Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose

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SARS-CoV-2 vaccine rollout has coincided with the spread of variants of concern. We investigated if single dose vaccination, with or without prior infection, confers cross protective immunity to variants. We analyzed T and B cell responses after first dose vaccination with the Pfizer/BioNTech mRNA vaccine BNT162b2 in healthcare workers (HCW) followed longitudinally, with or without prior Wuhan-Hu-1 SARS-CoV-2 infection. After one dose, individuals with prior infection showed enhanced T cell immunity, antibody secreting memory B cell response to spike and neutralizing antibodies effective against B.1.1.7 and B.1.351. By comparison, HCW receiving one vaccine dose without prior infection showed reduced immunity against variants. B.1.1.7 and B.1.351 spike mutations resulted in increased, abrogated or unchanged T cell responses depending on human leukocyte antigen (HLA) polymorphisms. Single dose vaccination with BNT162b2 in the context of prior infection with a heterologous variant substantially enhances neutralizing antibody responses against variants.

During worldwide rollout of SARS-CoV-2 vaccines it is vital to understand how vaccination influences immune responses and protection among those who have had prior natural SARS-CoV-2 infection. This is a knowledge-gap since a history of previous infection was an exclusion criterion in Phase III vaccine trials (*1*). Countries have adopted diverse approaches, including the UK policy to maximize deployment of first doses to the largest possible numbers by extending the time interval to second dose. At the end of 2020, it became apparent several virus variants had emerged (*2*, *3*) and that these might impact vaccine rollout. The B.1.1.7 variant, possessing the spike N501Y mutation, first emerged in the UK in December 2020 and spread rapidly (*4*). Additional variants of concern (VOC) include the B.1.351 variant, which emerged at about the same time in South Africa and the P.1 variant that emerged in January 2021 in Brazil. Both have the N501Y

mutation, in addition to E484K, the latter implicated in escape from neutralizing antibody (nAb) (*5*, *6*).

The Pfizer/BioNTech mRNA vaccine BNT162b2 encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein modified by two proline substitutions (*1*, *7*, *8*). A two-dose regimen of 30 μg BNT162b2, 21d apart, confers 95% protection against Wuhan-Hu-1 SARS-CoV-2 (*1*), eliciting high nAb titers, as well as CD4 cell and CD8 responses (*8*). When given as a single 60 μg dose, BNT162b1 induced virus Ab neutralization, but T cell responses were reduced compared with the standard prime-boost regime (*8*). Single 30 μg dose, BNT162b1 was not reported beyond d21. However, the cumulative incidence of COVID-19 cases among 21,676 placebo and 21,699 vaccine recipients diverged 12 days after the first dose, indicating possible early-onset first dose protection (*1*). For those who were previously infected, single

dose vaccination may act as a boost following natural infection. Therefore, we aimed to test the impact of prior SARS-CoV-2 infection on T cell and B cell responses to first dose vaccination.

To do this, we analyzed T and B cell immunity after the first 30 μg dose of the Pfizer/BioNTech mRNA vaccine BNT162b2, in a cohort of UK hospital healthcare workers (HCW) (9–12). The COVIDsortium HCW cohort has been studied longitudinally since the end of March 2020, providing accurate infection and immune history in the context of genotyping, including HLA imputation (10–12). Our aim was to compare T and B cell immunity after a first dose of vaccine in December 2020, in individuals post-infection (after natural infection), vaccinated post-infection (vaccination in the context of prior SARS-CoV-2 infection) and vaccinated naïve (single dose vaccination). We wished to explore if there is evidence for altered T cell recognition of the B.1.1.7 and B.1.351 variants, and in particular, of the N501Y mutation shared by several VOC.

The UK has deployed a heterodox vaccination regimen to maximize immune protection and slow spread of the B.1.1.7 lineage, giving an initial 30 μg dose of BNT162b2, followed by boosting up to 12 weeks later (13). A cross-sectional sub-study (n = 51) of the existing longitudinal HCW cohort (9–12) was recruited 22d (±2) after the first dose. After the start of the study, the majority of acute infections had already occurred among this cohort (11). At the time of receiving their first vaccine dose in December 2020, 25 individuals were approximately 39 weeks post SARS-CoV-2 infection with the Wuhan Hu-1 strain and prior to the emergence of VOC and 26 were confirmed uninfected, having tested negative in longitudinal serology for spike and nucleocapsid (N) (table S1 and fig. S1).

We first measured SARS-CoV-2 N antibody longitudinally up to 16–18 weeks, then at 28–30 weeks and finally 42 weeks post-recruitment to confirm that there was no laboratory evidence of new infection at the time of drawing blood for the vaccine study at 42 weeks: none of the previously uninfected HCW had become seropositive (Fig. 1A). T cell responses to spike protein and MEP (mapped epitope peptides) in either post infection, vaccinated post infection or vaccinated naïve individuals were compared (Fig. 1B). Ninety six percent (22/23) of vaccinated post infection individuals made a T cell response to spike protein compared to 70% (16/23) of vaccinated naïve individuals, with a 4-fold increase in magnitude of T cell response. Furthermore, while the T cell response to spike protein in vaccinated naïve individuals increased (p = 0.0440), it was lower than those of vaccinated post infection individuals (p = 0.0557) (Fig. 1C). As expected, there was no significant change in T cell response to N (a measure of immunity to natural infection) (fig. S2A).

Paired analysis of T cell immunity to spike protein in previously uninfected individuals, analyzed at the 16–18 week timepoint and 3 weeks after vaccination, showed a significantly increased response (p = 0.0089) (Fig. 1D). Three individuals who previously showed a response, despite lack of laboratory evidence for infection (therefore presumably a cross-reactive response to an endemic human coronavirus) showed an unchanged or decreased response to spike after vaccination.

The size of the SARS-CoV-2 S1 specific memory B cell pool was investigated by B cell ELISpot (Fig. 1E and fig. S2B). As for T cell responses, the number of S1 specific IgG⁺ antibody secreting cells (ASC) was far greater in vaccinated post infection individuals compared with vaccinated naïve individuals (p < 0.0001). Prior infection generated a 63-fold increase in S1 specific ASC. There were no pre-existing S1 specific ASC in uninfected HCW pre-vaccination. Twenty of 22 vaccinated naïve individuals had detectable S1 specific ASC comprising 0.02% to 1.54% of the memory B cell (MBC) pool. By comparison, all vaccinated post infection individuals had detectable S1 specific ASCs (1.90–50% of MBC pool). We previously reported (14), spike receptor binding domain (RBD) enhanced Ab responses in the vaccinated post infection group. In this work, the vaccination naïve group attained similar antibody titers to the post infection group at 16–18 and 28–30 weeks (Fig. 1F). Vaccinated naïve individuals made a lower nAb response to WT virus than seen following natural infection at 16–18 weeks although this did not achieve statistical significance. In line with the findings for MBC and RBD binding, there was a significantly enhanced nAb response in vaccinated post infection individuals compared with the vaccinated naïve group (Fig. 1G), the mean value being 25,273 compared to 420, that is, a 60-fold increase. To put this in context, these values are 43-fold higher than the values recorded after 2 vaccine doses in the Phase 1 trial (7). There was no correlation between the magnitude of the spike protein T cell response and the percentage of S1 specific ASC (Fig. 1H). As expected, there was a positive correlation between the percentage of S1 specific ASC and the serum titer of RBD antibody in the vaccinated post infection individuals (r = 0.6502; p = 0.0008) (Fig. 1I). After vaccination, two previously infected individuals showed lower percentages of S1 specific memory B cells and reduced serum RBD specific antibody levels than the rest of the group; prior infection involving case-definition symptoms tended to be associated with a higher specific B cell frequency than milder disease (Fig. 1F and fig. S2C). These individuals who, despite infection, had also not shown a detectable T cell response (one never seroconverted and the other rapidly became seronegative during longitudinal follow up) had a poor or absent response to infection that was only minimally overcome by vaccination.

The data in Fig. 1 indicate that there is a strong prime-boosting effect of prior infection on single dose vaccination. Augmentation is seen more strongly in MBC frequency, anti-

RBD, and nAb responses than for T cell response frequency. Furthermore, there was no correlation between S1 ASC frequency and T cell response frequency (Fig. 1H). There is, however, a correlation between S1 ASC and RBD antibody titers, indicating that individuals with higher numbers of MBC make higher antibody responses, and individuals who had experienced infection clustered at the higher end of this response (Fig. 1I).

Shortly before the vaccination program was initiated, several VOC emerged, including the B.1.1.7 VOC. This variant has nine mutations in the spike protein. Several studies have reported weaker nAb responses to B.1.1.7 relative to the previously circulating Wuhan-Hu-1 strain (*2–6*, *15–18*). The majority of SARS-CoV-2 immune naïve individuals made no nAb response to the B.1.1.7 (18/20) and B.1.351 (17/20) variants after single dose vaccination. In contrast, almost all vaccinated post infection individuals made a strong nAb response to the B.1.1.7 (24/24) and B.1.351 (23/24) variants after a single dose vaccination with a 46-fold (B.1.1.7) and 63-fold (B.1.351) increase in mean nAb IC50 in vaccinated post infection compared to vaccinated naïve individuals. In a paired analysis, we observed in vitro significantly reduced nAb potency to authentic B.1.1.7 variant virus (>24.7-fold lower than response to Wuhan Hu-1, p < 0.0001) in sera from individuals with a past medical history of natural infection (Fig. 2B). Worryingly, after single dose vaccination, 90% (18/20) of vaccinated naïve individuals showed no detectable nAbs (IC50 < 50) against B.1.1.7 (mean IC50 37, range 0 to 184; p = 0.2090), but did show demonstrable nAb responses to Wuhan Hu-1 SARS-CoV-2 virus (mean IC50 420, range 80 to 2,004; p = 0.0046). In contrast, all vaccinated post infection individuals responded to single dose vaccination with substantially enhanced nAb responses, neutralizing not just Wuhan Hu-1 SARS-CoV-2 (mean IC50 25,273; range IC50 581 to 76,369), but also the B.1.1.7 (mean IC50 1717; range: IC50 52 to 4919) and B.1.351 (mean IC50 5451; range: IC50 41 to 20,411) variants (Fig. 2, A and B, and fig. S3). We show a 14.7-fold reduction in neutralization (IC50) responses in SARS-CoV-2 B.1.1.7 variant compared to Wuhan Hu-1 virus in vaccinated post infection individuals. However, despite this fall the majority (22/24) remain within a "protective threshold." This was not the case for vaccinated naïve individuals. There was a 11.4-fold reduction in neutralization (IC50) responses against SARS-CoV-2 B.1.1.7 variant (mean, 37) compared to Wuhan Hu-1 virus (mean, 420) resulting in the majority of individuals (19/20) falling below the "protective threshold." This result was mirrored in the SARS-CoV-2 S1 specific memory B cell pool where reduced numbers of S1 specific IgG+ ASC are seen (in vaccinated naïve individuals compared to vaccinated post infection individuals) responding to S1 antigen containing the N501Y, K417N and E484K mutations. Prior infection substantially enhances the specific memory B

cell pool after single dose vaccination (Fig. 2C). We looked at correlations between RBD binding antibodies, B cell responses, T cell responses and IC50, comparing neutralization of Wuhan Hu-1, B.1.1.7 and B.1.315 live virus (Fig. 2D). Despite the lower neutralization of B.1.1.7 and B.1.315 variants, the pattern was retained of strong correlation between RBD antibody titer. S1 specific B cell frequency and neutralization and somewhat weaker correlation between T cell response and neutralization.

A lack of Ab-mediated protection in single-dose vaccinees could be mitigated by a broader repertoire of T cell responses (*18*). To investigate differences in T cell recognition, we designed peptide pools covering the affected regions of Wuhan-Hu-1, B.1.1.7, and B.1.351 variant sequence (table S2). We compared T cell responses to these peptide pools in PBMC from individuals vaccinated post infection and vaccinated naïve (Fig. 2E). Responses in post infection vaccinees were in general higher than in the vaccinated naïve individuals: note an enhanced response to the B.1.1.7 peptide pool. T cell responses were heterogeneous; responses to variant pools could be either higher or lower than to Wuhan Hu-1 pools. Alterations in affinity for the T cell receptor can lead to altered peptide ligand effects and differential polarization of cytokine effector programs, as we have previously observed in Zika virus infection (*19*). We wondered if this was also occurring for SARS-CoV-2; however, we found no evidence for immune deviation to interleukins (IL)-4, -5, -10,–13, 17A or 23 (fig. S3).

For B.1.1.7 and B.1.351, attention has centered on the N501Y mutation, as this is implicated in altered ACE2 binding and enhanced infectivity and transmission but is also a target for B and T cell recognition. We initially looked at T cell responses following natural infection and found that at 16–18 weeks post infection, the N501Y mutation appeared to have no substantial differential impact on the T cell response (Fig. 2F), unlike nAb recognition (*5*).

The specific impact of any T cell epitope changes on the immune response against VOC depends on changes in peptide binding to the peptide-presenting HLA molecules. Since the HLA complex is the most polymorphic part of the human genome, any alteration to core HLA binding motifs will differentially impact people with some HLA alleles over others. We performed *in silico* analysis (NetMHCIIpan) to predict which of the B.1.1.7 and B.1.351 mutations were found in HLA core binding motifs and how this might impact binding to common HLAII alleles (DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*1101, DRB1*1301, and DRB1*1501) (tables S3 and S4). Some of the mutations did not fall in a region predicted to bind the HLAII alleles tested (D3L, T716I, T1001I, A1708D and *3675-7* SGF del). Although several mutations were not predicted to significantly change affinity for the HLAII alleles, others did show predicted differential

affinities depending on host HLAII type (tables S3 and S4). Analyzing altered responses to the D1118H mutation, we noted that individuals who carried DRB1*0301 and DRB1*0401 showed enhanced T cell responses to the Wuhan Hu-1 peptide compared to those that did not (p = 0.0072) (Fig. 2G). T cell responses to the variant peptide appeared to be reduced in individuals carrying DRB1*0301 and DRB1*0401 (Fig. 2H). There is a basis for this in terms of differential HLAII binding as the D to H mutation is predicted to lose the T cell epitope for people carrying DRB1*0301 and DRB1*0401, but not for example in those who are DRB1*0701 or DRB1*1501 who would be predicted to show an enhanced response (table S3). People who are DRB1*1301 are predicted to gain a response as a consequence of this mutation. Analyzing responses to the E484K mutation seen in B.1.351 and P.1 variants, we noted that it did not fall in a region predicted to bind the HLAII alleles tested (table S4). The mutation appeared to have no substantial or differential impact on T cell responses (Fig. 2I).

When we primed transgenic mice expressing human HLA-DRB1*0401 with the Wuhan Hu-1 peptide pool, T cell responses to the B.1.1.7 variant peptide pool were significantly reduced (p = 0.0286) (Fig. 2J). Furthermore, the T cell response to the spike N501Y mutation common to all three of the current VOC was ablated.

In this HCW cohort, vaccinated naïve individuals made an anti S1 RBD Ab response with a mean titer of about 100 U/ml at d22 (±2d) after vaccination, roughly equivalent to the mean peak Ab response after natural infection (14). However, the spike T cell response after one dose was lower than after natural infection, and for 30% of vaccinees no response could be measured. However, T cell responses are enhanced 4-fold in those vaccinated post-infection. This T cell enhancement is small relative to the 63-fold change in ASC and the corresponding 140-fold change in Roche anti-S (RBD) Ab levels we observed after one vaccine dose in HCW vaccinated post infection (14). While much has been written about the impact of rapidly waning serum antibody, our findings confirm MBC are nevertheless primed and able to contribute a rapid, large response to repeat exposure. The rather large effect on B cell priming and restimulation, relative to T cells, in previously infected single-dose vaccinated individuals may reflect the fact that, among the nuanced differences between the licensed COVID-19 vaccines, aspects of the mRNA adjuvant effect appear to skew immunity to high nAb titers, which may underpin its high efficacy. Our evidence for enhanced vaccine responses after infection supports the case that only one vaccine dose is necessary to maximize immune protection for SARS-CoV-2 experienced individuals (14, 20).

It is striking that the high IC50 titers in those vaccinated after infection provide such a large protective margin that responses to authentic B.1.1.7 and B.1.351variants are also high.

In contrast, nAb responses in individuals several months on from mild infection show much lower IC50s against B.1.1.7 and B.1.351, often <100. Similarly, the majority of responses in naïve individuals after one dose show weak recognition of B.1.1.7 and B.1.351. This finding indicates potentially poor protection against B.1.1.7 and B.1.351 in individuals who have experienced natural infection or who have only had one vaccine dose.

It is important to map the effect of VOC mutations on any evasion of T cell immunity. The case has been made that reductions in antibody neutralization of mutant spike may be mitigated by protective T cells (8). A case has been made for the role of T cells as correlates of protection (21). Our evidence from this analysis 22d (±2) after one dose is that T cell immunity is mostly variably low but also relatively unperturbed by the N501Y mutation. The other mutations we considered that overlay CD4 epitopes were, as might be predicted, distributed across the range of HLAII polymorphisms. Those alleles associated with loss of CD4 response to the variant pool tended to be those with a lysine in pocket 4 of the groove (HLA-DR residue 71β), while those with an increased response to the variant pool tend to be those with a smaller, alanine. In HLA-DRB1*0401 transgenics we confirmed that in the context of a given HLAII heterodimer, the N501Y mutation can result in ablation of this part of the T cell response, demonstrating that HLA polymorphisms are likely to be significant determinants of responder/non-responder status with respect to vaccine escape.

SARS-CoV-2 immunity now encompasses post infection plus either zero, one or two vaccine doses and first and second dose naïve vaccinated. Single dose vaccination post infection achieves similar levels of S1 RBD binding antibodies to two doses in naïve vaccinated individuals and second dose vaccination in one dose vaccinated post infection individuals offers no additional enhancement (22). Moving forward, it will be important to resolve the quantitative and qualitative differences between these groups in terms of neutralizing antibody repertoire as well as phenotype and durability of memory B and T cell responses. Durability of immunity to natural infection and following vaccination, as well as sustained vaccine efficacy and vaccine escape need to be monitored over time.

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ACKNOWLEDGMENTS
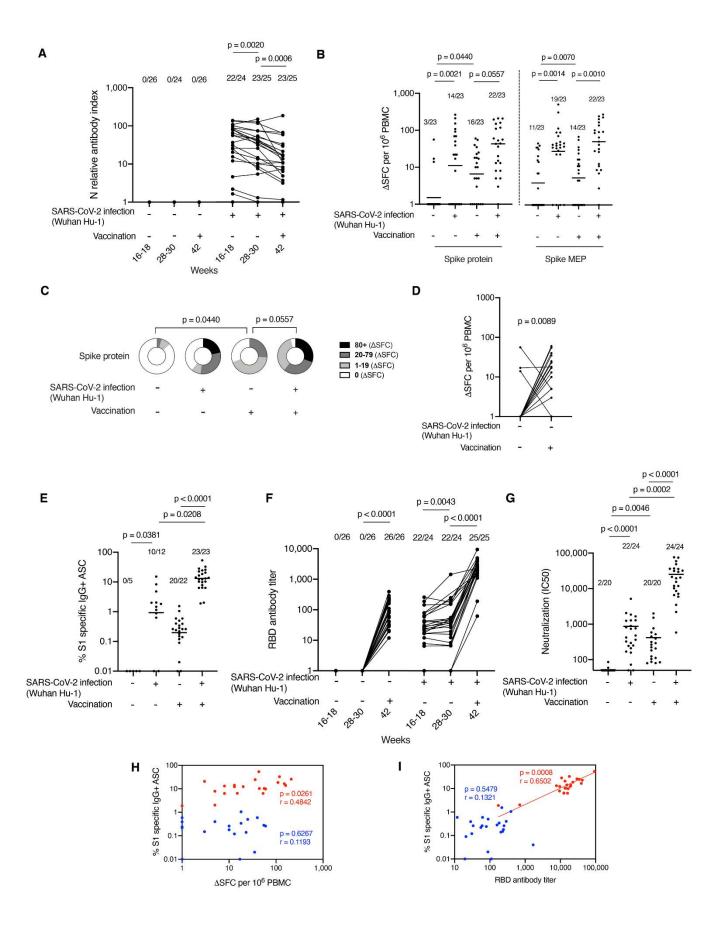
SUPPLEMENTARY MATERIALS

Fig. 1. Impact of prior natural infection with SARS-CoV-2 during the first wave on T and B cell responses to a single dose of the mRNA COVID-19 vaccine, BNT162b2. (A) N Ab measured by electrochemiluminescence immunoassay analyzer (ECLIA) in serum samples from HCW with (n = 25) and without (n = 26) laboratory confirmed SARS-CoV-2 infection (Wuhan Hu-1, during the first wave) 3 weeks after a single dose of the mRNA COVID-19 vaccine, BNT162b2. (B) Magnitude of T cell response to spike protein and spike mapped epitope peptides (MEP) in HCW with and without laboratory confirmed SARS-CoV-2 infection (n = 23 per group). Data are shown pre-vaccination (16–18 weeks after infection) and 3 weeks after the first dose vaccination (week 42) with line at geo mean. (C) Proportion of HCW with (n = 23) and without (n = 23) laboratory confirmed SARS-CoV-2 infection (during the first wave) with a T cell response to Spike protein within the range (0, 1–19, 20–79, >80 ΔSFC/10^6 PBMC) before and 3 weeks after first dose vaccination. (D) Magnitude of T cell response to spike protein in HCW without a history of SARS-CoV-2 infection, plotted pairwise at 16–18 weeks and 42 weeks (3 weeks after first dose vaccination). (E) Percentage of S1 specific IgG$^+$ antibody secreting cells (ASC) in vaccinated HCW with (n = 23) and without (n = 22) prior SARS-CoV-2 infection and in unvaccinated HCW with (n = 12) and without prior infection (n = 5). Line at geo mean. (F) RBD Ab titers measured by ECLIA in serum samples from HCW with (n = 25) and without (n = 26) laboratory confirmed SARS-CoV-2 infection following first dose vaccination. (G) Neutralizing antibody titer (IC50) against Wuhan Hu-1 authentic virus in HCW with (n = 24) and without (n = 20) laboratory confirmed SARS-CoV-2 infection. Line at arithmetic mean. (H) Correlation between percentage of S1 specific ASC and magnitude of T cell response to spike protein in vaccinated HCW with (n = 21, red) and without (n = 19, blue) a history of SARS-CoV-2 infection during the first wave. (I) Correlation between percentage of S1 specific ASC and RBD Ab titer in HCW with (n = 23, red) and without (n = 23, blue) a history of SARS-CoV-2 infection. [(A), (B), (E), and (F)] Numbers of HCW in each group with detectable responses are shown. [(F) and (G)] Data are shown pre-vaccination (16–18 weeks after infection) and 3 weeks after the first dose vaccination (week 42). [(A), (D), and (F)] Wilcoxon matched-pairs signed rank test. [(B), (C), (E), and (G)] Kruskal Wallis multiple comparison ANOVA with Dunn's correction. [(H) and (I)] Spearman's rank correlation. Ab, antibody; HCW, health care workers; RBD, receptor binding domain; S1, spike subunit 1; SFC, spot forming cells.
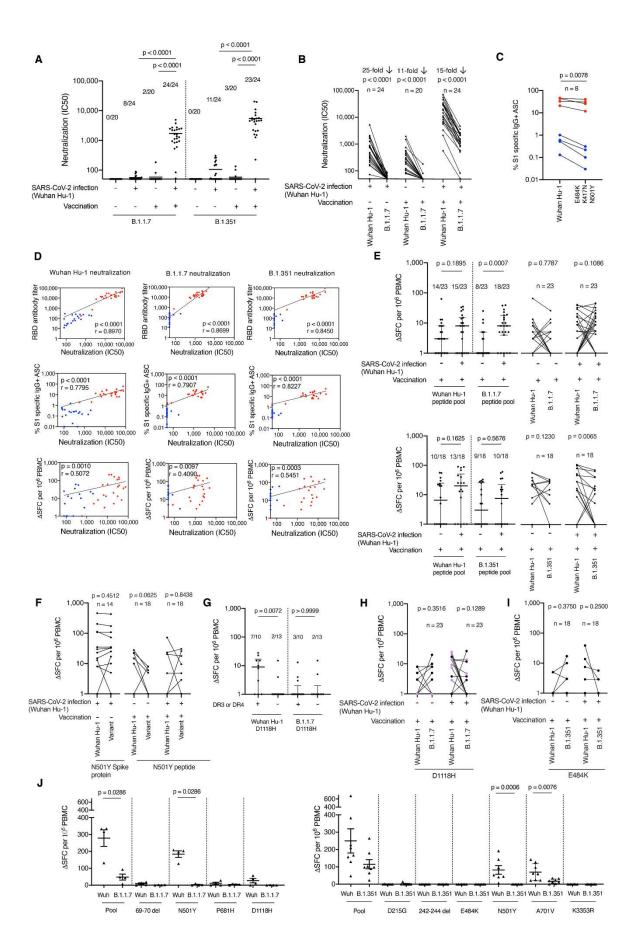
Fig. 2. Impact of vaccination and prior natural infection with SARS-CoV-2 during the first wave on T and B cell responses to the UK B.1.1.7 and South African B.1.315 variants. (**A**) Neutralizing antibody (nAb) titer (IC50) against B.1.1.7 and B.1.351 authentic virus in HCW with (n = 24) and without (n = 20) laboratory confirmed SARS-CoV-2 infection (Wuhan Hu-1). Lines at arithmetic mean. Data are shown pre-vaccination (16–18 weeks after infection) and 3 weeks after the first dose vaccination (week 42). (**B**) nAb (IC50) titers against Wuhan Hu-1 and B.1.1.7 authentic viruses plotted pairwise by individual. (**C**) Percentage of Wuhan Hu-1 S1 and S1 containing variant mutations (E484K, K417N and N501Y) specific IgG+ antibody secreting cells (ASC) in vaccinated HCW with (n = 4) and without (n = 4) prior SARS-CoV-2 infection. (**D**) Correlations between nAb (IC50) titers of Wuhan Hu-1, B.1.1.7 or B.1.351 authentic virus and RBD Ab titer, percentage of S1 specific ASC and magnitude of T cell response to S1 protein in vaccinated HCW with (n = 22–24, red) and without (n = 18–20, blue) a history of SARS-CoV-2 infection. (**E**) Magnitude of T cell response to Wuhan Hu-1, B.1.1.7 or B.1.351 peptide pools in vaccinated HCW with (n = 23 or 18) and without (n = 23 or 18) SARS-CoV-2 infection (Wuhan Hu-1), plotted as grouped data (median plus interquartile range) and pairwise for each individual. (**F**) Magnitude of T cell response to Wuhan Hu-1 S1 protein and N501Y variant spike RBD protein in unvaccinated HCW with laboratory confirmed SARS-CoV-2 infection (n = 14) or to Wuhan Hu-1 and N501Y mutated peptide in vaccinated HCW with (n = 18) and without (n = 18) a history of SARS-CoV-2 infection, plotted pairwise by individual. (**G**) Magnitude of T cell response to Wuhan Hu-1 or B.1.1.7 D1118H peptide in vaccinated HCW with a history of SARS-CoV-2 infection (n = 23), plotted by DRB1*0301/DRB1*0401 status. Lines at median plus interquartile range. (**H**) Magnitude of T cell response to Wuhan Hu-1 or B.1.1.7 D1118H peptide in vaccinated HCW with (n = 23) and without (n = 23) a history of SARS-CoV-2 infection plotted pairwise by individual and with individuals carrying DRB1*0301 or DRB1*0401 alleles marked in purple. (**I**) Magnitude of T cell response to Wuhan Hu-1 or B.1.351 E484K mutated peptide in vaccinated HCW with (n = 18) and without (n = 18) a history of SARS-CoV-2 infection, plotted pairwise by individual. (**J**) Magnitude of T cell response to Wuhan Hu-1 (Wuh), B.1.1.7 or B.1.315 peptide pools and individual peptides in Wuhan Hu-1 peptide immunized HLA-DRB1*04:01 transgenic mice (left-hand panel n = 4, right-hand panel n = 8. Lines at arithmetic mean + SEM). (A) Kruskal Wallis multiple comparison ANOVA with Dunn's correction. [(B), (C), (E) – right-hand panels, (F), (H), and (I)] Wilcoxon matched-pairs signed rank test. (D) Spearman's rank correlation. [(E) – left-hand panels, (G), and (J)] Mann-Whitney U test. ASC, antibody secreting cells; HCW, health care workers; RBD, receptor binding domain; S1, spike subunit 1; SFC, spot forming cells.