T cell responses to SARS-CoV-2 in healthy controls and primary immunodeficiency patients

List of authors:
Arnold Awuah, BSc*; Ava Zamani, MBBS†; Fariba Tahami, MSc*; Mark Davis, MBA*; Louis Grandjean, PhD††; Matthew Buckland, PhD*; Kimberly Gilmour, PhD*

*Department of Immunology, Great Ormond Street Hospital (GOSH)
†Affiliate of Department of Immunology, GOSH
††Department of Infectious Diseases, GOSH

Corresponding author:
Kimberly Gilmour
Camelia Botnar Laboratories
Great Ormond Street Hospital for Children NHS Foundation Trust
London
WC1N 3JH
020 7813 8466
kimberly.gilmour@gosh.nhs.uk

Word count:
1,147

Short Title – T-cell Response to COVID in Immunodeficiency

Keywords;
COVID19
SARS-COV2
T-cell
Proliferation
Immune Deficiency
Immune Response
Antibody Deficiency

Abbreviations;
SARS-COV2 – Severe Acute Respiratory syndrome Coronavirus -2
COVID19- Coronovirus related Immune Disorder -2019
S – Spike
M- Membrane
N- Nucleocapsid
PBMC – Peripheral Blood Mononuclear Cell
PID – Primary Immunodeficiency
PHA – Phytohaemagluttinin
CPM – Counts per Minute
XLA – X-Linked Agammaglobulinaemia
CD40L – CD40 Ligand Deficiency
SIOD - Schimke immune-osseous dysplasia
RAG – Recombinase Activating Gene
AIP – Actin Interacting Protein
WDR1 – WD domain repeat domain 1
CVID – Common Variable Immune Deficiencies

Summary
T-cell responses to COVID are now linked to improved outcomes. This paper demonstrates a method to robustly test responses for routine diagnostic use in healthy controls and those unable to make a detectable antibody response due to underlying immune deficiency (primary or acquired).

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel respiratory virus with a wide range of clinical presentations known collectively as COVID-19. The first wave of the COVID-19 pandemic saw a 65% hospitalisation rate and a 17% mortality rate amongst confirmed cases in the UK (1). Understanding the immune response to COVID-19 is a pre-requisite to identifying clinical correlates of exposure and immunity. This is of particular importance in vulnerable patients such as those with immunodeficiency, who may have more prolonged or severe infection (2,3). Detecting the antibody response to COVID-19 is essential to diagnostic testing, however the antibody response may wane over time (4), or may not

be detectable in patients with antibody deficiency (2,5) necessitating an examination of the role of the cell-mediated immunity. There is already evidence to suggest T cells may provide long-lasting immunity against the virus (6), and a T cell response has been detected in seronegative individuals post-COVID-19 (7). A simple and practical method is essential to assess the T cell response in the clinical setting.

Method

A functional [3H]-thymidine incorporation assay to assess the T cell response to SARS-CoV-2 was developed with the aim of analysing a cohort of primary immunodeficiency (PID) patients at Great Ormond Street Hospital. Peripheral blood mononuclear cells (PBMCs) were isolated from participants' venous blood samples, and stimulated with SARS-CoV-2 membrane (M), nucleocapsid (N) and spike (S) antigens at 1:2, 1:4 and 1:8 serial dilutions. All dilutions were duplicated. Where blood samples were insufficient in volume, they were stimulated with only S antigens. Antigens were supplied by Miltenyi-Biotech (Pro S 130-126-700, Pro N 130-126-698 & Pro M 130-126-702). Final concentrations of 1, 0.5 and 0.25 ug/ml were used. The mitogen phytohaemagglutinin (PHA) was used as a positive control and unstimulated samples (no added antigen or mitogen) were used as negative controls. After 4 days' incubation, the cells were pulsed with [3H]-thymidine for 4-6 hours. The incorporation of [3H]-thymidine by proliferating cells, in counts per minute (CPM), was measured for each suspension using the Harvester and MicroBeta2 counter as previously described (8). CPM values were derived from the dilution producing the peak average response between duplicates, unless there was poor agreement between duplicates, in which case the next highest reliable average response was used. Stimulation Index (SI) was also calculated for all conditions. The same materials, instruments and methods were used throughout to reduce inter-assay variability and the PHA mitogen stimulation was used as the quality control for each sample and results were not analysed if there was no detectable PHA response.
Statistical analysis was performed on Graphpad prism with comparison of groups by ANOVA and correction for multivariate analysis by MANOVA applied.

Participants in this study included healthy controls and patients with PID pre- and post-vaccination or with a history of natural infection. Vaccinated participants aged 40 and above had received either the Moderna, the Oxford/AstraZeneca or the Pfizer/BioNTech vaccine against COVID-19; participants under 40 years of age had received either the Moderna or the Pfizer/BioNTech vaccine, in-keeping with NHS England guidance (9). Ethical approval and consent from participants/parents or guardians was obtained for all participants included in the study (NRES London-Bloomsbury REC #06/Q508/16).

Results

Table I summarises participant characteristics and Table II summarises the data from 38 participants, including: 18 healthy controls (8 pre-vaccination with no known history of natural SARS-CoV-2 exposure, 6 post-vaccination, & 4 post-known infection), and 21 patients with PID (10 post-vaccination, 8 post-infection, and 3 of unknown SARS-CoV-2 status). Patients with PID included 12 with hypogammaglobulinaemia – 8 with common variable immunodeficiency (CVID), 4 with X-linked agammaglobulinaemia (XLA; and 8 with Combined Immune Deficiencies with predominantly T cell disorders – 1 with each of T cell activation disorder, ataxia-telangiectasia (A-T), Schimke immune-osseous dysplasia (SIOD), CD40 ligand (CD40L) deficiency, RAG1 severe combined immunodeficiency (SCID), and autoinflammatory syndrome secondary to AIP/WDR1 mutation, respectively, as well as 2 with Trisomy 21.

All healthy controls had minimal proliferation pre-vaccination but post-vaccination a statistically significant increase in proliferation to S antigen and post-infection increase in proliferation to M, N & S antigens which were not statistically different to the magnitude of the PHA response (p=ns) (Fig. 1). Average T cell proliferation was comparatively low in patients with hypogammaglobulinaemia post-exposure; however, within this group, patients with XLA had relatively high proliferation post-infection, including 2 of the highest proliferation responses of the entire cohort. There was no statistically significant difference between the magnitude of the PHA and M,N or S response in the XLA cohort (p=ns). As expected, patients with T

cell disorders had antigen specific proliferation responses near-equivalent to background despite a statistically significant PHA response vs background (P<0.06). In all participants, background CPM counts were below 3400 and PHA CPM counts were above 7000, including in those with PID and in all groups achieved statistical significance vs background (P<0.05 to <0.0005). A separate analysis of stimulation index had a concordant results (data not shown) with stimulation indices >3.0 for all conditions that had statistically elevated CPMs.

Discussion

T cell proliferation rates following exposure to SARS-CoV-2 antigens were assessed in individuals with immunodeficiency and healthy controls via utilisation of [3H]-thymidine incorporation assays, expanding our knowledge of the SARS-CoV-2 T cell response in a clinical setting. Patients with absent B cells (XLA) all mounted a robust T cell response post-infection; importantly, this always coincided with a negative serological response to COVID-19 (data not shown). Patients with XLA have been observed elsewhere to experience a milder COVID-19 disease course compared to patients with CVID, leading to speculation on the different roles of B and T lymphocytes in COVID-19 pathology (3).

In participants with CVID, T cell proliferative responses to PHA were comparable to healthy controls; however, T cell proliferation to SARS-CoV-2 S antigen was markedly reduced post-vaccination when compared with healthy controls post-vaccination. Serological responses were not reliably measured in this group given frequent concomitant treatment with immunoglobulin infusions; however, it should be noted a poor or absent serological response to vaccination forms part of the diagnostic criteria for CVID (10). Our data demonstrates that even though T cell numbers may be normal in patients with CVID, they may also have significant impairment of measurable T cell function consistent with the pathogenesis of these disorders. In XLA the defect is a block in the developmental of B-cells. In CVID the majority of defects are likely to be in pathways more essential to both T- and B- cell function, disorders removed from this group that now have a monogenic basis e.g. NFKB haplo-insufficiency highlight this dichotomy . The impaired antigen specific T-cell responses in CVID patients, raises concern about the effectiveness of vaccination in this

cohort, which suggests further exploration of protective strategies is needed in this group in larger studies.

On average, patients with T cell disorders had proliferation responses to SARS-CoV-2 antigens near-equivalent to background. Two patients with Trisomy 21 were included within this cohort. Trisomy 21 patients are known to have a variable maturational delay in adaptive immunity which manifests as low T cells, with a decrease in naïve T cells and impaired T cell proliferation (11). There is evidence that patients with Trisomy 21 experience a more severe COVID-19 disease course (12), highlighting this as an area where greater understanding of the immune responses to COVID-19 is needed.

Limitations of this study include small sample size, which was in part unavoidable due to the rarity of the studied disorders. SARS-CoV-2 status was unknown in 3 patients with combined immunodeficiency disorders, although 2 of the 3 had positive serological responses. In 2 paediatric patients, peripheral venous blood samples were small in volume and sufficient only for stimulation with S antigen.

[3H]-thymidine incorporation assays are ISO 15189-accredited in our laboratory and so appropriately standardised for inter- and intra- assay variability. There is a long history of routine use in clinical laboratories because they are robust and adaptable, although the use of radioactivity limits the use in some diagnostic laboratory settings. As a direct measure of T cell proliferation, these assays are highly applicable to a cohort of patients with PID in identifying those who mount a COVID T cell response and those who do not. Data from healthy controls confirms the robustness of this assay.

Acknowledgments

We thank the Immunology Department at Great Ormond Street Hospital for processing of samples in the laboratory and the members of staff who provided samples for analysis.

References

1. Merchant HA, Kow CS, Hasan SS. COVID-19 first anniversary review of cases, hospitalization, and mortality in the UK. https://doi-org.libproxy.ucl.ac.uk/101080/1747634820211890035. 2021;15(8):973–8.
2. Mullur J, Wang A, Feldweg A. A fatal case of coronavirus disease 2019 in a patient with common variable immunodeficiency. Ann Allergy, Asthma Immunol. 2021 Jan 1;126(1):90.
3. Quinti I, Lougaris V, Milito C, Cinetto F, Pecoraro A, Mezzaroma I, et al. A possible role for B cells in COVID-19? Lesson from patients with agammaglobulinemia. J Allergy Clin Immunol. 2020 Jul 1;146(1):211.
4. Seow J, Graham C, Merrick B, Acors S, Pickering S, Steel KJA, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol. 2020 Dec 1;5(12):1598–607.
5. Gupta S, Su H, Narsai T, Agrawal S. SARS-CoV-2-Associated T-Cell Responses in the Presence of Humoral Immunodeficiency. Int Arch Allergy Immunol. 2021 Feb 1;182(3):195–209.
6. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. Cell. 2020 Oct 1;183(1):158-168.e14.
7. Steiner S, Schwarz T, Corman VM, Sotzny F, Bauer S, Drosten C, et al. Reactive T Cells in Convalescent COVID-19 Patients With Negative SARS-CoV-2 Antibody Serology. Front Immunol. 2021 Jul 12;0:2557.
8. PerkinElmer I. Filter counting applications with the MicroBeta2 Microplate Counter [Internet]. 2009 [cited 2021 Jul 25]. Available from: https://resources.perkinelmer.com/lab-solutions/resources/docs/APP_Filter_counting_applications_MicroBeta.pdf
9. NHS. Coronavirus (COVID-19) vaccines - NHS [Internet]. NHS England. [cited 2021 Nov 30]. Available from: https://www.nhs.uk/conditions/coronavirus-covid-19/coronavirus-vaccination/coronavirus-vaccine/
10. Seidel M, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors

of Immunity. J allergy Clin Immunol Pract. 2019 Jul 1;7(6):1763–70.
11. Ram G, Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol. 2011 Apr;164(1):9.
12. Malle L, Gao C, Hur C, Truong HQ, Bouvier NM, Percha B, et al. Individuals with Down Syndrome Hospitalized with COVID-19 Have More Severe Disease. Genet Med. 2021 Mar 1;23(3):576.

Figure 1

CPM, Counts per minute per suspension; BKG, background – unstimulated samples; PHA, phytohaemagglutinin; HC, Healthy controls.

TABLE I Participant characteristics

Patient	Diagnosis	SARS-CoV-2 status
Healthy controls		
1	HC	Pre-vaccination*
2	HC	Pre-vaccination
3	HC	Pre-vaccination
4	HC	Pre-vaccination
5	HC	Pre-vaccination
6	HC	Pre-vaccination
7	HC	Pre-vaccination
8	HC	Pre-vaccination
9	HC	Post-vaccination
10	HC	Post-vaccination
11	HC	Post-vaccination
12	HC	Post-vaccination
13	HC	Post-vaccination
14	HC	Post-vaccination
15	HC	Post-infection
16	HC	Post-infection
17	HC	Post-infection
18	HC	Post-infection
Patients with hypogammaglobulinaemia		
19	CVID	Post-vaccination
20	CVID	Post-vaccination
21	CVID	Post-vaccination
22	CVID	Post-vaccination
23	CVID	Post-vaccination
24	CVID	Post-vaccination
25	CVID	Post-vaccination
26	CVID	Post-infection
27	XLA	Post-vaccination
28	XLA	Post-infection
29	XLA	Post-infection

30	XLA	Post-infection
Patients with Combined Immune Deficiency		
31	Down syndrome	Post-vaccination
32	Down syndrome	Post-infection
33	T cell activation disorder	Post-infection
34	A-T	Post-infection
35	SIOD	Post-infection
36	CD40L deficiency	Unknown
37	RAG1 SCID	Unknown
38	AIP/WDR1 mutation	Unknown

HC, Healthy controls; *CVID*, common variable immunodeficiency; *XLA*, X-linked agammaglobulinaemia; *A-T*, ataxia-telangiectasia; *SIOD*, Schimke immune-osseous dysplasia; *CD40L*, CD40 ligand; *SCID*, severe combined immunodeficiency.
*All 8 healthy controls pre-vaccination had no known history of natural SARS-CoV-2 exposure.

Supplementary material

TABLE II Summary of participant data

			CPM				
					SARS-CoV-2 antigens		
Pt.	**Diagnosis**	**SARS-CoV-2 status**	**BKG**	**PHA**	**M**	**N**	**S**
Healthy controls							
1	HC	Pre-vaccination	510	44742	892	556	1695
2	HC	Pre-vaccination	663	16328	1428	1432	2182
3	HC	Pre-vaccination	659	38723	653	817	739
4	HC	Pre-vaccination	1273	42686	2050	1508	2487
5	HC	Pre-vaccination	613	15825	618	579	706
6	HC	Pre-vaccination	456	16947	658	631	3307
7	HC	Pre-vaccination	610	42685	512	370	666
8	HC	Pre-vaccination	695	51356	1047	911	1372
9	HC	Post-infection	1901	35695	7273	1866	3085
10	HC	Post-infection	770	10037	2255	3788	5544
11	HC	Post-infection	425	18577	13811	12324	8265
12	HC	Post-infection	1880	15620	5702	4208	5813
13	HC	Post-vaccination	640	38550	939	1350	11712
14	HC	Post-vaccination	1177	29170	850	1063	10950
15	HC	Post-vaccination	849	25545	1506	1433	12301
16	HC	Post-vaccination	3359	18332	2040	3337	6962
17	HC	Post-vaccination	433	21918	825	907	6101

18	HC	Post-vaccination	429	13001	629	538	2999
Patients with hypogammaglobulinaemia							
19	CVID	Post-vaccination	2099	32708	2769	2007	2508
20	CVID	Post-vaccination	320	9852	ND	ND	512
21	CVID	Post-vaccination	1104	21164	ND	ND	1437
22	CVID	Post-vaccination	963	25869	1082	1030	1635
23	CVID	Post-vaccination	397	44820	557	2840	906
24	CVID	Post-vaccination	750	26451	1569	1008	4447
25	CVID	Post-vaccination	998	18379	945	1860	1322
26	CVID	Post-infection	625	35430	1608	1015	2379
27	XLA	Post-vaccination	552	14079	378	465	2495
28	XLA	Post-infection	2570	16137	20328	22269	21510
29	XLA	Post-infection	1138	7178	5925	3703	3576
30	XLA	Post-infection	2796	17042	5476	5037	12357
Patients with Combined Immune Deficiencies							
31	Trisomy 21	Post-vaccination	260	79543	1449	1047	2567
32	Trisomy 21	Post-infection	282	28839	1137	705	477
33	T cell activation disorder	Post-infection	706	43961	760	594	690
34	A-T	Post-infection	438	11048	854	554	854
35	SIOD	Post-infection	203	15513	447	385	341
36	CD40L deficiency	Unknown	704	17586	544	524	682
37	RAG1 SCID	Unknown	324	30326	1346	1146	1753
38	AIP/WDR1 mutation	Unknown	814	21214	208	281	428

Pt., Patient; *HC*, healthy controls; *CVID*, common variable immunodeficiency; *XLA*, X-linked agammaglobulinaemia; *A-T*, ataxia-telangiectasia; *SIOD*, Schimke immune-osseous dysplasia; *CD40L*, CD40 ligand; *SCID*, severe combined immunodeficiency; CPM, counts per minute per suspension; *BKG*, background – unstimulated samples; *PHA*, phytohaemagglutinin; *ND*, no data.

Data Availability statement

Anonymised data will be available subject to an appropriate request to the communicating author. This is not published online due to the patient sensitive data that is included in the paper.

Competing interests

The authors do not report any competing interests

Funding statement

This work was conducted at Great Ormond St Hospital NHS Foundation Trust as part of the development of an assay for routine NHS use and all work was funded by the Trust.

Author contributions

AA – designed the study and conducted the majority of the laboratory work and contributed to the writing and review of the data.

AZ – contributed to data analysis and manuscript preparation
FT - contributed to data analysis and manuscript preparation
MD - contributed to data analysis and manuscript preparation
LJ – contributed to study design, data analysis and manuscript preparation
MB - contributed to study design, patient recruitment data analysis and manuscript preparation
KG - contributed to study design, patient recruitment data analysis and manuscript preparation