Prevalence and functional profile of SARS-CoV-2 T cells in asymptomatic Kenyan adults

Taraz Samandari, … , Nina Le Bert, Antonio Bertoletti


**Graphical abstract**

Residents of rural Kenya (n = 80)
- November 2021
- No symptoms
- No contact with COVID-19 cases
- No COVID-19 vaccines

Prevalence of SARS-CoV-2 immunity
- Antibodies: 49%
- T cells: 81%

Immunogenicity of SARS-CoV-2 proteins
- Accessory
- Structural

Balanced inflammatory response
- IFN-γ
- IL-10

Find the latest version:
https://jci.me/170011/pdf
Prevalence and functional profile of SARS-CoV-2 T cells in asymptomatic Kenyan adults

Taraz Samandari,1 Joshua B. Ongalo,2 Kimberly D. McCarthy,1 Richard K. Biegon,3 Philister A. Madiega,2 Anne Mithika,2 Joseph Orinda,2 Grace M. Mboya,2 Patrick Mwaura,4 Omu Anzala,4 Clayton Onyango,1 Frederick O. Olouch,5 Eric Osoro,6,7 Charles-Antoine Dutertre,8,9 Nicole Tan,10 Shou Kit Hang,10 Smrithi Hariharaputran,10 David C. Lye,11,12,13,14 Amy Herman-Roloff,1 Nina Le Bert,10 and Antonio Bertoletti10,15

1US Centers for Disease Control and Prevention, Nairobi, Kenya. 2Kenya Medical Research Institute (KEMRI), Centre for Global Health Research, Kisumu, Kenya. 3Moi University School of Medicine, Immunology Section, Eldoret, Kenya. 4KAVI Institute of Research, University of Nairobi, Nairobi, Kenya. 5County Government of Kisumu, Department of Health and Sanitation, Kisumu, Kenya. 6Washington State University Global Health Kenya, Nairobi, Kenya. 7Paul G. Allen School of Global Health, Washington State University, Pullman, Washington, USA. 8Gustave Roussy Cancer Campus, Villejuif, France. 9Institut National de la Santé et de la Recherche Médicale (INSERM) U1015, Equipe Labellisée — Ligue Nationale contre le Cancer, Villejuif, France. 10Programme in Emerging Infectious Diseases, Duke University–National University of Singapore Medical School, Singapore. 11Tan Tock Seng Hospital, Singapore. 12Yong Loo Lin School of Medicine, National University of Singapore, Singapore. 13Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore. 14Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Singapore. 15Institut National de la Santé et de la Recherche Médicale (INSERM) U1015, Equipe Labellisée — Ligue Nationale contre le Cancer, Villejuif, France.

BACKGROUND. SARS-CoV-2 infection in Africa has been characterized by a less severe disease profile than what has been observed elsewhere, but the profile of SARS-CoV-2–specific adaptive immunity in these mainly asymptomatic patients has not, to our knowledge, been analyzed.

METHODS. We collected blood samples from residents of rural Kenya (n = 80), who had not experienced any respiratory symptoms or had contact with individuals with COVID-19 and had not received COVID-19 vaccines. We analyzed spike-specific antibodies and T cells specific for SARS-CoV-2 structural (membrane, nucleocapsid, and spike) and accessory (ORF3a, ORF7, ORF8) proteins. Pre-pandemic blood samples collected in Nairobi (n = 13) and blood samples from mild-to-moderately symptomatic COVID-19 convalescent patients (n = 36) living in the urban environment of Singapore were also studied.

RESULTS. Among asymptomatic Africans, we detected anti-spike antibodies in 41.0% of the samples and T cell responses against 2 or more SARS-CoV-2 proteins in 82.5% of samples examined. Such a pattern was absent in the pre-pandemic samples. Furthermore, distinct from cellular immunity in European and Asian COVID-19 convalescents, we observed strong T cell immunogenicity against viral accessory proteins (ORF3a, ORF8) but not structural proteins, as well as a higher IL-10/IFN-γ cytokine ratio profile.

CONCLUSIONS. The high incidence of T cell responses against different SARS-CoV-2 proteins in seronegative participants suggests that serosurveys underestimate SARS-CoV-2 prevalence in settings where asymptomatic infections prevail. The functional and antigen-specific profile of SARS-CoV-2–specific T cells in African individuals suggests that environmental factors can play a role in the development of protective antiviral immunity.

FUNDING. US Centers for Disease Control and Prevention, Division of Global Health Protection; the Singapore Ministry of Health’s National Medical Research Council (COVID19RF-0060, COVID19RF-001, COVID19RF-008, MOH-StaR17Nov-0001).

Introduction

SARS-CoV-2 infection in Africa is characterized by a low number of mild and severe cases of disease (1, 2). The incidence of severe COVID-19 has been particularly low in Kenya (3, 4). Even though an undercount of deaths from COVID-19 cannot be firmly excluded, Kenya’s National Emergency Operations Centre reported that approximately 90% of infected individuals were asymptomatic during the COVID-19 pandemic (3, 4). This might be mainly explained by the country’s youthful population (i.e., median of 19 years for Kenya versus 38 for the United States), but other factors such as cross-reactive immunity induced by other coronaviruses (5) or commensal microorganisms (6), trained immunity stimulated by live vaccines (i.e., bacille Calmette-Guérin [BCG] and live oral polio vaccines) (7, 8), or a downregulation of the inflammatory response via helminth coinfection (9) could play mitigating roles.

The prevalence of past infection is classically measured using serological assays and in Kenya, by January–March 2021, blood
donor anti-spike antibody seroprevalence ranged from 38% in rural western counties to 62% in the capital city, Nairobi (10). However, it has been repeatedly shown that among asymptomatic SARS-CoV-2-infected individuals, antibody levels are frequently low or absent, while T cell responses remain detectable (11-13). Furthermore, kinetics studies showed that memory T cells (14) persist longer than antibodies (15) in the blood, implying that seroprevalence as an indicator may underestimate the extent of asymptomatic SARS-CoV-2 exposure.

Here, we studied SARS-CoV-2–specific humoral and cellular immune responses in individuals from Kenya who never reported any symptoms of respiratory infection and who were not knowingly in contact with patients with COVID-19. We enlisted the guidance of locally-resident community health care workers to identify study participants residing in rural areas of the counties Kisumu and Elgeyo Marakwet, 2 regions of Kenya that by December 7, 2021, had reported 569 and 94 cases of COVID-19 per 100,000 population, respectively. Samples collected during November and December 2021 were studied in parallel for the presence of spike-specific antibodies and for T cells specific for SARS-CoV-2 structural (membrane, nucleoprotein [NP], spike) and accessory (ORF3a, ORF7, ORF8) proteins utilizing different methods of T cell characterization. Until now, SARS-CoV-2–specific T cells, which have been hypothesized to play a major role in the control of disease severity (16), have only been examined in African populations with convalescent COVID-19 (17, 18), while T cell response characteristics in asymptomatic Africans have never been studied.

Results

Study participants with no history of respiratory illness (cough, shortness of breath, fever, or sinus congestion) since December 2019, no contact with individuals known to have COVID-19, and no history of COVID-19 vaccination were recruited from Elgeyo Marakwet (n = 40) and Kisumu (n = 40; Figure 1A) counties. Among the participants, 42 of 80 (53%) were female and 65 of 80 (81%) were under the age of 50 years; 10 of 63 (16%) were HIV infected, the remainder (n = 17) having declined HIV testing, and 5 of 80 (6%) had hypertension (Table 1). All had negative nasal swab tests for SARS-CoV-2 by PCR. Antibodies specific for spike antigens were first tested using a surrogate neutralizing antibody test (GenScript cPass) (19) as well as anti-IgG-SARS-CoV-2 tests (InBios followed by EUROIMMUN assay; see Methods) measuring the level of IgG against the S1 region of the spike protein. We also tested NP-specific antibodies (anti-SARS-CoV-2 NCP ELISA, EUROIMMUN). Surrogate neutralizing antibodies were detected in 16 of 40 (40%) participants from Elgeyo Marakwet and in 18 of 40 (43%) participants from Kisumu (Figure 1B). Similar proportions were observed using the alternative InBios-EUROIMMUN assays (Figure 1B). To exclude the possibility that a primary infection with SARS-CoV-2 variants of concern (Delta, Omicron) might have induced spike-specific antibodies that do not cross-react with the Wuhan-Hu-1 spike protein, we tested for the presence of antibodies specific for the receptor-binding domain (RBD) region of Delta and Omicron. There was an almost complete concordance between the detection of antibodies against Wuhan-Hu-1 and Delta spike RBDs (Figure 1C). Only 2 individuals had antibodies specific for the Delta spike RBD in the absence of antibodies against the Wuhan spike RBD, while antibodies against the Omicron spike RBD were absent in all individuals except the 2 who had the higher values of pseudonutralizing activity. This observation is consistent with evidence that the Delta SARS-CoV-2 variant was circulating in Kenya in the second quarter of 2021, while Omicron started to be detected in Kenya only in late November 2021 (20).

We tested T cell reactivity against SARS-CoV-2 structural (spike, NP, membrane) and accessory (ORF3a, ORF7a/b, ORF8) proteins by stimulating whole blood within 8 hours from sample collection using 5 distinct peptide pools (Figure 2A, left). After overnight incubation, IFN-γ and IL-2 levels were measured in the supernatants of stimulated and unstimulated blood (Figure 2A, right) as previously reported (21). We detected IFN-γ and/or IL-2-producing, multi-specific anti-SARS-CoV-2 T cell responses (≥2 peptide pools) in 70% (28 of 40) of the individuals from Elgeyo Marakwet and 95% (38 of 40) of the individuals from Kisumu (Figure 2, B and C). Almost all individuals with positive anti-spike serology also showed cytokine responses against multiple SARS-CoV-2 proteins: 94% (15 of 16) of the Elgeyo Marakwet group and 100% (17 of 17) of the Kisumu group. Furthermore, peptide-induced IFN-γ and IL-2 secretion were not only detectable in antibody-seropositive individuals but also in the majority of seronegative ones: Elgeyo Marakwet, 54% (13 of 24) and Kisumu, 91% (21 of 23) (Figure 2, B and C). Thus, only 27.5% of asymptomatic individuals from Elgeyo Marakwet and 5% from Kisumu were negative for both SARS-CoV-2 serology and T cell cytokine analysis (Figure 2D).

Of note, some HIV-infected individuals displayed robust production of IFN-γ and IL-2, and the overall magnitude of SARS-CoV-2–specific T cell responses was not affected by HIV infection (Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/JCI170011DS1). The HIV status of participants is indicated with an asterisk in Figure 2, B and C; their median CD4+ T cell count was 596 cells/μL, with a range of 363–1,376 cells/μL.

The observation that serologically negative, asymptomatic individuals produced T cell cytokines after whole-blood stimulation with peptides covering different SARS-CoV-2 proteins sug-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Kisumu</th>
<th>Elgeyo Marakwet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, average (range)</td>
<td>38 (18–73)</td>
<td>37 (21–77)</td>
</tr>
<tr>
<td>Age ≥50 years, % (n/N)</td>
<td>20% (8/40)</td>
<td>18% (7/40)</td>
</tr>
<tr>
<td>Female, % (n/N)</td>
<td>53% (21/40)</td>
<td>53% (21/40)</td>
</tr>
<tr>
<td>Male, % (n/N)</td>
<td>47% (19/40)</td>
<td>47% (19/40)</td>
</tr>
<tr>
<td>Married, % (n/N)</td>
<td>58% (23/40)</td>
<td>70% (28/40)</td>
</tr>
<tr>
<td>Secondary ed., tech. or university, % (n/N)</td>
<td>38% (15/40)</td>
<td>53% (21/40)</td>
</tr>
<tr>
<td>HIV-infected, % (n/N)</td>
<td>19% (7/36)</td>
<td>11% (3/27)</td>
</tr>
<tr>
<td>Hypertension, % (n/N)</td>
<td>3% (1/40)</td>
<td>10% (4/40)</td>
</tr>
<tr>
<td>Diabetes, % (n/N)</td>
<td>0% (0/40)</td>
<td>0% (0/40)</td>
</tr>
</tbody>
</table>

*Some participants did not wish to be tested for HIV and did not share their HIV status. Of those who were HIV infected, the median CD4+ cell count was 596 cells/μL (range, 363–1,376). ed., education; tech., technical school.
suggests that these individuals possessed virus-specific T cells primed by SARS-CoV-2 infection. Indeed, the simultaneous presence of T cells specific for multiple SARS-CoV-2 proteins is characteristic of previous infection (22). However, since pre-pandemic cross-reactive T cells, usually specific for a single protein, have been observed in 40% to 70% of individuals worldwide (6, 23–27), we performed ELISPOT assays by exposing thawed pre-pandemic PBMCs from Nairobi as well as post-pandemic PBMCs from Kisumu and Elgeyo Marakwet.
were detected in 31% (4 of 13) of the volunteers. In contrast, the ELISPOT assays performed with post-pandemic PBMCs (collected at the same time as the whole blood for T cell assays between November 15 and December 2, 2021) confirmed the presence of T cell reactivity against multiple SARS-CoV-2 proteins in the great majority of the asymptomatic participants from Elgeyo Marakwet to peptide pools covering the entire lengths of 3 structural (spike, nucleocapsid, membrane) and 3 accessory proteins (ORF3a, ORF7, ORF8) of SARS-CoV-2 (Figure 3A). None of the PBMCs collected before 2019 demonstrated multi-specific T cell responses to SARS-CoV-2 (Figure 3B). Single responses (>5 spots × 10^6) to a peptide pool covering spike, membrane, ORF3a, or ORF7 were detected in 31% (4 of 13) of the volunteers. In contrast, the ELISPOT assays performed with post-pandemic PBMCs (collected at the same time as the whole blood for T cell assays between November 15 and December 2, 2021) confirmed the presence of T cell reactivity against multiple SARS-CoV-2 proteins in the great majority of the asymptomatic participants from Elgeyo Marakwet.
and Kisumu (Figure 3, C and D). As the viability of the PBMCs after freezing and thawing was suboptimal (low viability and/or failed positive controls), only 75% (60 of 80) of the samples were analyzable. ELISPOT data confirmed a pattern almost identical to the results obtained using the whole-blood rapid cytokine assays. T cells activated by at least 2 distinct peptide pools were detected in 73% and 74% of PBMCs, respectively, collected in Kisumu and Elgeyo Marakwet. Of note, the results obtained with the whole-blood assay and ELISPOT were identical in the Elgeyo Marakwet group (74% by ELISPOT versus 70% by whole blood), in which the viability of PBMCs was optimal (38 of 40 samples), whereas discrepancies in the frequency of positive responses in the Kisumu group (73% by ELISPOT versus 90% by whole blood) were associated with the poor viability of some samples, implying that the handling of samples can alter T cell immunological results (28).

Finally, to unequivocally demonstrate that peptide pools were activating T cells, selected PBMCs were stimulated with peptides, expanded in vitro, and then analyzed by flow cytometry for the presence of peptide-specific CD4+ or CD8+ T cells. SARS-CoV-2 peptide–specific CD4+ and CD8+ T cells were visualized, and their ability to recognize single peptides is shown in Supplemental Figure 2.

The utilization of peptide pools covering the whole length of the different structural (spike, membrane, nucleocapsid) and accessory (ORF3a, ORF7, ORF8) proteins in the ELISPOT assays (Figure 4A) permitted the evaluation of the relative T cell immunogenicity of the proteins in 44 asymptomatic Kenyan participants. We calculated the percentage of T cells recognizing each protein in individuals with multi-specific T cells. The bars in Figure 4B show the composition of the SARS-CoV-2 T cell response against different viral proteins for each individual. T cell immunogenicity was not proportional to the length of the protein tested.

For example, despite the fact that spike consisted of 51% of the length of all proteins tested (Figure 4A), anti-spike–specific T cells were the dominant T cell response in only 23% (10 of 44) of

Figure 3. T cells specific for different SARS-CoV-2 proteins in pre-pandemic samples and in the asymptomatic study participants. (A) SARS-CoV-2 proteome organization; analyzed proteins are highlighted in color. PBMCs were stimulated with 15 mer peptide pools covering SARS-CoV-2 spike, membrane, and NP structural proteins and ORF3a, ORF7, and ORF8 accessory proteins. IFN-γ–secreting cells (SFCs) in response to peptide stimulation were quantified by ELISPOT assay. The frequency of IFN-γ–secreting cells per 1 million PBMCs is shown for each peptide pool in (B) pre-pandemic samples from Nairobi (n = 13) and in samples collected in December 2021 from asymptomatic participants from (C) Kisumu (n = 22) and (D) Elgeyo Marakwet (n = 38). Participants are organized by level of neutralizing antibodies (percentage of inhibition by sVNT). (B–D) Pie chart insets show the percentage of participants with responses to 2–6 peptide pools (in red).
tested participants (Figure 4B). Interestingly, we noted a robust T cell response against the accessory proteins ORF3a and ORF8, uniquely present in sarbecoviruses (SARS-CoV-2 and SARS-CoV) (29). Although these proteins represented 15% of the length of all SARS-CoV-2 proteins tested (Figure 4A), ORF3a and ORF8 constituted the dominant response in 30% (13 of 44) of asymptomatic individuals. The T cell immunodominance pattern in asymptomatic individuals living in rural Kenya was, however, distinctive from that in mild-to-moderately symptomatic COVID-19 convalescents (n = 36) living in the urban environment of Singapore and tested 6 months after infection with identical methods and peptide pools (Figure 4C–E). Anti-spike activity clearly represented the dominant T cell response in Singaporean COVID-19 convalescents (Figure 4E). These results are consistent with observations by others who studied COVID-19 convalescents in the United Kingdom and the United States (30–32).

IL-10 production by virus-specific T cells has been associated with reduced inflammation in respiratory viral infections (33, 34). Therefore, we compared the functional cellular immune response of asymptomatic participants from Kenya with COVID-19 convalescents from Singapore. We used an unsupervised dimension reduction and clustering algorithm (uniform manifold approximation and projection [UMAP]) of the secretomes (IFN-γ, IL-2, and IL-10) of all peptide-stimulated samples (n = 495) after subtraction of cytokine levels present in corresponding dimethyl sulfoxide controls. This showed that the secretion of cytokines classically produced by Th1 cells, IFN-γ and IL-2, was overlapping. In contrast, samples with high levels of the regulatory cytokine IL-10 formed a cluster with only partial intersection (Figure 5A). The overall secretomes from the 3 groups of participants differed (Figure 5B). The UMAP from Elgeyo Marakwet displays more samples with no or low levels of cytokine release, consistent with Figure 2 showing that 30% of participants from this group had no SARS-CoV-2–specific cellular immunity. Many secretomes from both asymptomatic groups, Kisumu and Elgeyo Marakwet, clustered with high IL-10 levels, which was not seen for the secretomes from symptomatic COVID-19 convalescents from Singapore. Yet only samples from Kisumu and Singapore clustered on the UMAP with high IFN-γ and IL-2.

Deconvolution of the secretion profiles in response to the individual peptide pools covering the different SARS-CoV-2 proteins showed distinctive profiles between structural and accessory pro-
Figure 5. Cytokine secretion profile of SARS-CoV-2 peptide pool–stimulated whole blood from asymptomatic Kenyans and symptomatic convalescent Singaporeans. Whole blood was stimulated with SARS-CoV-2 peptide pools overnight, and the cytokine secretion profile (IFN-γ, IL-2, and IL-10) was analyzed using an unsupervised clustering algorithm UMAP. (A) UMAP plots with cytokine secretion heatmaps. (B) Concatenated cytokine secretion profiles from asymptomatic participants from Elgeyo Marakwet (left, green, n = 40 individuals, n = 200 tests) and from Kisumu (middle, brown, n = 40 individuals, n = 200 tests), and convalescent symptomatic COVID-19 patients from Singapore (right, blue, n = 19 individuals, n = 95 tests) overlaid on the global UMAP plot of all analyzed samples (black dots; each dot corresponds to 1 culture supernatant). (C) UMAP plots comparing the cytokine secretion profiles of whole blood from all individuals tested (n = 99) stimulated with the 5 different SARS-CoV-2 peptide pools shown individually. (D) Violin plots showing the quantity of IL-10, IL-2, and IFN-γ detected in the different culture supernatants from asymptomatic participants from Elgeyo Marakwet (left) and Kisumu (middle) and from symptomatic convalescents from Singapore (right). Friedman’s test followed by Dunn’s multiple-comparison test (line indicates the median). (E) Ratios of IL-10/IFN-γ quantities detected in the culture supernatants stimulated with the different peptide pools. (F) Ratios of IL-10/IFN-γ quantities detected in the culture supernatants stimulated with the different peptide pools are compared between the 3 cohorts. EM, Elgeyo Marakwet; Ki, Kisumu; SG, Singapore. (E and F) Kruskal-Wallis test, followed by Dunn’s multiple-comparison test (line indicates the median). *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.
teins (Figure 5C). Cytokine profiles in response to accessory proteins clustered with high IL-10 secretion. A side-by-side comparison of the cytokines produced by each peptide pool in the 3 cohorts showed that, while structural proteins had a robust Th1 response (high IFN-γ/IL-2, low IL-10), accessory proteins induced similar quantities of IFN-γ and IL-10 (Figure 5D). In the participants from Elgeyo Marakwet, ORF7/8 triggered significantly higher secretion of the antiinflammatory cytokine IL-10 than IFN-γ or IL-2.

The IL-10/IFN-γ ratio was highest for ORF7/8, followed by ORF3a in all 3 groups (Figure 5E). Comparison of the asymptomatic and convalescent groups revealed higher IL-10/IFN-γ ratios in the samples from Elgeyo Marakwet, followed by Kisumu, with the lowest ratios observed in the Singaporean group in response to all the tested proteins. The differences in IL-10/IFN-γ ratios in the Elgeyo Marakwet and Kisumu samples were statistically significant only for T cell responses against spike and membrane. However, T cell responses against all the tested proteins, except ORF3a, showed statistically significantly higher IL-10/IFN-γ ratios in asymptomatic participants from Elgeyo Marakwet compared with ratios in the Singaporean COVID-19 convalescents (Figure 5F).

Discussion

In a unique survey of both cellular and humoral immune responses against SARS-CoV-2 among asymptomatic Africans, we show that 78% of individuals living in 2 rural regions of Kenya, who had no reported respiratory symptoms since December 2019 and who were never knowingly in contact with individuals infected with SARS-CoV-2, possessed broadly reactive T cells specific to multiple SARS-CoV-2 proteins. Sixty percent of these asymptomatic individuals lacked anti-spike antibodies — an antibody response more durable than that of the anti-nucleocapsid antibody (15) — while, among these seronegative participants, 70% had multi-specific T cell responses. The simultaneous detection of a T cell response to different SARS-CoV-2 structural and accessory proteins in these individuals contrasts with the detection of T cell responses specific for single SARS-CoV-2 proteins that we observed in pre-pandemic samples collected in a different geographical area of Kenya. Thus, even though we cannot unequivocally claim that such multi-specific T cell responses demonstrate previous infection by SARS-CoV-2, it is essential to highlight that the cross-reactive T cells found in 30% to 70% of individuals tested before the pandemic (24, 25) were limited to single SARS-CoV-2 proteins (22). In addition, recent work showed that the CD4+ T cell response induced by NL-63 and OC-43 coronavirus infections largely do not overlap with SARS-CoV-2-induced CD4+ T cells (35). Therefore, we conclude that the detection of T cells specific for different SARS-CoV-2 proteins in the same individual is highly indicative of previous asymptomatic SARS-CoV-2 infection, as shown in seronegative health care workers in the United Kingdom who had been exposed to the virus (22).

Thus, in addition to strengthening the evidence that the rates of asymptomatic SARS-CoV-2 infection were already very high in Kenya before the advent of the highly transmissible Omicron variant of concern, our results strongly suggest that measurement of virus-specific T cells constitutes a more sensitive assay than the measurement of antibodies to detect past coronavirus infections. This is consistent with the differential waning of antibody titers and T cell frequencies, particularly in individuals with no or minimal symptoms (15) and with the detection of multi-specific SARS-CoV-2 T cell responses in the absence of antibodies in other studies of asymptomatic SARS-CoV-2 infection (11, 12). Furthermore, the persistence of virus-specific T cells over antibodies was also already observed in other coronavirus infections such as SARS-CoV-1 (36) and Middle East respiratory syndrome (MERS) (37, 38). Antibodies against SARS-CoV-1 are undetectable 2 to 3 years after infection (36), while SARS-CoV-1-specific T cells are detectable up to at least 17 years after infection (23). Similarly, T cell responses against MERS coronavirus were present in individuals with occupational exposure to camels in the absence of antibody responses (37, 38).

Despite such evidence, epidemiological assessments of the prevalence of SARS-CoV-2 have mainly utilized serologic assays, since antibodies are easier to measure than T cell responses. However, methodologies for the rapid detection of virus-specific T cells flourished during the COVID-19 pandemic (21, 39–41), including the use of whole blood, which appeared to correlate with protection from SARS-CoV-2 infection in 1 study (41). Here, we show directly that cytokine detection following whole-blood stimulation with peptides can be implemented in locations within a few hours’ distance from a facility with biosafety level-1 cabinets. As these whole-blood assays continue to be perfected for large-scale use, they could eventually be applied routinely for public health surveillance of exposure to microbes that are known to elicit seronegative responses in asymptomatic individuals or in individuals whose antibody levels wane quickly.

Suppose there is indeed a reduced antibody response to SARS-CoV-2, as observed in our cross-sectional study that ended in 2021. In that case, one may ask why there has been a steady increase in the seroprevalence of SARS-CoV-2 in Kenya and other countries of the African continent, reaching 90% or higher in many settings? Certainly, our study targeted asymptomatic individuals known to have reduced antibody positivity, whereas serosurveys encompass both symptomatic and asymptomatic persons. Nevertheless, there remains a wide discrepancy between the antibody and T cell responses. A clue comes from data in surveillance platforms in Kenya, where a 5% to 10% PCR positivity for SARS-CoV-2 has been observed among symptomatic individuals during interwave periods. We speculate that as SARS-CoV-2 transitioned from an epidemic to an endemic virus, continual reinfections have boosted the population’s antibody levels, especially as there has been very little promotion of nonpharmaceutical interventions in over a year. The fact that our participants were from a rural area and had at least some degree of isolation from urban centers may have allowed time for their antibody levels to decline rather than get boosted through reinfection.

We observed that the Kisumu group of asymptomatic participants had a 95% multi-specific T cell response to SARS-CoV-2 proteins, while the proportion was lower (70%) for participants from Elgeyo Marakwet. We attribute this difference to the greater interaction of residents of peri-urban Kisumu with the city of Kisumu, which boasts a population of approximately 350,000, whereas the residents of Elgeyo Marakwet whom we enrolled live a life distinctly more remote from urban centers.

These 2 rural communities have health and environmental characteristics that one may speculate influence the unique
immune responses we observed. In both communities, adminis-
tering a birth dose of BCG is routine, incident Mycobacterium tuber-
culosus (TB) disease, and helminthic infections are commonplace
(school children regularly receive anti-helminthics during biannu-
al mass drug administration events, and persons living with HIV
are routinely provided TB-preventive therapy). Although malaria
is uncommon in Elgeyo Marakwet, it is endemic in Kisumu. Fur-
thermore, while both groups often sleep in the same shelter as
their livestock to prevent their theft, those in Elgeyo Marakwet
typically own cows, prepare a fermented cow’s milk called mursik,
and exercise more because of the steep terrain that approximately
1,400 meters higher in altitude than Kisumu. Whether these envi-
ronmental differences explain some of the unexpected features of
SARS-CoV-2 T cell responses observed in asymptomatic rural
Kenyan will require additional investigation.

We observed 2 distinctive immunologic characteristics among
these asymptomatic rural Africans. The first was the observation
of an unexpectedly strong T cell immunogenicity against the viral
accessory proteins ORF3a and ORF8 that contrasted with the
SARS-CoV-2 T cells studied in convalescent urban Singaporeans
and Western (United Kingdom and United States) patients, who
instead showed a clear dominance of spike-specific T cell respons-
es (30–32). Of note, ORF3a and ORF8 proteins are unique to
SARS-CoV-2 and SARS-CoV (sarbecovirus), but are not present in
common seasonal coronaviruses (29). Hence, we can exclude the
notion that this peculiar T cell dominance is caused by cross-react-
ive memory T cells induced by seasonal coronaviruses. However,
exposure to commensal antigens can modulate the SARS-CoV-2
T cell repertoire (6). Thus, the possibility that differing microbi-
omes can alter the SARS-CoV-2 T cell immunodominance cannot
be excluded. An alternative hypothesis emerges from the kinetics
of SARS-CoV-2 protein synthesis. Accessory molecules (ORF3a
and ORF7a/b) are produced earlier after infection (42), prob-
ably because they play a role in suppressing innate immunity in
the infected cells (43). Furthermore, ORF3a, ORF7a/b (44), and
ORF8 (45) have been shown to reduce HLA class I presentation.
The hypothesis is that, in the early phases of infection, these pro-
teins might be more immunogenic than structural proteins, and
in asymptomatic infections, such abortive infections with limit-
ed virion production may predominate as compared with symp-
tomatic infections. Support for this hypothesis is the finding of an
early immune response against accessory proteins in patients with
acute COVID-19 (46).

The second intriguing feature was the skewed IL-10 produc-
tion by SARS-CoV-2 T cells detected preferentially in asymptom-
atic individuals living in rural Kenya, particularly in the rural com-
munities of Elgeyo Marakwet. While genetic factors might be the
basis of such a difference, another interpretation is that specific
environmental factors more common in rural areas of Kenya than
in very urbanized Singapore altered the cytokine profile of SARS-
CoV-2 T cells. The ability of helminths or mycobacteria (including
BCG) to skew the cytokine production of immune cells has been
well documented (47, 48), but other factors may also play a role.
For example, compared with Kisumu participants, the relatively
augmented levels of IL-10 production observed in participants
from Elgeyo Marakwet could be explained by the differences in
lifestyle and diet or by the absence of malaria in this high-altitude
region. Exercise (49) and fermented foods (50) are associated with
antiinflammatory effects on the human immune response, where-
as malaria has been shown to induce a Th1-like response (51). It
is worth pointing out that high levels of IL-10 were also detected in
a study of asymptomatic infection occurring in Singapore at the
beginning of the COVID-19 pandemic. In this case, the asymp-
tomatic individuals were all young guest laborers who had recently
arrived from Bangladesh (12). Whether the observed ability of
SARS-CoV-2 T cells to produce more IL-10 by can fully explain the
asymptomatic profile of SARS-CoV-2 infection observed in these
groups deserves further study.

Our study has several limitations. First, the study participants
were not selected at random from local communities but rather
were targeted by community health care workers because of the
likelihood of the individuals’ uninfected status and their minimal
contact with major urban centers. The small number of partici-
pants from these communities limits the generalizability of our
findings to the rest of Kenya or perhaps even within these com-
munities. Our comparison group for symptomatic convalescent
individuals consisted of hospitalized Singaporean patients with
mild-to-moderate COVID-19 and nonhospitalized individuals
from these same communities. Finally, as already highlighted,
our pre-pandemic PBMCs were from a healthy Nairobi cohort
and not from individuals from either Kisumu or Elgeyo Marakwet
because pre-pandemic PBMCs from healthy volunteers in these
counties were unavailable.

Our finding that cellular immune assays are more sensitive
than antibody assays in detecting SARS-CoV-2 infection in an
African population in which asymptomatic infections have pre-
dominated implies that seroprevalence surveys might underes-
timate the spread of COVID-19 in Africa (52). This observation,
coupled with the knowledge that coronaviruses have the propensi-
ty to evolve into diseases with pandemic potential, should spur on
the development of simple and scalable cellular immune assays to
test populations for public health purposes. To determine wheth-
er our findings are generalizable, we encourage the public health
research community to conduct similar studies on a much wider
scale. We also observed that the T cell responses of asymptom-
atic residents of these rural Kenyan communities were unusually
directed against SARS-CoV-2’s nonstructural proteins and were
skewed toward an antiinflammatory response. These data sup-
port the call for a more in-depth analysis of the impact of environ-
mental factors on the development of protective or pathological
antiviral immunity against SARS-CoV-2 to understand COVID-19
pathogenesis not only in Africa but around the world.

Methods

Study design
We worked with local health departments and 1 facility each in Kisu-
mu and Elgeyo Marakwet to identify a convenience sample of 40
potential volunteers who lived in rural parts of each county. Partici-
pants enrolled in this cross-sectional study had to be at least 18 years
of age and must not have received a COVID-19 vaccine. In addition,
because the enrollees were intended to be individuals not exposed
to SARS-CoV-2, they must have had no history of respiratory illness
(cough, shortness of breath, fever, or sinus congestion) since Decem-
The Journal of Clinical Investigation

CLINICAL MEDICINE

Abstract

Supernatants were transfected using the logical transformation function for a "multi-specific" response. T cells specific not for a single but multiple SARS-CoV-2 proteins (22), releasing cytokines like IFN-γ (23), IL-2, IL-10) above to 5 pg/mL from the corresponding levels in the peptide pool–stimulated samples. Following the manufacturer's instructions (ProteinSimple), the level of IFN-γ quantified using an Ella machine measuring IFN-γ in plasma was analyzed in the Singapore laboratory. Cytokine concentrations in the plasma were measured using the anti–SARS-CoV-2 NCP ELISA (IgG) test (EUROIMMUN) on samples from the corresponding levels in the peptide pool–stimulated samples. As did the Washington State University IRB. SiVET received ethics approval.

Laboratory procedures

Antibody assays. SARS-CoV-2-neutralizing antibodies were analyzed using a surrogate neutralization assay (cPass, GenScript Biotech) that measures how neutralizing antibodies in the serum bind to the HRP-labeled SARS-CoV-2 RBD and prevent it from binding to the hACE2 protein (19). RBds from 3 different SARS-CoV-2 variants were tested: the ancestral Wuhan strain, the Delta variant, and the Omicron variant. A threshold of 30% inhibition was considered a positive result. Additionally, a 2-step antibody detection test was conducted using the SCoV-2 Detect IgG ELISA (InBios) as the initial test, followed by the EUROIMMUN anti-SARS-CoV-2 ELISA as a confirmatory test for participants who were positive by the InBios test. NP-specific antibodies were tested with the anti-SARS-CoV-2 NCP ELISA (IgG) test (EUROIMMUN).

Cytokine release assays using whole blood. Details of this method are published elsewhere (21). Freshly drawn whole blood (320 μL) was stimulated with 5 distinct 15 mer peptide pools and controls (Figure 2A). After overnight culturing at 37°C with 5% CO₂, the supernatant (plasma) was collected and frozen at −80°C for later shipment to our Singapore laboratory. Cytokine concentrations in the plasma were quantified using an Ellia machine measuring IFN-γ, IL-2, and IL-10, following the manufacturer’s instructions (ProteinSimple). The level of cytokines present in the plasma of DMSO controls was subtracted from the corresponding levels in the peptide pool–stimulated samples. Samples with cytokine quantities (IFN-γ, IL-2, IL-10) above to 5 pg/mL were considered positive. Since SARS-CoV-2 infection usually induces T cells specific not for single but multiple SARS-CoV-2 proteins (22), we scored as “T cell-positive” only the individuals who had a positive response to at least 2 peptide pools for each cytokine tested or a "multi-specific" response.

Subsequently, concentrations of each cytokine in all culture supernatants were transformed using the logical transformation function, and UMAP was run using a 15 nearest neighbors (nn), min_dist of 0.5 and Euclidean distance (54). The results obtained from UMAP analyses were incorporated as additional parameters and converted to FCS files, which were then loaded into FlowJo (BD Biosciences) to generate heatmaps of cytokine secretion on the reduced dimensions.

SARS-CoV-2 peptide–specific T cell quantification by ELISPOT. The frequency of SARS-CoV-2 peptide–specific T cells was quantified as described previously (43). Briefly, cryopreserved PBMCs that had been shipped to Singapore were thawed and stimulated with the following 15 mer peptide pools overlapping by 10 amino acids in ELISPOT plates: structural (NP, membrane, spike) and accessory (ORF3a, ORF7, ORF8). The plates were then incubated with a human biotinylated IFN-γ detection antibody, followed by streptavidin–alkaline phosphatase (streptavidin-AP) and developed using the KPL BCIP/NBT phosphatase substrate (Seracare Life Sciences). The results are expressed as spot-forming cells (SFCs) per 10⁵ PBMCs.

Cell culture for T cell expansion. T cell lines were generated as follows: 20% of the PBMCs were pulsed with 10 μg/mL overlapping SARS-CoV-2 peptides for 1 hour at 37°C and then washed and cocultured with the remaining cells in AIM-V medium (Gibco, Thermo Fisher Scientific) supplemented with 2% AB human serum (Gibco, Thermo Fisher Scientific). T cell lines were cultured for 10 days in the presence of 20 U/mL recombinant IL-2 (R&D Systems).

Flow cytometry. PBMCs were stimulated with peptide pools and expanded in vitro for 10 days as described before (23). Expanded T cell lines were stimulated for 5 hours at 37°C with or without SARS-CoV-2 peptide pools (2 μg/mL). After 1 hour, 10 μg/mL brefeldin A (MilliporeSigma) and 1× monensin (BioLegend) were added. Cells were stained with the yellow LIVE/DEAD fixable dead cell staining kit (Invitrogen, Thermo Fisher Scientific) and the surface markers anti-CD3 (SK7 or OKT3; BioLegend), anti-CD4 (SK3, BD Biosciences), and anti-CD8 (SK1, BD Biosciences). Cells were subsequently fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) and stained with anti–IFN-γ (25723; R&D Systems) and anti–TNF-α (MAB11, BD Biosciences) antibodies and analyzed on a CytoFLEX (Beckman Coulter). Data were analyzed by FlowJo (BD Biosciences).

Statistics

Quantities of IL-10, IL-2, and IFN-γ detected in the different culture supernatants from asymptomatic participants from Elgeyo Marakwet and Kisumu and from symptomatic convalescents from Singapore were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D). Ratios of IL-10/IFN-γ quantities were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D). Ratios of IL-10/IFN-γ quantities were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D). Ratios of IL-10/IFN-γ quantities were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D). Ratios of IL-10/IFN-γ quantities were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D). Ratios of IL-10/IFN-γ quantities were compared using the Friedman test followed by Dunn’s multiple-comparison test (Figure 5D).

Study approval

Written informed consent was received from all participants. The Scientific and Ethics Review Unit of the KEMRI approved the protocol (KEMRI/RES/7/3/1, protocol no. 4186). The Ethics and Research Committee at the US Centers for Disease Control and Prevention relied on the KEMRI approved (CDC) protocol (CDC no. 7353) as did the Washington State University IRB. SiVET received ethics approval.

J Clin Invest. 2023;133(13):e170011 https://doi.org/10.1172/JCI170011
approval from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (P137/03/2015). Blood was also collected 6 months after SARS-CoV-2 infection from convalescent Singaporeans who had symptomatic COVID-19 in 2020 under a COVID-19 PROTECT study protocol, which was approved by the National Healthcare Group (NHG) Domain Specific Review Board (DSRB) (nos. 2012/00917 and NHG DSRB E 2020/00091). All participants provided written informed consent in accordance with the Declaration of Helsinki for Human Research.

Data availability
All data are available upon request.

Author contributions
TS, JBO, AB, and NLB conceptualized the study and designed the experiments. JBO, PAM, AM, JO, GMM, RKB, CO, PM, NT, SKH, and SH recruited participants, collected samples, and performed the experiments. NLB, AB, and TS analyzed the data. NLB, TS, and CAD prepared the figures and the table. AB, TS, AHR, and DCL acquired funding for the project. AB, TS, EO, NLB, KM, OA, FOO, and AHR were involved in project administration. The study was supervised by KM, CO, GMM, OA, AB, and NLB. TS and AB wrote the original draft of the manuscript, which was reviewed and edited by AB, TS, NLB, EO, JO, RKB, CO, and DCL.

Acknowledgments
We appreciate the participation of the volunteers, who made this study possible. Funding for this research was provided by the US Centers for Disease Control and Prevention, Division of Global Health Protection, the Singapore Ministry of Health’s National Medical Research Council under its COVID-19 Research Fund (COVID19RF3-0060, COVID19RF-001 and COVID19RF-008), and the Singapore Ministry of Health’s National Medical Research Council MOH-000019 (MOH-StaR17Nov-0001). The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Centers for Disease Control and Prevention.

Address correspondence to: Antonio Bertoletti, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore. Phone: 65.6601.3574; Email: antonio@duke-nus.edu.sg. Or to: Taraz Samandari, US Centers for Disease Control and Prevention, Kenya, c/o US Embassy, P.O. Box 606-00621, Village Market, Nairobi, 00621, Kenya. Phone: 254.730.485.481; Email: tss0@cdc.gov.


