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Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression

Maria Prendecki,[1,2] Candice Clarke,[1,2] Helena Edwards,[1,2] Stacey McIntyre,[1] Paige Mortimer,[1] Sarah Gleeson,[1,2] Paul Martin,[1,2] Tina Thomson,[2] Paul Randell,[3] Anand Shah,[4,5] Aran Singanayagam,[6,7] Liz Lightstone,[1,2] Alison Cox,[3] Peter Kelleher,[3,6] Michelle Willicombe,[1,2] Stephen P McAdoo [1,2]

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For numbered affiliations see end of article.

Correspondence to
Dr Maria Prendecki, Department of Immunology and Inflammation, Hammersmith Campus, London, UK; m.prendecki@imperial.ac.uk

MP and CC contributed equally.

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ABSTRACT

Objective There is an urgent need to assess the impact of immunosuppressive therapies on the immunogenicity and efficacy of SARS-CoV-2 vaccination.

Methods Serological and T-cell ELISpot assays were used to assess the response to first-dose and second-dose SARS-CoV-2 vaccine (with either BNT162b2 mRNA or ChAdOx1 nCoV-19 vaccines) in 140 participants receiving immunosuppression for autoimmune rheumatic and glomerular diseases.

Results Following first-dose vaccine, 28.6% (34/119) of infection-naïve participants seroconverted and 26.0% (13/50) had detectable T-cell responses to SARS-CoV-2. Immune responses were augmented by second-dose vaccine, increasing seroconversion and T-cell response rates to 59.3% (54/91) and 82.6% (38/46), respectively. B-cell depletion at the time of vaccination was associated with failure to seroconvert, and tacrolimus therapy was associated with diminished T-cell responses. Reassuringly, only 8.7% of infection-naïve patients had neither antibody nor T-cell responses detected following second-dose vaccine. In patients with evidence of prior SARS-CoV-2 infection (19/140), all mounted high-titre antibody responses after first-dose vaccine, regardless of immunosuppressive therapy.

Conclusion SARS-CoV-2 vaccines are immunogenic in patients receiving immunosuppression, when assessed by a combination of serology and cell-based assays, although the response is impaired compared with healthy individuals. B-cell depletion following rituximab impairs serological responses, but T-cell responses are preserved in this group. We suggest that repeat vaccine doses for serological non-responders should be investigated as means to induce more robust immunological response.

Key messages

What is already known about this subject?

- There are very few data relating to the effect of immunosuppression on immune responses to SARS-CoV-2 vaccination, as patients receiving immunomodulatory therapies were excluded from all vaccine trials.

What does this study add?

- When assessed by both serological and T cell-based assays, most patients (89.3%) develop immune responses following two doses of vaccine, despite immunosuppressive therapies.
- B-cell depletion following rituximab treatment was significantly associated with failure to seroconvert, although most of these patients developed T-cell responses to SARS-CoV-2.
- Tacrolimus use was associated with impaired T-cell responses.

How might this impact on clinical practice or future developments?

- Assessment of both serological and T-cell responses may be necessary to fully define responses to vaccination in immunosuppressed populations.
- Administration of additional vaccine ('booster') doses may be a potential strategy for serological non-responders.

INTRODUCTION

There is an urgent need to understand the impact of immunosuppressive therapies on the efficacy of vaccines to SARS-CoV-2.[1 2] Patients with autoimmune diseases have been considered clinically vulnerable to SARS-CoV-2 infection since the onset of the COVID-19 pandemic,[3] and population-based and registry-based studies suggest that they experience significant rates of hospitalisation, severe disease and death during its global spread.[4–6]

Several vaccine candidates have been shown to prevent severe disease in the general population,[7–10] although all clinical trials to date excluded patients receiving immunosuppression, who are at risk of diminished vaccine responses. The degree to which the immune response is altered may vary with the specific immunomodulatory regimen and the vaccine used. Published data, for example, indicate impaired humoral responses to influenza and pneumococcal vaccination, especially in those undergoing treatment with rituximab.[11–14] However, existing data derived from experience with other vaccine types may not translate to the novel vaccines deployed for COVID-19.

Here, we describe the serological and T-cell responses to first-dose and second-dose vaccines (with either BNT162b2 mRNA or ChAdOx1 nCoV-19 replication-deficient adenoviral vector vaccines) in a cohort of patients with autoimmune glomerular and rheumatic diseases treated with rituximab or other non-biological

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immunosuppressive therapies, in order to describe the impact of these treatments on vaccine response in this patient population.

METHODS

Study participants

Baseline samples were collected from 161 patients with immune-mediated glomerulonephritis and vasculitis who received their first-dose of SARS-CoV-2 vaccination (BNT162b2 mRNA or ChAdOx1 nCoV-19) between 17 January 2o21 and 9 March 2021. For assessment of immunological responses after the first-dose vaccine, 140 patients provided a first follow-up sample at a median of day 28 (IQR 28–30 days) after first-dose administration; 53 of these also provided paired samples for assessment of SARS-CoV-2 T-cell responses. To date, 103 patients in the study have received second-dose vaccine at a median of 30 days (IQR 28–42) after first dose and have provided a subsequent sample for serological analysis at a median of 21 days (IQR 19–28 days) after second-dose administration; 49 also provided paired samples for analysis of T-cell responses.

A group of healthy volunteer (HV) healthcare workers (HCWs) were used as a comparator group for the study (n=70). In this group, assessment of first-dose response was undertaken at a median of 21 days (IQR 19–25 days) after first-dose administration and at a median of 27 days (IQR 21.5–28.0 days) after second-dose administration. This group received second-dose vaccine at a median of 66 days after first-dose (IQR 61–69 days). To control for some of the differences between the cohorts of immunosuppressed (IS) patients (IS group) and the HV group, matching for age and vaccine type was performed.

Separate cohorts of HCWs were used to identify a threshold for positivity on the ELISpot assay in participants who were infection-naïve and unvaccinated (n=30).[15]

Serological testing

Serum was tested for antibodies to nucleocapsid protein (anti-NP) using the Abbott Architect SARS-CoV-2 IgG two-step chemiluminescent immunoassay (CMIA) according to the manufacturer's instructions. This is a non-quantitative assay and samples were interpreted as positive or negative with a threshold index value of 1.4. Spike (S) protein antibodies (anti-S IgG) were detected using the Abbott Architect SARS-CoV-2 IgG Quant II CMIA. Anti-S antibody titres are quantitative with a threshold value for positivity of 7.1 binding antibody units (BAU)/mL.

T-cell ELISpot

SARS-CoV-2-specific T-cell responses were detected using the T-SPOT Discovery SARS-CoV-2 (Oxford Immunotec) according to the manufacturer's instructions. In brief, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples with the addition of T-Cell Select (Oxford Immunotec) where indicated. A total of 250 000 PBMCs were plated into individual wells of a T-SPOT Discovery SARS-CoV-2 plate. The assay measures immune responses to five different SARS-CoV-2 structural peptide pools: S1 protein, S2 protein, NP protein, M protein (membrane), a mixed panel and positive (phytohaemagglutinin) and negative controls. Cells were incubated and interferon-γ secreting T cells were detected. Spot-forming units (SFUs) were detected using an automated plate reader (Autoimmun Diagnostika). Infection-naïve, unvaccinated participants were used to identify a threshold for a positive response using mean+3 SD SFU/10^6 PBMC for S peptide pools. This resulted in a cut-off for positivity of 40 SFU/10^6 PBMC for S protein responses.[15]

Statistical analysis

Statistical analysis was conducted using Prism V.9.0 (GraphPad Software, San Diego, California, USA). Unless otherwise stated, all data are reported as median with IQR. Where appropriate, Mann-Whitney U and Kruskal-Wallis tests were used to assess the difference between 2 or >2 groups, with Dunn's post hoc test to compare individual groups. For paired analysis, Wilcoxon test was used. Multivariate analysis was carried out using multiple logistic regression using variables which were found to be significant on univariate analysis.

Patient involvement

The initial study proposal was supported and funded by the West London Kidney Patient Association. Patients were not directly involved in the experimental design or in performing the study.

RESULTS

Sample collection and baseline data

A total of 140 IS patients provided samples at baseline and at 28-40 days after first vaccine dose; 103 patients provided a further sample 18–29 days after second-dose vaccine (administered at a median of 32 and 30 days after first dose for ChAdOx1 and BNT162b2, respectively). Clinical characteristics and immunosuppressive treatments are summarised in online supplemental table S1. One hundred and fourteen patients (81.4%) previously received rituximab, of whom 56.1% (64/114) were treated within the last 6 months, and 60.5% (69/114) were B-cell deplete (circulating CD19 <10 cells/μL) at the time of vaccination. All 69 patients who were B-cell deplete had received treatment with rituximab, 69.6% (48/69) within the last 6 months. Nineteen patients (13.6%) had evidence of previous SARS-CoV-2 infection on baseline testing—in keeping with the low prevalence of disease previously described in our cohort[16]—and these were analysed separately from those who were infection-naive. Two further patients developed anti-NP IgG after vaccination, indicating SARS-CoV-2 infection at or since vaccination and were excluded from analysis.

Immunological response to first-dose vaccine in infection-naïve patients

One hundred and nineteen infection-naïve patients were included in the analysis of response to first-dose vaccine. At 28–40 days, 28.6% (34/119) had detectable anti-S IgG (figure 1A; median 0.61 BAU/mL (IQR 0.03–9.8)). By univariate analysis, ChAdOx1 vaccine, prior cyclophosphamide treatment, prior rituximab treatment,and current B-cell depletion were all associated with a decreased likelihood of seroconversion (figure 1B,C). In the group of patients who had received rituximab, treatment within the last 6 months was associated with decreased rates of seroconversion (table 1), and the median anti-S titre was significantly lower in this group (0.12 and 1.1 BAU/mL in those treated <6 and >6 months, respectively, p=0.01). By multivariate analysis, B-cell depletion at the time of vaccination was associated with non-seroconversion (figure 1B; OR 0.3, p=0.03).

The rate and magnitude of serological responses in the IS group were significantly lower than those in an HV group (online supplemental table S2) at a similar time point after first-dose vaccine (figure 1D; 97.1% (68/70) seroconversion in the HV group, median anti-S titre 90 BAU/mL (IQR 40.7–199.8), p<0.0001 compared with IS cohort). In the IS cohort, we did not identify any correlation between serological

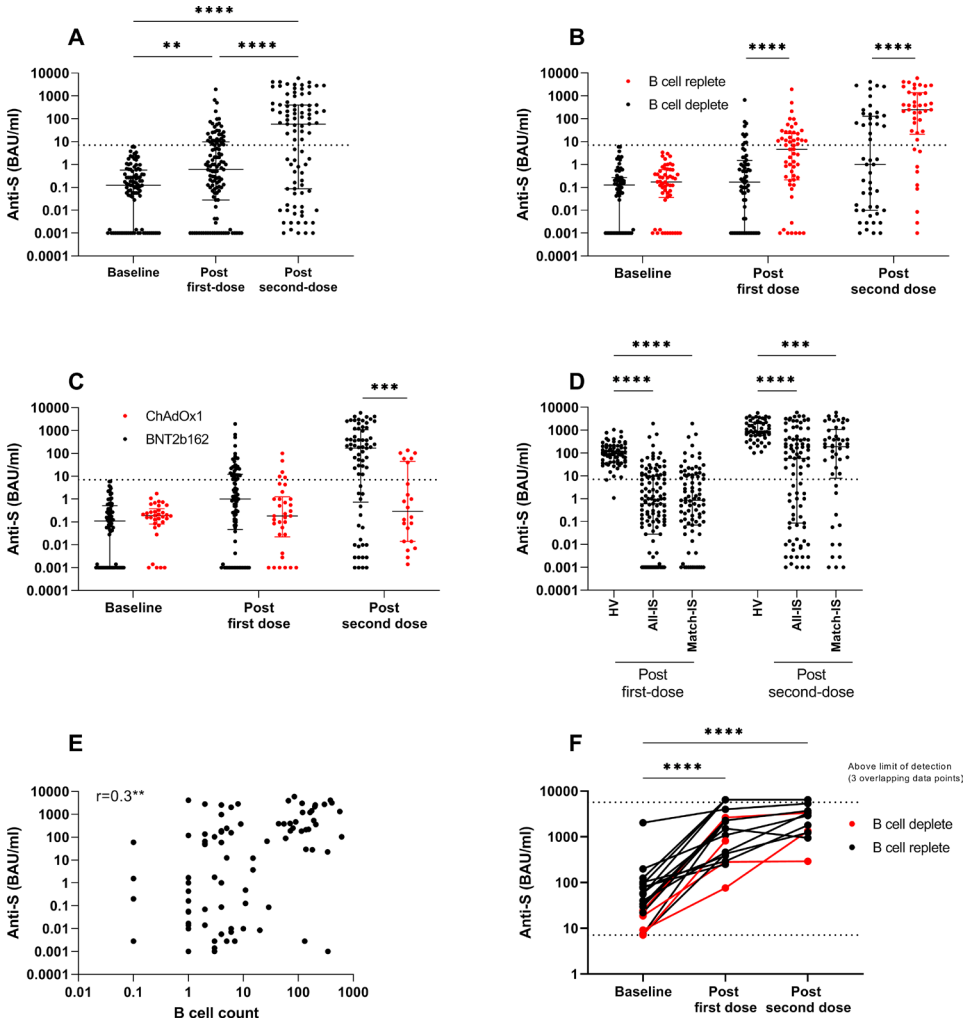

Figure 1 Humoral responses to SARS-CoV-2 vaccination in IS patients. (A) Anti-S titre at baseline, following first-dose and second-dose vaccine in patients who were infection-naïve. (B) Anti-S titre by B-cell status at the time of vaccination in infection-naïve patients at baseline, 28–40 days following first-dose vaccine and 18–29 days after second-dose vaccine. (C) Anti-S titre by vaccine type at the time of vaccination in infection-naïve patients at baseline, 28–40 days following first-dose vaccine and 18–29 days after second-dose vaccine. (D) Anti-S titre following first-dose and second-dose vaccinations in healthy volunteers (HVs), IS patients and a matched cohort of IS patients. (E) Correlation of anti-S titre after second-dose vaccination and B-cell count at the time of vaccination in IS patients. (F) Anti-S titre in patients with previous natural infection at baseline, following first-dose and second-dose vaccines. Dotted line indicates 7.1 BAU/mL, the threshold for detectable anti-S antibodies. For visualisation of data on a log scale, values=0 are represented by 0.001, which is below the lower limit of the assay (0.00142). HV, healthy volunteer; IS, immunosuppressed; S, spike. **p<0.01, ***p<0.001, ****p<0.0001.

response to first-dose vaccine and age, although we and others have reported this in healthy individuals.[15 17] The group of HVs included in this study is significantly younger than the IS group (online supplemental table S2; median age 41.4 and 53.7 years for HV and IS groups, respectively; p<0.0001). However, when an age-matched cohort of IS patients (median age 46.2 years) is used for comparison, serological responses were not significantly different from the whole IS cohort and remained lower than those in HV (figure 1D; median 0.85 BAU/mL (IQR 0.07–10.9), p<0.0001 compared with HV). This suggests that the overall younger age of our HV cohort does not fully account for the significant difference in serological response.

T-cell responses were assessed in 50/119 infection-naïve patients following first-dose vaccine. Only 26.0% (13/50) had detectable T-cell responses (>40 SFU/10^6 PBMC) (figure 2A and table 2). Patients receiving tacrolimus were less likely to have T-cell responses above the threshold for positivity: 0% (0/13) and 29.7% (11/37) of patients in T-cell responder and non-responder groups, respectively, were receiving tacrolimus (p=0.05) (figure 2B; median 6 and 16 SFU/10^6 PBMC in those receiving tacrolimus vs those who were not, p=0.003). Patients receiving ChAdOx1 were more likely to mount T-cell responses following first-dose vaccine: 69.2% (9/13) and 35.1% (13/37) of T-cell responders and non-responders, respectively, received ChAdOx1 vaccine (p=0.05) (figure 2C; median SFU/10^6 PBMC 8 and 29 for BNT162b2 and ChAdOx1, p=0.0007). Similar to serological responses after first-dose vaccine, T-cell responses were poorer in the IS group compared with HV (figure 2D; 61.1% (41/67) of HV had detectable responses, median 15 and 52 SFU/10^6 PBMC for IS and HV, respectively; p<0.0001).

In patients for whom both serological and T-cell assessments were available, 64.0% (32/50) did not have a demonstrable response to first-dose vaccine by either measure (online supplemental table S3).

Table 1 Patient characteristics by serological status in those with no evidence of previous natural infection

			First dose (n=119)				Second dose (n=91)		
			Non-seroconversion	Seroconversion			Non-seroconversion	Seroconversion	
Characteristics		n	n=85 (71.4%)	n=34 (28.6%)	P value	n	n=37 (40.7%)	n=54 (59.3%)	P value
Gender	Male	62	44 (71.0)	18 (29.0)		49	20 (40.8)	29 (59.2)	
	Female	57	41 (71.9)	16 (28.1)		42	17 (40.5)	25 (59.5)	
Age	Years (IQR)		52.0 (39.9–63.9)	56.2 (36.1–60.7)			60.5 (43.8–69.5)	51.8 (37.3–60.2)	0.05
Ethnicity	White	62	44 (71.0)	18 (29.0)		50	22 (44.0)	28 (56.0)	
	Black	10	7 (70.0)	3 (30.0)		7	1 (14.8)	6 (85.2)	
	South Asian	34	24 (70.6))	10 (19.6)		27	12 (44.4)	15 (55.6)	
	Mixed-race	7	6 (85.7)	1 (14.3)		3	1 (33.3)	2 (66.7)	
	Other	6	4 (66.7)	2 (33.3)		4	1 (25.0)	3 (75.0)	
Diagnosis	AAV and anti-GBM disease	45	35 (77.8)	10 (22.2)		34	17 (50.0)	17 (50.0)	
	Podocytopathy*	28	18 (64.3)	10 (35.7)		25	12 (48.0)	13 (52.0)	
	Membranous GN	23	15 (65.2)	8 (34.8)		21	6 (28.6)	15 (71.4)	
	SLE	19	14 (73.7)	5 (26.3)		8	1 (12.5)	7 (87.5)	
	Other†	4	3 (75.0)	1 (25.0)		3	1 (33.3)	2 (66.7)	
Comorbidities	Diabetes	19	17 (89.5)	2 (10.5)		16	9 (56.3)	7 (43.7)	
	Asthma/COPD	25	17 (68.0)	8 (32.0)		14	4 (28.6)	10 (71.4)	
	Previous malignancy	6	4 (66.7)	2 (33.3)		4	3 (75.0)	1 (25.0)	
Immunotherapy	Previous rituximab	99	77 (77.8)	22 (22.2)	0.002	75	35 (46.7)	40 (53.3)	0.01
	Last 6 months	56	49 (87.5)	7 (12.5)	0.016	44	26 (59.1)	18 (40.9)	0.0007
	Tacrolimus	23	17 (73.9)	6 (26.0)		21	7 (33.3)	14 (67.7)	
	Azathioprine	13	6 (46.1)	7 (53.9)		8	3 (37.5)	5 (62.5)	
	MMF	7	5 (71.4)	2 (29.6)		13	6 (46.2)	7 (53.8)	
	Methotrexate	3	2 (66.7)	1 (33.3)		2	0	2 (100)	
	Prednisolone	52	40 (76.9)	12 (23.1)		36	14 (38.9)	22 (61.1)	
	≥10 mg	19	13 (68.4)	6 (31.6)		11	5 (45.5)	6 54.5)	
	Belimumab	4	3 (75.0)	1 (25.0)		1	0	1 (100)	
	No current IS	4	1 (25.0)	3 (75.0)		4	1 (25.0)	3 (75.0)	
	Previous CYP	58	47 (81.0)	11 (19.0)	0.03	41	18 (43.9)	23 66.1)	
Vaccine	AZ/ChAdOx1	34	29 (85.3)	5 (14.7)	0.04	22	16 (72.3)	6 (37.7)	0.0009
	Pfizer/ BNT162b2	85	56 (65.9)	29 (34.1)		69	21 (30.4)	48 (69.6)	
Clinical parameter	B-cell depletion	64	54 (63.5)	10 (29.4)	0.001	49	28 (57.1)	21 (42.9)	0.0006
	Hypogammaglobulinaemia	25	19 (76.0)	6 (34.0)		22	12 (54.5)	10 (45.5)	

Comparison between groups by χ^2 test.
*Podocytopathy included minimal change disease and focal segmental glomerulosclerosis.
†Other diagnoses included C3 glomerulopathy and IgG4-related disease.
AAV, ANCA-associated vasculitis; COPD, chronic obstructive pulmonary disease; CYP, cyclophosphamide; GBM, glomerular basement membrane; IS, immunosuppressed; MMF, mycophenolate mofetil; SLE, systemic lupus erythematosus.

Immunological response to second-dose vaccine in infection-naïve patients

Ninety-one patients were included in the analysis of response to second-dose vaccine. At 18–29 days after second-dose vaccine, the proportion of patients with detectable anti-S IgG increased to 59.4% (54/91, figure 1A). In contrast, all HV individuals had detectable anti-S IgG after second-dose vaccine. The median anti-S titre after second-dose vaccine was significantly lower in IS patients than in HV, whether analysed as the whole cohort, or as an age-matched and vaccine-matched subgroup (figure 1D; median 58.7 (IQR 0.8–437.2), median 189.3 (IQR 7.9–1090) and median 877 (IQR 575–2203) BAU/mL for IS total cohort, IS matched grou and HV, respectively; p<0.0001).

Within the IS group, in those who had already seroconverted following first-dose vaccine, anti-S titres increased significantly in all patients. In those who were seronegative after first dose, a further 42.4% (28/66) now had detectable anti-S IgG. In keeping with our findings after first-dose vaccine, ChAdOx1 vaccine, prior rituximab treatment and current B-cell depletion were associated with a decreased likelihood of seroconversion, as was increasing age (figure 1B,C, and table 1). There was moderate correlation between serological response to second-dose vaccine and peripheral B-cell count at the time of vaccination (figure 1E). In the group of patients treated with rituximab, administration within the last 6 months was significantly associated with failure to seroconvert; 40.9% (18/44) vs 71.0% (22/31) seroconversion in those treated <6 months and >6 months previously, respectively (p=0.02). By multivariate analysis, B-cell depletion at the time of vaccination (OR 0.32, p=0.04) was significantly associated with non-seroconversion.

T-cell responses were assessed in 46/91 patients following second-dose vaccine and were detected in 82.6% (38/46, figure 2A). There were no differences in the rate or magnitude of T-cell response between those who seroconverted (81.2% (18/22), median SFU/10^6 PBMC 123) and those who did not (83.3% (20/24), median SFU/10^6 PBMC 148) (figure 2E and table 2). The number of patients without detectable T-cell responses following second-dose was small (n=8), and age

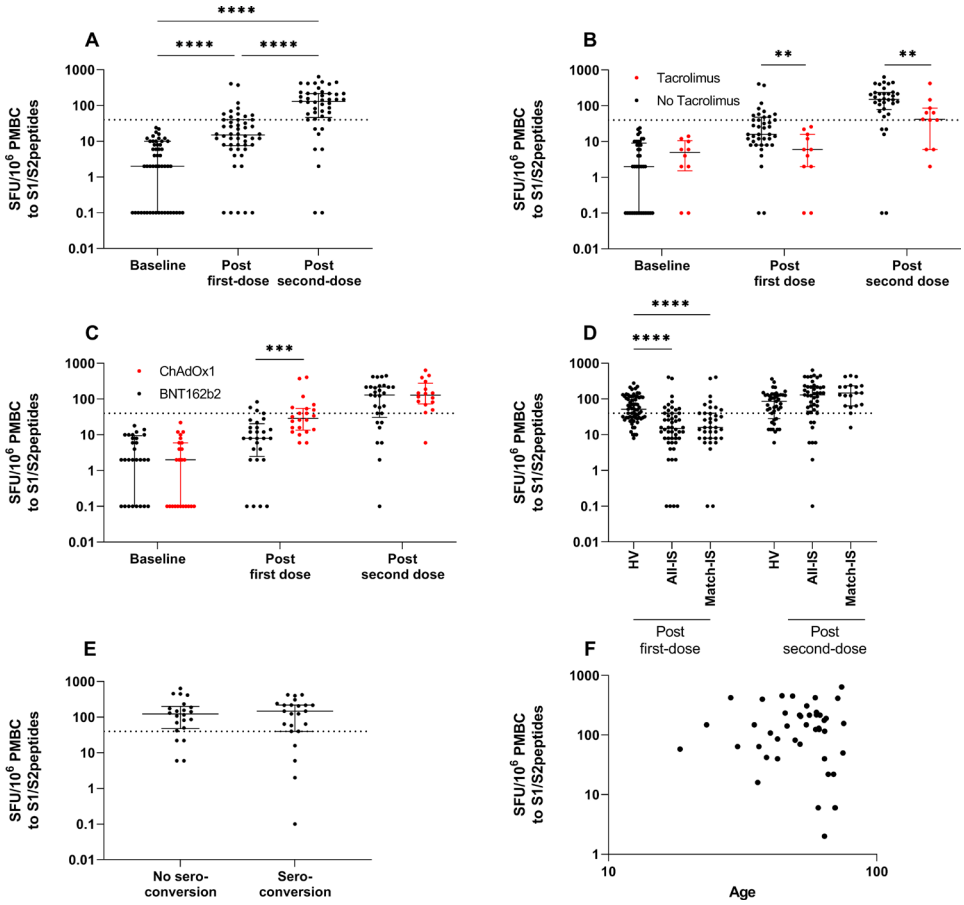

Figure 2 Cellular responses to SARS-CoV-2 vaccination in IS patients. (A) T-cell responses to spike protein peptides of SARS-CoV-2 in infection-naïve patients at baseline, 28–40 days following first-dose vaccine and 18–29 days after second-dose vaccine. (B) T-cell responses in those receiving tacrolimus therapy versus those who were not in infection-naïve participants at baseline, after first-dose vaccine and after second-dose vaccine. (C) T-cell responses by vaccine type in infection-naïve participants at baseline, after first-dose vaccine and after second-dose vaccine. (D) T-cell responses following first-dose and second-dose vaccinations in healthy volunteers (HVs), IS patients and a matched cohort of IS patients. (E) T-cell responses following second-dose vaccine in those who did and did not also seroconvert. (F) Correlation of T-cell responses after second-dose vaccination and age at time of vaccination. Dotted line indicates mean plus 3 SDs for spike peptide pool reactivity calculated from infection-naïve, non-vaccinated individuals (40 SFU/10^6 PBMC). For visualisation of data on a log scale, values=0 are represented by 0.1. HV, healthy volunteer; IS, immunosuppressed; PMBC, peripheral blood mononuclear cell; SFU, spot-forming unit.

was the only parameter significantly associated with absence of T-cell response to vaccination, although there was no correlation between age and magnitude of response (figure 2F and table 2; median age 51.9 and 61.5 years for those with T-cell responses above and below threshold, respectively; p=0.05). Although there was no significant difference in the proportion of patients with T-cell responses above threshold, the magnitude of response was significantly lower in patients treated with tacrolimus (figure 2B; median 53 and 152 SFU/10^6 PBMC for those treated with tacrolimus and not, p=0.01).

In infection-naïve patients for whom both serological and T-cell assessments were available, 47.8% (22/46) had negative serological responses after second-dose vaccine. Of these patients, 81.8% (18/22) had detectable T-cell responses. In patients who were B-cell deplete, an assessment of both serological and T-cell assessments were available in 30 patients, 60.0% (18/30) of whom had negative serological responses. In this B-cell deplete group with no serological response to vaccine, 83.3% (15/18) had detectable T-cell responses.

Comparing the HV and IS group, there was no significant difference in the proportion with T-cell responses to second-dose vaccine (figure 1D, 74.4% (32/43) of HV had T-cell responses above threshold) or in the magnitude of response (median 130 and 86 SFU/10^6 PBMC for IS and HV, respectively; p=not significant (ns)). Since second-dose vaccine samples in HV were limited to individuals who received BNT162b2; an analysis of age-matched and vaccine-matched IS patients was performed, and there were no significant differences in response (median 140 and 86 SFU/10^6 PBMC for matched IS and HV, respectively, p=ns; the numerical differences in T-cell number between these groups were not statistically significant and may reflect a degree of T cell enrichment in PMBC preparations from B-cell deplete IS patients).

In infection-naïve patients for whom both serological and T-cell assessments were available, the response rate (by one or both immunological parameters) increased significantly following each dose (36.0% (18/50) and 91.3% (42/46), respectively; p<0.0001). The four patients with no immunological response after second-dose were significantly older than those with a response by either measure; all four had received rituximab previously, although one was no longer B-cell deplete (online supplemental table S3).

Table 2 Patient characteristics by T-cell responses in those with no evidence of previous natural infection

		First dose (n=50)				Second dose (n=46)			
			No T-cell response	T-cell response	P		No T-cell response	T-cell response	
Characteristics		n	n=37 (74.0%)	n=13 (26.0%)	value	n	n=8 (17.4%)	n=38 (82.6%)	P value
Gender	Male	31	24 (77.4)	7 (22.5)		27	3 (11.1)	24 (88.9)	
	Female	19	13 (68.4)	6 (31.6)		19	5 (26.3)	14 (73.7)	
Age	Years (IQR)		54.9 (42.7–63.9)	49.4 (39.8–62.8)			65.1 (61.4–70.0)	51.9 (42.1–75.3)	0.02
Ethnicity	White	28	20 (71.4)	8 (28.6)		24	4 (16.7)	20 (83.3)	
	Black	1	1 (100)	0		2	0	2 (100)	
	South Asian	19	15 (78.9)	4 (21.1)		18	4 (22.2)	14 (77.8)	
	Mixed-race	2	1 (50.0)	1 (50.0)		1	0	1 (100)	
	Other	0	0	0		1	0	1 (100)	
Diagnosis	AAV and anti-GBM disease	24	14 (58.3)	10 (41.7)		19	4 (21.1)	15 (78.9)	
	Podocytopathy*	15	13 (86.7)	2 (13.3)		15	2 (13.3)	13 (86.7)	
	Membranous GN	10	9 (90.0)	1 (10.0)		9	2 (22.2)	7 (77.8)	
	SLE	0	0	0		2	0	2 (100)	
	Other†	1	1 (100)	0		1	0	1 (100)	
Comorbidities	Diabetes	10	7 (70.0)	3 (30.0)		8	3 (37.5)	5 (62.5)	
	Asthma/COPD	10	7 (70.0)	3 (30.0)		11	2 (18.2)	9 (81.8)	
	Previous malignancy	0	0	0		0	0	0	
Immunotherapy	Rituximab	44	31 (70.5)	13 (29.5)		41	7 (17.1)	34 (82.9)	
	Last 6 months	32	21 (65.6)	11 (34.4)		28	4 (14.3)	24 (85.7)	
	Tacrolimus	11	11 (100)	0	0.04	12	3 (25.0)	9 (75.0)	
	Azathioprine	4	2 (50.0)	2 (50.0)		3	0	3 (100)	
	MMF	5	3 (60.0)	2 (40.0)		4	1 (25.0)	3 (75.0)	
	Methotrexate	0	0	0		1	1 (100)	0	
	Prednisolone	17	14 (82.3)	3 (17.6)		14	2 (14.3)	12 (85.7)	
	≥10 mg	5	4 (80.0)	1 (20.0)		3	1 (33.3)	2 (66.7)	
	Belimumab	0	0	0		0	0	0	
	No IS	1	1 (100)	0		1	0	1 (100)	
	Previous CYP	25	15 (60.0)	10 (40.0)		20	3 (15.0)	17 (85.0)	
Vaccine	AZ/ChAdOx1	22	13 (59.1)	9 (40.9)	0.05	17	1 (5.9)	16 (94.1)	
	Pfizer/ BNT162b2	28	24 (85.7)	4 (14.3)		29	7 (24.1)	22 (75.9)	
Clinical parameter	B-cell depletion	33	22 (66.7)	11 (33.3)		30	5 (16.7)	25 (83.3)	
	Hypogammaglobulinaemia	13	10 (76.9)	3 (13.1)		9	3 (33.3)	6 (66.7)	

*Podocytopathy included minimal change disease and focal segmental glomerulosclerosis.
†Other diagnoses included C3 glomerulopathy and IgG4-related disease. Comparison between groups by χ^2 test.
AAV, ANCA-associated vasculitis; COPD, chronic obstructive pulmonary disease; CYP, cyclophosphamide; GBM, glomerular basement membrane; GN, glomerulonephritis; IS, immunosuppression; MMF, mycophenolate mofetil; SLE, systemic lupus erythematosus.

Immunological response to vaccination in patients with prior natural infection

In keeping with our previous report in healthy individuals,[15] the 19 participants with evidence of prior SARS-CoV-2 infection mounted robust serological responses to first-dose vaccination, including those who had previously received rituximab (n=13/19) or who were B-cell deplete (n=4/19, figure 1F). In 12 patients, serology was available following second-dose vaccine. Anti-S titre increased further following second-dose vaccine ('third' S protein challenge) in 8/12, remained above the limit of detection in 2/12, and declined or plateaued in only 2/12 (figure 1F). Due to the number of patients with responses above the threshold of detection of the assay, it was not possible to compare median anti-S titres following first-dose and second-dose vaccine in this group. T-cell responses were available for three patients in this cohort; all mounted robust cellular immunity to both first-dose and second-dose vaccines (60–616 and 300–580 SFU/10^6 PBMC after first and second doses, respectively).

DISCUSSION

The immune response to first-dose BNT162b2 mRNA or ChAdOx1 nCoV-19 vaccine was poor in patients receiving immunosuppression, with only 28.6% of patients having detectable humoral or T-cell responses. These rates compare poorly to a cohort of non-IS HVs. Reassuringly, immune responses were augmented by second-dose vaccine, increasing the seroconversion and T-cell response rates to 59.4% and 82.6%, respectively. Only 8.7% of patients had neither antibody nor T-cell responses following second-dose vaccine. These findings indicate that both vaccines are immunogenic in patients receiving immunosuppression, but that protocolised two-dose vaccination schedules are required. The augmented response to second-dose vaccine (and 'third' challenge in patients with prior natural infection) suggests that repeat boost strategies could be considered in this patient group, to induce more robust immune responses in the future.

B-cell depletion (following prior rituximab treatment) at the time of vaccination was the strongest predictor of failure to seroconvert, in keeping with data on impaired humoral responses to other

vaccines in patients treated with rituximab. These studies found that time since rituximab treatment was a determinant of serological response,[13 14] consistent with our finding of lower response rates in those who were currently B-cell deplete versus those who had repopulated peripheral B cells. Current guidelines differ regarding the timing of SARS-CoV-2 vaccination after rituximab.[18–20] While our data suggest that better serological responses may be achieved by delaying vaccination until B-cell reconstitution has occurred, it may not be ethical to do so when community transmission rates are high (or to defer rituximab treatment when needed for disease control). We therefore suggest that additional courses of vaccination should be made available to these patients between or after completed rituximab cycles.

While current vaccine efforts have focused on the induction of neutralising antibodies to SARS-CoV-2, T-cell immunity may also provide protection from infection. Experimental data suggest that CD8 + T-cell responses in particular may have a protective role in the presence of waning or subprotective antibody titres.[21] In addition, patients with agammaglobulinaemia have been described to recover from COVID-19 in the absence of a serological response, suggesting T-cell responses may be sufficient to mount protection or aid recovery from disease.[22–24] It is reassuring that vaccine-induced T-cell responses were detected in most of our study cohort, including those who were B-cell deplete at the time of vaccination, and those who failed to seroconvert. Tacrolimus use was associated with impaired T-cell response, and further studies are needed to investigate the impact of calcineurin inhibitors and other T cell-directed therapies on vaccine response in more detail.

The immune correlates of protection from disease, however, are not clearly defined. Published trials have not reported antibody measurements of participants who contracted COVID-19 following vaccination, and in vitro assessments of antibody neutralising activity have not been correlated with clinical outcomes. Robust CD8 and CD4 T-cell responses to BNT162b2/ChAdOx1 were reported in early-phase clinical studies,[25 26] although all participants also mounted neutralising antibody responses. Thus, further work is needed to determine whether the serological or T-cell response observed in our cohort will confer protection from clinical disease and whether the longevity of the immune response in this group is comparable to that in healthy individuals.

A limitation of our study is that only a small proportion of patients were treated with conventional synthetic disease-modifying antirheumatic drugs such as methotrexate or MMF, and some conditions such as systemic lupus erythematosus are under-represented. While we observed possible differences between vaccine types (with stronger serological responses in patients receiving BNT2b162 and better T-cell responses in those receiving ChAdOx1), our study is underpowered to determine if vaccine choice should be influenced by underlying disease or immunosuppressive treatment. Further studies in larger cohorts will be required to understand the impact of these factors and whether there are preferred vaccine types in these high-risk patient groups. In addition, the HV group in our study is not ideally matched to the IS cohort; individuals are younger, and an assessment of second-dose response was only available in participants receiving BNT162b2. The HV group also received second-dose vaccination after a longer time period than the IS cohort (67 and 30 days, respectively). We have undertaken limited matching based on age and vaccine type, but sufficiently detailed data for the HV cohort is not available to provide a more accurate comparator group.

Despite these limitations, our data confirm the immunogenicity of SARS-CoV-2 vaccination in an IS cohort, finding that B-cell depletion following rituximab impairs serological responses, but T-cell responses are preserved in this group. Reassuringly, our data confirm an immunological response in most patients, when assessed by a combination of serological and cell-based assays. Our findings support SARS-CoV-2 vaccination in this patient group; however, since the overall quality of response was impaired compared with healthy individuals, we suggest that repeat vaccine doses may be necessary to optimise the immunological response and to induce more robust serological responses in particular, for these vulnerable patients.

Author affiliations
[1]Centre for Inflammatory Disease, Department of Immunology and Inflammation, Imperial College London, London, UK
[2]Imperial College Renal and Transplant Centre, Imperial College Healthcare NHS Trust, Hammersmith Hospital, London, UK
[3]Department of Infection and Immunity, North West London Pathology NHS Trust, London, UK
[4]Respiratory Medicine, Royal Brompton Hospital, Guy's and St. Thomas' NHS Foundation Trust, London, UK
[5]MRC Centre of Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, London, UK
[6]Department of Infectious Diseases, Imperial College London, London, UK
[7]Department of Respiratory Medicine, Harefield Hospital, London, UK

Twitter Liz Lightstone @kidneydoc101

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ORCID iD
Stephen P McAdoo http://orcid.org/0000-0001-8260-8770

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