Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection

Catherine J. Reynolds1†, Leo Swadling2‡, Joseph M. Gibbons3‡, Corrina Pade3‡, Melanie P. Jensen4, Mariana O. Diniz5, Nathalie M. Schmidt2, David K. Butler1, Oliver E. Amin2, Sasha N. L. Bailey1, Sam M. Murray1, Franziska P. Pieper1, Stephen Taylor5, Jessica Jones5, Meleri Jones3,6, Wing-Yiu Jason Lee3, Joshua Rosenheim2, Aneesh Chandran4, George Joy4, Cecilia Di Genova7, Nigel Temperton8, Jonathan Lambourne8, Teresa Cutino-Mougé8, Mervyn Andiapen8, Marianna Fontana10, Angelique Smit10, Amanda Semper9, Ben O’Brien4,11,12, Benjamin Chain2, Tim Brooks5, Charlotte Manisty4,13, Thomas Treibell4,13, James C. Moon4,13, COVIDsortium Investigators†, Mahdad Noursadeghi2, COVIDsortium Immune Correlates Network‡, Daniel M. Altmann14, Mala K. Maini25, Áine McKnight26, Rosemary J. Boyton1†,15,16

Understanding the nature of immunity after mild/asymptomatic SARS-CoV-2 infection is crucial to controlling the pandemic. We analyzed T cell and neutralizing antibody responses in 136 health care workers (HCWs) 16 to 18 weeks after the start of the first U.K. lockdown, 76 of whom had mild/asymptomatic SARS-CoV-2 infection captured by serial sampling. Neutralizing antibodies (nAbs) were present in 89% of previously infected HCWs. T cell responses tended to be lower after asymptomatic infection than in those reporting case-definition symptoms of COVID-19, whereas nAb titers were maintained irrespective of symptoms. T cell and antibody responses were sometimes discordant. Eleven percent lacked nAb and had undetectable T cell responses to spike protein but had T cells reactive to other SARS-CoV-2 antigens. Our findings suggest that most individuals with mild or asymptomatic SARS-CoV-2 infection carry nAb complemented by multispecific T cell responses at 16 to 18 weeks after mild or asymptomatic SARS-CoV-2 infection.

INTRODUCTION

Studies of adaptive immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) include characterization of lethal, severe, and mild cases (1–8). Understanding how long immunity lasts in people who have had mild or asymptomatic infection is crucial. Health care worker (HCW) cohorts exposed to and infected by SARS-CoV-2 during the early stages of the pandemic are an invaluable resource to study this question (9–14). The U.K. COVIDsortium is a longitudinal, London hospital HCW cohort followed from the time of U.K. lockdown on 23 March 2020 (9, 10); weekly nasopharyngeal swabs for SARS-CoV-2 polymerase chain reaction (PCR), serology and serum collection for antibody (Ab) analysis, and a self-reporting health questionnaire allowed capture of mild/asymptomatic infection around the time of onset, so the duration of immunity could be tracked. Most of the healthy people infected with SARS-CoV-2 in the community have not been hospitalized and lack PCR confirmation of infection. A key public health concern is the extent to which immunity in mild or asymptomatic cases may confer protection from future infection (6, 15–18). In this cohort, 21.5% of the 731 HCWs studied had laboratory-confirmed infection, and all were asymptomatic or had mild disease. We conducted a cross-sectional case-controlled substudy (n = 136) to analyze T cell and neutralizing Ab (nAb) immunity at 16 to 18 weeks after U.K. lockdown (table S1). We collected samples from 76 HCWs with laboratory-defined evidence of SARS-CoV-2 infection and 60 HCWs matched for age, gender, and ethnicity that were consistently SARS-CoV-2 PCR-negative and serology-negative. Here, we set out to investigate whether asymptomatic or mild SARS-CoV-2 infection confers specific nAb and T cell responses lasting to 16 to 18 weeks.

RESULTS

SARS-CoV-2 multispecific T cell response

A number of T cell studies investigating SARS-CoV-2 infection have described the presence of T helper 1 (Th1) immunity (7). We assessed SARS-CoV-2 T cell frequencies by interferon-γ (IFNγ)– enzyme-linked immune absorbent spot (ELISpot) using three complementary approaches: whole protein (1), mapped epitope peptide (MEP) pools (4), and overlapping peptide (OLP) pools (table S2) (3). The use of whole protein allows assessment of CD4 T cell responses to naturally processed epitopes, whereas the MEP and OLP pools assessed a combination of CD4 and CD8 T cell responses directed against defined immunogenic regions and unbiased coverage of key viral proteins, respectively.
Analyzing T cell responses to spike and nucleocapsid (N) stimulating with whole protein in HCWs with mild or asymptomatic, laboratory-confirmed infection, only 49% responded to spike, whereas significantly more (85%) responded to N, showing a wide range of frequencies (Fig. 1A). Using MEP pools containing previously mapped immunogenic regions and offering good coverage for regional human leukocyte antigen (HLA) genotypes (4), responses of >80 spot-forming cells (SFCs) per 10^6 peripheral blood mononuclear cells (PBMCs) were found in 69% to peptide pools for spike, N, membrane (M), and open reading frame (ORF) 3a/7a, with the latter being at a significantly lower frequency. Eighty-seven percent of HCWs had detectable T cell responses to these MEP pools (Fig. 1B). A third T cell stimulation platform used OLP pools spanning the whole of N, M, and ORF3a, together with 15-mers spanning immunogenic regions of spike (fig. S2B); using this approach, we assessed multispecificity and cumulative SARS-CoV-2–specific T cell frequencies. This indicated a wide range of cumulative T cell response frequencies, from 0 to >1000 SFCs per 10^6 PBMCs, with 89% showing a detectable T cell response (Fig. 1C). With both the MEP and OLP platforms, responses to ORF3a/7a or ORF3a, respectively, were significantly lower than to other antigens (Fig. 1B and fig. S1A). Although T cell responses to individual regions were relatively weak, their cumulative frequencies across all pools tested were similar in magnitude to that of T cells directed against a pool of well-described CD8 epitopes from influenza, Epstein-Barr virus, and cytomegalovirus (FEC), assessed in parallel in the same donors (fig. S1A) and comparable with frequencies found against SARS-CoV-1 pools after SARS infection (19).

Responses to spike, N whole protein, and spike, N, and M MEP have significantly higher frequency in HCWs with laboratory-confirmed SARS-CoV-2 infection than those in the matched group without laboratory evidence of infection (Fig. 1D and fig. S1, C and D).

Fig. 1. T cell responses to SARS-CoV-2 antigens in HCW (laboratory-confirmed COVID-19) at 16 to 18 weeks after U.K. lockdown. (A to C) Magnitude of T cell response and proportion of HCW with a summed T cell response within the given ranges (0, 1 to 19, 20 to 79, and ≥80 SFCs per 10^6 PBMCs). (A) Spike and N protein (n = 75), (B) MEP (n = 75), and (C) OLP pools (n = 71), ordered by cumulative magnitude in HCW with laboratory-confirmed SARS-CoV-2 infection (n = 75). (D) Proportion of HCW with a T cell response to SARS-CoV-2 individual proteins or peptide pools within given ranges (0, 1 to 19, 20 to 79, and ≥80 SFCs per 10^6 PBMCs) in the following groups: HCW cohort with laboratory-confirmed infection (n = 75); HCW cohort with no laboratory-confirmed infection but with one or more case-definition symptoms (n = 26), non–case-definition symptoms (n = 24), or asymptomatic (n = 9); and pre–COVID-19 pandemic control cohort A (n = 20). (A and B) Bars at geomean. (A) Wilcoxon matched-pairs signed rank test. (B) Friedman multiple comparisons ANOVA with Dunn's correction.
For example, 85 and 49% of HCWs with laboratory-confirmed SARS-CoV-2 had T cell responses to N and spike protein, respectively, compared with 29 and 12% of SARS-CoV-2 PCR–negative and spike subunit 1 (S1) immunoglobulin G (IgG)–negative HCWs (P < 0.0001) (Fig. 1D). T cell recognition of these stimuli in HCWs without evidence of infection, irrespective of reported coronavirus disease 2019 (COVID-19)–like symptoms, was similar to that seen in pre–COVID-19 pandemic controls (Fig. 1D; fig. S1, C and D; and table S1B). The OLP pools (using increased cell numbers) showed detectable T cell responses in the PCR-negative, S1 IgG–negative HCW group (fig. S1B). With every T cell stimulation approach tested, responses were also seen in a proportion of prepandemic controls. Epitope mapping studies will be required to investigate possible cross-reactive components of these responses with other human coronaviruses as other studies have highlighted (2, 3, 20) and to assess the impact of any such cross-reactivity on disease outcome, whether positive or negative (21, 22).

In addition to IFNγ SFCs, we explored other cytokines indicative of non-T1,1 subset polarization by screening supernatants from spike and N protein–stimulated ELISpots; they showed no evidence of interleukin-4 (IL-4), IL-5, IL-13, IL-17, or IL-23 (fig. S1E). IL-2 release followed a similar pattern to that seen in the IFNγ ELISpots. However, tumor necrosis factor–α was released by antigen-stimulated cultures in response to N across the cohort spectrum from those with laboratory-confirmed infection to uninfected HCWs and prepandemic controls, presumably reflecting an amplified response from other cell types, including macrophages and natural killer cells.

In line with previous observations of SARS-CoV-2 T cells and ageing (23), T cell responses in HCWs (n = 75) with laboratory-confirmed SARS-CoV-2 correlated with age. There was a correlation with increasing age and T cell responses against spike MEP2, N1 OLP, and ORF3a/7a MEP (fig. S1E). IL-2 release followed a similar pattern to that seen in the IFNγ ELISpots. To better understand complementarity between nAb and T cells, we next compared the T cell, S1 IgG, and nAb responses in individual HCW with laboratory-confirmed SARS-CoV-2 (n = 76). T cell responses to N and spike protein correlated with peak S1 IgG titer but with weak correlation coefficients partly attributable to lack of T cell responses in some HCWs with positive Ab titers to spike and N protein (Fig. 3A and blue box in fig. S6, A and D). Correlations between peak N IgG/IgM titer and T cell responses to spike and N protein showed similar results (fig. S7A). Just more than half of the HCWs were discordant for T cell and S1 IgG responses, making no T cell response to spike protein, and 15% made no T cell response to N (fig. S6, A to F). Although we found no differences in terms of age, gender, ethnicity, symptom profile, clinical role, or PPE use, there tended to be more nonresponders among Black, Asian, and Minority Ethnic (BAME) HCWs. There was a correlation between the T cell response to spike protein (r = 0.482, P < 0.0001), spike MEP (r = 0.412, P < 0.0001), and peak S1 IgG titer across all the HCWs studied (those with and without laboratory-confirmed SARS-CoV-2 infection, n = 133) (fig. S7B). T cell responses to spike protein (r = 0.466, P < 0.0001) and spike MEP (r = 0.343, P < 0.0001) also correlated with nAb (IC50) (fig. S7B) but with some discordance.

In Fig. 2B, we showed that 11% of infected HCWs lacked detectable nAb at 16 to 18 weeks after U.K. lockdown. To understand the complementarity between T cell and nAb responses in individual HCW, we analyzed responses of all HCWs ranked either by nAb IC50 titer or cumulative T cell response. We first arrayed HCW...
responses ranked by magnitude of nAb response (Fig. 3B). Neutralization IC_{50} values for all HCWs were plotted in relation to an indicative, protective cutoff value of >200 (dashed horizontal red line in bottom panel). HCWs lacking detectable nAb are indicated by eight black arrows on the left. Their cumulative T cell response frequencies against viral antigens are shown in the panel above and are sometimes relatively low. Examining the converse, we then arrayed HCW responses ranked by magnitude of nAb response (Fig. 3C). From this plot, HCWs with the lowest cumulative T cell response (to the left of the plot) have a range of nAb responses from none to >200 IC_{50}. One young, asymptomatic, female HCW with a good peak S1 IgG titer had no T cell response to any antigens tested but made nAbs with a titer of 143 (Fig. 3, B and C, indicated by +). Another female HCW with a good S1 IgG titer also had no T cell response to any antigens tested but made nAbs with a titer of 747 (Fig. 3, B and C, indicated by *).

Of the 76 HCWs studied with mild or asymptomatic laboratory-confirmed SARS-CoV-2 infection, 64% had one or more case-defining symptoms, 25% had non-case-defining symptoms, and 11% were asymptomatic. Looking at T cell immunity and nAb levels across these symptom-stratified groups at 16 to 18 weeks, T cell responses tended to be higher in infected HCWs with case-defined symptoms. Responses to M MEP and ORF3a OLP were significantly higher in HCWs reporting case-definition symptoms than those that were asymptomatic (Fig. 3D) with 65, 68, and 63%,

### Fig. 2. nAb responses to SARS-CoV-2 antigens in HCW (laboratory-confirmed COVID-19) at 16 to 18 weeks after U.K. lockdown.

(A) Peak S1 IgG Ab titer and peak N IgG/IgM Ab titer across the study period in HCW with laboratory-confirmed SARS-CoV-2 infection (n = 76). (B) The distribution of nAb (IC_{50}) titers across the cohort of HCW with laboratory-confirmed infection and (C) the proportion of HCW with an undetectable (0 to 49), low (50 to 199), or high (200+) nAb titer (IC_{50}). (D) Correlation between peak S1 IgG Ab titer (left) or the peak N IgG/IgM Ab titer (right) and nAb titer (IC_{50}) in HCW with laboratory-confirmed SARS-CoV-2 infection. (E and F) Peak S1 IgG Ab titer (E) and nAb titer (IC_{50}) (F) in HCW with laboratory-confirmed infection, stratified by symptoms: ≥1 COVID-19 case-definition symptoms (red), non-case-definition symptoms (blue), or asymptomatic (gray) throughout the trial and within 3 months of trial initiation. (G) The proportion of HCW with an undetectable (0 to 49), low (50 to 199), or high (200+) nAb titer (IC_{50}) within specified age ranges: 20 to 29 years (n = 26), 30 to 39 years (n = 26), 40 to 49 years (n = 16), and ≥50 years (n = 21). (H) Correlations of age versus peak S1 IgG Ab titer (left) and nAb titer (IC_{50}) (right) in HCW with laboratory-confirmed SARS-CoV-2 infection separated by gender (female, black symbols; male, open symbols). (D and H) Spearman’s rank correlation, least-squares log-log lines shown. (A, B, E, and F) Bars at geometric mean. (E and F) Kruskal-Wallis multiple comparison ANOVA with Dunn’s correction. n.s., not significant.
Furthermore, there were OLP T cell responses in six of eight HCWs lacking nAb (Fig. 4D). Thus, HCWs lacking nAb tend to lack responses to spike while maintaining low-frequency T cells to other specificities. Assessing T cell responses ranked simply on the basis of presence or absence of recognition of proteins and peptide pools (rather than magnitude of response) indicates that those lacking a nAb response (black arrows) showed T cell responses against one to five antigens (Fig. 4E). Together, the data show discordance of nAb and T cell responses in individual HCW.

We looked in more detail at the data for some of the HCWs showing discordant elements of adaptive immunity (fig. S9). There was a strong correlation between nAb IC_{50} and contemporaneous EUROIMMUN S1 IgG titer, yet thorough scrutiny of the plot also revealed a significant minority of individuals with discordant responses (fig. S9A). This discordance between S1 binding Ab titers and neutralization may in part be due to conformational differences between S1 protein in ELISA and virally expressed spike protein or to the presence of nAbs targeting S2 (33, 34). Two individuals with laboratory-confirmed SARS-CoV-2 and a positive EUROIMMUN assay showed no detectable nAb response (fig. S9, B and C). One of these showed no T cell response to spike, N protein, or MEP, whereas the other showed a modest T cell response. Twelve HCWs with...
Fig. 4. Discordant T cell and nAb responses broken down by T cell antigen. (A to D) Top: Magnitude of the T cell response to spike protein \( (n = 75) \) (A); cumulative magnitude of T cell responses to spike protein and spike MEP and MEP2 pools \( (n = 70) \) (B); N protein and N, M, and ORF3a/7a MEP pools \( (n = 75) \) (C); or N1, N2, M, and ORF3a OLP pools \( (n = 70) \) (D) ordered by increasing cumulative magnitude of T cell responses in HCW with laboratory-confirmed SARS-CoV-2 infection. Bottom: nAb titers (IC50) in HCW with laboratory-confirmed SARS-CoV-2 infection, ordered by corresponding top panel. (E) The number of reactive SARS-CoV-2 proteins or peptide pools (top) and nAb titer (IC50) (bottom) in HCW with laboratory-confirmed SARS-CoV-2 infection \( (n = 70) \). Top panel ordered by cumulative magnitude; bottom panel ordered by top panel. HCW with no nAb (IC50 titer of less than 50) is indicated by black arrows. + and * denote two individuals with no T cell response to any protein or peptide pool.
A limitation of our study is the fact that T cell and nAb responses were only measured at the 16- to 18-week cross-sectional time point. Ideally, T cell and nAb responses would be measured longitudinally to capture peak responses occurring in the period after infection and any differential decline in responses resulting from variations across individuals as seen with Ab binding responses (24). Another caveat is the fact that because of limitations of blood sample volume, we only used one detection system to measure T cell responses, the IFNγ ELISpot. Other laboratories have opted to elicit low-frequency responses by prior expansion in peptides with IL-2. We considered that such expansion might obviate our ability to draw direct conclusions about response frequencies.

A cautionary note about the ephemeral nature of adaptive immunity to coronaviruses comes from data for annual reinfections with the four seasonal coronaviruses and emerging data for reinfection by SARS-CoV-2 (42, 43). Some studies have raised concern about the durability of serum Abs and B cell memory, with data pointing toward impaired germinal center reactions in severe acute COVID-19 (35). Other studies have focused on the potential for rapid waning of nAb after mild SARS-CoV-2 infection (14, 15). However, we find nAb detectable in most HCWs sampled 16 to 18 weeks after mild/asymptomatic infection. Some T cell data indicate that even asymptomatic people and household contacts develop low-frequency T cell responses, in line with results from the HCWs without laboratory-confirmed infection using one of our platforms with higher T cell numbers (6). We show here that infected HCWs can display highly heterogeneous T cell recognition of epitopes from diverse SARS-CoV-2 structural and nonstructural proteins, but it is not yet possible to decode the differential impacts of these responses for protection. Analysis of T cell response repertoire in convalescent, hospitalized patients with COVID-19 argues that the breadth of T cell response is a marker of mild disease (44).

Although Ab and/or T cell data have been reported in several settings (1–8, 13–16, 23–31), many studies lack the granularity to relate binding Ab, nAb, and broad-range T cell response analysis to long-term immunity after asymptomatic or mild disease—the common COVID-19 experience of most individuals. Our cohort study highlights the heterogeneity of immune memory in exposed individuals with mild or asymptomatic infection, cautioning against simple assertions about “typical” responses in most people. The cohort shows discordance between nAb and T cell responses with some individuals showing good nAb responses alongside low T cell responses and vice versa. When T cell responses are present, they are of variable frequency and specificity. There are also HCW lacking evidence of seroreactivity by SI EUROIMMUN assay yet with evidence of a positive nAb response. Although we find relative discordance of responses in our study comprising individuals at 4 months after infection, other reports of concordance either between SI Ab and neutralization or between neutralization and spike CD4 T cell responses tended to analyze within the first weeks after infection (2, 45).
In summary, we find that in most of these working adults, there is immunity at 16 to 18 weeks comprising nAb (often at a level likely to protect), usually complemented by multispecific T cell responses. Understanding protective immunity in the population will require simultaneous scrutiny of T cell and Ab responses.

MATERIALS AND METHODS

Study design

We conducted a cross-sectional case-controlled substudy of 136 hospital-based HCWs at 16 to 18 weeks after U.K. lockdown (Fig. 5). Seventy-six HCWs with mild/asymptomatic laboratory-confirmed SARS-CoV-2 infection captured by weekly SARS-CoV-2 PCR and EUROIMMUN/Roche Ab tests were recruited. An age-, sex-, symptom-, and ethnicity-matched group of 60 HCWs with similar exposure that remained SARS-CoV-2 PCR–negative and EUROIMMUN/Roche Ab test–negative throughout the 16-week follow-up period was also recruited. The HCWs completed a symptom diary and were divided into those that reported one or more case-definition symptoms, non–case-definition symptoms, or were asymptomatic during the 16-week follow-up period and in the 3 months before the start of the study. The main objective of the study was to investigate T cell and nAb immunity to SARS-CoV-2 infection in asymptomatic/mild COVID-19 in a working adult cohort.

Ethics statement

The COVIDsortium HCW bioresource was approved by the ethical committee of the U.K. National Research Ethics Service (20/SC/0149) and registered on ClinicalTrials.gov (NCT04318314). The study conformed to the principles of the Helsinki Declaration, and all participants gave written informed consent.

Prepandemic healthy donor samples were collected and cryopreserved before October 2019 (table S2). Prepandemic cohort A and B samples were recruited under ethics numbers 17/LO/0800 and 11/LO/0421, respectively.

COVIDsortium HCW participants

Adult HCWs (>18 years old) from a range of clinical settings who self-declared as fit to attend work were invited to participate via local advertisement of the project (see https://covid-consortium.com). Full study details of the bioresource (participant screening, study design, sample collection, and sample processing) have been previously published (10).

A cohort of 400 HCWs was initially recruited from St. Bartholomew’s Hospital, London, in the week of U.K. lockdown (23 to 31 March 2020). All participants were asymptomatic and self-declared fit to attend work in hospital. Recruitment was extended (27 April to 7 May 2020) to include 331 additional participants from multiple sites: St. Bartholomew’s Hospital (n = 101 additional), National Health Service (NHS) Nightingale Hospital (n = 10), and Royal Free NHS Hospital Trust (n = 220).

A prospective, observational, and longitudinal cohort design was used and consisted of questionnaires exploring demographics, clinical and exposure risks, and sample collection at baseline and weekly follow-up for 15 weeks from the start of each cohort. Participants were asked to provide details and timing of symptoms in the 3 months before baseline, and for those who were unable to attend follow-up visits (because of shift rostering, annual leave, or self-isolation), the reason for nonattendance was collected to ensure capture of information regarding isolation due to participant symptoms or household contacts. On return from self-isolation with symptoms, convalescent samples were collected. Further follow-ups at 6 and 12 months are planned.

Complete details of the sampling protocol have been previously published (10). Initial analysis of samples for determining SARS-CoV-2 infection included nasal RNA stabilizing swabs baseline and weekly with reverse transcription PCR (RT-PCR): Roche cobas SARS-CoV-2 test; Ab testing baseline and weekly: IgG Ab assay to spike protein S1 antigen (EUROIMMUN Anti–SARS-CoV-2 ELISA) and anti-N total Ab assay [Roche Elecsys Anti–SARS-CoV-2 electrochemiluminescence immunoassay (ECLI)]). Ab ratios of >1.1 were considered test positive for the EUROIMMUN SARS-CoV-2 ELISA, and those of >1 were considered test positive for the Roche Elecsys anti–SARS-CoV-2 ECLIA after published Public Health England (PHE) evaluations (46, 47).

At baseline, information relating to demographics and exposures was collected via a standardized questionnaire. Mean age of the cohort (n = 731) was 38 ± 11 years; 33% are male, 31% nurses, 20% doctors, and 19% work in intensive care units. COVID-19–associated risk factors were 37% BAME, 18% smokers, 13% obesity, 11% asthma, 7% hypertension,
and 2% diabetes mellitus (10). At weekly follow-up visits, information relating to symptom burden was recorded using a standardized questionnaire. Symptoms were classified as follows: “case-defining” (fever, new continuous dry cough, or a new loss of taste or smell), “non–case-defining” (specific symptoms other than case-defining symptoms or unspecified symptoms), or asymptomatic (no symptoms reported) [case definition for COVID-19, as of 29 May 2020 European Centre for Disease Prevention and Control (www.ecdc.europa.eu/en/covid-19/surveillance/case-definition)].

A total of 731 HCWs underwent 16 weeks of serial assessment (attending unless ill, self-isolating, on holiday, or redeployed). Across the main study cohort, 48 participants had positive RT-PCR results with 157 (21.5%) seropositive participants. Infections were asymptomatic or mild with only two hospital admissions (neither requiring intensive care admission, both discharged well). The cohort therefore represents working age community COVID-19 rather than hospitalized COVID-19.

In London, the case-doubling time in March 2020 was about 3 to 4 days. The number of nasal swabs testing positive for SARS-CoV-2 in our study peaked at 23 to 31 March 2020, suggesting that infections peaked on or around 23 March 2020, the day of U.K. lockdown. We thus observed approximately synchronous infections coincident with the peak epidemic transmission in London at the start of the study, U.K. lockdown on 23 March, and therefore used this as the benchmark starting point for our analysis of T cell and nAb responses in the first wave (24).

The cross-sectional case-controlled substudy (n = 136) collected samples at 16 to 18 weeks after U.K. lockdown (Fig. 5 and table S1). The cross-sectional case-controlled substudy included 76 HCWs (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys). Fifty-seven percent reported one or more case-defining COVID-19 symptoms. Twenty-four percent reported non–case-defining symptoms, and 19% were asymptomatic at baseline, during the 16-week follow-up, or in the 3 months before baseline. A second age-, gender-, and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 39 years; 36% male) who were SARS-CoV-2 PCR–negative and ethnicity-matched subgroup of 60 HCWs were recruited (mean age, 41 years; 36% male) with laboratory-defined evidence of SARS-CoV-2 either by SARS-CoV-2–positive PCR and/or positive for spike IgG (EUROIMMUN ELISA)/N IgG/IgM Ab (Roche Elecsys) tests throughout the 16-week follow-up. However, 44% reported one or more case-defining COVID-19 symptoms, 41% were non–case-defining symptoms, and 15% were asymptomatic at baseline, during the 16-week follow-up, and in the 3 months before baseline. There was no significant difference in T cell or nAb responses measured in HCWs with laboratory-confirmed SARS-CoV-2 that were recruited into the parent study via cohort 1 or 2 (fig. S10).

Isolation of PBMCs
PBMCs were isolated from heparinized blood samples using Pancell (Pan Biotech) or Histopaque-1077 Hybi-Max (Sigma–Aldrich) density gradient centrifugation in SepMate tubes (STEMCELL) according to the manufacturer’s specifications. Isolated PBMCs were cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO) and stored in liquid nitrogen.

Isolation of serum
Whole blood samples were collected in SST Vacutainers (VACUETTE, #455092) with inert polymer gel for serum separation and clot activator coating. After centrifugation at 1000g for 10 min at room temperature, serum layer was aliquoted and stored at −80°C for specific SARS-CoV-2 Ab titer detection by ELISA and for SARS-CoV-2 spike pseudo-typed virus neutralization assays.

SARS-CoV-2–specific Ab titer
SARS-CoV-2 Ab testing was carried out in the laboratories of PHE, UK using two commercial assays following the manufacturers’ instructions. The EUROIMMUN anti–SARS-CoV-2 ELISA IgG measures serum IgG against SARS-CoV-1 S1 antigen (46), and the Roche Elecsys Anti–SARS-CoV-2 ECLIA measures serum Ab (including IgG) directed against the SARS-CoV-2 N (47). The EUROIMMUN ELISA was carried out using a STRATEGEM GEMINI automated microplate processor. Raw optical density (OD) at 450-nm readings was adjusted by calculating the ratio of the OD of control or participant sample divided by the calibrator OD. A ratio of ≥1.1 was deemed positive. A ratio of 11 was taken to be the upper threshold as the assay saturates beyond this point. The Roche ECLIA was performed using a Roche Cobas e801 immunoassay analyzer. Results were expressed as a cutoff index (COI) calculated by the analyzer software as the electrochemiluminescence signal obtained from the participant sample divided by the lot-specific cutoff value. A COI value of ≥1 was deemed positive. Across their dynamic range, the semiquantitative indices of both assays approximate to a linear relationship with Ab level (24).

Recombinant proteins
The SARS-CoV-2 S1 spike antigen and N proteins were obtained from the Centre for AIDS Reagents (CFAR) and National Institute for Biological Standards and Control (NIBSC), UK and consisted of SARS-CoV-2 nucleoprotein and S1 spike antigen from P. Cherepanov, Francis Crick Institute, UK.

Mapped epitope pools
Pools of 13 to 20-mer peptides based on the protein sequences of SARS-CoV-2 S1 (spike), N, M, and ORF3a/7a described previously were synthesized (GL Biochem Shanghai Ltd., China) (4). To stimulate PBMCs, separate pools of sequences for spike (18 peptides), N (10 peptides), M (6 peptides), and ORF3a/7a (7 peptides) were used (table S2). A second mapped epitope pool of SARS-CoV-2 S1 peptides (spike MEP2) based on alignment of all sequences of published SARS-CoV-1 epitopes (www.iiedb.org; search criteria: positive assays only, T cells assays, host: human) with the spike–SARS-CoV-2 sequence and 15-mer peptides synthesized to cover the homologous sequences. In addition, we synthesized 15-mer peptides covering the predicted SARS-CoV-2 spike epitopes (3) to give a total of 55 peptides in this pool (spike MEP2) (table S2).

Overlapping peptide pools
Fifteen-mer peptides overlapping with 10 amino acids spanning the entire protein sequence of SARS-CoV-2 N, M, and ORF3a were synthesized (GL Biochem Shanghai Ltd.) (table S2). To stimulate PBMCs, the peptides were divided into four pools covering N (N1 and N2, 41 peptides each), M (43 peptides), and ORF3a (53 peptides).

IFNγ-ELISPOT assay
Unless otherwise stated, culture medium for human T cells was sterile 0.22 μm filtered RPMI 1640 medium (Gibco-BRL) supplemented with 10% by volume heat-inactivated FBS (1 hour at 64°C; HyClone) and 1% by volume 100× penicillin-streptomycin solution.
(Gibco-BRL). For experiments involving T cell stimulation with proteins or MEP peptide pools, precoated ELISpot plates (Mabtech, 3420-2APT) were washed four times with sterile phosphate-buffered saline (PBS) and were blocked with R10 for 1 hour at room temperature. Two hundred thousand PBMCs were seeded in R10 per well and were stimulated for 18 to 22 hours at 37°C with 5% CO2 with SARS-CoV-2 recombinant proteins (10 μg/ml) or MEP pools (10 μg/ml per peptide). Internal plate controls were R10 alone (without cells) and anti-CD3 (Mabtech, mAb CD3-2). At the end of the stimulation period, cell culture supernatants were collected and stored for later cytokine analysis by LumineX and ELISA. ELISpot plates were developed with human biotinylated IFNγ detection Ab, directly conjugated to alkaline phosphatase (1 μg/ml; Mabtech, 7-B6-1-ALP), diluted in PBS with 0.5% FBS, incubating 100 μl per well for 2 hours at room temperature. This was followed by 100 μl per well of sterile filtered bromochloroindolyl phosphate–nitro blue tetrazolium (BCIP-NBT)–plus phosphatase substrate (Mabtech) for 5 min at room temperature. Plates were washed in double-distilled water (ddH2O) and left to dry completely before being read on Autoimmun Diagnostika (AID) ELISpot plate reader. For experiments involving T cell stimulation with OLP peptide pools and spike MEP2 pool, ELISpot plates (Merck Millipore, MSIP4510) were coated with human anti-IFNγ Ab (10 μg/ml; Mabtech, 1-D1K) in PBS overnight at 4°C. Plates were washed six times with sterile PBS and were blocked with R10 for 2 hours at 37°C with 5% CO2. PBMCs were thawed and rested in R10 for 3 hours at 37°C with 5% CO2 before being counted. A total of 400,000 PBMCs were seeded in R10 per well and were stimulated for 16 to 20 hours with SARS-CoV-2 OLP pools or spike MEP2 pool (2 μg/ml per peptide). Internal plate controls were R10 alone (without cells) and two DMSO wells (negative controls), concanavalin A (ConA; positive control; Sigma-Aldrich) and FEC (HLAI-restricted peptides from influenza, Epstein-Barr virus, and cytomegalovirus; 1 μg/ml per peptide). ELISpot plates were developed with human biotinylated IFNγ detection Ab (1 μg/ml; Mabtech, 7-B6-1) for 3 hours at room temperature, followed by incubation with goat anti-biotin alkaline phosphatase (1:1000; Vector Laboratories) for 2 hours at room temperature, both diluted in PBS with 0.5% bovine serum albumin by volume (Sigma-Aldrich), and lastly with 50 μl per well of sterile filtered BCIP-NBT phosphatase substrate (Thermo Fisher Scientific) for 7 min at room temperature. Plates were washed in ddH2O and left to dry overnight before being read on an AID classic ELISpot plate reader (Autoimmun Diagnostika GmbH, Germany).

Analysis of ELISpot data was performed in Microsoft Excel. The average of two R10-alone wells or DMSO (Sigma-Aldrich) wells was subtracted from all peptide-stimulated wells, and any response that was lower in magnitude than 2 SDs of the sample specific control wells was not considered a peptide-specific response. Results were expressed as difference in ΔSFCs per 10⁶ PBMCs between the negative control and protein/peptide stimulation conditions. We excluded the results if negative control wells had >100 spot-forming cells per 10⁶ PBMCs or positive control wells (ConA or anti-CD3) were negative. Results were plotted using Prism v. 7.0e and 8.0 for Mac OS (GraphPad).

Cytokine measurement

Concentrations of IL-2 and tumor necrosis factor–α in cell culture supernatants in response to PBMC stimulation with spike or N protein were measured by ELISA using Duo-set Ab pairs and standards (Bio-Technne). OD measurements were performed on a FLUOStar Omega microplate reader (BMG LABTECH). Concentrations of IL-4, IL-5, IL-13, IL-17a, and IL-23 were measured by multiplex Luminex assay (Bio-Technne) on a Bio-Plex 200 instrument (Bio-Rad Laboratories Ltd). Cytokine levels were calculated in Microsoft Excel and concentrations for protein-stimulated samples obtained by subtracting values for medium-only controls. Standard curves were plotted using Prism 8.0 for Mac OS (GraphPad).

Cell lines

Human embryonic kidney (HEK) 293T and Huh7 [both American Type Culture Collection (ATCC)] were cultured and maintained in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) and supplemented with GlutaMAX, 10% (v/v) heat-inactivated FBS (56°C for 30 min), penicillin (100 IU/ml), and streptomycin (100 μg/ml). Cell lines were cultured at 37°C with 5% CO2.

Production and titration of SARS-CoV-2 pseudo-typed lentiviral reporter particles

Pseudo-type stocks were prepared by linear polyethyleneimine 25K (Polysciences) cotransfection of HEK-293T (ATCC) with SARS-CoV-2 spike pcDNA expression plasmid, HIV gag-pol p8.91 plasmid and firefly luciferase expressing plasmid pCMV6W at a 1:1.5 ratio (47, 48). A total of 2.5 × 10⁶ cells/cm² were plated 24 hours before transfection in 60-cm² cell culture dishes. Forty-eight and 72 hours after transfection, pseudo-type–containing culture medium was harvested and centrifuged at 300g for 5 min to clear cell debris. Aliquots were stored at −80°C. Tissue culture infectious dose assays were performed by transduction of Huh7 cells to calculate the viral titer and infectious dose for neutralization assays. p24 ELISA was also used to determine input concentration.

p24 ELISA

Pseudo-type stock concentrations were determined by ELISA for p24 protein concentration as previously described (49). White ELISA plates were precoated with sheep anti–HIV-1 p24 Ab (5 μg/ml; Aalto Bio Reagents) at 4°C overnight. Pseudo-viral supernatants were treated with 1% Empigen BB (Merck) for 30 min at 56°C and then plated at 1:10 dilution in tris-buffered saline (TBS) on precoated plates and incubated for 3 hours at room temperature. Alkaline phosphatase–conjugated mouse anti–HIV-1 p24 monoclonal Ab (Aalto Bio Reagents) in TBS, 20% (v/v) sheep serum, and 0.05% (v/v) Tween 20 was then added and incubated for 1 hour at room temperature. After four washes with PBS with 0.01% (v/v) Tween 20 and two washes with ELISA Light washing buffer (Thermo Fisher Scientific), CSPD substrate with Sulfate II enhancer (Thermo Fisher Scientific) was added and incubated for 30 min at room temperature before chemiluminescence detection using a CLARIOStar microplate reader (BMG LABTECH).

Pseudo-typed SARS-CoV-2 neutralization assays

SARS-CoV-2 pseudo-type neutralization assays were conducted using pseudo-typed lentiviral particles as previously described (48–51). The pseudo-type virus assay used here was developed, characterized, and validated to detect live virus by ourselves and one of the authors previously (36). Serum was heat-inactivated at 56°C for 30 min to remove complement activity. Serum dilutions in DMEM were performed in duplicate in white, flat-bottom, 96-well plates (Thermo Fisher Scientific, #136101) with a starting dilution of 1:20
and seven consecutive twofold dilutions to a final dilution of 1:2560 in a total volume of 100 µl. SARS-CoV-2 pseudo-typed lentiviral particles [1 × 10^5 relative light units (RLUs)] were added to each well and incubated at 37°C for 1 hour. Eight control wells per plate received pseudo-type and cells only (virus control), and another eight wells received cells only (background control). Negative controls of pooled prepanademic sera, collected before 2008, and a positive neutralizer were spaced throughout the plates. RLUs for each well were standardized against technical positive (virus control) and negative (cells only) controls on each plate to determine a percentage neutralization value. A total of 4 × 10^6 Huh7 cells suspended in 100 µl of complete media were added per well and incubated for 72 hours at 37°C and 5% CO2. Firefly luciferase activity (lumin 100 µl of 2.2 × 10^6 plaque-forming units in a volume of 10 ml of DMEM and 10% FBS. Flasks were observed daily, and vi-rus-containing cell culture medium was harvested when >80% of cells showed cytopathic effect. Supernatant was centrifuged at 500g for 5 min to clear cell debris, and aliquots were stored at −80°C. To determine the titer of SARS-CoV-2 virus stocks, VeroE6 cells were seeded at 3 × 10^4 cells per well in 48-well plates. After 24 hours, adherent cell monolayers were challenged with serial 1:10 duplicate dilutions of virus, and titer was assessed after 20 hours by extracellular staining to identify foci of infection. Cells were washed in PBS, fixed in ice-cold methanol:acetone (50:50), and virus antigen was stained using sera from convalescent individuals diluted 1:2000 in PBS and 1% FBS for 1 hour at 37°C. Cells were washed a further three times in PBS and incubated with goat anti-human IgG β-galactosidase–conjugated Ab (Southern Biotech, #2040-06) diluted 1:400 in PBS and 1% FBS for 1 hour at 37°C. After three further PBS washes, 300 µl of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside chromogenic substrate (0.5 mg/ml) in PBS containing 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, and 1 mM magnesium chloride was added to each well. Infected cells incubated at 37°C were stained blue within 1 and 4 hours after addition of substrate, and clusters of blue cells were counted as foci of infection to determine the virus titer defined as focus forming units (FFUs) per milliliter.

**Authentic SARS-CoV-2 and titration**

SARS-CoV-2 strain 2019-nCoV/BavPat1/2020 authentic virus cell culture supernatant (isolate collection date 1 January 2020) was purchased from the European Virus Archive Global (Charité Universitätsmedizin Berlin, Germany). VeroE6 were seeded in 75-cm² cell culture flasks 24 hours before inoculation with virus cell culture supernatant containing 2.2 × 10^6 plaque-forming units in a volume of 10 ml of DMEM and 10% FBS. Flasks were observed daily, and virus-containing cell culture medium was harvested when >80% of cells showed cytopathic effect. Supernatant was centrifuged at 500g for 5 min to clear cell debris, and aliquots were stored at −80°C. To determine the titer of SARS-CoV-2 virus stocks, VeroE6 cells were seeded at 3 × 10^4 cells per well in 48-well plates. After 24 hours, adherent cell monolayers were challenged with serial 1:10 duplicate dilutions of virus, and titer was assessed after 20 hours by extracellular staining to identify foci of infection. Cells were washed in PBS, fixed in ice-cold methanol:acetone (50:50), and virus antigen was stained using sera from convalescent individuals diluted 1:2000 in PBS and 1% FBS for 1 hour at 37°C. Cells were washed a further three times in PBS and incubated with goat anti-human IgG β-galactosidase–conjugated Ab (Southern Biotech, #2040-06) diluted 1:400 in PBS and 1% FBS for 1 hour at 37°C. After three further PBS washes, 300 µl of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside chromogenic substrate (0.5 mg/ml) in PBS containing 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, and 1 mM magnesium chloride was added to each well. Infected cells incubated at 37°C were stained blue within 1 and 4 hours after addition of substrate, and clusters of blue cells were counted as foci of infection to determine the virus titer defined as focus forming units (FFUs) per milliliter.

**Authentic SARS-CoV-2 microneutralization assays**

VeroE6 cells were seeded at 2 × 10^4 cells per well in a clear, flat-bottom, 96-well tissue culture plate 24 hours before infection. Participant serum was heat-inactivated for 30 min at 56°C to remove complement activity. Serum dilutions in DMEM were performed in duplicate in clear, U-bottom, 96-well plates with a starting dilution of 1:20 and seven consecutive twofold dilutions to a final dilution of 1:2560 in a total volume of 50 µl per well. SARS-CoV-2 virus (3 × 10^4 FFUs) was added to each serum dilution and incubated at 37°C for 1 hour. After incubation, serum/virus preparations were transferred into cell culture plates containing semiconfluent VeroE6 monolayers. Each plate had eight control wells with virus and cells only (virus control) and another eight wells with cells only (background only). Plates were incubated (37°C and 5% CO2) for 72 hours, after which supernatants were removed and wells were washed with PBS. Cells were fixed with 100 µl of 3.7% (v/v) formaldehyde for 1 hour. After two further PBS washes, cells were stained with 0.2% (w/v) crystal violet solution for 10 min. Plates were washed four times in distilled water to remove excess crystal violet and left to air dry. Crystal violet staining was resolubilized by addition of 100 µl of 1% (w/v) SDS solution to each well and incubated at 37°C for 10 min. Absorbance readings were taken at 570 nm using a CLARIOStar plate reader (BMG LABTECH). Negative controls of pooled prepanademic sera, collected before 2008, and serum from a neutralization-positive SARS-CoV-2 convalescent individual were spaced throughout the plates. Absorbance readings for each well were standardized against technical positive (virus control) and negative (cells only) controls on each plate to determine a percentage neutralization value. An average neutralization was calculated across the two sample replicates for each serum dilution. Neutralization curves for each serum tested were plotted, with the percentage neutralization modeled as a logistic function of the serum dilution factor (log10). A nonlinear regression (curve fit) method was used to determine the dilution fold that neutralized 50% (IC50). We classified positive samples as those with an IC50 of greater than 49. SARS-CoV-2 is classified as a hazard group level 3 facility.

**Statistics and reproducibility**

Data were assumed to have a non-Gaussian distribution. Nonparametric tests were used throughout. For single paired and unpaired comparisons, Wilcoxon matched-pairs signed-rank test and a Mann-Whitney U test were used. For multiple paired and unpaired comparisons, Friedman multiple comparisons, analysis of variance (ANOVA) with Dunn’s correction or Kruskall-Wallis one-way ANOVA with Dunn’s correction were used. For correlations, Spearman’s r test was used. P < 0.05 was considered significant. Prism v. 7.0e and 8.0 for Mac were used for analysis.

**SUPPLEMENTARY MATERIALS**

immunology.sciencemag.org/cgi/content/full/5/54/eabf3698/DC1

Figs. S1 to S10
Tables S1 to S4

View/request a protocol for this paper from Bio-protocol.

**REFERENCES AND NOTES**


Submitted 21 October 2020
Accepted 18 December 2020
Published First Release 23 December 2020
Final published 15 July 2021
10.1126/sciimmunol.abf3698

Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection


First published 23 December 2020
DOI: 10.1126/sciimmunol.eabf3698