Research Article

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

Arnold Awuah¹, Ava Zamani², Fariba Tahami¹, Mark Davis¹, Louis Grandjean³, Matthew Buckland¹,∗ and Kimberly Gilmour¹,∗

¹Department of Immunology, Great Ormond Street Hospital (GOSH), London, UK
²Affiliate of Department of Immunology, Great Ormond Street Hospital (GOSH), London, UK
³Department of Infectious Diseases, Great Ormond Street Hospital (GOSH), London, UK

∗Correspondence: Kimberly Gilmour, Camelia Botnar Laboratories, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK. Email: kimberly.gilmour@gosh.nhs.uk

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

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 prerequisite 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.
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 immunologically 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 Trissomy 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.

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

| 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

| Patient | Diagnosis | SARS-CoV-2 status |
|---------|-----------|------------------|
| 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

| Patient | Diagnosis | SARS-CoV-2 status |
|---------|-----------|------------------|
| 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.

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 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 proliferative 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.

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.
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.

References
1. Merchant HA, Kow CS, Hasan SS. COVID-19 first anniversary review of cases, hospitalization, and mortality in the UK. Expert Rev Respir Med 2021, 15, 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, 126, 90–2.
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, 146, 211–3.e4.
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, 5, 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, 182, 195–209.
6. Sekine T, Perez-Porti A, Rivera-Ballestero O, Strälin K, Gorin JB, Olsson A, et al.; Karolinska COVID-19 Study Group. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 2020, 183, 158–68.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, 12, 2557. https://www.frontiersin.org/article/10.3389/fimmu.2021.687449.
8. PerkinElmer I. Filter Counting Applications with the MicroBeta2 Microplate Counter [Internet]. 2009. https://resources.perkinelmer.com/lab-solutions/resources/docs/APP_Filter_counting_applications_MicroBeta.pdf (25 July 2021, date last accessed).
9. NHS. Coronavirus (COVID-19) Vaccines – NHS [Internet]. NHS England. https://www.nhs.uk/conditions/coronavirus-covid-19/coronavirus-vaccination/coronavirus-vaccine/ (30 November 2021, date last accessed).
10. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al.; ESID Registry Working Party and collaborators. The European Society for Immunodeficiencies (ESID) Registry working definitions for the clinical diagnosis of inborn errors of immunity. J Allergy Clin Immunol Pract 2019, 7, 1763–70.
11. Ram G, Chinen J. Infections and immunodeficiency in Down syndrome. Clin Exp Immunol 2011, 164, 9–16.
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, 23, 576–80.