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CMV Associated CD4+ TEMRA Cell Expansion and Vaccine Responsiveness in Older Nursing Home Residents

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Abstract

Older adults with advanced frailty exhibit strikingly heterogeneous immune responses. In 86 nursing home residents, clinical characteristics and viral exposure were not associated with longitudinal humoral responses to SARS-CoV-2 vaccination. Rather, expansion of immunosenescent CD4 + T_{EMRA} cells was robustly associated with poorer vaccine immunogenicity. Whilst CMV serostatus was not associated with vaccine response, it was strongly linked to CD4 + T_{EMRA} cell expansion, implicating CMV-associated immune remodelling in vaccine response heterogeneity.

MAIN TEXT

Both aging and frailty, a state of decreased physiological reserve in response to stressors, are characterised by marked inter-individual immune response heterogeneity. This is evident following vaccination, where substantial inter-individual variability in immunity exists, such as with influenza and SARS-CoV-2 vaccination.[1–3] Nursing Home Residents (NHRs), who typically have high levels of frailty and multimorbidity, are particularly vulnerable to infectious disease, yet remain under-represented in translational vaccinology/immunology studies, despite offering a unique opportunity to explore immune aging heterogeneity. Vaccination provides a controlled immune challenge through which the biological processes underlying immune-response variability in advanced age and frailty can be explored.[4]

Aging is accompanied by profound, but heterogeneous immune system changes. These include a low-grade, chronic inflammatory state (inflammaging), accumulation of terminally differentiated or senescent immune cell populations (immunosenescence), and the cumulative influence of lifelong infectious exposures (immunobiography).[5–7] These processes are interdependent. A canonical example is latent cytomegalovirus (CMV) infection, which can profoundly remodel the T-cell compartment and promote the accumulation of senescent T_{EMRA} populations.[8] However, the individual and collective influences of inflammaging, immunosenescence and immunobiography on vaccine responses in older adults with frailty remain poorly explored. For instance, whether CMV infection per se, or associated immune phenotypes, influences vaccine responsiveness is currently unclear.[9–11] Understanding these influences may provide important insights into biological determinants of immune aging and the significant inter-individual variation in vaccine responsiveness observed in later life. We therefore used a deeply phenotyped older NHR cohort, at the most extreme end of the age and frailty spectrum, to examine how clinical characteristics, inflammaging, immunosenescence and immunobiography contribute to heterogeneity in de novo vaccine responses in older adults.

We recruited 86 NHRs (81.1 ± 10.8 years; 65% female) from five nursing homes for assessment of SARS-CoV-2 vaccine-induced immune responses (Fig. 1A). Based on weekly PCR testing, serial anti-nucleocapsid antibody serology and clinical records, 45.4% (39/86) had evidence of prior SARS-CoV-2. There were no incident SARS-CoV-2 infections in the cohort during the study period. The cohort was characterised by high levels of frailty, multimorbidity, functional dependency and polypharmacy

(Supplementary Table S1). Anti-RBD IgG titres measured at baseline, 5 weeks and 6 months following Pfizer BNT162b2 mRNA vaccination demonstrated a classical boost-and-decay pattern (Fig. 1B). At 5 weeks 86.7% (72/83) of NHRs achieved titres above previously reported protective thresholds.[12] Despite this robust response, antibody titres varied markedly between NHRs (Fig. 1B). Notably, baseline clinical characteristics, including frailty, functional dependency, sarcopenia and major comorbidities, were not associated with antibody response at either 5 weeks or 6 months (Fig. 1C).

We next examined whether previous viral exposures contributed to this heterogeneity. Overall, 64% of the cohort were CMV seropositive, with higher or near universal exposure to Herpes Simplex Virus 1 (HSV1), Epstein-Barr Virus (EBV), influenza A and the seasonal coronaviruses NL63, 229E, HKU1 and OC43 (Fig. 1D). Neither seropositivity for common viruses nor baseline antibody seasonal coronavirus titres were associated with subsequent anti-RBD IgG titres at either timepoint (Fig. 1D). Thus, the striking heterogeneity in vaccine-induced immune response remained unexplained by either clinical characteristics or prior viral exposures.

We therefore examined whether this heterogeneity was related to baseline immune status differences. An unvaccinated, SARS-CoV-2 naive cohort of Midlife Healthy Controls (MHCs) provided a reference population for baseline assessment of immune status. Compared with MHCs, NHRs demonstrated marked elevations in circulating inflammatory and Senescence Associated Secretory Phenotype (SASP) mediators (Fig. 2A), together with marked differences in cellular immune composition. NHRs had higher CD8 + T_{EMRA}, CD8 + T_{CM}, T_{Reg} and significantly lower MAIT, NKT and γδ2 T cells (Fig. 2B). Despite these group level differences in immune markers of inflammaging and immunosenescence, substantial inter-individual heterogeneity was again evident across these markers within NHRs (Fig. 2C).

We then asked whether specific components of this heterogeneous inflammaging/immunosenescence phenotype were associated with subsequent humoral vaccine responses. Elastic-net regression with bootstrap stability selection was used to identify reproducible predictors from baseline immune cell populations and circulating inflammatory and immunosenescence-associated markers, accounting for baseline anti-RBD IgG titre and previous SARS-CoV-2 infection (Fig. 2D). At 5 weeks, only two features passed the pre-specified ≥ 80% stability threshold, namely greater γδ1 T cell abundance, associated with higher anti-RBD IgG titres, and greater CD4 + T_{EMRA} abundance, associated with lower titres. At 6-months, two soluble mediators exceeded the stability threshold, with higher YKL-40 associated with lower anti-RBD titres and higher MCP-1 associated with higher titres.

We next examined these predictors using multivariable linear regression adjusted for baseline anti-RBD titre and previous SARS-CoV-2. At 5 weeks both γδ1 T cells and CD4 + T_{EMRA} cells were significantly associated with vaccine-induced antibody titre (β: 0.12; 0.01, 0.24; p = 0.04 and β: -0.33; -0.59, -0.07; p = 0.01 respectively). Following adjustment for age, sex, BMI, frailty, only the association between greater CD4 + T_{EMRA} abundance and vaccine-induced immune response remained significant (β: -0.39; -0.66, -0.12; p = 0.01). Further adjustment for CMV serostatus strengthened this association (β: -0.54; -0.83, -0.24; p = 0.001), with little evidence of collinearity (VIFs ≤ 2.0). There was no statistically significant

evidence of interaction between CMV serostatus and CD4 + T_{EMRA} abundance, although power to detect interaction was limited. At 6-months, only the association between greater YKL-40 and lower anti-RBD titre persisted after covariate adjustment (β: -0.40; -0.68, -0.11; p = 0.007).

We next examined the determinants of CD4 + T_{EMRA} abundance in NHRs (Fig. 3A). Of the clinical factors and prior viral exposures examined, only CMV serostatus was associated with CD4 + T_{EMRA} abundance, with a marked CD4 + T_{EMRA} cell expansion in CMV-positive vs CMV-negative NHRs (median 2.36% [IQR:1.07–5.54%] vs 0.60% [IQR: 0.31–1.32%], respectively; Wilcoxon rank-sum p < 0.001; Fig. 3B). Further immunophenotypic examination demonstrated greater expression of KLRG-1 and CD57 with reduced CCR7 expression on CD4 + T_{EMRA} cells from CMV-positive NHRs (Fig. 3C), consistent with a terminally-differentiated, senescence-associated phenotype.

Thus, in this deeply-phenotyped cohort of older NHRs, substantial heterogeneity in vaccine responsiveness was not explained by clinical characteristics or previous viral exposures. NHRs exhibited marked features of immunosenescence and inflammaging. Most notably, greater baseline CD4 + T_{EMRA} cell abundance was associated with poorer early vaccine immunogenicity, whereas higher circulating YKL-40 was associated with lower antibody titres at six months. Our findings suggest that inter-individual heterogeneity in immune aging may be an important determinant of vaccine responsiveness that is not captured by conventional clinical measures of age, frailty, multimorbidity or infectious exposure history alone.

Our findings extend existing evidence linking age-associated T-cell differentiation with impaired vaccine responses. For instance, greater CD8 + T_{EMRA} cells expressing CD57 and KLRG-1 were associated with poorer responses to SARS-CoV-2 vaccination, although the study included relatively few older adults.[13] Similarly, in younger individuals with diabetes, greater CD4 + T_{EMRA} cells have been linked to poorer SARS-CoV-2 vaccine induced humoral immune responses.[14] In older adults specifically, a reduced vaccine-induced spike-specific CD4 + T-cell response is correlated with lower peak antibody responses to SARS-CoV-2 vaccination, underscoring the important role of CD4 + T cells in mounting protective vaccine responses in older adults.[15] Taken together, our findings support a role for age-associated T-cell compartment remodelling in de novo vaccine responses in older adults, with particular relevance in advanced age and frailty. Given the central role of CD4 + T cells in co-ordinating humoral immunity to SARS-CoV-2 vaccination[16], we provide evidence that senescent CD4 + T cell expansion may impair vaccine immunogenicity in NHRs.

The crucial driver of an expanded CD4 + T_{EMRA} population in the current study was CMV seropositivity. Associations between CMV serostatus and vaccine responsiveness have, however, been inconsistent.[11,17] In older adults, CMV-associated remodelling of the T-cell repertoire has been demonstrated without impairment of humoral or cellular responses to SARS-CoV-2 vaccination.[9] Consistent with this, CMV serostatus itself was not associated with vaccine responsiveness in our cohort. Rather, CMV was strongly associated with expansion of CD4 + T_{EMRA} cells exhibiting a terminally-

differentiated, senescent phenotype, while greater CD4 + T_{EMRA} abundance was associated with poorer vaccine immunogenicity. These findings suggest that heterogeneity in CMV-associated immune remodelling, rather than CMV exposure itself, may be more closely associated with vaccine responsiveness in older adults living with advanced frailty. More broadly, vaccination provides a controlled immune challenge through which to interrogate biological heterogeneity in the aging immune system, offering insight into why individuals of similar chronological age and frailty can exhibit markedly different immune responses. Whether the immune features identified here are also associated with responses to other vaccines or infectious challenges warrants consideration and further investigation.

Our findings should be interpreted as exploratory. Our study was a relatively small, single cohort without external validation and focused on humoral immune responses. It was not possible to assess clinical protection per se due to no incident infections during the study period, so clinical interpretation remains limited. Our study's strengths include the well-phenotyped nature of study participants, longitudinal follow-up and recruitment of those at the extreme end of the age and frailty spectrum – typically excluded from immunological and vaccinology research. Our findings add to growing evidence that chronological age and frailty alone incompletely capture the biological diversity of ageing. By identifying associations between CD4 + T-cell immunosenescence, CMV-associated immune remodelling, and vaccine responsiveness, this study highlights the potential value of immune-aging phenotypes as markers of immune resilience in later life.

METHODS

Study Design and Participants

NH-COVAIR (COVID-19 in Nursing Home Residents: Predicting Disease Severity, Outcomes and Vaccine-Induced Immune Responses) was a prospective observational longitudinal cohort study which included five separate nursing homes in Dublin, Ireland.[18] NHRs were recruited immediately before commencement of the national SARS-CoV-2 vaccination programme in January 2021. NHRs due to receive two doses of the Pfizer-BioNTech BNT162b2 mRNA vaccine were eligible for inclusion. Those receiving systemic immunosuppressive therapy/chemotherapy, or with systemic auto-immune disease, acute infection or active malignancy were excluded from participation. Individuals were classified as having prior SARS-CoV-2 based on review of clinical records, results of weekly NHR PCR testing conducted as part of national policy at the time of the study or based on a positive nucleoprotein result on the Roche Cobas Elecsys® anti-N assay (measured on all longitudinal serum samples).

Baseline clinical assessment and blood sampling was conducted prior to vaccination. Repeat blood sampling occurred at 5 weeks and 6 months following vaccination to assess vaccine immunogenicity and long-term response durability respectively. The primary outcome of interest in the current study was the 5 week timepoint – vaccine immunogenicity. Ethical approval was obtained from the Tallaght University Hospital-St James's Hospital Research Ethics Committee (20-NREC-COV-049). Written informed consent was obtained from participants or, where direct consent not possible due to cognitive

impairment, assent was obtained following discussion with family/carers in accordance with the study protocol. All protocols were conducted in accordance with the Declaration of Helsinki.

To enable comparison to a reference population of Midlife Healthy Controls (MHCs), samples were obtained from 25 healthy individuals (aged: 48 ± 15.5 years; 64% female) free from systemic illness and not on any regular medications recruited from phlebotomy clinics at St James's Hospital, Dublin as previously described and as approved by the Tallaght University Hospital-St James's Hospital Research Ethics Committee (JREC 2020-05 List 19).[19]

Clinical Assessment

A comprehensive geriatric assessment was performed on all participating NHRs at baseline. This included demographic characteristics, medical comorbidities, medication use and cognitive status through review of medical records. Multimorbidity was examined using individual diagnoses and total count of medical conditions. Medication exposure was also recorded. Frailty was assessed using the Clinical Frailty Scale as well as the nursing home specific FRAIL-NH scale.[20] Grip strength was assessed in a standardised fashion using a hand-held dynamometer. Functional dependency was assessed using the Barthel Index.

Blood Sampling and Processing

Thirty millilitres of peripheral blood was collected using standard aseptic technique at baseline before vaccination, at 5 weeks and 6 months following vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated from lithium-heparinised Vacuette® tubes by density-gradient centrifugation using Histopaque 1077 and Leucosep tubes, washed and cryopreserved in 10% dimethyl sulfoxide in fetal calf serum at -80^{o}C for future analysis. Plasma and serum were obtained from EDTA and serum clot activator tubes respectively, after centrifugation at 1,800 x g for 10 minutes and stored at -80^{o}C until analysis.

Assessment of Vaccine-Induced Humoral Immunity

Humoral responses to SARS-CoV-2 vaccination were assessed using serum collected at baseline, 5 weeks and 6 months. The V-PLEX assay from MesoScaleDiscovery (MSD) was used to quantify anti-Receptor Binding Domain (RBD) IgG antibodies using the MSD SARS-Cov-2 Panel 2 assay. This assay provides World Health Organization standardised Binding Antibody Units (BAU ml^{-1}). Assay protocols were followed exactly according to manufacturers' instructions with manufacturer-provided positive and negative pools included on each plate to ensure assay precision. All longitudinal samples from the same donor were included on the same plate run to eliminate inter-plate variability on longitudinal antibody titres. Positive and negative pools used in triplicate on each plate were used to assess intra and inter-

assay coefficient of variation, both of which were < 10%. A MESO QuickPlex SQ 120MM was used to read plates and results were analysed on the MSD Discovery Workbench (v4.0) software.

Assessment of Previous Viral Exposures

Seropositivity to common human herpesviruses and respiratory viruses was assessed using commercially-available qualitative ELISAs for nine common viruses. These included Herpes Simplex Virus 1 (HSV1; AssayGenie, HUFI08746), Herpes Simplex Virus 2 (HSV2; AssayGenie, HUFI08744), Varicella Zoster Virus (VZV; AssayGenie, HUFI08748), Epstein-Barr Virus (EBV; AssayGenie, HUFI04704), Cytomegalovirus (CMV; Abcam, ab108639), Human Herpesvirus 6A/B (HHV6; Abnova, KA1457), Influenza A (Abnova, KA6779), Influenza B (Abnova, KA6780), Respiratory Syncytial Virus (RSV; Abcam, ab108765) and Parainfluenza Virus (Abcam, ab108758). All assays were performed according to manufacturer's instructions. Optical density was measured using a plate reader with kit-specific positive, negative and cut-off controls used to classify participants as seropositive or seronegative for each virus.

Previous exposure to the endemic seasonal coronaviruses OC43, HKU1, 229E and NL63 was assessed using an in-house multiplex bead-based assay in the Infectious Diseases Epidemiology and Analytics Unit as previously described using a Luminex MagPix™ instrument.[21] Briefly, antigen coupled beads were used to quantify IgG responses to the spike antigens of each coronavirus. Median fluorescence intensity values were converted to relative antibody units (RAU) using standard curves generated from positive control donors and fitted using a five-parameter logistic regression. RAU values were subsequently natural log-transformed and standardised as z-scores for analysis, given that exposure was universal within the cohort.

Circulating Markers of Inflammaging and Immunosenescence

A panel of 45 markers of inflammaging and immunosenescence were derived based on prior literature demonstrating associations between biomarkers of inflammaging, immunosenescence and the SASP and important age-related health outcomes.[22] Based on availability and required sensitivity, analytes were assayed on either custom MSD S-PLEX/U-PLEX assays using an MSD QuickPlex 120MM plate reader or custom Luminex Discovery Assays (Bio-Techne; Catalog No: LXSAHM) analysed on a Luminex MagPix™ instrument as per manufacturers instructions. Twenty analytes were assayed using the MSD platform (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-18, IL-23, IL-33, TNF-α, G-CSF, TGF-β, IP-10, MCP-1, MIP-1β and YKL-40) and twenty-four on the Luminex platform (IL-8, IL-19, IL-21, IL-27, MIP-1α, MIP-3α, MIP-3β, MDC, Eotaxin, Eotaxin-3, MCP-2, TNFR1, Fas, MMP-1, MMP-7, SOST, OPN, RAGE, Angiopoietin-2, ADAMTS13, VCAM-1, ICAM-1, FGF-23 and GDF-15). Interferon-α was measured using a bespoke home-brew assay (BMS216C capture antibody; BMS216BK detection antibody; BMS305 calibrator; eBioscience) and Quanterix kits in Institut Pasteur Paris using a Simoa instrument as

previously described.[23] Notably, MHC comparator measurement were available only for the MSD analytes.

Spectral Flow Cytometry

Baseline cryopreserved PBMCs were analysed using high-parameter spectral flow cytometry. Briefly, PBMCs were thawed, washed and stained using a fluorochrome-conjugated panel optimised for overnight staining.[24] A 25-marker panel was designed to characterise conventional CD4 + and CD8 + T-cell subsets, regulatory T cells, natural killer cell populations, mucosal-associated invariant T (MAIT) cells, invariant Natural Killer T (iNKT) cells and γδ T-cell populations, together with markers of T-cell differentiation, activation, exhaustion and senescence. The panel included Vα24Jα18, Vδ1, Vδ2, Vα7.2, CD3, CD4, CD8, CD25, CD27, CD28, CD45, CD45RA, CD56, CD57, CD69, CD127, CD137, CD154, CD161, CCR7, KLRG-1, TIGIT, NKG2D, Ki67 and FoxP3 (Fluorophores, suppliers and catalogue numbers are supplied in Supplementary Table 2). Cryopreserved PBMCs were rapidly thawed at 37^{o}C, washed in complete IMDM supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin, and rested for 1 hour at 37^{o}C before staining. Samples were stained overnight with cell surface stains at 4^{o}C, after which they were washed and fixed using the eBioscience Ltd. FoxP3/Transcription Factor Staining Buffer Set. Cells were subsequently washed in permeabilization buffer and stained intracellularly (Ki67 and FoxP3) for 25 minutes in the dark. Cells were resuspended and analysed immediately on a Cytek Aurora™ spectral flow cytometer. Daily instrument quality control was performed using Cytek calibration beads, with spectral unmixing undertaken using SpectroFlo™ software. Raw .fcs files were subsequently analysed in FlowJo using a pre-defined manual gating strategy to identify 20 immune-cell populations of interest (gating strategy is provided in Supplementary Fig. 3).

Statistical Analysis

For descriptive statistics, mean ± standard deviation (SD) or median and interquartile range (IQR) were used as appropriate, and categorical variables were described as frequencies and percentages. For between group differences, t-tests and Wilcoxon rank-sum tests were used as appropriate. Antibody titres (anti-RBD) at all timepoints exhibited strong right-skew and so were natural-log transformed for all analyses.

In the first instance, associations between clinical variables and viral exposure history were examined using univariate linear regression, with log-transformed antibody titre at 5 weeks or 6 months as the dependent variable, adjusted for prior SARS-CoV-2 and baseline log-transformed antibody titre. VZV was not included in this analysis as seroprevalence was 100%. For the seasonal coronaviruses, as exposure was universal, total IgG titre in RAU was natural log-transformed and z-scored to examine the potential contribution of exposure burden to vaccine induced antibody responses.

Baseline differences in circulating immune-cell populations and soluble inflammatory markers between NHRs and MHCs were assessed using Wilcoxon rank-sum tests. Multiple comparisons were controlled using the Benjamini–Yekutieli false-discovery rate procedure, with adjusted $q < 0.05$ considered statistically significant.

To identify reproducible baseline immune features associated with subsequent vaccine response, elastic-net penalised regression was performed separately for natural-log transformed 5 week and 6-month antibody titres using the glmnet package in R. Prior to analysis, all 65 baseline immune features (20 cell populations and 45 circulating inflammatory markers) were natural-log transformed and z-scored as most exhibited strong right skew. These 65 candidate predictors were included as penalised and baseline anti-RBD IgG titre and previous SARS-CoV-2 infection as unpenalized and therefore retained in all models, given the profound impact of prior SARS-CoV-2 on vaccine-induced immune responses. Elastic net models were fitted using $\alpha = 0.5$, the penalty parameter (λ) was selected using 10-fold cross-validation based on mean squared error, with coefficients producing the minimum cross-validated error (lambda.min) used for feature selection. Participant-level bootstrap stability was used to assess robustness of feature selection. For each outcome, 1,000 bootstrap samples were generated by sampling participants with replacement. Within each sample, 10-fold cross validation was repeated and immune features with non-zero coefficients at lambda.min recorded as selected. A pre-specified threshold of ≥ 80% was considered evidence of stable feature selection.

Immune features exceeding the ≥ 80% stability threshold were subsequently examined using multivariate linear regression. Baseline models adjusted for baseline natural-log transformed anti-RBD IgG titre and previous SARS-CoV-2, with further models adjusted for age, sex, body mass index and frailty. For CD4 + TEMRA analysis, CMV serostatus was added to assess whether the assocaition with vaccine response was independent of CMV exposure. An interaction term between CD4 + TEMRA and CMV was used to assess potential interactions. Residual vs fit and Q-q plots were examined alongside Variance Inflation Factors (VIFs) to assess for multicollinearity. Missing data were omitted without replacement. For analysis of predictors of CD4 + TEMRA populations and flow cytometry markers of immune cell activation, proliferation and senescence in CD4 + TEMRA cels, multiple testing was applied using the Benjamini–Yekutieli false-discovery rate procedure, correcting for the total number of comparisons.

All statistical tests were two-sided, with $p < 0.05$ considered statistically significant. All analyses were conducted using R (version 4.6) and the packages used included tidyverse (including dplyr, tidyr, tibble, purrr, stringr, forcats and ggplot2), broom, glmnet, patchwork, ComplexHeatmap, circlize and e1071.

Declarations

DATA AVAILABILITY

Due to risk of participant de-identification from data in the NH-COVAIR study, full data are not publically available. Anonymised data are available from the corresponding author (dyera@tcd.ie) on reasonable request.

CODE AVAILABILITY

The R scripts used for data analysis and figure generation are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

AHD: Conceptualisation, Methodology, Formal Analysis, Investigation, Writing-Original Draft, Writing – Review & Editing, Project Administration; **DMM**: Conceptualisation, Writing – Review & Editing; **CR**: Investigation; Writing – Review & Editing; **IB**: Investigation; Writing – Review & Editing; **CN**: Project Administration; Investigation; Writing – Review & Editing; **LG**: Investigation; Writing – Review & Editing; **SP**: Investigation; Writing – Review & Editing; **FD**: Investigation; Writing – Review & Editing; **HD**: Investigation; Writing – Review & Editing; **HCH**: Investigation; Writing – Review & Editing; **OW**: Investigation; Writing – Review & Editing; **VB**: Investigation; Writing – Review & Editing; **JMF**: Conceptualisation, Writing – Review & Editing; **BM**: Conceptualisation, Writing – Review & Editing;**PGF**: Conceptualisation, Writing – Review & Editing; **DD**: Conceptualisation, Writing – Review & Editing; **MTW**: Conceptualisation, Writing – Review & Editing; **COF**: Conceptualisation, Writing – Review & Editing; **SPK:** Supervision, Conceptualisation, Writing – Review & Editing; **NMB**: Supervision, Conceptualisation, Writing – Review & Editing

COMPETING INTERESTS

The authors have no conflicts of interest to declare.

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Figures

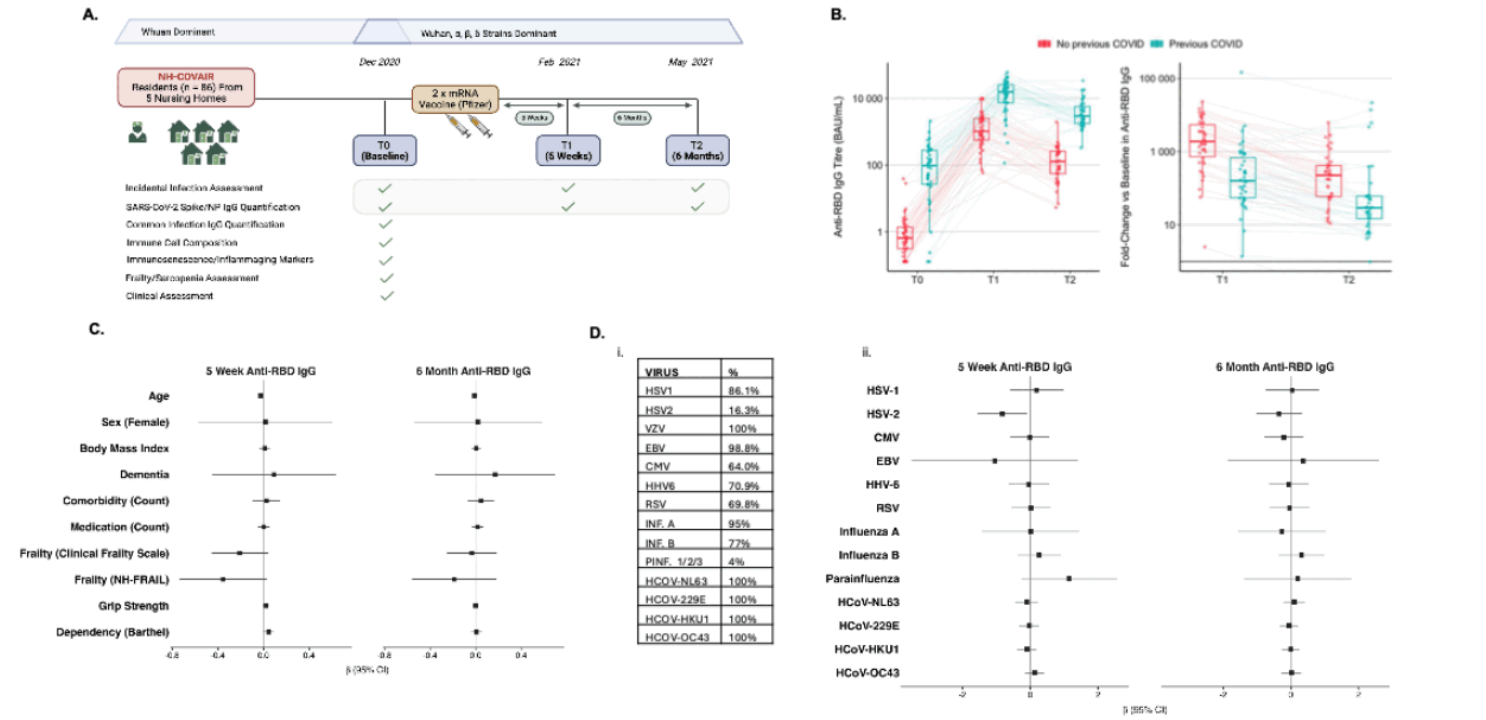


Figure 1

Baseline Clinical Characteristics and Prior Exposure to Common Viruses Were Not Associated with Vaccine-Induced Humoral Immune Responses in Older Nursing Home Residents. (A) Overview of the NH-COVAIR study and study design. Eighty-six older nursing home residents were assessed at baseline before two doses of the Pfizer mRNA SARS-CoV-2 vaccine with repeat assessment at 5 weeks and 6 months. Clinical characteristics including assessment of frailty and sarcopenia, immune cell composition, markers of immunosenescence and inflammaging, common viral exposures, and SARS-CoV-2 serology were assessed at baseline. (**B)**Anti-receptor binding domain (RBD) IgG titres were measured at baseline, 5 weeks, and 6 months following vaccination, stratified by previous SARS-CoV-2 infection. Left: absolute anti-RBD IgG titres; right: fold change relative to baseline. Lines connect repeated measurements from individual participants. (**C)**No associations were observed between baseline clinical characteristics and anti-RBD IgG titres either 5 weeks or 6 months. Square points represent β coefficients and horizontal lines the corresponding 95% CIs from separate linear regression models adjusted for baseline ln-transformed anti-RBD IgG titre and previous SARS-CoV-2 infection with ln-transformed anti-RBD titre as the dependent variable. (**D)**Immunobiography and vaccine responsiveness. **D (i)** Baseline seroprevalence of common viral infections in the cohort. **D (ii)** Associations between baseline immunobiography (seropositivity/z-scored titre for the four seasonal coronaviruses) and anti-RBD IgG titres at 5 weeks and 6 months. Points represent β coefficients and horizontal lines represent 95% CIs from separate linear regression models adjusted for ln-transformed baseline anti-RBD IgG titre and previous SARS-CoV-2 infection. For seasonal human coronaviruses, z-scored natural log-transformed baseline seasonal coronavirus antibody titres were analysed because seropositivity was near universal. **Abbreviations:**CMV: cytomegalovirus; EBV: Epstein-Barr virus; HCoV:

human coronavirus; HHV-6: human herpesvirus 6; HSV: herpes simplex virus; IgG: immunoglobulin G; RBD: receptor-binding domain; RSV: respiratory syncytial virus; VZV: varicella-zoster virus.

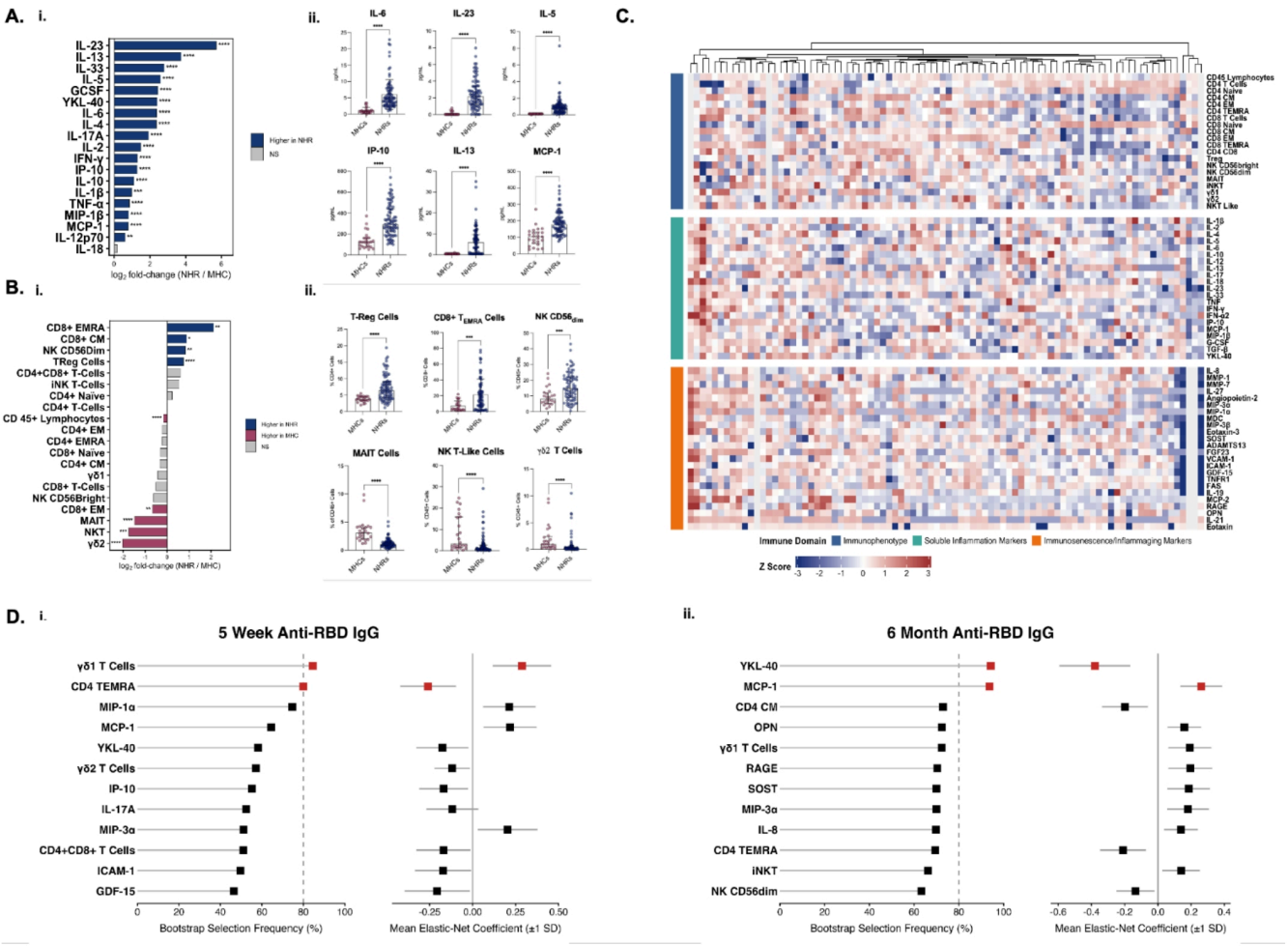


Figure 2

Circulating Inflammatory Markers and Immune Cell Populations in Older Nursing Home Residents and Association with Vaccine-Induced Humoral Immune Response . (A) Baseline soluble inflammatory profile in Nursing Home Residents (NHRs) compared with Midlife Healthy Controls (MHCs). **A (i)** Differences in circulating cytokine and inflammatory marker concentrations between NHRs and MHCs, expressed as $\log_2$ fold-change (NHR/MHC). **A (ii)** Representative distributions of selected inflammatory markers demonstrating prominent differences between groups. **(B)** Baseline cellular immune phenotype in NHRs compared with MHCs. **B (i)** Differences in immune cell populations expressed as $\log_2$ fold-change (NHR/MHC). **B (ii)** Representative distributions of selected cellular populations, including T-regulatory cells, CD8+ T_{EMRA} cells, $CD56_{dim}$ natural killer cells, MAIT cells, natural killer T-like cells and γδ2 T cells. In A(i) and B(i), coloured bars indicate significantly higher values in NHRs or MHCs and grey bars indicate non-significant differences. **(C)** Integrated baseline immune landscape of NHRs. Heatmap

displays z-standardised immune features across individual participants, grouped into immunophenotype, soluble inflammatory, and immunosenescence/inflammaging markers. Participants were hierarchically clustered according to their baseline immune profiles; white cells indicate missing measurements. **(D)** Elastic-net regression with bootstrap stability selection was used to identify baseline immune features associated with subsequent vaccine-induced humoral responses. Models incorporated the immune cell populations and soluble markers shown in C and were adjusted for baseline ln-transformed anti-RBD IgG titre and previous SARS-CoV-2 infection. The dependent variable was ln-transformed anti-RBD IgG titre at **D (i)** 5 weeks and **D (ii)** 6 months following vaccination. The 12 features with the highest bootstrap selection frequencies at each timepoint are displayed. Left panels show bootstrap selection frequency, with the dashed line denoting the prespecified 80% stability threshold; red squares identify features selected in ≥80% of bootstrap iterations. Right panels show mean elastic-net coefficients among bootstrap iterations in which each feature was selected, with horizontal lines representing ±1 SD. Continuous immune predictors were transformed where appropriate and z-standardised before modelling. **Abbreviations:** CM, central memory; IgG, immunoglobulin G; MAIT, mucosal-associated invariant T; NHR, nursing home resident; NKT: Natural Killer T-Like; MHC, Midlife Healthy Control; RBD, receptor-binding domain; TEMRA, terminally differentiated effector memory T cells re-expressing CD45RA. *p<0.05, **p<0.01, ***p<0.001

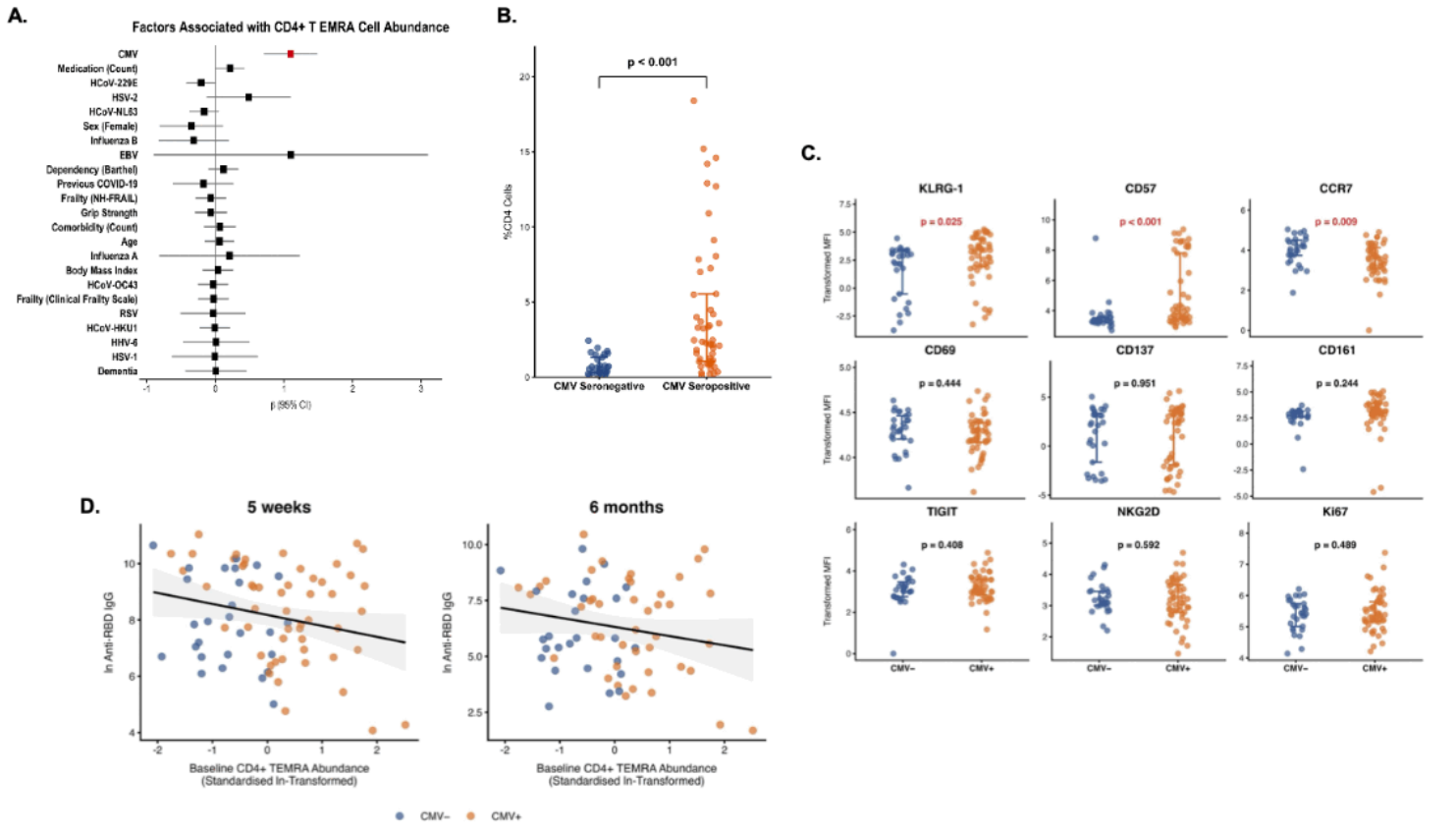


Figure 3

CMV-Associated Senescent CD4+ TEMRA Cell Expansion is Associated with Vaccine-Induced Humoral Immune Response in Nursing Home Residents. A. Factors associated with baseline CD4+ terminally differentiated effector memory T-cell re-expressing CD45RA (TEMRA) abundance. Points represent standardised β coefficients from regression models with 95% confidence intervals (CI). Variables are ordered by statistical significance; CMV is highlighted in red (the only significant association). **B.**Baseline CD4+ TEMRA cell abundance according to CMV serostatus. Points represent individual participants; horizontal lines and error bars indicate median and interquartile range**.**Wilcoxon rank-sum test. **C.**Phenotypic characterisation of CD4+ TEMRA cells according to CMV serostatus. Expression of KLRG-1, CD57, CCR7, CD69, CD137, CD161, TIGIT, NKG2D and Ki67 is presented as transformed median fluorescence intensity (MFI), using an inverse hyperbolic sine transformation with cofactor 150 [arcsinh(MFI/150)]. Points represent individual participants; horizontal lines and error bars indicate median and IQR. Wilcoxon rank-sum test. **d**, Association between baseline CD4+ TEMRA abundance and vaccine-induced anti-receptor-binding domain (RBD) IgG responses at 5 weeks and 6 months. CD4+ TEMRA abundance was natural-log transformed and standardised, Anti-RBD IgG titres were natural-log transformed. Points represent individual participants, coloured according to CMV serostatus (blue, CMV seronegative; orange, CMV seropositive). Black lines show the fitted association with shaded 95% confidence intervals. Associations were examined using multivariable linear regression adjusted for baseline anti-RBD IgG titre and previous COVID-19. **Abbreviations:**CI, confidence interval; CMV, cytomegalovirus; MFI, median fluorescence intensity; RBD, receptor-binding domain; TEMRA, terminally differentiated effector memory T cell re-expressing CD45RA.

Supplementary Files

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