






Basic science

Humoral and cellular immunity in patients with rare autoimmune rheumatic diseases following SARS-CoV-2 vaccination

Leher Gumber ¹, Nancy Gomez², Georgina Hopkins², Davis Tucis², Laura Bartlett², Kieran Ayling ³, Kavita Vedhara³, Graham Steers², Mithun Chakravorty⁴, Megan Rutter^{4,5}, Hannah Jackson², Patrick Tighe², Alastair Ferraro⁶, Sheila Power¹, Marie-Josèphe Pradère¹, David Onion², Peter C. Lanyon^{4,5,7}, Fiona A. Pearce ^{4,5,7,*}, Lucy Fairclough²

¹Nottingham University Hospitals NHS Trust, Nottingham, UK

²School of Life Sciences, University of Nottingham, Nottingham, UK

³School of Medicine, University of Nottingham, Nottingham, UK

⁴Department of Rheumatology, Nottingham University Hospitals NHS Trust, Nottingham, UK

⁵Lifespan and Population Health, School of Medicine, University of Nottingham, Nottingham, UK

⁶Department of Nephrology, Nottingham University Hospitals NHS Trust, Nottingham, UK

⁷NIHR Nottingham Biomedical Research Centre, Nottingham, UK

*Correspondence to: Fiona A. Pearce, Department of Rheumatology, Nottingham University Hospitals NHS Trust, Nottingham NG7 2UH, UK.

E-mail: fiona.pearce@nottingham.ac.uk

Abstract

Objectives: Coronavirus 2019 vaccine responses in rare autoimmune rheumatic diseases (RAIRDs) remain poorly understood; in particular there is little known about whether people develop effective T cell responses. We conducted an observational study to evaluate the short-term humoral and cell-mediated T cell response after the second severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination in RAIRD patients compared with healthy controls (HCs).

Methods: Blood samples were collected after the second dose and anti-spike, anti-nucleocapsid antibody levels and SARS-CoV-2-specific T cell responses were measured and compared with those of HCs. Activation-induced marker and deep phenotyping assays were used to identify differences in T cells between high and no/low antibody groups, followed by multidimensional clustering.

Results: A total of 50 patients with RAIRDs were included (31 with AAV, 4 with other systemic vasculitis, 9 with SLE and 6 with myositis). The median anti-spike levels were significantly lower in RAIRD patients compared with HCs ($P < 0.0001$). Fifteen (33%) patients had undetectable levels and 26 (57%) had levels lower than the lowest HC. Rituximab in the last 12 months ($P = 0.003$) was associated with reduced immunogenicity compared with a longer pre-vaccination period. There was a significant difference in B cell percentages ($P = 0.03$) and spike-specific CD4⁺ T cells ($P = 0.02$) between no/low antibody vs high antibody groups. Patients in the no/low antibody group had a higher percentage of terminally differentiated (exhausted) T cells.

Conclusions: Following two doses, most RAIRD patients have lower antibody levels than the lowest HC and lower anti-spike T cells. RAIRD patients with no/low antibodies have diminished numbers and poor quality of memory T cells that lack proliferative and functional capacities.

Keywords: rare autoimmune rheumatic diseases, SARS-CoV-2, vaccination, antibody, cell mediated, T cells

Rheumatology key messages

- A total of 57% of RAIRD patients had an insufficient antibody response (lower antibody levels than the lowest healthy control) following two vaccine doses.
- Patients with low or no antibodies also have significantly lower levels of memory T cells that lack both functional and proliferative capacities.
- Assessment of both serological and T cell responses is necessary to fully define responses to vaccination in immunosuppressed populations.

Introduction

The rapid development of vaccines and mass vaccination since the emergence of coronavirus disease 2019 (COVID-19) has helped control the transmission and severity of severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although these vaccines have a good efficacy and safety profile in the general population [1, 2], less is known about their effects in immunocompromised patients (ICPs). There is a

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particular gap in the literature related to people with rare autoimmune rheumatic diseases (RAIRDs) such as systemic vasculitis, who are thought to be at increased risk of severe poor outcomes and mortality from COVID-19 compared with the general population and compared with people with RA and other inflammatory arthritis [3–6]. Successful host protection from vaccination relies on a functional immune system including humoral and cell-mediated responses, which can be diminished in RAIRDs secondary to immunosuppressive therapy [7, 8]. Previous research has identified that high disease activity and high-dose glucocorticoids are associated with an increased risk of severe COVID-19 infection [9, 10]. In particular, rituximab, a monoclonal anti-CD20 B cell-depleting agent, has been shown to increase the severity of infection [11, 12] and the risk of COVID-19-related death [9] and reduce vaccine responsiveness [13]. Additionally, the time since the last rituximab treatment has been shown to impact humoral response, with a 7–9 month period prior to vaccine being the most significant predictor of impaired response [14, 15]. B cell numbers also influence response in rituximab-treated patients, with a minimum of 0.4% of circulating lymphocytes being required for seroconversion [16]. Methotrexate and glucocorticoids have also been shown to diminish immunogenicity of SARS-CoV-2 vaccines [7, 17–19].

The effect of vaccination on cellular immunity in patients with stable disease on long-term immunosuppressive therapy is less well described. A recent study on vasculitis and autoimmune glomerulonephritis patients found T cell responses in >80% of patients even in the absence of serological responses [14]. Another study, which aimed to characterize the phenotype of the T cell response, found a higher proportion of TNF- α -producing CD4 cells in seronegative autoimmune rheumatic disease patients [20]. However, both of these studies did not provide any data on memory T cells. As we know from previous research, memory T cells mediate a faster and more potent response upon repeat encounter with antigens and thereby underpin long-lasting immunity against infection [21]. In addition, some questions remain unanswered, including the short- and medium-term immune response to vaccination and vaccine response in different types of RAIRDs.

To address these research gaps, we conducted a prospective cohort study to evaluate the humoral and cell-mediated response to SARS-CoV-2 vaccination in patients with RAIRDs compared with healthy controls (HCs). Here we present the findings of the short-term response to two doses of SARS-CoV-2 vaccination with a focus on memory T cells, which have not been well described in previous studies.

Methods

Study design and population

We conducted a prospective, single-centre longitudinal cohort study in individuals with RAIRDs recruited from Nottingham University Hospitals NHS Trust in the UK from April to June 2021. Individuals were recruited through outpatient rheumatology and renal clinics either during clinic appointments or via e-mail, letter or telephone between appointments. Eligible individuals were adults ≥ 18 years of age with a diagnosis of a RAIRD (vasculitis, SLE, myositis, scleroderma and SS) and eligible to receive SARS-CoV-2 vaccinations. People were not eligible if they were <18 years old, ineligible to receive SARS-CoV-2 vaccinations, unable to provide blood samples, unable

to travel to the hospital for study visits, unable to consent or had low English proficiency. HCs were invited from a related study and were age- and sex-matched prior to invitation using a 1:1 ratio for comparison [22]. HCs who were invited were matched with the RAIRD group who were invited. More HCs (especially of older ages) did not wish to participate, hence the differences in numbers and ages. Based on previous similar research, a sample size of 50 per group was deemed sufficient to detect any significant differences in responses. A total of 102 RAIRD patients were identified, of whom 29 were ineligible and 21 declined to participate. A total of 52 RAIRD patients participated in the study, of whom 50 gave a blood sample 4 weeks or 3 months after their second vaccine. A total of 34 HCs agreed to participate, of which 2 were excluded, as they were taking immunosuppressants for RA, leaving 32 eligible to participate. All participants provided written informed consent.

Data and sample collection

A baseline questionnaire was administered to collect information on demographics, clinical factors (previous COVID-19 infection and tests), diagnosis, current and/or recent immunosuppressive medications, recent glucocorticoid use and vaccination details. Whole blood samples were collected 4 weeks after the second dose of the COVID-19 vaccine. In cases where the 4 week target could not be met due to appointment unavailability, blood samples were collected 3 months after the second dose ($n = 14$). Samples were taken at hospital sites and stored in accordance with the Human Tissue Authority and National Health Service guidelines. HCs had the same blood sample collections.

Patient involvement

Patients and members of the public were involved at all stages of the study design and conduct. The study proposal was peer reviewed by people with vasculitis and other RAIRDs and their feedback was incorporated into the study design. Study findings will be disseminated to patients and the public through the Vasculitis UK website and newsletters.

Antibody response

Heparinized whole blood was centrifuged to separate the plasma. Plasma was tested for nucleocapsid- and spike-specific antibodies in two separate ELISAs. Briefly, 384-well Maxisorp (Nunc) assay plates (Thermo Fisher Scientific, UK) were coated with 20 μ L/well of 1 μ g/mL of either Wuhan strain SARS-CoV-2 full-length spike protein or Wuhan strain SARS-CoV-2 nucleocapsid protein. Plates were sealed, incubated overnight and serially diluted, as per World Health Organization standards. Antibody titres were defined as positive if the value was >10 BAU. An antibody response was defined as sufficient if the IgG level was higher than that of the lowest HC. Further details are provided in the [supplementary methods](#) available at *Rheumatology* online.

T cell response

We examined the percentages of both T and B cells in 10 patients with no/low anti-spike IgG and 10 patients with high anti-spike IgG. Cryopreserved peripheral blood mononuclear cells were thawed and stimulated with SARS CoV-2-derived peptide pools ([Supplementary Table S1](#), available at *Rheumatology* online). An activation-induced marker assay was used to identify total CD4⁺ and CD8⁺ T cells to spike

and nucleocapsid and a deep phenotyping assay was used to determine cytokine responses and memory T cells (Supplementary Tables S2 and S3, available at *Rheumatology* online). Data analysis for flow cytometry was performed using Kaluza version 2.2 (Beckman Coulter, Indianapolis, IN, USA) and further multidimensional clustering analysis (FlowSOM) was then utilized to characterize the major phenotype of cells. Further details are provided in the supplementary methods.

Statistical analysis

Antibody responses were compared between individuals with RAIRDs and HCs using Stata version 14 (StataCorp, College Station, TX, USA). Differences between demographic and clinical characteristics and humoral immunogenicity were tested for significance using the chi-squared test. For outcome variables with low frequencies (<5), we used Fisher's exact test. All other outcome variables were incorporated into the multivariable logistic regression analysis to determine the influence of RAIRDs on the magnitude of response to the second dose of the vaccine. It has previously been suggested that age, sex and rituximab can influence antibody levels [18, 23, 24] and hence we adjusted for these as *a priori* confounders during the analysis. A 5% α level was used to determine the significance level. Only patients with complete outcome data were included in the models. Missing data were assumed as missing at random and no imputations were performed.

Study outcomes

The primary outcomes were the antibody and T cell responses to two doses of SARS-CoV-2 vaccination. Secondary outcomes included a comprehensive analysis of T cell activation, cytokine production and generation of memory T cells.

Ethical approval

The study was approved by the West Midlands–Black Country Research Ethics Committee (REC reference 21/WM/0097). The controls were obtained from a related study (REC reference 21/NW/0048).

Results

Patient characteristics

The demographics and clinical characteristics of the patients with RAIRDs ($n=50$) and HCs ($n=32$) are shown in Table 1. The median age of the RAIRD cohort was 53 years (IQR 42–61). The majority were female [$n=35$ (70%)] and White [$n=45$ (90%)]. The HC group was also predominantly female [$n=23$ (72%)] and White [$n=25$ (78%)] and had a median age of 51 years (IQR 42–62). The most common RAIRD was ANCA-associated vasculitis [$n=31$ (62%)], followed by SLE [$n=9$ (18%)], myositis [$n=6$ (12%)] and other systemic vasculitis [$n=4$ (8%)]. A total of 17 patients were taking glucocorticoids daily, of which 7 (14%) were on high doses (≥ 10 mg/day prednisolone equivalent). A total of 22 (44%) patients had rituximab in the 12 months prior to the first vaccination and 40 (80%) patients had a prior history of rituximab. One of these patients was taking a different anti-CD20 drug due to rituximab allergy. A total of 10 (20%) patients were currently taking immunosuppressive medications other than steroids and rituximab. Five (6%) patients had hypogammaglobulinemia (IgG <5.3 g/l) and four of these

Table 1. Demographic and clinical characteristics of patients with rare autoimmune rheumatic diseases and healthy age-matched controls

Characteristics	RAIRD, <i>n</i> (%) (<i>n</i> = 50)	Controls, <i>n</i> (%) (<i>n</i> = 32)
Age (years)		
Median (range)	53 (22–81)	53 (22–79)
18–49	20 (40.0)	14 (43.8)
50–64	19 (38.0)	14 (43.8)
≥ 65	11 (22.0)	4 (12.5)
Gender, <i>n</i> (%)		
Female	35 (70.0)	23 (71.9)
Male	15 (30.0)	9 (28.1)
Ethnicity, <i>n</i> (%)		
White	45 (90.0)	25 (78.1)
Non-white	5 (10.0)	7 (21.9)
Diagnosis, <i>n</i> (%)		
ANCA-associated vasculitis	31 (62.0)	
SLE	9 (18.0)	
Other systemic vasculitis	4 (8.0)	
Myositis	6 (12.0)	
Current immunosuppression, <i>n</i> (%)		
Methotrexate	4 (8.0)	
Mycophenolate	4 (8.0)	
Belimumab	2 (4.0)	
Previous rituximab	40 (80.0)	
≤ 6 months	17 (34.0)	
≤ 12 months	22 (44.0)	
Current glucocorticoids, <i>n</i> (%)		
≥ 10 mg/day	7 (14.0)	
<10 mg/day	10 (20.0)	
No steroids	33 (66.0)	
Hypogammaglobulinemia, <i>n</i> (%)	5 (6.0)	
Recent immunoglobulin therapy ^a , <i>n</i> (%)	4 (8.0)	
Vaccine type, <i>n</i> (%)		
Oxford-AstraZeneca	25 (50.0)	8 (23.5)
Pfizer-BioNTech	25 (50.0)	26 (76.5)

^a Excluded from the analysis on antibody response to vaccination.

patients had recently received Ig replacement therapy, thus their data were excluded from the antibody analysis. Half of the RAIRD cohort received the Pfizer-BioNTech vaccine and the other half received the Oxford-AstraZeneca vaccine.

Antibody responses

The median anti-spike IgG antibody response was significantly lower in RAIRD patients [median 34 (IQR 3–687)] compared with HCs [median 1453 (IQR 733–3405)] ($\chi^2=21.2$, $P<0.001$). Furthermore, 15 (33%) RAIRD patients had undetectable antibodies (Supplementary Table S4, available at *Rheumatology* online) and only 20 (43%) patients had a sufficient antibody response (IgG higher than the lowest HC) (Fig. 1a). Both the RAIRD and HC groups had virtually undetectable anti-nucleocapsid IgG responses (Fig. 1b), which is consistent with any previous antibody response to infection no longer being detectable and the responses observed being due to vaccination only. In general, older adults, males, patients with myositis and those on immunosuppressive or steroid treatment were more likely to have insufficient antibody responses (IgG levels lower than the lowest HC) as shown in Table 2. Additionally, none of the patients who had rituximab in the 6 months prior to the first vaccine and only 3 (16%) who had rituximab in the last 12 months had a sufficient antibody response. In the univariate analyses we found a significant inverse correlation

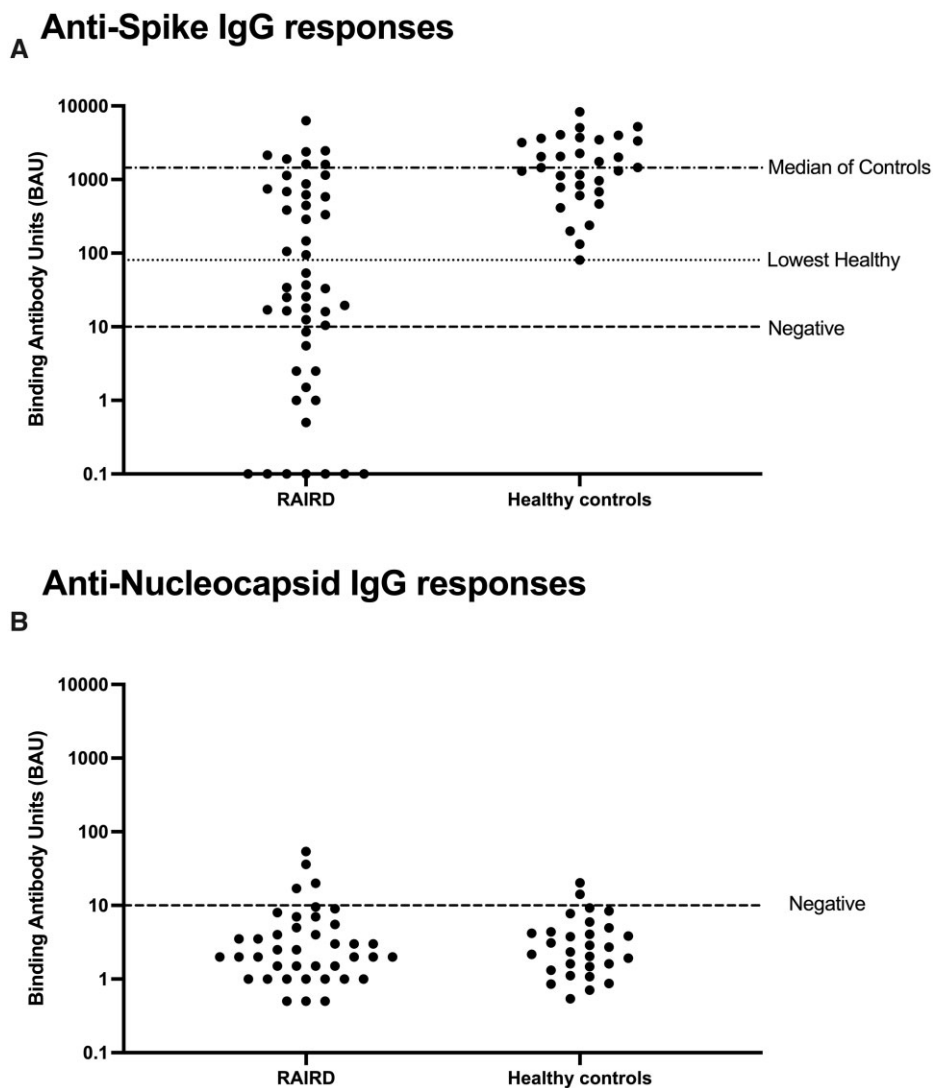


Figure 1. IgG responses to SARS-CoV-2 between RAIRD (vasculitis) patients and HCs. **(A)** Anti-spike IgG antibody responses in RAIRD (vasculitis) patients compared with HCs. The dashed line (negative) represents the cut-off for the assay, the dotted line (lowest healthy) shows the binding antibody units of the lowest HC and the semi-dashed line represents the median of the HCs. **(B)** Anti-nucleocapsid IgG responses were mainly below the limit of detection of the assay in both RAIRD patients and HCs. The dashed line (negative) represents the cut-off for the assay

between sufficient humoral response (Table 2) and rituximab therapy in the 12 months prior to receiving the first dose of the SARS-CoV-2 vaccine ($P = 0.003$). There was also a strong association between the diagnosis (ANCA-associated vasculitis, other systemic vasculitis, myositis or SLE) and humoral response, however, this did not reach statistical significance. In the multivariable analyses, rituximab in the last 12 months was associated with an insufficient humoral response [OR 0.11 (95% CI 0.03, 0.48); $P = 0.003$]. Age, gender and ethnicity did not have an influence on the humoral response, which reached statistical significance.

T and B cell responses in anti-spike antibody high and antibody low RAIRD patients

There was no significant difference in either total T cells (Fig. 2a), CD4⁺ T cells (Fig. 2b) or CD8⁺ T cells (Fig. 2c), but there was a significant difference in B cell percentages between the antibody no/low and high patients ($P = 0.0359$; Fig. 2d). Interestingly, two patients in the antibody high group had no

detectable B cells at the time of sampling, which is likely because both had treatment with rituximab after their second vaccination and before the blood sample was collected.

T cell responses to spike and nucleocapsid peptides in antibody high and antibody no/low RAIRD patients

In the antibody high group there were significantly more spike-specific CD4⁺ T cells than in the antibody no/low group ($P = 0.0217$; Fig. 3ai). There were no detectable nucleocapsid-specific CD4⁺ T cells in either group, consistent with no residual response to any prior infection with SARS-CoV-2 if exposed at all (Fig. 3aii). In addition, there were no significant differences in total cytokine-specific CD4⁺ T cells to spike (Fig. 3bi) between the antibody no/low and antibody high groups and no nucleocapsid-specific cytokine-secreting CD4⁺ T cells (Fig. 3bii). Analysis of cytokine patterns in spike-specific CD4⁺ T cells showed this was mainly IFN- γ for both the antibody no/low and antibody high group, with some cells

Table 2. Univariate and multivariate logistic regression analyses of sufficient antibody response following the second dose of vaccine in RAIRD patients^a

Variables	Sufficient antibody response ^b , <i>n</i> (%) (<i>n</i> = 20)	Insufficient antibody response, <i>n</i> (%) (<i>n</i> = 26)	Univariate analyses	Multivariate analyses	
			OR (95% CI) or two-sided <i>P</i> -value	OR (95% CI)	<i>P</i> -value
Age (for each additional year)			0.99 (0.95, 1.03)	1.00 (0.95, 1.04)	0.833
18–49	8 (50.0)	8 (50.0)			
50–64	8 (42.1)	11 (57.9)			
≥65	4 (36.4)	7 (63.6)			
Gender					
Female	15 (46.7)	17 (53.1)	1 (reference)	1 (reference)	
Male	5 (35.7)	9 (64.3)	0.63 (0.17, 2.30)	0.58 (0.14, 2.52)	0.471
Ethnicity					0.369 ^c
White	19 (46.3)	22 (53.7)			
Non-white	1 (20.0)	4 (80.0)			
Diagnosis					0.084 ^c
ANCA-associated vasculitis	13 (46.4)	15 (53.6)			
SLE	4 (50.0)	4 (50.0)			
Other systemic vasculitis	3 (75.0)	1 (25.0)			
Myositis	0	6 (100.0)			
Current immunosuppression					
Yes	5 (38.5)	8 (61.5)	0.75 (0.20, 2.78)		
No	15 (45.5)	18 (54.6)	1 (reference)		
Current glucocorticoids					
Yes	7 (41.2)	10 (58.8)	0.86 (0.26, 2.90)		
No	13 (44.8)	16 (55.2)	1 (reference)		
Previous rituximab					
≤6 months					
Yes	0	15 (100.0)	Omitted		
No	20 (64.5)	11 (35.5)	1 (reference)		
≤12 months					
Yes	3 (15.8)	16 (84.2)	0.11 (0.03, 0.47) ^d	0.11 (0.03, 0.48)	0.003 ^d
No	17 (63.0)	10 (37.0)	1 (reference)	1 (reference)	
Rituximab ever					
Yes	14 (37.8)	23 (62.2)	0.30 (0.65, 1.42)		
No	6 (66.7)	3 (33.3)	1 (reference)		

^a An antibody response was defined as sufficient if IgG levels were above that of the lowest HC.

^b Patients on Ig therapy were excluded from analysis on antibody response to vaccination.

^c *P*-value obtained from two-sided Fisher's exact test.

^d Statistically significant *P*-value.

also producing TNF- α in combination with IFN- γ in the no/low group and TNF- α alone in the antibody high group (Fig. 3ci). There was no detectable cytokine production by CD4⁺ T cells in response to nucleocapsid (Fig. 3cii).

We then examined CD8⁺ T cell responses and found no significant differences between the antibody high and antibody no/low groups for spike-specific CD8⁺ T cells (Fig. 3di and ei). Furthermore, there were very minimal CD8⁺ T cell responses to nucleocapsid (Fig. 3dii and eii). Analysis of cytokine patterns by spike-specific CD8⁺ T cells highlighted cytokine production in the antibody no/low group was primarily IL-2, whereas IFN- γ was the predominant cytokine in the antibody high group (Fig. 3fi). There was minimal cytokine production in response to nucleocapsid (Fig. 3fii).

The multidimensional clustering analysis using t-distributed stochastic neighbour embedding (t-SNE) showed a total of 27 clusters with different expression markers (Fig. 4a and b). Although not statistically significant, there was a higher percentage of clusters 9 and 12 in the no/low antibody group compared with the antibody high group (Fig. 4c, d and f). These clusters are associated with two terminally differentiated populations of T cells: effector memory 3 (EM3) and effector memory T cells re-expressing CD45RA (TEMRA) (Fig. 4e).

Discussion

Our study highlights several important findings about the immunological effects of COVID-19 vaccination in patients with RAIRDS. We found that that antibody responses were completely undetectable in 33% of RAIRD patients and insufficient in a further 24% of RAIRD patients after two doses of SARS-CoV-2 vaccine compared with HCs. Additionally, there were no statistically significant differences in antibody response between different types of RAIRDS. However, we had small numbers, and it is notable that none of the six people with myositis had a sufficient antibody response (IgG levels greater than the lowest HC). Interestingly, five of these six (83%) individuals had a prior history of rituximab treatment, so we hypothesize that this may have contributed in part to their diminished response.

Corticosteroids are commonly used in the treatment of RAIRDS, particularly during relapses. Our results suggest that concurrent use of corticosteroids does not significantly affect humoral response in RAIRDS. However, it is important to highlight that only seven RAIRD patients were on ≥ 10 mg steroids/day, so our sample may have been too small to make a firm conclusion. Previous studies have revealed conflicting evidence about the role of corticosteroids in immunogenicity. Observational studies have identified a decrease in serological

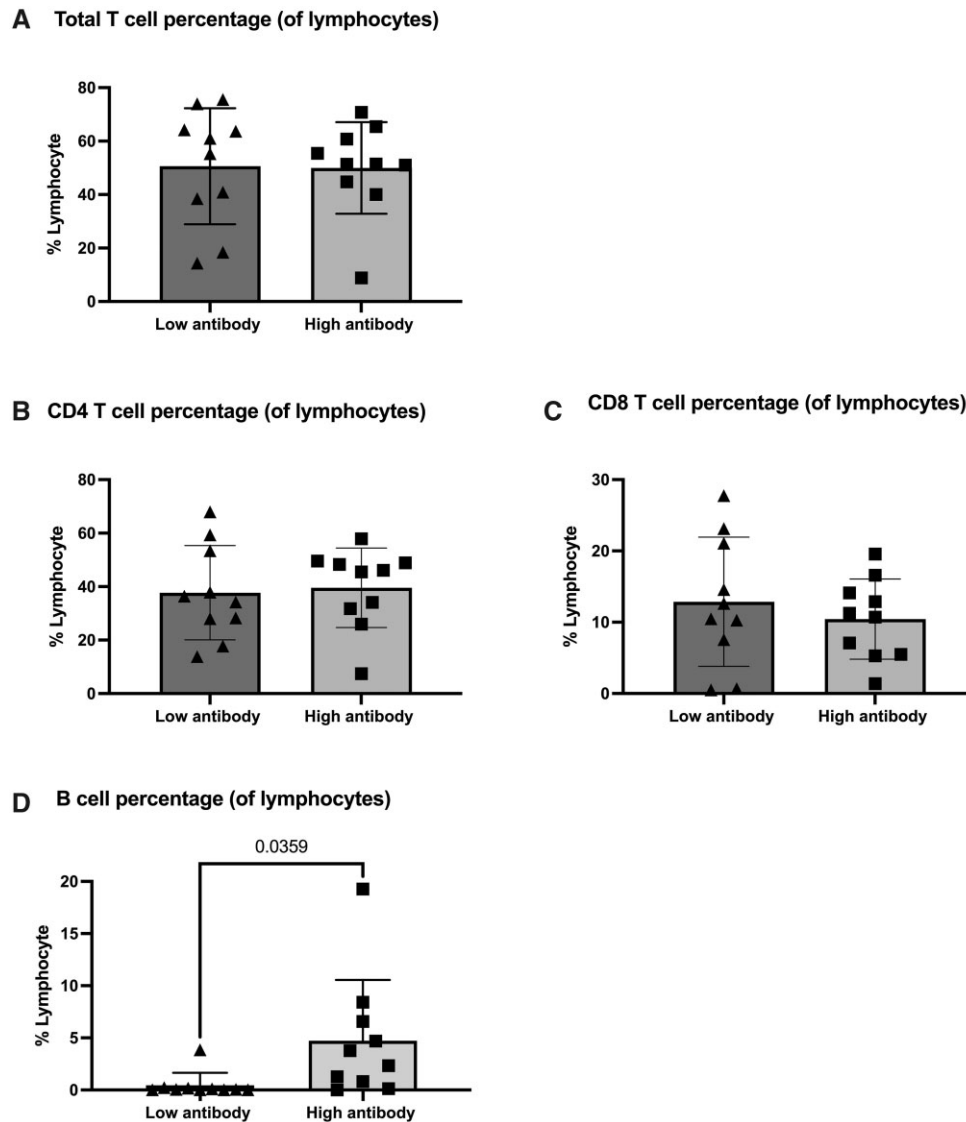


Figure 2. T and B cell responses in anti-spike antibody high and antibody low RAIRD patients. **(A)** Total percentage of T lymphocytes in anti-spike antibody high and antibody no/low RAIRD patients. **(B)** CD4 T cell and **(C)** CD8 T cell percentages of lymphocytes in anti-spike antibody high and antibody no/low RAIRD patients. **(D)** B cell percentage of lymphocytes was significantly lower in anti-spike antibody no/low RAIRD patients compared with anti-spike antibody high RAIRD patients

response to pneumococcal and hepatitis vaccines with long-term steroid use [25, 26]. The effect of corticosteroids on COVID-19 vaccines has not been thoroughly investigated. A recent study suggested that short-term use of low-dose steroids may not hinder antibody responses to COVID-19 vaccination [27]. However, this study was restricted to healthcare workers who did not have any significant comorbidities.

With the data from our cohort, we were able to demonstrate that antibody responses were significantly lower in RAIRD patients compared with HCs. This is similar to a recent Dutch study on patients with immune-mediated inflammatory disorders on concurrent immunosuppression. The authors identified that patients receiving rituximab, mycophenolate mofetil combination treatments and sphingosine 1-phosphate receptor modulators had lower rates of seroconversion following the second vaccine. These rates did improve after the third vaccine for all groups except rituximab [28]. Our results also showed that antibody response was diminished in patients receiving rituximab (anti-CD20) and the

interval between the administration of rituximab and vaccination was critical in predicting response. We found that all patients who received rituximab in the last 6 months prior to the first dose had insufficient antibody responses and 89% of those who had rituximab in the 12 months prior to the first vaccination had insufficient antibody responses. These findings correlate with previous studies that also demonstrate the negative impact of B cell-depleting therapies on response to vaccines [7, 11, 24, 29–32]. Conversely, some studies have shown that even individuals with low numbers of B cells secondary to rituximab treatment were able to mount a significant antibody response to COVID-19 vaccination provided T cell-mediated immunity was intact [33, 34]. Recent literature has suggested that to maximize response, the optimal timing for vaccination in rituximab-treated patients should be at least 9 months after the last infusion [33, 35]. However, our study shows that even patients who had been treated with rituximab in the 12 months prior to the first vaccination did not mount a sufficient response.

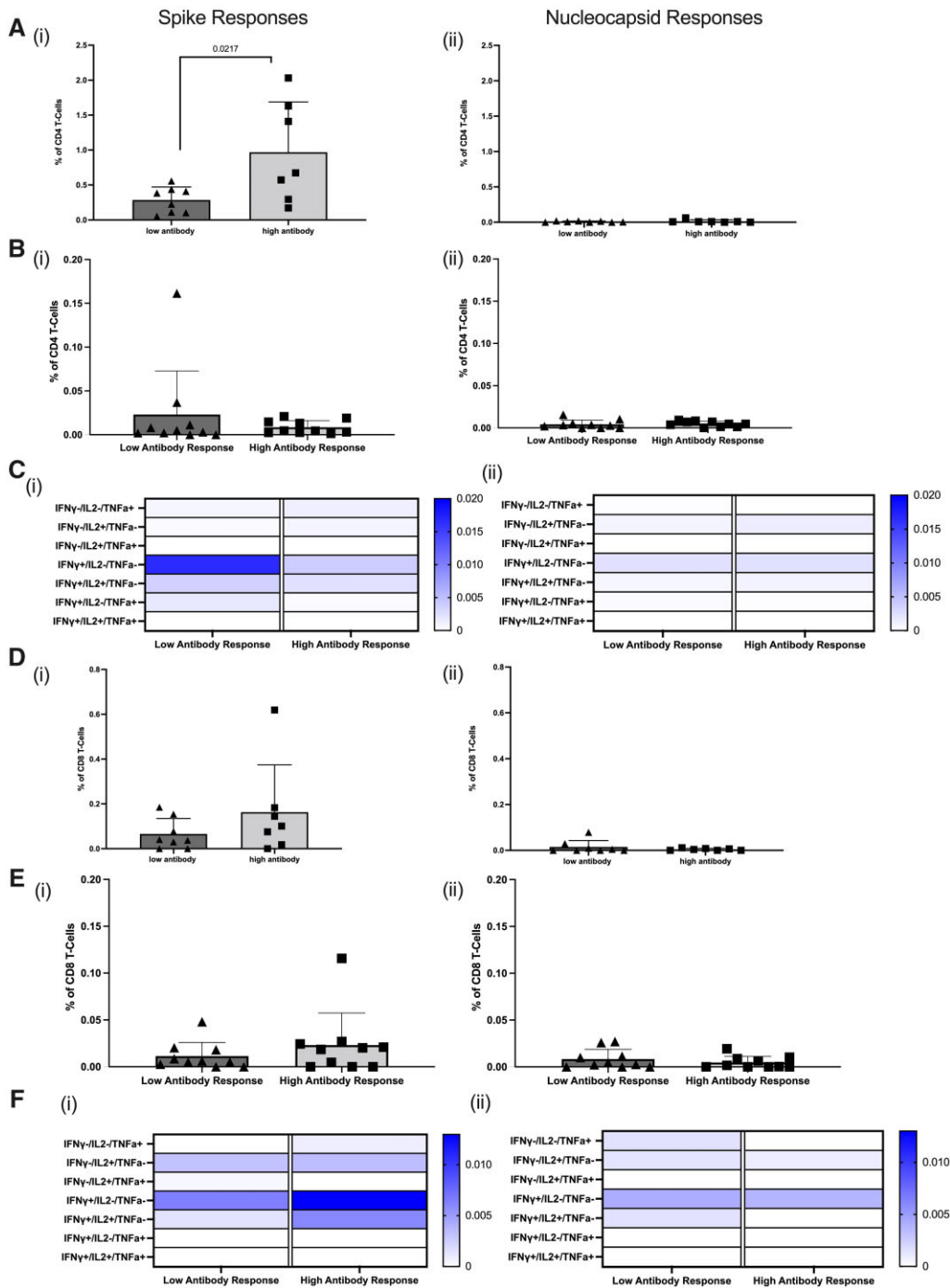


Figure 3. CD4⁺ and CD8⁺ T cell responses to SARS-CoV-2 peptides in antibody high and antibody no/low RAIRD patients. **(A)** Spike-specific CD4⁺ T cells were significantly higher in the antibody high patients and there were no detectable nucleocapsid-specific CD4⁺ T cells. **(B)** No difference in total cytokine-positive spike-specific CD4⁺ T cells. **(C)** Cytokine patterns expressed by spike and nucleocapsid-specific CD4⁺ T cells. **(D)** Higher spike-specific CD8⁺ T cells in antibody high patients. **(E)** No difference in total cytokine-positive spike-specific CD8⁺ T cells. **(F)** Cytokine patterns expressed by spike and nucleocapsid-specific CD8⁺ T cells

Cellular immune responses are essential in providing long-lasting immunity and underpin vaccine efficacy. Most current vaccines rely on the delivery of spike protein, and as a consequence the generation of spike-specific T cell response, in order to maintain immune memory after antibodies have waned [36]. Previous studies have shown that in healthy individuals, two doses of vaccine are sufficient to generate a T cell response similar to that after natural infection [37, 38]. However, our study revealed that RAIRD patients with

no/low antibody response had significantly fewer spike-specific CD4⁺ T cells, which are essential in coordinating and regulating antiviral immunity. Our results are in line with a recent study in kidney transplant recipients on immunosuppression, which also found a weak T cell response and positive SARS-CoV-2 antibodies in only 5–10% of patients following the first and second vaccine doses [39, 40]. However, this serological response improved to 36% after administration of the third dose [41]. This augmented response

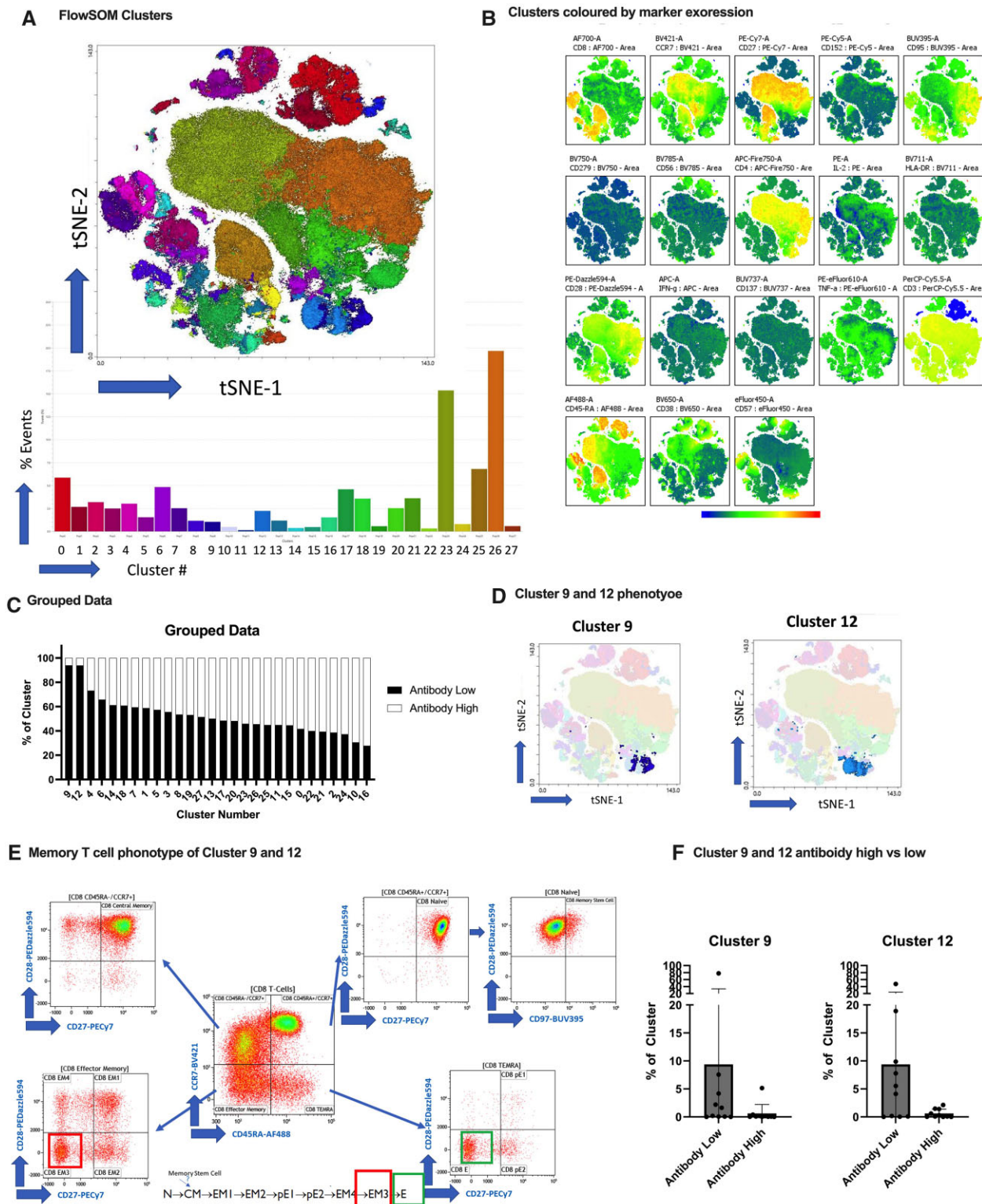


Figure 4. Multidimensional clustering analysis. **(A)** FlowSOM identified 27 clusters of major cell types from both antibody high and antibody no/low groups, which are colour-coded and displayed on a two-dimensional t-SNE plot. **(B)** t-SNE visualization coloured according to marker expression. **(C–E)** Clusters 9 and 12 were identified in the antibody no/low group and were associated with two terminally differentiated populations of T cells: EM3 and TEMRA. **(F)** No significant differences for clusters 9 and 12 were observed between the antibody no/low and antibody high group

suggests that repeated booster strategies could provide more long-term immunity in ICPs and warrants further research.

Our study also brings to light new findings about the function of memory T cells in people with RAIRDs. We observed the importance of an IFN- γ -predominant CD8⁺ T cell

response in RAIRD patients with high antibodies in coordinating the adaptive immune system. We also noted that this response was lower and predominantly IL-2-related in patients with no/low antibodies. This suggests that while CD8⁺ T cells may be activated, the main effector cytokines

for sustaining the antiviral response are not produced in RAIRD patients in the absence of antibodies. We also observed increased levels of clusters 9 and 12 encoding for EM3 (CD27⁺CD28⁻) and TEMRA in RAIRD patients with no/low antibodies. These cells lack expression of CD27 and CD28, suggesting immunosenescence and incompetence to vaccination [42, 43] and therefore increased susceptibility and greater probability of more severe SARS-CoV-2 infection.

The strengths of this study include the broad inclusion criteria, patients with a variety of RAIRD diagnoses and the use of age-matched HCs, which increases the generalisability of our findings. In addition, we evaluated both the humoral and cellular responses to two doses of SARS-CoV-2 vaccines. The limitations of this study include a small sample size and demographic differences between the RAIRD and control groups, some of which were adjusted for during our analysis. Furthermore, 14 patients were unable to have a blood sample taken 4 weeks post-vaccination, and in these cases we were only able to analyse their 3 month post-vaccination sample (however, in patients with both samples, we found no significant differences in the titres of antibodies). Additionally, some of the outcome variables had low frequencies and hence we could not adjust for these as potential confounders in our multivariate analysis.

In summary, we identified that patients with RAIRDS have significantly diminished antibody and T cell responses following two doses of SARS-CoV-2 vaccines. Receipt of rituximab in the last 12 months was associated with a reduced humoral response, so, where possible, vaccination should precede treatment with rituximab, as per clinical guidance. This also justifies the need for the additional booster vaccine doses, in line with national guidelines [44], and emphasizes the importance of assessing B and T cell responses in ICPs. We also recommend that for individuals requiring maintenance rituximab, shared decision making and risk assessments should be conducted by clinicians to review the timing of rituximab with future booster doses. It also raises questions about whether additional prophylactic measures such as antivirals may be required in addition to booster vaccine doses in individuals who do not mount a sufficient antibody response. Notably, some patients with no/low antibody response also have poor memory T cells that lack both proliferative and functional capacities and so future research is important to determine the long-term immune response to additional vaccine doses.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

PL, FP and LF conceived this research and revised the manuscript. LG wrote the manuscript. NG, GH, DT and LB processed the blood samples and ran the cell mediated assays. KA and KV provided the healthy control samples. GS provided help with the assays. AF, MC and MR identified patients to

invite to the study. HJ and PT ran the antibody assays. SP and M-J P recruited RAIRD patients and collected blood samples. DO supported the flow cytometry. All authors approved the final manuscript.

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