4[/nl]	CD8[/nl]	CD19[/nl]	NK[/nl]	EUROClass
CVID-1	56	m	0.30	0.06	0.10	0.60	0.90	0.14	0.08	smB-21low
CVID-2	60	m	1.66	0.06	0.15	0.32	0.33	0.19	0.10	smB-21norm
CVID-3	43	m	3.59	0.06	0.18	0.41	0.36	0.72	0.11	smB-21low
CVID-4	29	f	0.00	0.00	2.10	1.02	0.58	0.38	0.11	smB-21low
CVID-5	46	f	3.70	0.59	0.40	0.69	0.34	0.24	0.07	smB-21norm
CVID-6	56	f	3.12	0.06	0.20	0.45	0.36	0.13	0.14	smB-21low
CVID-7	58	m	2.00	0.06	0.05	0.40	0.26	0.12	0.23	smB-21low
CVID-8	51	f	3.60	0.25	0.18	0.43	0.23	0.06	0.02	smB-21norm
CVID-9	46	m	0.33	0.06	0.05	0.90	0.69	0.16	0.26	smB-21norm
CVID-10	74	m	1.30	0.00	0.09	0.50	1.30	0.32	0.26	smB-21low
CVID-11	31	m	0.00	0.00	0.00	1.06	0.60	0.00	0.28	B-
Median	51		1.66	0.06	0.15	0.5	0.36	0.16	0.11	

CVID, Common Variable Immunodeficiency; NK, natural killer cells; smB, switched memory B cells; norm, normal; B-, patients with equal or less than 1% B cells; f, female; m, male.

TABLE 1B | Characteristics of patients and controls.

(B) post COVID-19 HC.

ID	Age	Sex	pos. PCR	Time of analysis after first diagnosis by positive PCR [d]	Duration of symptoms [d]	WHO R&D Blueprint ordinal scale	IgG-ELISA [OR ratio]	IgA-ELISA [OR ratio]	PRNT50
Case-1	36	w	25.03.2020	51	19	2	1.10	0.42	1:20
Case-2	74	m	02.04.2020	60	4	2	1.83	5.03	1:160
Case-3	25	w	22.03.2020	70	5	2	1.80	1.66	1:80
Case-4	45	w	26.03.2020	75	25	2	7.96	5.02	1:80
Case-5	50	w	16.04.2020	48	14	2	2.09	1.56	<1:20
Case-6	28	m	28.03.2020	73	n.a.	2	2.48	2.40	1:20
Case-7	55	w	09.03.2020	85	13	3	6.23	5.08	1:320
Case-8	44	m	26.03.2020	75	8	2	1.31	1.20	<1:20
Case-9	22	m	24.03.2020	78	10	2	1.90	3	1:20
Case-10	43	m	12.03.2020	95	6	1	2.70	2.43	1:80
Case-11	75	m	20.03.2020	19	no symptoms	1	3.43	–	n.a.
Median	44			73	10		2.09	2.42	80

PCR, polymerase chain reaction; f, female; m, male; n.a., not applicable; WHO, World Health Organization; PRNT50, the dilution of serum to reduce the number of plaques, of the plaque reduction neutralization test, by 50% compared to the serum free virus.

–80°C and later transferred to liquid nitrogen. Samples from post COVID-19, unexposed HC and CVID patients were simultaneously analyzed. Thawed PBMCs were either incubated with DMSO (background control) or stimulated with 3µg/ml superantigen Staphylococcal enterotoxin B (SEB) (positive control) or 1 µg/ml of peptide pools SARS-CoV-2 Spike Glycoprotein (two vials with N-term and C-Term, PM-WCPV-S-1), SARS-CoV-2 NCAP (PM-WCPV-NCAP-1), HCoV-229E Spike Glycoprotein (two vials with N-term and C-term, PM-229E-S-1) and HCoV-OC43 Spike Glycoprotein S1 (two vials with N-term and C-Term, JPT Peptide Technologies GmbH, Berlin), respectively, for 16h at 37°C and 5% CO_2. After 2h of stimulation, brefeldin A (BFA) was added as secretion inhibitor. Cells were then stained extracellularly with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific) and lyzed and permeabilized using FoxP3 transcription factor staining buffer set (eBioscience). Afterwards, intracellular staining was performed for CD3 BV650, CD4 PerCp-Cy5.5, CD8 BV510, CD137 PE, CD154 BV421, IL-2 APC, IFNγ BV605, and TNFα AF700 (Biolegend). The stained cells were measured at a CytoflexLX (Beckman Coulter) and analyzed using FlowJo software version 10.6.2 (BD). Reactive CD154+CD137+CD4+ or CD137+CD8+ T cells $\geq$ 0.005% within total CD4+ or CD8+ T cells and with a $\geq$ 1.2-fold response of the background control were considered as positive. This threshold corresponds to the range in which 95% of all negative samples are. Unspecific stimulation was excluded by subtracting the background signal of the DMSO sample from the peptide stimulated samples. Single, double (dp) or triple (tp) cytokine producing T cell subsets were analyzed using Boolean combination gates.

Statistical Analysis

Statistical data analyses were done using GraphPad Prism 6 software. Nonparametric statistical methods were used. Continuous variables were expressed as median and interquartile range (IQR). Univariate comparisons of T cell responses in two independent groups were done using the Mann-Whitney-U test. Distribution of T cell response between the three cohorts was analyzed using a 2 × 2 contingency table. Significance was tested by χ^2-square test. Correlation between the T cell responses toward the different peptides was analyzed by Spearman's rank correlation coefficient and linear regression.

A two-tailed p-value of <0.05 was considered statistically significant. Due to multiple testing p-values are considered descriptive.

RESULTS

Patient Characteristics and IgG Responses to SARS-CoV-2

11 CVID patients, 11 post COVID-19, and 12 unexposed HC participated in this study. The characteristics of CVID patients are shown in **Table 1A**. The median age for CVID was 51 years (range 29–74), for unexposed HC 35 years (range 25–65) and for post COVID-19 HC 44 years (range 22–75). All CVID patients and 11/12 unexposed and 10/11 Post COVID-19 HC are Caucasian, two are Asian. All CVID patients were under continuous IgG replacement therapy for a minimum of 2 years (median 7, range 2–30 years). Post COVID-19 HC had previous mild COVID-19 (WHO) and a median of 73 days (range 48–95) after diagnosis before T cell analysis (**Table 1B**). Patients with CVID and unexposed HC had no history of COVID-19. All post COVID-19 HC had specific IgG against SARS-CoV-2, while all unexposed HC and CVID patients were seronegative (**Supplementary Figure 1**). In addition, 10 Post COVID-19 HC had neutralizing IgG against SARS-CoV-2.

Further, CD3+, CD4+ and CD8+ T cell frequencies of the three groups are shown in **Supplementary Figure 2**. CVID patients have higher frequencies of CD3+ and CD8+ T cells compared to unexposed HC and post COVID-19 HC, which is already described for CVID patients (16, 17). Frequencies of CD4+ T cells were comparable between the three groups.

Groups Analysis of SARS-CoV-2 and HCoV-Reactive T Cells

In order to study the T cell response to SARS-CoV-2 and two common HCoV strains we analyzed the frequency of SARS-CoV-2 spike and NCAP, HCoV-229E and –OC43 spike peptide-reactive CD154+CD137+CD4+ and CD137+CD8+ T cell responses *in vitro*

by flow cytometry. Only T cell responses above the threshold of 20% above background activation were included in this study (**Supplementary Table 1**). Cytokine producing capacity of the reactive T cells was assessed by percentages of virus peptide-reactive IFNγ, TNFα and IL-2-producing T cells. **Figure 1** shows the gating strategy in a representative convalescent patient in response to SARS-CoV-2 C-terminal spike peptide pool who had a mild COVID-19 infection.

CD154+CD137+CD4+ and CD137+CD8+ Activated T Cell Responses to SARS-CoV-2, HCoV-229E and –OC43 and SEB

In 7 of 11 CVID patients, reactive CD4+ T cells against at least one spike peptide pool of SARS-CoV-2 were detectable and in 4 of these 7 also against HCoV-229 and/or –OC43, but none against NCAP (**Figure 2A**). Altogether, there were fewer CD4+ and CD8+ T cells reactive to the 7 spike and NCAP peptide pools in comparison to unexposed HC ($p<0.0005$ for 1, $p<0.005$ for 6, $p<0.05$ for two of 14 peptide responses, **Table 2**). Activated CD4+ T cells reactive against at least one of the spike peptide pools of SARS-CoV-2 were found in 75% of unexposed HC, 81% of post COVID-19 and in 75% and 63% of the HCoVs, respectively (**Figures 2A, B**, **Table 2**, and **Supplementary Tables 1**, **2**). No CVID patient showed a CD4+ T cell response and fewer patients a CD8+ T cell response against SARS-CoV-2 NCAP compared to HC.

In CVID patients and HC with positive T cell responses, peptide-reactive CD4+ and CD8+ T cells were found in all three cohorts in a similar frequency (**Figures 2A, B**). Further, activated CD4+ and CD8+ T cells in response to SEB had comparable frequencies in all individuals in the three cohorts (**Figures 2C, D**).

As the median age of the three groups differs, we analyzed if there is an association between age and SARS-CoV-2 spike peptide response. We observed no significant differences in age and response to peptides in all three cohorts (**Supplementary Figure 3**).

Next, we correlated the frequencies of T cells reactive with corresponding peptide pools from SARS-CoV-2 and HCoV. In CVID patients, no correlation analysis could be performed due to too few individuals with reactive T cells. However, all CVID patients with HCoV-reactive T cells had also SARS-CoV-2-reactive T cells. In unexposed HC we found significant correlations for most CD4+ and CD8+ responses against spike peptide pools of N- and C-terminal from all three coronaviruses, suggesting cross-reactive SARS-CoV-2 T cells (**Tables 3A**, **B**). In contrast, in post COVID-19 HC only a correlation of the CD4+ responses against spike of HCoV -OC43, but not with SARS-CoV-2 was found (**Table 3A**). No correlation of frequencies of spike reactive CD4+ T cells with spike specific IgG was found (data not shown).

CD4+ and CD8+ Cytokine Responses in Activated T Cells

The percentage of cytokine producing T cell responses in CD154+CD137+CD4+ and CD137+CD8+ was analyzed by intracellular staining. Using Boolean combination gating, seven subsets of IFNγ, TNFα and IL-2 single positive, IFNγ/TNFα,

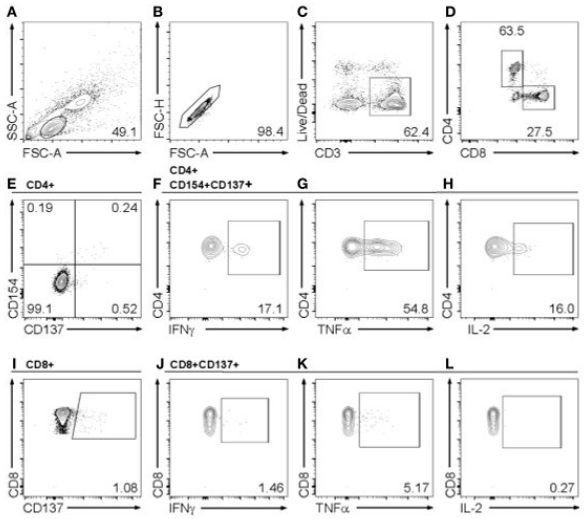

FIGURE 1 | Gating strategy for flow cytometry analysis of activated CD4+ and CD8+ T cells and their cytokine expression profile. Example of a gating strategy in a post coronavirus disease 2019 (COVID-19) healthy control (HC) in response to stimulation with the SARS-CoV-2 C-terminal spike peptide pool. Shown are **(A)** lymphocytes, **(B)** single cells, **(C)** living CD3+ T cells, **(D)** CD4+ and CD8+ T cells, **(E)** activated CD154+CD137+CD4+ T cells, **(F–H)** production of IFNγ **(F)**, TNFα **(G)** and IL-2 **(H)** in CD154+CD137+ activated CD4+ T cells **(I)** and in CD137+CD8+ activated T cells **(H–J)** production of IFNγ **(J)**, TNFα **(K)** and IL-2 **(L)** in activated CD8+ T cells. Single, double (dp) or triple (tp) cytokine producing activated T cell subsets were analyzed using Boolean combination gates.

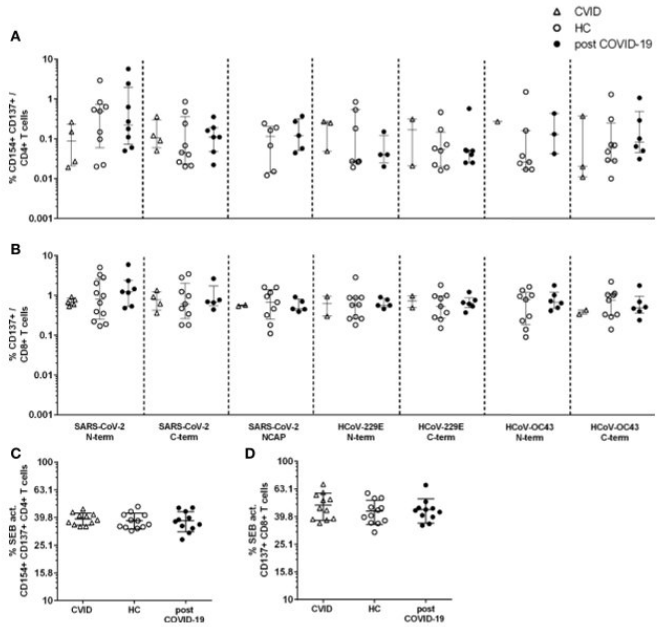

FIGURE 2 | CD154+CD137+CD4+ and CD137+CD8+ T cell response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and human endemic corona viruses (HCoV) peptides **(A, B)** and Staphylococcal enterotoxin B (SEB). **(C, D)** Peripheral blood mononuclear cells (PBMCs) of CVID (n=11. triangle), HC (n=12. empty black dots) and post-COVID-19 (n=11. filled black dots) were stimulated with 1 µg/ml CoV peptides or 3 µg/ml SEB for 16 h. Frequencies of activated CD154+CD137+CD4+ **(A)** and CD137+CD8+ **(B)** T cells after stimulation with the different CoV peptides. Frequencies of activated CD154+CD137+CD4+ **(C)** CD137+CD8+ **(D)** after stimulation with SEB. Only T cell responses above the threshold of 20% above background activation are shown. CVID patients lacked a response to SARS-CoV-2 NCAP peptide pool in activated CD4+ T cells and are hence not shown **(A)**. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant.

TABLE 2 | T cell response to peptides of SARS-CoV-2 and HCoV in common variable immunodeficiency disorder (CVID), unexposed and post coronavirus disease 2019 (COVID-19) healthy control (HC).

Peptides	CVID (n=11)	unexposed HC (n=12)	post COVID-19 HC (n=11)
	n of individuals with activated CD4+ T cells		
SARS-CoV-2 N-term	4 (p=0.06)	9	8
SARS-CoV-2 C-term	4 (p=0.06)	9	7
SARS-CoV-2 NCAP	**0 (**)**	6	5
HCoV-229E N-term	3	7	4
HCoV-229E C-term	**2 (*)**	8	7
HCoV-OC43 N-term	**1 (*)**	7	3
HCoV-OC43 C-term	3 (p=0.06)	8	6
	n of individuals with activated CD8+ T cells		
SARS-CoV-2 N-term	**5 (**)**	12	7
SARS-CoV-2 C-term	4 (p=0.06)	9	5
SARS-CoV-2 NCAP	**2 (**)**	9	5
HCoV-229E N-term	**2 (**)**	10	5
HCoV-229E C-term	**2 (**)**	9	6
HCoV-OC43 N-term	**0 (***)**	9	6
HCoV-OC43 C-term	**1 (**)**	9	6

For statistical analysis of CVID vs unexposed HC a 2 × 2 contingency table was used and tested for significance by χ^2-square test (two-tailed). A two-tailed p-value of p<0.05= (p<0.005=**; p<0.0005=***) was considered statistically significant. Significant values are bolded.*

TABLE 3A | Correlation of the frequency of CD4+ T cells activated by N- or C-terminal spike peptides of SARS-CoV-2 or the endemic corona viruses HCoV-229E and -OC43.

	HCoV-229E N-term	HCoV-229E C-term	HCoV-OC43 N-term	HCoV-OC43 C-term
		healthy controls		
SARS-CoV-2 N-term	**r=0.8649** **p=0.0159** n=7		r=0.7714 p=0.1028 n=6	
SARS-CoV-2 C-term		**r=0.9429** **p=0.0167** n=6		**r=0.8214** **p=0.0341** n=7
HCoV-229E N-term			*r=0.7827* *p=0.0722* n=6	
HCoV-229E C-term				**r=0.8214** **p=0.0341** n=7
		post COVID-19		
SARS-CoV-2 N-term	r=−0.316 p>0.9999 n=4		r=0.5 p>0.9999 n=3	
SARS-CoV-2 C-term		r=−0.403 p=0.4333 n=6		r=0.6156 p=0.3 n=5
HCoV-229E N-term			n.d. n=2	
HCoV-229E C-term				**r=0.8827** **p=0.0444** n=6

Non-parametric spearman correlation was performed. A two-tailed p-value of p<0.05 was considered statistically significant. Significant values are bolded. [r, correlation coefficient; n, number of tested pairs].

TABLE 3B | Correlation of the frequency of CD8+ T cells activated by N- or C-terminal spike peptides of SARS-CoV-2 or the endemic corona viruses HCoV-229E and -OC43.

	HCoV-229E N-term	HCoV-229E C-term	HCoV-OC43 N-term	HCoV-OC43 C-term
		healthy controls		
SARS-CoV-2 N-term	**r=0.8842** **p=0.0013** n=10		**r=0.7167** **p=0.0369** n=9	
SARS-CoV-2 C-term		**r=0.9461** **p=0.0013** n=8		**r=0.8929** **p=0.0123** n=7
HCoV-229E N-term			**r=0.7699** **p=0.0193** n=9	
HCoV-229E C-term				**r=0.7904** **p=0.0251** n=8
		post COVID-19		
SARS-CoV-2 N-term	r=0.6669 p=0.2667 n=5		r=0.7714 p=0.1028 n=6	
SARS-CoV-2 C-term		r=0.6 p=0.35 n=5		r=0 p>0.9999 n=4
HCoV-229E N-term			r=0.7182 p=0.1667 n=5	
HCoV-229E C-term				r=0.1 p=0.95 n=5

Non-parametric spearman correlation was performed. A two-tailed p-value of p<0.05 was considered statistically significant. Significant values are bolded. [r, correlation coefficient; n, number of tested pairs].

IFNγ/IL-2 and IL-2/TNFα double positive (dp) and IFNγ/TNFα/IL-2 triple positive (tp) cells were depicted (**Figure 1** for gating strategy). In the entire cohort, the most frequent CD4+ peptide reactive cytokine subsets were IL-2/TNFα dp and tp T cells (**Figures 3A, C**). CVID patients had significantly lower tp T cells against the spike peptides of SARS-CoV-2 and HCoV-OC43 vs unexposed HC (SARS-CoV-2: N-terminal p=0.0020, C-terminal p=0.036; HCoV-OC43 C-terminal p=0.05; **Figure 3A**), while there were no differences among the post COVID-19 and unexposed HC cohorts. Interestingly, post COVID-19 patients had significantly higher frequencies of tp SARS-CoV-2 NCAP-reactive CD4+ T cells clearly distinguishing them from unexposed HC (p=0.0043, **Figure 3A**). In CVID patients, no CD154+CD137+CD4+ T cell response to SARS-CoV-2 NCAP were found (**Figure 2B** and **Table 2**). The other cytokine subsets are shown in Supplements (**Supplementary Figure 4**). Strongest cytokine responses in CD8+ activated T cells were observed in IFNγ/TNFα dp and tp subsets, but no significant differences among the cohorts were found (**Figures 3B, D**).

Of note, comparable frequencies of SEB-reactive CD154+CD137+CD4+ and CD137+CD8+ cytokine producing activated T cell subsets were observed in all three cohorts, implicating that there is not a general impaired T cell cytokine production in CVID patients (**Figures 3E, F**).

DISCUSSION

In this study, we provide first evidence of endemic HCoV- and SARS-CoV-2-cross-reactive T cells in CVID patients. However, fewer reactive CD4+ and CD8+ T cells to spike peptide pools and fewer multifunctional CD4+ compared to HC and no NCAP-reactive CD4+ cells were detected.

Our finding of normal frequencies of HCoV and SARS-CoV-2-reactive T cells in a subset of CVID patients is in line with previous studies showing that anti-viral T cell immunity is not relevantly impaired in most CVID patients (13, 18, 19). CVID patients had less frequent T cells reactive against spike peptides of the common cold corona viruses HCoV-229E and -OC43. Possible reasons for this could be that IgG replacement therapy may protect from infections with common cold HCoVs or that patients with CVID avoid contacts with acutely infected persons. Normal T cell reactivity in CVID patients was demonstrated by responses to SEB stimulation, arguing against an obvious underlying T cell defect in non-responders, although an impaired T cell response due to CVID-related immune dysfunction cannot be excluded.

There is increasing evidence, that the majority of HC have T cells reactive to human endemic corona viruses. Our data also provides further evidence for frequent pre-existing T cells reactive against SARS-CoV-2 in unexposed healthy individuals. The presence of cross-reactive T cells to peptide pools of SARS-CoV-2 in unexposed healthy individuals was already reported by different groups ranging from 35% to 90% (7–10, 20, 21). These differences likely depend on the sensitivity of different assays used, and the type of peptide pools. We observed a high correlation of T cells reactive against spike N- or C-terminus of the two HCoVs and SARS-CoV-2 in unexposed but not post COVID-19 HC suggesting cross-reactivity of pre-existing T cells. This finding is in accordance with recent studies from Mateus and Nelde (9, 10). While the RBD is poorly conserved, they provide evidence for homology of many MHC epitopes of the spike protein between HCoV and SARS-CoV-2. We found most unexposed and post COVID-19 HC to have SARS-CoV-2 reactive CD4+ and CD8+ T cells in similar frequencies. In contrast to most other studies, T cell analyses in our convalescent HC was performed median 2.5 months after infection. This could explain why in our study the frequency of SARS-CoV-2 reactive T cells did not differ between COVID-19 recovered patients and unexposed HC.

Virus-specific memory T cells have been shown to persist for many years after infection with SARS-CoV-1 (21–23). In line with these observations, we found that SARS-CoV-2-reactive T cells in convalescent patients acquired a multifunctional (triple positive for INFγ, IL-2 and TNFα) phenotype, which is considered as correlate of protective immunity (24). We found much higher frequencies of tp NCAP reactive CD4+ T cells in post COVID-19 compared to unexposed HC, while high tp spike reactive CD4+ T cells were found in both groups. In CVID patients, no NCAP reactive CD4+ T cells could be detected and spike reactive CD4+ T cells showed little to no tp. A possible explanation for the different cytokine profile is that these CVID patients had contact with HCoV longer time ago. This hypothesis would be supported by comparable frequencies of TNFα single and TNFα/IL-2 dp spike reactive T cells in unexposed CVID and HC belonging to less differentiated and longer lasting memory T cells (16). An alternate explanation would be an impaired ability to mount tp T cells. This is, however, less likely, as we found similar frequencies of tp SEB T cells and tp influenza-specific T cells in CVID vs HC after vaccination (13).

Our cross-sectional study is limited with regards to low numbers of donors and a median higher age in CVID than HC. However, we observed no influence of age on T cell responses. Taken into consideration, that approximately 10 % of patients with mild or asymptomatic SARS-CoV-2 infections fail to mount a detectable antibody response, we cannot exclude that one of our HC had an unrecognized infection with SARS-CoV-2 although it is rather unlikely due to the low number of documented infections in our area in spring 2020. Furthermore, it is of critical importance to evaluate SARS-CoV-2 T cell responses in post COVID-19 CVID patients, too. One limitation of our study is that we could not analyze the T cell response in CVID patients post COVID-19, as until now none of our CVID patients at Charité had COVID-19. Unfortunately, given the continuing spread of the pandemic, SARS-CoV-2 infections in our cohort are very likely to occur and might contribute in evaluating the potential role of the here detected pre-existing SARS-CoV-2 T cells in this patient group. It further remains to be clarified, if SARS-CoV-2 reactive T cells after infection or after vaccination are able to protect or ameliorate the infection in the absence of a humoral immune response as it was reported from previous studies of MERS and SARS-CoV-1 (25–28). The biological relevance of a pre-existing immunity to SARS-CoV-2 remains unclear and could be beneficial or even detrimental. In

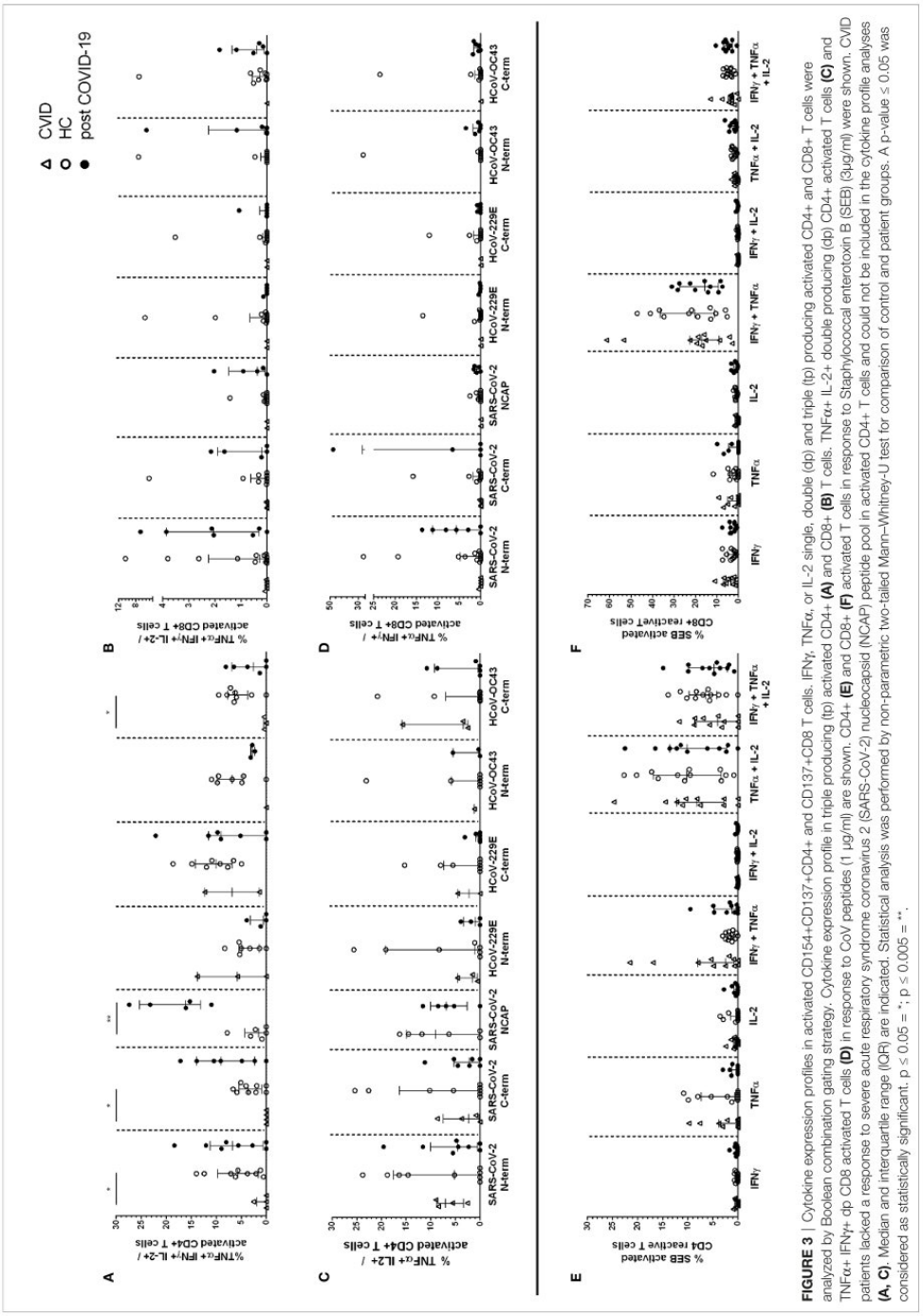

FIGURE 3 | Cytokine expression profiles in activated CD154+CD137+CD4+ and CD137+CD8 T cells. IFNγ, TNFα, or IL-2 single, double (dp) and triple (tp) producing activated CD4+ and CD8+ T cells were analyzed by Boolean combination gating strategy. Cytokine expression profile in triple producing (tp) activated CD4+ **(A)** and CD8+ **(B)** T cells. TNFα+ IL-2+ double producing (dp) CD4+ activated T cells **(C)** and TNFα+ IFNγ+ dp CD8 activated T cells **(D)** in response to CoV peptides (1 µg/ml) are shown. CD4+ **(E)** and CD8+ **(F)** activated T cells in response to Staphylococcal enterotoxin B (SEB) (3µg/ml) were shown. CVID patients lacked a response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid (NCAP) peptide pool in activated CD4+ T cells and could not be included in the cytokine profile analyses **(A, C)**. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **.

pandemic influenza H1N1, pre-existing T cell immunity was found to be beneficial (29, 30), so it is tempting to speculate that (cross-) reactive SARS-CoV-2 T cells may provide at least partial protection against COVID-19 disease.

Taken together, our data provides evidence for cross-reactive SARS-CoV-2 cells in a subset of CVID patients as well as a rationale for SARS-CoV-2 vaccination and has implications for the monitoring of vaccine-induced T cell responses.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Charité Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments (EA2/092/20 from June 4th, 2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS, I-KN, MS-H, UB, and LH made substantial contributions to conception and design. LH made patient samples available. SS and SB performed acquisition and analysis of data. SS, FS, LH, and CS performed interpretation of data. CD, VC, and TS and performed analysis of neutralizing IgG. DW and MS-H helped with analyzing data. CS and LH wrote the article. H-DV, MS-H, DW, CD, VC, TS, FS, and SS reviewed the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.607918/full#supplementary-material

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Conflict of Interest: VC is named together with Euroimmun on a patent application filed recently regarding detection of antibodies against SARS-CoV-2.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material Study 1:

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Supplementary Material

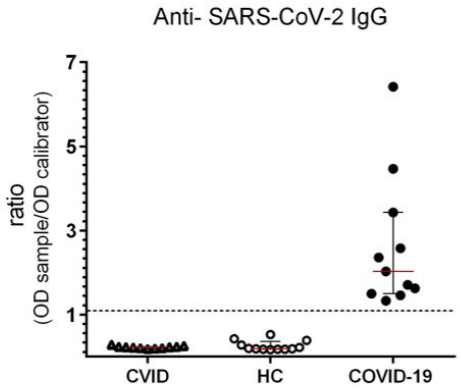
1 Supplementary Figures and Tables

Supplementary tables can be found as Excel sheets under the following names:

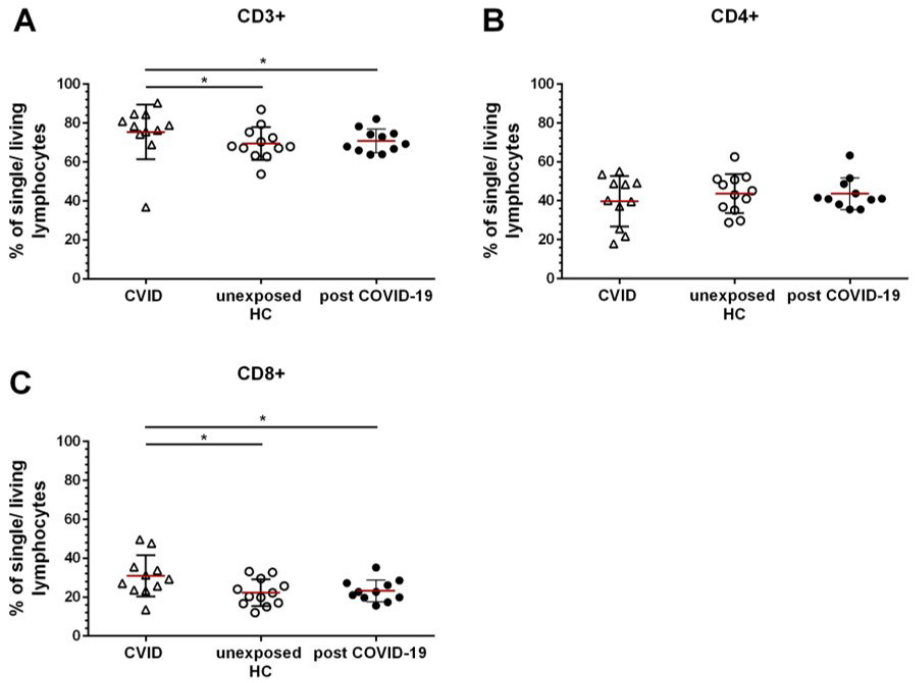
Supplementary Table 1: Raw data of CD154+CD137+CD4+ and CD137+ CD8+ T cell frequencies

Supplementary Table 2: Summary of CD154+CD137+CD4+ and CD137+CD8+ T cell frequencies in CVID, post COVID-19 and unexposed HC

1.1 Supplementary Figures



Supplementary Figure 1: Anti SARS-CoV-2 ELISA (IgG) of CVID patients, unexposed HC and post COVID-19 HC. Serum IgG against the N-terminal domain of the spike protein including the immunologically relevant receptor binding domain (RBD) of SARS-CoV-2 analyzed by EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG). All CVID patients and unexposed HC are negative for anti-SARS-CoV-2 IgG. Results are evaluated by calculating a ratio of the OD of the control or patient sample over the OD of the calibrator. A ratio of <0.8 is negative, ratio ≥ 0.8 to <1.1 is borderline, ratio ≥1.1 is positive (dotted line). Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **; $p \leq 0.0005$ = ***; $p < 0.0001$ = ****.



Supplementary Figure 2: T cell frequencies of CVID patients unexposed and post COVID-19 HCs. Frequencies of CD3+. CD4+ and CD8+ T cell frequencies were analyzed by flow cytometry and calculated as percentage of single, living lymphocytes. CVID patients show higher frequencies of CD3+ **(A)** and CD8+ **(C)** T cells compared to unexposed and post COVID-19 HC. No differences in CD4+ T cells was observed **(B)**. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **

Supplementary Table 1a: Raw data of $CD154^+CD137^+CD4^+$ T cell frequency. Reactive T cells > 0.005% within total $CD4^+$ T cells and with a ≥ 1.2-fold response of the background control (DMSO) were considered as positive.

unexposed HC	Raw Data CD4+ CD154+ CD137+	-Background CD4+ CD154+ CD137+
HC-1-SARS-CoV-2 N-term	0,099	0,0000
HC-1-SARS-CoV-2 C-term	0,120	0,0000
HC-1-SARS-CoV-2 NCAP	0,100	0,0000
HC-1-HCoV-229E N-term	0,120	0,0000
HC-1-HCoV-229E C-term	0,170	0,050
HC-1-HCoV-OC43 N-term	0,120	0,0000
HC-1-HCoV-OC43 C-term	0,120	0,0000
HC-1-SEB	38,200	38,080
HC-1-00 DMSO	0,120	0,000
HC-2-SARS-CoV-2 N-term	0,58	0,5350
HC-2-SARS-CoV-2 C-term	0,0900	0,0450
HC-2-SARS-CoV-2 NCAP	0,0570	0,0120
HC-2-HCoV-229E N-term	0,0720	0,0270
HC-2-HCoV-229E C-term	0,0500	0,0000
HC-2-HCoV-OC43 N-term	0,0690	0,0240
HC-2-HCoV-OC43 C-term	0,0550	0,0100
HC-2-SEB	37,7000	37,6550
HC-2-00 DMSO	0,0450	0,0000
HC-3-SARS-CoV-2 N-term	0,083	0,0000
HC-3-SARS-CoV-2 C-term	0,077	0,0000
HC-3-SARS-CoV-2 NCAP	0,061	0,0000
HC-3-HCoV-229E N-term	0,096	0,015
HC-3-HCoV-229E C-term	0,100	0,019
HC-3-HCoV-OC43 N-term	0,098	0,017
HC-3-HCoV-OC43 C-term	0,150	0,069
HC-3-SEB	41,700	41,619
HC-3-00 DMSO	0,081	0,000
HC-4-SARS-CoV-2 N-term	0,9200	0,831
HC-4-SARS-CoV-2 C-term	0,5700	0,481
HC-4-SARS-CoV-2 NCAP	0,3300	0,241
HC-4-HCoV-229E N-term	0,6200	0,531
HC-4-HCoV-229E C-term	0,1600	0,071
HC-4-HCoV-OC43 N-term	0,100	0,011
HC-4-HCoV-OC43 C-term	0,1600	0,071
HC-4-SEB	34,4000	34,311
HC-4-00 DMSO	0,0890	0,000
HC-5-SARS-CoV-2 N-term	0,0480	0,0000
HC-5-SARS-CoV-2 C-term	0,0320	0,0000
HC-5-SARS-CoV-2 NCAP	0,0320	0,0000
HC-5-HCoV-229E N-term	0,0470	0,0000
HC-5-HCoV-229E C-term	0,0630	0,0000
HC-5-HCoV-OC43 N-term	0,0410	0,0000
HC-5-HCoV-OC43 C-term	0,0640	0,0000
HC-5-SEB	33,2000	33,1330
HC-5-00 DMSO	0,0670	0,0000
HC-6-SARS-CoV-2 N-term	0,160	0,0200
HC-6-SARS-CoV-2 C-term	0,160	0,0200
HC-6-SARS-CoV-2 NCAP	0,120	0,0000
HC-6-HCoV-229E N-term	0,150	0,0000
HC-6-HCoV-229E C-term	0,140	0,0000
HC-6-HCoV-OC43 N-term	0,130	0,0000
HC-6-HCoV-OC43 C-term	0,150	0,0000
HC-6-SEB	32,000	31,8600
HC-6-00 DMSO	0,140	0,0000

HC-7-SARS-CoV-2 N-term	0,800	0,6300
HC-7-SARS-CoV-2 C-term	0,410	0,2400
HC-7-SARS-CoV-2 NCAP	0,360	0,1900
HC-7-HCoV-229E N-term	0,350	0,1800
HC-7-HCoV-229E C-term	0,340	0,1700
HC-7-HCoV-OC43 N-term	0,330	0,1600
HC-7-HCoV-OC43 C-term	0,480	0,3100
HC-7-SEB	41,500	41,3300
HC-7-00 DMSO	0,170	0,0000
HC-8-SARS-CoV-2 N-term	0,6700	0,4900
HC-8-SARS-CoV-2 C-term	0,2200	0,0400
HC-8-SARS-CoV-2 NCAP	0,1500	0,0000
HC-8-HCoV-229E N-term	0,1400	0,0000
HC-8-HCoV-229E C-term	0,1400	0,0000
HC-8-HCoV-OC43 N-term	0,1100	0,0000
HC-8-HCoV-OC43 C-term	0,1600	0,0000
HC-8-SEB	33,9000	33,7200
HC-8-00 DMSO	0,1800	0,0000
HC-9-SARS-CoV-2 N-term	0,1700	0,0970
HC-9-SARS-CoV-2 C-term	0,1400	0,0670
HC-9-SARS-CoV-2 NCAP	0,1400	0,0670
HC-9-HCoV-229E N-term	0,1000	0,0270
HC-9-HCoV-229E C-term	0,1300	0,0570
HC-9-HCoV-OC43 N-term	0,1100	0,0370
HC-9-HCoV-OC43 C-term	0,1200	0,0470
HC-9-SEB	47,8000	47,7270
HC-9-00 DMSO	0,073	0,0000
HC-10-SARS-CoV-2 N-term	0,1800	0,1480
HC-10-SARS-CoV-2 C-term	0,0580	0,0260
HC-10-SARS-CoV-2 NCAP	0,0470	0,0150
HC-10-HCoV-229E N-term	0,0510	0,0190
HC-10-HCoV-229E C-term	0,0540	0,0220
HC-10-HCoV-OC43 N-term	0,0580	0,0260
HC-10-HCoV-OC43 C-term	0,0600	0,0280
HC-10-SEB	34,0000	33,9680
HC-10-00 DMSO	0,032	0,0000
HC-11-SARS-CoV-2 N-term	0,0500	0,0220
HC-11-SARS-CoV-2 C-term	0,0490	0,0210
HC-11-SARS-CoV-2 NCAP	0,027	0,0000
HC-11-HCoV-229E N-term	0,0540	0,0260
HC-11-HCoV-229E C-term	0,0440	0,0160
HC-11-HCoV-OC43 N-term	0,0450	0,0170
HC-11-HCoV-OC43 C-term	0,0570	0,0290
HC-11-SEB	43,9000	43,8720
HC-11-00 DMSO	0,028	0,0000
HC-12-SARS-CoV-2 N-term	2,9500	2,8920
HC-12-SARS-CoV-2 C-term	0,9100	0,8520
HC-12-SARS-CoV-2 NCAP	0,22	0,1620
HC-12-HCoV-229E N-term	0,9000	0,8420
HC-12-HCoV-229E C-term	0,52	0,4620
HC-12-HCoV-OC43 N-term	1,5500	1,4920
HC-12-HCoV-OC43 C-term	1,3500	1,2920
HC-12-SEB	35,5000	35,4420
HC-12-00 DMSO	0,058	0,0000

post COVID-19 HC	Raw Data CD4+ CD154+ CD137+	-Background CD4+ CD154+ CD137+
COVID-1-SARS-CoV-2 N-term	0,260	0,110
COVID-1-SARS-CoV-2 C-term	0,260	0,110
COVID-1-SARS-CoV-2 NCAP	0,420	0,270
COVID-1-HCoV-229E N-term	0,190	0,040
COVID-1-HCoV-229E C-term	0,200	0,050
COVID-1-HCoV-OC43 N-term	0,160	0,010
COVID-1-HCoV-OC43 C-term	0,250	0,100
COVID-1-SEB	37,400	37,250
COVID-1-00 DMSO	0,150	0,000
COVID-2-SARS-CoV-2 N-term	0,3900	0,2700
COVID-2-SARS-CoV-2 C-term	0,2800	0,1600
COVID-2-SARS-CoV-2 NCAP	0,4900	0,3700
COVID-2-HCoV-229E N-term	0,2700	0,1500
COVID-2-HCoV-229E C-term	0,1700	0,0500
COVID-2-HCoV-OC43 N-term	0,2500	0,1300
COVID-2-HCoV-OC43 C-term	0,4100	0,2900
COVID-2-SEB	46,8000	46,6800
COVID-2-00 DMSO	0,1200	0,0000
COVID-3-SARS-CoV-2 N-term	0,2100	0,0000
COVID-3-SARS-CoV-2 C-term	0,2100	0,0000
COVID-3-SARS-CoV-2 NCAP	0,2000	0,0000
COVID-3-HCoV-229E N-term	0,1800	0,0000
COVID-3-HCoV-229E C-term	0,1900	0,0000
COVID-3-HCoV-OC43 N-term	0,1700	0,0000
COVID-3-HCoV-OC43 C-term	0,2000	0,0000
COVID-3-SEB	39,7000	39,4900
COVID-3-00 DMSO	0,2100	0,0000
COVID-4-SARS-CoV-2 N-term	0,240	0,177
COVID-4-SARS-CoV-2 C-term	0,110	0,0470
COVID-4-SARS-CoV-2 NCAP	0,120	0,0570
COVID-4-HCoV-229E N-term	0,064	0,0000
COVID-4-HCoV-229E C-term	0,088	0,025
COVID-4-HCoV-OC43 N-term	0,069	0,0000
COVID-4-HCoV-OC43 C-term	0,094	0,0310
COVID-4-SEB	38,400	38,337
COVID-4-00 DMSO	0,063	0,000
COVID-5-SARS-CoV-2 N-term	0,290	0,050
COVID-5-SARS-CoV-2 C-term	0,260	0,020
COVID-5-SARS-CoV-2 NCAP	0,280	0,040
COVID-5-HCoV-229E N-term	0,210	0,0000
COVID-5-HCoV-229E C-term	0,180	0,0000
COVID-5-HCoV-OC43 N-term	0,240	0,0000
COVID-5-HCoV-OC43 C-term	0,180	0,0000
COVID-5-SEB	27,800	27,560
COVID-5-00 DMSO	0,240	0,000
COVID-6-SARS-CoV-2 N-term	0,2600	0,060
COVID-6-SARS-CoV-2 C-term	0,3900	0,190
COVID-6-SARS-CoV-2 NCAP	0,1800	0,0000
COVID-6-HCoV-229E N-term	0,1800	0,0000
COVID-6-HCoV-229E C-term	0,2300	0,030
COVID-6-HCoV-OC43 N-term	0,1700	0,0000
COVID-6-HCoV-OC43 C-term	0,1900	0,0000
COVID-6-SEB	47,0000	46,800
COVID-6-00 DMSO	0,2000	0,000

COVID-7-SARS-CoV-2 N-term	0,6800	0,6240
COVID-7-SARS-CoV-2 C-term	0,4100	0,3540
COVID-7-SARS-CoV-2 NCAP	0,1000	0,0440
COVID-7-HCoV-229E N-term	0,0760	0,0200
COVID-7-HCoV-229E C-term	0,0810	0,0250
COVID-7-HCoV-OC43 N-term	0,0980	0,0420
COVID-7-HCoV-OC43 C-term	0,1200	0,0640
COVID-7-SEB	35,5000	35,4440
COVID-7-00 DMSO	0,0560	0,0000
COVID-8-SARS-CoV-2 N-term	0,0850	0,0000
COVID-8-SARS-CoV-2 C-term	0,1100	0,0220
COVID-8-SARS-CoV-2 NCAP	0,0820	0,0000
COVID-8-HCoV-229E N-term	0,0850	0,0000
COVID-8-HCoV-229E C-term	0,1400	0,0520
COVID-8-HCoV-OC43 N-term	0,0950	0,0000
COVID-8-HCoV-OC43 C-term	0,0810	0,0000
COVID-8-SEB	30,9000	30,8120
COVID-8-00 DMSO	0,0880	0,0000
COVID-9-SARS-CoV-2 N-term	2,540	2,4100
COVID-9-SARS-CoV-2 C-term	0,240	0,1100
COVID-9-SARS-CoV-2 NCAP	0,250	0,1200
COVID-9-HCoV-229E N-term	0,170	0,0400
COVID-9-HCoV-229E C-term	0,170	0,0400
COVID-9-HCoV-OC43 N-term	0,140	0,0100
COVID-9-HCoV-OC43 C-term	0,180	0,0500
COVID-9-SEB	44,100	43,9700
COVID-9-00 DMSO	0,130	0,0000
COVID-10-SARS-CoV-2 N-term	6,050	5,6600
COVID-10-SARS-CoV-2 C-term	0,220	0,0000
COVID-10-SARS-CoV-2 NCAP	0,160	0,0000
COVID-10-HCoV-229E N-term	0,100	0,0000
COVID-10-HCoV-229E C-term	0,960	0,5700
COVID-10-HCoV-OC43 N-term	0,820	0,4300
COVID-10-HCoV-OC43 C-term	1,450	1,0600
COVID-10-SEB	35,000	34,6100
COVID-10-00 DMSO	0,390	0,0000
COVID-11-SARS-CoV-2 N-term	0,670	0,0000
COVID-11-SARS-CoV-2 C-term	0,600	0,0000
COVID-11-SARS-CoV-2 NCAP	0,560	0,0000
COVID-11-HCoV-229E N-term	0,540	0,0000
COVID-11-HCoV-229E C-term	0,580	0,0000
COVID-11-HCoV-OC43 N-term	0,440	0,0000
COVID-11-HCoV-OC43 C-term	0,580	0,0000
COVID-11-SEB	34,000	33,2100
COVID-11-00 DMSO	0,790	0,0000

CVID	Raw Data CD4+ CD154+ CD137+	-Background CD4+ CD154+ CD137+
CVID-1-SARS-CoV-2 N-term	0,1900	0,0000
CVID-1-SARS-CoV-2 C-term	0,2500	0,0500
CVID-1-SARS-CoV-2 NCAP	0,1800	0,0000
CVID-1-HCoV-229E N-term	0,2300	0,0300
CVID-1-HCoV-229E C-term	0,2100	0,0100
CVID-1-HCoV-OC43 N-term	0,2200	0,0200
CVID-1-HCoV-OC43 C-term	0,1800	0,0000
CVID-1-SEB	38,4000	38,2000
CVID-1-00 DMSO	0,2000	0,0000
CVID-2-SARS-CoV-2 N-term	0,065	0,0000
CVID-2-SARS-CoV-2 C-term	0,068	0,0000
CVID-2-SARS-CoV-2 NCAP	0,057	0,0000
CVID-2-HCoV-229E N-term	0,048	0,0000
CVID-2-HCoV-229E C-term	0,050	0,0000
CVID-2-HCoV-OC43 N-term	0,055	0,0000
CVID-2-HCoV-OC43 C-term	0,055	0,0000
CVID-2-SEB	34,300	34,228
CVID-2-00 DMSO	0,072	0,000
CVID-3-SARS-CoV-2 N-term	0,0800	0,0190
CVID-3-SARS-CoV-2 C-term	0,0560	0,0000
CVID-3-SARS-CoV-2 NCAP	0,0420	0,0000
CVID-3-HCoV-229E N-term	0,1100	0,0490
CVID-3-HCoV-229E C-term	0,0820	0,0210
CVID-3-HCoV-OC43 N-term	0,0540	0,0000
CVID-3-HCoV-OC43 C-term	0,0810	0,0200
CVID-3-SEB	35,3000	35,2390
CVID-3-00 DMSO	0,0610	0,0000
CVID-4-SARS-CoV-2 N-term	0,1800	0,1490
CVID-4-SARS-CoV-2 C-term	0,1200	0,0890
CVID-4-SARS-CoV-2 NCAP	0,0260	0,0000
CVID-4-HCoV-229E N-term	0,0320	0,0000
CVID-4-HCoV-229E C-term	0,0360	0,0000
CVID-4-HCoV-OC43 N-term	0,0270	0,0000
CVID-4-HCoV-OC43 C-term	0,0420	0,0110
CVID-4-SEB	40,6000	40,5690
CVID-4-00 DMSO	0,0310	0,0000
CVID-5-SARS-CoV-2 N-term	0,300	0,0200
CVID-5-SARS-CoV-2 C-term	0,400	0,1200
CVID-5-SARS-CoV-2 NCAP	0,210	0,0000
CVID-5-HCoV-229E N-term	0,280	0,0000
CVID-5-HCoV-229E C-term	0,310	0,0300
CVID-5-HCoV-OC43 N-term	0,260	0,0000
CVID-5-HCoV-OC43 C-term	0,250	0,0000
CVID-5-SEB	46,000	45,7200
CVID-5-00 DMSO	0,280	0,0000
CVID-6-SARS-CoV-2 N-term	0,064	0,0000
CVID-6-SARS-CoV-2 C-term	0,085	0,0000
CVID-6-SARS-CoV-2 NCAP	0,092	0,0000
CVID-6-HCoV-229E N-term	0,100	0,0000
CVID-6-HCoV-229E C-term	0,097	0,0000
CVID-6-HCoV-OC43 N-term	0,080	0,0000
CVID-6-HCoV-OC43 C-term	0,099	0,0000
CVID-6-SEB	36,800	36,7000
CVID-6-00 DMSO	0,100	0,0000

CVID-7-SARS-CoV-2 N-term	0,0940	0,0270
CVID-7-SARS-CoV-2 C-term	0,0580	0,0000
CVID-7-SARS-CoV-2 NCAP	0,0610	0,0000
CVID-7-HCoV-229E N-term	0,0660	0,0000
CVID-7-HCoV-229E C-term	0,0510	0,0000
CVID-7-HCoV-OC43 N-term	0,0650	0,0000
CVID-7-HCoV-OC43 C-term	0,0670	0,0000
CVID-7-SEB	43,4000	43,3330
CVID-7-00 DMSO	0,0670	0,0000
CVID-8-SARS-CoV-2 N-term	0,0460	0,0000
CVID-8-SARS-CoV-2 C-term	0,0330	0,0000
CVID-8-SARS-CoV-2 NCAP	0,0530	0,0000
CVID-8-HCoV-229E N-term	0,0370	0,0000
CVID-8-HCoV-229E C-term	0,0430	0,0000
CVID-8-HCoV-OC43 N-term	0,0550	0,0000
CVID-8-HCoV-OC43 C-term	0,0400	0,0000
CVID-8-SEB	34,3000	34,2390
CVID-8-00 DMSO	0,0610	0,0000
CVID-9-SARS-CoV-2 N-term	1,390	0,2200
CVID-9-SARS-CoV-2 C-term	1,530	0,3600
CVID-9-SARS-CoV-2 NCAP	1,210	0,0400
CVID-9-HCoV-229E N-term	1,440	0,2700
CVID-9-HCoV-229E C-term	1,480	0,3100
CVID-9-HCoV-OC43 N-term	1,440	0,2700
CVID-9-HCoV-OC43 C-term	1,540	0,3700
CVID-9-SEB	42,200	41,0300
CVID-9-00 DMSO	1,170	0,0000
CVID-10-SARS-CoV-2 N-term	1,070	0,2600
CVID-10-SARS-CoV-2 C-term	0,900	0,0900
CVID-10-SARS-CoV-2 NCAP	0,840	0,0300
CVID-10-HCoV-229E N-term	1,060	0,2500
CVID-10-HCoV-229E C-term	0,810	0,0000
CVID-10-HCoV-OC43 N-term	0,270	0,0000
CVID-10-HCoV-OC43 C-term	0,091	0,0000
CVID-10-SEB	42,600	41,7900
CVID-10-00 DMSO	0,810	0,0000
CVID-11-SARS-CoV-2 N-term	0,680	0,0300
CVID-11-SARS-CoV-2 C-term	0,630	0,0000
CVID-11-SARS-CoV-2 NCAP	0,630	0,0000
CVID-11-HCoV-229E N-term	0,710	0,0600
CVID-11-HCoV-229E C-term	0,690	0,0400
CVID-11-HCoV-OC43 N-term	0,630	0,0000
CVID-11-HCoV-OC43 C-term	0,630	0,0000
CVID-11-SEB	37,100	36,4500
CVID-11-00 DMSO	0,650	0,0000

= negative after substraction of background signal

= positive after substraction of background signal and value above threshold of 1.2-fold above background control

e.g. 0,015 = negative signal because it does not exceed the threshold of 1.2-fold above the background control

SEB = Superantigen Staphylococcal enterotoxin B

00 DMSO = background control only stimulated with DMSO

Supplementary Table 1b: Raw data of $CD137^{+}CD8^{+}$ T cell frequency. Reactive T cells > 0.005% within total $CD4^{+}$ T cells and with a ≥ 1.2-fold response of the background control (DMSO) were considered as positive.

unexposed HC	Raw Data CD8+ CD137+	-Background CD8+ CD137+
HC-1-SARS-CoV-2 N-term	6,250	2,820
HC-1-SARS-CoV-2 C-term	6,230	2,800
HC-1-SARS-CoV-2 NCAP	5,030	1,600
HC-1-HCoV-229E N-term	4,310	0,880
HC-1-HCoV-229E C-term	4,390	0,960
HC-1-HCoV-OC43 N-term	4,770	1,340
HC-1-HCoV-OC43 C-term	4,550	1,120
HC-1-SEB	49,300	45,870
HC-1-00 DMSO	3,430	0,000
HC-2-SARS-CoV-2 N-term	6,4900	5,0200
HC-2-SARS-CoV-2 C-term	2,7100	1,2400
HC-2-SARS-CoV-2 NCAP	2,4100	0,9400
HC-2-HCoV-229E N-term	2,3400	0,8700
HC-2-HCoV-229E C-term	2,4600	0,9900
HC-2-HCoV-OC43 N-term	2,2600	0,7900
HC-2-HCoV-OC43 C-term	2,4900	1,0200
HC-2-SEB	60,2000	58,7300
HC-2-00 DMSO	1,4700	0,0000
HC-3-SARS-CoV-2 N-term	8,180	3,300
HC-3-SARS-CoV-2 C-term	8,320	3,440
HC-3-SARS-CoV-2 NCAP	6,460	1,580
HC-3-HCoV-229E N-term	7,710	2,830
HC-3-HCoV-229E C-term	6,700	1,820
HC-3-HCoV-OC43 N-term	6,520	1,640
HC-3-HCoV-OC43 C-term	7,100	2,220
HC-3-SEB	51,300	46,420
HC-3-00 DMSO	4,880	0,000
HC-4-SARS-CoV-2 N-term	1,4100	0,9800
HC-4-SARS-CoV-2 C-term	0,9000	0,470
HC-4-SARS-CoV-2 NCAP	1,6100	1,1800
HC-4-HCoV-229E N-term	1,0100	0,580
HC-4-HCoV-229E C-term	0,9600	0,530
HC-4-HCoV-OC43 N-term	0,5200	0,0900
HC-4-HCoV-OC43 C-term	0,7600	0,330
HC-4-SEB	35,9000	35,4700
HC-4-00 DMSO	0,4300	0,0000
HC-5-SARS-CoV-2 N-term	1,9100	0,650
HC-5-SARS-CoV-2 C-term	0,9700	0,0000
HC-5-SARS-CoV-2 NCAP	1,2600	0,0000
HC-5-HCoV-229E N-term	1,5200	0,2600
HC-5-HCoV-229E C-term	1,5400	0,2800
HC-5-HCoV-OC43 N-term	1,5600	0,3000
HC-5-HCoV-OC43 C-term	1,5900	0,3300
HC-5-SEB	37,1000	35,8400
HC-5-00 DMSO	1,2600	0,0000
HC-6-SARS-CoV-2 N-term	1,0200	0,390
HC-6-SARS-CoV-2 C-term	0,8100	0,1800
HC-6-SARS-CoV-2 NCAP	0,9500	0,320
HC-6-HCoV-229E N-term	0,9200	0,2900
HC-6-HCoV-229E C-term	0,8800	0,2500
HC-6-HCoV-OC43 N-term	0,8600	0,2300
HC-6-HCoV-OC43 C-term	0,7100	0,0800
HC-6-SEB	40,0000	39,3700
HC-6-00 DMSO	0,6300	0,0000

HC-7-SARS-CoV-2 N-term	0,8400	0,2200
HC-7-SARS-CoV-2 C-term	0,6600	0,0400
HC-7-SARS-CoV-2 NCAP	0,6500	0,0300
HC-7-HCoV-229E N-term	0,8800	0,2600
HC-7-HCoV-229E C-term	0,7200	0,1000
HC-7-HCoV-OC43 N-term	0,7200	0,1000
HC-7-HCoV-OC43 C-term	0,7600	0,1400
HC-7-SEB	44,9000	44,2800
HC-7-00 DMSO	0,6200	0,0000
HC-8-SARS-CoV-2 N-term	0,8400	0,1900
HC-8-SARS-CoV-2 C-term	0,5200	0,0000
HC-8-SARS-CoV-2 NCAP	0,6700	0,0200
HC-8-HCoV-229E N-term	0,6600	0,0100
HC-8-HCoV-229E C-term	0,5900	0,0000
HC-8-HCoV-OC43 N-term	0,6800	0,0300
HC-8-HCoV-OC43 C-term	0,5500	0,0000
HC-8-SEB	37,9000	37,2500
HC-8-00 DMSO	0,6500	0,0000
HC-9-SARS-CoV-2 N-term	0,6900	0,1700
HC-9-SARS-CoV-2 C-term	0,7000	0,1800
HC-9-SARS-CoV-2 NCAP	0,6300	0,1100
HC-9-HCoV-229E N-term	0,7000	0,1800
HC-9-HCoV-229E C-term	0,6700	0,1500
HC-9-HCoV-OC43 N-term	0,6600	0,1400
HC-9-HCoV-OC43 C-term	0,8100	0,2900
HC-9-SEB	54,8000	54,2800
HC-9-00 DMSO	0,5200	0,0000
HC-10-SARS-CoV-2 N-term	1,2600	0,3500
HC-10-SARS-CoV-2 C-term	1,2500	0,3400
HC-10-SARS-CoV-2 NCAP	1,3700	0,4600
HC-10-HCoV-229E N-term	1,0000	0,0900
HC-10-HCoV-229E C-term	0,9900	0,0800
HC-10-HCoV-OC43 N-term	1,0500	0,1400
HC-10-HCoV-OC43 C-term	0,9800	0,0700
HC-10-SEB	41,3000	40,3900
HC-10-00 DMSO	0,9100	0,0000
HC-11-SARS-CoV-2 N-term	2,2600	1,1500
HC-11-SARS-CoV-2 C-term	2,0800	0,9700
HC-11-SARS-CoV-2 NCAP	1,7800	0,6700
HC-11-HCoV-229E N-term	1,9800	0,8700
HC-11-HCoV-229E C-term	1,9400	0,8300
HC-11-HCoV-OC43 N-term	2,1200	1,0100
HC-11-HCoV-OC43 C-term	1,8700	0,7600
HC-11-SEB	55,0000	53,8900
HC-11-00 DMSO	1,1100	0,0000
HC-12-SARS-CoV-2 N-term	2,7900	2,0300
HC-12-SARS-CoV-2 C-term	1,3700	0,6100
HC-12-SARS-CoV-2 NCAP	0,9400	0,1800
HC-12-HCoV-229E N-term	1,3300	0,5700
HC-12-HCoV-229E C-term	1,1300	0,3700
HC-12-HCoV-OC43 N-term	1,7100	0,9500
HC-12-HCoV-OC43 C-term	1,8000	1,0400
HC-12-SEB	31,4000	30,6400
HC-12-00 DMSO	0,7600	0,0000

post COVID-19 HC	Raw Data CD8+ CD137+	-Background CD8+ CD137+
COVID-1-SARS-CoV-2 N-term	1,890	0,480
COVID-1-SARS-CoV-2 C-term	2,100	0,690
COVID-1-SARS-CoV-2 NCAP	2,320	0,910
COVID-1-HCoV-229E N-term	1,970	0,560
COVID-1-HCoV-229E C-term	2,070	0,660
COVID-1-HCoV-OC43 N-term	1,820	0,410
COVID-1-HCoV-OC43 C-term	1,500	0,000
COVID-1-SEB	36,700	35,290
COVID-1-00 DMSO	1,410	0,000
COVID-2-SARS-CoV-2 N-term	3,2500	1,230
COVID-2-SARS-CoV-2 C-term	2,4600	0,4400
COVID-2-SARS-CoV-2 NCAP	2,4800	0,4600
COVID-2-HCoV-229E N-term	2,8000	0,7800
COVID-2-HCoV-229E C-term	2,5500	0,5300
COVID-2-HCoV-OC43 N-term	3,8100	1,790
COVID-2-HCoV-OC43 C-term	2,4900	0,4700
COVID-2-SEB	53,0000	50,9800
COVID-2-00 DMSO	2,0200	0,0000
COVID-3-SARS-CoV-2 N-term	2,5800	0,0000
COVID-3-SARS-CoV-2 C-term	2,6500	0,0000
COVID-3-SARS-CoV-2 NCAP	2,7800	0,0000
COVID-3-HCoV-229E N-term	2,4600	0,0000
COVID-3-HCoV-229E C-term	2,8900	0,0000
COVID-3-HCoV-OC43 N-term	3,1100	0,2000
COVID-3-HCoV-OC43 C-term	3,3100	0,4000
COVID-3-SEB	42,4000	39,4900
COVID-3-00 DMSO	2,9100	0,0000
COVID-4-SARS-CoV-2 N-term	2,520	1,170
COVID-4-SARS-CoV-2 C-term	3,990	2,640
COVID-4-SARS-CoV-2 NCAP	1,740	0,390
COVID-4-HCoV-229E N-term	1,810	0,460
COVID-4-HCoV-229E C-term	1,960	0,610
COVID-4-HCoV-OC43 N-term	1,840	0,490
COVID-4-HCoV-OC43 C-term	1,860	0,510
COVID-4-SEB	46,900	45,550
COVID-4-00 DMSO	1,350	0,000
COVID-5-SARS-CoV-2 N-term	1,100	0,000
COVID-5-SARS-CoV-2 C-term	1,110	0,010
COVID-5-SARS-CoV-2 NCAP	1,320	0,220
COVID-5-HCoV-229E N-term	1,170	0,070
COVID-5-HCoV-229E C-term	1,060	0,0000
COVID-5-HCoV-OC43 N-term	1,190	0,090
COVID-5-HCoV-OC43 C-term	1,040	0,0000
COVID-5-SEB	35,400	34,300
COVID-5-00 DMSO	1,100	0,000
COVID-6-SARS-CoV-2 N-term	2,2800	0,3700
COVID-6-SARS-CoV-2 C-term	2,0600	0,1500
COVID-6-SARS-CoV-2 NCAP	1,9800	0,0700
COVID-6-HCoV-229E N-term	2,1200	0,2100
COVID-6-HCoV-229E C-term	2,0100	0,1000
COVID-6-HCoV-OC43 N-term	1,8800	0,0000
COVID-6-HCoV-OC43 C-term	2,2900	0,3800
COVID-6-SEB	42,5000	40,5900
COVID-6-00 DMSO	1,9100	0,0000

COVID-7-SARS-CoV-2 N-term	2,7700	1,4500
COVID-7-SARS-CoV-2 C-term	2,0900	0,770
COVID-7-SARS-CoV-2 NCAP	2,0200	0,700
COVID-7-HCoV-229E N-term	2,2200	0,900
COVID-7-HCoV-229E C-term	2,0800	0,760
COVID-7-HCoV-OC43 N-term	2,0400	0,720
COVID-7-HCoV-OC43 C-term	1,7200	0,4000
COVID-7-SEB	44,2000	42,8800
COVID-7-00 DMSO	1,3200	0,0000
COVID-8-SARS-CoV-2 N-term	1,1100	0,1400
COVID-8-SARS-CoV-2 C-term	0,9700	0,0000
COVID-8-SARS-CoV-2 NCAP	1,1500	0,1800
COVID-8-HCoV-229E N-term	1,1600	0,1900
COVID-8-HCoV-229E C-term	1,0600	0,0900
COVID-8-HCoV-OC43 N-term	1,0700	0,1000
COVID-8-HCoV-OC43 C-term	1,0300	0,0600
COVID-8-SEB	45,0000	44,0300
COVID-8-00 DMSO	0,9700	0,0000
COVID-9-SARS-CoV-2 N-term	3,3300	2,3400
COVID-9-SARS-CoV-2 C-term	1,0500	0,0600
COVID-9-SARS-CoV-2 NCAP	1,1400	0,1500
COVID-9-HCoV-229E N-term	1,0600	0,0700
COVID-9-HCoV-229E C-term	0,9700	0,0000
COVID-9-HCoV-OC43 N-term	1,1300	0,1400
COVID-9-HCoV-OC43 C-term	1,2300	0,2400
COVID-9-SEB	46,9000	45,9100
COVID-9-00 DMSO	0,9900	0,0000
COVID-10-SARS-CoV-2 N-term	7,1400	5,9300
COVID-10-SARS-CoV-2 C-term	1,0000	0,0000
COVID-10-SARS-CoV-2 NCAP	0,9400	0,0000
COVID-10-HCoV-229E N-term	0,9000	0,0000
COVID-10-HCoV-229E C-term	2,4300	1,2200
COVID-10-HCoV-OC43 N-term	2,2200	1,0100
COVID-10-HCoV-OC43 C-term	2,9800	1,7700
COVID-10-SEB	45,9000	44,6900
COVID-10-00 DMSO	1,2100	0,0000
COVID-11-SARS-CoV-2 N-term	2,070	0,5300
COVID-11-SARS-CoV-2 C-term	2,200	0,6600
COVID-11-SARS-CoV-2 NCAP	1,990	0,4500
COVID-11-HCoV-229E N-term	2,100	0,5600
COVID-11-HCoV-229E C-term	1,910	0,3700
COVID-11-HCoV-OC43 N-term	2,180	0,6400
COVID-11-HCoV-OC43 C-term	2,230	0,6900
COVID-11-SEB	68,7000	67,1600
COVID-11-00 DMSO	1,540	0,0000

CVID	Raw Data CD8+ CD137+	-Background CD8+ CD137+
CVID-1-SARS-CoV-2 N-term	2,7300	0,7800
CVID-1-SARS-CoV-2 C-term	2,8700	0,9200
CVID-1-SARS-CoV-2 NCAP	2,4900	0,5400
CVID-1-HCoV-229E N-term	2,8900	0,9400
CVID-1-HCoV-229E C-term	2,9200	0,9700
CVID-1-HCoV-OC43 N-term	2,2000	0,2500
CVID-1-HCoV-OC43 C-term	2,1800	0,2300
CVID-1-SEB	40,4000	38,4500
CVID-1-00 DMSO	1,9500	0,0000
CVID-2-SARS-CoV-2 N-term	2,070	0,590
CVID-2-SARS-CoV-2 C-term	2,820	1,340
CVID-2-SARS-CoV-2 NCAP	2,050	0,570
CVID-2-HCoV-229E N-term	1,780	0,300
CVID-2-HCoV-229E C-term	1,970	0,490
CVID-2-HCoV-OC43 N-term	1,660	0,180
CVID-2-HCoV-OC43 C-term	1,820	0,340
CVID-2-SEB	47,100	45,620
CVID-2-00 DMSO	1,480	0,000
CVID-3-SARS-CoV-2 N-term	1,0400	0,0900
CVID-3-SARS-CoV-2 C-term	1,0600	0,1100
CVID-3-SARS-CoV-2 NCAP	0,8300	0,0000
CVID-3-HCoV-229E N-term	0,9500	0,0000
CVID-3-HCoV-229E C-term	1,1000	0,1500
CVID-3-HCoV-OC43 N-term	1,0100	0,0600
CVID-3-HCoV-OC43 C-term	1,0600	0,1100
CVID-3-SEB	38,5000	37,5500
CVID-3-00 DMSO	0,9500	0,0000
CVID-4-SARS-CoV-2 N-term	2,8900	0,0000
CVID-4-SARS-CoV-2 C-term	2,9700	0,0000
CVID-4-SARS-CoV-2 NCAP	2,8000	0,0000
CVID-4-HCoV-229E N-term	2,8000	0,0000
CVID-4-HCoV-229E C-term	2,8100	0,0000
CVID-4-HCoV-OC43 N-term	3,0200	0,0500
CVID-4-HCoV-OC43 C-term	2,7600	0,0000
CVID-4-SEB	55,9000	52,9300
CVID-4-00 DMSO	2,9700	0,0000
CVID-5-SARS-CoV-2 N-term	3,1000	0,5300
CVID-5-SARS-CoV-2 C-term	3,0400	0,4700
CVID-5-SARS-CoV-2 NCAP	2,4600	0,0000
CVID-5-HCoV-229E N-term	2,8200	0,2500
CVID-5-HCoV-229E C-term	2,9200	0,3500
CVID-5-HCoV-OC43 N-term	2,5600	0,0000
CVID-5-HCoV-OC43 C-term	2,9400	0,3700
CVID-5-SEB	54,6000	52,0300
CVID-5-00 DMSO	2,5700	0,0000
CVID-6-SARS-CoV-2 N-term	2,5200	0,1300
CVID-6-SARS-CoV-2 C-term	2,4000	0,0100
CVID-6-SARS-CoV-2 NCAP	2,3600	0,0000
CVID-6-HCoV-229E N-term	2,6100	0,2200
CVID-6-HCoV-229E C-term	2,4500	0,0600
CVID-6-HCoV-OC43 N-term	2,4400	0,0500
CVID-6-HCoV-OC43 C-term	2,3100	0,0000
CVID-6-SEB	46,6000	44,2100
CVID-6-00 DMSO	2,3900	0,0000

CVID-7-SARS-CoV-2 N-term	1,41000	0,00000
CVID-7-SARS-CoV-2 C-term	1,88000	0,36000
CVID-7-SARS-CoV-2 NCAP	1,61000	0,09000
CVID-7-HCoV-229E N-term	1,54000	0,02000
CVID-7-HCoV-229E C-term	1,53000	0,01000
CVID-7-HCoV-OC43 N-term	1,52000	0,0000
CVID-7-HCoV-OC43 C-term	1,64000	0,12000
CVID-7-SEB	69,60000	68,08000
CVID-7-00 DMSO	1,52000	0,00000
CVID-8-SARS-CoV-2 N-term	2,72000	0,68000
CVID-8-SARS-CoV-2 C-term	2,42000	0,38000
CVID-8-SARS-CoV-2 NCAP	2,10000	0,06000
CVID-8-HCoV-229E N-term	1,95000	0,0000
CVID-8-HCoV-229E C-term	2,24000	0,20000
CVID-8-HCoV-OC43 N-term	2,20000	0,16000
CVID-8-HCoV-OC43 C-term	2,27000	0,23000
CVID-8-SEB	61,40000	59,36000
CVID-8-00 DMSO	2,04000	0,00000
CVID-9-SARS-CoV-2 N-term	2,940	0,1700
CVID-9-SARS-CoV-2 C-term	3,390	0,6200
CVID-9-SARS-CoV-2 NCAP	2,800	0,0300
CVID-9-HCoV-229E N-term	2,800	0,0300
CVID-9-HCoV-229E C-term	3,150	0,3800
CVID-9-HCoV-OC43 N-term	3,260	0,4900
CVID-9-HCoV-OC43 C-term	3,250	0,4800
CVID-9-SEB	59,7000	56,9300
CVID-9-00 DMSO	2,770	0,0000
CVID-10-SARS-CoV-2 N-term	27,700	1,200
CVID-10-SARS-CoV-2 C-term	28,200	1,700
CVID-10-SARS-CoV-2 NCAP	27,300	0,800
CVID-10-HCoV-229E N-term	25,000	0,0000
CVID-10-HCoV-229E C-term	23,300	0,0000
CVID-10-HCoV-OC43 N-term	22,900	0,0000
CVID-10-HCoV-OC43 C-term	24,600	0,0000
CVID-10-SEB	62,1000	35,6000
CVID-10-00 DMSO	26,500	0,0000
CVID-11-SARS-CoV-2 N-term	5,190	0,9100
CVID-11-SARS-CoV-2 C-term	4,660	0,3800
CVID-11-SARS-CoV-2 NCAP	4,800	0,5200
CVID-11-HCoV-229E N-term	4,770	0,4900
CVID-11-HCoV-229E C-term	4,910	0,6300
CVID-11-HCoV-OC43 N-term	4,780	0,5000
CVID-11-HCoV-OC43 C-term	4,980	0,7000
CVID-11-SEB	42,3000	38,0200
CVID-11-00 DMSO	4,280	0,0000

= negative after substraction of background signal

= positive after substraction of background signal and value above threshold of 1.2-fold above background control

e.g. 0,015 = negative signal because it does not exceed the threshold of 1.2-fold above the background control

SEB = Superantigen Staphylococcal enterotoxin B

00 DMSO = background control only stimulated with DMSO

Supplementary Table 2a: Summary of $CD154^+CD137^+CD4^+$ and $CD137^+CD8^+$ T cell frequencies in CVID patients. Reactive T cells > 0.005% within total CD4+ T cells and with a ≥ 1.2-fold response of the background control (DMSO) were considered as positive.

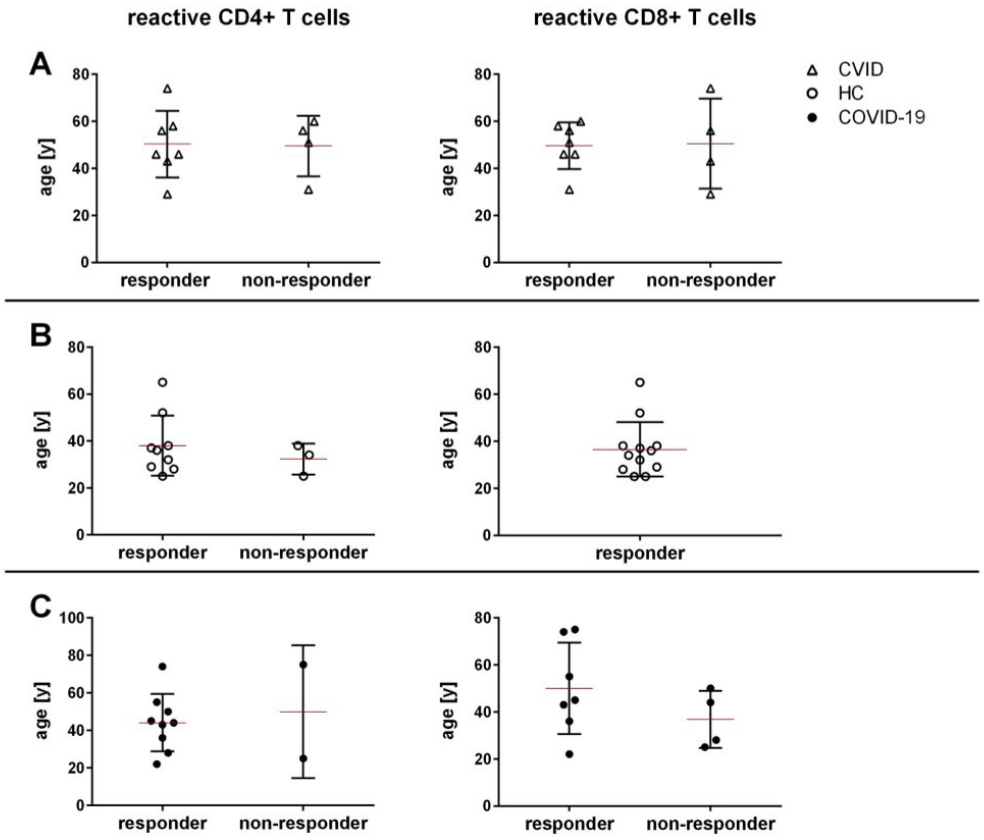
CVID CD4+ CD154+ CD137+								
	SARS-CoV-2 N-term	SARS-CoV-2 C-term	SARS-CoV-2 NCAP	HCoV-229E N-term	HCoV-229E C-term	HCoV-OC43 N-term	HCoV-OC43 C-Term	SEB
CVID-1		0,0500						38,2000
CVID-2								34,2280
CVID-3	0,0190			0,0490	0,0210		0,0200	35,2390
CVID-4	0,1490	0,0890					0,0110	40,5690
CVID-5		0,1200						45,7200
CVID-6								36,7000
CVID-7	0,0270							43,3330
CVID-8								34,2390
CVID-9		0,3600		0,2700	0,3100	0,2700	0,3700	41,0300
CVID-10	0,2600			0,2500				41,7900
CVID-11								36,4500
n=	4	4	0	3	2	1	3	11
% =	36,4	36,4	0,0	27,3	18,2	9,1	27,3	100,0
CD8+ CD137+								
CVID-1	0,7800	0,9200	0,5400	0,9400	0,9700			38,4500
CVID-2	0,5900	1,3400	0,5700	0,3000	0,4900		0,3400	45,6200
CVID-3								37,5500
CVID-4								52,9300
CVID-5	0,5300							52,0300
CVID-6								44,2100
CVID-7		0,3600						68,0800
CVID-8	0,6800							59,3600
CVID-9		0,6200						56,9300
CVID-10								35,6000
CVID-11	0,9100							38,0200
n=	5	4	2	2	2	0	1	11
% =	45,5	36,4	18,2	18,2	18,2	0,0	9,1	100,0

Supplementary Table 2b: Summary of $CD154^+CD137^+CD4^+$ and $CD137^+CD8^+$ T cell frequencies in post COVID-19 HC. Reactive T cells > 0.005% within total $CD4^+$ T cells and with a ≥ 1.2-fold response of the background control (DMSO) were considered as positive.

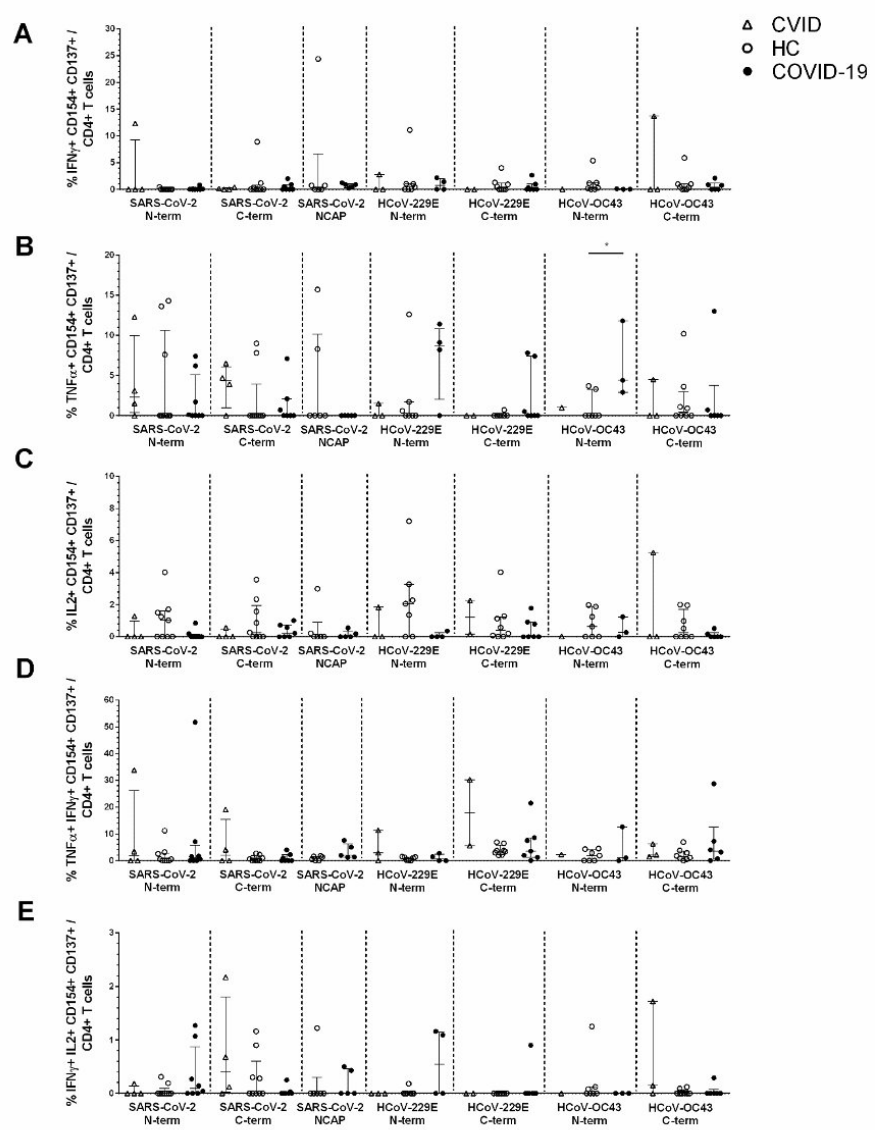
post COVID-19 CD4+ CD154+ CD137+								
	SARS-CoV-2 N-term	SARS-CoV-2 C-term	SARS-CoV-2 NCAP	HCoV-229E N-term	HCoV-229E C-term	HCoV-OC43 N-term	HCoV-OC43 C-Term	SEB
COVID-1	0,1100	0,1100	0,2700	0,0400	0,0500		0,1000	37,2500
COVID-2	0,2700	0,1600	0,3700	0,1500	0,0500	0,1300	0,2900	46,6800
COVID-3								39,4900
COVID-4	0,1770	0,0470	0,0570		0,0250		0,0310	38,3370
COVID-5	0,0500							27,5600
COVID-6	0,0600	0,1900						46,8000
COVID-7	0,6240	0,3540	0,0440	0,0200	0,0250	0,0420	0,0640	35,4440
COVID-8		0,0220			0,0520			30,8120
COVID-9	2,4100	0,1100	0,1200	0,0400	0,0400		0,0500	43,9700
COVID-10	5,6600				0,5700	0,4300	1,0600	34,6100
COVID-11								33,2100
n=	8	7	5	4	7	3	6	11
% =	72,7	63,6	45,5	36,4	63,6	27,3	54,5	100,0
post COVID-19 CD8+ CD137+								
COVID-1	0,4800	0,6900	0,9100	0,5600	0,6600	0,4100		35,2900
COVID-2	1,2300	0,4400	0,4600	0,7800	0,5300	1,7900	0,4700	50,9800
COVID-3								39,4900
COVID-4	1,1700	2,6400	0,3900	0,4600	0,6100	0,4900	0,5100	45,5500
COVID-5								34,3000
COVID-6								40,5900
COVID-7	1,4500	0,7700	0,7000	0,9000	0,7600	0,7200	0,4000	42,8800
COVID-8								44,0300
COVID-9	2,3400						0,2400	45,9100
COVID-10	5,9300				1,2200	1,0100	1,7700	44,6900
COVID-11	0,5300	0,6600	0,4500	0,5600	0,3700	0,6400	0,6900	67,1600
n=	7	5	5	5	6	6	6	11
% =	63,6	45,5	45,5	45,5	54,5	54,5	54,5	100,0

Supplementary Table 2c: Summary of $CD154^+CD137^+CD4^+$ and $CD137^+CD8^+$ T cell frequencies in unexposed HC. Reactive T cells > 0.005% within total $CD4^+$ T cells and with a ≥ 1.2-fold response of the background control (DMSO) were considered as positive.

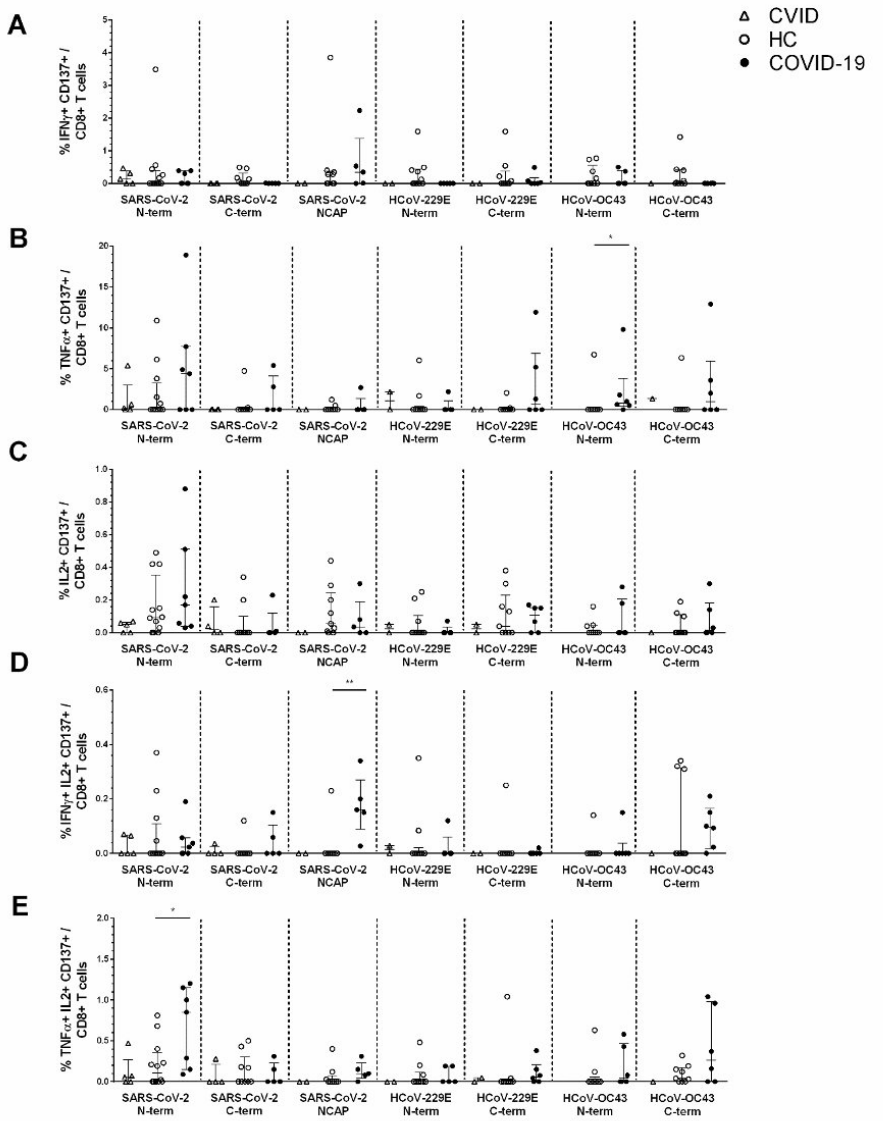
unexposed HC CD4+ CD154+ CD137+								
	SARS-CoV-2 N-term	SARS-CoV-2 C-term	SARS-CoV-2 NCAP	HCoV-229E N-term	HCoV-229E C-term	HCoV-OC43 N-term	HCoV-OC43 C-Term	SEB
HC-1					0,0500			38,0800
HC-2	0,5350	0,0450	0,0120	0,0270		0,0240	0,0100	37,6550
HC-3					0,0190	0,0170	0,0690	41,6190
HC-4	0,8310	0,4810	0,2410	0,5310	0,0710		0,0710	34,3110
HC-5								33,1330
HC-6	0,0200	0,0200						31,8600
HC-7	0,6300	0,2400	0,1900	0,1800	0,1700	0,1600	0,3100	41,3300
HC-8	0,4900	0,0400						33,7200
HC-9	0,0970	0,0670	0,0670	0,0270	0,0570	0,0370	0,0470	47,7270
HC-10	0,1480	0,0260	0,0150	0,0190	0,0220	0,0260	0,0280	33,9680
HC-11	0,0220	0,0210		0,0260	0,0160	0,0170	0,0290	43,8720
HC-12	2,8920	0,8520	0,1620	0,8420	0,4620	1,4920	1,2920	35,4420
n=	9	9	6	7	8	7	8	12
% =	75,0	75,0	50,0	58,3	66,7	58,3	66,7	100,0
unexposed HC CD8+ CD137+								
	SARS-CoV-2 Spike I	SARS-CoV-2 Spike II	SARS-CoV-2 NCAP	HCoV-229E SI	HCoV-229E SII	HCoV-OC43 SI	HCoV-OC43 SII	SEB
HC-1	2,8200	2,8000	1,6000	0,8800	0,9600	1,3400	1,1200	45,8700
HC-2	5,0200	1,2400	0,9400	0,8700	0,9900	0,7900	1,0200	58,7300
HC-3	3,3000	3,4400	1,5800	2,8300	1,8200	1,6400	2,2200	46,4200
HC-4	0,9800	0,4700	1,1800	0,5800	0,5300	0,0900	0,3300	35,4700
HC-5	0,6500			0,2600	0,2800	0,3000	0,3300	35,8400
HC-6	0,3900	0,1800	0,3200	0,2900	0,2500	0,2300		39,3700
HC-7	0,2200			0,2600			0,1400	44,2800
HC-8	0,1900							37,2500
HC-9	0,1700	0,1800	0,1100	0,1800	0,1500	0,1400	0,2900	54,2800
HC-10	0,3500	0,3400	0,4600					40,3900
HC-11	1,1500	0,9700	0,6700	0,8700	0,8300	1,0100	0,7600	53,8900
HC-12	2,0300	0,6100	0,1800	0,5700	0,3700	0,9500	1,0400	30,6400
n=	12	9	9	10	9	9	9	12
% =	100,0	75,0	75,0	83,3	75,0	75,0	75,0	100,0



Supplementary Figure 3: Analysis of age distribution in SARS-CoV-2 peptide responders and non-responders. The age of responders and non-responders to SARS-CoV-2 spike peptides was analyzed for reactive CD4+ and CD8+ T cells in CVID **(A)**, unexposed HC **(B)** and post COVID-19 HC **(C)**. No differences of age between responders and non-responders was observed. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **.



Supplementary Figure 4a: Cytokine expression profiles in activated CD154+ CD137+ CD4 T cells. IFNγ, TNFα, or IL2 single and double producing (dp) activated CD4 T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)**, IL2 **(C)** TNFα + IFNγ **(D)**, IFNγ + IL2 **(E)** producing CD4 activated T cells in response to CoV peptides (1 µg/ml, 16h). CVID patients lacked a response to SARS-CoV-2 NCAP peptide pool in activated CD4 T cells and could not be included in the cytokine profile analyses. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **



Supplementary Figure 4b: Cytokine expression profiles in activated CD137+ CD8 T cells. IFNγ, TNFα, or IL2 single and double producing (dp) activated D8 T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)**, IL2 **(C)** IFNγ + IL2 **(D)**, TNFα + IL2 **(E)** producing CD8 activated T cells in response to CoV peptides (1 µg/ml, 16h). Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **

8.2 Study 2

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SARS-CoV-2 T Cell Response in Severe and Fatal COVID-19 in Primary Antibody Deficiency Patients Without Specific Humoral Immunity

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Morbidity and mortality of COVID-19 is increased in patients with inborn errors of immunity (IEI). Age and comorbidities and also impaired type I interferon immunity were identified as relevant risk factors. In patients with primary antibody deficiency (PAD) and lack of specific humoral immune response to SARS-CoV-2, clinical disease outcome is very heterogeneous. Despite extensive clinical reports, underlying immunological mechanisms are poorly characterized and levels of T cellular and innate immunity in severe cases remain to be determined. In the present study, we report clinical and immunological findings of 5 PAD patients with severe and fatal COVID-19 and undetectable specific humoral immune response to SARS-CoV-2. Reactive T cells to SARS-CoV-2 spike (S) and nucleocapsid (NCAP) peptide pools were analyzed comparatively by flow cytometry in PAD patients, convalescents and naïve healthy individuals. All examined PAD patients developed a robust T cell response. The presence of polyfunctional cytokine producing activated $CD4^+$ T cells indicates a

memory-like phenotype. An analysis of innate immune response revealed elevated CD169 (SIGLEC1) expression on monocytes, a surrogate marker for type I interferon response, and presence of type I interferon autoantibodies was excluded. SARS-CoV-2 RNA was detectable in peripheral blood in three severe COVID-19 patients with PAD. Viral clearance in blood was observed after treatment with COVID-19 convalescent plasma/monoclonal antibody administration. However, prolonged mucosal viral shedding was observed in all patients (median 67 days) with maximum duration of 127 days. PAD patients without specific humoral SARS-CoV-2 immunity may suffer from severe or fatal COVID-19 despite robust T cell and normal innate immune response. Intensified monitoring for long persistence of SARS-CoV-2 viral shedding and (prophylactic) convalescent plasma/specific IgG as beneficial treatment option in severe cases with RNAemia should be considered in seronegative PAD patients.

Keywords: primary immunodeficiencies (PID), primary antibody deficiency (PAD), coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), convalescent plasma (CP), type I interferons, innate immunity

INTRODUCTION

Severity of COVID-19 is associated with increased age, male sex, and comorbidities, such as diabetes, arterial hypertension or pulmonary disease (1). In patients with inborn errors of immunity (IEI), morbidity and mortality of COVID-19 is increased (2–9). However, IEI form a very heterogeneous group of patients (10). With <700 COVID-19 cases in IEI patients reported worldwide (11), a higher risk for severe disease courses was confirmed to occur in older patients and in those with comorbidities (2). Identified immunological host factors include type I interferon (IFN) autoantibodies or disruption of type I IFN signaling, affecting innate immune response to SARS-CoV-2 (12, 13). These autoantibodies are found frequently in APS-1 (autoimmune polyendocrine syndrome) patients (14), however general frequency of type I IFN autoantibodies in PAD patients are currently unknown and other immunological mechanisms underlying the predisposition to severe disease courses remain to be determined.

With regard to humoral immunity, early clinical data from XLA patients suggested that lack of SARS-CoV-2 humoral immunity may be sufficiently compensated by innate and T cell immunity in order to prevent severe COVID-19 (2, 15–20). On the other hand, patients with Good's syndrome, a thymoma-associated hypogammaglobulinemia with B and/or T cell deficiency and lack of specific humoral immune response, were reported to be at significantly increased risk for severe COVID-19 and a high case fatality rate (12). Observations in immunocompetent patients with mild COVID-19 disease, where SARS-CoV-2-specific antibodies are undetectable in 10–15% (21), further highlighted the relevance of non-antibody mediated immunity and levels of specific T cellular immunity were found to be similar, regardless of the presence or absence of SARS-CoV-2 antibodies (22).

Data on SARS-CoV-2-specific T cell immunity in patients with primary antibody deficiency (PAD) is still rare. (Cross) reactive T cells against other endemic human coronaviruses were found in CVID patients (23), and a group of five mild COVID-19 cases in PAD patients, with largely preserved specific antibody response, was recently reported to generate a robust T cell response (24). In addition, studies in PAD patients reported detectable specific T cell responses after COVID-19 vaccination (25–28).

Analyzing T cellular and innate immune response in severe COVID-19 in PAD patients without specific humoral immunity may help to identify necessary and redundant aspects of immune response to COVID-19 and is important to understand the different clinical outcomes in patients with antibody deficiency.

In the present study, we report clinical and immunological findings of 5 PAD patients with severe COVID-19 that failed to generate a specific humoral immune response to SARS-CoV-2. All patients presented with respiratory insufficiency due to COVID-19 pneumonia and two patients developed a fatal disease course. SARS-CoV-2 reactive T cells to spike (S) and nucleocapsid (NCAP) peptide pools were analysed comparatively by flow cytometry in PAD patients, convalescents, and naïve healthy individuals. We analyzed innate immune response by assessing CD169 (also termed: sialic acid-binding immunoglobulin-like lectin 1 or SIGLEC1) expression on monocytes. The activation of the type I IFN pathway results in a rapid increase of SIGLEC1 expression on the surface of macrophages and monocytes. Hence, type I IFN levels correlate with expression of SIGLEC1 on monocytes (29). In addition, presence of type I IFN autoantibodies was examined. Clinical and virological outcomes after COVID-19 convalescent plasma (CP) and monoclonal antibody therapy in three PAD patients with RNAemia are presented.

METHODS

Study Subjects

Five patients with severe COVID-19 and confirmed diagnosis of CVID (n:3; fulfilling the European Society for Immunodeficiencies (ESID) criteria) and Good's syndrome (n:2), six mild COVID-19 convalescent healthy controls (CHC) and naïve healthy controls (HC) were included (**Table 1**). All cases were diagnosed

TABLE 1 | Baseline characteristics of COVID-19 PAD patients.

	Age	Sex	Underlying PAD	Comorbidities	IgG in g/l (before IgRT)	IgA g/l	IgM g/l	$CD4^+$/ nl	$CD8^+$/ nl	NK cells/nl	$CD19^+$/ nl	csmBc (in % of CD19+)
Patient 1	56	m	CVID	GLILD, cachexia (BMI: 16), thalassemia minor, low dose steroid treatment (2.5mg/d)	0.0	<0.1	<0.05	0.36	0.72	0.08	0.10	1.1
Patient 2	48	f	CVID	Bronchiectasis, DM type 2, thalassemia minor	3.2	<0.1	<0.05	0.60	1.17	0.08	0.01	–
Patient 3	49	m	CVID	obesity (BMI 31.2)	0.97	<0.1	<0.05	0.59	0.34	0.06	0.34	0.0
Patient 4	43	m	Good's syndrome	none (thymectomy due to thymoma in 2016)	6.7	1.2	<0.05	0.37	0.31	0.26	0.00	–
Patient 5	49	m	Good's syndrome	New diagnosed and untreated thymoma	0.65	0.15	0.07	0.72	2.17	0.08	0.00	–

BMI, body mass index; csmBc, class-switched memory B cells; CVID, common variable immunodeficiency disorder; DM, diabetes mellitus; f, female; GLILD, granulomatous–lymphocytic interstitial lung disease; IgRT, immunoglobulin replacement therapy; m, male.

by RT-PCR in nasopharyngeal swab. All patients, CHC and HC were not vaccinated against COVID-19. Blood samples from naïve HCs were drawn from the laboratory staff. Those naïve controls did not have a history of prior SARS-CoV-2 infection. Our study was approved by the Ethics Committee of Charité Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments (EA2/092/20 from June 4, 2020). All patients and controls gave written informed consent.

SARS-CoV-2 Antibody Serology

SARS-CoV-2 IgG against the S1 and N-terminal domain of SARS-CoV-2 spike was analyzed in serum by ELISA according to the manufacturer's instructions (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) and also by using fully automated Euroimmun Analyzer I (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). A positive IgG and IgA antibody response was determined by optical density (OD) ratios above 1.1. Titer of SARS-CoV-2 neutralizing IgG was assessed by the use of plaque reduction neutralization test (PRNT) as it has been previously described (30).

SARS-CoV-2 RT-PCR in Plasma

RT-PCR testing for SARS-CoV-2 RNA in plasma or serum samples was done as previously described (31) and by using a preformulated oligonucleotide mixture (Tib-Molbiol, Berlin, Germany).

CD169/SIGLEC1 Expression on Monocytes

In all severe COVID-19 PAD patients, SIGLEC1 expression on monocytes was analyzed in EDTA whole blood based on a method described previously (32). SIGLEC1 levels on monocytes were investigated using an admitted flow cytometry protocol at the clinical diagnostics laboratory (Labor Berlin GmbH). Samples were stained with mouse anti-human antibodies against SIGLEC1 (clone 7–239), CD14 and CD45 (antibodies purchased from Beckman Coulter).

Detection of Anti-Type I IFN Autoantibodies

Presence of anti-type I IFN autoantibodies was analyzed using an electrochemiluminescence immunoassay-platform (MSD, Rockville, U.S.) as described previously (33). Light signal count (LSC) levels <1,980 for anti-Interferon-α antibodies and LSC levels <1,961 for anti-interferon-ω antibodies are considered negative.

Generation of Convalescent Plasma

Plasma was collected from convalescent COVID-19 patients with confirmed neutralizing antibody titer in PRNT50 of at least 1:320 (21) and fulfilling all national standards for blood donation. Single-donor apheresis was conducted with Trima Accel System (Automated Blood Collection) Terumo BCT, Inc. Depending from body weight, two to three bags of 220 ml each were collected and frozen rapidly at <−30°C in less than 8 h after collection. Dosing of CP was conducted to achieve an expected PRNT50 of ≥1:40 in the recipient.

Cell Isolation and Culture

Time point of sampling for T cell analysis is indicated in **Table 2**. Patients were off Dexamethasone treatment at least 10 days prior to sampling. Median time post symptom onset in healthy convalescent controls was 87 days (60–157 days). Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood using density gradient centrifugation and transferred to liquid nitrogen. PBMCs were thawed and rested for 24 h in IMDM medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. Cells were seeded with a concentration of 2×10^6/ml. The background control was incubated with DMSO only and the positive control was stimulated with 3 µg/ml superantigen staphylococcal enterotoxin B (SEB). Stimulation for SARS-CoV-2 responsive T cells was performed using 1 µg/ml of peptide pools for S (two vials with N-term and C-term, PM-WCPV-S-1) and NCAP (PM-WCPV-NCAP-1) proteins (JPT Peptide Technologies GmbH, Berlin). Samples were incubated for 18 h under standard conditions (37°C, 5% CO_2). After 2 h of incubation with peptide pools, brefeldin A (BFA), a secretion inhibitor, was added to the cell culture.

Detection of SARS-CoV-2 Antigen Specific T Cells

PBMCs were stained on their surface with LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific) following fixation and permeabilization (FoxP3 transcription factor staining buffer set, eBioscience). Staining for intracellular markers was conducted using human anti-CD3 BV650 (OKT3), -CD4 PerCp-Cy5.5 (SK3), -CD8 BV510 (RPA-T8), -CD137 PE (4B4-1), CD154 BV421 (24–31), IL-2 AP (MQ1-17H12), -IFNγ BV605 (4S.B3),

TABLE 2 | Clinical and laboratory parameters specific for COVID-19 of PAD patients.

	Underlying PAD	RT-PCR in nasal swab	RT-PCR in peripheral blood	Spike-IgG and -IgA	SIGLEC1 [molecules/ monocyte] (norm. <2,400)	Clinical severity according to WHO R&D blueprint scale	COVID-19 treatment	Clinical outcome	Duration of RT-PCR positivity in nasal swab [d]	Time point of blood sampling for T cell response after symptom onset [d]
Patient 1	CVID	pos.	pos. (max. viral load: 3.2×10^4 copies/ml)	neg.	9,771	6→8	dexamethasone, IVIG, CP	deceased	fatal COVID-19 (40)	39
Patient 2	CVID	pos.	neg.	neg.	15,485	4	dexamethasone, IVIG,	recovered	62	40
Patient 3	CVID	pos.	neg.	neg.	11,741	4	dexamethasone	recovered	61	24
Patient 4	Good's syndrome	pos.	pos. (max. viral load: 7×10^4 copies/ml)	neg.	1,423	5	dexamethasone, IVIG, CP	recovered	127	128
Patient 5	Good's syndrome	pos.	pos. (max. viral load: 8.8×10^4 copies/ml)	neg.	11,758	7→8	dexamethasone, IVIG, mAb	deceased	47	not done

CP, convalescent plasma; CVID, common variable immunodeficiency disorder; IVIG, intravenous immunoglobulin; mAb, monoclonal antibodies; neg., negative; pos, positive; SIGLEC1, sialic acid-binding immunoglobulin-like lectin 1.

-TNFα AF700 (MAb11) (Biolegend). Samples were analyzed using CytoflexLX Flow Cytometer (Beckman Coulter) and FlowJo software (version 10.6.2, BD). A positive T cell response was defined as frequency of $CD154^+CD137^+CD4^+$ T cells being ≥0.005% within total $CD4^+$ T cells. Moreover, samples signals had to exceed the background signal by a minimum of 20%. This threshold value refers to the range in which 95% of all negative samples are. In order to exclude unspecific activation the background signal (DMSO sample) was subtracted from the peptide stimulated samples. Frequencies of effector cytokine producing antigen specific T cells were analyzed for IFNγ, TNFα, IL-2 and seven different subsets were examined by Boolean combination gating strategy to identify single (sp), double (dp) or triple (tp) cytokine producing activated T cell populations.

Statistical Analyses

Data analyses was performed using GraphPad Prism 6 software. Nonparametric statistical methods were used. Continuous variables were expressed as median and interquartile range (IQR). Univariate comparisons of T cell responses in two independent groups were performed using Mann–Whitney-U test. A p-value of <0.05 was considered statistically significant. Because of multiple testing p-values are considered descriptive.

RESULTS

Study Subjects and Humoral Immune Response

PAD patients fulfilled ESID criteria for CVID (n:3) and Good's syndrome (n:2) (see **Table 1** for detailed patient baseline characteristics). All five PAD patients had SARS-CoV-2 infections confirmed by RT-PCR in nasopharyngeal swab. Severity was graded according to the Centre of Disease Control (34). Extensive bilateral COVID-19 pneumonia was detected by chest CT in four cases (patient #1, #3, #4, #5), with onset of respiratory insufficiency occurring between days 8 and 20 PSO. Patient #2 had milder respiratory insufficiency (SpO2: 91% at room air) together with infiltrates in chest X-ray. Please refer to **Table 2** and **Supplementary Text 1** for detailed clinical case descriptions.

All PAD patients (patient #1–5) failed to mount a specific antibody response to SARS-CoV-2 (negative for IgG and IgA antibodies) (**Table 2**). Serological response in RT-PCR-confirmed COVID-19 convalescent cohort showed positive SARS-CoV-2-IgG in three, borderline positive tests in two and a negative test result in one patient. SARS-CoV-2-IgA was positive in five individuals, while one convalescent individual was tested negative. As expected, SARS-CoV-2-IgG and -IgA was not detected in healthy controls (**Supplementary Figure 1**).

PAD Patients With COVID-19 Show High Frequencies of SARS-CoV-2 Specific T-Cells

For specific T cell responses we analyzed 4 PAD patients, 6 CHC and 6 naïve HC. For detailed gating strategy please refer to **Supplementary Figure 2**.

All analyzed PAD patients had SARS-CoV-2 S reactive $CD154^+CD137^+CD4^+$ T cells either to the N- (n:4) or C-terminal (n:3) part. 6/6 CHC had reactive $CD4^+$ T cells to both SARS-CoV-2 S peptide pools, whereas naïve HCs had fewer N- (n = 4) and C-terminal (n = 2) responses. PAD patients showed significantly higher frequencies of $CD4^+$ antigen specific T cells to the N-terminus compared to CHC (p = 0.005) and naïve HC (p = 0.014) (**Figure 1A**). CHC also showed significantly higher Spike N-terminal reactive $CD4^+$ T cells compared to naïve HC (p = 0.02).

Regarding C-terminal S reactive $CD4^+$ T cells, PAD patients had higher frequencies compared to CHC (p = 0.02). Moreover, PBMC stimulation with SARS-CoV-2 NCAP peptide pool elucidated higher frequencies of antigen specific $CD4^+$ T cells in 4/4 PAD patients compared to CHC (p = 0.03) and also in CHC compared to naïve HC (p = 0.04) (**Figure 1A**). Stimulation with the positive control SEB resulted in similar frequencies of $CD4^+$ activated T cells among the investigated groups (**Figure 1B**).

Patient #1 already expressed SARS-CoV-2-S-reactive T cells seven months prior to his severe COVID-19 disease as reported in our previous study on SARS-CoV-2 cross-reactive T cells in CVID patients (23). Before SARS-CoV-2 infection patient #1 only expressed low levels of activated T cells ($CD154^+CD137^+CD4^+$) reactive to N-terminus of SARS-CoV-2 S protein and also against S of human endemic corona viruses. During severe COVID-19 disease, activated $CD4^+$ T cells reactive to SARS-CoV-2 C-terminal S and NCAP were also detectable and frequencies of activated, reactive T cells were generally much higher (1.8–2.2% $CD154^+CD137^+CD4^+$ during COVID-19) (**Supplementary Table 1**).

$CD8^+$ T cell responses to peptide pools of SARS-CoV-2 structural proteins were observed in all 4 PAD patients. However, frequencies showed no differences compared to CHC and HC (**Supplementary Figure 3A**). SEB stimulation serving as positive control resulted in comparable frequencies of activated $CD137^+CD8^+$ T cells among the investigated groups (**Supplementary Figure 3B**).

SARS-CoV-2 Antigen Specific $CD4^+$ T Cells of PAD Patients and Convalescent Healthy Controls Express a Distinct Cytokine Profile

In all analyzed PAD patients and CHC we observed triple-positive (tp) antigen specific $CD4^+$ T cells following stimulation with SARS-CoV-2 Spike (S) N- and C-terminal peptide pools (see **Figure 2A** and **Supplementary Figure 2** for gating strategy and representative flow cytometry plots). PAD patients showed a higher expression of triple cytokine producing activated $CD4^+$ T cells compared to CHC (C-terminal, p = 0.02) and naïve HC (N-terminal, p = 0.014) in response to SARS-CoV-2 S. Frequencies of S- and NCAP-reactive triple cytokine producing activated $CD4^+$ T cells were significantly higher in CHC compared to HC (N-terminal, p = 0.03; C-terminal, p = 0.04) (**Figure 2A**). CHC further expressed higher NCAP reactive triple cytokine producing activated $CD4^+$ T cells compared to HC (p = 0.04). Overall highest responses to specific SARS-CoV-2 stimulation were observed for TNFα + IL-2 double cytokine producing activated $CD4^+$ T cells with PAD patients showing significantly higher S N-terminal specific TNFα + IL-2 dp $CD4^+$ T cells compared to CHC (p = 0.014) and HC (p = 0.005). This was as well observed for PAD patients compared to CHC (p = 0.03) after NCAP specific stimulation (**Figure 2B**). T cell responses for the remaining single and double cytokine producing $CD4^+$ T cell subsets did not show a distinct pattern and are shown in **Supplementary Figure 4**.

$CD8^+$ T cells showed no differences in frequencies of cytokine reactive T cells (**Supplementary Figures 5A–G**).

Detection of Viral Load in Blood, Prolonged Viral Shedding and Treatment With Specific SARS-CoV-2 Antibodies in PAD Patients

In three severe cases of COVID-19 (patient #1, #4 and #5), SARS-CoV-2-RNA was detected in peripheral blood with 3.2×10^4, 7×10^4 and 8.8×10^4 copies/ml respectively (**Table 2**). Transfusion of 440 ml AB0-compatible COVID-19 CP with confirmed levels of neutralizing antibodies (PRNT50 ≥1:320) was conducted in patient #1 and #4 and was well tolerated. Patient #5 received 8 g of Casirivimab/Imdevimab. Rapid decline of SARS-CoV-2 viral load in peripheral blood was observed after CP treatment within 3 days post treatment. Decrease of viral load

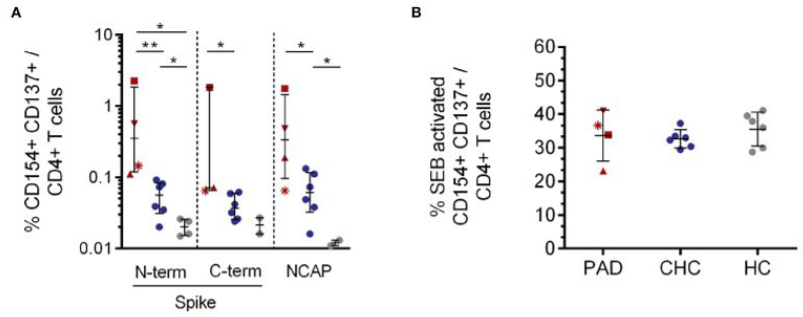

FIGURE 1 | $CD154^+CD137^+CD4^+$ T cell response to SARS-CoV-2 Spike and NCAP peptide pools and SEB positive control. PBMCs of PAD (n = 4, red), CHC (n = 6, blue) and naïve HC (n = 6, grey) were stimulated with 1 µg/ml SARS-CoV-2 peptides or 3 µg/ml SEB. Frequencies of activated $CD154^+CD137^+CD4^+$ **(A)** T cells after stimulation with the different SARS-CoV-2 peptides and SEB **(B)**. Only T cell responses above the threshold of 20% above background activation are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤0.05 was considered as statistically significant. p ≤0. 05 = *; p ≤0.001 = **. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

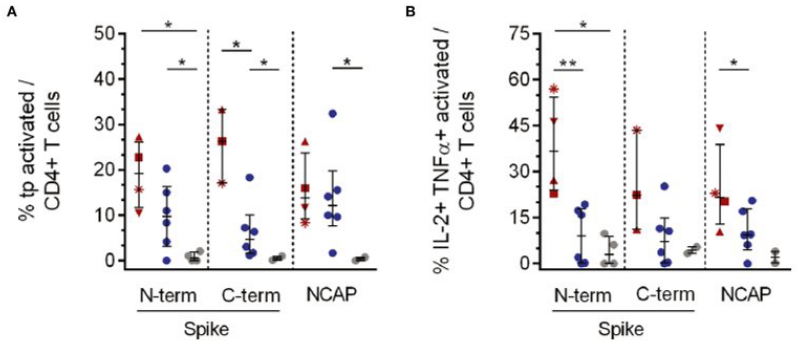

FIGURE 2 | Triple and double cytokine producing activated CD4$^+$ T cell subsets in response to SARS-CoV-2 specific peptide stimulation. PBMCs of PAD (n = 4, red), CHC (n = 6, blue) and naïve HC (n = 6, grey) were stimulated with 1 µg/ml SARS-CoV-2 peptides or 3 µg/ml SEB. IFNγ, TNFα and IL-2 tp activated CD4$^+$ T cells were analyzed by Boolean combination gating strategy. Cytokine expression profile in tp activated CD4$^+$CD154$^+$CD137$^+$ **(A)** T cells, and also TNFα + IL-2 dp **(B)** activated CD4$^+$ T cells in response to SARS-CoV-2 peptide pools are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤0.05 was considered as statistically significant. p ≤0. 05 = *; p ≤0.001 = **. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

in peripheral blood was seen 6 days after treatment with monoclonal antibodies Casirivimab/Imdevimab and RT-PCR in plasma turned negative after 14 days. Serological follow-up showed detectable SARS-CoV-2-specific IgG (#1, #4, #5) and IgA (#1 and #4) respectively (see **Table 3**).

Prolonged viral shedding (>40 days) was confirmed by RT-PCR in nasopharygeal swabs in all patients with patient #4 remaining positive for 127 days PSO (**Table 2**).

Innate Immune Response by SIGLEC1 Expression on Monocytes and Anti-Type I IFN Autoantibodies

SIGLEC1 on monocytes is a downstream molecule in IFN signaling serving as a surrogate marker of type I IFN signature (35). Apart from patient #4, all patients had elevated levels of SIGLEC on monocytes, indicating a normal type I IFN response. Patient #4 expressed low levels of SIGLEC1 (1,423 molecules/monocyte) despite RNAemia (**Table 2**). However, no autoantibodies against IFN-α or IFN-ω were detected in any of our patients (**Supplementary Table 2**). Reanalysis of previously conducted whole exome sequencing of patient #1 revealed a heterozygous mutation in *IFNAR1* (V307I), which was reported to be of no functional relevance [(12) and personal communication with JL Casanova and Q Zhang]. WES data of patient #3 did not reveal any suspect or disease causing mutations. In the remaining patients WES was not conducted.

DISCUSSION

In the present study of severe COVID-19 cases in PAD patients with undetectable SARS-CoV-2-specific antibodies, a robust T cell response was observed. Similar to convalescent immunocompetent patients, where polyfunctional SARS-CoV-2-specific T cells have been described (36), triple cytokine-producing, activated T cells were observed in our PAD patients, indicating the generation of a memory-like phenotype (36, 37). T cell response to COVID-19 was even stronger in PAD patients than in convalescent healthy controls (CHC), which is probably due to the generally milder disease course and slightly later median time point of sampling in the control group.

Innate immune response by type I IFN was shown to be of pivotal importance, and patients with defects in type I IFN signaling or autoantibodies against interferon-α or -ω are at increased risk for severe COVID-19 (12, 13). In IEI patients, type I IFN autoantibodies were reported for APS-1 patients (14). Although described previously (38, 39), impact of type I IFN autoantibodies on the high case fatality rate in Good's syndrome was never examined.

By assessing SIGLEC1 on monocytes, a robust *ex vivo* marker of type I IFN response, we observed an expected rise of expression levels in 4/5 patients. Low levels in patient #4 (Good's syndrome) and decline of SIGLEC1 expression levels in patient #1, despite RNAemia, prompted evaluation of anti-cytokine antibodies. However, we could not find anti-IFN-α or -IFN-ω autoantibodies in any of our severe COVID-19 cases. Decreasing SIGLEC-1 expression during disease course might be related to concurrent medication with dexamethasone.

SARS-CoV-2 viral load, and in particular viral load in blood, was shown to be associated with increased risk of mortality (40, 41). In the here reported severe COVID-19 patients without specific antibodies but detectable RNAemia, viral clearance in blood, and in part also clinical improvement, was clearly associated with administration of specific antibodies in convalescent plasma (CP) and monoclonal antibodies. COVID-19 CP has been used previously in IEI patients and its

TABLE 3 | SARS-CoV-2 antibody serology in serum and viral load in peripheral blood of PAD patients treated with convalescent plasma and monoclonal antibodies.

	SARS-CoV-2-Spike-IgG ratio	SARS-CoV-2-Spike-IgA ratio	PRNT50	SARS-CoV-2 RT-PCR in peripheral blood
Patient 1 (before 1st CP treatment)	0.18 (neg.)	0.06 (neg.)	<1:20 (neg.)	pos. (3.2×10^4 copies/ml)
Patient 1 (d1 after 1st CP treatment)	2.16 (pos.)	0.37 (neg.)	1:20	pos. (6.1×10^3 copies/ml)
Patient 1 (d3 after 1st CP treatment)	1.16 (pos.)	1.3 (pos.)	not done	neg.
Patient 1 (before 2nd CP treatment)	0.49 (neg.)	0.08 (neg.)	not done	neg.
Patient 1 (d2 after 2nd CP treatment)	1.88 (pos.)	1.16 (pos.)	1:40	not done
Patient 4 (before CP treatment)	0.07 (neg.)	0.39 (neg.)	<1:20 (neg.)	pos. (7×10^4 copies/ml)
Patient 4 (d1 after CP treatment)	4.22 (pos.)	1.08 (borderline pos.)	1:80	not done
Patient 4 (d3 after CP treatment)	2.43 (pos.)	0.42 (neg.)	not done	<500 copies/ml (negative)
Patient 5 (before mAb treatment)	0.04 (neg.)	0.06 (neg.)	not done	pos. (8.8×10^4 copies/ml)
Patient 5 (d6 after mAb treatment)	10.94 (pos.)	0.18	not done	pos. (5×10^3 copies/ml)
Patient 5 (d14 after mAb treatment)	>11 (pos.)	0.21	not done	neg.

CP, convalescent plasma; mAb, monoclonal antibody; neg., negative; pos., positive.

efficacy is usually evaluated clinically and according to viral load in nasopharyngeal or respiratory tract specimens (42), but evaluation of virus neutralizing capacity in blood in IEI patients is missing (17, 43, 44).

It is unknown, whether a lack of specific humoral immunity to SARS-CoV-2 per se leads to an increased risk of RNAemia. Based on numerous mild COVID-19 cases in XLA patients (2, 15–20), at least generally persisting and high viral loads seem unlikely.

Data obtained during acute SARS-CoV-2 infection from larger cohorts of mild and severe COVID-19 cases in antibody deficient patients are needed to approach this question. Our data suggest a more differentiated approach to CP and SARS-CoV-2 monoclonal antibody treatment, which should include testing of viral load in blood and assessing antibody titer in the plasma recipient in order to detect accelerated reduction of transfused antibody levels related to virus neutralization and antibody consumption.

Despite a robust T cell response, we observed prolonged viral shedding > 40 days after initial positive RT-PCR in all our patients. Patient #4, suffering from Good's syndrome, even remained positive for 127 days. Prolonged viral shedding of SARS-CoV-2 in IEI patients was reported previously (2). Although longer periods of persisting viral shedding are also reported in the general (45) and elderly seropositive population (46), the extended duration of viral shedding in primary and secondary antibody deficiency suggests a role for antibodies in viral clearance. Of note, prolonged shedding was shown to be associated with marked within-host genomic evolution of SARS-CoV-2 with continuous turnover of dominant viral variants (47), warranting prolonged surveillance and testing of seronegative PID patients with COVID-19.

Overall, our observations of a robust T cellular immunity and detectable type I IFN innate response in severe COVID-19 patients without specific SARS-CoV-2 antibodies support the relevance of humoral immunity.

Our study included one CVID patient where SARS-CoV-2-reactive T cells had been described prior to his COVID-19 disease (23). These preexisting cross-reactive T cells to SARS-CoV-2, were described in 35–90% of unexposed healthy individuals (36, 37, 48–52), however their role during infection is a matter on ongoing debate. At least in our patient, pre-existing reactive T cells in the absence of specific SARS-CoV-2 antibodies did not prevent severe COVID-19. However, the level of preexisting T cell immunity was rather low and increased substantially during infection. Therefore this observation does not allow to draw conclusions on possible protective levels of T cell immunity after vaccination.

As shown previously for XLA and CVID patients after vaccination against seasonal influenza or hepatitis B (53–55), induction of specific T cell immunity is also detectable after COVID-19 vaccination in IEI patients (25–28).

The increased levels of NCAP-reactive T cells suggest that patients unable of generating specific antibodies, might possibly benefit from vaccines immunizing against spike and nucelocapsid structures.

Having in mind the recent data on prophylactic monoclonal antibody treatment (56, 57), our observations support the use of prophylactic antibody therapy in patients with known failure to mount a specific antibody response against SARS-CoV-2.

Although current data indicate, that commercially available immunoglobulin products already contain neutralizing SARS-CoV-2 antibodies to a certain extent (58), neutralizing SARS-CoV-2 antibodies might not be detectable in commercially available products before Spring/Summer 2022 due to the long production process and safety regulations. Clinical effectiveness of SARS-CoV-2-specific antibodies in immunoglobulin products against present and emerging SARS-CoV-2 variants remain to be investigated.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Charité Universitätsmedizin Berlin in accordance with the 1964 Declaration of Helsinki and its later amendments (EA2/092/20 from June 4, 2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LH and CS made substantial contributions to conception and design of the study and lead the project. LH, MK, AW, SG, JK, DZ, KW, and AP made patient samples available. NA, UK and APr made convalescent plasma available. SS planned and performed all experiments on T cell assays, analyzed results, composed all figures and interpreted data. SS and LH wrote the manuscript. LG and SB provided support during experimental implementation. SS, CS, and LH interpreted data. VC, TS, and CD and performed analysis of SARS-CoV-2 serology. CM, OS, and TM analyzed type I IFN autoantibodies. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.840126/full#supplementary-material

Supplementary Figure 1 | SARS-CoV-2 antibody serology in COVID-19 PAD patients, convalescent (CHC) and naïve (HC) healthy controls. Serum IgG **(A)** and IgA **(B)** against the N-terminal domain of the spike protein, including the immunologically relevant receptor binding domain (RBD) of SARS-CoV-2, were analyzed by EUROIMMUN Anti-SARS-CoV-2 ELISAs. Results are evaluated by calculating a ratio of the OD of the control or patient sample over the OD of a calibrator. A ratio <0.8 is negative, ratio ≥ 0.8 to <1.1 is borderline, ratio ≥1.1 is positive (dotted lines). Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼, patient #5 ♦.

Supplementary Figure 2 | Representative flow cytometry plots for analysis of activated CD4+ and CD8+ T cells and their expression of effector cytokines. Example of a gating strategy in presence of a specific stimulation with SARS-CoV-2 Spike N-terminal peptide pool. PBMCs were gated on lymphocytes. Douplets as well as dead cells were excluded. Living CD3+ T cells were distinguished in CD4+ helper and CD8+ effector T cells. Within those T cell populations activated CD4+CD154+CD137+ and CD8+CD137+ T cells were gated and the expression of IFNγ, TNFα and IL-2 analyzed. Single, double or triple cytokine producing activated T cell subsets were analyzed using Boolean combination gates.

Supplementary Figure 3 | CD137+CD8+ T cell response to SARS-CoV-2 Spike and NCAP peptide pools and SEB positive control. PBMCs of PAD (n=4, red), CHC (n=6, blue) and naïve HC (n=6, grey) were stimulated with 1 µg/ml SARS-CoV-2 peptides or 3 µg/ml SEB. Frequencies of activated CD137+ CD8+ T cells **(A)** after stimulation with the different SARS-CoV-2 peptides. Frequencies of activated CD137+ CD8+ T cells **(B)** after stimulation with SEB. Only T cell responses above the threshold of 20% above background activation are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

Supplementary Figure 4 | Single and double cytokine producing activated CD4+ T cells in response to SARS-CoV-2 specific peptide stimulation. IFNγ, TNFα, or IL2 single (sp) or double (dp) producing activated CD4+ T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)** and IL2 **(C)** sp and IFNγ+IL-2+ **(D)** and IFNγ+ TNFα **(E)** dp activated CD4+ T cells in response to SARS-CoV-2 peptide pools are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

Supplementary Figure 5 | Single, double and triple cytokine producing activated CD8+ T cells in response to SARS-CoV-2 specific peptide stimulation. IFNγ, TNFα, or IL2 single (sp) or double (dp) or triple (tp) producing activated CD8+ T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)** and IL2 **(C)** sp and IFNγ+IL-2 **(D)**, IFNγ+TNFα **(E)** IL-2+TNFα **(F)** dp and IFNγ+TNFα+IL2 tp activated CD8+ T cells in response to SARS-CoV-2 peptide pools are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

Supplementary Table 1 | Activated CD4+ T cell frequencies and cytokine response of patient #1 before and during SARS-CoV-2 infection. Please refer to Excel sheet **Table 1**.

Supplementary Table 2 | Light signal count (LSC) for anti-IFN α and -ω autoantibodies determined by electrochemiluminescence immunoassay-platform.

Supplementary Text 1 | Detailed clinical case descriptions of PAD Patients.

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Conflict of Interest: VC is named together with Euroimmun on a patent application filed recently regarding detection of antibodies against SARS-CoV-2. Authors CM, TM and OS are employed by Labor Berlin GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Online Supplementary Text 1: Detailed clinical case descriptions of PAD patients

Patient #1

Patient #1 is a 56-year old male patient of Libanese provenance with CVID (EUROclass smB-21low) diagnosed 6 years earlier. Under immunoglobulin replacement therapy (IgRT) patient 1 was clinically stable with no frequent respiratory infections despite mild GLILD. Patient #1 suffered from recurrent *Campylobacter jejuni* infections with weight loss (BMI: 16) and thalassemia minor.

Patient #1 was tested positive for SARS-CoV-2 in nasal swab by RT-PCR in October 2020. He presented initially with fever and gastrointestinal symptoms and was admitted to hospital at day 3 post symptom onset (PSO). Chest CT showed bilateral pneumonia. Day 8 PSO he developed respiratory insufficiency requiring supplemental oxygen. Treatment with high dose IVIG and Dexamethasone was initiated. Despite broad antimicrobial treatment due to suspected bacterial superinfection, respiratory condition deteriorated and patient was admitted to ICU for high flow oxygen treatment at day 22 PSO. SARS-CoV-RT PCR detected viral load in peripheral blood ($3{,}2x10^4$ copies/ml) and remained positive in sputum without presence of specific SARS-CoV-2 antibodies at day 22 PSO. Therefore 440ml of convalescent plasma (including neutralizing antibodies tested in PRNT50 with 1:320) was administered at day 23 PSO and resulted in viral clearance in peripheral blood at day 26 PSO. With continued requirement for high flow oxygen treatment, patient #1 showed only a mild clinical improvement. In the context of low neutralizing antibody titer, another 440ml of COVID-19 convalescent plasma was infused. Serological data showed an expected rise of SARS-CoV-2-IgG and –IgA antibodies and patients (see table 3) respiratory condition improved from 40 l/min oxygen via highflow to 4 l/min via nasal cannula within 3 days. Viral detection in nasopharyngeal swab persisted. At day 37 PSO patient #1 developed a massive intracerebral hemorrhage and died at day 40 PSO despite immediate neurosurgical intervention.

Post mortem analysis excluded presence of type I interferon autoantibodies. Reanalysis of previously conducted whole exome sequencing revealed a heterozygous mutation in *IFNAR1* (V307I), which was reported to be of no functional relevance (Zhang et al.).

Patient #2

Patient #2 is 48-year old female CVID (Euroclass B-) patient of Turkish origin. Patient #2 presented with fever, general fatigue, nausea and vomiting and was tested positive for SARS-

CoV-2 by RT-PCR in nasal swab in 11/2020 and 2 days PSO. Patient #2 had mild respiratory symptoms (SpO2:92% at room air) and discrete streaky pattern in chest X-ray. Due to respiratory symptoms supplemental oxygen and treatment with dexamethasone was started at day 8 PSO. Clinical condition improved rapidly, however patient 2 remained positive for SARS-CoV-2 by RT-PCR until day 62 PSO. SARS-CoV-2-Spike-IgG and –IgA were not detectable at any time during COVID-19. Type I interferon autoantibodies were not detected. Presence of SARS-CoV-2 by RT-PCR in peripheral blood was not assessed.

Patient #3

Patient 3 is a 49-year old male Caucasian patient with CVID (EUROclass smB-21norm). Patient 3 is under subcutaneous immunoglobulin replacement therapy without relevant infectious or non-infectious complications. Patient 3 presented with fever and diarrhea in January 2021 and was tested positive for SARS-CoV-2 by RT-PCR the same day. On day 14 PSO patient 3 was admitted to hospital with continuing fever up to 40°C and increasing coughing and dyspnea. Bilateral COVID-19 pneumonia was detected by chest CT and supplemental oxygen therapy was initiated at a low flow rate of 3-4 Liters/min. Patient 3 required supplemental oxygen for the following 12 days and was discharged from hospital at day 27 PSO. SARS-CoV-2 RT-PCR resulted negative in peripheral blood but remained positive in nasal swab for 61 days. SARS-CoV-2-Spike-IgG and –IgA were not detectable at any time during COVID-19. Type I interferon autoantibodies were not detected.

Patient #4

Patient 4 is a 43-year old Caucasian patient with Good's syndrome diagnosed 5 years earlier. Patient 4 has a mild IgG reduction, no detectable IgM and a complete loss of CD19+ B cells. Patient 4 presented with symptoms of a common cold and was tested positive for SARS-CoV-2 by RT-PCR in November 2020. At day 20 PSO patient 4 was admitted to hospital due to respiratory insufficiency presenting bilateral pulmonary infiltrates in chest CT. High dose IVIG and dexamethasone had no clinical benefit and patient 4 was transferred to ICU for non-invasive ventilation (NIV).

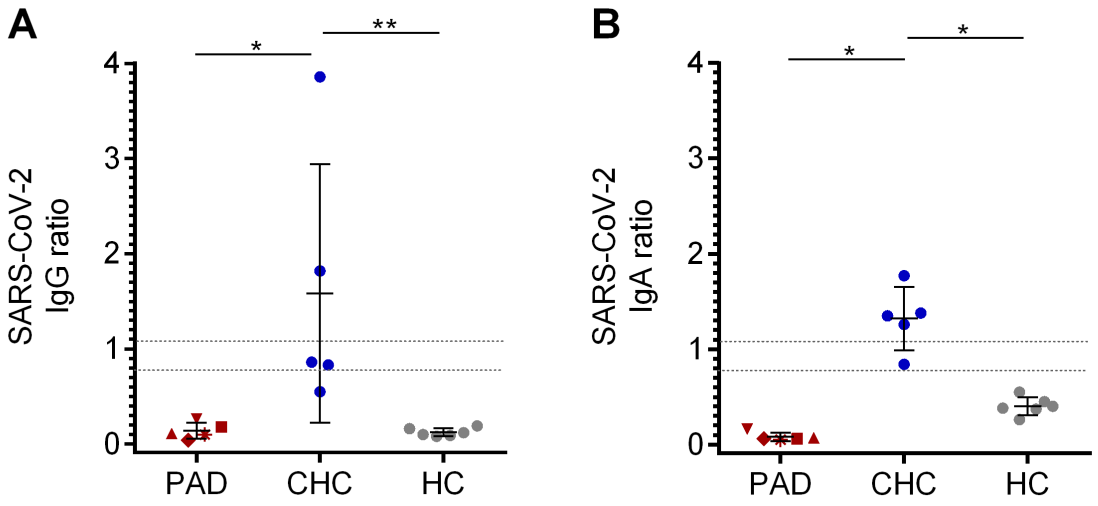
Patient 4 was tested positive for SARS-CoV-2 in peripheral blood by RT-PCR on day 30 and day 45 PSO. Due to viremia with continuing respiratory insufficiency with requirement for NIV and persistent fever patient 4 received 440ml of COVID-19 convalescent plasma (including neutralizing antibodies tested in PRNT50 with 1:320) on day 46 PSO. Patient 4 improved significantly within 12h after convalescent plasma administration, presenting improved

oxygenation and normal temperature. Viral load in peripheral blood declined from $7x10^{4}$ to negative on day 50 PSO.

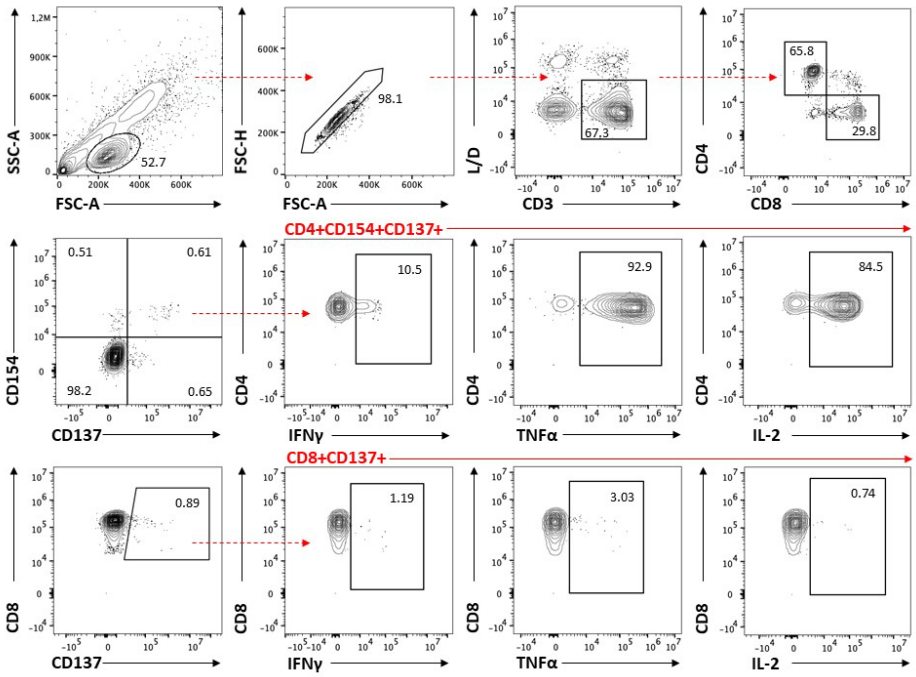
SARS-CoV-2-Spike-IgG and –IgA were not detectable at any time during COVID-19 but showed an expected increase and detectable neutralizing antibodies after infusion of convalescent plasma. Type I interferon autoantibodies were not detected. Patient 4 remained SARS-CoV-2 positive by RT-PCR in nasal swab for 127 days PSO.

Patient #5

Patient #5 is a 48-year old patient of Turkish descendance and was diagnosed with Good's syndrome during COVID-19. Seven days after symptom onset, patient #5 was admitted to hospital due to respiratory insufficiency. Mechanical ventilation was started on day 20 PSO. After unsuccessful treatment with dexamethasone, immunoglobulins and different antibiotics patient #5 was transferred to our center. He received 8g of Casirivimab/Imdevimab and ECMO treatment was initiated on day 30 PSO. Serological and virological follow-up showed detectable SARS-CoV-2-specific IgG but no specific IgA and viral clearance of SARS-CoV-2 in peripheral blood within 14 days post treatment. Patient #5 deceased 47 days after disease onset despite ECMO due septic shock.



Supplementary Figure 1 | SARS-CoV-2 antibody serology in COVID-19 PAD patients, convalescent (CHC) and naïve (HC) healthy controls. Serum IgG **(A)** and IgA **(B)** against the N-terminal domain of the spike protein, including the immunologically relevant receptor binding domain (RBD) of SARS-CoV-2, were analyzed by EUROIMMUN Anti-SARS-CoV-2 ELISAs. Results are evaluated by calculating a ratio of the OD of the control or patient sample over the OD of a calibrator. A ratio <0.8 is negative, ratio ≥ 0.8 to <1.1 is borderline, ratio ≥1.1 is positive (dotted lines). Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric two-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. $p \leq 0.05$ = *; $p \leq 0.005$ = **. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼, patient #5 ♦.



Supplementary Figure 2 | Representative flow cytometry plots for analysis of activated $CD4^+$ and $CD8^+$ T cells and their expression of effector cytokines. Example of a gating strategy in presence of a specific stimulation with SARS-CoV-2 Spike N-terminal peptide pool. PBMCs were gated on lymphocytes. Douplets as well as dead cells were excluded. Living $CD3^+$ T cells were distinguished in $CD4^+$ helper and $CD8^+$ effector T cells. Within those T cell populations activated $CD4^+CD154^+CD137^+$ and $CD8^+CD137^+$ T cells were gated and the expression of IFNγ, TNFα and IL-2 analyzed. Single, double or triple cytokine producing activated T cell subsets were analyzed using Boolean combination gates.

Supplementary Table 1 | Activated CD4⁺ T cell frequencies and cytokine response of patient #1 before and during SARS-CoV-2 infection. Please refer to Excel sheet **Table 1**.

Activated CD4+ T cell frequencies before COVID-19

ID	Raw Data CD4+ CD154+ CD137+	-Background CD4+CD154+CD137+
IEI_1_SARS-CoV-2 N-Term	0,0800	0,0190
IEI_1_SARS-CoV-2 C-Term	0,0560	0,0000
IEI_1_SARS-CoV-2 NCAP	0,0420	0,0000
IEI_1_HCoV-229E N-Term	0,1100	0,0490
IEI_1_HCoV-229E C-Term	0,0820	0,0210
IEI_1_HCoV-OC43 N-Term	0,0540	0,0000
IEI_1_HCoV-OC43 C-Term	0,0810	0,0200
IEI_1_SEB	35,3000	35,2390
IEI_1_00 DMSO	0,0610	0,0000

Activated CD4+ T cell cytokine response before COVID-19

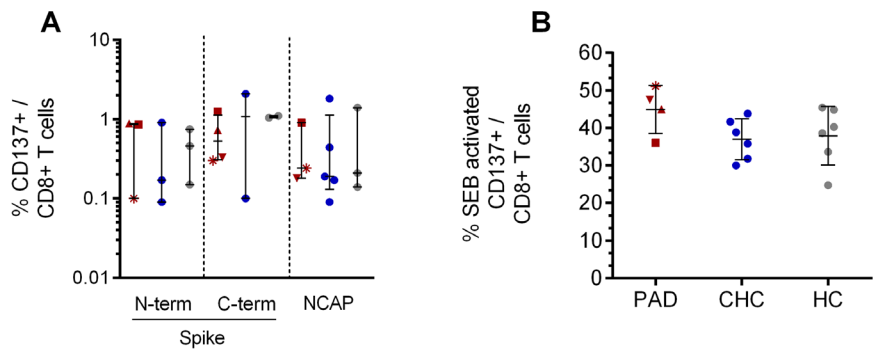
CD4+ act TNFa+ only	SARS-CoV-2 N-Term	SARS-CoV-2 C-Term	SARS-CoV-2 NCAP	HCoV-229E N-Term	HCoV-229E C-Term	HCoV-OC43 N-Term	HCoV-OC43 C-Term	SEB	00 DMSO
	1,5000	-	-	1,5000	0,0000	-	4,5000	9,8000	0,0000
CD4+ act IL2+ TNFa+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	8,4500	-	-	4,5900	4,5500	-	3,4500	8,1900	0,0000
CD4+ act IL2+ only	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	0,0000	-	-	1,8300	2,2700	-	0,0000	0,5700	0,0000
CD4+ act IFNg+ TNFa+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	3,4000	-	-	11,3000	30,2000	-	1,4000	16,9000	0,0000
CD4+ act IFNg+ only	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	12,3100	-	-	2,8000	0,0000	-	13,7100	0,4000	0,0000
CD4+ act IFNg+ IL2+ TNF	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	0,0000	-	-	13,8300	12,3300	-	0,0000	2,8400	0,0000
CD4+ act IFNg+ L2+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**HCoV-229E N-Term**	**HCoV-229E C-Term**	**HCoV-OC43 N-Term**	**HCoV-OC43 C-Term**	**SEB**	**00 DMSO**
	0,0000	-	-	0,0000	0,0000	-	1,7200	0,1200	0,0000

Activated CD4+ T cell frequencies after SARS-CoV-2 infection

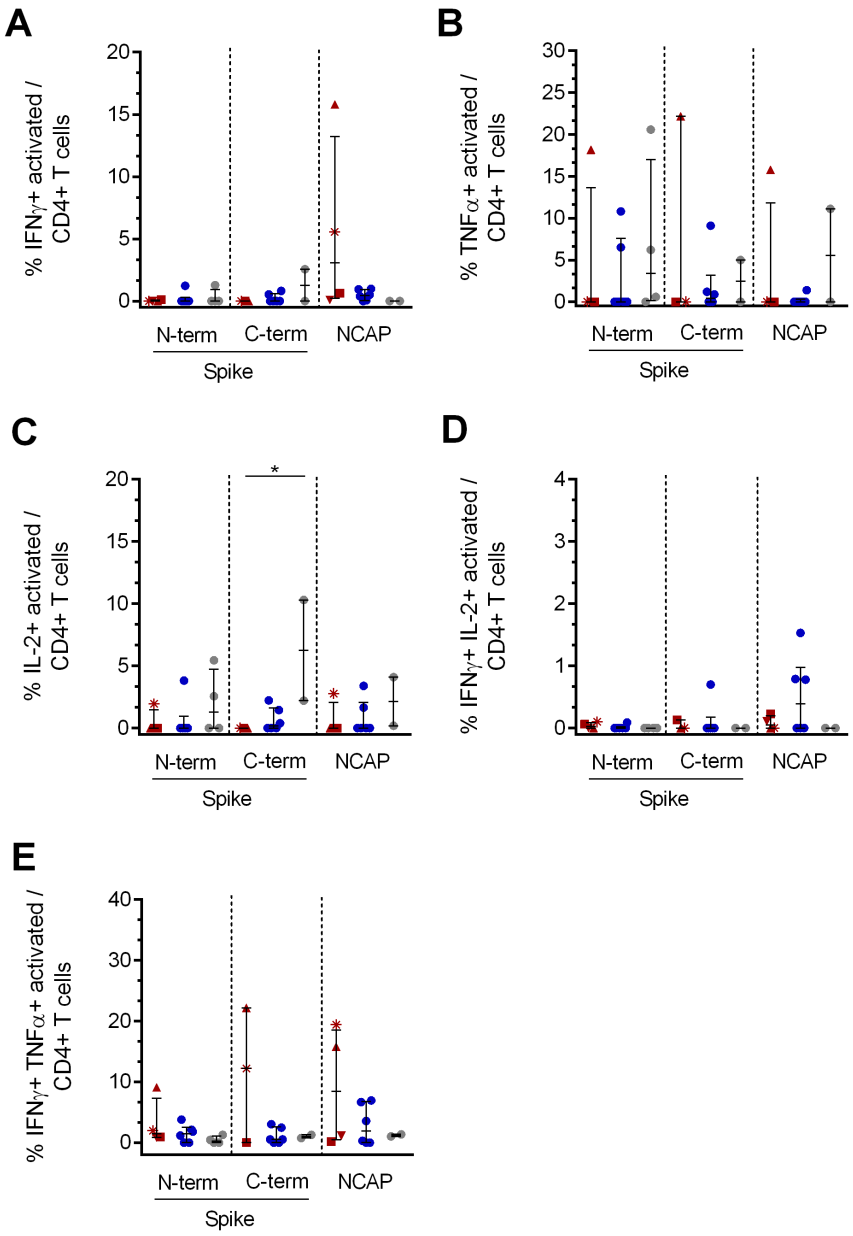
ID	Raw Data CD4+ CD154+ CD137+	-Background CD4+CD154+CD137+
IEI_1_SARS-CoV-2 N-Term	2,300	2,271
IEI_1_SARS-CoV-2 C-Term	1,870	1,841
IEI_1_SARS-CoV-2 NCAP	1,790	1,761
IEI_1_SEB	33,800	33,771
IEI_1_00 DMSO	0,029	0,000

Activated CD4+ T cell Cytokine response w/ COVID-19

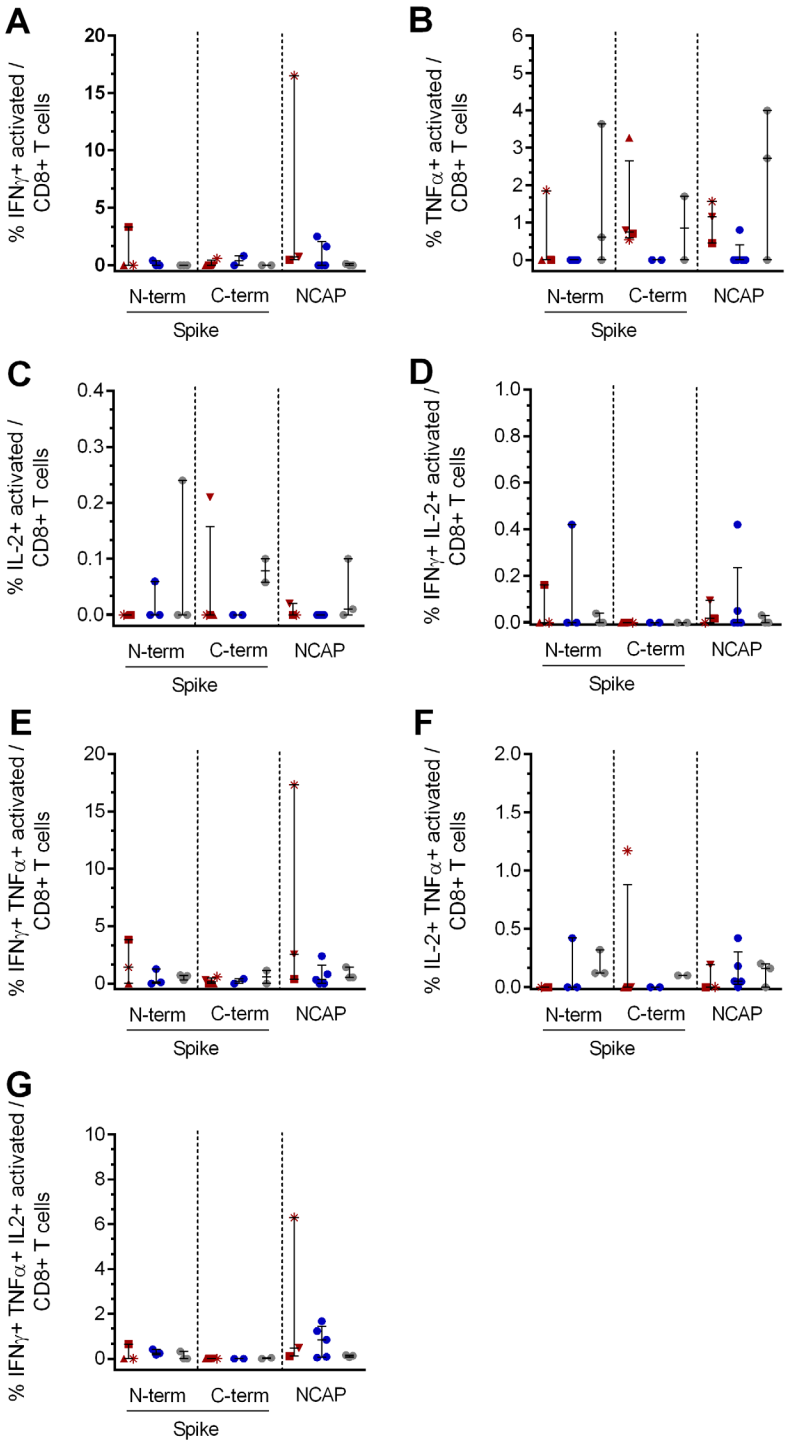
CD4+ act TNFa+ only	SARS-CoV-2 N-Term	SARS-CoV-2 C-Term	SARS-CoV-2 NCAP	SEB	00 DMSO
	0,000	0,000	0,000	0,000	0,000
CD4+ act IL2+ TNFa+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	22,900	22,400	20,300	0,000	0,000
CD4+ act IL2+ only	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	0,000	0,000	0,000	0,000	0,000
CD4+ act IFNg+ TNFa+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	0,910	0,000	0,200	15,870	0,000
CD4+ act IFNg+ only	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	0,130	0,000	0,630	1,330	0,000
CD4+ act IFNg+ IL2+ TNF	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	22,770	26,370	15,970	0,000	0,000
CD4+ act IFNg+ L2+	**SARS-CoV-2 N-Term**	**SARS-CoV-2 C-Term**	**SARS-CoV-2 NCAP**	**SEB**	**00 DMSO**
	0,066	0,130	0,230	0,140	0,000



Supplementary Figure3 | CD137⁺CD8⁺ T cell response to SARS-CoV-2 Spike and NCAP peptide pools and SEB positive control. PBMCs of PAD (n=4, red), CHC (n=6, blue) and naïve HC (n=6, grey) were stimulated with 1 µg/ml SARS-CoV-2 peptides or 3 µg/ml SEB. Frequencies of activated CD137⁺ CD8⁺ T cells **(A)** after stimulation with the different SARS-CoV-2 peptides. Frequencies of activated CD137⁺ CD8⁺ T cells **(B)** after stimulation with SEB. Only T cell responses above the threshold of 20% above background activation are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.



Supplementary Figure 4 | Single and double cytokine producing activated CD4⁺ T cells in response to SARS-CoV-2 specific peptide stimulation. IFNγ, TNFα, or IL2 single (sp) or double (dp) producing activated CD4⁺ T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)** and IL2 **(C)** sp and IFNγ⁺IL-2⁺ **(D)** and IFNγ⁺ TNFα **(E)** dp activated CD4⁺ T cells in response to SARS-CoV-2 peptide pools are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. p ≤ 0.05 = *. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.



Supplementary Figure 5 | Single, double and triple cytokine producing activated CD8⁺ T cells in response to SARS-CoV-2 specific peptide stimulation. IFNγ, TNFα, or IL2 single (sp) or double (dp) or triple (tp) producing activated CD8⁺ T cells were analyzed by Boolean combination gating strategy. IFNγ **(A)**, TNFα **(B)** and IL2 **(C)** sp and IFNγ⁺IL-2 **(D)**, IFNγ⁺TNFα **(E)** IL-2⁺TNFα **(F)** dp and IFNγ⁺TNFα⁺IL2 tp activated CD8⁺ T cells in response to SARS-CoV-2 peptide pools are shown. Median and interquartile range (IQR) are indicated. Statistical analysis was performed by non-parametric one-tailed Mann–Whitney-U test for comparison of control and patient groups. A p-value ≤ 0.05 was considered as statistically significant. PAD patients are depicted by the following symbols: patient #1 *; patient #2 ▲; patient #3 ■; patient #4 ▼.

Supplementary table 2: Light signal count (LSC) for anti-interferon-alpha and -omega autoantibodies determined by electrochemiluminescence immunoassay-platform.

Patient	alpha Biotynilation	light signal count (LSC) IFN-alpha antibody	omega Biotynilation	light signal count (LSC) IFN-omega antibody
Patient #1	1:1000	129 (negative)	1:500	141 (negative)
Patient #2	1:1000	162 (negative)	1:500	96 (negative)
Patient #3	1:1000	72 (negative)	1:500	78 (negative)
Patient #4	1:1000	333 (negative)	1:500	108 (negative)
Patient #5	1:1000	< 1980 (negative)	1:500	< 1961 (negative)

8.3 Study 3

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ORIGINAL ARTICLE

Impaired B Cell Recall Memory and Reduced Antibody Avidity but Robust T Cell Response in CVID Patients After COVID-19 Vaccination

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Abstract

Purpose Humoral and cellular immune responses were described after COVID-19 vaccination in patients with common variable immunodeficiency disorder (CVID). This study aimed to investigate SARS-CoV-2-specific antibody quality and memory function of B cell immunity as well as T cell responses after COVID-19 vaccination in seroresponding and non-responding CVID patients.

Methods We evaluated antibody avidity and applied a memory B cell ELSPOT assay for functional B cell recall memory response to SARS-CoV-2 after COVID-19 vaccination in CVID seroresponders. We comparatively analyzed SARS-CoV-2 spike reactive polyfunctional T cell response and reactive peripheral follicular T helper cells (pT_{FH}) by flow cytometry in seroresponding and non-seroresponding CVID patients. All CVID patients had previously failed to mount a humoral response to pneumococcal conjugate vaccine.

Results SARS-CoV-2 spike antibody avidity of seroresponding CVID patients was significantly lower than in healthy controls. Only 30% of seroresponding CVID patients showed a minimal memory B cell recall response in ELISPOT assay. One hundred percent of CVID seroresponders and 83% of non-seroresponders had a detectable polyfunctional T cell response. Induction of antigen-specific $CD4^+CD154^+CD137^+CXCR5^+$ pT_{FH} cells by the COVID-19 vaccine was higher in CVID seroresponder than in non-seroresponder. Levels of pT_{FH} did not correlate with antibody response or avidity.

Conclusion Reduced avidity and significantly impaired recall memory formation after COVID-19 vaccination in seroresponding CVID patients stress the importance of a more differentiated analysis of humoral immune response in CVID patients. Our observations challenge the clinical implications that follow the binary categorization into seroresponder and non-seroresponder.

Keywords CVID · COVID-19 · vaccination · memory · T cell response · antibody response

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Introduction

Coronavirus disease (COVID-19) is caused by severe respiratory syndrome coronavirus-2 (SARS-CoV-2), and has caused more than 500 million infections and over 6 million deaths worldwide since its emerging in late 2019 [1].

Patients with common variable immunodeficiency disorder (CVID), the most frequent clinically relevant primary immunodeficiency, are at higher risk for COVID-19-associated hospitalization and mortality [2] as well as for increased risk of prolonged or recurrent (breakthrough) SARS-CoV-2 infections [3]. Vaccination is considered to be the most effective and safest prophylactic measure in patients with primary immunodeficiency including genetically defined inborn errors of immunity (IEI) [4]. Cumulative data on COVID-19 vaccine response from more than 1500 patients with IEI have been reported with CVID being the most frequent underlying immunodeficiency [5]. Findings on humoral immune response after COVID-19 vaccination are variable with frequencies of seroresponding CVID patients ranging from 20 to 95% [6–12]. A positive T cellular immune response was reported in 46–83% of CVID patients [8, 10–15]. Reasons for the observed variability might include differences in methodology and different vaccination regimens as well as clinical, immunological, and genetic heterogeneity of CVID patients.

More importantly, the detection of specific antibodies after COVID-19 vaccination raises important questions on the quality and longevity of the humoral immune response, since the seroconversion state has potential clinical implications for treatment in SARS-CoV-2-infected CVID patients. Functional assessments of humoral immunity already revealed that CVID patients express lower neutralizing antibody levels than healthy individuals [8–11, 15]. Avidity of generated antibodies was analyzed in two recent studies, reporting similar levels of antibody avidity in CVID patients and healthy individuals 4 weeks after second COVID-19 vaccination but without significant increase after more than two vaccinations [9, 16]. Detailed characterizations regarding B cell memory formation are limited and suggest an atypical memory formation [17]. Without affecting seroconversion rates, booster vaccination in CVID patients was shown to further increase antibody levels in some seroresponder [9, 18], while effects of boosting on specific T cell immunity is variable [9, 10].

In the present study, we evaluated antibody avidity and functional B cell recall memory responses to SARS-CoV-2 vaccination in CVID seroresponders. In addition, specific polyfunctional T cell response and the generation of SARS-CoV-2 specific follicular T helper cells (T_{FH}) were assessed by flow cytometry in CVID seroresponder and non-seroresponder. All included CVID patients had a previously documented impaired specific antibody response to conjugated pneumococcal vaccination.

Methods

Study Subjects

Samples of 16 CVID patients before first and after the second SARS-CoV-2 vaccination were collected from the outpatient clinic for immunodeficiencies at the Institute for Medical Immunology, Charité Universitätsmedizin Berlin. All CVID patients were adults and diagnosed according to the criteria defined by the European Society for Immune Deficiency (ESID) [19]. CVID patients had an impaired vaccine response to pneumococcal conjugate vaccine (specific IgG antibodies below protective levels or low specific antibodies without increase after vaccination). Samples of 8 healthy controls (HC) before and after the second dose of SARS-CoV-2 vaccination were collected from laboratory employees at Charité Universitätsmedizin Berlin. All samples were collected between June and October 2021. During this period, SARS-CoV-2 B.1.617.2 (Delta) was the most predominant strain in Germany. All CVID patients and HC were infection naïve with no clinical history of SARS-CoV-2 infection, expressing negative spike antibodies before first vaccination (Table 2) and remaining seronegative for nucleocapsid (NP) after vaccination, to address possible infections between sampling time points (Supplementary Table S2).

Sample Preparation

Serum and heparinized whole blood was collected at a median of 133 days for CVID seroresponder (IQR: 24) and 128 days for CVID non-seroresponder (IQR: 34) after second COVID-19 vaccination. Samples from healthy individuals were collected at 32 days (IQR: 5) after second COVID-19 vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation over Pancoll (PAN-Biotech, Germany) using Leucosep tubes (Greiner Bio-One). PBMCs were cryopreserved and stored in liquid nitrogen.

SARS-CoV-2 Antibody Serology

SARS-CoV-2 spike serum IgG against the S1 domain was assessed by ELISA according to the manufacturer's instructions (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) using fully automated Euroimmun Analyzer I (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). To confirm results obtained by ELISA, a microarray-based multiparametric immunoassay

for detection of IgG antibodies against SARS-CoV-2 spike and NP (SeraSpot® Anti-SARS-CoV-2 IgG, Seramun Diagnostica GmbH, Heidesee, Germany) was applied.

IgG Avidity Assay

To measure avidity of SARS-CoV-2 spike IgG antibodies, serum samples were analyzed by a modified SARS-CoV-2-S1 ELISA (Euroimmun) [20]. Serum samples were diluted 1:101 with sample buffer and incubated on plates pre-coated with recombinant SARS-CoV-2 spike (S1) proteins. After incubation for 1 h at 37 °C, wells were washed and 200 µL urea (5.5 M.) or 200 µL phosphate-buffered saline (PBS) was added to the plates and incubated for 10 min at 37 °C. After a washing step, conjugate and substrate were added according to the manufacturer's instructions. OD was detected at 450 nm, and the relative avidity index was calculated by dividing the observed OD of the urea-treated sample by that of the PBS-treated sample, multiplied by 100 [20].

SARS-CoV-2 Interferon-Gamma Release Assay (IGRA)

IGRA (Euroimmun) for quantitative IFNγ release by SARS-CoV-2-specific T cells following second dose SARS-CoV-2 vaccine was performed according to the manufacturer's instructions. In summary, 500 µl heparinized whole blood was added to three stimulation tubes coated with specific SARS-CoV-2 S1 peptide pool, mitogen control, and uncoated blank, respectively. Blood was incubated for 24 h at 37 °C, 5% CO_2. Collected plasma was stored at − 20 °C until analysis by Quan-T-Cell ELISA (Euroimmun). According to manufacturer values ≥ 200 IU/ml are positive, values between 100 and 200 IU/ml are considered borderline.

T Cell Phenotyping for SARS-CoV-2 Spike Reactive T Cells by Flow Cytometry

For each experimental approach, patient and control samples before and after vaccination were simultaneously assessed. Cryopreserved PBMCs were thawed and rested for 24 h in IMDM/10% FCS/1% P/S at 37 °C, 5% CO_2. Stimulation was performed with 1 µg/ml of SARS-CoV-2 S peptide pools for N- and C-terminal domains (PM-WCPV-S-1, JPT Peptide Technologies GmbH, Berlin). Superantigen staphylococcal enterotoxin B (SEB) was used (3 µg/ml) as positive and DMSO as background control. Secretion inhibitor brefeldin A (BFA) (15 µg/ml) was added to each condition after 2 h. Stimulation continued for 16 h. Cells were washed and extracellular markers for anti-human CCR7 AF488, CD45RA PE-Cy7, and Live/Dead Fixable Blue stained for 30 min at 37 °C, 5% CO_2. After repeated washing, fixation/permeabilization buffer was applied (FoxP3 transcription factor staining buffer set, eBioscience) and incubated for 30 min at 4 °C. Intracellular staining was performed for anti-human CD3 BV650, CD4 PerCp-Cy5.5, CD8 BV510, CD137 PE, CD154 BV421, IL-2 APC, IFNγ BV605, TNFα AF700, and CXCR5 PE-Dazzle (Supplementary Table S3) for 30 min at 4 °C. CytoflexLX flow cytometer and FlowJo software version 10.6.2 were used for analysis. Unspecific activation was excluded by subtracting the background signal (DMSO only) from the peptide and SEB activated samples. A positive T cell response was defined as $CD154^+CD137^+CD4^+$ T cells > 0.005% within total $CD4^+$ T cell population and 20% above the background. Boolean combination gating was used for analysis of single and polyfunctional cytokine producing T cell subsets.

Memory B Cell ELISPOT Assay

Cryopreserved PBMCs were thawed and seeded in a 6-well plate at a concentration of 4×10^6 cells in 3 ml RPMI/10% FCS/1% P/S (culture medium) per well in the presence of 5% CO_2 at 37 °C. B cell proliferation was induced by the protocol from Crotty et al. [21] with 6 µg/ml CpG, 100 ng/ml of Pokeweed mitogen (PWM), staphylococcus aureus Cowan (SAC) (1:10 000), and 50 µM β-Mercaptoethanol for 7 days (called SAC protocol hereafter).

For the detection of antibody secreting cells (ASC) in HC and seroresponsive CVID patients after the second dose of SARS-CoV-2 vaccine, ELISPOT assay (enzyme-linked immuno spot assay) was performed with expanded cells after the 7-day cell culture. 96-well MultiScreen Filter Plates (Merck Millipore) were coated overnight (ON) at 4 °C with 1 µg/ml stabilized trimeric spike protein SARS-CoV-2 (wild type Excell Gene), as well as 1.2 µg/ml goat anti-human IgG (Jackson ImmunoResearch), serving as positive control. PBS was applied as negative control to exclude unspecific antibody binding.

Expanded cells were plated in the 96-well MultiScreen Filter Plates in duplicate at dilutions of 2.5×10^5/100 µl (IgG) and 1×10^6/100 µl for SARS-CoV-2 S protein, 6.25×10^3/100 µl for IgG positive controls and incubated for 6 h. Wells were then thoroughly washed six times with PBS supplemented with 1% bovine serum albumin (BSA) and 0.05% Tween. 100 µl/well, goat anti-human IgG-HRP (1:500) (Invitrogen) secondary antibody was applied and incubated ON at 4 °C. Afterwards, wells were washed three times with PBS. Substrate buffer (0.3 M sodium acetat solution, 0.2 M acetic acid solution, Auqa dest., pH = 5.0), 3-amino-9-ethyl-carbazole (AEC)-dimethylformamide (DMF) solution (1:30) and 3% H_2O_2 (1:100)) was applied to reach spot development. AID ELISPOT reader and AID ELISPOT 7.0 iSpot software were used for analysis. Results were manually verified to exclude artificial spots and multiple counting.

B Cell Phenotyping by Flow Cytometry

FACS analysis of B cell subsets was performed on PBMCs of day 0 (ex vivo) and on day 7 after cell culture (in vitro). Cells were incubated with a LIVE/DEAD fixable Aqua (Thermo Fisher) for 30 min at RT. Extracellular staining with fluorescently conjugated antibodies CD3 PB, CD19 PE-Cy7, CD21 PE, CD24 PerCp-Cy5.5, CD27 FITC, CD38 AF700, IgM APC, and IgD APC-Cy7 (Supplementary Table S4) for 30 min at 4 °C was performed. CytoflexLX flow cytometer and FlowJo software version 10.6.2 were used for analysis. Gating for B cell phenotyping was performed according to EUROClass classification (Supplementary Table S5, Supplementary Fig. S1) [22].

Statistical Analyses

Unpaired comparisons across multiple groups were performed using the Kruskal–Wallis test with Dunn's post-test for multiple comparisons to find significant differences among multiple investigated groups. If a significance was detected, two-tailed Mann–Whitney *U* test was performed for unpaired comparisons across two groups. In order to analyze responses before and after vaccination, the Wilcoxon matched pairs signed-rank test was applied for paired comparisons within a group. Correlation analyses were performed using Spearman's rank correlation coefficient. Continuous variables are shown as median and interquartile range (IQR). A p-value of < 0.05 was considered statistically significant. GraphPad Prism version 9.3.1 was used for statistical analyses.

Results

Study Cohort Characteristics

Ten seroresponding (CVID R), 6 non-responding (CVID NR) CVID patients, and 8 healthy controls (HC) were analyzed unvaccinated and after second COVID-19 vaccination. Seroresponse was defined according to manufactures instruction by a ratio of $> 1{,}1$ for spike-specific SARS-CoV-2 IgG antibody response in Euroimmun ELISA (Table 1). Data from SeraSpot analysis confirmed humoral immune response to spike (S1, S full, and RBD) in CVID R (Supplementary Table S2). Basic immunological parameters included IgG, IgA, IgM, $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cells as well as NK cells and T and B cell subsets (see Table 2) and showed no significant differences between the CVID groups. In addition, clinical characterization included non-infectious (immune cytopenia, autoimmunity, lymphoproliferation, granulomatous lymphocytic interstitial lung disease) and infectious manifestations (recurrent pneumonia and bronchiectasis) which showed no significant differences (see Table 3). Age and gender were similar in CVID R and CVID NR (median age 57 years).

Table 1 Serological data of anti-SARS-CoV-2 spike IgG in CVID patients and healthy controls (EUROIMMUN ELISA©)

	Before COVID-19 vaccination		After COVID-19 vaccination	
	IgG OD ratio	Result	IgG OD ratio	Result
CVID R 1	0.13	Negative	6.48	Reactive
CVID R 2	0.16	Negative	3.86	Reactive
CVID R 3	0.13	Negative	2.88	Reactive
CVID R 4	0.1	Negative	1.94	Reactive
CVID R 5	0.14	Negative	2.82	Reactive
CVID R 6	0.12	Negative	3.21	Reactive
CVID R 7	0.14	Negative	7.75	Reactive
CVID R 8	0.14	Negative	3.56	Reactive
CVID R 9	0.9	Negative	3.04	Reactive
CVID R 10	0.28	Negative	2.24	Reactive
CVID NR 1	0.14	Negative	0.14	Negative
CVID NR 2	0.15	Negative	0.66	Negative
CVID NR 3	0.14	Negative	0.28	Negative
CVID NR 4	0.25	Negative	0.23	Negative
CVID NR 5	0.14	Negative	0.48	Negative
CVID NR 6	0.23	Negative	0.15	Negative
HC-1	0.16	Negative	7.67	Reactive
HC-2	0.12	Negative	7.4	Reactive
HC-3	0.12	Negative	6.42	Reactive
HC-4	0.19	Negative	6.85	Reactive
HC-5	0.08	Negative	7.36	Reactive
HC-6	0.09	Negative	7.22	Reactive
HC-7	0.09	Negative	8.12	Reactive
HC-8	0.08	Negative	8.3	Reactive

COVID-19, coronavirus disease 2019; *CVID*, common variable immunodeficiency disorder; *HC*, healthy control; *NR*, non-seroresponder; *OD*, optical density; *R*, seroresponder

Impaired SARS-CoV-2 Spike Antibody Response in CVID Patients

Spike-specific SARS-CoV-2 IgG antibody response was analyzed in two different systems (ELISA and SeraSpot). ELISA indicated a significant increase of spike-specific SARS-CoV-2 IgG antibodies after COVID-19 vaccination in HC (IgG: $p = 0.008$) and CVID R patients (IgG: $p = 0.002$), but antibody levels were significantly lower in CVID R patients compared to HC (IgG: $p = 0.002$) (Fig. 1A). Data from SeraSpot analysis indicated negativity for NP, which is not induced by the spike-based COVID-19 vaccination but by a previous SARS-CoV-2 infection (Supplementary Table S2).

Table 2 Description of immunological parameter before COVID-19 vaccination comparing CVID seroresponder with CVID patients that failed to mount specific antibodies after completed COVID-19 vaccination

	IgG in g/L prior to IgRT	IgA in g/L	IgM in g/L	$CD4^+$ (cell count per nl)	$CD8^+$ (cell count per nl)	$CD19^+$ (cell count per nl)	NK (cell count per nl)	Naïve $CD4^+$ $CD45RA^+$ in % of $CD4^+$	Naïve Bc in % of $CD19^+$	MZ-like Bc in % of $CD19^+$	IgM + MBC in % of $CD19^+$	CS MBC in % of $CD19^+$	Transitional Bc in % of $CD19^+$	Activated Bc in % of $CD19^+$	CS PB in % of $CD19^+$
Normal range	7–16	0.7–4.0	0.4–2.3	0.5–1.2	0.3–0.8	0.1–0.4	0.1–0.4	> 15	42.6–82.3	7.4–32.5	–	6.5–29.1	0.6–3.4	0.9–7.6	0.4–3.6
CVID R 1	1.30	0.06	0.11	0.59	0.72	0.96	0.39	10	71.9	4.3	0.3	0.5	8.7	12.6	0
CVID R 2	2.66	< 0.1	0.06	0.41	0.14	0.14	0.09	34	67.3	14.5	1.4	0.5	2.4	13.2	0.1
CVID R 3	< 0.3	0.06	0.05	0.57	0.61	0.28	0.09	21	86.2	5.7	0.3	1	4	1.6	0
CVID R 4	< 0.3	< 0.1	0.05	0.41	0.36	0.22	0.28	10	81.3	12.2	0.3	0.6	3.5	1.5	0
CVID R 5	2.00	0.06	0.1	0.55	0.23	0.07	0.06	6	71.4	3.5	0.3	1.3	11.4	12.3	0.1
CVID R 6	1.86	0.14	0.15	0.37	1.23	0.22	0.13	24	76.9	8.4	0.6	2.7	1.6	7.7	0.3
CVID R 7	3.70	0.59	0.4	0.69	0.34	0.24	0.07	62	79.8	7.6	0.4	0.8	1.7	9.1	0.1
CVID R 8	3.00	0.04	0.16	0.55	0.33	0.37	0.13	27	56.51	31.73	2.36	1.78	2.47	6.06	0.89
CVID R 9	0.77	< 0.1	0.05	0.26	0.76	0.14	0.41	6	53.7	9	3.8	0.7	9.2	21.6	0.8
CVID R 10	1.69	0.06	0.28	0.33	0.5	0.12	0.07	15	64.4	12.9	1.9	1.6	0.5	18.7	0
Median values CVID R 1–10	**1.78**	**0.06**	**0.13**	**0.48**	**0.43**	**0.22**	**0.11**	**18**	**71.65**	**8.7**	**0.5**	**0.9**	**2.985**	**10.7**	**0.1**
CVID NR 1	1.66	0.06	0.15	0.32	0.33	0.19	0.1	21	76	3.5	2.5	0.2	6.6	3.3	0
CVID NR 2	3.60	0.25	0.18	0.43	0.23	0.06	0.02	5	70.9	4.7	0	1.3	8	14.7	0.5
CVID NR 3	0.33	0.06	0.05	0.5	0.28	0.16	0.26	21	79.3	11.2	0.6	0.8	1.6	4.8	0.4
CVID NR 4	< 0.30	0.06	0.05	0.64	0.62	0.04	0.05	47	81.4	7.3	1.1	0.3	4.2	4.1	0
CVID NR 5	< 0.3	< 0.1	2.2	0.84	0.44	0.38	0.11	60	59.3	33.8	0	0.6	0.7	5.3	0.1
CVID NR 6	< 0.3	< 0.1	0.05	0.93	0.55	0.08	0.05	2	67.5	8.8	2.6	1.1	1.8	15.8	0
Median values CVID NR 1–6	**0.32**	**0.08**	**0.1**	**0.57**	**0.385**	**0.12**	**0.08**	**21**	**73.45**	**8.05**	**0.85**	**0.7**	**3**	**5.05**	**0.05**
p-value* CVID R vs. CVID NR	0.21	0.52	0.94	0.30	0.54	0.21	0.17	0.94	0.87	0.74	0.99	0.28	0.73	0.56	0.80

Values are in bold to highlight that this line is a summary of the group (CVID R and CVID NR respectively)

Bc, B cell; *CS*, class-switched; *CVID*, common variable immunodeficiency disorder; *g/L*, grams per liter; *HC*, healthy control; *IgRT*, immunoglobulin replacement therapy; *MBC*, memory B cells; *MZ*, marginal zone; *nl*, nanoliters; *NK*, natural killer cells; *NR*, non-seroresponder; *OD*, optical density; *PB*, plasmablasts; *R*, seroresponder; bolded values = median; *p*-value calculated using Mann–Whitney *U* test

SARS-CoV-2 Spike Antibody Avidity Is Significantly Diminished in Seroresponding CVID Patients

SARS-CoV-2 spike antibody avidity of CVID R patients was significantly lower than in HC ($p < 0{,}001$; Fig. 1B). Avidity correlated with levels of SARS-CoV-2 spike IgG in HC ($p = 0.01$; $r = 0.833$, Fig. 1C) but not in CVID R patients ($p = 0.2$; $r = 0.45$ Fig. 1D).

Formation of B Cell Memory Is Impaired in CVID Patients Despite the Presence of Circulating Antibodies

It is unknown whether seroconversion in CVID patients could also result in the development of a functional B cell memory. Here, we aimed to study the functional memory B cell (MBC) response after COVID-19 vaccination in CVID R patients and HC. ELISPOT results were analyzed in combination with B cell subsets (for gating see Supplementary Fig. S1). For comparability, data was calculated per B cell and PB proportion within CVID patients and HC. IgG secreting cells, detected in the ELISPOT assay, were calculated per 10,000 PBs and 10,0000 MBC which were used on day 0 for in vitro stimulation.

Memory B Cell and Plasmablast Phenotype in Seropositive CVID Patients and HC

Based on flow cytometry staining at day 0 ex vivo and day 7 after in vitro stimulation using the SAC protocol, a decrease in both percentage and count of class-switched (CS) MBC was observed in HC (Fig. 2A). As expected, CVID R patients had initial significantly lower levels of CS MBC compared to HC ($p < 0.0001$). After expansion, CVID R patients elicited again significantly lower levels of CS MBC compared to HC ($p = 0.003$, Fig. 2A). CVID R patients also had significantly lower frequencies of CS PB ex vivo ($p < 0.0001$), and CS PB after in vitro stimulation ($p < 0.0001$, Fig. 2B). The decrease in frequency of MBC and increase in PB in HC indicates successful differentiation after in vitro stimulation. MBC of CVID R patients were also able to differentiate into PB following SAC stimulation, but to a much lower extent.

Deficient Memory B Cell Recall Response in Seropositive CVID Patients After COVID-19 Vaccination

Only 3/10 CVID R patients showed a minimal SARS-CoV-2 MBC recall response in the ELISPOT assay, whereas all HC showed a response (see Fig. 2C for exemplarily ELISPOT wells). CVID R patients had significantly lower SARS-CoV-2 ASCs per 10,000 CS MBC for S specific IgG ($p = 0.0007$) compared to HC (Fig. 2D). Regarding CS PB, ELISPOT elucidated a lower MBC recall response for S IgG ($p = 0.001$) in CVID R compared to HC as well (Fig. 2F). Whole IgG positive controls elicited high levels of ASCs in HC. Of note, whole IgG control responses were as well detectable in 7/10 CVID R patients. After normalizing spots per 10,000 CS PB and 10,000 CS MBC, counts did not differ significantly between the groups ($p = 0.1$ and $p = 0.4$ respectively, Fig. 2E and G).

SARS-CoV-2 T Cellular Immune Response Is Robustly Induced in CVID Patients After COVID-19 Vaccination

In addition to humoral immune responses, the T cellular response was examined to evaluate if a SARS-CoV-2-specific T cell response is induced as a result of COVID-19 vaccination. Seroresponsive, non-seroresponsive CVID patients and HCs were comparatively analyzed.

Quantitative IFNγ Release by SARS-CoV-2-Specific T Cells

The IGRA enables the quantitative determination of IFNγ release by SARS-CoV-2 T cells after pathogen-specific stimulation. IGRA revealed a positive response in 6 CVID R and 3 CVID NR patients. One patient of each group was borderline positive. Negative results were obtained from 3 CVID R and 2 CVID NR patients. Stimulation with PMA served as positive control and showed positive results in all CVID patients despite one of the seroresponding individuals, which in contrast had a positive SARS-CoV-2-specific response (Table 4).

SARS-CoV-2 Spike Reactive Polyfunctional T Cell Responses

SARS-CoV-2 S peptide–activated T cell subsets were assessed by flow cytometry. Antigen-specific $CD4^+$ T cells were investigated using activation markers CD137 and CD154 along with expression of cytokines IFNγ, TNFα, and IL-2. An activated T cell response was defined as > 0.005% of total $CD4^+$ T cells and 20% above the background signal. Polyfunctional cytokine subsets were obtained by Boolean combination gating. Moreover, formation of T_{FH} cells was investigated by staining of CXCR5 in activated $CD4^+$ T cells (for gating, see Supplementary Fig. S2).

SARS-CoV-2 S reactive $CD4^+CD154^+CD137^+$ T cells were induced in all HCs (N-term $p = 0.016$; C-term $p = 0.008$) and CVID R (N-term $p = 0.004$) after COVID-19 vaccination. In the group of CVID NR patients, 5 of 6 were able to generate a $CD4^+$ T cell response to a similar extent than the two other groups (Fig. 3A). SEB positive control revealed comparable levels of $CD4^+CD154^+CD137^+$ T cells before and after vaccination among all groups investigated indicating an intact T cell response. CVID NR patients showed slightly higher levels of activated $CD4^+$ T cells

Table 3 Clinical characterization of CVID seroresponder (CVID R) and CVID non-seroresponder (CVID NR) before COVID-19 vaccination in % of affected patients

	Immune cytopenia	Autoimmunity	Splenomegaly/ lympho-proliferation	GLILD	Bronchiectasis	Rec. pneumonias	Immuno-suppression	Genetic diagnostics
CVID R	10%	20%	60%	10%	0%	30%	0	Negative in all patients
CVID NR	33%	0%	100%	0%	33%	50%	0	Negative in all patients
p-value	0.52	0.5	0.23	1	0.125	0.61	1	

CVID, common variable immunodeficiency disorder; *GLILD*, granulomatous lymphocytic interstitial lung disease; *NR*, non-seroresponder; *R*, seroresponder; *rec*., recurrent

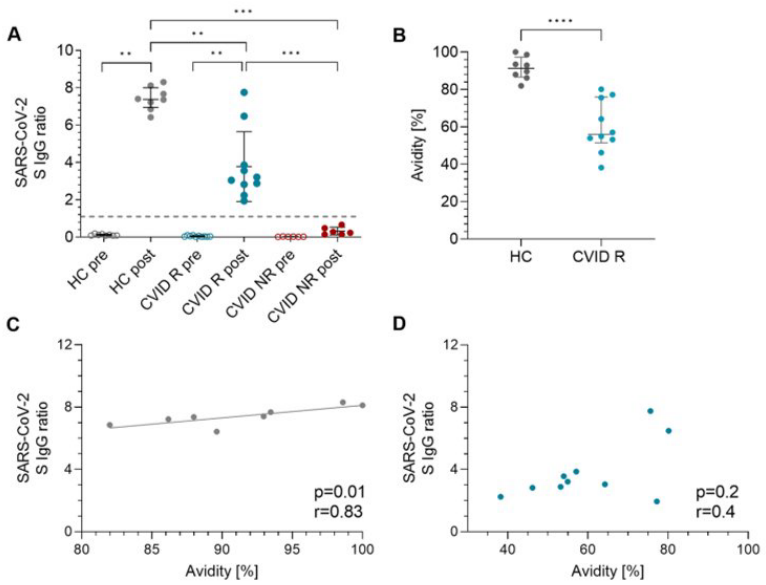

Fig. 1 SARS-CoV-2 IgG antibody serology and avidity in COVID-19 vaccinated CVID patients and HC. **A** Serum IgG against the N-terminal domain of SARS-CoV-2 spike protein (EUROIMMUN Anti-SARS-CoV-2 ELISAs). Results displayed as OD ratio of the control or patient sample over the OD of a calibrator. Ratio < 0.8 = negative. Ratio ≥ 0.8 to < 1.1 = borderline. Ratio ≥ 1.1 = positive (dashed line). **B** SARS-CoV-2 IgG antibody avidity in HC and CVID R patients assessed by SARS-CoV-2 RBD avidity ELISA. Median and interquartile range (IQR) are indicated. Unpaired comparisons across two groups were done by two-tailed non-parametric Mann–Whitney *U* test; paired comparisons within a group were done using the Wilcoxon matched pairs signed-rank test; $p \le 0.05$; **: $p \le 0.01$; ***: $p \le 0.001$; ****: $p \le 0.0001$. **C–D** Correlation of SARS-CoV-2 S IgG with avidity in HC (**C**) and CVID R patients (**D**). Correlation analysis was performed using Spearman's rank correlation coefficient

post vaccination after SEB stimulation compared to CVID R patients ($p = 0.02$) (Fig. 3B).

Vaccination in our infection naïve study cohort induced comparable frequencies of polyfunctional activated $CD4^+CD154^+CD137^+$ T cells in all groups. $IFN\gamma^+TNF\alpha^+IL\text{-}2^+$ triple-positive (tp) SARS-CoV-2 S N- and C-terminal reactive T cells significantly increased post vaccination in HC and CVID R (N-term: HC $p = 0.008$, CVID R $p = 0.002$; C-term: HC $p = 0.008$, CVID R $p = 0.04$). In the group of CVID NR, tp-activated $CD4^+$ T cells were induced in 5/6 patients. Post vaccination tp cytokine responses did not differ between the three groups (Fig. 3C).

COVID-19 Vaccination Induces Spike-Specific Circulating TFH Cells

Higher frequencies of $CD4^+CD45RA^-CXCR5^+$ peripheral T_{FH} cells were observed in CVID patients than in HC

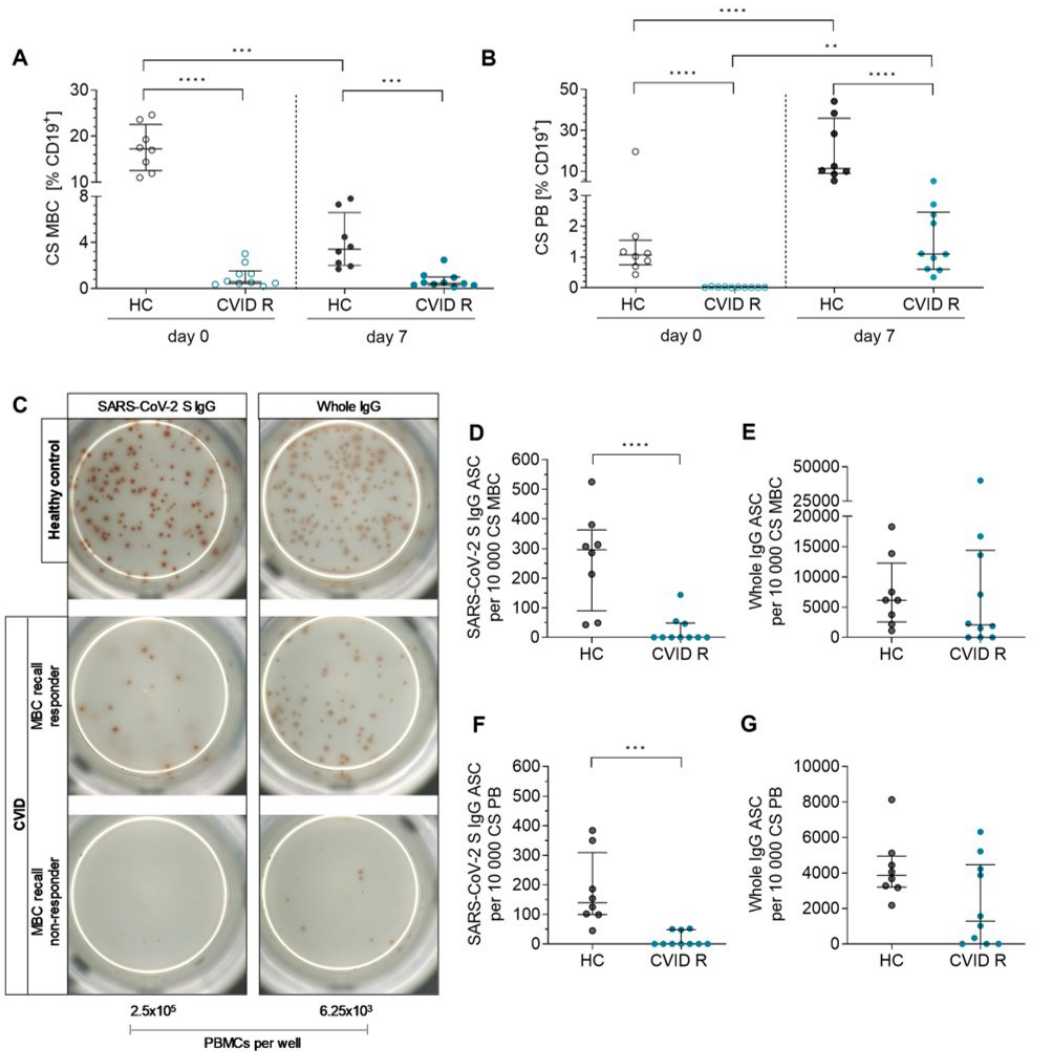

Fig. 2 Impaired SARS-CoV-2 memory B cell recall response in CVID R patients after COVID-19 vaccination. Memory B cell (MBC) response was analyzed using a combination of flow cytometry to identify B cell subpopulations and ELISPOT assay for capacity of MBC to differentiate into antibody secreting cells (ASC). **A** Class-switched (CS) MBC and **B** CS PB frequencies assessed by flow cytometry ex vivo and after in vitro stimulation. **C** ELISPOT plate of SARS-CoV-2 MBC recall response and whole IgG positive control exemplarily shown for a HC and a CVID MBC recall responder and a CVID MC recall non-responder. SARS-CoV-2 S IgG ELISPOTS calculated per 10,000 CS MBC (**D**) and 10,000 CS PB (**F**). Whole IgG positive control for 10,000 CS MBC (**E**) and 10,000 CS PB (**G**). Unpaired comparisons across two groups were done by two-tailed non-parametric Mann–Whitney *U* test; paired comparisons within a group were done using the Wilcoxon matched pairs signed-rank test. $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$

(Fig. 3D; for gating, see Supplementary Fig. S2). Induction of antigen reactive $CD4^+CD154^+CD137^+CXCR5^+$ peripheral T_{FH} cells by the COVID-19 vaccine was detected in all three groups (Fig. 3E). Frequencies of T_{FH} cells significantly increased in response to stimulation with SARS-CoV-2 S N-terminal peptide pool in HC ($p = 0.008$) and CVID R ($p = 0.02$). In CVID NR, 5/6 patients showed an increase but did not reach statistical significance. Regarding stimulation

with the C-terminal peptide, pool frequencies were higher in HC after vaccination ($p = 0.008$). Moreover, SARS-CoV-2 S C-terminal reactive pT_{FH} were higher in HC compared to CVID NR ($p = 0.008$) as well as in CVID R compared to CVID NR ($p = 0.02$). Moreover, levels of SARS-CoV-2 spike–specific pT_{FH} cells did not correlate with antibody levels, antibody avidity or frequency of SARS-CoV-2 spike reactive polyfunctional $CD4^+$ cells (see Supplementary Fig. S3).

Discussion

Evaluation of humoral COVID-19 vaccine response revealed variable and in part surprisingly high rates of seroresponders among CVID patients. However, in a disease, which is defined by impaired antibody and B cell memory formation, quality and longevity of humoral immune response need to be considered.

In the present study, SARS-CoV-2 seroresponding and non-responding CVID patients as well as HC were comparatively analyzed for their antibody avidity and for the functional longevity of their humoral immune response by using a SARS-CoV-2 spike–specific MBC ELISPOT assay. In addition, T cellular immune response, including flow cytometric detection of SARS-CoV-2 reactive polyfunctional $CD4^+$ T cells and T_{FH} cells, was assessed.

Table 4 Post COVID-19 vaccination IFNγ-release assay of SARS-CoV-2 peptide and PMA stimulated whole blood in CVID seroresponder and CVID non-seroresponder

ID	SARS-CoV-2 IFNγ [mIU/ml]	Mitogen control PMA IFNγ [mIU/ml]
CVID R 1	79.80	2403.20
CVID R 2	64.32	511.42
CVID R 3	147.15	2396.39
CVID R 4	677.59	2488.77
CVID R 5	227.29	2421.58
CVID R 6	618.80	44.74
CVID R 7	2483.27	2483.27
CVID R 8	202.30	2484.13
CVID R 9	2435.33	2435.33
CVID R 10	23.88	860.71
Median CVID R 1–10	214.78	2412.39
CVID NR 1	56.20	358.26
CVID NR 2	124.73	2306.53
CVID NR 3	466.27	2499.50
CVID NR 4	2499.50	2499.50
CVID NR 5	1128.41	938.55
CVID NR 6	34.58	2308.46
Median CVID NR 1–6	295.50	2307.50

CVID, common variable immunodeficiency disorder; *mIU/ml*, milli-international units per milliliter; *NR*, non-seroresponder; *R*, seroresponder; *rec.*, recurrent, *PMA*, Phorbol-12-myristat-13-acetat

Despite detectable SARS-CoV-2 antibodies after two COVID-19 vaccinations, humoral immune response in CVID patients differed substantially from healthy individuals as SARS-CoV-2 spike antibodies in CVID patients showed a significantly reduced avidity in comparison to HC. Our study complements recently published data on avidity in CVID patients after COVID-19 vaccination [9, 16]. In contrast to our data, Sauerwein et al. observed similar levels of specific SARS-CoV-2 antibody levels and avidity in HC and CVID patients after two vaccinations, but reported lower avidity and antibodies after (3^{rd}) booster vaccination [16]. Conflicting results may be due to different methodologies or might be related to the later time point of analysis and waning antibody levels. However, data from a kinetic study in CVID patients showed relatively stable anti-spike IgG antibody levels 4 weeks and 20 weeks after 2^{nd} COVID-19 vaccination and a positive trend for increased avidity after 3^{rd} vaccination [9]. Immunological and genetic heterogeneity within the group of COVID-19 seroresponding CVID patients are likely to contribute to the different observations.

SARS-CoV-2-specific IgG antibodies in peripheral blood are not informative about the source or MBC functionality and may arise from short- or long-lived plasma cells or from MBC after differentiation into ASC.

While conventional ELISPOT assay provides a qualitative and quantitative readout and can be designed to detect specific antibody responses [23, 24], the use of ELISPOT following in vitro stimulation and differentiation of MBC into ASC enables a functional analysis of specific B cell memory. Using an in-house SARS-CoV-2 spike–specific MBC ELISPOT assay, all HC showed a detectable response; however, only 3 (30%) seroresponding CVID patients had minimally detectable SARS-CoV-2 spike–specific IgG from ASCs after in vitro simulation and differentiation. Ratio of specific ASC per CS MBC as well as per CS PB was as significantly lower in CVID patients, indicating that the majority of seroresponding CVID patients failed to develop a robust humoral memory response.

Data on B cell memory in CVID patients after COVID-19 vaccination are very limited. Using flow cytometry, SARS-CoV-2–specific atypical MBC (defined as $CD19^+CD24^-CD27^-CD38^-$) with proposed low affinity were reported [17]. The present study provides additional functional data, showing an impaired specific recall memory response in seroresponding CVID patients. In the general population, SARS-CoV-2 mRNA-based vaccination induces both, a persistent germinal center (GC) B cell response and a robust but transient extra-follicular (EF) immune response resulting in antibodies of lower affinity from circulating PB [25, 26]. Lower avidity and impaired humoral memory

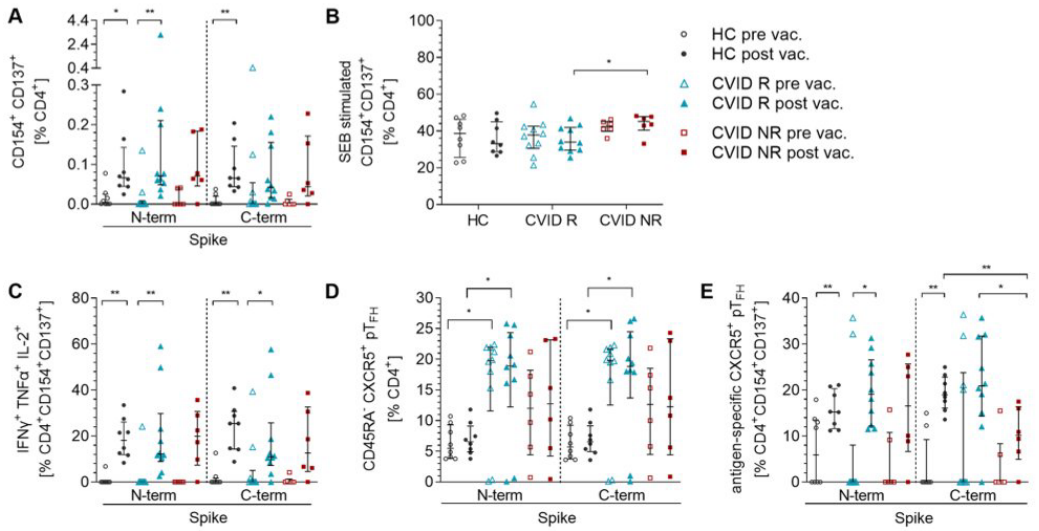

Fig. 3 SARS-CoV-2 specific T cell responses in CVID patients and HC before and after COVID-19 vaccination. PBMCs were stimulated with 1 µg of SARS-CoV-2 S peptide pools or 3 µg SEB. Activated $CD4^+$ T cell subsets were analyzed by multicolored flow cytometry. $CD4^+CD154^+CD137^+$ T cells stimulated with SARS-CoV-2 S peptide pools (**A**) and SEB (**B**). Polyfunctional ($IFN\gamma^+TNF\alpha^+IL\text{-}2^+$) SARS-CoV-2 reactive $CD4^+CD154^+CD137^+$ T cells (**C**). Peripheral TFH subset derived from $CD4^+CD45RA^-CXCR5^+$ (**D**) and from SARS-CoV-2 reactive $CD4^+CD154^+CD137^+CXCR5.^+$ T cells (**E**). Unpaired comparisons across multiple groups were done by the Kruskal–Wallis test with Dunn's post-test for multiple comparisons; unpaired comparisons across two groups were done by two-tailed non-parametric Mann–Whitney *U* test; paired comparisons within a group were done using the Wilcoxon matched pairs signed-rank test. $p \le 0.05$; **: $p \le 0.01$; ***: $p \le 0.001$; ****: $p \le 0.0001$

formation argue for a predominantly EF and impaired GC response in our cohort of COVID-19-vaccinated CVID patients.

In addition to B cell differentiation and maturation, GC reaction involves multiple B cell extrinsic factors including specific (follicular) T cell interactions. Previous data suggested a correlation between reduced specific humoral immune response and impaired specific T cellular immunity in CVID patients [17, 27]. Our findings contrast this observation, with all CVID seroresponder and 5/6 non-seroresponder showing a robust polyfunctional $CD4^+$ T cell immune response thus complementing previous reports of robust specific SARS-CoV-2 T cell responses in CVID patients with mild [28] and severe SARS-CoV-2 infections [29] as well as to COVID-19 vaccination [8–10] and other vaccines, such as influenza [30, 31]. A limitation of our study is the relatively long period of collecting samples in CVID patients and HC. While healthy control was analyzed earlier, all participating individuals were evaluated at least 4 weeks after 2nd vaccination. Multiple studies show a stable specific SARS-CoV-2 CD4 + cellular immune response between 2 weeks and 6 months after second COVID-19 vaccination in healthy individuals [9, 32, 33] and for CVID patients [8].

The relatively broad range in specific T cell immunity in CVID patients may at least partly attributable to the applied methodology of analyzing SARS-CoV-2-specific T cell immunity. This is exemplified in our cohort by the variability of T cell responses ranging from 31 to 44% no or low responders when using commercially available IGRA assay and reaching 94% patients with polyfunctional triple-positive activated T cells by flow cytometry. In addition to methodological aspects, the general clinical and immunological heterogeneity of CVID patients may help to reconcile different observations. Higher frequencies of activated T cells in non-seroresponding CVID patients to SEB as positive control challenge the hypothesis of a generally impaired T cell immunity leading to a lower specific T cell response in our cohort of CVID patients [13].

T_{FH} cell and B cell interaction during GC reaction are a prerequisite for high-affinity antibody formation and levels of specific T_{FH} were reported to correlate positively with vaccine-induced antibodies against conjugated pneumococcal, hepatitis B, and influenza [34, 35]. In SARS-CoV-2, mRNA vaccination was shown to induce a robust specific

T_{FH} response with stable persistence for at least 6 months after 2nd vaccination [36].

The role of T_{FH} cells in CVID patients remains poorly understand. While a preserved T_{FH} response was observed upon influenza vaccination [31], lower levels of specific T_{FH} cells were reported in a cohort of COVID-19-vaccinated CVID patients [13]; however, analysis of T_{FH} cell response was not differentiated into seroresponding and seronegative patients in this study. In our cohort, we could successfully identify a T_{FH} response after stimulation with SARS-CoV-2 spike peptide pools with CVID seroresponder and HC expressing similar frequencies. However, in seronegative CVID patients, T_{FH} cells did not increase significantly after stimulation with N-terminal spike and also T_{FH} response to C-terminal SARS-CoV-2 spike peptide pool was significantly lower in seronegative than in seroresponding CVID patients. This observation suggests an important role of T_{FH} cells during COVID-19 vaccine response. However, we did not observe a correlation between levels of specific T_{FH} cells with antibody levels or avidity.

Higher levels of activated $CXCR5^+$ peripheral T_{FH} cells were reported previously for CVID patients [37] in particular in patients with non-infectious manifestations (autoimmunity and granulomatous disease), suggesting a functional significance of this association. However, T_{FH} cells form a functionally and phenotypically heterogeneous group. Although high expression of CXCR5 is one of the defining hallmarks of T_{FH}, CXCR5 is also expressed on 20–25% of peripheral blood human central memory $CD4^+$ T cells [38]. Detection of this subgroup prior to SARS-CoV-2 spike stimulation may therefore represent an unspecific state of activation. Of note, in CVID patients, peripheral T_{FH} cells with phenotypical markers of activation were recently shown to express an mRNA signature of exhaustion, apoptosis, and senescence [39]. These observations should prompt caution when interpreting phenotypical findings in the context of T_{FH} functionality. To further understand the role of T_{FH} cells would require a detailed analysis, including functional assays and an expanded marker profile, in larger cohorts. Given the heterogeneity of changes in GC reactions in CVID patients, a uniform pathomechanism of impaired COVID-19 vaccine response is unlikely [40–44].

Limitations of our study include the slight heterogeneity and timing of administered COVID-19 vaccines and that interpretation is restricted to patients after receiving two COVID-19 vaccinations. While more than two vaccinations were shown to increase humoral immune response in CVID patients [18], it remains uncertain whether repeated vaccinations could also translate into B cell memory formation. Due to increased levels of SARS-CoV-2-IgG antibodies in commercially available immunoglobulins [45], a mere serological evaluation does no longer allow to distinguish between passive immunization and active antibody generation. Using SARS-CoV-2-specific MBC ELISPOT assays may enable researchers to circumvent this uncertainty. HC were significantly younger; however by providing data before and after vaccination, we were able to analyze comparatively intra-individual responses within each group.

The identification of possible predictive factors of a strong or impaired immune response to COVID-19 vaccination would improve risk stratification and support an individual prophylactic management for CVID patients. Previous vaccination studies in CVID patients described the potential impact of the type of vaccine and distribution of B cell subsets affecting vaccination responses [46]. Regarding COVID-19 vaccine response in CVID, a range of immunological and clinical factors have been described, including non-infectious complications and ongoing immunosuppressive therapy as well as elevated $CD21^{low}$ B cells, low B cells, low naïve T cells, and reduced IgA and IgM levels [7, 11, 47]. In our cohort, CVID seroresponder and non-seroresponder did not differ in any key immunological parameter; however the small number of patients limits the interpretation. Of note, and in line with recent observations [9], previous failure to mount a specific antibody response to pneumococcal conjugate vaccine could not predict COVID-19 vaccine response in our cohort of CVID patients. This discrepancy might be related to the antigen structure, but immunogenicity might also depend on the mode of immunization (mRNA vs conjugated vaccine) [48]. While humoral immune response to pneumococcal conjugated vaccine requires GC reaction, response to COVID-19 involves both GC and EF structures [49]. Immunological and genetic aspects that shape the humoral immune response to SARS-CoV-2 vaccination in CVID patients remain incompletely understood. However, the observation of seroresponding and non-seroresponding CVID patients may help to shed light onto the diverse pathomechanisms of CVID. Further studies in larger cohorts are required to evaluate possible underlying B cell differentiation defects in CVID patients.

Conclusions

Reduced avidity of SARS-CoV-2 IgG and significantly impaired recall memory formation after COVID-19 vaccination in seroresponding CVID patients stress the importance of a more differentiated analysis of humoral immune response in CVID patients. Our observations challenge the binary categorization into seroresponder and non-seroresponder and potentially impact on clinical decisions for the prophylactic management of COVID-19.

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Author Contribution Conceptualization: Leif G. Hanitsch; sourcing of patient samples: Leif G. Hanitsch; methodology: Sophie Steiner; formal analysis and investigation: Sophie Steiner, Sandra Bauer; Tatjana Schwarz, Lara M. Jeworowski; analysis of results and composition of figures: Sophie Steiner; interpretation of data: Sophie Steiner, Leif G. Hanitsch, Carmen Scheibenbogen, Victor M. Corman, Tatjana Schwarz; writing—original draft preparation: Sophie Steiner, Leif G. Hanitsch; writing—review and editing: all authors. All authors gave final approval of the version to be published.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Charité, Universitätsmedizin Berlin (EA2/092/20 from June 4th 2020) and with the 1964 Helsinki Declaration and its later amendments.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication The authors affirm that human research participants provided informed consent for publication of acquired data.

Conflict of Interest VMC is named together with Charité—Universitätsmedizin Berlin and Euroimmun GmbH on a patent application (Methods and reagents for diagnosis of SARS-CoV-2 infection. Pub number 20210190797) filed recently regarding the diagnostic of SARS-CoV-2 by antibody testing. The other authors have no relevant financial or non-financial interests to disclose.

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Supplementary Material Study 3:

Supplementary Tables

Table S1: Cohort Characteristics for healthy controls and CVID patients

ID	Age	Gender	Ethnicity	Administered vaccine
HC-1	34	f	caucasian	ChAdOx1-S / BNT162b2
HC-2	39	f	caucasian	ChAdOx1-S / BNT162b2
HC-3	44	m	caucasian	ChAdOx1-S / BNT162b2
HC-4	35	f	caucasian	ChAdOx1-S / BNT162b2
HC-5	39	f	caucasian	ChAdOx1-S / BNT162b2
HC-6	30	f	caucasian	2 x BNT162b2
HC-7	34	m	caucasian	ChAdOx1-S / BNT162b2
HC-8	28	m	caucasian	2 x mRNA-1273
Median	35			
ID	**Age**	**Gender**	**Ethnicity**	**Administered vaccine**
CVID R 1	41	f	caucasian	2 x BNT162b2
CVID R 2	42	f	caucasian	2 x BNT162b2
CVID R 3	62	f	caucasian	2 x BNT162b2
CVID R 4	56	m	caucasian	2x ChAdOx1-S
CVID R 5	32	f	caucasian	ChAdOx1-S / mRNA-1273
CVID R 6	62	f	caucasian	2 x BNT162b2
CVID R 7	49	f	caucasian	2 x BNT162b2
CVID R 8	62	f	caucasian	2 x ChAdOx1-S
CVID R 9	59	m	caucasian	2 x BNT162b2
CVID R 10	62	m	caucasian	2 x BNT162b2
Median	57			
CVID NR 1	63	m	caucasian	2 x BNT162b2

CVID NR 2	54	f	caucasian	ChAdOx1-S / BNT162b2
CVID NR 3	48	m	caucasian	2 x BNT162b2
CVID NR 4	54	m	caucasian	ChAdOx1-S / BNT162b2
CVID NR 5	31	f	caucasian	2 x BNT162b2
CVID NR 6	69	f	caucasian	2 x BNT162b2
Median	57			

CVID = common variable immunodeficiency disorder; f = female; m = male; NR = non-seroresponder; R = seroresponder

Table S2: Serological data of healthy controls and CVID patients after COVID-19 vaccination (SeraSpot©)

after COVID-19 vaccination						
	Ratio NP IgG	**Ratio RBD IgG**	**Ratio S1 IgG**	**Ratio S full IgG**	**Anti-SARS-CoV-2 NP IgG**	**Anti-SARS-CoV-2 Spike IgG**
CVID R 1	0.05	7.77	5.59	6.64	negative	reactive
CVID R 2	0.11	4.7	3.22	3.97	negative	reactive
CVID R 3	0	4.56	3.32	3.32	negative	reactive
CVID R 4	0	2.5	1.57	2.0	negative	reactive
CVID R 5	0.05	1.43	1.1	1.37	negative	reactive
CVID R 6	0.06	4.56	2.66	3.59	negative	reactive
CVID R 7	0.07	7.07	5.87	6.47	negative	reactive
CVID R 8	0.08	3.64	3.08	3.11	negative	reactive
CVID R 9	0.06	5.88	4.59	5.32	negative	reactive
CVID R 10	0	3.86	2.36	2.64	negative	reactive
CVID NR 1	0.11	0.97	0.47	0.67	negative	negative
CVID NR 2	0	0.31	0.08	0.19	negative	negative
CVID NR 3	0.08	0.03	0	0.03	negative	negative
CVID NR 4	0.2	0.1	0	0.17	negative	negative
CVID NR 5	0.39	0.19	0.03	0.23	negative	negative
CVID NR 6	0.08	0	0	0	negative	negative
HC-1	0.03	6.29	5.86	5.89	negative	reactive
HC-2	0.05	5.95	5.57	5.73	negative	reactive
HC-3	0	6.09	5.29	5.53	negative	reactive
HC-4	0.06	6.14	5.29	5.6	negative	reactive
HC-5	0.14	5.19	4.69	4.98	negative	reactive
HC-6	0.17	7.13	6.43	6.47	negative	reactive
HC-7	0.1	5.39	5.2	5.12	negative	reactive
HC-8	0	5.94	4.83	5.17	negative	reactive

COVID-19 = coronavirus disease 2019; CVID = common variable immunodeficiency disorder; HC = healthy control; NP = nucleocapsid protein; NR = non-seroresponder OD = optical densitiy; R = seroresponder; RBD = receptor binding domain; S = Spike; S1 = S1 subunit of spike protein

Table S3: Marker for T cell phenotyping

Target	Conjugate	Clone	Dilution	Company
Anti-human CD3	BV650	OKT3	1:100	Biolegend
Anti-human CD4	PerCp-Cy5.5	SK3	1:100	Biolegend
Anti-human CD8	BV510	RPA-T8	1:100	Biolegend
Anti-human CD137	PE	4B4-1	1:100	Biolegend
Anti-human CD154	BV421	24-31	1:200	Biolegend
Anti-human IL-2	APC	MQ1-17H12 (RUO)	1:200	BD
Anti-human IFNγ	BV605	4S.B3	1:20	Biolegend
Anti-human TNFα	AF700	MAb11	1:20	Biolegend
Anti-human CCR7	AF488	G043H7	1:100	Biolegend
Anti-human CD45-RA	PE-Cy7	HI100	1:100	Biolegend
Anti-human CXCR5	PE-Dazzle	J252D4	1:100	Biolegend
Dead Cell Staining	Fixable Blue		1:100	Thermo Fisher

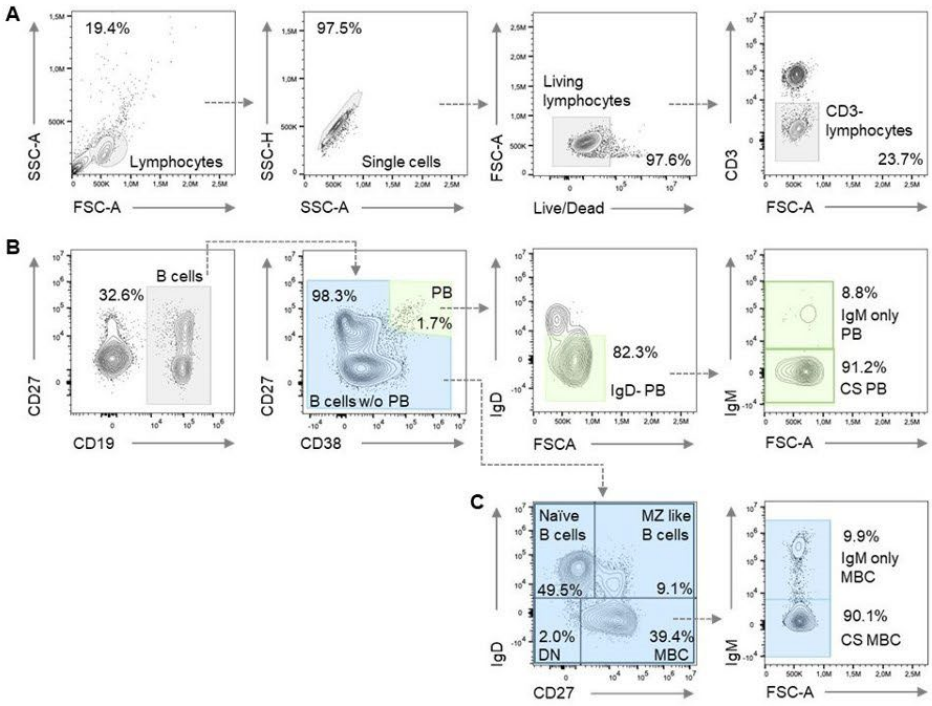
Table S4: Marker for B cell and plasmablast phenotyping

Target	Conjugate	Clone	Dilution	Company
Anti-human CD3	Pacific Blue	UCHT1	1:50	Biolegend
Anti-human CD19	PE-Cy7	HIB19	1:66	Biolegend
Anti-human CD21	PE	Bu32	1:50	Biolegend
Anti-human CD24	PerCp-Cy5.5	ML5	1:66	Biolegend
Anti-human CD27	FITC	M-T271	1:40	Biolegend
Anti-human CD38	Alexa Fluor 700	HIT2	1:50	Biolegend
Anti-human IgM	APC	MHM-88	1:40	Biolegend
Anti-human IgD	APC-Cy7	IA6-2	1:50	Biolegend
Dead Cell Staining	Fixable Aqua		1:50	Thermo Fisher

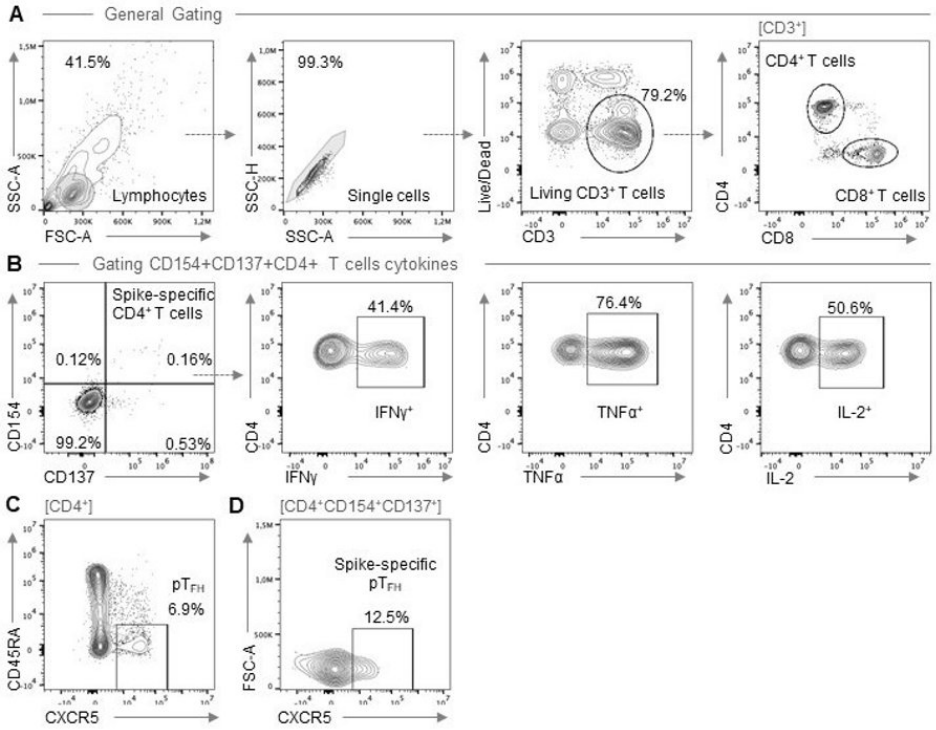
Table S5: Subsets of B cells and plasmablasts within single, living $CD3^{-}CD19^{+}$ lymphocytes

B cell subset	Marker
Naïve B cells	$IgD^{+}CD27^{-}$
Marginal zone like B cells (MZ-like)	$IgD^{+}CD27^{+}$
Memory B cells	$IgD^{-}CD27^{+}$
IgM only memory B cells	$IgD^{-}CD27^{+}IgM^{+}$
Class-switched memory B cells	$IgD^{-}CD27^{+}IgM^{-}$
Plasmablasts	$CD38^{++}CD27^{++}IgD^{-}$
IgM only Plasmablasts	$CD38^{++}CD27^{++}IgD^{-}IgM^{+}$
Class-switched Plasmablasts	$CD38^{++}CD27^{++}IgD^{-}IgM^{-}$

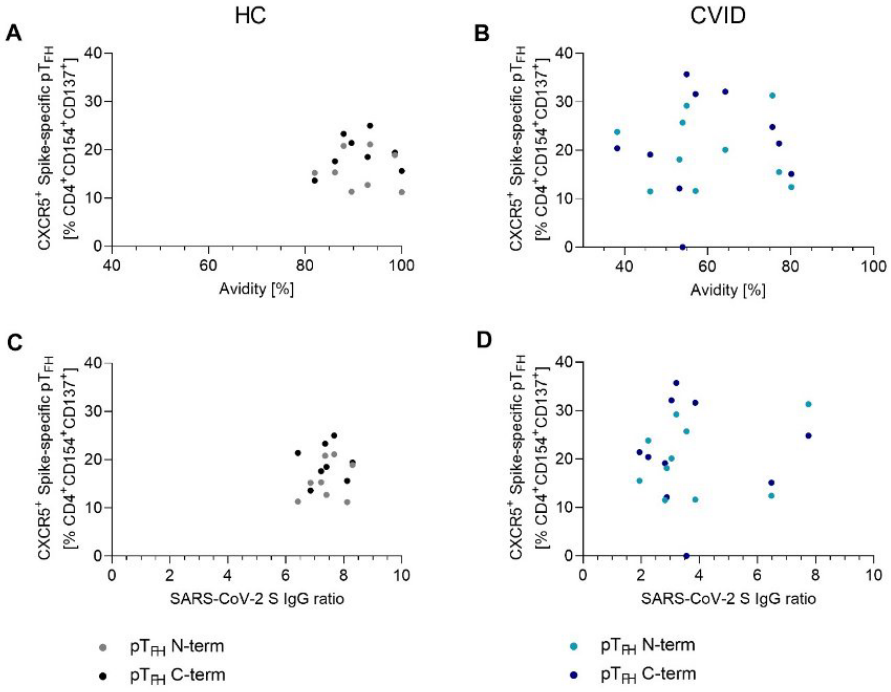
Supplementary Figure S1: Gating strategy for B cell phenotyping.



Supplementary Figure S2: Gating strategy for SARS-CoV-2 spike-specific T cell subsets and their cytokine expression.



Supplementary Figure S3: Correlation of SARS-CoV-2 antibody avidity and SARS-CoV-2 spike reactive $CD4^+CD154^+CD137^+CXCR5^+$ peripheral T_{FH} cells in COVID-19 vaccinated CVID patients and healthy controls.



Online Resource Legends

Supplementary Figure S1: Gating strategy for B cell phenotyping. Gating is exemplary shown in thawed PBMCs of a healthy individual in *ex vivo* condition. **(A)** Strategy for analyzing B cell subsets in PBMCs starting from lymphocytes followed by exclusion of duplets together with dead cells and selection of $CD3^-$ lymphocytes. **(B)** Strategy for assessment of plasmablasts (PB) derived from $CD19^+$ B lymphocytes. PBs are defined as $CD38^{++}CD27^{++}$. Following identification of PB. cells were gated on IgD^- cells and their expression of IgM to identify IgM only and class-switched (CS) PB. **(C)** Gating strategy for B cells without PB to analyze and IgD^-CD27^+ memory B cells (MBC) and their expression of IgM for IgM only and CS MBC.

Supplementary Figure S2: Gating strategy for SARS-CoV-2 Spike-specific T cell subsets and their cytokine expression. Gating is exemplary shown for a healthy individual after peptide stimulation. **(A)** Strategy for analyzing T cell subsets in PBMCs starting from lymphocytes followed by exclusion of duplets together with dead cells and selection of $CD3^+$ T cells. which were further subdivided into $CD4^+$ and $CD8^+$ T cells. **(B)** $CD4^+$ T cells were further gated on $CD154^+CD137^+$ activated T cells and their expression of IFNγ. TNFα and IL-2. **(C)** Gating of $CD45RA^-CXCR5^+$ pT_{FH} cells derived from $CD4^+$ T cells. **(D)** Gating of SARS-CoV-2 Spike specific $CXCR5^+$ pT_{FH} cells derived from $CD4^+CD154^+CD137^+$ T cells.

Supplementary Figure S3: Correlation of SARS-CoV-2 antibody avidity and SARS-CoV-2 spike reactive $CD4^+CD154^+CD137^+CXCR5^+$ peripheral T_{FH} cells in COVID-19 vaccinated CVID R patients and healthy controls. (A-B) Correlation analysis of SARS-CoV-2 spike-specific pT_{FH} with SARS-CoV-2 S IgG antibody ratio in HC **(A)** and CVID R patients **(B)** and of SARS-CoV-2 Spike-specific pT_{FH} with antibody avidity in HC **(C)** and CVID R patients **(D)**. Correlation analysis was performed using Spearman's rank correlation coefficient.

Table S1: Cohort characteristics for healthy controls and CVID patients

Table S2: Serological data of CVID patients after COVID-19 vaccination (SeraSpot©)

Table S3: Marker for T cell phenotyping

Table S4: Marker for B cell and plasmablast phenotyping

Table S5: Subsets of B cells and plasmablasts within single, living $CD3^-CD19^+$ lymphocytes

9 CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

10 PUBLICATION LIST

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