Wanjiku *et al. BMC Infectious Diseases* (2026) 26:174
https://doi.org/10.1186/s12879-026-12582-6

BMC Infectious Diseases

RESEARCH

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Pre-COVID-19 ex vivo cross-reactive IFN-γ cellular response to SARS-CoV-2 spike overlapping peptides is more prevalent among Kenyan compared to Swedish adults

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Abstract

Background Global WHO data indicate that Sub-Saharan African (SSA) countries, such as Kenya, experienced reduced coronavirus disease 2019 (COVID-19) severe-morbidity and mortality burdens relative to their more affluent counterparts in Europe, Asia, and North America.

Methods We analysed peripheral blood mononuclear cells (PBMC) samples collected from Kenya and Sweden before and during COVID-19. Pre-COVID-19 samples were available for 80 adults and 10 infants from Kenya, and 20 adults from Sweden. COVID-19 samples were available for 39 Kenyan adults. The samples were analysed for ex vivo IFN-γ secretion using an Enzyme-Linked Immunosorbent (ELISpot) assay following in vitro stimulations with overlapping SARS-CoV-2 spike-protein peptides. T-cells expressing IFN-γ, IL-2, TNF-α, CD154, and CD107a were assessed following similar stimulations, using intracellular cytokine staining (ICS) and multiparameter flow cytometry.

Results 55.7% of the Kenyan pre-COVID-19 adult samples were classified as responders by ELISPOT responses to spike-protein peptides, compared with 28% of Swedish pre-COVID-19 adult sample ($p = 0.04$). The frequencies for SARS-CoV-2 spike-specific TNF-α CD4+, TNF-α CD8 + and IFN-γ CD8 + T-cell responses, tended to be higher in the Kenyan adults although these differences did not reach statistical significance.

Conclusion Pre-COVID-19 T-cell responses could contribute to lower morbidity and mortality associated with SARS-CoV-2 infections in SSA relative to Europe, Asia, and North America.

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Keywords Cross-reactive responses, Pre-pandemic, IFN-γ-secreting cells, T-cells, COVID-19, SARS-CoV-2, Sub-Saharan Africa

Background

Most SARS-CoV-2 infections result in either asymptomatic or mild disease, but a small proportion of individuals develop severe COVID-19 requiring hospitalisation, and some of these cases result in death [1]. Compared to North America, Asia and Europe, SSA experienced relatively fewer severe cases and fatalities despite many reports of similar SARS-CoV-2-infection rates [2]. This paradoxical observation has puzzled many public health experts [3–5]. The suggested hypotheses for the reduced burden of severe and fatal COVID-19 in SSA include potential underreporting of cases and deaths, a relatively younger population, warmer climatic conditions, and immune regulation induced from either BCG vaccinations [6–8] or previous exposures to highly inflammatory diseases like malaria [9], or cross protection from a higher level of exposure to other coronaviruses [10–16].

There is evidence for cellular and humoral cross-reactivity to SARS-CoV-2 [10–16], and the role for pre-existing hCoV reactive CD4 + T-cell responses in providing SARS-CoV-2 cross protection [17]. A high incidence of exposures to hCoVs and other pathogens could have induced pre-COVID-19 cross protective T-cell responses, or could have downregulated inflammation after infections of SSA residents with SARS-CoV-2 [11–16]. SARS-CoV-2 cross neutralising antibodies are undetectable, even in recent confirmed cases of hCoV [18]. However, antigen-specific memory T-cells are more durable and frequently cross-reactive, and may play a key role in the clearance of SARS-CoV-2 infected cells [13, 19, 20]. Recent studies, predominantly conducted in high income countries (HICs), have reported the presence of pre-COVID-19 cross-reactive T-cell immune responses to SARS-CoV-2 with endemic hCoVs [11–15, 20]. Studies from SSA countries like Uganda [21, 22], Senegal [23], and South Africa [24] have provided some evidence regarding pre-COVID-19 cross-reactive T-cell to SARS-CoV-2 in adults.

This study examined ex vivo pre-COVID-19 IFN-γ cellular responses using ELISpot assay and the frequency of functional T-cells, expressing either IFN-γ, IL-2, TNF-α, CD154, and CD107a by multiparametric flow cytometry following in vitro stimulation with overlapping SARS-CoV-2 spike peptides. Importantly, we compared results from PBMC samples collected before 2019 from Kenyan and Swedish adults, undertaking standardised assays as a single batch in the same laboratory in Kenya. We further included additional control samples from 10 Kenyan infants (< 1 year) and 39 adults COVID-19 patients.

Methodology

Study design and participants

Kenyan pre-COVID-19 blood samples were collected from 80 adults and 10 infants aged below 1 year during an annual malaria surveillance cross-sectional survey conducted in March 2018 in Kilifi, Kenya. The Swedish pre-COVID-19 blood samples had been collected from 20 healthy adult volunteers living in Sweden between March 2012 and June 2019. At the time of pre-COVID-19 sampling, all the individuals had no symptomatic or acute infection. Post-COVID-19 positive control samples were collected from 39 Kenyan adults for studies investigating the kinetics of the immune response to SARS-CoV-2 in COVID-19 patients in Kilifi and Nairobi between 1st August 2020 and 15th February 2022. Sampling was conducted between the day of diagnosis and day 180 following infection. The study participants were COVID-19 vaccine–naïve, as COVID-19 vaccines were not yet available in Kenya during the sampling period. Blood was separated into plasma and PBMCs and stored at -80 °C and liquid nitrogen, respectively, until used for analyses.

Overlapping SARS-CoV-2 spike peptides and in vitro stimulants for PBMC in the elispot assay

Pools of peptides covering the spike region 1 (S1) and spike region 2 (S2) of the SARS-CoV-2 spike protein were synthesised by ProImmune. A total of 253 peptides, each 15-mers long and overlapping with the next by 10 amino acid residues, were used. These were organized into 12 mini-pools, P1 to P12, each comprising 18–24 peptides corresponding to SARS-CoV-2 spike regions S1 (P1 to P6) and S2 (P7 to P12). The Peptide sequences and pooling strategy were described previously [25]. Two positive controls, phytohemagglutinin-L (PHA-L, Sigma) and anti-CD3, were used to induce a mitogenic response and demonstrate the viability of lymphocytes and T-cells, respectively. A negative control (i.e., no peptide but with the Dimethyl sulfoxide [DMSO] carrier) was also included to detect non-specific IFN-g release. The first 10 samples with sufficient cell numbers to test against all 12 peptide pools were included in a preliminary analysis to guide peptide pool prioritization when participants' PBMCs, after thawing, yielded insufficient numbers for testing across all pools. Following this analysis, 4 peptide pools—P3 and P4 representing the S1 region, and P8 and P10 representing the S2 region—were prioritized whenever the numbers of PBMCs were inadequate (Figure S1).

ELISpot assay

We used an IFN-g ELISpot assay, which quantifies the frequency of antigen-specific effector cells producing IFN-g after an overnight co-culture of PBMC with either specific peptides or a non-antigen specific positive control [26]. Briefly, 96 well ELISpot plates (Millipore, UK) were coated with a monoclonal antibody against IFN-g (Mabtech, AB, Sweden) at 10 µg/mL overnight. 200,000 PBMCs per well were plated in triplicates for each individual and plates incubated at 37 °C in a humidified carbon dioxide (5%) incubator for 16–18 h of stimulation with overlapping peptide pools at 10 µg/mL in triplicate wells. PHA-L and anti-CD3 were used as positive controls at final concentrations of 10 µg/mL and 2 µg/mL per well, while DMSO was included as a negative control at a similar concentration to peptides. After incubation, PBMCs were discarded, the plate washed, and the detector for the secreted IFN-g footprints on the plate membrane, anti- IFN-g biotinylated monoclonal antibody (Mabtech), added at 1 µg/mL and the plate incubated for 2 h at room temperature. The plate was then washed, and streptavidin alkaline phosphatase (Mabtech) added at 1 µg/mL, before another incubation for 1 h at room temperature. Following washing, the 1-StepTM NBT/BCI (nitro blue tetrazolium/5-bromo-4-chloro-3-phosphatase) substrate (Thermo Scientific) was added, and the plate left in the dark for 7 min until the developing spots were clearly visible. The plate was then washed three times using tap water. Following overnight drying, the spots in each well were enumerated with an AID ELISpot Reader (v.4.0). Counts were presented as IFN-g Spot-Forming Units (SFUs) per million PBMC, which is determined by taking an average of the triplicate wells. Results are reported here as SFUs per million PBMCs after subtracting the background (mean of individual negative wells) from the antigen-specific responses. We summed up the responses from pools P3 and P4 (representing S1) and pools P8 and P10 (representing S2). For the spike region, we combined the aggregated responses from S1 and S2. Individuals' assays were considered failed, and their results were excluded if the negative control wells had > 150 SFUs per million PBMCs or the positive control wells had < 200 SFUs per million PBMCs. An ELISpot assay limit of detection of 10 SFUs per million PBMCs was also applied, and all wells with values < 10 SFUs per million PBMCs replaced with a possible minimal result equal to 5 SFUs per million PBMCs. Individuals' IFN-γ cellular responses were considered positive, if their SFUs were > 71 SFUs per million PBMCs after background subtraction (corresponding to the 25th percentile of Kenyan COVID-19 patients).

In total, 79 of 80 pre-COVID-19 Kenyan adult samples, 10 of 10 of the Kenyan pre-COVID-19 infant samples, 18 of 20 Swedish pre-COVID-19 adult samples and 37 of 39 post-COVID-19 Kenyan adult patient samples diagnosed with COVID-19 were included in the analysis. Of participants included in the analysis, 78 (99%) pre-COVID-19 Kenyan adult samples, 3 (30%) Kenyan infant samples, 5 (28%) Swedish adult samples and 14 (38%) COVID positive Kenyan patient samples had all the 12 peptide pools tested (Table S1). Since only a small number of infants, Swedish adults and COVID cases had sufficient PBMCs to test all 12 peptide pools, subsequent analyses were restricted to the prioritised peptide pools 3 and 4 for S1 and peptide pools 8 and 10 for S2, and a summation of pools 3, 4, 8 and 10 for total spike responses (Figure S1).

Synthetic peptide pools for intracellular cytokine staining stimulation assay

A total of 178 peptides (15–18-mers with a ten amino acid overlap) spanning the entire SARS-CoV-2 spike protein (S: positions 1–178) were synthesised by Mimotopes Pty Ltd and pooled together into one pool for PBMC stimulations. Peptide sequences and pooling strategy were described previously [27]. DMSO was used as a negative control and PMA/ionomycin as a positive control.

Intracellular cytokine staining and multi-parametric flow cytometry

To determine T-cell function, we used ICS and multiparametric flow cytometry to identify and quantify effector T-cells responding to in vitro stimulation by overlapping SARS-CoV-2 spike peptides with IL-2, IFN-γ and TNF-α cytokine secretion, and upregulation of CD154 and CD107a activation makers. Briefly, cryopreserved PBMCs were thawed and rested for 2–4 h and thereafter plated at 1 × 10^6 live cells per well for SARS-CoV-2 spike peptide stimulations and the DMSO, and at 0.5 × 10^6 live cells per well for PMA/ionomycin in 96-well round-bottom plates. SARS-CoV-2 spike peptide stimulation were added at a final concentration of 2 µg/mL, PMA at 0.05 µg/mL and ionomycin (Sigma) at 0.5 µg/mL, and DMSO (Sigma) at 2 µg/mL. All the stimulations and controls were done in the presence of the co-stimulatory anti-CD28 and anti-CD49 (1 µg/mL, BD Biosciences) antibodies. CD107a BV421 (0.04 µg/ml, clone H4A3, BD Biosciences) was then added to all wells and plates incubated for 1 h at 37 °C temperature, 5% CO_2, 95% humidity for surface staining. Following incubation, Brefeldin A (5 µg/mL, MP Biomedicals) was added, and cells incubated for a further 15 h. After washing the cells with 1X PBS, dead cells were labelled using live/dead fixable aqua dye (Invitrogen) for 30 min at 25 °C. The cells were then washed with 1X Perm/Wash before fixation and permeabilisation using Cytofix-Cytoperm for 20 min at 4 °C. Cells were washed three times with 1X Perm/Wash and then stained with APC 'Fire' 750 anti- CD3

(clone SK7, Biolegend), PE Dazzle 594-anti- CD4 (clone RPA T4, Biolegend), PercpCy5.5-anti- CD8 (clone RPA T8, Biolegend), PECy7- anti-CD154 (clone 24-31, Biolegend), APC-anti- IFN-γ (clone B27, BD Biosciences), PE-anti- IL2 (clone MQ1-17HI2, Biolegend) and FITC-anti- TNF-α (clone Mab11, BD Biosciences) for 20 min at 4 °C. Cells were washed twice with 1X Perm/Wash and resuspended in 200 µl 1X PBS and stored at 4 °C in the dark until acquisition. Samples were acquired on a BD LSR Fortessa (BD Biosciences) flow cytometer, and data analysed using FlowJo version 10.10.0 software. Gating strategies used are detailed in (Figure S2). Results are presented as the proportion of IFN-γ, IL-2 or TNF-α secreting CD4 or CD8 T-cells, or as the proportion of CD154 expressing CD4 T-cells or CD107a expressing CD8 T-cells after subtracting the background (values from negative control wells for each sample). A limit of detection of 0.02% was applied, and all wells with values below 0.02% were replaced with the lowest possible value of 0.01%. Boolean gates were used to examine the frequency of polyfunctional T-cells defined as CD4 or CD8 T-cells co-expressing any of the cytokines IFN-γ, TNF-α, IL-2 and surface markers CD154 or CD107a.

Statistical analysis

Data management and statistical analysis were performed using GraphPad Prism version 10.2.3 [28]. IFN-γ SFUs detected by ELISPOT were log-transformed prior to analysis. For the primary analysis, comparisons were performed between Kenyan and Swedish pre-COVID-19 adult samples to assess differences in IFN-γ SFUs, as well as the proportions of cytokine secreting T-cells and T-cell activation markers in response to SARS-CoV-2 spike peptides or its subregions (S1 or S2). A two-tailed t-test was used to compare IFN-γ SFUs as continuous variables, while Fisher's exact test was applied to compare the proportions of responders to SARS-CoV-2 spike peptides between Kenyan and Swedish pre-COVID-19 adult samples. The Mann Whitney test was used for comparisons of the proportions of cytokine-secreting T-cells and T-cell activation markers. For the secondary analysis, a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test was used to evaluate differences in IFN-γ SFUs across all four study groups. The Kruskal Wallis test with Dunn's multiple comparisons test was used to compare the proportions of cytokine secreting T-cells and T-cell activation markers in response to SARS-CoV-2 spike peptides or subregions across the study groups. A paired t-test was used to assess interindividual variability in responses to peptides corresponding to S1 and S2. All tests were performed at 5% significance level.

Results

Participants characteristics

The median ages of the pre-COVID-19 study participants were 31 years (interquartile range (IQR) 24–40 – for Kenyan adults, 33 years (IQR 31–41) for Swedish adults, and 0.75 years (IQR 0.58–0.83) for Kenyan infants. The proportions of male participants were 29% for Kenya adults, 5% for Swedish adults and 50% for Kenyan infants. Kenyan COVID-19 patients had a median age of 43 years (IQR 34–55), with about 49% being male (Table S2).

IFN-γ responses by ELISpot

For the S1 region, Kenyan pre-COVID-19 adult samples tended to have higher IFN-γ responses (with a geometric mean of 42 [95% CI 31–56] SFUs per million PBMCs) than Swedish pre-COVID adult samples (geometric mean of 22 [95% CI 12–39] SFUs per million PBMCs; $p = 0.05$). For the S2 region, Kenyan and Swedish pre-COVID-19 adult samples had similar geometric mean responding T-cell numbers at geometric mean of 24 (95% CI: 19–30) and 27 (95% CI: 16–45), SFUs per million PBMCs, respectively ($p = 0.66$; Fig. 1a).

With S1 and S2 regions combined, Kenyan pre-COVID-19 adult samples had higher responses (geometric mean of 70 [95% CI 53–93]) than Swedish pre-COVID-19 adult samples (49 [95% CI 29–84]; $p = 0.26$; Fig. 1b). Amongst the Kenyan pre-COVID-19 adult samples there were higher S1 responses compared to S2 responses (geometric mean of 42 [95% CI 31–56] vs. 24 [95% CI 19–30] SFUs per million PBMCs; $p < 0.0001$) (Figure S3a). For the Swedish pre-COVID-19 and infants pre-COVID-19 samples, the magnitude of S1 and S2 responses were similar (Figures S3a–c).

We next analysed the proportion of responders within each subgroup. Responders were defined as individuals with IFN-γ SFUs responses exceeding 71 SFU per million PBMCs after background subtraction, corresponding to the 25th percentile of Kenyan COVID-19 patients. Notably, 55.7% of the Kenyan pre-COVID-19 adult samples were classified as responders, which was higher than 28% of Swedish pre-COVID-19 adult samples ($p = 0.04$) in response to SARS-CoV-2 spike peptides. Among Kenyan pre-COVID-19 infants, 40% were responders. As expected, a large proportion, 75.7% of the COVID-19 positive individuals were classified as responders (Fig. 1c).

Cross reactive cytokine responses to SARS-CoV-2 peptides by ICS

To establish whether the IFN-γ responses observed by ELISpot were from memory T-cells and whether other cytokines were secreted, we incorporated ICS assay followed by multiparametric flow cytometry. Our data show that both CD4+ and CD8+ T-cells from the different

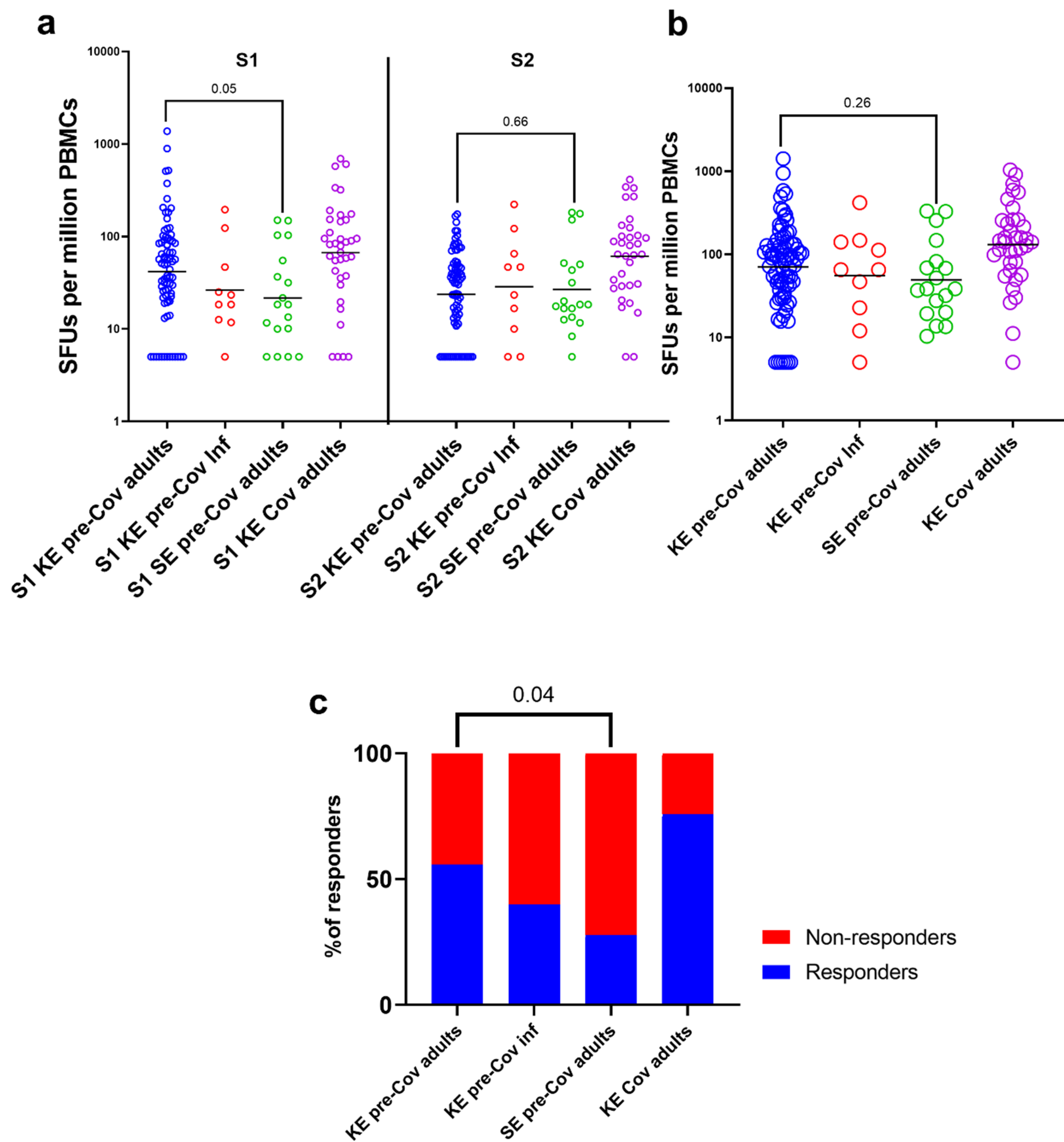

Fig. 1 Comparison of the frequencies of IFN-gamma secreting cell responses to SARS-CoV-2 Spike regions 1 and 2 among study groups**. a** frequencies of Spike regions 1 and 2 separately, **b** aggregated frequencies of Spike regions 1 and 2, **c** Responders (Cut off for responders is 25th percentile of Spike in CoV-adults). Comparisons were performed on log-transformed data using two-tailed *t*-tests and ordinary one-way ANOVA with Tukey's multiple comparisons test to assess differences in responses to S1, S2, and spike peptides. Fisher's exact test was used to compare the proportions of responders to SARS-CoV-2 spike peptides between Kenyan and Swedish pre-COVID-19 adult samples. Significance was set at $p < 0.05$. Black line shows Geometric mean. KE- pre-cov adults is Kenyan pre-COVID-19 adult samples, KE- pre-cov Inf is Kenyan pre-COVID-19 infants samples, SE- pre-cov adults is Swedish pre-COVID-19 adult samples and KE-Cov adults is Kenyan COVID-19 adults samples

groups produced IFN-γ, TNF-α and IL-2 after incubation with overlapping SARS-CoV-2 spike peptides. CD4 + T-cells also upregulated CD154 an activation marker. Kenyan pre-COVID-19 adult samples exhibited higher cytokine levels for IFN-γ and TNF-α responses, though not statistically significant (Figure S4). For the IFN-γ CD8 + T-cell responses, Kenyan pre-COVID-19 adult samples had a higher median of 0.06% (IQR

0.01–0.54%) compared to Swedish pre-COVID-19 adult samples (median: 0.01% [IQR 0.01–0.4%]; $p = 0.54$). The TNF-α CD4 + T-cell responses were higher in Kenyan pre-COVID-19 adult samples (median: 0.03% [IQR 0.01–0.095%]) than Swedish pre-COVID-19 adult samples (median: 0.015% [IQR 0.01–0.055%]; $p = 0.39$). Similarly, TNF-α CD8 + T-cell responses were elevated in Kenyan pre-COVID-19 adult samples (median: 0.06% [IQR 0.01–0.17%]) compared to Swedish pre-COVID-19 adult samples (median: 0.03% [IQR 0.01–0.05%]; $p = 0.13$) (Figure S4b, c–d). The Kenyan COVID-19 patients had notably higher TNF-α CD4 + T-cells levels (median: 0.26% [IQR 0.11–0.73%]) compared to Kenyan pre-COVID-19 adult samples, (median: 0.03% [IQR 0.01–0.095%]; $p = 0.01$), Kenyan pre-COVID-19 infant samples (median: 0.02% [IQR 0.01–0.04%]; $p = 0.007$) and Swedish pre-COVID-19 adult samples (median: 0.015% [IQR 0.01–0.055%]; $p = 0.005$) (Figure S4c). IL-2 CD4 + T-cell frequencies were also higher in Kenyan COVID-19 patients (median: 0.09% [IQR 0.015–0.515%]) compared to Kenyan (median: 0.01% [IQR 0.01–0.025%]; $p = 0.02$) and Swedish pre-COVID-19 adult samples (median: 0.01% [IQR 0.01–0.01%]; $p = 0.004$) (Figure S4e).

Our analysis of the number of functional markers co-expressed by these T-cells showed that only a small proportion of individuals showed polyfunctionality before COVID-19, hence statistical testing could not be performed. Individuals analysed post-COVID-19 had better CD4 and CD8 T-cell polyfunctionality. Compared to Swedish pre-COVID-19 adult samples, a higher proportion of Kenyan pre-COVID-19 adult samples had polyfunctional CD4 + and CD8 + T-cells (Figure S5a, b). Collectively, these data suggest that the pre-COVID-19 CD4 + and CD8 + T-cells from Swedish adult samples were less primed to secrete TNF-α and IFN-γ compared to those of the Kenyan pre-COVID-19 adult samples.

Discussion

We compared the frequencies and prevalence of SARS-CoV-2 cross-reactive T-cell responses by measuring IFN-γ responses by ELISPOT and phenotypic and functional characterization by flow cytometry in pre-COVID-19 PBMC collected in Kenya, and in Sweden. We observed higher S1 specific and full spike frequencies, and a greater prevalence, of pre-pandemic SARS-CoV-2 spike peptide specific cross-reactive IFN-γ secreting cells measured by an ELISpot assay in Kenyan adults, compared to Swedish adults. Overall, these data suggest that Kenyan pre-COVID-19 adult samples had higher IFN-γ SFU responses to the S1 and spike regions but similar S2 responses compared to Swedish pre-COVID-19 adult samples. A larger proportion of Kenyan pre-COVID-19 adult samples also responded to SARS-CoV-2 spike peptides. Responses to S1-specific S2-specific, and to the full spike region increased following contact with COVID-19. These findings suggest a greater pre-pandemic exposure to S1-like epitopes in Kenya contributing to SARS-CoV-2 cross-reactive T-cell responses compared to Sweden.

We confirmed, using ICS assay, that the spike-peptide specific IFN-γ secreting cells included both CD4 + and CD8 + T-cells. Furthermore, we did not identify evidence that the Kenyan pre-COVID-19 adult samples had statistically significantly higher frequencies of pre-pandemic SARS-CoV-2 spike-specific TNF-α, or IFN-γ responses either in CD4 + T-cell responses or CD8 + T-cell responses compared to their Swedish counterparts. However, the ELISPOT assay is more sensitive than the ICS assay we used, and therefore the power to detect differences between populations is more limited in the ICS work [29].

The higher frequencies and prevalence of IFN-γ secreting cells among the Kenyan pre-pandemic adult-PBMCs compared to their Swedish counterparts is consistent with higher levels of pre-existing anti-SARS-CoV-2 cross protective immunity in SSA [30]. These higher frequencies of pre-pandemic SARS-CoV-2 cross reactive cells may contribute to the lower prevalence of severe COVID-19 and deaths in Kenya relative to European countries like Sweden. This idea is consistent with previous observations of protection from severe COVID-19 by recent exposures to endemic hCoVs [31]. Furthermore, mechanistic studies in mice demonstrated that hCoV-specific, SARS-CoV-2–cross-reactive T -cells contribute to SARS-CoV-2 immune responses upon infection and vaccination [17].

There is little evidence for a role for cross-reactive neutralising antibodies as these are rare, even where there is PCR evidence for recent hCoV infection [18]. The mechanism by which SARS-CoV-2 cross reactive CD4 + T-cells lower COVID-19 severity may include various mechanisms such as the direct killing of virus infected cells, or through their ability to offer CD8 T cells help and protecting against infection, or both, thus reducing viral loads [32–35]. Alternatively (or in addition), SARS-CoV-2 cross reactive memory CD4 + T-cells may provide the necessary costimulatory molecules for helping rapid and amnestic production of protective antibodies [17].

This study has a few limitations. First, for the ICS assays, we only had access to pre-COVID-19 PBMC from 21 Kenyan adults,10 Kenyan infants, 10 Swedish adults and 9 Kenyan COVID-19-19 patients, which reduced the statistical power of comparing responses among the groups. Second, we were only able to use spike peptides to stimulate PBMCs as the number of cells was limited; it would have been more comprehensive to include other SARS-CoV-2 proteins. Third, in the ELISpot assays, the unavailability of adequate numbers of PBMCs meant that only a subset of samples could be tested across all

12 peptide pools, particularly for infants, Swedish adults, and COVID-19 cases. This may have led to loss of information from the untested pools and reduced the statistical power for some comparisons. Lastly, we didn't have data on the sex of some of the Swedish participants, which limits our discussion on the role of sex in the immune responses reported here.

Conclusion

We report higher frequencies and prevalence of pre-COVID-19 SARS-CoV-2 spike cross-reactive IFN- γ secreting cells, some of which include CD8 + T -cells, among Kenyan, compared to Swedish adults. This raises the possibility that higher levels of cross-protective cellular responses in SSA could explain, at least in part, why there was less severe morbidity and mortality on the continent relative to Europe, Asia and North America. Nonetheless, ours is an observational study, and lacks a longitudinal follow-up linking pre-existing cross-reactive immune responses and disease severity. Subsequent studies, including relatively higher sample sizes of pre-COVID-19 PBMC from SSA and a few more countries from Europe and/or America, are required to confirm these findings and extend the research into investigating the actual mechanism of the SARS-CoV-2 cross-protection in longitudinal studies. Importantly, such studies will provide new insights on how to develop novel cross-protective vaccines against similar pathogens, like coronaviruses.

Abbreviations

SSA	Sub-Saharan African
COVID-19	Coronavirus disease 2019
PBMC	Peripheral blood mononuclear cells
ELISpot	Enzyme-Linked Immunosorbent
ICS	Intracellular cytokine stainin
S1	Spike region 1
S2	Spike region 2
L-PHA-L	Phytohemagglutinin
DMSO	Dimethyl sulfoxide
SFUs	Spot-Forming Units

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-026-12582-6.

Supplementary Material 1

Acknowledgements

We thank the field, laboratory, and healthcare workers involved in the blood sampling. We appreciate all the study participants. This manuscript is published with the permission of the Director General of KEMRI.

Author contributions

P.W., P.B., F.M.N., and E.N. designed the study. P.W., J.M., J.C., H.K.K., B.K.D., O.K., D.W., L.I.O.O., C.S., S.D., G.M.W., and A.F. contributed to sample and reagent acquisition, methodology, and data compilation. P.W. and B.O. conducted the statistical analysis. P.B., F.M.N., and E.N. contributed to the interpretation of results. P.W. and B.O. drafted the initial manuscript. All authors reviewed, revised, and approved the final manuscript.

Funding

This study/project is funded by the EDCTP2 Programme (grant number RIA2020EF-3042) which is supported by the European Union, and the Swedish International Development Cooperation Agency. SD is supported by an NIHR Global Health Research Professorship (NIHR300791). AF is supported by Swedish Research Council (MH 2018–02688). CS is funded from Swedish Research Council (2019–05438 and 2023 – 01943). FMN is an MRC/UKRI, UK, African Research Leader (MR/P020321/1). EN is funded from the Gates Foundation (INV 064558). This research was funded in part by the Wellcome Trust core support (grant number 203077/Z/16/Z) to the KEMRI–Wellcome Trust Research Programme, Kilifi, Kenya.

Data availability

Data is available upon a reasonable request. Requests are made by completing a "data request form from" downloaded from https://kemri-wellcome.org/data/ and sending it via email to the data governance committee dgc@kemri-wellcome.org.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the ethical principles for medical research involving humans, including research on identifiable human material, as declared in Helsinki in 1964. We received ethical approval from the Kenya Medical Research Institute's (KEMRI) Scientific and Ethics Review Unit (SERU) under protocols SERU 3149, 4081 and 4085. Ethical approval for the collection of the Swedish samples was obtained from the regional Ethics Committee in Stockholm, Sweden under protocols 2006/893 – 31/4 and 2019–03436. Each participant, or their parent or legal guardian, provided informed consent for their respective samples to be used for both the contemporaneous and future research.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 19 March 2025 / Accepted: 9 January 2026
Published online: 17 January 2026

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