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

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Abstract

Understanding the T-cell response to SARS-CoV-2 is key in patients who lack antibody production. We demonstrate the applicability of a functional assay to measure the T-cell response in a cohort of patients with immunodeficiency.

Keywords: COVID-19, 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

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% hospitalization 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 are 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, and Pro M 130-126-702). Final concentrations of 1, 0.5, and 0.25 μg/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 h. 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.

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Statistical analysis was performed on Graphpad prism with a 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 were obtained for all participants included in the study (NRES London-Bloomsbury REC #06/Q508/16).

Results

Table 1 summarises participant characteristics and Supplementary Table S1 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, and 4 post-known infections), 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 a minimal proliferation pre-vaccination but post-vaccination had a statistically significant increase in proliferation to S antigen and post-infection had increase in proliferation to M, N, and 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 the stimulation index had concordant results (data not shown) with stimulation indices >3.0 for all conditions that had statistically elevated CPMs.

Table 1: 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

*All 8 healthy controls pre-vaccination had no known history of natural SARS-CoV-2 exposure.

Discussion

T-cell proliferation rates following exposure to SARS-CoV-2 antigens were assessed in individuals with immunodeficiency and healthy controls via utilization 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

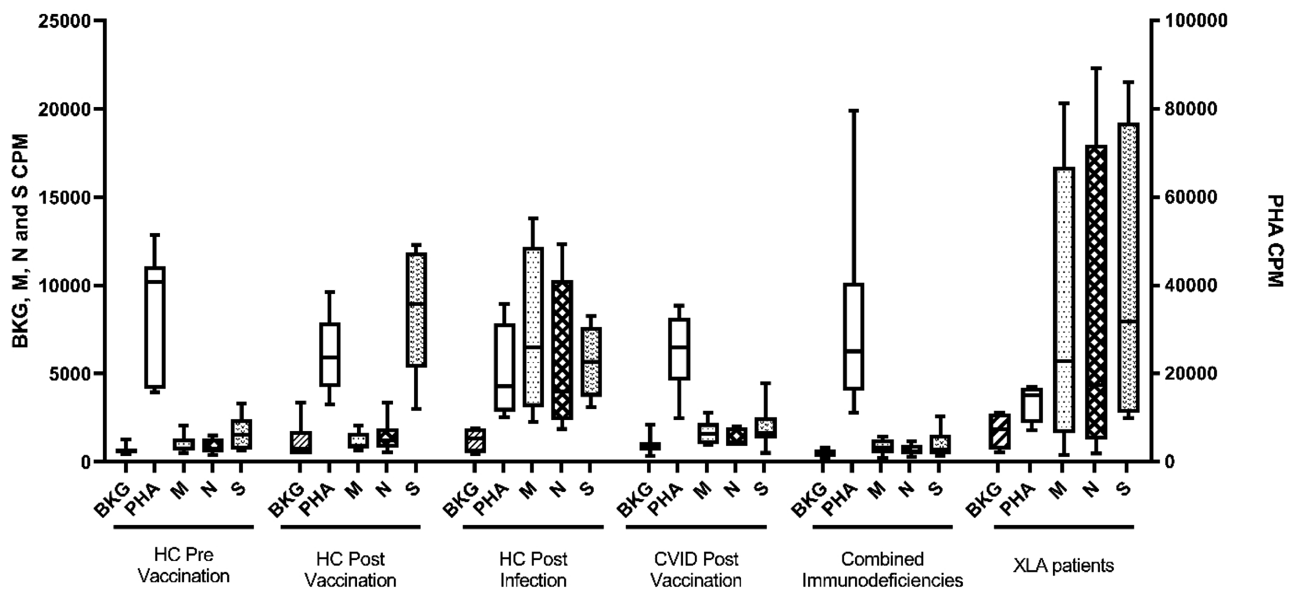

Figure 1: Healthy controls with proliferative responses pre-vaccination, post-vaccination or post-infection. Patient responses are post-infection or post-vaccination as in Table 1. For patients and Health Controls a background and PHA response are given as well as responses to M, N, and S antigens. CPM, Counts per minute per suspension; BKG, background – unstimulated samples; PHA, phytohaemagglutinin; HC, 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 demonstrate 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 development 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 are 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 the background. Two patients with Trisomy 21 were included within this cohort. Patients with Trisomy 21 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 a greater understanding of the immune responses to COVID-19 is needed.

Limitations of this study include a 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 standardized 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 confirm the robustness of this assay.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

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Competing interests

The authors do not report any competing interests

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.

Data availability

Anonymized 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.

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