Patients with ESKD are vulnerable to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Multiple studies report that patients on hemodialysis (HD) mount variable SARS-CoV-2–specific serologic and cellular responses after natural infection. There is increasing evidence that neutralizing antibody and anti-S1 IgG titers are correlates of protection. However, the relationship of cellular immunogenicity and protection has not been established. Because emerging variants of concern are reported to reduce vaccine efficacy, it will be critical to understand which immune responses are likely to predict protection within vulnerable patient cohorts. We observed high incidence of SARS-CoV-2 reinfection (RI) in patients on HD who were vaccine naive (25%, nine of 36 patients) and so we investigated whether cellular immune responses correlated with risk of RI in this cohort.

We prospectively recruited 36 patients on HD who were vaccine-naive and had SARS-CoV-2 infection within a single center in the United Kingdom. Details of recruitment, sample collection, and demographics are in Supplemental Appendixes 1 and 2, Supplemental Figure 1, and Supplemental Table 1. Primary infection was diagnosed by positive SARS-CoV-2 PCR or positive SARS-CoV-2 anti-nucleoprotein IgG (n=36). RI was diagnosed by positive SARS-CoV-2 PCR (n=9; Supplemental Table 2). Of the patients who were convalescent and vaccine naive, 25% developed PCR-confirmed RI within 47–264 days of first infection (median [interquartile range (IQR)], 206 [140–228] days; Supplemental Figure 2, Supplemental Table 2). Clinical descriptions and sequencing data defining the RI cohort are included in Supplemental Appendix 1 and Supplemental Tables 2 and 3. Proliferative SARS-CoV-2–specific T cell responses to 15–18mers overlapping peptide pools spanning SARS-CoV-2 proteins S1, S2, M, NP, ORF3, and ORF8 were assessed as previously described (Supplemental Appendix 1, Supplemental Figure 3). SARS-CoV-2–specific serologic responses were measured by multiplexed MSD immunoassays (Supplemental Appendix 1).

To first establish whether recruited patients on HD could mount SARS-CoV-2–specific T cell responses after natural infection, we used an assay that can detect such cellular responses in unvaccinated, younger healthcare workers (HC) infected with SARS-CoV-2 (Supplemental Table 4). To match the range of days after primary infection in the HD cohort, we used HC data from day 28 and day 182 after the first infection. However, comparisons between the HD and HC cohorts are limited by the lack of matching of age, disease severity, and duration of viral shedding. There were no differences in T cell responses to most SARS-CoV-2 proteins, S1, S2, M and ORF8 (CD8+), and S1, NP, and ORF8 (CD4+), between the two cohorts after the first infection. Notably, CD4+ and CD8+ T cell responses to ORF3 were decreased in the HD population when compared with the HC cohort. ORF3 has recently been shown to inhibit autophagic flux and has been suggested to be a method by which SARS-CoV-2 escapes degradation. The mechanistic relevance of this in the HD population merits further study. Our observations demonstrate patients on HD can mount detectable SARS-CoV-2 T cell responses to natural infection using this M.W.C. and P.K. contributed equally to this work.

Published online ahead of print. Publication date available at www.jasn.org.

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To determine if T cell responses correlated with protection against RI within the HD cohort, we compared the magnitude of SARS-CoV-2–specific CD4+ and CD8+ T cell responses after primary infection in the RI cohort versus patients on HD who had developed a single infection only during the same follow-up period (SI) (Figure 1, C–D, Supplemental Figures 4 and 5). Comparing the latest time point available before RI, there were no differences in T cell proliferative responses to any peptide pools tested between the RI and SI cohorts (Figure 1, C–D). Longitudinal analysis of individuals’ responses before and after RI further illustrated the lack of correlation of cellular responses with protection: two RI patients mounted robust and relatively broad T cell responses in primary infection, before RI episodes, and showed no evidence of waning responses over time (Supplemental Figure 6, ID-003 and ID-019); conversely, two different RI patients made very narrow or nonexistent T cell responses during primary infection (Supplemental Figure 6, ID-006 and ID-017). Nor was a boost in T cell responses observed within the RI cohort after the second infection (Figure 1, C–D).

In contrast, serologic analysis demonstrated that RI patients demonstrated poor SARS-CoV-2 IgG responses, equivalent to prepandemic levels, before RI (median [IQR] RI spike titer, 187 [143–3432] AU/ml; P=0.96), unlike SI patients (median [IQR] SI spike titer, 22,826 [1255–63,811] AU/ml; P<0.001). IgG titers increased after RI when compared with prepandemic sera of the same cohort (median [IQR] RI spike titer, 22,611 [4488–75,509] AU/ml; P=0.0006; Supplemental Appendix 1, Supplemental Figure 7). Examination of spike IgG titers relative to either SI-specific CD4+ or CD8+ T cell proliferation revealed no correlation of serologic and cellular responses across the SI or RI cohorts (Figure 1, E–F).

A comprehensive study of SARS-CoV-2 T cells in older, healthy subjects (>65 years) has demonstrated reduced coordination between CD4+ and CD8+ T cell responses and with serologic titers. Our observations support these early findings. Taken together, our data show that SARS-CoV-2–specific CD4+ or CD8+ T cell responses do not predict protection against RI in patients on HD. Consistent with other reports, serologic responses correlate with immune protection.

Limitations of this study include a small sample size and the lack of age-matched, healthy controls with similar disease severity. With such small patient numbers, it is difficult to confidently attribute statistical significance to these observations. However, to the best of our knowledge, this is the largest cohort study of T cell responses in patients with SARS-CoV-2 RI, irrespective of HD. Because study recruitment ranged between March and November 2020, and vaccines were administered from January 2021, the follow-up duration of convalescent patients who were vaccine naive was variable: RI rates may have been underestimated (Supplemental Figure 2). The risk of RI may also vary with the emergence of new variants. This reinforces the importance of continuing to study immune responses to breakthrough infection in vulnerable cohorts as they occur during the pandemic. Although this study has examined immune responses in a vaccine-naive cohort, the serologic findings after natural infection are consistent with those in vaccinated cohorts.

The correlates of immune protection are likely to be similar after exposure to antigen whether by infection or vaccination. However, this warrants further study in vaccinated HD populations.

Despite these constraints, our observations are the first to demonstrate the inability of CD4+ or CD8+ T cell responses to predict protection against future infection from SARS-CoV-2 in HD. Early reports of a rapidly emerging variant of concern describe an association with increased RI rates and reduced vaccine-induced neutralizing capacity of antibodies in healthy individuals. An appreciation of which immunologic responses correlate with protection from SARS-CoV-2 infection and severe disease, or indeed those which do not, will be crucial when assessing clinical efficacy of vaccination programs in vulnerable HD cohorts.

**DISCLOSURES**

E. Barnes reports having consultancy agreements with, and receiving research funding from, Roche and Vaccitech. E. Barnes and P. Klenerman report being National Institute for Health Research (NIHR) senior investigators. K. Bull reports having other interests in, or relationships with, the 100,000 Genomes Project (as member), Exeter College Oxford, Renal Genomics England Clinical Interpretation Partnership, Staines (as a research fellow), and the University of Oxford Medical Sciences Division Research Ethics Committee (as member); having consultancy agreements with DJS Antibodies Ltd.; receiving honoraria from Exeter College Oxford; and receiving research funding from Kidney Research UK, Medical Research Council, and Novo-Nordisk. P. Klenerman reports receiving research funding from Johnson and Johnson.

**Significance Statement**

Patients on hemodialysis (HD) are vulnerable to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and mount poor neutralizing antibody responses after two-dose vaccination. Although serologic responses have been associated with reduced rates of reinfection, the relationship between cellular immunogenicity and protection has not been established. We report, for the first time, high incidence of reinfection in patients on HD who are vaccine naive (25%), which identifies that T cell responses do not predict protection against reinfection. Instead, patients on HD who went on to become reinfected had mounted highly variable and sometimes robust proliferative T cell responses to a broad array of SARS-CoV-2 peptide pools during the primary infection. The understanding that SARS-CoV-2–specific T cell responses are not predictive of protection against future infection will be a critical issue when measuring clinical efficacy of vaccination in these vulnerable cohorts, particularly when facing rapidly emerging variants of concern.
**Figure 1. SARS-CoV-2–specific T cell responses are not associated with protection against RI in patients on HD.** (A and B) T cell proliferative responses were compared between HCs (two time points after initial primary infection: day 28 [d28] and d182) versus patients on HD with a single infection (33–153 days after infection; median [IQR], 67 [58–92] days). Each dot is an individual response and bar shows median with IQR; y axes are log transformed so 0% is not represented. (C and D) T cell responses were compared between patients on HD who become reinfected with SARS-CoV-2 (RI) with those with a single infection (SI). T cell responses after first infection (37–188 days after first infection; median [IQR], 108 [62–182] days) and after second infection (17–72 days after second infection; median [IQR], 30 [23–51] days) within the RI cohort were also compared. Each dot is an individual response and bar shows median with IQR; y axes are log transformed so 0% is not represented. (E and F) No correlation between T cell proliferative responses to S1 peptide pool and S1 IgG titers after primary infection. Each dot is an individual response. Red dots are RI patients (n=6), gray dots are SI patients (n=15). R and P values in black are for the whole HD cohort; R and P values in gray are for the SI cohort; R and P values in red are for the RI cohort. (A and B) Statistical analyses to perform unpaired comparisons across multiple groups were performed using the Kruskal–Wallis test with Dunn post-test for multiple comparisons (adjusted P values displayed). (C and D) Statistical analysis done using Mann–Whitney two-tailed t test. (E and F) Statistical analysis done using Spearman rank correlation, two-tailed test. ID, patients’ study identification number; M, membrane; NP, nucleocapsid; ORF3, open reading frame 3; ORF8, open reading frame 8; post 1st inf, after first infection in an RI patient; post 2nd inf, after second infection in an RI patient; Pt, patient; S1, spike 1; S2, spike 2.

and Pfizer Inc., and having consultancy agreements with UCB. A. Ogbe reports serving on a speakers bureau for Take-Two Interactive to speak on COVID-19. S. Shankar reports having the patents EP3619297A1 (PCT National Phase United States) filed, and serving as an NIHR clinical lecturer. A. Richter reports Research Funding: The Binding Site – fund joint PhD; and Speakers Bureau: CSL Behring, and Oxford Immunotec. L. Turtle reports Patents or Royalties: shareholder of intellectual property in a Zika vaccine developed by the University of Liverpool, University of Manchester and the UK Health Security Agency; and Advisory or Leadership Role: UK Health

JASN 33: 883–887, 2022  
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FUNDING
This work was supported by the Oxford Transplant Foundation grant HJR01740; the Oxfordshire Health Services Research Committee grant REF: 1392; and the UK Department of Health and Social Care, UK Research and Innovation (UKRI), and NIHR COVID-19 Rapid Response Grant (COV19-RECLPA). J. Becket is funded by the Wellcome Trust Institutional Strategic Support Fund grant ISSF0007267 and the NIHR Research Capability Fund grant RCF20/055. F. Isa is a Wellcome Trust Clinical Research Career Development Fellow funded by grant 211122/Z/18/Z. J. Hester is funded by the EU Horizon 2020 Research and Innovation Programme grant 825392, RESHAPE. T. Tipton, S. Longet, L. Turtle, and M.W. Carroll are supported by the US Food and Drug Administration Medical Countermeasures Initiative contract 75F40120C00085. P. Klenerman is funded by the NIHR grant WT109985MA. S.J. Dunachie is funded by an NIHR Global Research Professorship (NIHR300791). T. de Silva is funded by a Wellcome Trust Intermediate Clinical Fellowship 110058/Z/15/Z. R.P. Payne is funded by a Wellcome Trust Career Re-entry Fellowship 204721/Z/16/Z. C.J.A. Duncan is funded by a Wellcome Trust Clinical Research Career Development Fellowship 211153/Z/18/Z. L. Turtle is supported by the Wellcome Trust grant 205228/Z/16/Z. L. Turtle and P. Klenerman are supported by the NIHR Health Protection Research Unit in Emerging and Zoonotic Infections at the University of Liverpool, in partnership with Public Health England, in collaboration with Liverpool School of Tropical Medicine and the University of Oxford (grant NIHR200607).

AUTHOR CONTRIBUTIONS

SUPPLEMENTAL MATERIAL
This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2021121587/-/DCSupplemental.

Supplemental Table 1. Haemodialysis patient characteristics: Single versus re-infections.
Supplemental Table 2. Re-infection patient demographics.
Supplemental Table 3. Viral sequencing of SARS-CoV-2 in 1st infection.
Supplemental Table 4. Demographics for healthy healthcare workers (HC) infected with SARS-CoV-2 who are included in T cell analysis.
Supplemental Figure 1. Study flowchart describing haemodialysis (ICHHD) patient recruitment following diagnosis with SARS-CoV-2 infection by either positive nasopharyngeal swab PCR test or positive IgG nucleocapsid (NP) Abbott Architect test.
Supplemental Figure 2. SARS-CoV-2 PCR-positive reinfection-free survival in HD patients following a primary infection.
Supplemental Figure 3. Gating strategy showing how gates are set to determine the frequency of proliferative T cells.
Supplemental Figure 4. Summary of T cell proliferative responses from all samples within the reinfection and single infection HD cohorts presented as a heatmap.
Supplemental Figure 5. Summary of T cell proliferative responses from all samples within the reinfection and single infection HD cohorts following primary infection and before reinfection episodes, presented as scatter plots relative to timepoint of sampling.
Supplemental Figure 6. Magnitude of T cell responses across all available timepoints for 4 patients who became re-infected.
Supplemental Figure 7. S1 and RBD IgG titres in single infection and reinfection patients in comparison to pre-pandemic levels.

Supplemental Appendix 2. Supplemental References.

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