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Broad spectrum SARS-CoV-2-specific immunity in hospitalized First Nations peoples recovering from COVID-19

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Abstract

Indigenous peoples globally are at increased risk of COVID-19-associated morbidity and mortality. However, data that describe immune responses to SARS-CoV-2 infection in Indigenous populations are lacking. We evaluated immune responses in Australian First Nations peoples hospitalized with COVID-19. Our work comprehensively mapped out inflammatory, humoral and adaptive immune responses following SARS-CoV-2 infection. Patients were recruited early following the lifting of strict public health measures in the Northern Territory, Australia, between November 2021 and May 2022. Australian First Nations peoples recovering from COVID-19 showed increased levels of MCP-1 and IL-8 cytokines, IgG-antibodies against Delta-RBD and memory SARS-CoV-2-specific T cell responses prior to hospital discharge in comparison with hospital admission, with resolution of hyperactivated HLA-DR$^+$CD38$^+$ T cells. SARS-CoV-2 infection elicited coordinated ASC, Tfh and CD8$^+$ T cell responses in concert with CD4$^+$ T cell responses. Delta and Omicron RBD-IgG, as well as Ancestral N-IgG antibodies, strongly correlated with Ancestral RBD-IgG antibodies and Spike-specific memory B cells. We provide evidence of broad and robust immune responses following SARS-CoV-2 infection in Indigenous peoples, resembling those of non-Indigenous COVID-19 hospitalized patients.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the infectious agent that causes coronavirus disease 2019 (COVID-19), has led to a substantial health burden globally.[1] High-risk groups, including the elderly,[2] pregnant women,[3] individuals with underlying comorbidities[2] and Indigenous peoples globally[4] are more susceptible to SARS-CoV-2 infections and severe diseases. Higher COVID-19 cases and case fertility rates in Indigenous peoples have been reported in the USA and Peru (Ucayali region), but this was not the case for Australia, Canada and Ecuador during the early phase of the pandemic.[1] During 2020–2021, COVID-19 cases were very low in Australian First Nations peoples due to early and strict public health measures, interstate border closures and a "zero COVID-19 policy" in Australia. However, even after the lifting of COVID-19 restrictions, based on nationwide high COVID-19 vaccination targets (> 80–90% state-to-state), Australian First Nations peoples still have a COVID-19 mortality rate 1.6 times higher than non-Indigenous people.[5]

Socioeconomic factors can contribute to the high risk of infectious diseases in Indigenous populations.[6] Chronic conditions, including diabetes and renal diseases, which are highly prevalent in Indigenous populations,[7] increase the severity of viral infections.[2] However, it is not yet fully understood whether immunological factors associated with chronic comorbidities are playing a role in disease severity. Our recent study showed robust immune responses towards COVID-19 vaccination in Australian First Nations peoples, although reduced antibody responses were found in individuals with diabetes and renal diseases.[8] Similarly, previously we highlighted that Australian First Nations peoples can mount robust immune responses towards seasonal influenza vaccination.[9] Yet, Australian First Nations peoples are still disproportionately affected by severe influenza disease, as evidenced by the 2009 influenza pandemic and seasonal influenza epidemics.[10,11] Therefore, scant data exist on whether immunological factors contribute to disease susceptibility and severity towards SARS-CoV-2 infection or other respiratory viral infections in Indigenous peoples, but are urgently needed to inform rational strategical planning and to better protect Indigenous populations globally.

In this study, we recruited Australian First Nations peoples hospitalized with COVID-19 to assess their immune responses during acute illness and at the recovery phase. We performed in-depth immune analyses, including antibody responses towards the Ancestral SARS-CoV-2 strain and variants of concern strains, cytokines and chemokines, cellular activation, memory-Spike-specific B cell responses, activating Spike-specific T cell responses (activation-induced marker, AIM) and Spike-epitope-specific T cell responses *ex vivo* using peptide-human leukocyte antigen (p-HLA) class-I and class-II tetramers. Our study provides evidence for robust immune responses following SARS-CoV-2 infection in Indigenous peoples. During hospitalization and recovery of Australian First Nations COVID-19 patients, antibodies directed towards receptor-binding domain (RBD) and nucleocapsid (N), antibody-secreting cells (ASCs), T follicular helper (Tfh) cells, tetramer-specific T cells and peptide-stimulated AIM^+ T cell frequencies resembled those reported for non-Indigenous COVID-19 hospitalized patients.

RESULTS

LIFT cohort of Australian First Nations peoples hospitalized with COVID-19

To investigate immune responses towards SARS-CoV-2 infection in Indigenous peoples, we recruited 13 PCR-confirmed COVID-19 Australian First Nations peoples hospitalized at the Royal Darwin Hospital between 16 November 2021 and 13 May 2022 (Supplementary table 1). We collected blood samples at study enrolment (median 3 days post disease onset) and at (or just prior to) hospital discharge (median 11 days post disease onset) (Figure 1a). The median age was 53 years (range 33–73 years) and 31% of COVID-19 patients were female (Figure 1b). As the Northern Territory in Australia had a zero COVID-19 policy prior to opening its borders in December 2021, SARS-CoV-2 infections in our cohort reflected patients' first SARS-CoV-2 exposures, with Delta and Omicron variants circulating at the time. COVID-19 patients included nine ward patients (six requiring supplemental oxygen support) and four ICU patients, all requiring non-invasive oxygen support. Eleven patients (7/9 ward, 4/4 ICU) were previously vaccinated with the BNT162b2 COVID-19 vaccine with one ward patient receiving one dose, six receiving two doses (four ward, two ICU) and four receiving three doses (two ward, two ICU). Patients received a combination of treatments including antivirals ($n = 8$), monoclonal antibody (Sotrovimab, targeting the Spike protein; $n = 2$), antibiotics ($n = 3$), immunomodulators ($n = 13$) and/or bronchodilator ($n = 1$). No patients died in the study (Supplementary table 1). All patients had comorbidities with 10/13 exhibiting three or more (Figure 1c). 12/13 patients had diabetes and/or renal disease, which we have recently described to be associated with lower humoral immune responses following BNT162b2 vaccination.[8]

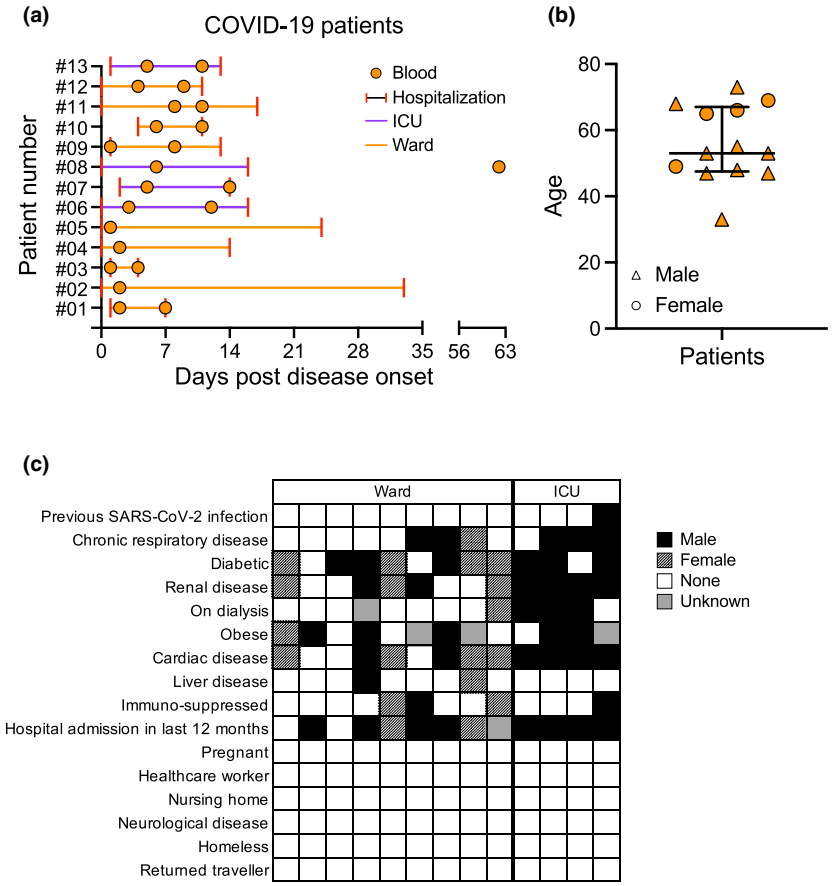

Figure 1. Australian First Nations COVID-19 patient cohort. **(a)** Timeline of 13 First Nations hospitalized COVID-19 patients. **(b)** Age and gender of COVID-19 patient cohort. **(c)** Comorbidities and risk factors of patients.

Cytokine and antibody responses to SARS-CoV-2 infection in Australian First Nations peoples

Heightened inflammatory responses such as IL-6 and IL-18 and an increase in IL-8 during hospitalization were observed in hospitalized severe COVID-19 patients prior to COVID-19 vaccination but not healthy, mildly acute or convalescent individuals.[12,13] Here, in the COVID-vaccination era, plasma cytokine and chemokine levels were fairly stable in Indigenous peoples at enrolment (V1) and discharge (V2), except for MCP-1 (mean 1596 pg mL^{-1}, range 102.7–12 039 pg mL^{-1}; involved in regulating migration and infiltration of monocytes/macrophages) and IL-8 (mean 2765 pg mL^{-1}, range 250.1–17 204 pg mL^{-1}; an interferon-gamma inducing proinflammatory cytokine), which increased by 14-fold and 6-fold at hospital discharge, respectively (Figure 2a, b). Indeed, these peak MCP-1 and IL-8 levels were 1.5–7.2-fold higher than what we have observed previously in our earlier hospitalized cohorts of unvaccinated non-Indigenous patients (mean MCP-1 level of 1047 and 222.3 pg mL^{-1}, mean IL-8 level of 1041 and 678.6 pg mL^{-1}).[12,13] Conversely, our patients had similar V2 but 0.2-fold V1 (mean 449.9 pg mL^{-1}) IL-8 levels compared with baseline levels of Indigenous and non-Indigenous people with chronic comorbidities (mean 2263 pg mL^{-1}),[8] perhaps due to the usage of IL-8 at acute infection.

IgG antibody levels directed at Ancestral RBD and Delta RBD were generally high at enrolment (V1) with $\log_{10}$ median titers of 3.623 and 3.612, respectively, with the majority of patients having antibodies above the seropositive cut-off lines (mean + 2SD of COVID-19 pre-exposed individuals),[14,15] 11/13 and 9/13, respectively (Figure 2c). Only antibodies against Delta RBD increased significantly at discharge ($P = 0.0371$), perhaps reflecting the most closely matched circulating strain. Omicron RBD antibody levels ($\log_{10}$ median titer of 3.237) were significantly lower than the Ancestral and Delta RBD levels at enrolment (V1) and discharge (V2)

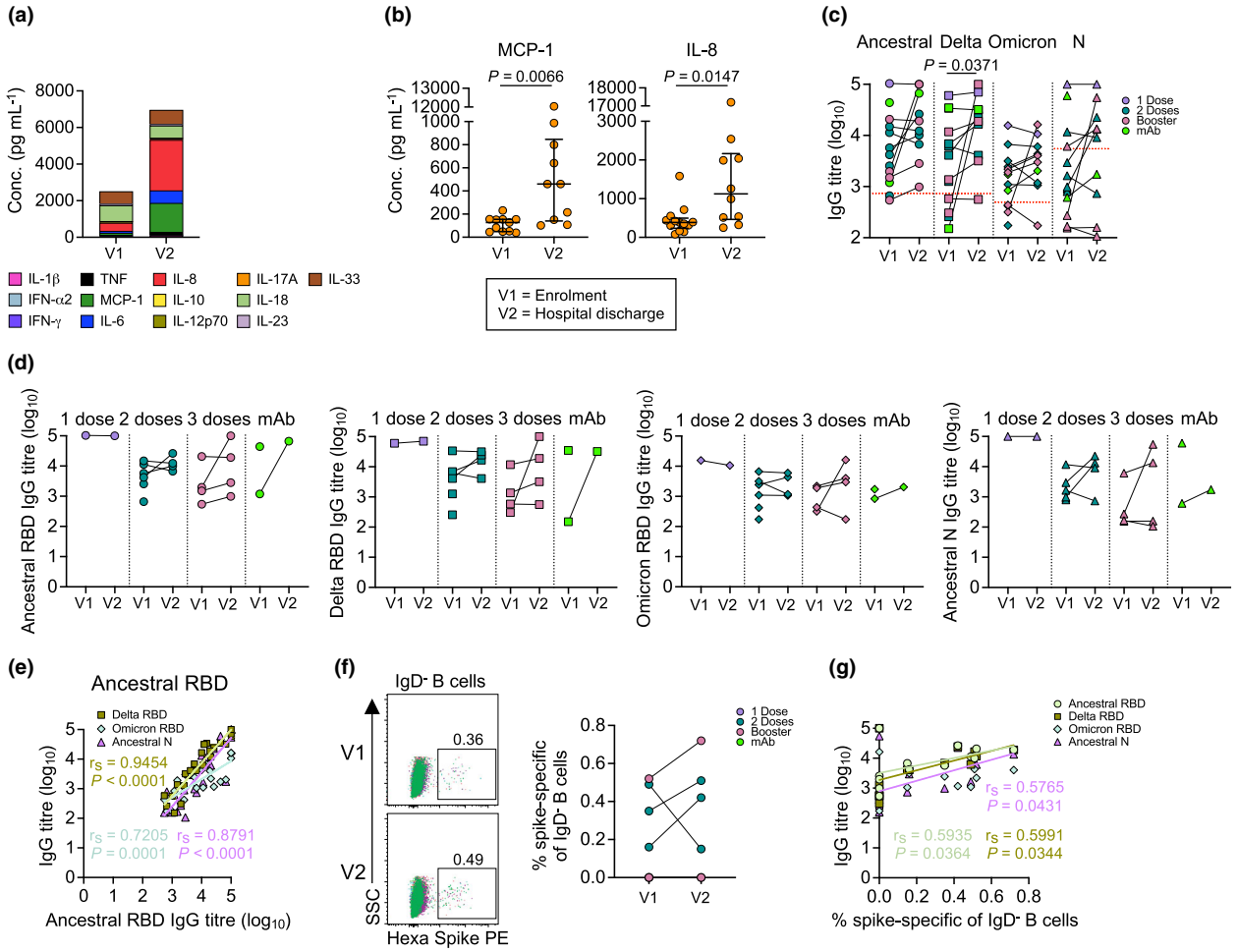

Figure 2. Cytokine and IgG responses towards RBD and N following SARS-CoV-2 infection in Australian First Nations peoples. **(a)** Concentration of 13 cytokines and chemokines at enrolment (V1) and hospital discharge (V2). **(b)** Concentration of MCP-1 and IL-8. Bars indicate median with interquartile range. **(c)** IgG titer against Ancestral, Delta and Omicron RBD and Ancestral N. Red horizontal dotted lines indicate seropositivity defined as mean + 2SD of unexposed/pre-vaccination samples.[14,15] **(d)** IgG titer against Ancestral, Delta and Omicron RBD and Ancestral N grouped by dose of COVID-19 vaccination or monoclonal antibody (mAb) treatment. **(e)** Correlation between Ancestral RBD IgG titer and Delta RBD, Omicron RBD and Ancestral N. **(f)** Concatenated representative FACS plots and frequency (%) of Spike-specific IgD$^-$ B cells. **(g)** Correlation between Spike-specific IgD$^-$ B cells and Ancestral, Delta and Omicron RBD and Ancestral N. Correlation was determined with Spearman's correlation. Statistical significance was determined with the two-tailed Wilcoxon test. Exact *P*-values are shown unless $P < 0.0001$.

($0.0007 < P < 0.0398$), but 9/13 had seropositive responses. IgG antibody levels against Ancestral N were more variable (V1 $\log_{10}$ median titer of 2.993), where only 4/13 showed seropositive antibody responses at enrolment (V1), increasing to 5/10 at discharge (Figure 2c).

Antibody responses were comparable when patients were stratified into having one dose, two doses, third dose booster or monoclonal antibody treatment (Sotrovimab) (Figure 2d). IgG titers specific for Ancestral RBD were highly correlated with IgG titers specific for Delta RBD, Omicron RBD and Ancestral N (Figure 2e). *Ex vivo* assessment of Spike probe-specific B cells revealed an increase in cell frequency from enrolment (V1) to discharge (V2) in 3/6 patients (Figure 2f; Supplementary figure 1a). Moreover, the frequency of Spike-specific B cells correlated positively with IgG antibody titers (Figure 2g).

Cellular responses in Australian First Nations peoples infected with SARS-CoV-2

In hospitalized patients with SARS-CoV-2 infection and other viral infectious diseases such as influenza, direct *ex vivo* whole blood flow cytometry analyses of immune responses and their activation profiles provided important insights into patients' recovery and disease

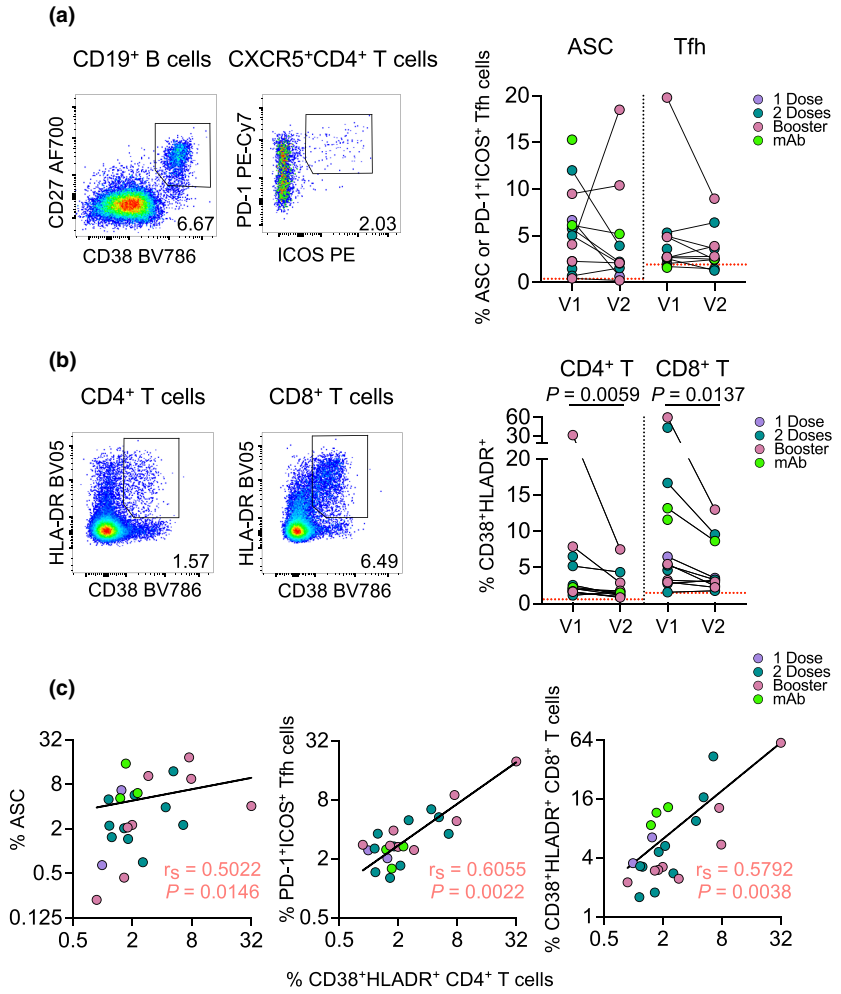

Figure 3. Prototypic cellular responses in Australian First Nations peoples infected with SARS-CoV-2. **(a)** Representative FACS plots and frequency (%) of $CD27^+CD38^+$ antibody-secreting cells (ASC) and activated $ICOS^+PD\text{-}1^+CXCR5^+CD4^+$ T follicular helper cells (Tfh). **(b)** Representative FACS plots and % of activated $CD38^+HLA\text{-}DR^+$ $CD4^+$ and $CD8^+$ T cells. Red horizontal dotted lines indicate healthy cutoff with the median of % activated cells from healthy individuals.[16] **(c)** Correlation between % activated $CD4^+$ T cells with % ASC, activated Tfh cells and activated $CD8^+$ T cells. Correlation was determined with Spearman's correlation. Statistical significance was determined with the two-tailed Wilcoxon test. Exact *P*-values are shown.

severity.[12,13,16,17] Our previous data in hospitalized non-Indigenous patients, who recovered from SARS-CoV-2 infection, demonstrated concomitant increases in ASC and activated Tfh responses, which both peaked during acute infection before declining to lower levels (~ < 1% of parental population) at convalescence.[13] We also observed transient activation of $CD4^+$ T cells, and more notably $CD8^+$ T cells, during acute SARS-CoV-2 infection, by co-expression of cell surface HLA-DR and CD38 markers.[13,16] However, prolonged expression of HLA-DR and CD38 can be detrimental and associated with increasing disease severity, potentially leading to fatal disease outcomes during SARS-CoV-2, influenza, Ebola and HIV.[16,18–23]

In First Nations COVID-19 patients, whole blood analysis of $CD27^{hi}CD38^{hi}$ ASCs and $PD\text{-}1^+ICOS^+CXCR5^+CD4^+$ activated Tfh cells showed high frequencies of both ASC (5.5%, 0.4–15.3%) and Tfh cells (4.4%, 1.6–19.8%), well above healthy control levels (median 0.39% for ASC and 1.92% for activated Tfh cells)[16] in 13/13 and 11/13 patients at enrolment (V1) respectively, which remained relatively stable during their hospital stay (Figure 3a; Supplementary figure 1b). Activated $CD38^+HLA\text{-}DR^+$ $CD4^+$ and $CD8^+$ T cells peaked at enrolment (V1) before decreasing at the time of hospital discharge (V2), indicating transient activation of T cells (Figure 3b). As per our previous studies in non-Indigenous COVID-19 patients,[13,16] activated

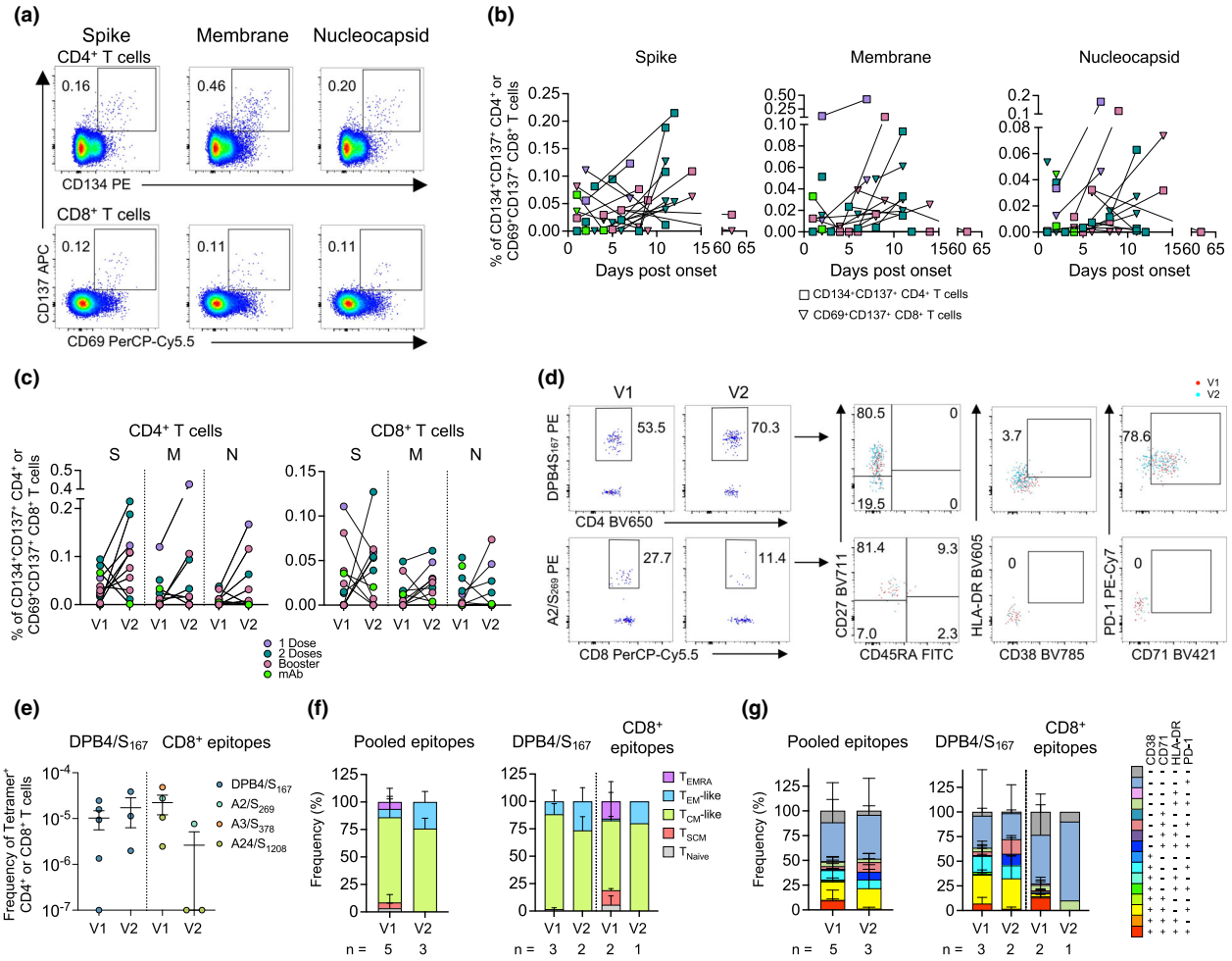

Figure 4. SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in COVID-19 Australian First Nations patients. **(a)** Representative FACS plots of Spike/membrane/nucleocapsid-specific $CD134^+CD137^+CD4^+$ and $CD69^+CD137^+CD8^+$ T cells. **(b)** Frequency (%) of Spike/membrane/nucleocapsid-specific $CD134^+CD137^+CD4^+$ and $CD69^+CD137^+CD8^+$ T cells *versus* days post disease onset or **(c)** at enrolment (V1) and discharge (V2). **(d)** Representative FACS plots of tetramer$^+$ $CD4^+$ and $CD8^+$ T cells and expression of phenotype and activation markers. **(e)** % of tetramer$^+$ $CD4^+$ and $CD8^+$ T cells. Bars indicate mean with SEM. **(f)** Phenotypes of tetramer$^+$ $CD4^+$ and $CD8^+$ T cells including $T_{Naïve}$ ($CD27^+CD45RA^+CD95^-$), $T_{Stem\ cell\text{-}like\ memory\ (SCM)}$ ($CD27^+CD45RA^+CD95^+$), $T_{CM\text{-}like}$ ($CD27^+CD45RA^-$), $T_{EM\text{-}like}$ ($CD27^-CD45RA^-$) and T_{EMRA} ($CD27^-CD45RA^+$) subsets. **(g)** Activation of tetramer$^+$ $CD4^+$ and $CD8^+$ T cells based on the activation markers CD38, CD71, HLA-DR and PD-1. Only samples with ≥ 10 tetramer$^+$-enriched events were included for phenotyping and activation analysis. Mean with SD is shown in stacked plots. Statistical significance was determined with the two-tailed Wilcoxon test.

$CD38^+HLA\text{-}DR^+CD4^+$ T cell responses correlated with activated $PD\text{-}1^+ICOS^+$ Tfh, ASC and $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cell responses (Figure 3c).

We further defined SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in COVID-19 Australian First Nations patients using the AIM assay and stimulation with overlapping peptide pools spanning Ancestral Spike, Membrane and Nucleocapsid (Figure 4a; Supplementary figure 1c). Measuring the expression of CD69, CD134 and CD137 activation markers, SARS-CoV-2-reactive $CD4^+$ and $CD8^+$ T cells directed at Spike, Membrane and Nucleocapsid generally increased during hospital stay (Figure 4b), although this was more apparent for AIM-specific $CD4^+$ T cells (Figure 4c).

To investigate SARS-CoV-2 epitope-specific $CD4^+$ and $CD8^+$ T cells, we performed direct *ex vivo* tetramer staining combined with tetramer-associated magnetic enrichment (TAME), which was directed at four known immunogenic SARS-CoV-2 epitopes: $DPB4/S_{167}$, $A2/S_{269}$, $A3/S_{378}$ and $A24/S_{1208}$ (Figure 4d; Supplementary figure 1d).[24–28] HLA-DPB4, -A2, -A3 and -A24 represented 61.5% (8/13), 7.7% (1/13), 7.7% (1/13) and

38.5% (5/13) of our cohort, respectively (Supplementary table 2), representing similar HLA frequencies to our previous Indigenous cohort.[29] Following SARS-CoV-2 infection, tetramer-specific $CD4^+$ and $CD8^+$ T cell responses were detected in 4/5 and 4/4 patients respectively and tetramer$^+$ frequencies ranged between 1.35×10^{-6} and 4.84×10^{-5} (Figure 4e), and remained stable between enrolment and discharge. Pooled mean frequencies of patients at enrolment (mean frequency 1.02×10^{-5} and 2.23×10^{-5} for DPB4/S_{167} and $CD8^+$ epitopes, respectively) were similar to those of Australian First Nations peoples after two doses of BNT162b2 vaccine (mean frequency 3.72×10^{-5} and 7.26×10^{-5} for DPB4/S_{167} and $CD8^+$ epitopes, respectively), while baseline (unvaccinated, pre-exposed) mean frequencies were on average 10-fold lower (1.08×10^{-6} and 5.29×10^{-6}) with more of a naive-like phenotype.[8] In agreement with previously published reports,[24,25,27,30] the majority of SARS-CoV-2-specific T cells were of central memory (T_{CM})-like phenotype (Figure 4f). Approximately 50% of SARS-CoV-2-specific T cells expressed at least two of the activation markers: CD38, CD71, HLA-DR and PD-1 (Figure 4g), similar to that observed in influenza-specific tetramer$^+$ T cell responses in hospitalized influenza virus-infected patients,[17] where co-expression of all four activation markers was more prominent at enrolment (V1) compared with discharge (V2).

Overall, our study demonstrated broad and concurrent anti-viral immune responses in Australian First Nations peoples hospitalized with SARS-CoV-2 infection.

DISCUSSION

Our study provides key insights into immune responses following SARS-CoV-2 infection in Indigenous peoples. It demonstrates that Australian First Nations peoples hospitalized with COVID-19, and predominantly vaccinated, elicited broad immunity, including RBD/N-antibodies towards the Ancestral, Delta and Omicron strains, ASCs, Tfh cells and tetramer-specific $CD4^+$/$CD8^+$ T cell responses.

Age, male gender and chronic conditions are independent and co-risk factors for contributing to COVID-19 disease severity,[2] as well as to other infectious viral diseases such as influenza.[31,32] This may explain why the majority of our First Nations hospitalized patients were middle-aged or older, male and with chronic conditions (8/13 or 61.5% having all three risk factors). Moreover, we have shown previously that individuals with diabetes and renal diseases have perturbed antibody responses towards COVID-19 vaccines.[8] Given that 12 out of 13 First Nations COVID-19 patients had diabetes and/or renal diseases, our hospitalized cohort may be more vulnerable than healthy individuals even after COVID-19 vaccination. Indeed, the patients had a median $\log_{10}$ titer of 3.623 against Ancestral RBD at enrolment (V1), which is lower than the median of 4.072 observed in First Nations individuals without comorbidities after the 2nd COVID-19 vaccine dose.[8]

Previous studies showed that severe COVID-19 was associated with hypercytokinemia and hyperactivation of innate and adaptive immune cells,[13,33–36] together with high titers of antibodies.[36] In the current study, an increase in levels of MCP-1 and IL-8 from enrolment (V1) to discharge (V2) was still observed, whereas other cytokines and chemokines remained stable. Prolonged co-expression of HLA-DR and CD38 on $CD8^+$ and $CD4^+$ T cells can be detrimental leading to disease outcomes, as was the case for a cohort of H7N9 influenza A virus-infected patients in China in 2013.[37] Co-expression of HLA-DR^+CD38^+ on T cells has been described previously to be largely contributed by non-specific bystander activation of T cells during acute influenza virus infection in a mouse model.[38] However, in our study, activated $CD38^+$HLA-DR^+ $CD4^+$ and $CD8^+$ T cells were transiently elevated at enrolment (V1), with levels significantly decreased prior to discharge (V2), most likely reflecting the patients' recovery. This is consistent with the robust and transient immune responses that precede the patients' recovery in mild to moderate cases[16,33,35,39] with prominent SARS-CoV-2-specific antibodies, B cell and $CD4^+$/$CD8^+$ T cell responses.[13,25,40–43]

In terms of SARS-CoV-2-specific T cell responses, AIM^+ $CD4^+$ and $CD8^+$ T cell responses were elevated in multiple patients during hospitalization. The increase of AIM^+ T cells is similar to those reported in non-Indigenous COVID-19 patients, which occurred gradually over time at early timepoints post disease onset.[43] A previous study also reported a negative correlation of AIM^+ T cells with viral loads, indicating viral clearance.[43] The frequency of tetramer-specific T cells was stable during hospitalization, which has been reported to be stable up to 9 months post SARS-CoV-2 infection for the immunodominant B7/N_{105} $CD8^+$ T cell epitope.[24] These T cells at enrolment were at frequencies comparable to Australian First Nations peoples after two COVID-19 vaccine doses and exhibited a T_{CM}-like phenotype.[8] Similar to our previous study in influenza-infected hospitalized patients,[17] epitope-specific $CD4^+$ and $CD8^+$ T cells mainly expressed CD38, CD71 and/or PD-1.

Our study has some limitations. We acknowledge that samples were collected from a small number of hospitalized patients. Thus, the results might not apply to patients who do not require hospitalization. However, given the very low case numbers in Darwin during the study recruitment (November 2021–May 2022), where

interstate borders had started to lift, we were still able to capture a small cohort of patients for immune analyses including those critically ill in ICU. As discussed earlier, the patients had several known risk factors for severe COVID-19, which might predispose them to hospitalization. Further studies are needed to investigate immune responses in Indigenous peoples infected with SARS-CoV-2 that cover broader demographical ranges.

Despite the limitations, in the current study, we performed broad immune analyses in Australian First Nations peoples hospitalized who had recovered from COVID-19. During hospitalization, Australian First Nations patients with COVID-19 elicited prototypical antibodies directed towards RBD and N, ASCs, activated Tfh cells, tetramer-specific $CD4^+$/$CD8^+$ T cells and peptide-stimulated AIM^+ T cells. As the majority of our COVID-19 patient cohort (85%) was vaccinated, we have not observed greatly exacerbated cytokine profiles (with the exception of increased IL-8 and MCP-1 levels) or hyperactivation of T cells depicted by HLA-DR and CD38 expression, both previously associated with severe COVID-19 in unvaccinated patients.

METHODS

LIFT study participants and specimens

We enrolled 13 Australian First Nations patients hospitalized with COVID-19 in Darwin between November 2021 and May 2022. Samples were taken at Visit 1 (V1, enrolment, $n = 13$) and Visit 2 (V2, hospital discharge, $n = 10$). Heparinized peripheral blood and serum were collected for isolating peripheral blood monocular cells (PBMCs), plasma, sera and granulocytes.[9] Bloods were shipped overnight from the Menzies School of Health Research to the University of Melbourne for immediate blood processing and whole blood analyses. Demographic, clinical and sampling information are described in Supplementary table 1. All donors consented to genetic analysis of HLA-I and HLA-II which was performed on genomic DNA isolated from granulocytes by the Victorian Transplant and Immunogenetics Service (Melbourne, Australia) and the results are shown in Supplementary table 2.

Ethics statement

Experiments conformed to the Declaration of Helsinki Principles and the Australian National Health and Medical Research Council Code of Practice. Written informed consent was obtained from all blood donors prior to the study. The study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (#2012-1928, LIFT) and the University of Melbourne Human Research Ethics Committees (#21864).

Cytokine analysis

Cytokines and chemokines including IL-1β, IFN-α2, IFN-γ, TNF, MCP-1 (CCL2), IL-6, IL-8 (CXCL8), IL-10, IL-12p70, IL-17A, IL-18, IL-23 and IL-33 were measured using the LEGENDplex™ Human Inflammation Panel 1 kit (BioLegend, San Diego, USA), according to manufacturer's instructions. Samples were acquired on a FACSCanto™ II cytometer (BD Biosciences, Franklin Lakes, USA) and analyzed with the online QOGNIT LEGENDplex™ program.

Antibodies, memory B cells and T-cell assays

IgG ELISAs for Ancestral, Delta and Omicron RBD, as well as Ancestral N were performed as described.[30,40] A volume of 200 µL of whole blood was used to measure ASCs ($CD3^-CD19^+CD27^{hi}CD38^{hi}$), activated Tfh cells ($CD3^+CD4^+CXCR5^+ICOS^+PD\text{-}1^+$) and activated $CD8^+$ and $CD4^+$ T cells ($HLA\text{-}DR^+CD38^+$), as described previously.[16] Memory Spike-specific B cell responses were measured on thawed PBMCs or TAME-flow through fractions using Ancestral Spike recombinant probes, essentially as described including full staining panel.[30] The SARS-CoV-2-specific AIM assay was performed essentially as described[15] using thawed PBMCs cultured at 37°C/5% CO_2 for 24 h with 10 µg mL^{-1} lots of Spike (181 peptides, 0.06 µg mL^{-1} per peptide), Membrane (31 peptides, 0.32 µg mL^{-1} per peptide) or Nucleocapsid (59 peptides, 0.17 µg mL^{-1} per peptide) peptide pools (all BEI Resources, Rockville, USA), or DMSO (Sigma-Aldrich, St Louis, USA) as a negative control. The full staining panel of antibodies has been described in detail.[15] *Ex vivo* TAME was performed, as essentially described,[15,30] on donors expressing matched HLA to the following p-HLA tetramers: $HLA\text{-}A*02:01/S_{269}$ (YLQPRTFLL), $HLA\text{-}A*03:01/S_{378}$ (KCYGVSPTK), $HLA\text{-}A*24:02/S_{1208}$ (QYIKWPWYI) and $HLA\text{-}DPA1*01:03/DPB1*04:01/S_{167}$ (TFEYVSQPFLMDLE). Monomers were generated by the Rossjohn Laboratory (Monash University, Melbourne, Australia) and conjugated to streptavidin-labeled PE or APC fluorochromes (both BD) to form tetramers. TAME was performed using anti-PE and/or anti-APC microbeads, LS columns and QuadroMACS™ Separator (all Miltenyi Biotec, Bergisch Gladbach, Germany) to enrich for $tetramer^+$ cells. Full antibody staining panel has been described in detail.[30] Enriched $tetramer^+$ cells below 10 were not analyzed by phenotype (CD45RA, CD27, CD95) or activation status (CD71, CD38, HLA-DR, PD-1). The cells were analyzed on LSRII Fortessa (BD), and FCS files were analyzed using FlowJo v10 software (BD). Flow cytometry gating strategies are shown in Supplementary figure 1.

Statistical analyses

Statistical significance was assessed using the two-tailed Wilcoxon signed-rank test and Spearman's correlation coefficient (r_s) in Prism v10 (GraphPad, Dotmatics, Boston, USA) unless stated otherwise.

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Wuji Zhang: Data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **E Bridie Clemens:** Data curation; investigation; project administration; writing – original draft. **Lukasz Kedzierski:** Data curation; formal analysis; investigation; methodology; writing – review and editing. **Brendon Y Chua:** Data curation; formal analysis; methodology; writing – review and editing. **Mark Mayo:** Investigation; methodology; supervision. **Claire Lonzi:** Data curation; writing – review and editing. **Alexandra Hinchcliff:** Data curation; formal analysis; writing – review and editing. **Vanessa Rigas:** Data curation; formal analysis; writing – review and editing. **Paula Binks:** Data curation; investigation; writing – review and editing. **Bianca F Middleton:** Data curation; formal analysis; writing – review and editing. **Louise C Rowntree:** Data curation; formal analysis; methodology; writing – review and editing. **Lily F Allen:** Data curation; formal analysis; methodology; writing – review and editing. **Hyon-Xhi Tan:** Data curation; formal analysis; methodology; writing – review and editing. **Jan Petersen:** Data curation; formal analysis; methodology; writing – review and editing. **Priyanka Chaurasia:** Data curation; formal analysis; methodology; writing – review and editing. **Florian Krammer:** Data curation; methodology; writing – review and editing. **Adam K Wheatley:** Data curation; methodology; writing – review and editing. **Stephen J Kent:** Data curation; resources; supervision; writing – review and editing. **Jamie Rossjohn:** Conceptualization; data curation; methodology; resources. **Adrian Miller:** Supervision; writing – original draft. **Sarah Lynar:** Data curation; investigation; writing – review and editing. **Jane Nelson:** Data curation; project administration; software. **Thi HO Nguyen:** Conceptualization; investigation; methodology. **Jane Davies:** Conceptualization; data curation; formal analysis; investigation; writing – review and editing. **Katherine Kedzierska:** Conceptualization; methodology; project administration; resources; supervision; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. All authors declare no other competing interests.

DATA AVAILABILITY STATEMENT

The published article includes all datasets generated or analyzed during the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.