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Research Article

Persistence of the immune response after two doses of ChAdOx1 nCov-19 (AZD1222): 1 year of follow-up of two randomized controlled trials

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Abstract

The trajectory of immune responses following the primary dose series determines the decline in vaccine effectiveness over time. Here we report on maintenance of immune responses during the year following a two-dose schedule of ChAdOx1 nCoV-19/AZD1222, in the absence of infection, and also explore the decay of antibody after infection. Total spike-specific IgG antibody titres were lower with two low doses of ChAdOx1 nCoV-19 vaccines (two low doses) ($P = 0.0006$) than with 2 standard doses (the approved dose) or low dose followed by standard dose vaccines regimens. Longer intervals between first and second doses resulted in higher antibody titres ($P < 0.0001$); however, there was no evidence that the trajectory of antibody decay differed by interval or by vaccine dose, and the decay of IgG antibody titres followed a similar trajectory after a third dose of ChAdOx1 nCoV-19. Trends in post-infection samples were similar with an initial rapid decay in responses but good persistence of measurable responses thereafter. Extrapolation of antibody data, following two doses of ChAdOx1 nCov-19, demonstrates a slow rate of antibody decay with modelling, suggesting that antibody titres are well maintained for at least 2 years. These data suggest a persistent immune response after two doses of ChAdOx1 nCov-19 which will likely have a positive impact against serious disease and hospitalization.

Keywords: vaccine, antibodies, anti-viral immunity, vaccination

Abbreviations: AIC: Akaike's Information Criteria; ELISA: enzyme-linked immunosorbent assay; ELISpot: enzyme-linked immunospot; EU: ELISA units; IgG: immunoglobulin G; LDLD: two low doses; LDSD: low dose followed by standard dose; mRNA: messenger ribonucleic acid; ND50: dilution to achieve 50% virus neutralization; SARS CoV-2: severe acute respiratory syndrome coronavirus 2; SDSD: 2 standard doses; SEAP: secreted embryonic alkaline phosphatase; UKHSA: United Kingdom Health Security Agency; VE: vaccine efficacy; VoC: variant(s) of concern

Introduction

The SARS CoV-2 pandemic continues to place a significant burden on healthcare systems and economies worldwide.

The ChAdOx1 nCoV-19 (AZD1222) vaccine is a replication deficient adenoviral vectored vaccine which encodes the SARS CoV-2 spike protein from the circulating strain first identified in late 2019. ChAdOx1 nCoV-19 has been distributed for vaccination in more than 180 countries across six continents and over 3 billion doses have been released for supply to vaccination programmes worldwide.

Vaccine efficacy (VE) against symptomatic infection in a pre-specified pooled analysis of trials conducted in the UK, South Africa, and Brazil, was 66.7% (95% CI 57·4–74·0) and in the US Phase 3 study VE was 74% (95% CI 65.3–80.5%) [1, 2] Data continue to accrue demonstrating that vaccination against COVID-19 prevents hospitalization and death with positive outcomes demonstrated after vaccination with a single dose of ChAdOx1 nCoV-19 [2–4]. Real-world data demonstrate that vaccination protects against serious disease and hospitalization following infection with variants of concern (VoC) [5, 6], and it has been estimated that the ChAdOx1 nCoV-19 vaccine has saved 6.3 million lives in the first year of the global vaccine rollout.

After peaking 4–6 weeks post-vaccination, vaccine-induced immunity, and VE against symptomatic infection begin to wane [7, 8]. The trajectory of antibody decay after a two-dose schedule has been published for some COVID-19 vaccines with 6 months of follow-up [9–12]. Antibody decay is often modelled using an exponential model with the assumption that antibody waning assumes a linear trajectory. This assumption may not hold when modelling the trajectory of antibody decay over an entire year and longer.

Currently, there are few data available on the persistence of antibody titres for one year after vaccination due to on-going booster vaccination and virus transmission. Here we present persistence data for both antibody and T-cell responses during 1 year of follow-up after a two-dose schedule of ChAdOx1 nCoV-19, excluding those participants with a known or suspected case of COVID-19. We model the decay process to determine whether antibody continues to decay at a similar rate in the second half of the year as is observed in the first 6 months. We also investigate if decay rates vary by the dose of ChAdOx1 nCoV-19/AZD1222 vaccine received and the interval between first and second doses, and after infection.

Materials and methods

Participants and study procedures

Participants are from two study sites (Oxford and Southampton) from the multi-centre single-blind randomized controlled trials of ChAdOx1 nCoV-19 in the UK (COV001, NCT04324606 and COV002, NCT04400838). Participants were recruited to either safety and immunogenicity groups or efficacy cohorts and randomized to receive either ChAdOx1 nCoV-19 vaccine or a control product (MenACWY). A full description of the safety, immunogenicity (up to 28 days after second vaccination), and interim efficacy of these and related studies has been previously published, including full study protocols [2, 13, 14].Written informed consent was received from all participants.

Participants were enrolled between April and August, 2020. Participants included in this analysis received either two low-dose vaccines (LDLD) or two standard doses of vaccine (SDSD), or a low dose followed by a standard dose (LDSD) of vaccine, or three standard doses. Blood was sampled at day 90, 182, or 364 second dose (±30 days), or 28 days earlier (days 154 and 336) in those who were received two doses 28 days apart. Control group participants were not included in the analysis which is focussed on the persistence of immune responses to ChAdOx1 nCoV-19 vaccine only.

Immunogenicity assessments

Total anti-spike IgG was measured using an in-house standardized total IgG ELISA against trimeric SARS CoV-2 spike protein. Live virus microneutralization titres were measured by Health Security Agency (Public Health England) using the Victoria strain of SARS-CoV-2 [15]. Cellular responses were assessed using an *ex vivo* interferon-γ enzyme-linked immunospot (ELISpot) assay to enumerate antigen-specific T cells [15].

Anti-ChAdOx1 vector neutralization titres were measured using a secreted embryonic alkaline phosphatase (SEAP)-reporter assay, which measures the reciprocal of the serum dilution required to reduce in-vitro expression of vector-expressed SEAP by 50%, 24 h after transduction [16].

Statistical methods

Log-transformed anti-spike IgG data were analyzed in a mixed effects log–log regression model regressing log-transformed IgG values on log-transformed days from vaccination. Participant-level random intercepts were included along with fixed effect terms for participant age (years, centred on age 40 years), the interval between first and second doses (< 8 weeks, 9–12 weeks, >12 weeks), and the vaccine doses received (LDLD, LDSD, SDSD). Log–log models were compared with log–linear models using Akaike's Information Criteria (AIC).

A similar approach was applied to the analysis of ELISpot and neutralization data. However, terms for interval between first and second doses, and vaccine received were not included as these were not applicable to the data, which was available on a subset of SDSD participants only and without peak immunogenicity timepoint of day 14 post primary vaccination.

Participants who had evidence of SARS-CoV-2 infections during the follow-up period were excluded from the main models, as were those who received third doses of vaccine or additional vaccines outside of trial procedures. For each participant, an arbitrary threshold of a more than 2-fold increase in antibody titres between two study visits was considered evidence of possible infection or unreported vaccinations outside the trial and these were excluded.

A separate model was fitted to antibody values from timepoints subsequent to a positive PCR test in vaccinated participants and the decay slope modelled with a similar log–log mixed effects regression model with participant-level random effects and no additional fixed effects.

Analysis was done using R version 4.1.1. R code and output are shown in the Appendix.

Ethical review was provided by the South Central Berkshire Research Ethics Committee (20/SC/0179).

Results

There were 600 participants included in the analysis of anti-spike IgG for whom between one and four blood samples were available from timepoints post second dose of vaccine.

Over a 1-year follow-up period log–log models fit the serology data better than log–linear (exponential decay) models due to faster decay of antibody in the first half of the year than later in the year.

Total spike-specific IgG antibody titres were lower with two low doses of ChAdOx1 nCoV-19/AZD1222 vaccines (LDLD) (*P* = 0.0006) than with SDSD or LDSD regimens

(Fig. 1, Table S1). However, serological responses after SDSD and LDSD vaccines were not significantly different from each other ($P = 0.47$). Longer intervals between first and second doses resulted in higher antibody titres ($P < 0.0001$); however, there was no evidence that the decay trajectory differed by interval or by vaccine dose, and therefore the model without the interaction was retained. Older age was associated with lower titres irrespective of dose received (Fig. 2), the age by days interaction was non-significant and removed from the model (Table S1). Following a third dose of ChAdOx1

nCoV-19/AZD1222, the decay of IgG antibody titres followed a similar trajectory as that observed after two doses. No differences between males and females were observed, as previously published [17].

A similar modelling approach was applied to the ELISpot response (Fig. 3). Data were available from participants who received two standard doses < 8 weeks apart and there was more rapid reduction in spot forming cells (SFCs) in the first 6 months after vaccination than in subsequent time periods. Geometric mean SFCs per 10^6 PBMCs (95% CI) were 364
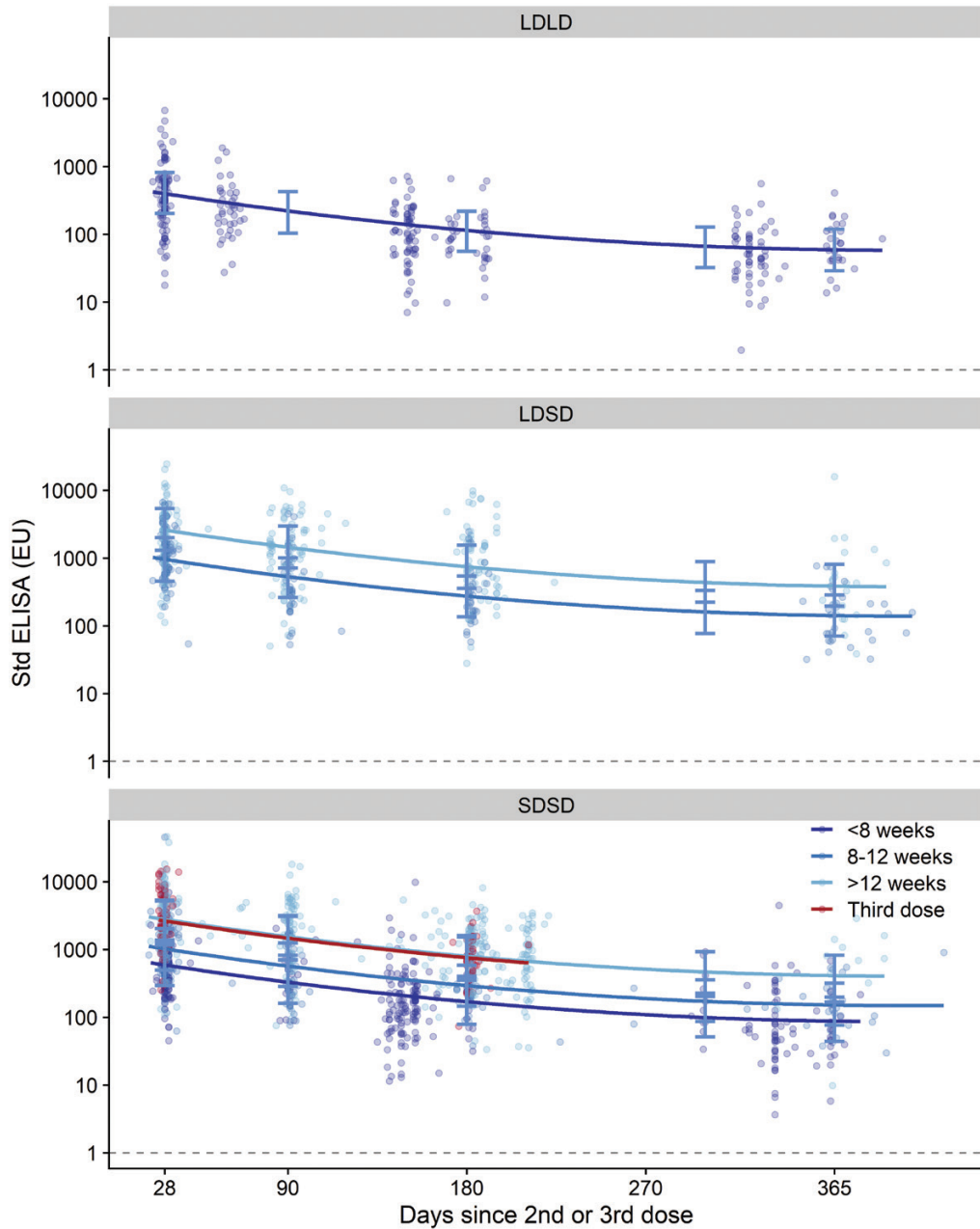
Figure 1. Anti-spike IgG by standardized ELISA, by vaccine and dose interval. LDLD: two low doses; LDSD: low dose followed by standard dose vaccines; SDSD: two standard dose vaccines. Each dot represents one sample. Regression lines show estimates from mixed effects regression model of log-transformed ELISA values regressed against log-transformed days since vaccination (log-log model), adjusted for age (continuous), vaccine schedule (LDLD, LDSD, SDSD) and interval (< 8 weeks; 8-12 weeks; > 12 weeks). A model testing the interaction term between days and interval was a poorer fit to the data (based on AIC) therefore the interaction was not retained and the slopes of regression lines are fixed showing parallel decay. Decay of antibody after a third dose is consistent with the pattern of decay after two doses. Dotted line shows lower limit of the assay (EU=1). Error bars show bootstrapped confidence intervals for model predicted values at day 28, 90, 180, 300 and 365 from the model output. These are also shown in Tables S1 and S2. The dataset contains 1796 samples from 704 participants
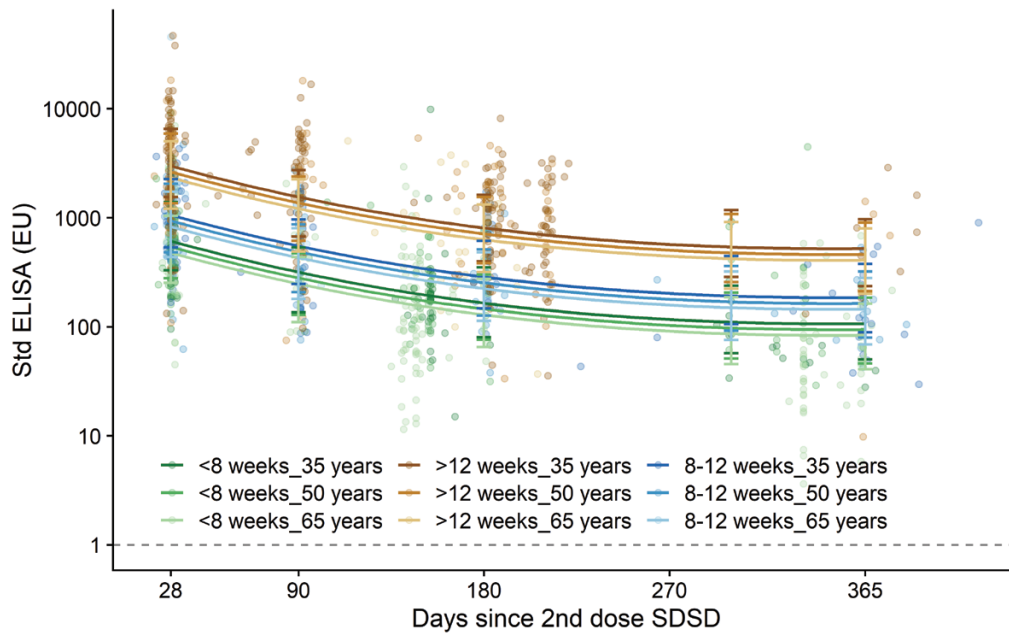
Figure 2. Anti-spike IgG by standardized ELISA, by age and dose interval (SDSD recipients only. SDSD: two standard dose vaccines. Each dot represents one sample. Regression lines show estimates from mixed effects model shown in figure 2 for those who received SDSD vaccines only. Age was significant in the model (P=0.0008), but the magnitude of the effect was less than the effect of the timing of the first two doses. Dotted line shows lower limit of the assay (EU=1). Error bars show bootstrapped confidence intervals for model predicted values at day 28, 90, 180, 300 and 365 from the model output. The dataset contains 1796 samples from 704 participants
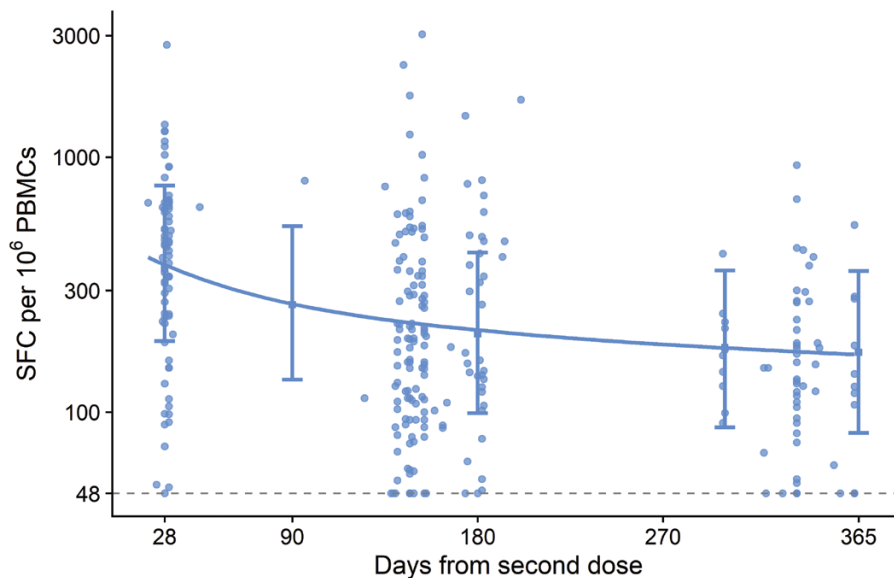
Figure 3. Interferon-γ ELISpot response to peptides spanning the SARS-CoV-2 spike insert in participants who received two standard doses. Each dot represents one sample. The dataset contains 292 samples from 167 participants. Dotted line shows lower limit of the assay at 48 SFC per 10^6 PBMCs. Error bars show bootstrapped 95% confidence intervals from the model output. These are also shown in Table S3

(190, 773) at day 28, 203 (99, 423) at day 180, and 172 (83, 358) at day 365. (Fig. 3, Table S3). Of note, the peak in t-cell responses is generally 14 days after vaccination and so these responses represent decay from a previous higher peak at an unmeasured timepoint.

Limited data were available for measuring neutralization using a live microneutralization assay in participants who received SDSD regimens. Trends were similar to the analysis of both total anti-spike IgG antibody and ELISpot responses

with initial decay more rapid than at later time points (Fig. 4, Table S4).

Data from assays measuring anti-ChAdOx1 vector neutralizing titre demonstrate that these titres are well maintained out to day 180 and fell from a geometric mean titre of 345 at days 28 to 203 at day 180, with substantially overlapping confidence intervals (Fig S2, Table S5).

Total spike-specific IgG antibody titres in vaccinated participants with positive PCR tests after vaccination showed

a similar pattern of decay although fewer data points were available for robust model comparisons (Fig. 5).

Discussion

In the absence of infection, our analysis demonstrates that waning of antibody is more rapid in the first 6 months after vaccination with ChAdOx1-nCoV-19/AZD122 than from 6 to 12 months after vaccination. Importantly, antibody waning is slower at later time periods and titres are maintained a year after vaccination with a primary series (two doses) and have a similar rate of decay in individuals who were infected after vaccination. Extrapolation of antibody levels out to 2 years post vaccination predicts levels that are well maintained. In addition, the T-cell response is well-maintained out to a year with a slow rate of decay. At a population level, the
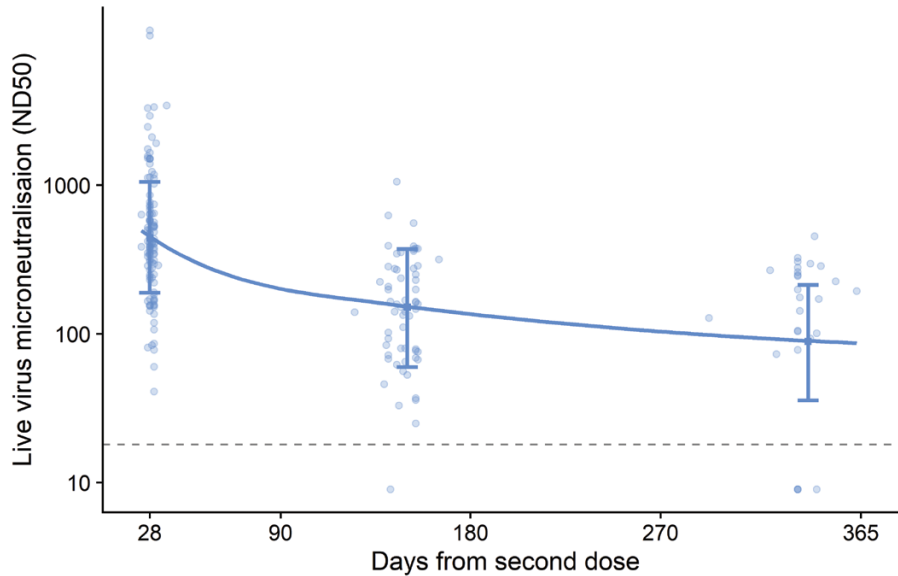
Figure 4. Live virus microneutralization titre (ND50) in participants who received two standard doses. ND50: Dilution to achieve 50% virus neutralization. Each dot represents one sample. Dotted line shows lower limit of the assay at a titre of 18. Error bars show bootstrapped 95% confidence intervals for model predicted values at day 28, 150, and 340 from the model output. These are also shown in Table S4. The dataset contains 198 samples from 114 participants
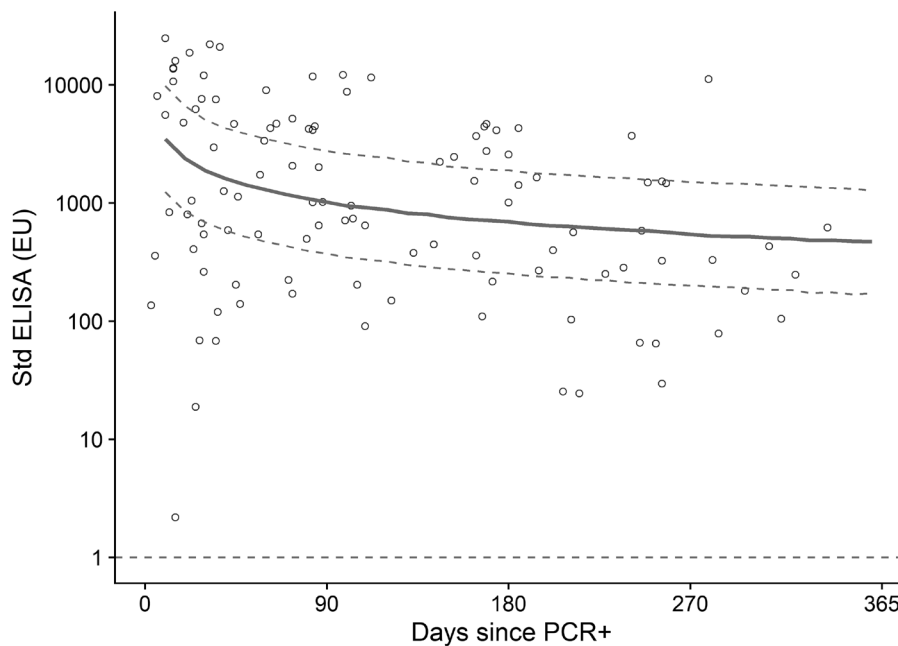
Figure 5. Anti-spike IgG by standardized ELISA in participants who were vaccinated and then had a positive PCR test. Data shown are ELISA values at timepoints after a positive PCR test, in participants who received two doses of vaccine prior to a positive PCR test. Each dot represents one sample. The solid line is the regression curve from a linear mixed model of log-transformed ELISA values regressed on log-transformed days since PCR test, with a participant-level random effect. Dotted curves show the bootstrapped predicted 95% confidence limits. Dotted horizontal line shows lower limit of the assay (EU=1)

high levels of immune persistence seen will have a positive impact on serious disease and hospitalization. Not surprisingly, in those regimens that achieved higher antibody titres (two SD vaccinations in younger adults), the titres are higher at later time-points.

While mRNA-based vaccines have been shown to induce higher titre antibodies after primary series vaccination than viral vectors, there is rapid onset of antibody decay after mRNA vaccination [18]. The slow rate of immune decay after two doses of ChAdOx1 nCoV-19 is similar to that observed after one dose of Ad26 CoV-2 [18, 19]. The mechanism underpinning antibody persistence across vaccine regimens is unclear—data are accruing which suggest vaccination against COVID-19 with mixed vaccine modalities (e.g. viral vectors and RNA) results in significantly better immune persistence and a wider repertoire of antibody responses [20, 21]. A diverse and well-maintained memory response, capable of recognizing VoC, will be the preferred option to best protect individuals going forward.

UKHSA have shown rapid waning of protection from infection in the 6 months after vaccination, for all COVID-19 vaccines. Our results are consistent with some waning of efficacy in the first 6 months but between 6 and 12 months efficacy against severe disease and hospitalization may be well maintained. Antibody waning followed a similar trajectory after a primary series vaccination and after vaccination followed by infection. Sequential exposure to VoC, which will happen in periods of high virus circulation and transmission, will provide a natural boost to pre-existing immune responses against SARS CoV-2 and may augment the persistence of high titres of antibodies for sustained periods of time. Efficacy estimates can be underestimated in test-negative case control studies because it is difficult to remove all previous (and unknown) infections from the analysis, causing what is often referred to as 'deletion of susceptibles' bias. This is particularly problematic later in the pandemic, when an increasing proportion of unvaccinated controls is immune following prior undetected infection.

In our analysis, the interval between vaccine doses had a larger impact on the resulting antibody titres at later timepoints than the type of dose received (e.g. SD vs LD). Both real world and randomized controlled trial data from mRNA vaccine regimens also support this finding, demonstrating that antibody levels were higher after the extended dosing interval (6–14 weeks) compared with the conventional 3- to 4-week regimen [12, 22] supporting the extended vaccination interval used in the UK. Regardless, it will be important to investigate the optimum interval for booster vaccinations and while it is unclear if booster doses will be an ongoing requirement it will be important to assess in high-risk individuals, including older adults.

Third and fourth dose booster vaccination programmes have been rolled out in many countries and as such, the protection provided by two-doses of vaccines 12 months after cannot be easily determined, particularly as immunity will also be influenced by the SARS-CoV-2 variants in circulation. We have demonstrated that a third dose ChAdOx1 nCoV-19/ AZD1222 can augment antibodies titres and we demonstrate here the kinetics of antibody decay following a third dose of ChAdOx1 nCoV-19 is similar to that after the primary series. Boosters may help reset the resting immune response to a higher level resulting in augmented protection against VoC and resulting in better persistence of class-switched, high affinity long-lived memory B cells.

Importantly, T-cell responses are also well maintained, and there is increasing evidence that cellular immunity and non-neutralizing antibodies are key mediators in protecting against disease caused by VoC [23, 24]. The starting point for analysis of decay of cellular responses was measured at day 28, not at the peak response time for T-cell responses of day 14. This may affect model estimates, making decay appear slower than it otherwise would if the peak response time could be observed. Real world data are demonstrating the protective impact of hybrid immunity induced post-vaccination and infection.

Limitations

There are some limitations to these analyses. These are exploratory analyses and data were not available for the same participants for every assay as these are secondary time points. Although all participants with suspected infections or unreported vaccinations were removed from the analysis, some cases may have been missed and these participants may have antibodies that are affected by their infection.

Our data demonstrate that after a primary series vaccination with ChAdOx1 nCoV-19/AZD1222 the immune response persists for at least 12 months, and given the decay follows a non-linear trajectory, immunity is likely detectable for a significant period of time thereafter; modelling performed herein predicts a relatively high level of antibodies can be detected two years after vaccination. Given the ongoing transmission of SARS CoV-2, it is important to have a well-maintained immune response to protect against severe disease and minimize the burden on healthcare system.

Supplementary data

Supplementary data is available at *Clinical and Experimental Immunology* online.

Ethics approval

Ethical review was provided by the South Central Berkshire Research Ethics Committee (20/SC/0179).

Conflict of interests

Oxford University has entered into a partnership with AstraZeneca for further development of ChAdOx1 nCoV-19. AstraZeneca reviewed the final manuscript before submission, but the authors retained editorial control. SCG is a cofounder of and shareholder in Vaccitech (collaborators in the early development of this vaccine candidate) and named as an inventor on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and a patent application covering this SARS-CoV-2 vaccine. T.L. is named as an inventor on a patent covering use of ChAdOx1-vectored vaccines (PCT/GB2012/000467) and was a consultant to Vaccitech. P.M.F. is a consultant to Vaccitech. A.J.P. is Chair of the UK Department of Health and Social Care's JCVI, but does not participate in policy advice on coronavirus vaccines. A.J.P. is an NIHR Senior Investigator.

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Data availability

Anonymized participant data will be made available when the trials are complete, upon requests directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

Author contributions

A.J.P. is chief investigator. A.F., J.A., P.A., S.B.-R., S.B., M.B., F.C., S.C., E.A.C., S.D., C.D., N.J.E., K.J.E., S.N.F., P.M.F., J.F., C.G., S.C.G., L.G., B.H., H.E.H., D.J., S.K., Y.F.M., E.P., M.H.R., H.R., H.S., M.D.S., R.S., K.M.T., M.U., D.W.o., D.W.r. contributed to implementation of the study and/or laboratory experimentation. M.V. performed the statistical analysis. A.F., T.L., A.J.P., and M.V. contributed to the preparation of the report. All authors critically reviewed and approved the final version.

Clinical trial registration

The clinical trials described in this manuscript are COV001, registered at clinicaltrials.gov as NCT04324606 and COV002 registered as NCT04400838.

Patient consent

Written informed consent was received from all participants.

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