Vaccine-elicited B- and T-cell immunity to SARS-CoV-2 is impaired in chronic lung disease patients

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Patients with chronic lung disease show impaired B- and T-cell immunity after SARS-CoV-2 vaccination https://bit.ly/3OyVlEH

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Abstract

Background While vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) provides significant protection from coronavirus disease 2019, the protection afforded to individuals with chronic lung disease is less well established. This study seeks to understand how chronic lung disease impacts SARS-CoV-2 vaccine-elicited immunity.

Methods Deep immune phenotyping of humoral and cell-mediated responses to the SARS-CoV-2 vaccine was performed in patients with asthma, COPD and interstitial lung disease (ILD) compared to healthy controls.

Results 48% of vaccinated patients with chronic lung diseases had reduced antibody titres to the SARS-CoV-2 vaccine antigen relative to healthy controls. Vaccine antibody titres were significantly reduced among asthma ($p<0.035$), COPD ($p<0.022$) and a subset of ILD patients as early as 3–4 months after vaccination, correlating with decreased vaccine-specific memory B-cells in circulation. Vaccine-specific memory T-cells were significantly reduced in patients with asthma ($CD8^+$ $p<0.004$; $CD4^+$ $p<0.023$) and COPD ($CD8^+$ $p<0.008$) compared to healthy controls. Impaired T-cell responsiveness was also observed in a subset of ILD patients ($CD8^+$ 21.4%; $CD4^+$ 42.9%). Additional heterogeneity between healthy and disease cohorts was observed among bulk and vaccine-specific follicular T-helper cells.

Conclusions Deep immune phenotyping of the SARS-CoV-2 vaccine response revealed the complex nature of vaccine-elicited immunity and highlights the need for more personalised vaccination schemes in patients with underlying lung conditions.

Introduction

Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targeting the ancestral (Wuhan-Hu-1/2019) viral spike (S) protein has been broadly effective at limiting infection and severe coronavirus disease 2019 (COVID-19) [1–6]. With respect to SARS-CoV-2 infection, both the humoral and cell-mediated arms of the adaptive response are important for achieving optimal control of COVID-19 [7]. As such, generating effective B-cell and T-cell immunity against SARS-CoV-2 remains the goal during vaccination. Much of the protection afforded by both the Pfizer/BioNTech BNT162b2 and the Moderna mRNA-1273 mRNA vaccines is mediated by increased serum neutralising antibodies to the viral spike protein [8]. The efficacy of such neutralising antibodies depends on their titre, avidity and half-life [9–17]. In infected individuals, the half-lives of IgG anti-spike and anti-receptor-binding domain (RBD) have been reported to be 103–126 and 83–116 days, respectively [18, 19]. The half-life of antibodies in vaccinated individuals may be shorter, as titres are significantly decreased after 6 months [20–25].

The difference in antibody half-life between infected and vaccinated individuals may depend on the half-lives of the plasma cells or differences in the memory B-cells that produce them [26]. Memory B-cells do not constitutively secrete soluble antibody, but, after re-exposure to virus or vaccine, rapidly convert to plasma cells and can thus contribute to production of high levels of protective antibodies [27]. The importance of memory B-cells to lasting immunity to SARS-CoV-2 infection after vaccination is highlighted by findings showing that RBD specific memory B-cells survive even after anti-RBD antibodies are absent from serum [25, 28].

In addition to humoral immunity, SARS-CoV-2-specific T-cells provide protection against the virus and may be particularly relevant in the case of SARS-CoV-2 variants of concern, such as B.1.617.2 delta and B.1.1.529 omicron, which express mutated spike proteins that can more effectively evade neutralising antibodies [24, 29–33]. The ability of the virus to escape antibody but not T-cell immunity stems from the nature of the different antigenic targets on the spike protein recognised by B-cells (proteins) and T-cells (peptides) [7, 32, 34–37]. Underlying their potential importance, the relative expansion of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cells associates with COVID-19 disease severity, and T-cell memory appears more durable than serum antibody titres [18, 25, 35, 38, 39]. The rapidity of T-cell responses after infection and vaccination also provides important protective benefits [35, 40, 41]. Circulating $CD4^+$ follicular T-helper (Tfh) cells are also found in the memory T-cell pool. While SARS-CoV-2-specific Tfh cells are less durable than other memory T-cell subsets after vaccination and may not be required for the generation of antibodies against the virus, these cells are probably important in orchestrating a productive T- and B-cell response to SARS-CoV-2 infection [25, 34, 42–46].

Although we have gained significant understanding of natural immunity and response to SARS-CoV-2 infection and vaccination, informative data were not generated in chronic lung disease patients, who are at highest risk of mortality and morbidity due to COVID-19 [47]. Patients with lung diseases may suffer more than healthy subjects from SARS-CoV-2 infections because of underlying pulmonary limitations and/or abnormal lung immune function. Immunosuppressant drugs taken by patients with chronic lung disease can also reduce their immune responses to the SARS-CoV-2 vaccine as reported in other disease contexts [48–52]. Indeed, certain conditions and treatments may significantly reduce the ability of patients to produce anti-SARS-CoV-2 antibody [53–60]. Therefore, it is critical to understand the vaccine response in high-risk chronic lung disease patients to help identify subsets of individuals who may be at greatest risk of poor outcomes. To reveal whether limitations in vaccine responsiveness exist within chronic lung disease patients and to understand better the heterogeneity of responses across different chronic lung diseases, we performed deep phenotyping of the humoral and cell-mediated immune response to SARS-CoV-2 vaccination in a select, investigative cohort of patients with interstitial lung disease (ILD), COPD and asthma, compared to healthy subjects.

Methods

Study population

Chronic lung disease and healthy control blood samples were collected as part of two institutional review board-approved protocols under which subjects provided informed consent: 1) a prospective study of response to SARS-CoV-2 vaccinations that recruited from National Jewish Health clinics and 2) the National Jewish Health BioBank which recruits patients undergoing normal clinical laboratory testing or from a healthy donor pool. Patient information regarding vaccine status, medicine and infection status was collected at time of sample collection or as part of their normal medical record. For samples used, no prior SARS-CoV-2 infection was noted in medical records at time of blood draw. All samples were taken between 17 June 2021 and 31 August 2021.

Serum and peripheral blood mononuclear cell sample preparation

Blood was collected from multiple 10 mL blood draws into EDTA tubes. Serum was processed after density gradient centrifugation and peripheral blood mononuclear cells (PBMCs) (post-red blood cell lysis) were resuspended in 10% dimethyl sulfoxide + 90% fetal bovine serum (FBS) in cryovials prior to storage in liquid nitrogen.

SARS-CoV-2 RBD, nucleocapsid and human angiotensin-converting enzyme 2 generation

SARS-CoV-2 spike RBD (amino acids 319–541) with C-terminal 6× histidine tag was expressed in 293F cells as described previously [61]. The SARS-CoV-2 nucleocapsid (amino acids 247–364) was expressed in bacteria using plasmid NR-52434 (BEI Resources) and purified with Ni Sepharose. The RBD protein was purified with nickel column. Both RBD and nucleocapsid were eluted (20 mM Tris pH 8.0, 150 mM sodium chloride and 250 mM imidazole) and purified proteins concentrated with Amicon centrifugal tubes with a 10 kDa cutoff filter. Proteins were then separated on the Superdex 200 10/300 GL size exclusion

column with PBS as running buffer to collect monomers. Human angiotensin-converting enzyme (ACE)2 (1–615) fused to mouse IgG γ2a Fc region (ACE2-mFc) was expressed in the human 293F cell line as reported previously [62]. The fusion protein was purified with protein A and eluted with 0.1 M glycine buffer pH 3.0. The eluted protein was immediately neutralised with 1 M Tris pH 9.0.

ACE2-mFc competition assay

We used an ACE2-mFc competition assay to assess the function of serum antibody in blocking ACE2 binding to spike RBD with bamlanivimab as standard [62, 63]. Briefly, 1 μg·mL^{-1} ACE2-mFc was pre-mixed with different concentrations of bamlanivimab or serum at 1:10 dilution. The mixture was then incubated with spike RBD-coated ELISA plate. The binding of ACE2-mFc to RBD was detected by alkaline phosphatase (AP)-conjugated anti-mouse IgG γ2a specific antibody (BD Biosciences).

RBD-tetramer generation

SARS-CoV-2 spike RBD (aa319 to aa541) with C-terminal histidine tag and Avitag was expressed and purified as described earlier. The RBD was biotinylated by BirA enzyme. The biotinylated RBD was conjugated to the streptavidin labelled with different fluorescent dyes.

ELISA for RBD or nucleocapsid serum antibody

20 μg·mL^{-1} 6×histidine-tagged spike RBD or 10 μg·mL^{-1} nucleocapsid was used for coating the ELISA plate. After blocking, human serum at different dilutions was incubated on the plates. The bound IgG was detected with 1:5000 dilution of goat anti-human IgG, Fcγ fragment specific conjugated with alkaline phosphatase (Jackson ImmunoResearch). This was similarly done for bound IgM using an AP-conjugated secondary antibody (Jackson ImmunoResearch). Bamlanivimab was used as standard for converting ELISA optical density value to serum antibody amount [64]. Pre-pandemic biobank sera were used as a negative control. The value for positive nucleocapsid antibody testing is set as two-fold negative control.

QuantiVac EUROIMMUN assay

Serum was diluted 1:100 according to manufacturer instructions and IgG anti SARS-CoV-2 spike protein antibodies were detected by ELISA, using the EUROIMMUN QuantiVac anti-SARS-CoV-2 (IgG) kit (EUROIMMUN Medizinische Labordiagnostika). This kit uses the recombinant S1 domain of the SARS-CoV-2 spike protein to coat the wells. Results were reported as a qualitative interpretation (positive, negative or borderline for antibody) and as a semi-quantitative value expressed as RU·mL^{-1} (relative units). The linear range of the assay was established in the laboratory as 8–95 RU·mL^{-1} and the reference range as <8 RU·mL^{-1}. Samples with values above the linear range were further diluted 1:4 and rerun. If results were above the linear range when run at this dilution, they were reported as >380 RU·mL^{-1}.

Staining of RBD-specific B-cell subsets by flow cytometry

Human PBMC samples were obtained from the biobank at National Jewish Health. PBMC were thawed, and stained with 2 μg·mL^{-1} double-coloured RBD tetramers (conjugated with BV421 and phycoerythrin (PE)), human Fc block and fluorescein isothiocyanate (FITC)-ovalbumin first on ice for 30 min. CD19 APCcy7, IgD BV510, dump (CD4, CD8, CD14, CD16) PerCP antibodies were then added for staining. Cells were washed and stained with Ghost UV450 dye and fixed with 1% paraformaldehyde for flow cytometry analysis.

PBMC cultures and antigen-specific T-cell stimulation

PBMCs were thawed and resuspended in complete RPMI-1640 (10% FBS, 10 mM HEPES, 50 μM 2-β mercaptoethanol, 2 mM L-glutamine, and 1% penicillin and streptomycin). After counting, PBMCs were stained with 5 μM cell proliferation dye eFluor 670 (CPD; #65-0840, Thermo Fisher). CPD-labelled cells were plated at 2×10^5 PBMCs per well in cRPMI + 2 ng·mL^{-1} (10 U·mL^{-1}) recombinant human interleukin (IL)-2 (Biolegend). For RBD stimulation, wells were incubated with 2.5 μg·mL^{-1} of RBD protein (amino acids 319–541 of spike protein) or media alone for 5 days. For cytokine analysis, on day 5 of culture, cells were left unstimulated or were stimulated with 50 ng·mL^{-1} phorbol 12-myristate 13-acetate (Sigma) and 1 μg·mL^{-1} of ionomycin (Sigma-Aldrich) 4 h before harvest. All wells were provided with 10 μg·mL^{-1} of brefeldin A (Sigma-Aldrich) and 1× dilution of monensin (GolgiStop; BD Biosciences) to prevent cytokine secretion.

Staining of T-cell subsets by flow cytometry

PBMC were labelled with LIVE/DEAD fixable violet dye (L34955; Invitrogen), followed by surface antibody staining (CD4, clone RPA-T4; CD8, clone SK1; CD3, clone OKT3; CXCR5, clone J252D4; Biolegend). After surface staining, cells were fixed and permeabilised using FOXP3/transcription factor staining buffer set (#00-5523-00; Invitrogen) per manufacturer's instructions. Fixed cells were stained for

intracellular cytokines anti-IL-2 (clone MQ1-17412; Biolegend) and anti-interferon-γ (clone 4S.B3; Biolegend). Data were collected by flow cytometric analysis on a LSR II (BD Biosciences) cytometer and analysed using FlowJo (BD Biosciences).

Statistical analysis

All comparisons were made using paired and unpaired t-tests with Prism 9 (GraphPad). Where possible, p-values and r correlations are provided directly in figures. p-values in grouped graphs represent unpaired, two-tailed t-tests.

Results

A subset of patients with chronic lung disease exhibit reduced serum antibody titres after vaccination against SARS-CoV-2

Serum samples were used to assess SARS-CoV-2 vaccine (Pfizer-BioNTech BNT162b2, Moderna mRNA-1273 and Janssen) responsiveness in a cohort of nine asthma patients, eight COPD patients and 15 ILD patients and 31 healthy controls (table 1). To investigate the humoral response, we performed an in-house quantitative ELISA for serum SARS-CoV-2 nucleocapsid and spike RBD-specific antibodies. Serum collected between 14 and 231 days after the last vaccination/boost was analysed. Asthma ($p<0.035$) and COPD ($p<0.022$) patients showed significantly reduced antibody titres 3–4 months after vaccination compared to healthy controls (figure 1a). 40% (six out of 15) of ILD patients also exhibited reduced antibody titres compared to healthy subjects. To validate these findings, serum titres from the in-house anti-RBD assay and QuantiVac ELISA (semiquantitative Spike protein IgG) were compared. As expected, samples with the highest serum anti-RBD titres, including 100% of healthy controls, were most prominent in the highest anti-spike titre bin (>350 BAU·mL^{-1} (binding antibody units)) while those showing low anti-RBD titres were enriched in the lowest bin (<150 BAU·mL^{-1}) (figure 1b).

Although no prior infection was noted at the time of blood collection, 16% of the samples showed evidence of prior SARS-CoV-2 infection based on anti-nucleocapsid antibody titres in the serum that were two-fold higher than a pre-pandemic control sample (figure 1c). To investigate how prior infection was influencing anti-RBD antibody titres in figure 1a, we compared where individuals with prior infection (open circles) fell on the graphs. While some asthma and ILD patients with prior viral exposure showed similar serum anti-RBD titres to healthy controls, some asthma and COPD patients with prior

TABLE 1 Investigative cohort of severe acute respiratory syndrome coronavirus 2-vaccinated patients with chronic lung disease

	Asthma	COPD	ILD	Healthy controls	Total
Subjects	9	8	15	31	63
Age at sample years	58 (43–71)	64 (57–73)	62 (47–73)	50 (25–72)	56 (25–73)
Females	5 (56)	5 (62)	9 (60)	14 (45)	33 (52)
Males	4 (44)	3 (38)	6 (40)	17 (55)	30 (48)
Days from last vaccination to sample	117 (87–156)	138 (112–163)	122 (84–144)	121 (14–231)	123 (14– 231)
Immunosuppressants	6 (66)	4 (50)	9 (60)	0 (0)	19 (30)
FEV_1 pre-bronchodilator % pred	76 (49–106)	63 (27–98)	77 (33–109)	NA	NA
Subjects	8	7	15		
FVC pre-bronchodilator % pred	78 (61–99)	81 (55–111)	75 (34–97)	NA	NA
Subjects	8	7	15		
FEV_1/FVC pre-bronchodilator % pred	94 (78–109)	75 (48–101)	101 (88–115)	NA	NA
Subjects	8	7	15		
Meets GINA 4 criteria	3				
Meets GINA 5 criteria	3				
Pfizer-BioNTech COVID-19 vaccine 30 µg/0.3 mL intramuscular suspension	4	2	12	28	46
Moderna COVID-19 vaccine 100 µg/0.5 mL intramuscular suspension	3	3	2	3	11
Janssen COVID-19 vaccine 0.5 mL intramuscular suspension	1	3	0	0	4
Combination of vaccines	1	0	1	0	2
Nonsmokers	4	1	7	NA	

Data are presented as n, mean (range) or n (%). ILD: interstitial lung disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GINA: Global Initiative for Asthma; COVID-19: coronavirus disease 2019; NA: not available.

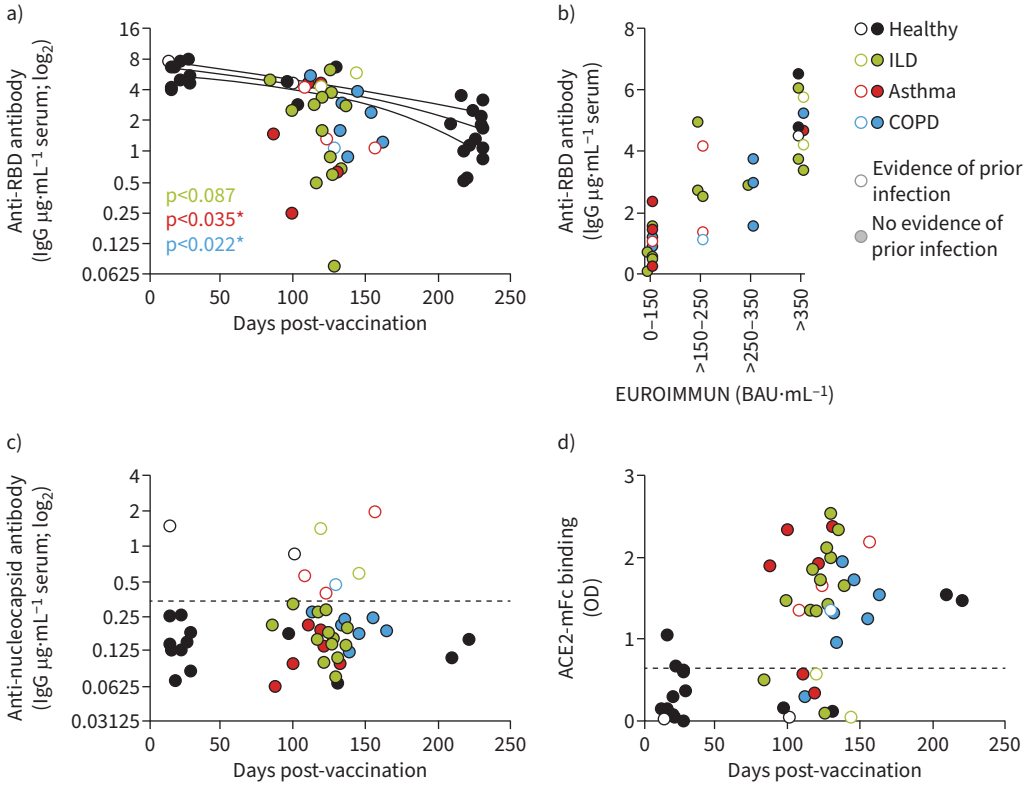

FIGURE 1 Impaired serum antibody titres against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (RBD) in a subset of patients with chronic lung disease after vaccination. a) ELISA for serum IgG binding to SARS-CoV-2 RBD in healthy, interstitial lung disease (ILD), asthma and COPD patients 14–231 days post-SARS-CoV-2 vaccination. Line represents simple linear regression for healthy subjects flanked by 95% confidence intervals. b) Serum anti-RBD antibodies detected 75–175 days after vaccination of healthy and chronic lung disease patients using the in-house ELISA were compared to antibody titres against the SARS-CoV-2 spike protein S1 domain using QuantiVac ELISA (EUROIMMUN) IgG binding antibody units (BAU). c) Serum IgG binding to SARS-CoV-2 nucleocapsid. Dotted line represents values two-fold greater than pre-pandemic negative control. d) Optical density of angiotensin-converting enzyme (ACE)2-mFc binding to RBD in competition assay with patient serum. Dotted line represents optical density (OD) 0.65, which corresponds to the OD that 0.1 µg·mL^{-1} of the positive control bamlanivimab anti-RBD antibody outcompetes ACE2-Fc binding of RBD. OD values below this threshold represent serum with good blocking activity. p-values represent unpaired t-test comparing titres taken 75–175 days after last vaccination. *: p<0.05.

SARS-CoV-2 exposure still exhibited low anti-RBD antibody titres. Thus, while natural infection may complement the vaccine response in some patients with chronic lung disease, it does not boost vaccine titres in others, suggesting that hybrid immunity (natural infection+vaccination=improved protection) may be compromised in at least some patients with chronic lung disease [65, 66].

Because individuals with ILD, asthma and COPD were more likely to exhibit reduced antibody responses after vaccination, we investigated whether serum from these individuals was less likely to neutralise virus. To do this we performed an ACE2-mFc competition assay. In this assay, the patient's serum anti-RBD antibodies compete with recombinant ACE2-mFc (the receptor that binds RBD) for RBD binding. More neutralisation capacity of the serum results in better blocking of ACE2-mFc. This manifests as less ACE2-mFc detection in the assay. Consistent with our prior work and others, 94% of serum from vaccinated healthy controls exhibited good blocking activity over the first 3–5 months post-vaccination (figure 1d). In contrast, only 22% of patients with ILD, asthma or COPD showed good blocking activity over this same time span. The limited blocking activity in patients with chronic lung disease was independent of prior infection history, as some patients with nucleocapsid antibodies also showed limited blocking activity in this assay. Together, these investigative findings suggest that many patients with ILD, asthma, and COPD may not achieve or maintain the same level of humoral protection after vaccination as healthy subjects.

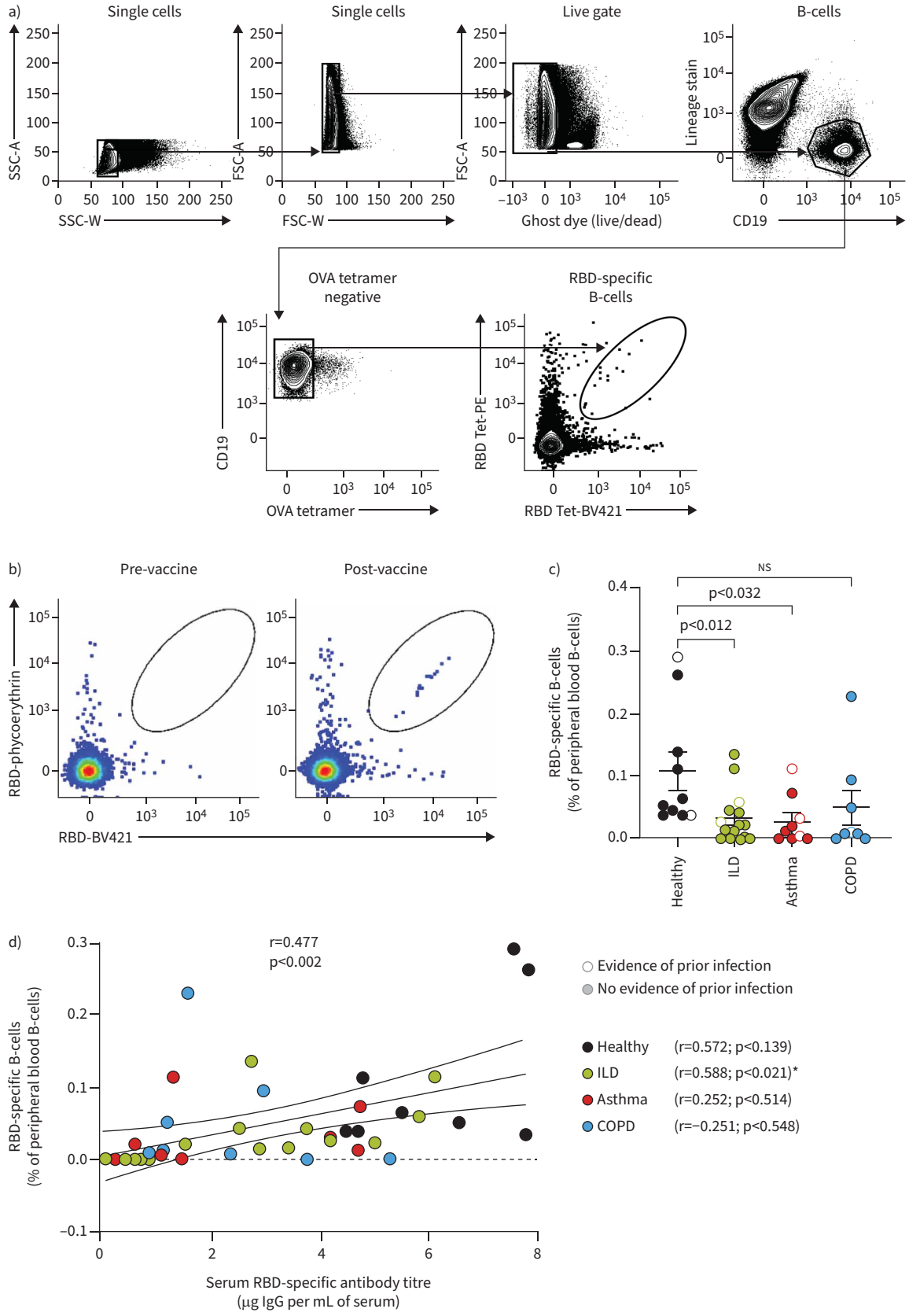

FIGURE 2 Decreased circulating receptor-binding domain (RBD)-reactive memory B-cells in patients with chronic lung disease after vaccination compared to healthy controls. Peripheral blood mononuclear cells (PBMCs) collected 14–175 days post-vaccination. a) Gating scheme for

RBD-specific B-cells from PBMCs. b) Representative pseudocolour plots of circulating B-cells from blood of patients pre- and post-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination. Gate represents dual RBD-tetramer binding B-cells. c) Graph represents the percentage of RBD^+ B-cells within the total circulating B-cell pool of healthy, interstitial lung disease (ILD), asthma and COPD patients. d) Correlation of serum anti-RBD antibody titres and circulating RBD-binding B-cells detected in healthy and chronic lung disease patients after SARS-CoV-2 vaccination. Lines represent best-fit simple linear regression with flanking lines demarcating 95% confidence intervals. n=8–14; error bars represent SEM. *: $p<0.05$; NS: nonsignificant.

Circulating spike-specific B-cells are reduced in patients with chronic lung disease

To investigate vaccine-specific memory B-cells, we enriched PBMCs for B-cells and identified RBD-specific B-cells using double-coloured RBD-tetramers (figure 2a,b) [67]. We minimised contamination of non-RBD-specific B-cells by eliminating B-cells that bound an "irrelevant" ovalbumin-FITC protein (figure 2a) [68, 69]. RBD-specific B-cells were not observed in PBMCs taken pre-pandemic (figure 2b). Individuals with ILD ($p<0.012$) and asthma ($p<0.032$) had significantly fewer circulating RBD-specific B-cells than healthy controls (figure 2c). COPD patients on average had fewer RBD-specific B-cells within the circulating B-cell population than observed in healthy controls (figure 2c). Of note, recent SARS-CoV-2 infection based on anti-nucleocapsid antibodies in the serum did not appear to impact the percentage of RBD-specific memory B-cells (figure 2c). This appears consistent with reports showing that recent viral infection can impede B-cell responses after boosting with the vaccine [70].

When RBD-specific B-cells from all patients were compared to their RBD-specific serum antibody titres, a significant correlation ($r=0.477$; $p<0.002$) was observed (figure 2d). While the strongest positive correlation was observed in healthy subjects ($r=0.572$), ILD patients ($r=0.588$; $p<0.021$) also correlated. Together, these data indicate that many individuals with chronic lung disease fail to generate a robust pool of circulating vaccine-specific B-cells compared to healthy controls.

T-cell response to SARS-CoV-2 vaccination is impaired in patients with chronic lung disease

To investigate the RBD-specific $CD8^+$ and $CD4^+$ T-cell responses in a way that was agnostic to a patient's human leukocyte antigen type, we used a modified approach previously described to efficiently detect spike-responsive T-cells in the blood of patients with mild COVID-19 [42]. This approach identifies RBD-reactive T-cells based on their loss of cell proliferation dye (figure 3a). Using this approach, subsets of individuals with underlying lung conditions exhibited diminished RBD-specific T-cell responses compared to healthy controls (figure 3b,c). Specifically, $CD8^+$ ($p<0.004$) and $CD4^+$ ($p<0.023$) T-cell responses in asthma patients were significantly reduced, as were $CD8^+$ ($p<0.008$) T-cell responses in COPD patients. Of note, 21% of ILD patients showed limited $CD8^+$ T-cell responses and 42% failed to evoke a robust $CD4^+$ T-cell response after vaccination. Similarly, 33–37.5% of asthmatic and COPD patients had no observable $CD4^+$ and $CD8^+$ T-cell responses to the vaccine antigen. Similar to our findings with RBD-specific antibodies and memory B-cells, prior SARS-CoV-2 infection did not have an obvious impact on the percentage of RBD-specific $CD8^+$ or $CD4^+$ T-cells in this exploratory cohort. This indicates that prior infection in some patients with chronic lung disease does not enhance T-cell-mediated immunity after vaccination. This again may suggest that hybrid immunity is impaired in a subset of ILD, asthma and COPD patients.

To find out if humoral and cell-mediated immunity were impacted similarly in our patient pool after vaccination, we compared RBD-specific T-cell responses to serum RBD-specific antibodies. While $CD4^+$ T-cell responsiveness correlated strongly ($r=0.728$; $p<0.0001$) with $CD8^+$ T-cell vaccine responses across disease cohorts, no correlation was observed between RBD-specific T-cell responses and RBD-specific antibody titres (figure 3d–f). This suggests that an individual's humoral response to vaccine can be independent of their vaccine-elicited T-cell immunity and *vice versa*.

Vaccine-specific T-cells in patients with chronic lung conditions have impaired cytokine potential

To address T-cell function, the cytokine potential of T-cells in our patient cohorts was assayed by intracellular cytokine staining. While the percentages of bulk $CD8^+$ T-cells that were interferon (IFN)-γ competent were significantly ($p<0.012$) elevated among vaccinated COPD patients compared to healthy controls, the percentage of such cells in asthmatic and ILD patients were not significantly different (figure 4a). Conversely, the percentage of bulk CD8 T-cells from asthmatic patients that could produce IL-2 were significantly ($p<0.014$) reduced relative to healthy controls (figure 4a). While this suggests that some heterogeneity exists in the cytokine profiles of patients with chronic lung disease, for the most part, bulk T-cell function appears similar across disease groups. Even less heterogeneity was observed in the cytokine potential of $CD4^+$ T-cells across disease groups and healthy patients (figure 4b).

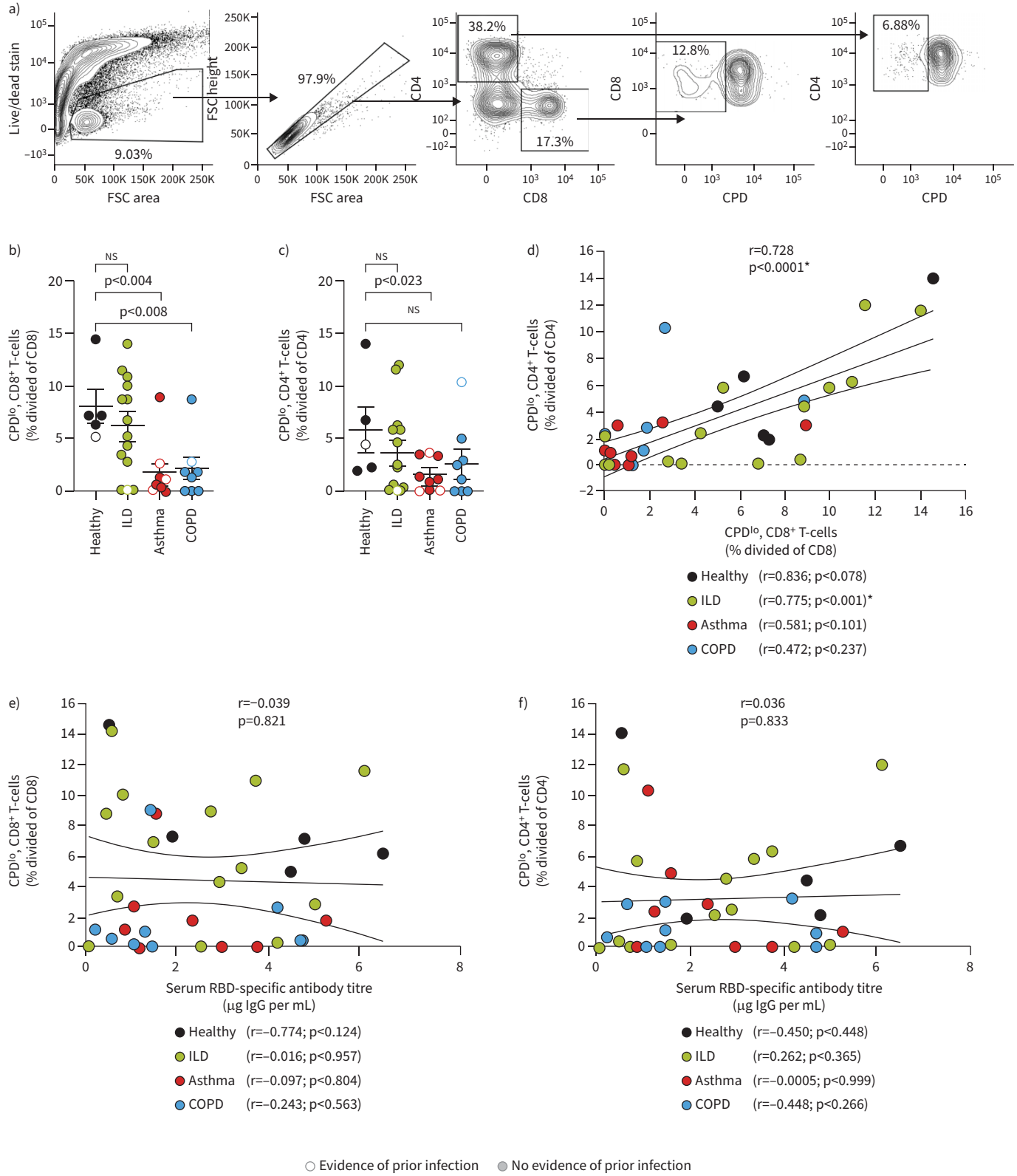

FIGURE 3 Decreased circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD)-specific T-cells in a subset of patients with chronic lung disease after vaccination. Peripheral blood mononuclear cells (PBMCs) collected 75–220 days post-vaccination. a) Gating scheme to identify RBD-specific $CD4^+$ and $CD8^+$ T-cells based on loss of cell proliferation dye (CPD). b) Graph represents the percentage of CPD-low (divided) RBD-specific $CD8^+$ T-cells within the total circulating $CD8^+$ T-cell populations after culture and stimulation with RBD-protein in healthy, interstitial lung disease (ILD), asthma and COPD patients. Numbers are normalised by subtracting CPD-low population in PBMC cultures

that received no protein. c) Graph represents the percentage of CPD-low (divided) $CD4^+$ T-cells within the total circulating $CD4^+$ T-cell populations after culture and stimulation with RBD-protein in healthy patients and patients with chronic lung disease. Numbers are normalised by subtracting CPD-low population in PBMC cultures that received no protein. d) Correlation of CPD-low (divided) $CD4^+$ and $CD8^+$ T-cells in circulation in healthy, ILD, COPD and asthma patients after SARS-CoV-2 vaccination. Central line represents best-fit simple linear regression; flanking lines demarcate 95% confidence intervals. e) Correlation between CPD-low (divided) $CD8^+$ T-cells in circulation and serum antibody titres against RBD in healthy, ILD, COPD and asthma patients after SARS-CoV-2 vaccination. Central solid line represents best-fit simple linear regression; flanking lines demarcate 95% confidence intervals. f) Correlation between CPD-low (divided) $CD4^+$ T-cells in circulation and serum antibody titres against RBD in healthy, ILD, COPD and asthma patients after SARS-CoV-2 vaccination. Central line represents best-fit simple linear regression; flanking lines demarcate 95% confidence intervals. n=5–14; error bars represent SEM. FSC: forward scatter. *: $p<0.05$; NS: nonsignificant.

In contrast to bulk T-cell populations, heterogeneity in cytokine potential was observed in vaccine-responsive T-cell populations. In these experiments, vaccine responsive T-cells were defined by loss of the cell staining dye CPD, indicative of cells that had divided in response to RBD antigen. In patients with chronic lung disease, the percentage of RBD-responsive $CD8^+$ T-cells from asthma and COPD patients that could produce IFN-γ and/or IL-2 was significantly reduced compared to similar T-cells obtained from healthy subjects (figure 4c,d). A similar finding was observed in RBD responsive $CD4^+$ T-cells from asthma and COPD patients (figure 4e,f). Of note, while asthma and COPD patients showed more homogeneity in their T-cell functionality, a subset of patients with ILD also exhibited decreased IFN-γ and IL-2 within RBD-specific $CD4^+$ and $CD8^+$ T-cells compared to healthy controls. This suggests that at least some patients within each disease cohort exhibit reduced T-cell functionality to the vaccine. No obvious difference was observed when individuals within each disease group were binned based on prior or recent SARS-CoV-2 infection (presence of anti-nucleocapsid antibodies).

When looking at total T-cell responsiveness, patients mounting a productive $CD4^+$ T-cell response generally exhibited a productive $CD8^+$ T-cell response ($r=0.703$; $p<0.0001$) (figure 4g). To understand whether T-cell function similarly tracked with humoral immunity after vaccination, we compared IFN-γ^+ RBD-specific T-cells in each patient to their serum anti-RBD titres. In all patient groups, no significant correlation was observed (figure 4h,i). Together with serum antibody and memory B-cell data, these findings indicate that the SARS-CoV-2 vaccine may differentially promote T-cell and humoral immunity in some ILD, asthma and COPD patients.

SARS-CoV-2-specific Tfh cells exhibit decreased cytokine potential in patients with chronic lung conditions compared to healthy controls

Given that Tfh cells are important in driving humoral vaccine responses, we next investigated the Tfh response in vaccinated patients with pulmonary disease. The percentage of $CXCR5^+$ $CD4^+$ circulating Tfh (cTfh) cells among the total $CD4^+$ T-cell pool was decreased across all disease cohorts reaching significance within asthma ($p<0.011$) and COPD ($p<0.006$) patients (figure 5a). While IL-2 production remained comparable to healthy controls, the relative percentage of IFN-γ expressing cTfh cells was increased across all chronic lung disease cohorts (figure 5b). Increased IFN-γ production was most evident in COPD ($p<0.027$) patients; however, at least some ILD and asthma patients also exhibited increased IFN-γ expression within bulk cTfh cells relative to healthy controls. Despite the increased IFN-γ production observed in bulk cTfh cells in patients with chronic lung disease, RBD-responsive (CPD^{lo}) cTfh cells on average exhibited decreased IFN-γ production compared to vaccinated, healthy controls reaching significance in asthma patients (figure 5c). In fact, 21% of ILD patients, 44% of asthma patients and 25% of COPD patients in this investigative cohort lacked IFN-γ-expressing RBD-responsive Tfh cells above background (figure 5c). This mirrors the decreased functionality of vaccine responsive T-cells within non-Tfh cell populations.

Discussion

This study highlights the significant heterogeneity that exists in the vaccine response to SARS-CoV-2 in individuals with ILD, COPD and asthma compared to healthy controls. In our assessment of vaccine-induced antibody titres, memory B-cell subsets and T-cells in patients with asthma, COPD and ILD, we found that 48.3% of patients with chronic lung disease exhibited serum antibody titres to the vaccine antigen below the expected titres observed in healthy controls 3–4 months after the last vaccine administration. This correlated with decreased RBD-specific circulating memory B-cells. Importantly, while serum antibodies generated by healthy controls showed good neutralisation activity, the vast majority of ILD, COPD and asthma patients showed poor neutralisation activity, even during the first 3–4 months post-vaccination. In addition, most patients with asthma and COPD and a subset of patients with ILD had reduced circulating RBD-responsive $CD4^+$ T-cells, $CD8^+$ T-cells and Tfh cells. Of note, while most

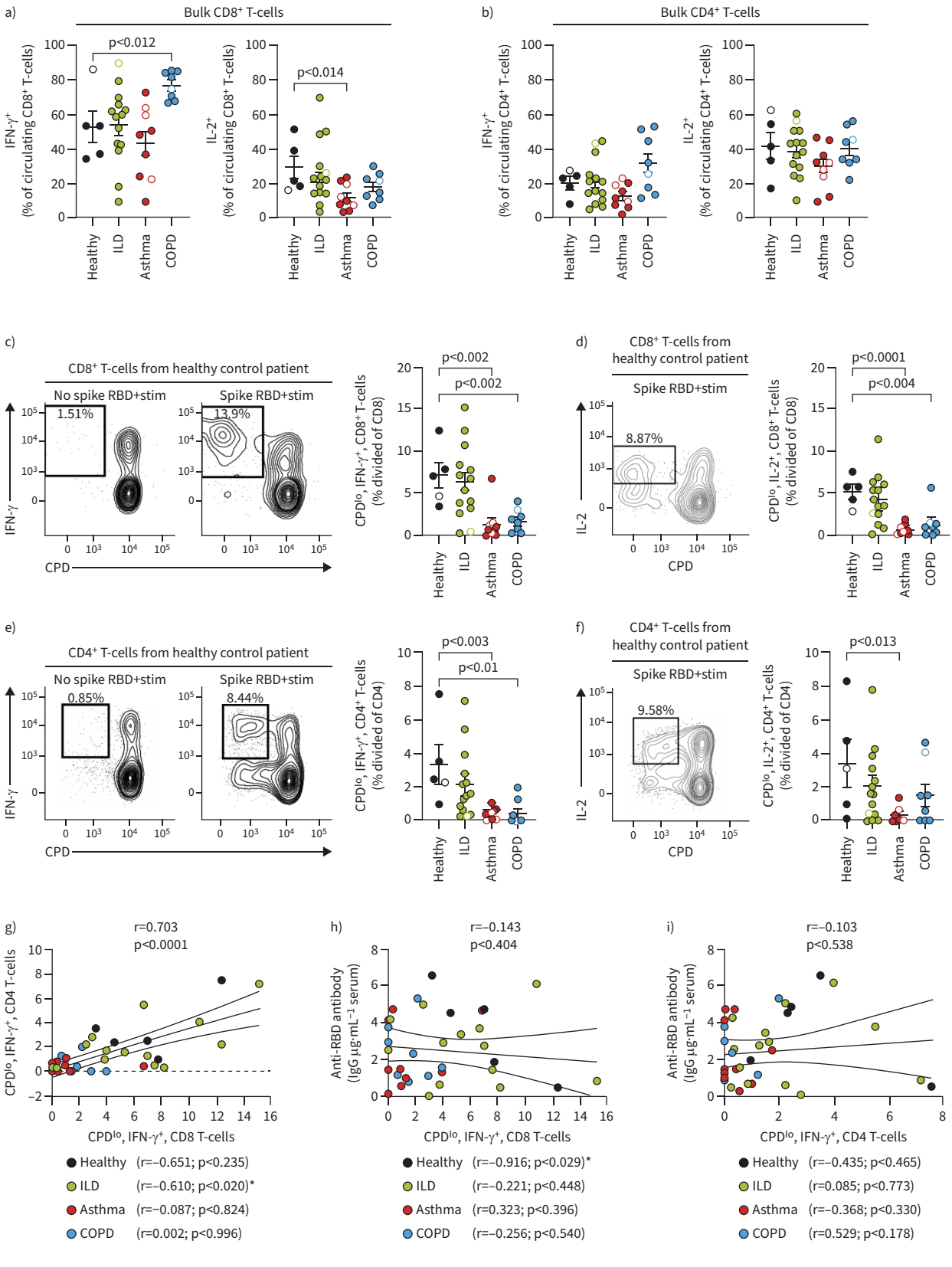

FIGURE 4 Impaired cytokine potential among severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor-binding domain (RBD)-specific T-cells after vaccination of patients with chronic lung disease. Peripheral blood mononuclear cells (PBMCs) collected 75–220 days

post-vaccination. a) Graph represents the percentage of interferon (IFN)-γ- and interleukin (IL)-2-expressing $CD8^+$ T-cells within the total circulating $CD8^+$ T-cell population after stimulation (stim) with RBD protein in healthy patients and patients with chronic lung disease. Numbers based on gates set in unstimulated cultures. b) Graph represents the percentage of IFN-γ- and IL-2-expressing $CD4^+$ T-cells within the total circulating $CD4^+$ T-cell population after stimulation with RBD protein in healthy patients and patients with chronic lung disease. Numbers based on gates set in unstimulated cultures. c) Contour plots and graph identifying the percentage of IFN-γ^+ cell proliferation dye (CPD)-low (divided) $CD8^+$ T-cells within the total $CD8^+$ T-cells pool. Gate in contour plot identifies circulating CPD-low (divided) $CD8^+$ T-cells that express IFN-γ. Dividing cells above background are only found in cultures stimulated with RBD. d) Representative contour plot and graph identifying the percentage of IL-2^+ CPD-low (divided) $CD8^+$ T-cells within the total $CD8^+$ T-cells pool. Numbers in graph are normalised by subtracting CPD-low (dividing) population in PBMC cultures that received no protein. e) Contour plots and graph identifying the percentage of IFN-γ^+ CPD-low (divided) $CD4^+$ T-cells within the total $CD4^+$ T-cells pool after vaccination. Gate in contour plot identifies circulating CPD-low (divided) $CD4^+$ T-cells that express IFN-γ. Notable CPD^{low} population that falls outside of gate represents RBD-responsive T-cells that are not expressing IFN-γ. Numbers in graph are normalised by subtracting CPD-low (dividing) population in PBMC cultures that received no protein. f) Representative contour plot and graph identifying the percentage of IL-2^+ CPD-low (divided) $CD4^+$ T-cells within the total $CD4^+$ T-cells pool. Numbers in graph are normalised by subtracting CPD-low (dividing) population in PBMC cultures that received no protein. g,h,i) Correlation between g) IFN-γ expressing CPD-low (divided) $CD4^+$ and $CD8^+$ T-cells, h) IFN-γ expressing CPD-low (divided) $CD8^+$ T-cells and serum RBD antibody titres and i) IFN-γ expressing CPD-low (divided) $CD4^+$ T-cells and serum RBD antibody titres in healthy, interstitial lung disease (ILD), asthma and COPD patients after SARS-CoV-2 vaccination. Central solid line represents best-fit simple linear regression; flanking lines demarcate 95% confidence intervals. n=5–14; error bars represent SEM. *: $p<0.05$.

chronic lung disease patients showed impaired vaccine responsiveness, many patients exhibited defects in only one arm of the adaptive response (humoral and cell-mediated) to SARS-CoV-2 vaccination. This highlights the considerable variability in vaccine responses among patients with chronic lung disease and illustrates the importance of deep immunophenotyping of high-risk patients to determine their overall immunity to SARS-CoV-2 after vaccination.

While the limited sample size prevents firm conclusions, an interesting observation within this small exploratory study was a failure to see enhanced vaccine responses in individuals who had evidence of a recent SARS-CoV-2 exposure. It has been shown that prior infection can promote more robust B- and T-cell response to the vaccine, and this hybrid immunity is associated with less severe COVID-19 [65, 66]. It is possible that natural exposures to SARS-CoV-2 in this setting occurred after vaccination, which has been linked to reduced antibody responses and memory B-cell generation [70]. If and how hybrid immunity is impacted in patients with ILD, asthma and COPD warrants further investigation, as these individuals would appear to be at greater risk of developing more severe disease if hybrid immunity is impaired. This evident lack of robust hybrid immunity in patients with chronic lung disease is likely to become more clinically relevant over time, as it appears that ongoing vaccination efforts are transitioning to a yearly dose, similar to traditional vaccination for influenza. In that regard, understanding how immune responses to both natural infection and vaccination differ between chronic lung disease patients and healthy controls is likely key to understanding how to evoke lasting protection.

While not designed or powered to address safety, efficacy or durability of the vaccine response in patients with chronic lung disease, the current data suggest that what we understand regarding vaccination in healthy subjects may not be directly applicable to patients with chronic lung disease. Furthermore, the data also show that vaccine responses may differ depending on the type of underlying lung condition. For example, as a group, individuals with COPD and asthma were more likely to exhibit impaired antibody and T-cell responses than ILD patients, who instead exhibited greater heterogeneity in their mRNA vaccine response. Factors that separate responders from nonresponders within a particular disease group may reflect distinct disease-associated endotypes within COPD, asthma and ILD, including the possibility that subsets of each of these lung diseases are associated with broadly abnormal immunity, a concept that finds support in previous studies [71]. Another likely factor contributing to the varied vaccine responses observed across these pulmonary disease cohorts are medicines used to treat chronic lung disease. Immunosuppressants (*e.g.* biologics and/or prednisone) represent one class of drugs often prescribed to patients with ILD (*e.g.* sarcoidosis, hypersensitivity and connective tissue disorder-ILD) and asthma to control disease pathobiology and symptoms. Future studies are needed to dissect the intrinsic impact of the disease from extrinsic factors that are impeding vaccination efficacy in patients with ILD, asthma and COPD.

A key caveat in the current study is the lack of a longitudinal assessment within these different disease cohorts. We know from healthy controls that each arm of the immune system varies over time after vaccination. For example, while anti-RBD antibody titres and cTfh numbers wane 6 months after vaccination, vaccine-specific T-cell responses and memory B-cell responses remain relatively stable over

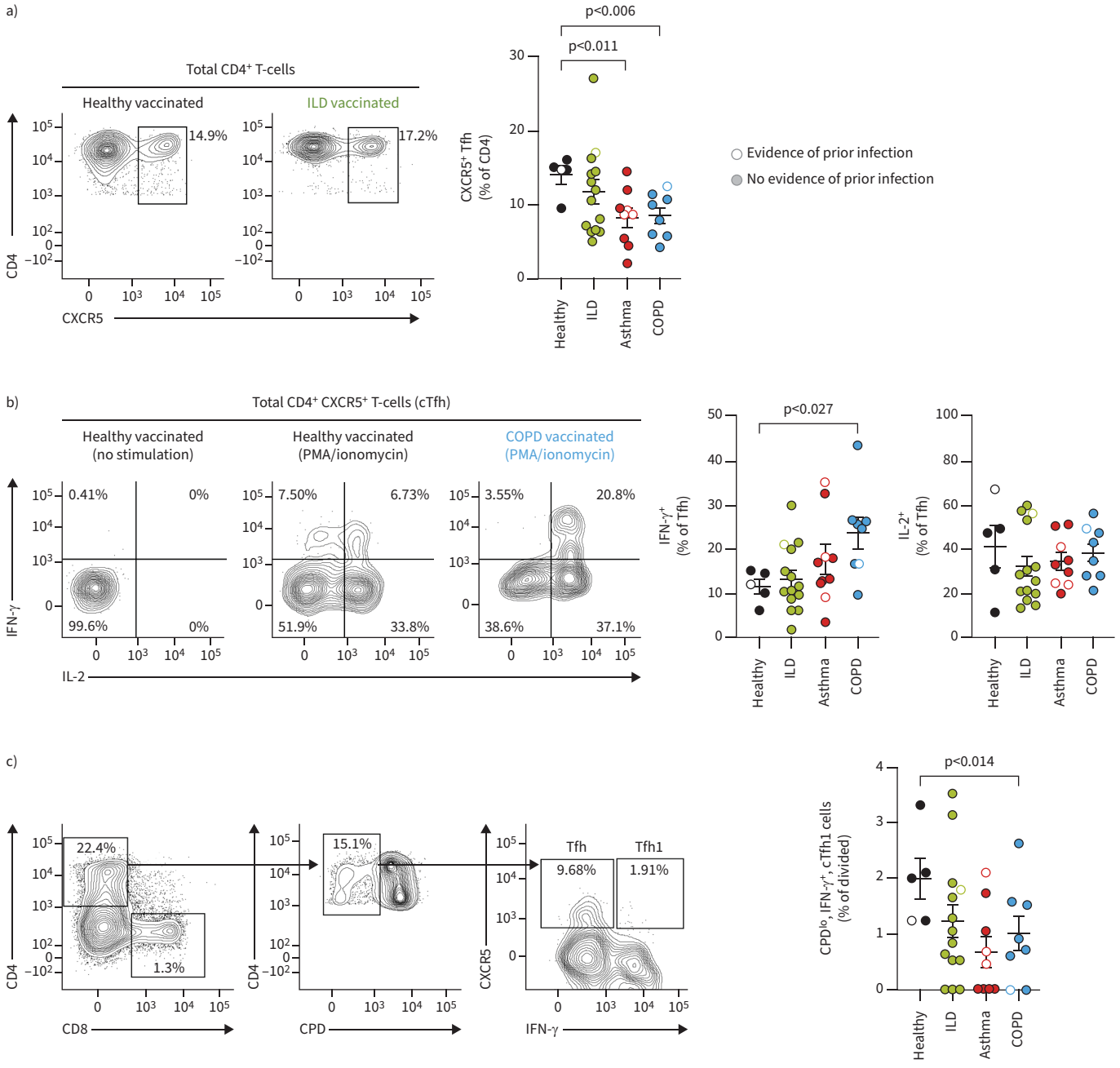

FIGURE 5 Patients with chronic lung disease have heterogeneous follicular T-helper (Tfh) cell responses after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination compared to healthy controls. Peripheral blood mononuclear cells (PBMCs) collected 75–220 days post-vaccination. a) Contour plots from representative PBMC cultures from healthy and interstitial lung disease (ILD) vaccinated patients. Contour plot and graph reveal the percentage of $CXCR5^+$ Tfh cells among total circulating $CD4^+$ T-cells. b) Representative contour plots from a) of $CXCR5^+$ Tfh cells from PBMC cultures of healthy and COPD vaccinated patients with or without stimulation with phorbol 12-myristate 13-acetate (PMA)/ionomycin. Gates reveal the percentage of $CXCR5^+$ Tfh cells expressing one or both interferon (IFN)-γ and interleukin (IL)-2 cytokines. Graphs show the percentage of circulating Tfh (cTfh) cells in these distinct disease cohorts and healthy controls that express IFN-γ or IL-2. c) Gating scheme and graph show the percentage of IFN-γ expressing cell proliferation dye (CPD)-low (divided) $CXCR5^+$ Tfh cells within the receptor-binding domain (RBD)-specific Tfh cell population in healthy patients and patients with chronic lung conditions. Numbers in graph are normalised by subtracting CPD-low (dividing) population in PBMC cultures that received no protein. n=5–14; error bars represent SEM.

that same period in healthy subjects [25, 72]. Whether similar kinetics occur in individuals with chronic lung disease remains unknown. The investigative data provided herein suggest that a large percentage of individuals with chronic lung disease fail to mount productive humoral and cell-mediated immunity during

the first and second dosing of the vaccine. What remains unclear is whether such nonresponders remain impaired after subsequent vaccination attempts. While there is evidence that a third booster can be effective in providing some protection against SARS-CoV-2 in other high-risk populations [56, 73–75], some seronegative individuals who did not respond to the first two doses of vaccine also fail to respond to the third boost [76]. Thus, the benefit of multiple boosts or more frequent boosting in subsets of patients with asthma, ILD and COPD that show inadequate vaccine responsiveness should be explored.

Another caveat of the current study is that it focused solely on the circulating RBD-responsive IgG antibody, memory B-cell and T-cell responses post-vaccination. While the data are certainly consistent with impaired vaccine responses within these circulating populations, natural infection can induce tissue-resident and mucosal immunity that may not be adequately reflected in the current data [77]. Indeed, serum IgA (a reflection of mucosal immunity) against SARS-CoV-2 generated after natural infection or vaccination corresponds to protection against the virus [78, 79]. Furthermore, while protective antibody responses to the vaccine largely concentrate to the RBD portion of the spike protein, the T-cell repertoire to the vaccine is more heterogeneous [80]. Many vaccine-responsive T-cells recognise antigens outside of the RBD, and these would not have been identified based on the protocols used in this study. Whether individuals with ILD, asthma and COPD possess impaired mucosal responses in addition to those observed here and whether such individuals exhibit similarly impaired T-cell responses to vaccine epitopes that lie outside of the RBD regions remain important areas of future investigation.

In conclusion, while limited in sample size, this exploratory study suggests that efficacy of the vaccine and vaccine-induced immunity in healthy individuals should not be uniformly extrapolated to individuals with chronic lung disease. This finding has clinical relevance, as these individuals are considered at high risk for contracting severe COVID-19. Patients with COPD, for example, have increased odds of hospitalisation, intensive care unit admission and mortality compared to healthy controls if exposed to SARS-CoV-2 [47]. Given the relatively high percentage of patients with chronic lung disease showing some form of impaired vaccine responsiveness and the high degree of heterogeneity in the responses observed across individuals with ILD, asthma and COPD, chronic lung disease patients may benefit from personalised vaccination schemes and deeper assessment of immune responses to ensure optimal protection in this vulnerable population.

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