Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses.

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Abstract

More than 190 vaccines are currently in development to prevent infection by the novel coronavirus SARS-CoV-2. Animal studies suggest that while neutralising antibody (NAb) against the viral spike protein may correlate with protection, additional antibody functions may also be important in preventing infection. Previously, we reported early immunogenicity and safety outcomes of a viral vector coronavirus vaccine, ChAdOx1 nCoV-19 (AZD1222), in a single-blinded phase I/II randomised controlled trial of healthy adults aged 18-55 years (NCT04324606). Now we describe safety and exploratory humoral and cellular immunogenicity of the vaccine, from subgroups of volunteers in that trial, who were subsequently allocated to receive a homologous full (SD/SD D56 n=20) or half-dose ChAdOx1 booster vaccine (SD/LD D56 n=32) 56 days following prime. Previously reported immunogenicity data from the open-label 28 day interval prime-boost group (SD/SD D28 n=10) are also presented to facilitate comparison. Additionally, we describe volunteers boosted with the comparator vaccine (MenACWY n=10). In this interim report, we demonstrate that a booster dose of ChAdOx1 nCoV-19 is better tolerated than priming doses and, using a systems serology approach, we demonstrate anti-spike neutralising antibody titres (NAb) as well as Fc-mediated functional antibody responses, including antibody dependent neutrophil/monocyte phagocytosis, complement activation and NK cell activation are substantially enhanced by a booster dose of vaccine. A booster dose of vaccine induced stronger antibody responses than a dose-sparing half-dose boost, although the magnitude of T cell responses did not increase with either boost dose. These data support the two-dose vaccine regime that is now being evaluated in phase III clinical trials.

Introduction

Of 198 COVID-19 vaccine candidates at various development stages, 44 are in clinical trials, 10 of which are in late stage clinical development [1]. The majority of SARS-CoV-2 vaccine candidates in development are designed to induce immune responses against the spike surface antigen with many demonstrating early encouraging immunogenicity readouts from clinical trials [2–6].

Much work has been carried out to characterise the immune response to infection with SARS-CoV-2 and in this manner delineate potential correlates of protection. Importantly in a Rhesus macaque challenge model, neutralising antibodies levels following vaccination using the spike antigen correlated with protection against SARS-CoV-2 [7,8] . It is therefore generally accepted that neutralising antibody against spike protein is likely to be critically important in protecting against overt disease and these antibodies are detected in many but not all convalescent individuals. The magnitude of antibody responses appear to relate to antigen load, with higher responses associated with more severe disease, especially in older adults [9–12]. Beyond direct neutralisation through blocking of viral host cell entry, other antibody functions against SARS-CoV-2 are likely to be important determinants of the course of infection. Systems serology approaches have demonstrated differences in the induction of anti-spike antibody subclasses and fc-mediated functions between protected and susceptible animals following vaccination[7,13], as well as between convalescent and deceased patients when sampled early disease[14]. Anti-spike antibodies with diverse effector functions including antibody-dependent neutrophil phagocytosis, antibody-dependent natural killer (NK) cell degranulation, antibody-dependent complement deposition and antibody-dependent cellular phagocytosis were induced following initial SARS-CoV-2 infection in rhesus macaques that were subsequently protected against reinfection [13]. Similarly, anti-spike antibodies with broad functionalities were enriched in convalescent compared with deceased individuals[14]. Robust T cell responses against SARS CoV-2 have been described during acute disease and in recovery [15]. Other studies have also demonstrated a highly activated cytotoxic T cell

phenotype in acute disease and a polyfunctional response during convalescence [16]. It should be noted, however, that vaccine mediated immune correlates of protection have yet to be defined for SARS-CoV-2 or any human coronaviruses.

Although clinical trials of multiple SARS-CoV-2 vaccine candidates [2–4,6,12,17–19]have reported the induction of antibodies that bind to the spike protein, including neutralising antibodies, at the time of writing, no detailed characterisation of vaccine-induced antibodies have thus far been reported in humans. We recently reported preliminary safety and immunogenicity of the adenoviral-vectored SARS-CoV-2 vaccine, ChAdOx1 nCoV-19 (AZD1222)[17]. Here we describe further evaluation of the quantity and quality of post-vaccination humoral and cellular immune responses of individuals recruited to the phase I/II clinical trial of the ChAdOx1 nCoV-19 vaccine. We compare immunogenicity and reactogenicity in trial participants with different booster doses and schedules.

Results

Participants

Following an amendment to the trial (Dated 22 June 2020) 52 participants aged between 18 and 55 years, who had already received an initial standard dose of ChAdOx1 nCov-19, were boosted with a second dose of ChAdOx1 nCov-19 at a 56 day interval. 20 of these participants received a standard dose boost (SD/SD D56) and 32 received a dose-sparing half-dose boost (SD/LD D56). In order to maintain blinding, 10 volunteers in the comparator vaccine arm also received a booster dose of MenACWY vaccine at day 56 (MenACWY) (Figure S1). We previously reported on adverse events, anti-spike binding and neutralising antibody titres, and IFNγ ELISPOT responses in 10 subjects who received two standard doses 28 days apart (SD/SD D28)[17]. These data are reproduced here (Figure 2) to enable comparison between day 28 and day 56 boost intervals. Recruitment for the trial took place between April 23 and May 21, 2020 and no withdrawals have occurred from these groups to date. Analysis of baseline blood samples from the entire trial population (n=1077) showed that n=30 ChAdOx1 nCov-19 prime recipients were seropositive to SARS-CoV-2 spike protein (n=30/544) prior to vaccination.

Safety and Tolerability

As previously reported, systemic reactogenicity following ChAdOx1 nCov-19 prime vaccination was observed[17] with 15/52 (28.8%) participants reporting post-vaccination pyrexia (Figure 1). However, a booster dose of ChAdOx1 nCov-19 in the same participants resulted in minimal reactogenicity, with no episodes of pyrexia (0/52) occurring. Additionally, the rates of volunteers experiencing any moderate or severe solicited systemic reactions within 7 days of vaccination were higher following prime (moderate 27/52d, 51.9%, severe 10/52, 19.2%) compared with boost (moderate 10/52, 19.2%, severe 2/52, 3.8%). Local reactions were also higher following prime vaccinations, with moderate local reactions reported in 12/52 (23.1%) participants following prime compared with only

2/52 (3.8%) following boost. These results are consistent with the previously reported open-label 28-day interval ChAdOx1 boost group (SD/SD D28 n=10)[17].

No serious adverse events (SAE) have occurred in either SD/SD D56, SD/LD D56 or SD/SD D28 ChAdOx1 nCov-19 prime-boost groups to date. The overall reactogenicity profile after the second dose was lower than after the first, and the pattern was consistent between groups with different doses and time intervals between doses. The most frequently reported local and systematic reactions were tenderness and fatigue, respectively (Figure 1). We also report here local and systemic reactions for participants that received ChAdOx1 nCov-19 and were found to be seropositive to SARS-CoV-2 spike antigen at baseline on a mesoscale discovery multiplexed assay platform (MIA) (n=30, Extended Data Figure S1). There was no appreciable difference among those who were seropositive compared with those who were seronegative at baseline in either local or systemic reactogenicity (Extended Data Figure S2).

Mesoscale Discovery Multiplexed Assay (MIA) and neutralising antibody titres

Anti-spike IgG antibodies to SARS-CoV-2 spike and receptor binding domain (RBD) were measured in a multiplex serology immunoassay. In both cases antibody titres rose after the first vaccination with a further increase after the second (figure 2A). At 14 days after the second dose anti-spike IgG titres were not significantly different between those who received the booster at 28 days (GMT 35990, 95% CI 24408, 53068 ;previously published as median and IQR in Folegatti et al[17]) or at 56 days (SD/SD D56: GMT 44485, 95% CI 31714, 62400, p=0.426 and SD/LD D56: GMT 25667, 95% CI 18814, 35015, p=0.250). However, those who received a half-dose boost had lower titres 14 days post-boost than those who received the standard dose (p=0.020). Similar findings were also seen for anti-RBD IgG using MIA (Figure 2A). Following prime vaccination, anti-spike IgG titres were increased 10-fold in these individuals after 28 days (Figure 2A, Supplementary Table S2).

Neutralising antibodies were assayed using a microneutralisation assay reporting the reciprocal of the serum dilution required to reduce live SARS-CoV-2 infection of single cells by 80% (MNA_{80}). NAb

were induced following prime vaccinations and significantly increased after a booster dose in ChAdOx1 nCoV-19 groups. Median normalised NAb titres at 14 days post boost were 274 (IQR 232, 542) for SD/SD D28, 170 (IQR 226, 368) for SD/LD D56 and 395 (IQR 259, 640) for SD/SD D56 (Figure 2B, Supplementary Table S3). No NAb activity was observed in the MenACWY group. Neutralising antibodies were also determined in a pseudovirus neutralisation assay reporting IC_{50}. Median NAb titres measured with the pseudovirus assay at 14 days post boost were 253 (IQR 100, 391) for SD/LD D56 and 424 (IQR 229, 915) for SD/SD D56 (previously reported values for SD/SD D28 were 451 IQR: 212, 627[17]) (Figure 2A and Supplementary Table S4).

Anti-Spike Antibody Class and Subclass

Antibody classes and subclasses within the anti-spike response were determined. Vaccination with ChAdOx1 nCoV-19 increased anti-spike IgM and IgA titres with a peak response measured 28 days after prime for IgM. There was no difference in the response measured 14 days after SD or LD boost, and the post-boost response was of a similar magnitude to SD/SD with a 28 day interval (Figure 3A). Serum samples that were IgG positive 28 days after prime vaccination were assayed for anti-spike IgG subclasses. IgG1 and IgG3 responses were readily detectable at day 28 and were at a similar level, prior to boosting, on day 56 in regimens with a 56 day interval. Following booster vaccination, the median IgG1 response did not increase in participants who received the standard dose regimen with a 28 day interval, although this may be limited by the small group size (n=10). IgG1 responses did increase 14 days after a SD or LD boost in regimens with a 56 day interval, with no measured difference in the magnitude of the response due to dose. IgG3 responses were increased following booster vaccination across all three regimens regardless of interval or dose. The response was predominantly IgG1 and IgG3, with low levels of IgG2 and IgG4 (Figure 3B). This Th1-biased IgG response is in agreement with other studies investigating adenoviral vectored vaccine priming in humans [20–22]. These analyses highlight the similarity in antibody response induced after ChAdOx1 nCoV-19 vaccination regardless of interval or booster dose [20]. Note that antibody isotype data for

SD/SD D28 (n=10) is also presented in Ewer et al 2020 (submitted) in order to facilitate comparison with other groups presented in that paper.

Anti-spike antibody functionality

Anti-spike antibody function was explored further to determine the ability of antibodies induced by vaccination to support antibody-dependent monocyte phagocytosis (ADMP), and neutrophil phagocytosis (ADNP). Both functions were induced by the first vaccination and substantially increased by the second, with a trend towards a larger increase when the interval between the doses was 56 rather than 28 days, and when the booster dose was SD rather than LD (Figure 4A and 4B). In comparison with serum and plasma samples taken from convalescent COVID patients between 28 and 91 days after a positive PCR test, both ADMP and ADNP were higher in the vaccinated group after the second dose. Biobanked serum samples collected from healthy volunteers prior to 2020 were negative in both assays and there was no change in these functions in participants who received the MenACWY vaccine (Supplementary Table S5).

Antibody-dependent complement deposition (ADCD) was also induced by prime vaccination and increased following booster doses at D56 with higher median fluorescence intensity (MFI), indicating greater complement deposition, observed in recipients of a standard booster dose compared to those receiving half dose (Figure 4C, Supplementary Table S5).

The capacity of the ChAdOx1 nCoV-19 vaccine to induce anti-spike antibody-dependent NK cell activation (ADNKA) in humans was also explored, and reported as the capacity to trigger CD107a expression (Figure 4D). Results demonstrate that single dose ChAdOx1 nCoV-19 induced low ADNKA responses which were boosted by the second dose given either at day 28 or 56. The dose used for boosting at day 56 had no impact on the resulting ADNKA measured at day 70 (SD/SD 56 median 5.78 IQR 4.33, 7.7, SD/LD 56 median 5.29 IQR 3.61, 6.13), whereas ADNKA measured at day 42 after boosting at D28 was lower (median 3.96 IQR 3.44, 5.36). The responses observed after two doses of vaccines were within similar range to those detected in a cohort of 21 convalescent COVID patients

(median 5.31 IQR 2.97, 8.77) whereas no change was detected after MenACWY vaccination (Supplementary Table S5).

Cellular Response

As reported previously [17], total antigen specific T cell responses measured by interferon-γ ELISpot were induced and peaked 14 days after the first vaccination. In 10 people who received a booster dose at day 28 (SD/SD D28), responses after a further 28 days post boost (i.e. day 56) were the same as in a group of previously reported[17] participants (n=43) who had not received a booster dose at the same timepoint (p=0.1126). Similarly, there was no significant difference in the magnitude of spike specific T cell responses at 28 days following boost vaccination between the 28 or 56 day interval groups (p=0.736, ANOVA comparing 3 boosted groups) Figure 2C, Supplementary Table S6.

Anti-vector immunity

Anti-ChAdOx1 neutralising antibodies were induced after the first vaccination and remained elevated above baseline until 84 days post enrolment (last timepoint assessed). In the groups receiving the second vaccination at day 56 there was a slight decline between day 28 and day 56 followed by a slight boost after the second vaccination, again resulting in similar responses after the first and second vaccinations (p=0.351) and no differences between groups 28 days after the booster dose regardless of interval or dose (Supplementary Figure S3A, Supplementary Table S7). Anti-ChAdOx1 neutralising antibody titres at the time of the second dose did not correlate with spike-specific antibody response following the second vaccination measured by standardised ELISA 28 days after the boost (p=0.195), or T cell response measured by IFN-γ ELISpot 28 days after the boost (p=0.994), for any vaccination regimen (Supplementary Figure S3B). There were 9 participants with positive (>1) anti-ChAdOx1 neutralisation titres at baseline. In this small sample no correlation with anti-ChAdOx1 neutralising titres at day 28 was evident. Additionally, there was no correlation between pre-existing immunity to the ChAdOx1 vector and reactogenicity at second vaccination (Supplementary Figure S4).

Discussion

We present strong evidence that that a second dose of ChAdOx1 nCoV-19 enhances both the titre and the functionality of the antibody response, when measured at early times after the boost[17]. Additionally, we show clear evidence that a booster dose is less reactogenic than the first dose. The data presented here were key to supporting the decision to change from a one dose to a two dose regimen for the phase III trials of ChAdOx1-nCoV19, which are now underway. Tolerability of vaccines is important for public acceptance, and the expected reactogenicity profile of the different vaccine products which may be used to control SARS-CoV-2 has to be fully characterised and communicated to future vaccine recipients before successful deployment [17]. We note that vaccinations were temporarily paused following the triggering of a holding rule in another clinical trial of ChAdOx1 nCov-19. Vaccinations were resumed in this trial shortly afterward, following review by the independent trial data safety monitoring board (DSMB) and UK regulatory agency, the Medicines and Healthcare products Regulatory Agency (MHRA). Here we show clearly that, although systemic reactogenicity is prominent after a priming dose of ChAdOx1 nCoV-19, a second dose is consistently less reactogenic, regardless of dose interval. We note that as participants in SD/SD D56 and SD/LD D56 groups were asked to volunteer for a second dose after initially being recruited for one dose only, there was a possibility of selection bias in these groups towards individuals who experienced milder post prime side-effects. However, local and systemic reactions seen after priming doses in these groups were comparable to those seen in the ChAdOx1 nCov-19 participants in the phase I/II trial as previously reported[17], suggesting this to be a representative group of the wider trial population. The observation of reduced second dose reactogenicity is in contrast to reported profiles of two mRNA vaccines for COVID19 and a protein-adjuvant vaccine technology, in which, though generally well tolerated, reactogenicity increased with the second dose [4,18,23]. This phenomenon is noteworthy since it is conceivable that additional doses may be required in the future to sustain immunity. Schedules that mix the different vaccine technologies in heterologous

prime boost regimens may maximise immunogenicity, whilst limiting reactogenicity, and could result in innovative strategies that harness the strengths of the different technologies.

There was no association between reactogenicity and presence or absence of antibodies to either SARS-CoV-2 or ChAdOx1 at the time of vaccination. This is an important finding when considering extended use of the vaccine post licensure, when antibody screening will not be performed prior to vaccination and a variable proportion of the population will already have been exposed to SARS-CoV-2. Antibodies against the ChAdOx1 vector are induced by the first vaccination but do not prevent boosting and are not further increased by the second vaccination with either a 4 week or 8 week interval. These observations are also important for further development of viral vectors in general, particularly as multiple different vaccines are being developed that use the same viral vectors as those in development for SARS-CoV-2 vaccines, including Ad26, Ad5 and ChAdOx1[2,3,24–26].

T cell responses, here measured by IFN-γ Elispot, were induced rapidly after vaccination and are well maintained in all dosing regimens and intervals between vaccinations. Although neutralising antibodies are frequently considered a critical immune response post-viral infection, T cells have been shown to play an important roles during COVID-19. Studies have shown that SARS-CoV-2-specific CD4+ and CD8+ T cells were associated with milder disease in acute and convalescent subjects and (PMID: 33010815). It has previously been shown that during influenza infection, T cells do mitigate against overt disease and play a key role in disease mitigation[27–29]. Furthermore, an intriguing study in COVID19 suggests that T cells may correlate with protection[30]. Additionally, adoptive transfer experiments have demonstrated that coronavirus specific T cells are sufficient to protect immunodeficient mice from a mouse-adapted SARS strain challenge[31] as well as chicks from infectious bronchitis virus[32]. IFN-γ Elispot results demonstrate that across all regimens responses are equally well maintained out to 28 days post final vaccination irrespective of vaccination interval. Cytotoxic T cells are responsible for destroying virus-infected cells, preventing further spread of the

virus after infection. The finding that SARS-CoV-2 is capable of cell-to-cell spread [33] highlights the importance of this arm of the immune response as once infection has taken place, the virus is shielded from neutralising antibody binding. Helper T cells are also critical to support B cell function for the initial and continued production of antibody. However, the types of T cell responses elicited by this, and other SARS-CoV-2 vaccines, and their roles in protection from infection or disease severity require further investigation.

Correlates of protection against SARS-CoV2 in humans are currently unknown, but the assumption from animal studies is that neutralising antibodies directed against the spike protein may be critically important [7,13]. Here we have shown that neutralising antibodies are consistently induced across two different dosing intervals, as demonstrated across two assays using either live virus neutralisation or pseudotyped virus neutralisation as a readout, although we acknowledge that we do not yet have data on the durability of these responses. These observations are similar to those reported for several other COVID19 vaccines, in which higher titres of neutralising antibodies are produced following 2-dose vaccination regimens [3,4,18,23]. As has been seen previously after adenovirus-vectored vaccination, data presented here show that IgG subclasses IgG1 and 3 are induced by ChAdOx1 nCoV-19 [20–22,34,35]. Here we see induction of IgA and IgM in addition to antibody-dependent functional activities ADMP, ADNP, ADCD and ADNKA, and are higher following a second dose.

Whilst the titre of neutralising antibodies capable of preventing cellular invasion has emerged as the strongest correlate of protection in pre-clinical SARS-CoV-2 vaccine studies, non-neutralising functional activities are increasingly recognised as important mediators of viral control, working in tandem with CD8+ T cells to kill virally infected cells in the host [36,37]. In preclinical studies of SARS-CoV-2 vaccination, Fc-mediated antibody functions including ADCD and ADNKA correlated with protection against infection following viral challenge, and, in combination with neutralising antibodies, enhanced the ability to distinguish fully protected Rhesus macaques from those which become infected [7,8]. In this study, ADNP, ADMP and ADNKA responses induced by two doses of

ChAdOx1 nCoV-19 were in the same range or higher than that observed in a set of samples from convalescent individuals collected more than one month after disease. As shown on the polar plots, broadly similar functional antibody profiles were induced post vaccination, with mixed IgG1, IgG3 and phagocytosis. In contrast to responses in vaccinated individuals, higher titres of anti-spike IgA and IgM antibody isotypes and ADCD were observed in convalescent samples suggesting a qualitative difference in vaccine-induced humoral immunity compared with immune responses following natural infection. One previous study of hospitalised COVID-19 patients demonstrated that the presence of broadly functional anti-spike antibodies in early disease correlated with survival[14]. This suggests a possible protective effect of Fc-mediated anti-spike antibody effector functions against COVID-19, which although requiring further investigation as to their role in SARS-CoV-2 immunity, we have now also demonstrated here may be induced through prime-boost ChAdOx1 nCoV-19 vaccination.

It has been hypothesized that pre-existing anti-vector immunity may affect immune responses induced by human adenoviral vectored vaccinations and their resulting efficacy[2,38]. However, in other studies it has been shown that viral vectored vaccines are less likely to be a significant concern[39–41]. Furthermore, there is some evidence to suggest the negative impact of pre-existing vector immunity can be overcome by booster schedules[42]. In the case of ChAdOx1, a previous seroprevalence study has demonstrated a low prevalence of anti-ChAdOx1 neutralising antibodies in adults in both the UK and Gambia[43], suggesting this is likely to be less of an issue with this vector though further seroprevalence studies in different populations may be helpful.

Phase III trials evaluating safety, immunogenicity and efficacy of ChAdOx1 nCoV-19 after two doses in adults are now underway. If these trials are successful in determining vaccine efficacy, the immune functions induced by vaccination and described here will be evaluated in relation to vaccine efficacy. In summary, ChAdOx1 nCoV-19 is well tolerated in a 2-dose regimen and induces

multifunctional antibody responses that are enhanced by a booster dose, in addition to T cell responses. The data strongly support evaluation of a two dose vaccine regimen in the phase III trials.

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Author contributions

SCG and AJP conceived the trial and AJP is the chief investigator. AJP, PMF, DJ, HR, and MV contributed to the protocol and design of the study. AVSH is the study site principal investigator. JB. SBR, CD, KE, CG, RH, JH, LS, MV, DB, SB, MB, MC, BC, EC, NE, AF, AG, BH, AL, RM, JM, CR, KT, DW, TL were responsible for laboratory testing and assay development. PKA, DJ, HR, PMF, AMM, MR, BA, MF, SK, AL, MM, MM, YM, EP, IP, RS, MW and MS contributed to the implementation of the study and/or data collection. MV and XL conducted the statistical analysis. CG, ADD, and RT were responsible for vaccine manufacturing. TL, MV, SCG, AJP, DJ, PMF, CR, and LS contributed to the preparation of the report. All authors critically reviewed and approved the final version.

Competing Interests Statement

Oxford University has entered into a partnership with Astra Zeneca for further development of ChAdOx1 nCOv-19. SCG is co-founder of Vaccitech (collaborators in the early development of this vaccine candidate) and named as an inventor on a patent covering use of ChAdOx1-vectored vaccines and a patent application covering this SARS-CoV-2 vaccine. TL is named as an inventor on a patent application covering this SARS-CoV-2 vaccine and was a consultant to Vaccitech for an unrelated project. PMF is a consultant to Vaccitech. AJP is Chair of UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI), but does not participate in discussions on COVID19 vaccines, and is a member of the WHO's SAGE. AJP is an NIHR Senior Investigator. The views expressed in this article do not necessarily represent the views of DHSC, JCVI, NIHR or WHO. AVSH reports personal fees from Vaccitech, outside the submitted work and has a patent ChAdOx1 licensed to Vaccitech, and may benefit from royalty income to the University of Oxford from sales of this vaccine by AstraZeneca and sublicensees. MS reports grants from NIHR, non-financial support from AstraZeneca, during the conduct of the study; grants from Janssen, grants from GlaxoSmithKline, grants from Medimmune, grants from Novavax, grants and non-financial support from Pfizer, grants from MCM, outside the submitted work. CG reports personal fees from the Duke Human Vaccine

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Figure Legends (For main text only)

Figure 1: Solicited local and systemic reactions by different doses and time intervals between doses

Top panel: Local solicited reactions following prime and boost at different dose regimens. Bottom panel: Systemic solicited reactions following prime and boost at different dose regimens. Day 0 is the

day of vaccination. P= post-vaccination observation period in the clinic. Fever = Mild: 38.0°C to <38.5°C; moderate: 38.5°C to <39.0°C; severe: ⩾ 39.0°C. Feverish = Self-reported feeling of feverishness.

Figure 2: SARS-CoV-2 Antibody and T cell response following prime-boost vaccination

(2A) Multiplex SARS-CoV-2 IgG response by multiplex immunoassay after Prime-Boost. Time course of IgG responses are shown for three ChAdOx1 nCoV-19 prime-boost groups; SD/SD: two standard doses administered either 28 (n=10 participants, except for day 42 timepoint where samples from only 9 participants were available) or 56 days (n=20 participants, except for days 28, 70 and 84 timepoints where samples from only 19 participants were available) apart, SD/LD: standard dose prime followed by low dose boost 56 days apart (n=32 participants, except for days 70 and 84 timepoints where samples from only 29 and 31 participants were available, respectively) and for two doses of MenACWY comparator vaccine (n=10 participants). Dotted vertical lines show timepoints at which boosting occurred. Plot shows median and interquartile range. AU/ml = Arbitrary units/ml. Left panel anti-RBD (Receptor Binding Domain) responses. Right panel anti-Spike (SARS-COV-2 spike protein) responses. Dashed line indicates responses in 30 participants who received only one dose of ChAdOx1 nCoV-19 and were seropositive at baseline (seropositivity threshold defined as anti-spike IgG > 1000 AU/ml). (**(2B) Live SARS-CoV-2 microneutralisation IC_{80} (MNA80) after Prime-Boost:** Time course of Microneutralisation titre at IC_{80} is shown for three ChAdOx1 nCoV-19 prime-boost groups; SD/SD: two standard doses administered either 28 (n=10 participants, except for day 42 timepoint where samples from only 9 participants were available) or 56 days apart (n=19 participants), SD/LD: standard dose prime followed by low dose boost 56 days apart (n=24 participants) and for two doses of MenACWY comparator (n=10 participants). Error bars show medians and inter-quartile ranges. To normalise data across assay runs, a reference sample was included in all assay runs and test samples normalised to this value by generating log10 ratios.

Dotted lines shows upper limit of assay (values outside this range set to 640). The lower limit of the normalised data was set to 5. **(2C) IFNγ ELISpot response to peptides spanning the SARS-CoV-2 spike vaccine insert after vaccination with ChAdOx1 nCoV-19:** The total ex vivo T cell response to the SARS-CoV-2 spike vaccine insert encoded within the vaccine is shown over time (IFNγ ELISpot; spot forming cells per 10^6 PBMC; calculated by summing the responses to peptide pools corrected for background; materials and methods). Response is shown as median with interquartile range per vaccination regimen; D56 (n=4 at days 0 and 28 timepoints, n=10 participants at day 35, n=20 at day 70 and 19 at day 84) , SD/LD: standard dose prime and low-dose boost (n=9 for days 0 and 28 timepoints, n=31 at day 70, n=32 at day 84) . Participants received a full or half-dose booster dose of ChAdOx1 nCoV-19 at day 56 (SD/SD D56, SD/LD D56). The LLD is 48 SFC and is denoted by a dotted line. MenACWY (n=79 at day 0, n=43 at day 7, n=44 at day 14, n=69 at day 28, n=42 at day 56, n=10 at days 70 and 84 timepoints). **(2D) Neutralising antibody measured in pseudovirus assay (Monogram IC50)** Pseudovirus neutralisation titres. Dots represent individual datapoints. Box plots represent median titres and IQR. Left: standard dose given 28 days apart (n=10). Middle: standard dose given 56 days apart (n=20). Right: dose sparing regime (standard followed by half dose n=32) given 56 days apart. (SD/SD D28: D0 n=10, D28 n=10, D35 n=7, D42 n=9; SD/SD D56 D0 n=20, D28, D35, D42 not performed, D56 n=20, D70 n=19; SD/LD D56 D0 n=32, D28, D35, D42 not performed, D56 n=32, D70 n=29). *Data for SD/SD D28 (panels 2A-D) are previously published and reproduced here from The Lancet, 396, Folegatti, P. M. et al., Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial, 467–478 (2020)* [17] *with permission from Elsevier.*

Figure 3a: SARS-CoV-2 spike-specific immunoglobulin isotype responses induced by prime-boost regimens of ChAdOx1 nCoV-19 and Figure 3b: SARS-CoV-2 spike-specific IgG subclass responses induced by prime-boost regimens of ChAdOx1 nCoV-19

Volunteers received a standard dose (SD) of ChAdOx1 nCoV-19 at day 0 followed by a second vaccination with SD at day 56 (left panel) or low dose (LD) at day 56 (middle panel) or SD at day 28 (right panel) of ChAdOx1 nCoV-19. **A)** SARS-CoV-2 spike trimer-specific IgA and IgM responses were quantified by ELISA and expressed as ELISA units. Solid lines connect samples from the same participant. Bold solid lines show median with IQR. Different groups had different planned timepoints for blood sampling. Assays were performed at D0, D28, D56 and D70 (SD/SD D56 – n=20 participants, except for D28 where samples from only 19 volunteers were available; and SD/LD D56 – n=32 participants, except for D70 where samples from only 31 volunteers were available) or D0, D7, D14, D28, D35, D42 or D56 (SD/SD D28 – n=10 participants except for D42 where samples from only n=9 participants were available). **B)** Volunteers with measurable SARS-CoV-2 spike-specific IgG at day 28 were assayed for IgG subclasses. Assays were performed at D28, D56 and D70 (SD/SD D56 - n=20 participants, except for D28 where samples from only 19 volunteers were available; and SD/LD D56 – n=32 participants, except for D70 where samples from only 31 volunteers were available) or D7, D14, D28, D35, D42 or D56 (SD/SD D28 – n=10 participants except for D42 where samples from n=9 participants were available). SARS-CoV-2 spike-specific antibody responses were quantified by ELISA. IgG1 and IgG3 responses were expressed as ELISA units and IgG2 and IgG4 responses expressed as OD at 405nm. Solid lines connect samples from the same participant. Bold solid lines show median with IQR.

Figure 4: Antibody dependent monocyte phagocytosis (A) and neutrophil phagocytosis (B), complement deposition (C), and natural killer cell activation (D) in trial participants, convalescent plasma, and pre-pandemic plasma. Anti-spike antibody attributes at 14 days post boost in trial participants (E, F, G) and convalescent plasma (H)

Longitudinal Fc-dependent antibody functionality in ChAdOx1-nCoV19 vaccine recipients, convalescent COVID-19 patients and pre-pandemic samples.

(a) Antibody-dependent monocyte phagocytosis (ADMP) scores for vaccine recipients receiving either 2 standard doses 28 days apart (SD/SD D28 n=10) or 56 days apart (SD/SD D56 n=19), or one standard and one low dose 56 days apart (SD/LD D56 n=24). Convalescent COVID-19 patients (Conv, n=48) and pre-pandemic controls (Pre-2020, n=19) are also shown. Median and interquartile range of normalised responses are shown for each timepoint studied.

(b) Antibody-dependent neutrophil phagocytosis (ADNP) scores for vaccine recipients, convalescent COVID-19 patients and pre-pandemic controls. SD/SD D28 n=10, SD/SD D56 n=18, SD/LD D56 n= 24, Conv n=45, pre-2020 n=14. Median and interquartile range of normalised responses are shown for each timepoint studied.

(c) Antibody-dependent complement deposition (ADCD). Background subtracted median fluorescence intensity (MFI) medians and interquartile ranges are shown for vaccine recipients receiving 2 standard doses 56 days apart (n=10), or one standard and one low dose 56 days apart (n=12). Convalescent COVID-19 patients (Conv, n=37) are also shown.

(d) Antibody-dependent natural killer cell activation (ADNKA). Median and interquartile range of percentage CD107a+ NK cells relative to control wells is shown for vaccine recipients receiving either 2 standard doses 28 days apart (n=10) or 56 days apart (n=19), or one standard and one low dose 56 days apart (n=22), and convalescent COVID-19 patients (n=28), and pre-2020 controls (n=16).

(E-H) Polar plots of data normalised across all timepoints and groups using min-max normalisation. Each wedge represents an anti-spike antibody isotype/subtype or function. For boosted groups (E,F,G) the median value of each antibody assay at 14 days post-booster vaccine is displayed and is represented by the size of the wedge. Note, ADCD was not performed on the SD/SD D28 group and is omitted from figures C and E. Values obtained from convalescent samples taken from a group of

patients and asymptomatic individuals between 28 and 91 days from PCR positivity individuals are also shown (H).

Online Methods

Study Design and Procedures

As previously described, a phase 1/2 participant-blinded, multicentre, randomised controlled trial is currently underway at five UK sites (ClinicalTrials.gov identifier: NCT04324606)[17]. We now report further on participants in the same trial that received two doses of vaccine, describing solicited adverse events, SAEs and secondary or exploratory humoral and cellular immunology endpoints from these groups.

Healthy volunteers between the ages of 18 and 55 were initially randomised to receive a single dose of either ChAdOx1 nCov-19 at a standard dose of 5×10^{10} vp or a comparator vaccine in a 1:1 ratio. Randomisation lists, using block randomisation stratified by study group and study site, were generated by the study statistician (MV). Block sizes of two and four were chosen to align with the study group sizes and the sequence of enrolment, and varied across study groups. Randomisation was performed electronically with full allocation concealment within a secure web platform used for the study (REDCap version 9.5.22; Vanderbilt University, Nashville, TN, USA). A non-randomised open-label group consisting of 10 participants was also recruited and received a 2-dose schedule of ChAdOx1 nCov-19 28 days apart at a standard dose (SD) of 5×10^{10} viral particles (SD/SD D28). Following encouraging preliminary NAb data from that group, the trial was amended (amendment date 22nd June 2020) in order to administer a homologous booster dose at 56 days to a selection of volunteers that had originally been allocated to receive a single dose of vaccine. We selected 62 participants from protocol group 2 (n=408) that had originally been assigned to receive either a single dose of ChAdOx1 nCov-19 (n=52) or MenACWY (n=10) only and who were also seronegative for SARS-CoV-2 spike antigen at baseline to be boosted at the day 56 timepoint. Group 2 participants that were seronegative were given the choice to either opt in or decline a boost vaccination. The 52

ChAdOx1 nCov-19 primed participants were randomised to receive either a standard dose boost (n=20 SD/SD D56, 5×10^{10} vp) or dose sparing half-dose boost (n=32, SD/LD D56, 2.5×10^{10} vp) ChAdOx1 nCov-19 at D56. The 10 participants that had been primed with MenACWY were administered a second dose of MenACWY at D56, primarily to maintain blinding of vaccine allocation. Participants in group SD/SD D28 were followed up on days 1, 3, 7, 14, 28, 31, 35, 42, 56. A standard single dose (prime only) was administered to 44 participants randomised to ChAdOx1 nCoV-19, making up the single SD group and were followed up on days 1, 3, 7, 14, 28, and 56. The 62 volunteers boosted at D56 were followed up on days 28, 56, 70 and 84 from prime. Laboratory capacity meant that, for some assays, not all samples from each participant could be run at every timepoint. None of the participants in Single SD, SD/SD D28, SD/SD D56, SD/LD D56 groups or in the MenACWY arm received prophylactic paracetamol following either prime or boost vaccination.

Reactogenicity and immunogenicity data from all 30 participants in the trial population that were retrospectively found to be seropositive at baseline against SARS-CoV-2 spike antigen and received ChAdOx1 nCov-19 (Figure S1, Group 1 n=2, Group 2/4 n=27, Group 3 n=1) are also shown.

Safety bloods and exploratory immunology samples were collected for all participants. All volunteers were observed for adverse events for 60 minutes after prime dose and a minimum of 15 minutes after the booster vaccination. Self-reported solicited and unsolicited adverse events were recorded via electronic diary for up to 28 days following each vaccine administration.

Written informed consent was obtained from all participants prior to enrolment. The trial is being conducted according to the principles of Good Clinical Practice (GCP) and is approved by a national ethics committee (South Central Berkshire Research Ethics Committee, reference 20/SC/0145) and the UK regulatory agency (the Medicines and Healthcare products Regulatory Agency). An independent data safety monitoring board (DSMB) was appointed before the commencement of recruitment as previously described [17]. Screening and randomisation procedures were as previously described [17]. Participants with a history of possible but unconfirmed COVID-19 infection or those at

high risk of exposure were initially excluded from the trial until SARS-CoV-2 serology became available for screening, from which point only volunteers seropositive at screening were excluded. Despite these steps, several participants were later found to be seropositive to SARS-CoV-2 at the baseline pre-vaccination timepoint and analysed as the 30 baseline seropositive ChAdOx1 nCov-19 recipient group in this manuscript.

The trial staff preparing and administering the vaccine were not blinded to treatment allocation, however, the vaccine was prepared out of participant's sight and was administered using syringes covered with opaque material to ensure participants remained blinded. Only participants enrolled in SD/SD 28 were not randomised or blinded. Clinical investigators and the laboratory team remained blinded to group allocation.

Convalescent plasma samples from adults ($\geqslant$18 years) with PCR-positive SARS-CoV-2 infection were obtained from symptomatic patients admitted to hospital or from surveillance on health-care workers who did not have symptomatic infection, as previously described[17]. These samples were tested using ADNK, ADNP, ADMP and ADCA and anti-spike antibody isotypes were quantified. Different samples were analysed across the assays, dependent on sample availability, laboratory capacity, and assay-specific requirements. Where multiple longitudinal samples were available for the same participant, only one timepoint is included in the analyses in this article and the earliest timepoint (at least 28 days after initial symptoms) was selected. Pre-pandemic plasma samples were acquired from clinical study participants' visits undertaken in 2012-2013 stored, with individuals informed consent, in the Oxford Vaccine Centre Biobank (National Research Ethics Service reference 10/H0504/2)

Statistical Analysis

Summary statistics are presented by study groups, including medians with interquartile ranges, or geometric means (95% confidence interval). A limited number of statistical comparisons are included due to a desire not to over-analyse groups with small sample sizes. For comparisons between

groups, independent samples t-tests. or analysis of variance was applied to log-transformed antibody data. For the analysis of the relationship between anti-vector neutralisation titres and anti-spike IgG or t-cell responses, an unadjusted linear regression was applied. All analyses were conducted using R version 3.6.1.

Power and sample size calculations were done for the primary efficacy only, which is not presented here. No specific power calculations were carried out for these immunogenicity subgroups which are secondary endpoints in the study and mostly descriptive in nature. The results presented in this manuscript are from an interim analysis of secondary or exploratory outcomes for the described groups of this phase I/II randomised control trial (NCT04324606).

Vaccines

ChAdOx1 nCoV-19 consists of a replication-deficient adenovirus viral vector, derived from the simian adenovirus Y25 serotype, that expresses a full-length codon-optimised SARS-CoV-2 spike protein (GenBank accession number MN908947) with a tissue plasminogen activator leader sequence. The recombinant adenovirus ChAdOx1 nCoV-19 was produced as previously described [43]. The vaccine supplied for prime doses was formulated and vialed by the Clinical Biomanufacturing Facility (CBF), University of Oxford, Oxford, UK, as previously described [17]. Booster doses were supplied by either the CBF (volunteers in SD/SD D28) or were manufactured at Advent Srl, Pomezia, Italy (volunteers in SD/SD D56 and SD/LD D56). Both products were manufactured to current Good Manufacturing Practice (cGMP). The licenced comparator meningococcal group $ACW_{135}Y$ Conjugate Vaccine (MenACWY , NIMENRIX®, Pfizer) was administered at the standard dose of 0.5 mL. All vaccines were administered intramuscularly as a single injection into the deltoid.

Mesoscale Discovery Multiplexed Immunoassay (MIA)

Antigen-specific responses to ChAdOx1 nCoV19 vaccination and/or natural SARS-CoV-2 infection were measured using a multiplexed immunoassay (MIA). The MIA was developed and performed by

Meso Scale Discovery (MSD), Rockville, MD, and is described in Folegatti *et al* [17]. Briefly, dried plates coated with SARS-CoV-2 Spike and RBD were blocked, washed and incubated with samples, reference standards, and controls. Internal quality controls and reference standard reagents were developed from pooled human serum. Following incubation and washing steps, detection antibody was added (MSD SULFO-TAG™ Anti-Human IgG Antibody), incubated and plates washed again. MSD GOLD™ Read Buffer B was added and plates read using a MESO® SECTOR S 600 Reader. Samples at the lower limit of quantitation were set to 2.58 for Spike and 2.60 for RBD, while samples at the upper limit were set to 320000 for Spike and 317073 for RBD.

Public Health England Microneutralisation Assay (PHE MNA80)

Using a similar method to that described above for PHE MNA80 (and described in [17]), the microneutralisation assay (MNA) measures microplaques using the ImmunoSpot® S6 Ultra-V Analyzer. Briefly, serum and SARS-CoV-2 (Victoria/01/2020, Doherty Institute, Melbourne, Australia) virus mixtures were added to monolayers of virus-susceptible Vero/E6 cells for one hour before replacement of inoculum with overlay (1% w/v CMC in complete media). Following a 24-hour incubation, cells were fixed with formaldehye. Microplaques were detected using a SARS-CoV-2 antibody specific for the SARSCoV-2 RBD Spike protein and a rabbit HRP conjugate. Infected microplaques were detected using TrueBlueTM substrate. Resulting counts analysed in SoftMax Pro v7.0 software. Data presented for the MNA80 microneutralisation assays in this manuscript have been normalised to allow comparison across assay runs, including previously published timepoints.

Monogram Biosciences pseudotype neutralisation assay (PseudoNA)

Neutralizing antibody (Nab) titres were determined using a lentivirus-based SARSCoV-2 pseudovirus particle expressing spike protein (Accession number: MN908947.3). The pseudotype neutralisation CoV nAb assay is described in [17] and based on previously described methodologies using HIV-1

pseudovirions [44–46].Briefly, heat inactivated, diluted serum samples were incubated with SARS-CoV2 pseudotyped virus. Nab titres were determined by creating 9 serial three-fold dilutions of test samples. Irrelevant pseudotyped virus was used as a control. Following incubation of diluted sera and pseudovirus particle, HEK 293 ACE2-transfected cells were added, plates were incubated and luciferase expression measured. Nab titres are reported as the reciprocal of the serum dilution conferring 50% inhibition (ID50) of pseudovirus infection. %Inhibition = 100% – (((RLU(Vector+Sample+Diluent) – RLU(Background))/(RLU(Vector+Diluent) – RLU(Background))) x 100%). SARS CoV-2 nAb Assay Positive and Negative Control Sera are included on each 96-well assay plate.

Antibody-dependent Natural Killer cell Activation assay (ADNK)

To assess the antigen-specific antibody-dependent NK cell activation (ADNKA), 96-well Nunc Maxisorp ELISA plates (Thermo Fisher) were coated with recombinant SARS-CoV-2 spike protein at 2.5ug/ml in carbonate/bicarbonate solution (Sigma Aldrich) for 16 hours at 4°C. Plates were washed with phosphate buffer saline (PBS) and blocked with 5% BSA in PBS. Serum and plasma samples were plated undiluted in duplicate. Following incubation for two hours at 37 ° C, the plates were washed and the natural killer NK-92® cell line retroviral transduced to express human CD16 (PTA-8836 American Type Culture Collection, and described in Binyamin et al. [47]) was added at 10^5 cells per well in the presence of Brefeldin A (10ug/ml, Sigma Aldrich), Golgi Stop (BD Biosciences) and CD107a (1:20, PE, clone H4A3, BD Biosciences). A sample of cells were separately stained with CD56 (1:1000, BV786, clone NCAM16, BD Biosciences) and CD16 (1:10, AF594, clone GRM-1, Santa Cruz Biotechnology) in order to verify consistent expression of CD16. After 5 hours incubation, cells were transferred to V-bottom plates and stained for FACS analysis (Extended Data Figure S5). Live NK cells were identified by fixable LIVE/DEAD staining (1:500, R780, BD Biosciences). Cells were fixed and data acquired using a BD Fortessa. Percentages of CD107a+ NK cells relative to control wells with

spike protein and blocking buffer only were determined in FlowJo software (version 10.7.1). A pre-pandemic pool of three donors and a pool of six hospitalised SARS-CoV-2 infected individuals were plated in triplicate on each plate, for quality control of each assay.

Bead coupling for ADMP and ADNP assays

Red fluorescent (580/605) NeutrAvidin-labelled microspheres (Thermo Fisher, F-8875) were freshly coupled to biotinylated SARS-CoV2 spike protein for each assay. Spike protein (at a concentration of 0.388µl/mL) was coupled to the beads at a 3:1 ratio and incubated for 2 hours at 37°C. Beads were washed twice with 0.1% BSA and diluted 100-fold in 0.1% BSA 10µl was added to each well in the ADNP and ADMP assays.

Antibody dependent neutrophil phagocytosis (ADNP)

The ADNP assay is based on a previously described protocol [48] with some modifications. Whole donor blood, collected in sodium heparin tubes, was treated with Ammonium-Chloride-Potassium (ACK) lysing buffer (Thermo Fisher, A1049201) for 5 minutes followed by centrifugation to collect white blood cells. Cells were washed with DPBS (Sigma, D8537), counted and adjusted to $2.5x10^5$ cells/mL in media consisting of Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, R5886) supplemented with 100 U/mL penicillin/streptomycin (Sigma, P4458) and 20 mmol/L L-glutamine (Sigma, G7513).

Serum diluted 100x in RPMI was added to antigen-coupled beads in a 96-well plate and incubated for 2 hours at 37°C. All samples were assayed in duplicate and each plate contained 2 quality control (QC) samples in addition to appropriate negative controls. Wells were washed with DPBS and a total of 500,000 white blood cells were added per well followed by a further 1 hour incubation at 37°C. Cells were then stained using a cocktail of Mouse Anti-human CD3 Alexa Fluor 700, BD Pharmingen, Clone UCHT1, cat: 557943, lot: 9185576, 1:80, Mouse anti-human CD14 APC Cy7, BD Pharmingen, Clone MΦP9, cat:557831, lot: 0044497, 1:80 and Mouse anti-human CD66b Pacific Blue, Biolegend

clone G10F5, cat 305112, lot: B285068, 1:80 and incubated for 15 minutes at room temperature in the dark. Following washing and fixation using 4% paraformaldehyde (PFA, Santa Cruz Biotechnology, SC-281692), the proportion of cells containing fluorescently-labelled beads was ascertained using flow cytometry (BD, Fortessa X20).

Data were analysed with Flowjo (BD, Version 10), using a gating strategy to select neutrophils (Extended Data figure S6). First neutrophils were gated based on forward and side scatter. Doublets were excluded in the following two steps. Furthermore, T cells and monocytes are excluded using a double-negative gate for CD3 and CD14. The final neutrophil gate is based on CD66b-positivity, after which bead-positive cells are gated. In all cases there is a clear separation between positive and negative positive populations. Gates were kept consistent between samples but were checked for each sample.

Normalised phagocytic scores were calculated by multiplying the percentage of bead-positive cells by the mean fluorescence intensity (MFI) of the beads within these cells and normalising against a QC sample set to 1. As multiple plates were run during an experiment, plates failed if any of the QC sample averages fell outside of the mean +/- 2 SD range of that particular QC across plates. In addition, samples were excluded from further analysis if the replicates showed a coefficient of variation (CV) over 25%. The data in the current paper are all derived from one experiment.

Antibody dependent monocyte phagocytosis (ADMP)

The ADMP assay is based on a previously described protocol [49] with some modifications. Briefly, a human monocytic THP-1 cell line (ATCC) was grown and maintained using supplier instructions. Serum was diluted 4000x in RPMI and was added to antigen-coupled beads in a 96-well plate and incubated for 2 hours at 37°C. All samples were assayed in duplicate and each plate contained 2 quality control (QC) samples in addition to appropriate negative controls. At the end of the 2 hours incubation period, wells were washed with RPMI and 250,000 THP-1 cells diluted in media consisting of Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, R5886) supplemented with 100

U/mL penicillin/streptomycin (Sigma, P4458) and 20 mmol/L L-glutamine (Sigma, G7513) were added to each well. Cells were incubated with the antibody-coated beads for 18 hours at 37°C. After the 18 hour incubation period, cells were washed with PBS and fixed using 4% PFA. The proportion of cells containing fluorescently-labelled beads was measured using flow cytometry (BD, Fortessa X20).

Data were analysed with Flowjo (BD, Version 10), using a gating strategy to select the THP-1 cells (Extended Data Figure S7). First THP-1 cells were gated based on forward and side scatter to exclude debris. Doublets were excluded in the following two steps, after which bead+ cells were gated. There is a very clear separation between the positive and negative population. Gates are kept consistent between samples but were checked for each sample.

Normalised phagocytic scores were calculated by multiplying the percentage of bead-positive cells with the mean fluorescence intensity (MFI) of the beads within these cells and normalising against a QC sample set to 1. As multiple plates were run during an experiment, plates failed if any of the QC sample averages fell outside of the mean +/- 2 SD range of that particular QC across plates. In addition, samples were excluded from further analysis if the replicates showed a coefficient of variation (CV) over 25%. The data in the current paper are all derived from one experiment.

Antibody dependent complement deposition (ADCD)

SPHERO^{TM} Carboxyl magnetic blue fluorescent beads (Spherotech, USA) were coupled with SARS-CoV-2 whole spike protein (Lake Pharma, USA, ref 46328) using a two-step Sulpho-NHS/EDC process detailed in Brown *et al* [50]. Spike protein was included at saturation levels and coupling confirmed by the binding of IgG from a Covid-19 convalescent donor known to have high levels of anti-spike protein IgG.

Heat-inactivated test serum (4µl of 1:10 dilution, in duplicate) was added to 16µl blocking buffer (PBS, 2% BSA, BB). This was followed by 20µl of SARS-CoV-2 spike protein-coated magnetic beads (50

beads per µl) to give a final serum dilution of 1:100, and the mixture incubated at 25°C for 30min with shaking at 900rpm. The beads were washed twice in 200µl wash buffer (BB + 0.05% Tween-20) then resuspended in 50µl BB containing 10% IgG- and IgM-depleted human plasma (prepared as per [51]) and incubated at 37°C for 15min with shaking at 900rpm. Beads were next washed twice with 200µl wash buffer and resuspended in 100µl FITC-conjugated rabbit anti-human C3c polyclonal antibody (Abcam, UK) diluted 1:500 in BB and incubated at room temperature in the dark. After two more washes with 200µl wash buffer, the samples were resuspended in 60µl Hank's Balanced Salt Solution and analysed using a CytoFLEX S flow cytometer (Beckman Coulter, USA) and CytExpert software (Extended Data Figure S8). For each sample, a minimum of 100 beads were collected. Conjugated beads were gated based on Forward Scatter and Violet Side Scatter then presented on an APC-histogram. The APC peak was gated and displayed on a FITC-histogram which represents deposition of C3b/iC3b. The median fluorescence intensity (MFI) of a complement-only, no serum control was subtracted from each test sample to give the antibody-dependent median FITC fluorescence. Test sera were run in duplicate and data presented are from a single experiment.

Ex-vivo IFN gamma ELISPOT

Ex-vivo ELISPOTS were carried out to measure SARS-CoV-2 spike antigen specific cellular immune responses as previously described [17]. Briefly, fresh peripheral blood mononuclear cells (PBMCs) were separated from whole blood with lithium heparin by density centrifugation within four hours of venepuncture. A total of 253 synthetic peptides (15mers overlapping by 10 amino acids) spanning the entire vaccine insert, including the tPA leader sequence were used to stimulate PBMC (Pro-Immune, Oxford UK). Peptides were pooled into 12 pools for the SARS-CoV-2 spike protein containing 18 to 24 peptides, plus a single pool of 5 peptides for the tPA leader. Peptide sequences and pooling were previously described [17]. Data were analysed according to a quality control standard operational procedure.

Anti-ChAdOx1 Neutralisation Assay

Chimpanzee adenovirus ChAdOx1-specific neutralizing antibody titers were assessed using a secreted placental alkaline phosphatase (SEAP) quantitation assay as described [43] with the following minor modifications. Briefly, GripTite 293 MSR cells (Invitrogen, catalogue no. R795-07, cultured as per manufacturer's instructions) were seeded in 96 well plates the previous day at $3x10^4$ cells per well. Serial dilutions in 0% FBS–DMEM (phenol red free; GibcoBRL catalog no. 31053-028), of heat inactivated test serum or plasma were mixed 1:1 with the ChAdOx1–SEAP (secreted alkaline phosphatase) reporter vector (at a fixed concentration) and incubated for 1 hour to allow antibody binding. Multiple virus/media only controls, as well as previously titred serum/plasma controls were included. Serum/Plasma-virus mixes were applied to cells for 1 hour to allow infection, before replacement with 10% FBS –DMEM (phenol red free) for 24 hours. Final serum dilutions were 1:18 to 1:4608 and each serum sample was tested in duplicate. For each sample, SEAP concentration was assessed in 50 µl aliquots of culture supernatant, with CPSD as an indicator substrate (Tropix Phospha-Light Chemiluminescent Assay Kit, Life Technologies, T1017). Luminescence intensity was measured using a Varioskan Flash luminometer (Thermo Fisher Scientific). Serum dilution neutralization titers were measured by linear interpolation of adjacent values (to 50% inhibition) to determine the serum dilution required to reduce SEAP concentration by 50% compared to wells with virus alone, using a standardised spread sheet.

IgG Standardised ELISA

Antigen specific total IgG was detected using an in-house indirect ELISA using trimeric SARS-CoV-2 spike protein, as described previously[17]. Briefly, ELISA plates were coated with 2ug/ml spike protein for at least 16 hours at 4˚C. After a washing step, diluted serum from vaccinees and relevant controls was added and incubated for 2 hours at room temperature. Secondary antibody conjugated to alkaline phosphatase was added after a further washing step. Development was performed using 4-nitrophenyl phosphate in diethanolamine substrate buffer. Optical density was measured at 405nm and data is presented as standardised ELISA units.

Isotype & Subclass Standardised ELISA (IgA, IgM, IgG1 and IgG3)

Standardised ELISA was performed on donor serum to quantify circulating SARS-CoV-2 spike-specific IgG1, IgG3, IgA and IgM. Nunc MaxiSorp™ ELISA plates (ThermoFisher Scientific) were coated overnight (≥16 hours) at 4 °C with 50 µL per well of 5 µg/mL SARS-CoV-2 full – length trimeric spike protein (FL-S) diluted in PBS. This protein was produced as follows. A soluble SARS-CoV-2 FL-S protein (GenBank MN908947 Wuhan-Hu-1) construct encoding residues 1-1213 with two sets of mutations that stabilise the protein in a pre-fusion conformation (removal of a furin cleavage site and the introduction of two proline residues; K983P, V984P) was expressed as described [52]. The endogenous viral signal peptide was retained at the N terminus (residues 1-14), a C-terminal T4-foldon domain incorporated to promote association of monomers into trimers to reflect the native transmembrane viral protein, and a C-terminal His6 tag included for nickel-based affinity purification. FL-S was transiently expressed in Expi293™ (Thermo Fisher Scientific) and protein purified from culture supernatants by immobilised metal affinity followed by gel filtration in Tris-buffered saline (TBS) pH 7.4 buffer.

Plates were washed 3x with PBS/Tween (0.05%) (PBS/T) and tapped dry. Plates were blocked for 1 hour with 100 µL per well of Blocker™ Casein in PBS (ThermoFisher Scientific) at 20 °C. Test samples were diluted in blocking buffer (minimum dilution of 1:50) and 50 µL per well was added to the plate in triplicate. For each immunoglobulin isotype or subclass being tested, the respective reference serum (made from a pool of high titre donor serum) was diluted in blocking buffer in a 2-fold dilution series to form a 10-point standard curve. 3 independent dilutions of the reference serum were made (with a dilution factor corresponding to the 4th point in the standard curve) to serve as internal controls. The standard curve and internal controls were added to the plate at 50 µL per well in duplicate. Plates were incubated for 2 hours at 37 °C with 300 rpm shaking and then washed 3x with PBS/T and tapped dry. Secondary antibody was diluted in blocking buffer and 50 µL per well was added. The secondary antibody used was dependent on the immunoglobulin subclass or isotype

being detected. These were Mouse Anti-Human IgG1 Hinge-AP, Mouse Anti-Human IgG3 Hinge-AP, Goat Anti-Human IgA-AP and Goat Anti-Human IgM-AP (Southern Biotech). Plates were incubated for 1 hour at 37 °C with 300 rpm shaking. Plates were washed 3x with PBS/T and tapped dry. 100 µL per well of PNPP alkaline phosphatase substrate (ThermoFisher Scientific) was added and plates were incubated for 1-4 hours at 37 °C with 300 rpm shaking. Optical density at 405 nm (OD_{405}) was measured using an ELx808 absorbance reader (BioTek) until the internal control reached an OD_{405} of 1. The reciprocal of the internal control dilution giving an OD_{405} of 1 was used to assign an ELISA unit (EU) value of the standard. Gen5 ELISA software v3.04 (BioTek) was used to convert the OD_{405} of test samples into EU by interpolating from the linear range of standard curve fitted to a 4-parameter logistics model. Any samples with an OD_{405} below the linear range of the standard curve at the minimum dilution tested were assigned a minimum EU according to the lower limit of quantification of the assay.

Isotype and Subclass OD ELISA (IgG2 and IgG4)

Antigen-specific IgG2 and IgG4 responses were detected in the absence of an antigen-specific serum control. Nunc MaxiSorp™ ELISA plates (ThermoFisher Scientific) were coated with 50 µL per well of 5 µg/mL SARS-CoV-2 trimeric spike protein (The Jenner Institute, University of Oxford). Plates were also coated with a specified concentration of a commercial human immunoglobulin control: recombinant Human IgG2 Lambda or recombinant Human IgG4 Lambda (Bio-Rad Laboratories Ltd). Plates were left overnight (≥16 hours) at 4 °C. Plates were washed 3x with PBS/Tween (0.05%) (PBS/T) and tapped dry. Plates were blocked for 1 hour with 100 µL per well of Blocker™ Casein in PBS (ThermoFisher Scientific) at 20 °C. Test samples and 5 pre-pandemic negative control samples were diluted 1:50 in blocking buffer and 50 µL was added to antigen-coated wells in duplicate. 50 µL of blocking buffer was added to immunoglobulin-coated wells and blank wells. Plates were incubated for 2 hours at 37 °C with 300 rpm shaking and then washed 3x with PBS/T and tapped dry. Secondary antibody was diluted in blocking buffer and 50 µL per well was added. The secondary

antibody used was dependent on the immunoglobulin subclass being detected: Mouse Anti-Human IgG2 Fd-AP or Mouse Anti-Human IgG4 Fc-AP (Southern Biotech). Plates were incubated for 1 hour at 37 °C with 300 rpm shaking. Plates were washed 3x with PBS/T and tapped dry. 100 µL per well of PNPP alkaline phosphatase substrate (ThermoFisher Scientific) was added and plates were incubated for 1-4 hours at 37 °C with 300 rpm shaking. Optical density at 405 nm (OD_{405}) was measured using an ELx808 absorbance reader (BioTek) until the immunoglobulin control reached a specified OD_{405}. Negative cut-offs were calculated using the formula Mean + 7.858*SD of the OD_{405} readings of the pre-pandemic negative control serum samples, where 7.858 is the SD multiplier with a 99.9% confidence level for n=5 controls [53].

Role of funder

The funder had not role in the design, execution or analysis of the study presented here.

Data Availability

The University of Oxford is committed to providing access to anonymised data for non-commercial research at end of the clinical trial, which is currently scheduled to be one year after the last participant is enrolled, unless granted an extension. Oxford will collaborate with AstraZeneca UK Limited of such requests prior to disclosure.

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