Oxford Transplant Foundation

Pump-Priming Grant

Report

Sushma Shankar

Title: Analysis of longitudinal immunity in SARS-CoV-2-convalescent patients with a renal transplant or haemodialysis

Objective:

To determine whether convalescent patients with RRT who have recovered from SARS-CoV-2, develop a sustained and protective adaptive immune response to COVID-19 based on existing surrogate markers of protection, and to compare this response to that of the convalescent general population. RRT is defined as regular HD or a functioning renal transplant.

Method:

Sample collection

HD and renal transplant patients who were vaccine-naïve and had recovered from SARS-CoV-2 infection underwent phlebotomy during in-hospital haemodialysis sessions, at regular 30 day intervals following initial infection. Transplant patients who were vaccine-naïve underwent phlebotomy only when attending clinic appointments 28-192 days following SARS-CoV-2 infection, in order to minimise hospital visits whilst shielding. Nasopharyngeal swab and RT-PCR testing was undertaken as part of dialysis unit screening programmes, on average 2.1 (SD 0.4) times per month throughout the follow-up period of the study. Nasopharyngeal swab and RT-PCR testing in the transplant cohort was undertaken upon symptomatic presentation.

Clinical information including date of SARS-CoV-2 infection defined by a positive PCR test and/or detection of antibodies to nucleocapsid protein, presence or absence of symptoms, age, gender, ethnicity, immunosuppression, duration of RRT, diabetes and primary renal disease of participants were recorded. Clinical serological assays to detect IgG to nucleocapsid protein was performed using the Abbott Architect i2000 chemiluminescent microparticle immunoassay (Abbott, Maidenhead, UK). Antibody levels ≥1.40 arbitrary units were considered positive.

Peripheral blood mononuclear cells (PBMCs) were separated from heparinised whole blood using density gradient centrifugation with Lymphoprep (StemCell Technology, 07861) and cryopreserved in liquid nitrogen for later use. Extracted serum was stored at -80℃ until further analysis.

T Cell proliferation assays

T cell proliferation assays were conducted as previously described [1]. Briefly, cryopreserved PBMCs are thawed in R10 (RPMI - Sigma-Aldrich, 10% Fetal bovine serum (FBS) – Sigma-Aldrich, 1% L-Glutamine and 1% Penicillin – Streptomycin (Pen-Strep)). Cells were then labelled with CTV cell dye for 10 minutes at room temperature followed by quenching with FBS. Labelled cells were plated in RAB10 (RPMI + 10% Human AB serum – Sigma-Aldrich) at 0.25 x 10^6 cells per well and stimulated with 15 – 18nmers overlapping peptide pools (OLP) spanning the SARS-CoV-2 proteins of interest namely: S1, S2, M, NP, ORF3 and ORF8. 2µg/ml Phytohemagglutinin L (PHA-L, Sigma) and DMSO (0.2%) were used as positive and negative controls respectively. Cells were left to incubate for 7 days at 37°C with a media change on day 4. After 7 days, cells were harvested and stained with cocktail of anti-human CD3 – FITC (Biolegend), anti-human CD4 – APC (Biolegend), anti-human CD8 – PECy7 (Biolegend) and Live/dead Aqua (Invitrogen). Cells were then fixed in 4% paraformaldehyde (PFA - Sigma-Aldrich) and acquired on a MACSquant X (Miltenyi Biotec). Data was analysed on FlowJo v10 and all datapoints are presented as background subtracted data. Positivity threshold of 1% was established from historical data using mean responses in DMSO only wells + 3x standard deviation (SD) [1].

Mesoscale Discovery (MSD) binding assays

IgG responses to SARS-CoV-2 were measured using a multiplexed MSD immunoassay: The V-PLEX COVID-19 Coronavirus Panel 3 (IgG) Kit (cat. no. K15399U) from Meso Scale Diagnostics, Rockville, MD USA. To measure IgG antibodies, 96-well plates were blocked with MSD Blocker A solution for 30 minutes. Following washing with Wash Buffer, the diluted samples (1:500-1:10000 in diluent buffer) were added to wells for 2 hours at RT alongside with the reference standard and internal controls. After another wash, wells were incubated with the detection antibody (MSD SULFO-TAG™ Anti-Human IgG Antibody, 1:200 in diluent buffer) for 1 hour at RT. Following washing, the electrochemiluminescent signal was detected by adding MSD GOLD™ Read Buffer B and the plates were read using a MESO® SECTOR S 600 Reader.

Results:

22% vaccine-naive HD and transplant patients demonstrated PCR-positive re-infection (RI) at median 212 days (IQR 140-239) post 1st infection. Comparisons of T cell responses between convalescent ESRD and healthcare workers (HC) confirmed that ESRD patients were able to mount broad and robust SARS-CoV-2-specific cellular immune responses following primary infection. Strikingly, there were no differences in magnitude of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell responses mounted by HD and renal transplant patients who developed reinfection (RI) when compared to those who only developed a single infection (SI) during the same follow-up period. No boost in T cell responses was observed following reinfection episodes. In contrast, RI patients demonstrated poor SARS-CoV-2 IgG responses equivalent to pre-pandemic levels, prior to RI (median RI Spike: 187AU/ml, IQR 143-3432, p=0.96), unlike patients who developed single infection only (SI) (median SI Spike: 22826AU/ml, IQR 1255-63811, p<0.0001). IgG titres increased following RI vs. pre-pandemic (median RI Spike:

22611AU/ml, IQR 4488-75509, p=0.0006). Thus whilst IgG responses correlated with immune protection against re-infection with SARS-CoV-2, $CD4^+$ and $CD8^+$ T cell responses did not correlate with such protection.

Outputs: (publications/presentations)

This work has now been published in the Journal of the American Society of Nephrology (JASN) [2] (link included below).

https://jasn.asnjournals.org/content/33/5/883

Next Steps (what is it leading to)

This work led onto a much larger national collaboration to study SARS-CoV-2 vaccine responses in Haemodialysis patients, funded by Kidney Research UK. The findings were published in the Lancet [3] and also informed JCVI policy on prioritisation of booster vaccinations in vulnerable patient groups, in September 2021. We will now evaluate SARS-CoV-2-specific B cell populations in these immune-incompetent patients from samples collected during the OTF-funded project, to better understand the mechanisms behind such poor serological responses. We are extremely grateful to the Oxford Transplant Foundation for their support, without which this study would not have been possible.

References

1. Ogbe, A., et al., *T cell assays differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses.* Nat Commun, 2021. **12**(1): p. 2055.
2. Shankar, S., et al., *SARS-CoV-2-Specific T Cell Responses Are Not Associated with Protection against Reinfection in Hemodialysis Patients.* J Am Soc Nephrol, 2022. **33**(5): p. 883-887.
3. Carr, E.J., et al., *Neutralising antibodies after COVID-19 vaccination in UK haemodialysis patients.* Lancet, 2021. **398**(10305): p. 1038-1041.