T cell and antibody responses induced by a single dose of ChAdOx1 nCoV-19 (AZD1222) vaccine in a Phase 1/2 clinical trial.

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Abstract

SARS-CoV-2, the causative agent of COVID-19, has caused a global pandemic and safe, effective vaccines are urgently needed[1]. Strong, Th1-skewed T cell responses can drive protective humoral and cell-mediated immune responses[2], and may reduce the potential for disease enhancement[3]. Cytotoxic T cells clear virus-infected host cells and contribute to control of infection[4]. Studies of patients infected with SARS-CoV-2 have suggested a protective role for both humoral and cell-mediated immune responses in recovery from COVID-19[5,6].

ChAdOx1 nCoV-19 (AZD1222) is a candidate SARS-CoV-2 vaccine comprising a replication-deficient simian adenovirus expressing full-length SARS-CoV-2 spike protein. We recently reported preliminary safety and immunogenicity data from a Phase 1/2 trial of the ChAdOx1 nCoV-19 vaccine (NCT04400838)[7] given as either a one or two dose regimen. The vaccine was tolerated, with induction of neutralising antibodies and antigen-specific T cells against the SARS-CoV-2 spike protein. Here we describe in detail exploratory analyses of the immune responses in adults, aged 18-55 years, up to eight weeks after vaccination with a single dose of ChAdOx1 nCoV-19 in this trial, demonstrating an induction of a Th1-biased response characterised by IFN-γ and TNFα cytokine secretion by $CD4^+$ T cells and antibody production predominantly of IgG1 and IgG3 subclasses. $CD8^+$ T cells, of monofunctional, polyfunctional and cytotoxic phenotypes, were also induced. Taken together, these results suggest a favourable immune profile induced by ChAdOx1 nCoV-19 vaccine, supporting the progression of this vaccine candidate to ongoing Phase 2/3 trials to assess vaccine efficacy.

Introduction

Efforts to develop a vaccine against SARS-CoV-2 to control the global COVID-19 disease pandemic have been underway since January 2020 with more than forty vaccine candidates in clinical trials by October 2020[1]. The past decade has seen an expansion and acceleration in the development of tools to support pandemic preparedness, including the development of vaccines against novel and emerging pathogens[8,9]. This acceleration, spurred on by numerous outbreaks of diseases including SARS-CoV, MERS-CoV, Ebola and Zika, has leveraged the use of platform technologies and blueprints for target product profiles for priority diseases[10]. Replication-deficient adenoviruses[11] are attractive for use as COVID-19 vaccine candidates as they can be manufactured at scale, have favourable safety profiles and are highly immunogenic. Importantly, viral vectored vaccines can induce strong immune responses in older adults and immunocompromised individuals[12,13]. Replication-deficient adenovirus vectors are also potent inducers of both antibodies as well as cytotoxic T cells; the latter can clear virus-infected host cells and contribute to the control of infection, alleviating disease symptoms[4,14]. Importantly, high frequency T cell responses targeting the SARS-CoV-2 spike protein have been detected in patients that recover from COVID-19 with recent data suggesting a role for T cells during COVID-19[15-17].

Previous efforts to develop vaccines against human coronaviruses have faced challenges with several preclinical studies demonstrating disease enhancement in vaccinated animals after viral challenge. This was characterized by eosinophilic infiltrates resulting in immunopathology, following the induction of a T helper cell type 2 (Th2) biased response, or a weak neutralizing antibody response that might contribute to antibody-dependent enhancement of infection (ADE)[3]. In-depth analysis of SARS-CoV-2 vaccines are being conducted to determine whether responses are Th1 or Th2 dominated; these types of studies are being implemented in a number of COVID-19 vaccine trials[18-21].

ChAdOx1 nCoV-19 (AZD1222) is a replication-deficient simian adenoviral vector that expresses the full-length SARS-CoV-2 spike protein. In preclinical studies, either a single dose or two doses of ChAdOx1 nCoV-19 vaccination prevented SARS-CoV-2 mediated pneumonia in rhesus macaques[22]. We previously reported safety data from Phase 1/2 studies and demonstrated induction of SARS-CoV-2 spike-specific antibodies after vaccination, with boosting of binding and neutralising titres following a second dose[7]. These data supported progression to Phase 3 trials with a 2-dose regimen and we have now expanded our immunogenicity analysis to explore a wider range of the immunological phenotypes induced. In an accompanying paper (Barrett et al., *In Press*), we present detailed functional antibody profiling of responses to prime-boost regimes with differing doses and intervals.

Currently there are no defined correlates of protection against COVID-19 infection and the immunological thresholds required for vaccine efficacy remain undefined[23]. Clinical studies have suggested a protective role for both humoral and cell-mediated immunity in recovery from SARS-CoV-2 infection[5,6,24]. Here we provide a detailed description of the immune response following administration of one dose of ChAdOx1 nCoV-19. We define in detail the isotypes, subclasses and antibody avidity induced post vaccination, and also perform multiplex cytokine profiling and intracellular cytokine staining (ICS) analysis demonstrating that ChAdOx1 nCoV-19 vaccination induces a predominantly Th1-type response.

Results

Study participants

Recruitment, vaccination and demographics of the study participants were previously reported with interim safety and immunogenicity data[7]. Healthy adults aged 18-55 (n=88) were randomized to receive either 5 x 10^{10} viral particles of ChAdOx1 nCov-19 or control vaccine (MenACWY) (group 1, Supplementary Figure 1). Blood samples were collected on the day of vaccination and 7-, 14-, 28- and 56-days post vaccination. Supplementary Table 1 summarises the number of individuals assessed in each assay.

Immune cell activation induced by ChAdOx1 nCoV-19 vaccination with Th1-biased cytokine secretion.

An unbiased approach was applied to measure gross phenotypic and cellular changes on days 7, 14, 28 post vaccination (figure 1A-E). Flow cytometric and combined tSNE analysis of 26 randomly selected ChAdOx1 nCoV-19 vaccinated volunteers showed discrete populations of T cells, NK cells, and B cells. Within these clusters, distinct populations of proliferating (Ki-67$^+$) or activated (CD69$^+$) cells were identified (figure 1B-E). B cells, especially the IgG$^+$ B cell population, up-regulated Ki-67 at all post-vaccination time points (figure 1F and G). Within the total B cell population, the shift towards an activated phenotype peaked on day 7 to day 28, and for the IgG$^+$ B cell population on days 7-14 (figure 1F and G).

CD4$^+$ T cells had increased expression of CD69 on days 7 to 28 post-vaccination and a trend towards increased Ki-67 expression at days 7 and 14 post-vaccination (figure 1F and G). [25 25] CD8$^+$ T cells expressed a similar pattern of Ki-67 and CD69 expression between days 7 and 28 post-vaccination (figure 1F and G). We did not detect increases in expression of terminal differentiation markers CD57 and KLRG1 in post-vaccination CD8$^+$ T cells (Supplementary Figure 2), which would indicate a

reduction in post-vaccination cytotoxic capacity[26]. After peptide stimulation, an increase in TNFα and IFNγ production by $CD4^+$ T cells was also observed at day 14 (figure 1H).

NK cells can elicit a cytotoxic response to viral infection or vaccination[27,28]. Total expression of Ki-67 by NK cells increased steadily to a peak at day 28 (figure 1F). There was no significant change in the expression of CD57, or the activating receptor NKG2C (Supplementary Figure 2).

Multiplex cytokine analysis was performed on day 7 post-vaccination following antigen-specific stimulation of PBMC with pooled SARS-CoV-2 spike peptides. Of the nine cytokines analysed, five (IL-1β, IL-12p70, IL-4, IL-13 and IL-8) showed no difference in expression levels following stimulation. IFNγ and IL-2 levels following PBMC stimulation were significantly increased in ChAdOx1 nCoV-19 vaccinees compared with MenACWY controls (***$p = 0.0009$ and **$p = 0.0027$ respectively, two-tailed Mann-Whitney test). IL-4 and IL-13 levels following PBMC stimulation were not elevated in ChAdOx1 nCoV-19 vaccinees following stimulation of PBMC ($p > 0.05$ for both, two-tailed Mann-Whitney test), but a modest increase in IL-10 was measured (*$p = 0.045$, two-tailed Mann-Whitney test). The magnitude of cytokine secretion measured in PBMC supernatant in ChAdOx1 nCoV-19 vaccinees was greater for IFNγ (median 36.4 pg/mL, IQR 15-67) and IL-2 (median 10.7 pg/mL, IQR 1.7-22), than for IL-10 (median 1.4 pg/mL, IQR 0.9-2.6) indicating a strong potential bias towards secretion of Th1 cytokines in blood in response to stimulation with SARS-CoV-2 spike peptides (figure 1I).

Humoral and cellular immune responses to ChAdOx1 nCoV-19 do not differ by sex

Robust immunity induced by ChAdOx1 nCOV-19 against the SARS-CoV-2 spike antigen, measured by *ex vivo* IFNγ ELISpot and total IgG ELISA was previously reported[7]. We analysed these two main immunological outcome measures by sex and age (Supplementary Figure 3). We found no sex difference in vaccine response at any of the time points measured Supplementary Figure 3A and B, $p > 0.05$, two-tailed Mann-Whitney test). We detected no association between age and magnitude of

immune response for either outcome measure (Supplementary Figure 3C) in this population aged between 18 and 55 years.

ChAdOx1 nCoV-19 vaccination induces SARS-CoV-2-specific IgM and IgA levels

Anti-SARS-CoV-2 IgG responses were detectable at day 14, peaked at day 28 and were maintained at day 56 as reported previously[7]. Here we show that vaccination with ChAdOx1 nCoV-19 also generated increased levels of SARS-CoV-2 spike-specific IgM and IgA with peak responses at day 14 or day 28, respectively (figure 2A and B; Supplementary Table 2). Low SARS-CoV-2 spike-specific IgE was detected following vaccination with ChAdOx1 nCoV-19, similar to that in convalescent COVID-19 patients (Supplementary Figure 4).

Anti-SARS-CoV-2 spike-specific IgG avidity increased significantly between day 28 (median 0.66, IQR 0.60-0.76; n=44) and day 56 after vaccination (median 0.88, IQR 0.74-0.94; n=44) (***p <0.001, two-tailed Wilcoxon test) (figure 2C). At day 56, IgG avidity induced by ChAdOx1 nCoV-19 vaccination was comparable to that measured in plasma from convalescent COVID-19 patients (median 0.77, IQR 0.62-0.92; n=49).

Subclass analysis after vaccination with ChAdOx1 nCoV-19

Specific IgG1 and IgG3 responses were readily detectable at day 14, increased by day 28 and returned to a similar level to that measured on day 14 by day 56 (figure 3A and B; table S2). While IgG3 responses were quantifiable in nearly all vaccinees (day 14 39/44; day 28 42/44 and day 56 39/44), IgG1 responses were quantifiable in approximately half (day 14 24/44; day 28 23/44 and day 56 22/44). Median levels of IgG2 and IgG4 were low across all time points (figure 3C and D). A similar IgG3/IgG1 profile with low levels of IgG2 and IgG4 was measured in convalescent plasma samples. In agreement with previously reported data[29], SARS-CoV-2 spike-specific IgG1 was below the limit of quantitation in some convalescent plasma samples (figure 3A).

ChAdOx1 nCoV-19 induces a broad T cell response to the S1 and S2 subunits of the SARS-CoV-2 spike antigen

Vaccine-specific T cell responses were measured by IFNγ ELISpot before and after vaccination with ChAdOx1 nCoV-19, peaking at day 14[7] and summed T cell responses to the peptide pools for this cohort have been previously reported[7]. Responses were assayed against 13 pools of overlapping peptides (table S3) spanning the length of the vaccine antigen insert, which includes the S1 and S2 subunits, and an exogenous human tissue plasminogen activator (tPA) leader signal sequence peptide previously shown to enhance immunogenicity of a MERS-CoV vaccine candidate in mice[30]. There was a significant increase in response against both subunits between D0 and D14 (Figure 4A, n=42 participants, $p<0.0001$ for both S1 and S2 comparing D0 to D14, 2-tailed Wilcoxon matched pairs test). All pools except tPA elicited a positive response in at least 24% of participants, (defined as the median of the negative control plus 4 SDs) indicating recognition of multiple epitopes across the spike antigen (figure 4B, n=42). The most frequently recognized pools were 4 and 2 which span amino acids 311 to 430 and 101 to 200 of the S1 domain and generated a positive response by IFNγ ELISpot in 35/42 (83%) and 33/42 (78%) of participants, respectively. Responses at D14 were also plotted as fold-change from D0 (figure 4B and Supplementary Figure 5) and the greatest increases were to pools 4 and 5. These pools elicited a median response of 146 SFC/10^6 PBMCs and 80 SFC/10^6 PBMCS respectively at day 14, equating to a median of a 27 and 18-fold change from baseline.

Vaccination induces a Th1-biased $CD4^+$ and $CD8^+$ T cell response against SARS-CoV-2 spike peptides

Flow cytometry with intracellular cytokine staining of PBMC stimulated with peptides spanning the S1 and S2 subunits of SARS-CoV-2 spike protein demonstrated antigen-specific cytokine secretion from both $CD4^+$ (median 0.12, IQR 0.061-0.16) and $CD8^+$ (median 0.074 IQR 0.036-0.12) T cells 14 days after a single dose of ChAdOx1 nCoV-19 (figure 4C). $CD8^+$ T cells expressing the degranulation marker CD107a indicating cytotoxic function were detected after vaccination (median 0.038, IQR 0.012-0.066, figure 4D). $CD4^+$ responses were heavily biased towards secretion of Th1 cytokines (IFNγ and IL-2) rather than Th2 (IL-5 and IL-13, figure 4E) The frequency of cytokine positive cells was generally higher in the $CD4^+$ T cell population than the $CD8^+$ T cell population and cytokine responses were detected at day 14 from participants with positive pre-vaccination T cell and antibody responses to SARS-CoV-2 (figure 4F). When combinations of cytokines were assessed, few multifunctional T cells were detected in either the $CD4^+$ or $CD8^+$ T cell populations (figure 4G). Responses were dominated by T cells expressing single cytokines, particularly monofunctional $IFN\gamma^+$ $CD8^+$ T cells.

Discussion

An effective vaccine against COVID-19 will likely require both neutralising antibodies and a Th1-driven cellular component. Analysing the induction of immune responses post-vaccination is driven in part by concerns about enhanced disease from potentially immunopathologic Th2 responses, as seen in animal studies of vaccines against other coronaviruses[3,18-21]. Vaccine enhanced disease was also observed in early development of inactivated vaccines against respiratory syncytial virus (RSV), wherein pathology was associated with a high ratio of non-neutralising antibodies to neutralising antibodies, infiltration of neutrophils and eosinophils, and predominantly a Th2 biased response[31]. We have shown that antibodies induced after the first dose of ChAdOx1 nCoV-19 are neutralising and are further increased following a second dose[7] and were associated with reduced disease in vaccinated and challenged NHPs[22].

We have described here the profile of cytokine expression from both $CD4^+$ and $CD8^+$ T cells and the IgG subclass composition of the antibody response after administration of a single dose of the ChAdOx1 nCoV-19 vaccine. Robust B cell activation and proliferation was observed after vaccination and anti-IgA and IgG antibodies to the SARS-CoV-2 spike protein were readily detected in sera from vaccinated volunteers[7]. Anti-spike IgG responses at the peak of the response post-vaccination show a polarized IgG1 response, consistent with naturally acquired antibodies against SARS-CoV-2, as well as an IgG3 response in the majority of vaccinees. Produced early after viral infections, IgG3 coordinates multiple antibody effector functions and may contribute to recovery after SARS-CoV-2 infection[32,33]. A mixed IgG1 and IgG3 response, with low levels of IgG2 and little detectable IgG4 is in agreement with previously published reports describing the induction of Th1-type human IgG subclasses (IgG1 and IgG3) following adenoviral priming[34,35].

ChAdOx1 nCoV-19 induces a broad and robust T cell response to both S antigen subunits. The functionality of the T cell response observed here is similar in phenotype to that observed with other

replication deficient adenoviral vectors, with responses dominated by individual T cells secreting single, rather than multiple cytokines[20]. Whether vaccine-induced monofunctional or polyfunctional T cells are of greater protective value appears to vary by disease[53,54] and is unclear for SARS-CoV-2 infection. Analysis of cytokine secretion following peptide stimulation of PBMC demonstrated that IFNγ and IL-2 secretion were increased in ChAdOx1 vaccinees compared with controls and, importantly, IL-4 and IL-13 levels were not increased. Similarly, phenotyping by flow cytometry demonstrated that $CD4^+$ T cells secreted predominantly Th1 cytokines (IFNγ, IL-2 and TNFα) rather than Th2 (IL-5 and IL-13). Importantly we demonstrate with several methodologies (multiplex cytokine profiling, ICS analysis and antibody isotype profiling) that vaccination with ChAdOx1 nCoV-19 induces a predominantly Th1 response.

An important aspect in the epidemiology of COVID-19 disease is the marked difference in the mortality rates from disease between males and females, despite similar case rates[36]. We therefore disaggregated the data by sex and demonstrated no difference in the magnitude of either cellular or total IgG antibody responses between male and female participants. Other significant demographic risk factors for COVID-19 disease have been shown to include age and ethnicity[37]. The sample size in this cohort was relatively small, age was limited to 18-55 years and the vast majority of participants were white, limiting the ability to investigate these variables. It will be necessary to continue disaggregated analysis of the larger Phase 2 and 3 cohorts, powered for sub-group analysis. It will also be important to continue to assess immune response durability over time, with consideration given to comorbidities which may further influence vaccine-induced immunity[38].

Although there are no defined immune correlates of protection against COVID-19, it is generally accepted that high-titre neutralising antibodies with a robust cytotoxic $CD8^+$ T cell response and Th1 biased $CD4^+$ effector response will be optimal for protective immunity following SARS-CoV-2 exposure[39]. Determining the precise threshold and phenotype of immune responses associated with protection will be crucial for bridging between populations and vaccines for any vaccine that

demonstrates useful efficacy against infection or disease. If the immunogenicity of current vaccine candidates is insufficient, alternative prime boost regimens using technologies that are rapidly and sustainably scalable, such as heterologous adenoviral prime-boost regimens, or combinations of viral vectors with approaches such as mRNA vaccines, may be implemented. While adenovirus-based viral vectors and mRNA vaccines have been in preclinical development for some time, few have progressed to Phase 3 and subsequent market authorisation therefore relatively little is known about effectiveness when compared with traditional vaccine platforms.

Although the number of participants studied here is relatively small, the detailed immunophenotyping of vaccine-induced immunity described here demonstrates strong humoral and cellular immune responses after a single dose, characterised by a Th1-dominated response. Importantly, several other COVID-19 vaccine candidates in clinical development have also reported neutralising antibody responses[40] and induction of Th1-biased cell-mediated immunity.

This data further supports the ongoing evaluation of the ChAdOx1 nCoV-19 vaccine candidate in Phase 2 and 3 clinical trials.

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Author contributions (CRediT taxonomy)

Conceived and designed experiments-AF, KE, TL, HS

Methodology – SB-R, JB, KE

Formal analysis – SB-R, JB, HS, AF, IC,RAM,RIM,KE,DB, MV

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Project administration – PA, MW

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Reviewed and edited the paper -all authors

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

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Figure Legends

Figure 1. Activation of lymphocyte populations post ChAdOx1 nCoV-19 vaccination.

(**A-E**) t stochastic neighbour embedding (tSNE) analysis of 9,600,000 live lymphocytes from 26 ChAdOx1 nCoV-19 vaccine trial participants across four time points (D0 n=24, D7 n=23, D14 n=25, D28 n=24). 2 samples were not available and 6 samples with <100,000 live lymphocytes were excluded. The tSNE plot was generated by concatenation of samples containing 100,000 randomly selected live lymphocytes from each sample.

(**A**) global clustering of immune cells across all samples. (**B-E**) tSNE population analysis at day 0 and days 7, 14, and 28 post-vaccination. Areas of Ki-67$^+$ activity (yellow) cluster in IgG$^+$ B cells (1), NK cells (2), and CD4$^+$ T cells (3) post-vaccination. Analysis conducted on unstimulated cells.

(**F-H**) Heatmap analysis of activation markers expressed by immune cells at days 0, 7, 14 and 28 post-ChAdOx1 nCoV-19 vaccination. (D0 n=24, D7 n=23, D14 n=25, D28 n=24)

(**F**) Expression of Ki-67 by IgG$^+$ B cells and NK cells (top two rows), and NK cells (top three rows). Expression of CD69 by CD4$^+$ T cells and CD8$^+$ T cells (bottom two rows). G) Expression of Ki-67 by B cells, CD4$^+$ T cells and CD8$^+$ T cells.

(**H**) Expression of TNFα and IFNγ by CD4$^+$ T cells. Analysis of TNFα and IFNγ expression conducted on cells stimulated with spike glycoprotein peptide pools with unstimulated values subtracted. All other analysis conducted on unstimulated cells

(**I**) A multiplex cytokine analysis was performed on day 7 post-vaccination using supernatants following antigen specific stimulation of PBMC from ChAdOx1 nCov-19 (red) and MenACWY (blue). Number of samples presented MenACWY-ChAdOx1 nCov-19: IFNγ (n =40-40); IL-2 (n=42–42); TNFα (n=40-41); IL-1β (n=41-42); IL-12p70 (n=38-28); IL-4 (n=38-38); IL-10 (n=41-39); IL-13 (n=31-36); IL-8 (n=42-41). Individual data points shown here as aligned dot plot with lines showing median with IQR.

Significant differences determined by two-tailed Mann-Whitney test with Bonferroni correction for multiple comparison (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).

Figure 2. Immunoglobulin isotype responses induced by ChAdOx1 nCoV-19 vaccination.

Volunteers received ChAdOx1 nCoV-19 (red) or MenACWY (blue) vaccination at day 0. SARS-CoV-2 spike trimer-specific IgM (**A**) and IgA (**B**) responses were quantified by standardised ELISA. Individual data points were expressed as ELISA units (EU) and shown here as aligned dot plot with lines showing median with IQR. Assays were performed at D0, D7, D14, D28 and D56 post vaccination (n=44 for all time points in both groups of volunteers, except for D56 in ChADOx1 nCoV19 where there were only 43 volunteers available). Dotted lines are shown at the limit of quantification of each assay.

(**C**) Avidity of SARS-CoV-2 spike trimer-specific IgG responses was measured using a NaSCN chemical displacement ELISA. Individual data points were expressed as an IC50 and shown here as scatter dot plots with lines showing median with IQR. Assay was performed at D28 (n=44) and D56 post vaccination with ChAdOx1 nCoV-19 (n=43). Significant difference determined by two-tailed Wilcoxon test (***$p < 0.001$).

CONV = convalescent plasma samples from recovered SARS-CoV-2 patients shown here as scatter dot plot with line showing median with IQR (SARS-CoV-2 spike trimer-specific IgM, IgA n=50; IgG avidity n=49). CONV symbols are coloured by disease severity, green - asymptomatic, grey - mildly symptomatic, yellow - moderately symptomatic, orange - severely symptomatic and red - critically symptomatic.

Figure 3. IgG subclass responses induced by ChAdOx1 nCoV-19 vaccination.

Volunteers with measurable SARS-CoV-2 spike-specific IgG at day 14 were assayed for IgG subclasses. SARS-CoV-2 spike trimer-specific IgG1 (**A**) and IgG3 (**B**) responses were

quantified by standardised ELISA and expressed as ELISA units (EU) IgG2 (**C**) and IgG4 (**D**) were measured by indirect ELISA and expressed as OD_{405}. Individual data points shown here as aligned dot plot with lines showing median with IQR. Assays were performed at D14, D28 and D56 post vaccination (n=44 for all time points, except for D56 where there were only 43 volunteers available). Dotted lines are shown at the limit of quantification of each assay.

CONV = convalescent plasma samples from recovered SARS-CoV-2 patients shown here as scatter dot plot with lines showing median with IQR (n=50). CONV symbols are shown as diamonds and coloured by disease severity, green - asymptomatic, grey - mildly symptomatic, yellow - moderately symptomatic, orange - severely symptomatic and red - critically symptomatic.

Figure 4: T cell responses to SARS-CoV-2 spike peptides measured by IFNγ ELISpot and flow cytometry with intracellular cytokine staining.

Only data from ChAdOx1 nCoV-19 vaccinees is shown.

(**A**) Total response to S1 and S2 (sum of 6 peptide pools each) at D0 and D14 post-vaccination. Individual data points shown here as scatter dot plot with lines showing median with IQR (n=42 for all time points). The dashed line represents the lower limit of detection of the assay (48 spot-forming cells (SFC)). Significant difference determined by two-tailed Wilcoxon test (***$p < 0.001$).

(**B**) Heat map of fold-change in SFC to each peptide pool for every participant from D0 to D14 post-vaccination (n=42 for all time points).

(**C**) Frequency of $CD4^+$ or $CD8^+$ T cells expressing IFNγ, IL-2 or TNFα (n = 34) and (**D**) frequency of CD8+ T cells expressing CD107a+ at D14 post-vaccination (n=34). Individual data points shown here as scatter dot plot with lines showing median with IQR.

(**E**) Frequency of Th1 and Th2 cytokine secretion by $CD4^+$ T cells at D14 post-vaccination (n=34). Individual data points are shown as box plot with whiskers from the minimum to the maximum value.

(**F**) Frequency of $CD4^+$ or $CD8^+$ T cells expressing relevant individual cytokines at D14 post-vaccination. Individual data points shown here as scatter dot plot with lines showing median with IQR (n=32). Dotted lines show lower limit of detection (LLD). Dark green circles indicate participants with an ELISPOT response to the summed spike peptides pools >200 SFC/10^6 PBMC prior to vaccination (n=2), light green circles indicate participants with an ELISpot response to the summed spike peptides pools >200 SFC/10^6 PBMC who were also seropositive prior to vaccination (n=3). Clear circles represent participants with an ELISpot response <200 SFC/10^6 PBMC who were also seronegative prior to vaccination (n=27).

(**G**) Pie charts indicating the expression of cytokine combinations from $CD4^+$ and $CD8^+$ T cells (n=32, all panels).

Online Methods

Study procedures and sample processing

Full details on the conduct of the Phase I/II randomised controlled trial of ChAdOx1 nCoV-19 (AZD1222), including the trial protocol, were previously published[7]. This study was registered at ISRCTN [15281137] and ClinicalTrials.gov [NCT04324606]. Only data from single-dose vaccinated volunteers is included in this paper. Prior to enrolment, all participants gave written informed consent. The trial is conducted according to the principles of Good Clinical Practice (GCP) and approval was obtained by a national ethics committee (South Central Berkshire Research Ethics Committee, reference 20/SC/0145) and regulatory agency in the UK (the Medicines and Healthcare products Regulatory Agency). An independent data safety monitoring board (DSMB) was appointed before recruitment began.

Blood samples were collected on the day of vaccination and 7-, 14-, 28- and 56-days post vaccination. At timepoints for immunological analyses, blood samples were taken in both plain and heparinised collection tubes. Samples were processed within 4 hours of the blood draw. Plain tubes were processed for the collection of blood serum. Tubes were centrifuged at 1800 rpm for 5 minutes and the serum harvested for storage at -80°C until required. Heparinised tubes were processed for the collection of peripheral blood mononuclear cells (PBMCs) and blood plasma by density gradient centrifugation. Blood was decanted into Leucosep tubes (Greiner Bio-One) containing Lymphoprep (STEMCELL Technologies) and centrifuged at 1000 x g for 13 minutes with the brake off. A fraction of blood plasma was collected and stored at -80°C, whilst the remaining sample was decanted into a fresh falcon tube and topped up with R0 media (RPMI-1640 cell culture media containing 1% penicillin/streptomycin and 2 mM L-glutamine (all Sigma-Aldrich). Samples were centrifuged again at 1800 rpm for 5 mins, the supernatant poured off and the cell pellet resuspended once more in R0 for washing. After centrifugation, the cell pellet was resuspended in 10 ml of R10 media (RPMI-1640

containing 1% penicillin/streptomycin, 2 mM L-glutamine and 10% foetal calf serum (FCS, Labtech Intl.) for counting.

Cells were counted using a CasyCounter (OMNI Life Science) for use in fresh assays or for cryopreservation. The assays performed on fresh cells were ELISPOT and intracellular cytokine staining only (described below). All remaining cells were frozen at a concentration of 8-12 x 10^6 PBMCs per ml. After centrifugation (1800 rpm, 5 mins) cells were resuspended in cold FCS at half the total freeze-down volume. Cells were placed in a refrigerator (2-8°C) for 20 mins before an equal volume of cold FCS containing 20% dimethylsulphoxide was added. 1 ml aliquots were prepared and quickly transferred to CoolCells (Corning) for freezing at -80°C overnight. Tubes were then transferred to a -150°C ultra-low temperature freezer until required.

Convalescent plasma samples were obtained from hospitalised adult (≥18 years) patients admitted with PCR-positive SARS-CoV-2 infection or from health-care workers enrolled in COVID-19 surveillance studies. Studies were approved by the following committees: (Gastrointestinal Illness in Oxford: COVID substudy [Sheffield Research Ethics Committee reference: 16/YH/0247], ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections [Oxford Research Ethics Committee C reference 13/SC/0149], and Sepsis Immunomics project [Oxford Research Ethics Committee C, reference 19/SC/0296]). Both asymptomatic and symptomatic participants were tested for each assay. Additional details on experimental procedures performed on convalescent plasma samples was described previously[7].

Peptides and stimulations

Peptides spanning the full length of the SARS-CoV-2 spike protein sequence were synthesised for use in antigen-specific T cell assays (Proimmune Ltd.). A total of 253 peptides were synthesised as 15-mers overlapping by 10 amino acids. Peptides were also synthesised for the N-terminal tissue plasminogen activator (tPA) leader sequence which is included to increase expression of the vaccine

antigen from the adenoviral vector. Details of peptide sequences and pooling for assays are shown in table S3. Briefly, for the Cytek™ Aurora flow cytometry assay, MSD Th1/Th2 cytokine profiling assay and intracellular cytokine staining, two separate peptide pools were made spanning the S1 (134 peptides) and S2 (119 peptides) subunits of the SARS-CoV-2 spike protein. For the ELISPOT assay, 12 pools of between 18-24 peptides were made consisting of 6 pools each for the S1 and S2 subunits. A separate tPA leader sequence pool (5 peptides) was included in this assay.

Flow Cytometry conducted on Cytek Aurora spectral analyser

Flow cytometry was performed from frozen aliquots of peripheral blood mononuclear cells (PBMCs) of donors from days 0, 7, 14 and 28 after vaccination with ChAdOx1 nCoV19 (D0 n=24, D7 n=23, D14 n=25, D28 n=24). Cells were defrosted in media containing >5 U/mL benzonase and re-suspended in complete RPMI media supplemented with 10% FCS, L-glutamine and Penicillin/streptomycin at a concentration of $2x10^7$cells/mL. $2x10^6$ PBMCs per well were plated in a 96-well plate and stimulated with synthetic peptides spanning the SARS-CoV-2 spike protein split into two separate pools for the S1 and S2 subunits (table S3) at a final concentration of 2 µg/mL, or media as a control. One well per donor was stimulated with Phorbol 12-myristate 13-acetate and ionomycin (Cell Activation Cocktail, BioLegend) as a positive control. PBMCs were co-stimulated in the presence of anti-human CD28, CD49d (1 µg/mL, Life Technologies Ltd), and CD107a-BV785 (BioLegend) for two hours at 37°C with 5% CO_2, and then incubated for a further 16 hours after the addition of 1 µg/mL Brefeldin A and Monensin to each well (BioLegend).

PBMCs were washed in FACS buffer (phosphate Buffered Saline with 0.5% bovine serum albumin and 1% EDTA) and stained with a cocktail of surface antibodies including anti-human Live/Dead-Zombie UV, CD4-AF700, CD19-Spark NIR 685, CD56-APC, CCR7-PerCP/Cy5.5, PD1-PE/Dazzle 594, CD57-PE/Cy7(BioLegend) CD8-AF405, CD45RA-SuperBright 702, CD27-PerCP eF710, CD20-AF532 (ThermoFisher Scientific) CD16-BUV495, CD3-BUV661, CD138-BUV805, NKG2A-BV480, IgM-BB515

(BD Biosciences), NKG2C-PE, KLRG1-VioBlue (Miltenyi) in FACS buffer with 10% Brilliant Stain buffer Plus (BD Biosciences). PBMCs were incubated at 4°C in the dark for 30 minutes, then washed twice in FACS buffer. PBMCs were then incubated in CytoFix/CytoPerm solution (BD Biosciences) at 4°C in the dark for 30 minutes, then washed twice in Perm/Wash buffer, and then stained with a cocktail of intracellular antibodies including: anti-human IFNγ-BV650, IL-2-BV605 (BioLegend), IgG-BV421, TNFα-BUV395, CD69-BV750, CD71-BUV563, CD25-BV737 (BD Biosciences) Ki-67-APC eF780 (ThermoFisher Scientific) in Perm/Wash. PBMCs were incubated at 4°C in the dark for 30 minutes, washed twice in Perm/Wash buffer, once in FACS buffer, then re-suspended in 200 µL FACS buffer for acquisition on a custom four-laser Cytek Aurora spectral analyser using SpectroFlo v2.2 (Cytek biosciences).

Single-fluorochrome compensation was calculated on beads (BD Biosciences, Miltenyi) or human PBMCs. Analysis of data was conducted on FlowJo (v10.6.2) by a hierarchical gating strategy (figure S6) and Prism 8 (GraphPad). Peptide-specific responses were calculated by subtraction of the unstimulated controls from the peptide stimulated samples.

Down-sampling and t-SNE analysis was conducted on gated live lymphocytes in FlowJo v.10.7.1. A random sample of 100,000 cells per donor and time point were collected and concatenated into a single file. All fluorochrome colours and the sample time point were included as parameters. The t-SNE analysis was implemented on FlowJo v.10.7.1 with 100,000 iterations, a perplexity of 30, and using Barnes-Hut gradient algorithm.

MSD –Th1/Th2 cytokine profiling

Th1/Th2 cytokine responses were measured in tissue culture supernatants from the stimulation of PBMCs with synthetic peptides covering the spike protein. $5x10^5$ freshly isolated PBMCs were resuspended in 250 µL of R10 media in 96 well U-bottom plates and supplemented with 1 µg/mL anti-human CD28 and CD49d. Peptides spanning the S1 and S2 subunits of the SARS-CoV-2 spike

protein (table S3) were added to separate wells at a concentration of 2 µg/mL. Each sample also included an unstimulated (media only) control. Following a 16–18 hour incubation at 37°C with 5% CO_2, cells were pelleted by centrifugation (1800 rpm, 5 min) and 200 µLof supernatant was harvested. Supernatants from the S1 and S2 stimulations were combined and stored at –80°C until required.

Cytokine responses were analysed using MSD (Meso Scale discovery) V-plex proinflammatory cytokine (human) Panel 1 kit, validated by MSD. Each plate is coated with 9 different capture mAbs against 9 different cytokines arranged in independent spots on the base of each well. Cytokines IFN-γ, IL-1β, IL-2, IL-4, IL-8, IL-10, IL-12p70, IL-13 and TNFα are associated with either a Th1 or Th2 type T-cell response.

Supernatants were diluted 1:2 for unstimulated sample and 1:10 for S1/S2 stimulated sample in MSD diluent 2. The kit provides a multi-analyte lyophilised calibrator that when reconstituted will be used the standard curve using a 4-fold serial dilution to form an 8-point standard curve plated out in duplicate. Cytokine measurements were carried out according to manufacturer's instructions. Plates are read on MSD reader within 15 mins of adding Read buffer.

Data was analysed using MSD discovery workbench 4.0. Samples were repeated if any sample a replicate with a coefficient of variations (CV) greater than 20%. Replicates were read off the standard curve, multiplied by dilution factor, and concentration was reported as an average of the replicates in pg/mL. Concentration from unstimulated sample was subtracted from concentration from stimulated (background subtract). Negative values of background subtract have been replaced by zeros. An arbitrary value of 0.0001 has been added to the background subtracts across all the samples to overcome the presence of null values raised from samples too low to be read off the standard curve.

Isotype & Subclass Standardised ELISA

Samples from participants vaccinated with ChAdOx1 nCoV-19 and convalescent plasma samples were assayed for anti-spike IgG1, IgG3, IgA and IgM. Samples from participants vaccinated with MenACWY were assayed for anti-spike IgA and IgM antibodies only. Standardised ELISA was used to quantify circulating SARS-CoV-2 spike-specific IgG1, IgG3, IgA and IgM responses. Full methodological details for this assay were previously published (Barrett et al, *In Press*). Briefly, ELISA plates were coated overnight with 5 µg/mL SARS-CoV-2 full-length spike protein. After blocking with Blocker™ Casein in PBS (ThermoFisher Scientific), samples (minimum 1:50 dilution) were incubated for 2 hours at 37°C with 300 rpm shaking. Standard curve and internal controls were created from reference serum using a pool of high titre donor serum. An alkaline phosphatase-conjugated secondary antibody (dependent on the immunoglobulin subclass or isotype being detected) was then added and incubated for 1 hour at 37°C with 300 rpm shaking. Plates were developed using PNPP alkaline phosphatase substrate (ThermoFisher Scientifc) for 1-4 hours at 37°C with 300 rpm shaking and read at 405nm when the internal control reached an OD_{405} of 1. Plate pass/fail criteria are described in Barrett et al, *In Press*.

Isotype and Subclass OD ELISA

Antigen-specific IgG2, IgG4 and IgE responses were detected in the absence of an antigen-specific serum control by OD ELISA. Detailed procedures for this assay were previously described (Barrett et al, *In Press*). Briefly, ELISA plates were coated overnight with 5 µg/mL SARS-CoV-2 full-length spike protein, plus a commercial human immunoglobulin control for the antibody isotype or subclass being assayed. After blocking with Blocker™ Casein in PBS, test samples and pre-pandemic negative controls (minimum 1:50 dilution) were plated out for 2 hours at 37°C with 300 rpm shaking. Different alkaline–phosphatase-conjugated secondary antibodies were added depending on the immunoglobulin isotype or subclass being assayed for 1 hour at 37°C with 300 rpm shaking. Plates

were developed using PNPP alkaline phosphatase substrate for 1-4 hours at 37°C with 300 rpm shaking and read at 405 nm when the immunoglobulin control reached a specified OD_{405}. Negative cut-off calculations are described in (Barrett et al, *In Press*).

Avidity ELISA

The avidity of SARS-CoV-2 spike-specific IgG from volunteers who had a quantifiable response at day 28 was assessed. Anti-SARS-CoV-2 spike-specific total IgG antibody avidity of donor serum was assessed by sodium thiocyanate (NaSCN)-displacement ELISA. Nunc MaxiSorp™ ELISA plates (ThermoFisher Scientific) were coated overnight (≥16 hours) at 4°C with 50 µL per well of 2 µg/mL SARS-CoV-2 trimeric spike protein diluted in PBS. 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 a positive control serum pool, we diluted in blocking buffer to normalise them to an OD_{405} of 1 and 50 µL per well was added in duplicate to each row of the plate (except the last row where only blocking buffer was added). Plates were incubated for 2 hours at 20°C and then washed 3x with PBS/T and tapped dry. Increasing concentrations of NaSCN (Sigma-Aldrich) diluted in PBS were added at 50 µL per well to each row down the plate (1M, 2M, 3M, 4M, 5M, 6M) except for the first and last row where only PBS was added. Plates were incubated for 15 minutes at 20°C and then washed 6x with PBS/T and tapped dry. Anti-Human IgG (γ-chain specific)−alkaline phosphatase antibody produced in goat (Sigma-Aldrich) was diluted 1:1000 in blocking buffer and 50 µL per well was added to the plate. Plates were incubated for 1 hour at 20°C and then 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 20°C. Optical density at 405 nm (OD_{405}) was measured using an ELx808 absorbance reader (BioTek) until the untreated sample wells reached an OD_{405} of 1 (0.8-2.0). Gen5 ELISA software v3.09 (BioTek) was used to plot the test sample OD_{405} against concentration of NaSCN and a spline function with smoothing factor 0.001 was fitted to the data. For each sample, concentration of NaSCN required to

reduce the OD_{405} to 50% of that without NaSCN (IC_{50}) was interpolated from this function and reported as a measure of avidity.

Ex vivo IFNγ ELISpot assays

ELISpot assays were performed on freshly isolated PBMCs prior to, and 14 days after vaccination with ChadOx1 nCoV19 as previously described [7]. Assays were performed using Multiscreen IP ELISpot plates (Millipore) were coated overnight at 4°C with 10 µg/mL of human anti-IFNγ coating antibody (clone 1-D1K, Mabtech) in carbonate buffer, before washing 3 times with PBS and blocking with R10 media for 2-8 hours. 2.5×10^5 PBMCs were added to each well of the plate along with 13 pools of peptides covering the SARS-CoV-2 spike protein and the N-terminal tissue plasminogen activator leader sequence at a final concentration of 10 µg/mL (table S3). Each assay was performed in triplicate and incubated for 16–18 hours at 37°C with 5% CO_2.

Plates were then developed by washing 6 times with PBS/T, followed by addition of 1 µg/mL anti-IFNγ detector antibody (7-B6-1-Biotin) to each well. After a 2–4 hour incubation, plates were washed again and 1:1000 SA-ALP added for 1-2 hours. After a final wash step, plates were developed using BCIP NBT-plus chromogenic substrate (Moss Inc.).

ELISpot plates were counted using an AID automated ELISpot counter (AID Diagnostika GmbH, algorithm C), using identical settings for all plates, and spot counts were adjusted only to remove artefacts. Responses were averaged across triplicate wells and the mean response of the unstimulated (negative control) wells were subtracted. Results are expressed as spot forming cells (SFC)/10^6 PBMCs. Responses to a peptide were considered positive if background subtracted responses were >40 SFU/10^6 PBMCs. If responses were >80 SFC/10^6 PBMC in the negative control (PBMC without antigen) or <800 SFC/10^6 PBMC in the positive control wells (pooled Staphylococcal

enterotoxin B at 0.02 µg/mL and phytohaemagglutinin-L at 10 µg/mL), results were excluded from further analysis.

Intracellular cytokine staining

Intracellular cytokine staining (ICS) was performed on freshly isolated PBMCs stimulated with pooled S1 and S2 peptides. 3 x 10^6 PBMCs were resuspended in 5 mL polypropylene FACS tubes to a volume of 1 mL in R10 media supplemented with 1 µg/mL anti-human CD28 and CD49d and 1 µl CD107a PE-Cy5 (eBioscience). S1 and S2 peptide pools (table S3) were added at a concentration of 2 µg/mL. Each sample also included a positive control (Staphylococcal enterotoxin B at 1 µg/mL, Sigma Aldrich) and an unstimulated (media only) control. Cells were incubated at 37°C with 5% CO_2 for 16-20 hours with Brefeldin A (3 µg/mL) and monensin (2 mM) (eBioscience) added after 2 hours.

At the end of the incubation, cells were washed in FACS buffer (PBS containing 0.1% bovine serum albumin and 0.01% NaN_3) and transferred to a 96 well U-bottom tissue culture plate for staining. A surface staining cocktail was first added containing 2.5 µL of a 1:40 dilution of Aqua Live/Dead stain (ThermoFisher Scientific) and 1 µL of BV711 CCR7 (Biolegend) in 46.5 µL FACS buffer. Cells were incubated in the dark for 20 minutes and washed with FACS buffer. 100 µL CytoFix/CytoPerm solution (BD Biosciences) was added to each well and left to incubate for a further 20 minutes. Cells were then washed with Perm/Wash buffer before intracellular cytokine staining. The ICS cocktail contained 0.025 µL CD45RA BV605, 0.025 µl TNFα PE-Cy7, 0.1 µl IFNγ FITC, 0.025 µl CD14 e450, 0.025 µl CD19 e450, 0.5 µl CD3 AF700, 1 µL IL-2 BV650, 1.25 µL IL-5 PE, 2.5 µL IL-13 APC, 3.5 µL CD4 PerCP Cy5.5 and 5 µL CD8 APC-eF780 to a total volume of 50 µL diluted in FACS buffer. Samples were stained in the dark for 30 minutes. Cells were washed twice with perm/wash buffer and twice with FACS buffer before being resuspended in 100 µL of 1% paraformaldehyde.

Compensation controls were prepared fresh for each batch using OneComp eBeads (eBioscience). Cells were kept on ice and strained through a 35 µm filter before acquisition. Cells were acquired on a 5-laser BD LSRFortessa flow cytometer (BD Biosciences) using FACSDiva v8.02 (BD BioSciences) and data analysed in FlowJo v10.7. A hierarchical gating strategy was applied for sample analysis (figure S7). A QC process was applied to remove samples with fewer than 100,000 events in the live $CD3^+$ gate, samples with <1% cytokine response to SEB ($CD4^+$ and $CD8^+$ $IFN\gamma^+$, $CD8^+$ $TNF\alpha^+$). A lower limit of detection was applied and only samples with an ELISPOT response greater than 200 SFC/10^6 PBMC were included in the analysis.

Statistical analysis

All statistical tests as well as all graphical representation of the data were performed in GraphPad Prism 8.4.3. Data are presented as medians with interquartile ranges (IQR). To check for the normality of the data, d'Agostino-Pearson tests were used. Unpaired samples were compared using a Mann-Whitney U tests, while paired samples were compared with Wilcoxon test. All tests were two-tailed, with a 5% per-comparison error rate. Bonferroni correction was used to correct for multiple comparisons. Correlations were analysed using Spearman's rank test. *p* values of less than 0.05 were considered significant.

Data Availability Statement

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 on such requests prior to disclosure.