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Randomised, double-blind, controlled phase 1 trial of the candidate tuberculosis vaccine ChAdOx1-85A delivered by aerosol versus intramuscular route

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SUMMARY

Background: A BCG booster vaccination administered via the respiratory mucosa may establish protective immune responses at the primary site of *Mycobacterium tuberculosis* infection. The primary objective of this trial was to compare the safety and immunogenicity of inhaled versus intramuscular administered ChAdOx1-85A.

Methods: We conducted a single-centre, randomised, double-blind, controlled phase 1 study (Swiss National Clinical Trials Portal number SNCTP000002920). After a dose-escalation vaccination in nine BCG-vaccinated healthy adults, a dose of 1×10^{10} vp of ChAdOx1-85A was administered to twenty BCG-vaccinated adults that were randomly allocated (1:1) into two groups: aerosol ChAdOx1-85A with intramuscular saline placebo or intramuscular ChAdOx1-85A with aerosol saline placebo, using block randomisation. A control group of ten BCG-naïve adults received aerosol ChAdOx1-85A at the same dose. Primary outcomes were solicited and unsolicited adverse events (AEs) up to day 16 post-vaccination and Serious AEs (SAEs) up to 24 weeks; secondary outcomes were cell-mediated and humoral immune responses in blood and bronchoalveolar lavage (BAL) samples.

Findings: Both vaccination routes were well tolerated with no SAEs. Intramuscular ChAdOx1-85A was associated with more local AEs (mostly pain at the injection site) than aerosol ChAdOx1-85A. Systemic AEs occurred in all groups, mainly fatigue and headaches, without differences between groups. Respiratory AEs were not different between BCG-vaccinated groups. Aerosol ChAdOx1-85A vaccination induced Ag85A BAL and systemic cellular immune responses with compartmentalisation of the immune responses: aerosol ChAdOx1-85A induced stronger BAL cellular responses, particularly IFNγ/IL17+CD4+ T cells; intramuscular ChAdOx1-85A induced stronger systemic cellular and humoral responses.

Interpretation: Inhaled ChAdOx1-85A was well-tolerated and induced lung mucosal and systemic Ag85A-specific T-cell responses. These data support further evaluation of aerosol ChAdOx1-85A and other viral vectors as a BCG-booster vaccination strategy.

Research in context

Evidence before this study

Tuberculosis disease caused by *Mycobacterium tuberculosis* is a major cause of infectious disease mortality globally. Neonatal BCG vaccination fails to completely protect adults. One potential

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vaccination strategy is to boost the efficacy of BCG vaccination by targeting the respiratory mucosa using an aerosol vaccine. We searched Pubmed and Google Scholar, combining the terms '*Mycobacterium tuberculosis*', 'aerosol vaccination' and 'clinical trial' for original research articles and systematic reviews published in any language up to March 2024. We identified three clinical trials, all of which investigated aerosol administration of MVA85A or AdHu5Ag85A, recombinant viral vector vaccines for tuberculosis in BCG-vaccinated participants. Aerosol vaccines were safe and induced/boosted antigen-specific systemic and mucosal cellular responses.

Added value of this study

This is the first report on the safety and immunogenicity of the aerosol administration of ChAdOx1-85A in humans compared to the intramuscular route. The aerosol ChAdOx1-85A was well-tolerated in both BCG-vaccinated and BCG naïve adults, induced/boosted robust Ag85A-specific mucosal cellular responses and peripheral cellular responses.

Implications of all the available evidence

We have demonstrated the safety and immunogenicity profile of ChAdOx1-85A administered via the mucosal route in humans. This study supports further investigation to fully characterise the mucosal and systemic response to ChAdOx1 expressing multiple protective antigens from *M. tuberculosis* and other respiratory pathogens.

Introduction

It is a century since the only currently licensed tuberculosis vaccine, Bacillus Calmette–Guérin (BCG), was first developed. Despite widespread worldwide use of BCG, *Mycobacterium tuberculosis* (*M. tuberculosis*) remains one of the leading global causes of death from any single infectious pathogen, and the leading cause of death in low and middle-income countries. This may be attributed to the highly variable efficacy of BCG against pulmonary tuberculosis (ranging from 0 % to 80 %).[1] An effective vaccination strategy that prevents pulmonary tuberculosis in adults remains the most economical and durable tuberculosis control strategy. Modelling of tuberculosis epidemiological data suggests that targeting adolescents or adults could have a greater and more rapid effect on diminishing disease transmission.[2] Recently, two clinical trials investigating boosting BCG vaccination in tuberculosis-endemic countries showed promising results. Intradermal BCG revaccination of adolescents led to a 45 % reduction in sustained *M. tuberculosis* infection as measured by QuantiFERON (QFT) conversion compared to placebo.[3] Additionally, vaccinating *M. tuberculosis* latently infected adults, with the subunit protein/adjuvant candidate vaccine, M72/AS01e, demonstrated a 49.7 % (95 % CI 2.1–74.2) reduction in active pulmonary tuberculosis disease.[4,5] These studies demonstrate that an effective prime-boost vaccination strategy can improve the efficacy of BCG vaccination. Further research into how this efficacy can be further improved, such as the route of immunisation, is needed. The route of *M. tuberculosis* infection is primarily via aerosol inhalation. Vaccination via the respiratory mucosa may establish protective immune responses at the primary site of infection. Evidence from mice and non-human primate (NHP) *M. tuberculosis* challenge studies suggests that vaccine delivery via the respiratory mucosa may be more protective than intradermal or intramuscular routes.[6–8] Protective immunity can be associated with the presence of antigen-specific lung resident T cells after vaccination via the respiratory mucosa.[7,9,10] In clinical trials, aerosol delivery of the candidate tuberculosis vaccine MVA85A induced high levels of lung mycobacterial specific T cell immune responses.[11,12] Intramuscular administration of the candidate vaccine ChAdOx1-85A was safe and induced Ag85A-specific systemic cellular and humoral responses.[13]

In this proof-of-concept double-blind, randomised phase 1 clinical trial, we compared the safety and immunogenicity of aerosol and intramuscular administered ChAdOx1-85A in healthy BCG-vaccinated adults. Additionally, we compared responses to healthy BCG-naïve adults who received aerosol ChAdOx1-85A as a prime vaccination. We here showed that aerosol ChAdOx1-85A was well-tolerated and induced lung mucosal and systemic Ag85A-specific T cell responses.

Methods

Study design

This phase I clinical trial was designed as an open-label, dose-escalation study in BCG-vaccinated adults followed with a randomised, paired-placebo, double-blind study in BCG-vaccinated and BCG-naïve adults to compare aerosol and intramuscular routes of immunisation with ChAdOx1-85A. The study took place at the Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland. It was approved by the local Competent Ethics Committee (CER-VD, ref. 2017-02308) and the Regulatory Authority, Swissmedic ref. 2018GT1002. This trial was registered with ClinicalTrials.gov (number NCT04121494), on the Swiss National Clinical Trials Portal (number SNCTP000002920) and was conducted according to the principles of the Declaration of Helsinki and ICH Good Clinical Practice.

Participants

BCG-vaccinated and BCG-naïve, healthy adults aged 18–55, were screened and enroled at the Clinical Trial Unit, CHUV, Lausanne. The selection was based on the vaccination record, scar examination and year of birth in Switzerland (after 1980) since BCG vaccination was practiced in newborns there until the 1970s. Enroled participants were in good health and had normal baseline haematology, coagulation, and biochemistry; a normal chest radiograph; no substantial abnormality of pulmonary function tests; and negative serological testing for hepatitis B, hepatitis C, and HIV. Latent *M. tuberculosis* infection was excluded by a negative ex-vivo interferon-gamma (IFN-γ) ELISpot response to *M. tuberculosis* early secreted antigen of 6 kDa (ESAT-6) and 10-kDa culture filtrate protein (CFP-10) peptides, by a validated assay routinely performed in the context of the diagnosis for *Mtb* infection at CHUV.[14,15] Current smokers (more than three cigarettes per day), people using nasal or inhaled drugs, and those with a history of asthma or other respiratory diseases were excluded (Appendix A, p3). All participants provided written informed consent.

Randomisation and masking

For the route comparison, we randomly allocated eligible BCG-vaccinated participants (1:1) to aerosol ChAdOx1-85A and intramuscular saline (aerosol group) or intramuscular ChAdOx1-85A and aerosol saline (intramuscular group). Blocks of four in the randomisation sequence ensured allocation concealment. Individual sealed opaque envelopes, provided by the CHUV Pharmacy and kept by the investigator in a secure location at the study site, were available in case of need of unblinding. Study participants, clinicians, pulmonologists, and immunologists were masked to treatment allocation, which was achieved through the paired placebo trial design.

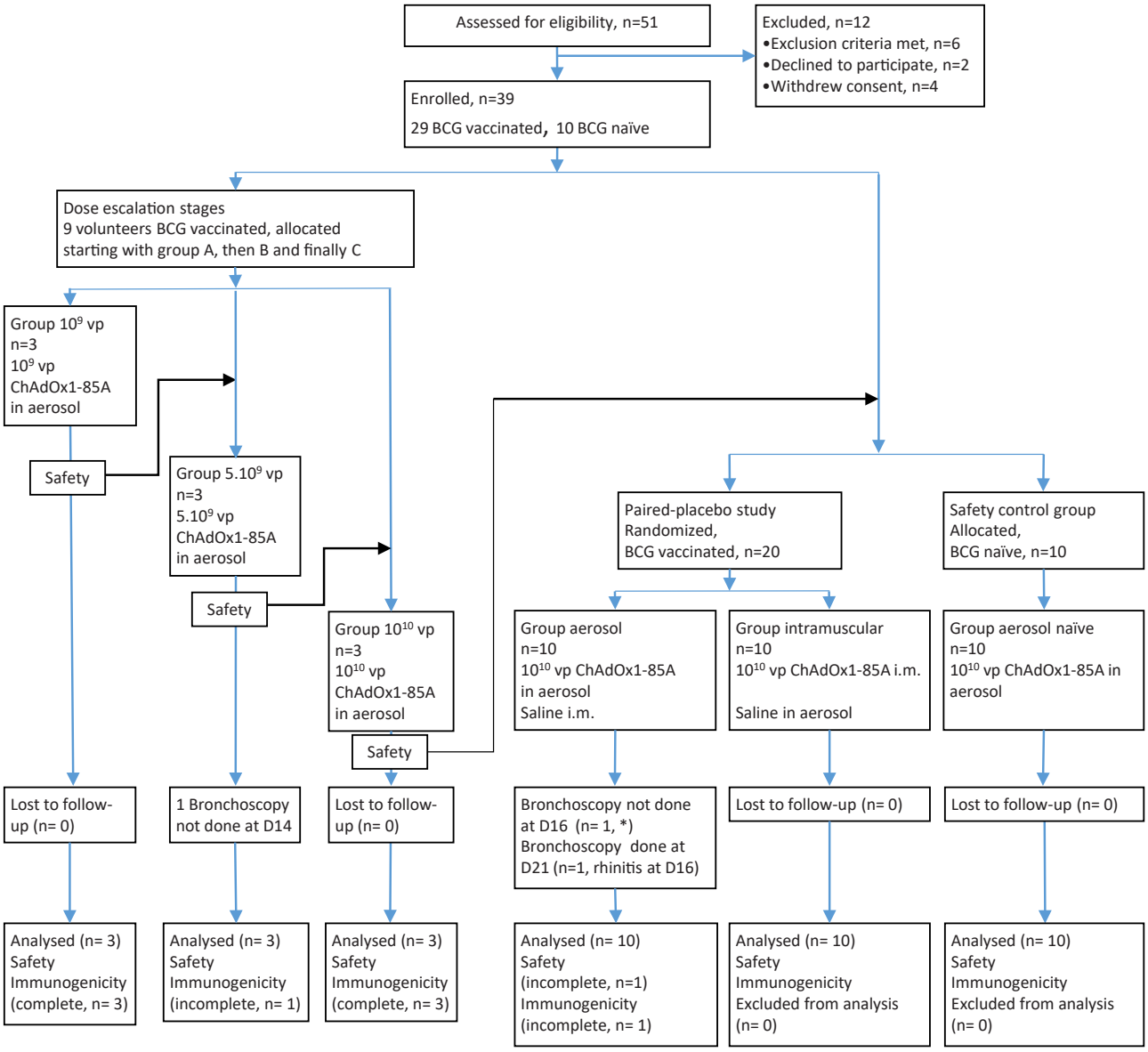

Fig. 1. Study design. All the volunteers completed the 24-week follow-up. In the 5×10^9 vp group, a bronchoscopy was not performed due to pregnancy, the participant completed the safety follow-up but was excluded from lung immunogenicity. *The last bronchoscopy in the aerosol group, was cancelled because of the Covid-19 lock-down.

Procedures

Enroled participants received one dose of vaccine ChAdOx1-85A. Aerosol administration (1 mL volume) was performed using an ultrasonic mesh nebuliser (MicroAir U22, Omron Healthcare, Japan), and intramuscular injection (0.5 mL volume) was delivered to the deltoid region of the arm. The first nine BCG-vaccinated participants received escalating doses of aerosol inhaled ChAdOx1-85A, starting with 1×10^9 viral particles (vp) (n = 3), 5×10^9 vp (n = 3) and 1×10^{10} vp (n = 3). The following 20 BCG-vaccinated participants were randomised to receive aerosol ChAdOx1-85A (and intramuscular placebo) or intramuscular 1×10^{10} vp ChAdOx1-85A vaccine (and inhaled placebo) (aerosol and intramuscular groups, respectively). The BCG-naïve participants (naïve aerosol group, n = 10) received 1×10^{10} vp aerosol ChAdOx1-85A vaccine without intramuscular placebo. Bronchoscopy (with or without lymph node (LN) aspiration) and bronchoalveolar lavage (BAL) were performed two weeks after vaccination, and blood samples were taken at each visit (screening, D0, D7, D14/16, D28, D84 and D168).

Outcomes

The primary safety outcomes were the frequency and intensity of vaccination-related, solicited and unsolicited, local (injection site), respiratory and systemic AEs up to day 16 after vaccination, and SAEs up to 24 weeks. The secondary outcome was immunogenicity and we assessed cellular and humoral responses to Ag85A vaccine antigen, ChAdOx1 vector, and PPD as a natural source of Ag85A. We performed an *ex vivo* interferon gamma (IFNγ) ELISpot assay on PBMC taken at D0, D16 and D168, and an intracellular cytokine staining (ICS) to assess the antigen-specific CD4+ and CD8+ T-cell production of IFNγ, TNF, IL2 and IL17 in BAL and LN lymphocytes at D14/16, and in PBMC taken at D0, D16, D28, D84 and D168. Memory/naïve T cell subsets were defined by the expression of CD45RA and CCR7. Markers used are indicated in Appendix Table 1. In addition, we measured antigen-specific serum and BAL IgG, IgM and IgA responses by ELISA. Details of clinical monitoring and procedures, including vaccine, bronchoscopy, and immunological assays, are provided in Appendices B and C, respectively (Appendix pp 4–10, Appendix Fig. 1, p. 10, and Appendix Tables 1 and 2, p. 9).

Table 1
Study population characteristics at screening.

	1×10^9 Group	5×10^9 Group	1×10^{10} Group	Aerosol group	Intramuscular group	Naïve aerosol group	.
Route	Aerosol	Aerosol	Aerosol	Aerosol	i.m.	Aerosol	.
Dose of ChAdOx1-85A	1×10^9 vp	5×10^9 vp	1×10^{10} vp	1×10^{10} vp	1×10^{10} vp	1×10^{10} vp	*p Value
n	3	3	3	10	10	10	.
Median age (years) [95 % CI]	33	50	44	46	39	†27	0.0003
	[26–41]	[31–54]	[37–54]	[42–51]	[31–46]	[21–30]	.
BCG vaccinated	Yes	Yes	Yes	Yes	Yes	No	.
Gender, female, n (%)	3 (100 %)	2 (66 %)	3 (100 %)	5 (50 %)	6 (60 %)	5 (50 %)	.
Ethnicity, African, n (%)	2 (66 %)	0 (0 %)	0 (0 %)	1 (10 %)	0 (0 %)	0 (0 %)	.
Ethnicity, Caucasian, n (%)	1 (33 %)	3 (100 %)	3 (100 %)	9 (90 %)	10 (100 %)	10 (100 %)	.
Median BMI (kg/m^2) [95 % CI]	23.9	22	23.5	23.4	24.65	23.05	0.6811
	[22.1–25.8]	[19.1–25]	[17.4–25.1]	[22.5–29.4]	[20.7–28.4]	[20.4–26.8]	..
% Predicted FEV1 [95 % CI]	100.7	83.35	84.76	93.49	87.65	88.43	0.173
	[98.5–117.1]	[77.8–114.4]	[80.67–101.6]	[84.27–101.6]	[80.46–95.9]	[80.86–96.89]	.
% Predicted PEF [95 % CI]	97.3	92.3	129.3	110.6	100.2	105.8	0.255
	[95.7–126]	[87.4–103.3]	[98.9–132.6]	[89.1–125.2]	[91.2–110]	[89.3–114.5]	.
Smoker n (cpd)	0	1 (1)	0	1 (2)	2 (2)	1 (2)	.

cpd: median cigarette per day per smoker; FEV1, forced expiratory volume in 1 s; PEF, peak expiratory flow.
* Kruskal-Wallis p value.
† Age in the naïve aerosol group was less than the age in the aerosol group.

Statistical analysis

The frequency of AEs, as categorical variables, was summarised by vaccine groups as numbers of participants presenting AEs and frequency of AEs per participant. Comparison between groups used two-way RM ANOVA or mixed-effects model (REML). Immune responses per group were presented as medians and 95 % CI or interquartile ranges at each time-point, fold change from baseline and overall responses using Area under the Curve over time (AUC). Comparisons were performed using nonparametric tests: intra-group comparisons used the Friedman with post-hoc multiple comparison test or Wilcoxon matched pairs test; inter-group comparisons used the Kruskal-Wallis with post-hoc multiple comparison test or Mann-Whitney tests. Cross correlations were investigated using nonparametric Spearman correlation. Statistical analyses were performed using GraphPad Prism (v9.0).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Fifty-one people were screened and 12 were excluded before enrolment (six met exclusion criteria, two did not consent and four withdrew consent before vaccination). Between January 2019 and March 2020, we enroled 29 BCG-vaccinated and 10 BCG-naïve participants (Fig. 1). One participant from the group that received 5×10^9 vp was excluded at D14, before bronchoscopy, because of a positive pregnancy test. Two bronchoscopies were not performed: one in the group that received 5×10^9 vp (exclusion), one in the aerosol group (unavailability of clinical facilities due to the Covid-19 pandemic). The baseline characteristics of the participants were not different between groups, except for gender and age (Table 1). Dose-escalation groups were 88.9 % female. The naïve aerosol group included younger participants due to their BCG-naïve status in relation to the cessation of neonatal BCG vaccination in the general Swiss population in the 1970s.

Reactogenicity

There were no serious AEs during the trial. In the dose-escalation phase (groups 1×10^9, 5×10^9 and 1×10^{10}), there were no safety issues (Appendix D, p. 11 and Appendix Figs. 2 and 3, pp. 12–13). The highest dose (1×10^{10} vp ChAdOx1-85A) was chosen for the vaccinations in the randomised phase, for both aerosol and intramuscular groups and the BCG-naïve aerosol group. In these groups, vaccine-related AEs were mild to moderate (Fig. 2). As expected, participants from the intramuscular group experienced more local AEs, mostly pain at the injection site, than participants from the aerosol group (12 vs 3 AE; ANOVA, $p = 0.0093$, nAEs/participant). Solicited systemic AEs occurred in all groups, mainly fatigue and headaches, without differences between groups. Twenty-five of 29 (86.2 %) participants presented an alveolar leucocytosis that was mainly mild (22/25). This consisted of BAL lymphocytosis (three moderate, 22 mild) accompanied by BAL neutrophilia (9/25, one in the aerosol group, four in the intramuscular group and BCG-naïve aerosol group) or BAL eosinophilia (8/25, four in the aerosol group, two in the intramuscular and BCG-naïve aerosol group each). Nine of the 29 (31 %) participants presented signs of mild bronchial inflammation (one in the aerosol group, three in the intramuscular group and five in the BCG-naïve aerosol group). No wheezing was observed. Sore throat was observed in eight participants (three in the aerosol group, four in the intramuscular group and one in the BCG-naïve aerosol group). Ten of the 30 (33.3 %) participants experienced transient unsolicited dysgeusia (two in the aerosol group and intramuscular group and six in the BCG-naïve aerosol group), dry mouth (n = 1, aerosol group), rhinitis (n = 2, aerosol and BCG-naïve aerosol) or oral pruritus (n = 1, BCG-naïve aerosol). Evaluation of lung function showed no change over time in FEV1 and peak expiratory flow (PEF) in all groups, except in one volunteer from the BCG-naïve aerosol group who displayed a transient 17.5 % decrease of PEF at D28 thought to be associated with a concurrent upper respiratory airway infection (Appendix Fig. 4, p. 14). Overall, there was no difference between aerosol and intramuscular groups in the number of respiratory AEs. Interestingly, participants from the BCG-naïve aerosol group had more respiratory AEs than those in the aerosol group (mainly cough and dysgeusia) (36 vs 22 AE; REML, $p = 0.0422$, nAEs/group, data not shown).

Inhaled ChAdOx1-85A induced a robust peripheral IFNγ response

There was no difference between any groups in peripheral blood mononuclear cells (PBMC) Ag85A- and ChAdOx1-specific IFNγ baseline responses (Fig. 3a and Appendix Fig. 5, p. 15, respectively). During the dose-escalating phase, there was no difference between groups in peripheral blood mononuclear cells (PBMC) IFNγ responses to Ag85A, PPD or ChAdOx1 at baseline (Appendix Fig. 5a, p. 15). The three doses induced a significant Ag85A specific response, (mixed

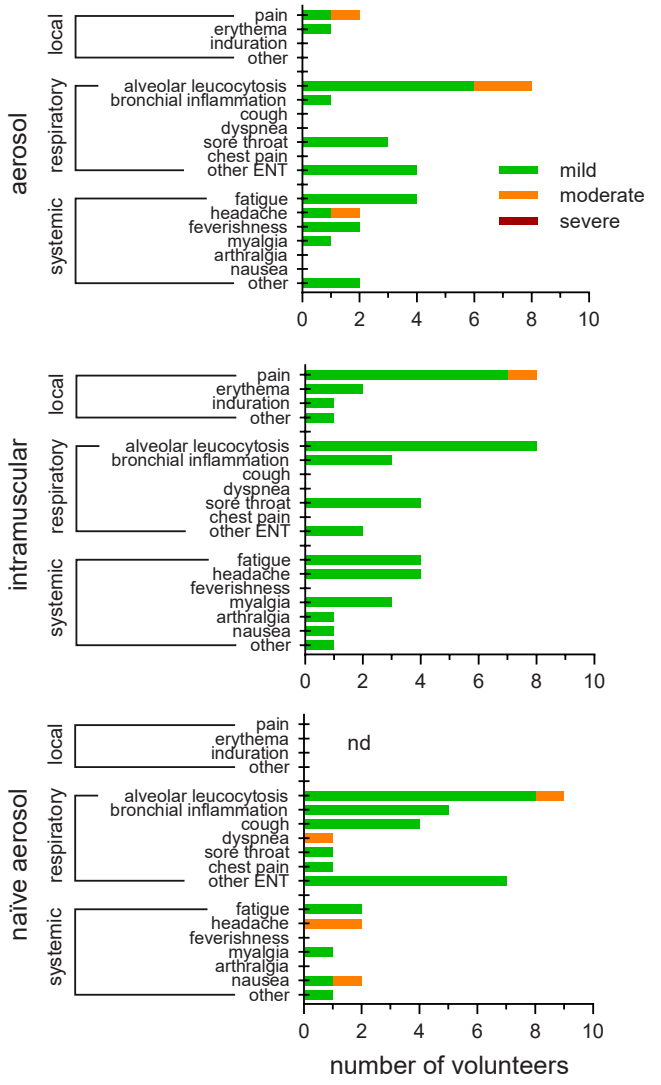

Fig. 2. ChAdOx1-85A vaccine related adverse events. A dose of 1×10^{10} vp ChAdOx1-85A was administered by aerosol or intramuscular injection in BCG-vaccinated participants and by aerosol in BCG-naïve participants; n = 10 per group. Frequencies are expressed as number of volunteers who experienced vaccine related, solicited or unsolicited (other), at least once, at peak severity grade, in the 2 weeks after vaccination and including bronchoscopy linked observations of lung inflammation. n = 10 per group, with the exception of a bronchoscopy not performed in one volunteer in the aerosol group. Alveolar leucocytosis includes alveolar lymphocytosis, neutrophilia, and eosinophilia. Bronchial inflammation includes hyperaemia of bronchial mucosa, erythema, and mucoid secretion. Other AEs consist of: haematoma (n = 1) for local; dysgeusia (n = 10), rhinitis (n = 2), dry mouth (n = 1) and pruritus (n = 1) for other ENT; chills (n = 1), lymphopenia (n = 1), tingling in hand (n = 1) and dizziness (n = 1) for systemic. Bronchoscopy related AEs are excluded. nd, not done. Participants from the intramuscular group received aerosol saline.

effect model p = 0.0051), significant at D14 (p = 0.0199), without a dose effect. No significant change in PPD- or ChAdOx1-specific responses was observed. At baseline, in aerosol and intramuscular (BCG-vaccinated) groups, 13 of 20 participants responded to PPD, ie, with responses above the baseline values of the BCG-naïve aerosol group. One participant from the BCG-naïve aerosol group presented a positive response to PPD, probably due to non-tuberculous mycobacterial exposure (Appendix Fig. 5b, p. 15). AUC analysis of Ag85A-specific IFNγ responses did not show differences any groups (Kruskal-Wallis, p = 0.4218). In these three groups, all participants responded with Ag85A-specific responses peaking at D16 (Friedman, $p < 0.0001$), maintained above the baseline at D168 in most of the participants (22/30, 73 %). Peak Ag85A-specific responses after intramuscular vaccination were significantly higher than those obtained after aerosol (Fig. 3a, intramuscular: 489.7 [352.3–748.7], aerosol: 242.5 [142.5–330], BCG-naïve aerosol: 123.1 [−6.2–552.7] SFU/10^6 PBMC, median [95 %CI]).

Intramuscular ChAdOx1-85A induced higher peripheral responses than inhaled ChAdOx1-85A

The kinetics of cytokine production (IFNγ, IL2, TNF and IL17) was different in the intramuscular and aerosol groups (Fig. 3b). Intramuscular vaccination induced cytokine+ Ag85A-specific CD4+ and CD8+ T cells at D16 with a peak at D28, especially CD4+ TNF+ cells. In contrast, after aerosol administration, peripheral cytokine+ Ag85A-specific T cells first reduced and then increased, particularly IL2+ cells at D7. IL17+ Ag85A-specific T cells decreased in the aerosol group while increasing in the other groups.

Tem and Tcm subsets principally composed Ag85A-specific CD4+ memory responses (Appendix Fig. 6, p.16), particularly after intramuscular injection, with responses shared between Tem IFNγ+, IL2+ or TNF+ and Tcm TNF+ and IL2+ (Appendix Fig. 7, pp. 17–18). After aerosol, modulation of precursors followed that of Tcm, with a decrease of Tcm IL2+ at D7 and, at D84 in the aerosol group only, a decrease of the latter plus Tem IFNγ+, TNF+ and IL17+. Intramuscular ChAdOx1-85A induced Ag85A-specific CD8+ Tem cells, mainly IFNγ+ (Appendix Fig. 8, pp. 19–20). In the aerosol group, there was a trend for a decrease in Tem IL2+ and TNF+ from D7.

Peripheral Ag85A-specific CD4+ and CD8+ T cell responses were mostly monofunctional and the four studied cytokines were represented (Appendix Fig. 9, p. 21). Vaccination with ChAdOx1-85A induced polyfunctional Th1 Ag85A-specific CD4+ and CD8+ T cell responses, especially in the intramuscular and BCG-naïve aerosol groups (Appendix Fig. 10, p. 22), with subsets that persisted until D84 or D168 after intramuscular vaccination. At D28, polyfunctional CD8+ IFNγ+IL2+TNF+ T cells increased in the intramuscular group, and CD8 + IFNγ+TNF+ T cells in the intramuscular and naïve aerosol groups. In the aerosol group, there was a non-significant trend for the frequency of Ag85A-specific CD4 + and CD8 + IFNγ + IL2 + TNF + T cells to increase.

Inhaled ChAdOx1-85A induced higher lung T cell responses

Inhaled ChAdOx1-85A induced higher lung T cell responses than intramuscular ChAdOx1–85A. At D16 post vaccination, the level of CD4+ T cells in BAL was increased in the aerosol group compared to other groups (Appendix Fig. 11, p. 23). BAL Ag85A-specific CD4+ and CD8+ T cell cytokine expressions were dominated by IFNγ, followed by TNF, IL2 and IL17 responses. Only the magnitude of Ag85A-specific CD4+ IL17+ T cells was significantly higher in both aerosol groups compared to intramuscular vaccination, irrespective of BCG vaccination status (Appendix Fig. 12, p. 24). However, IFNγ, TNF and IL2 cytokine expression levels by Ag85A-specific CD4+ T cells were two to five times higher after aerosol vaccination (Appendix Table 3, p. 25). Furthermore, aerosol vaccination (both groups) induced higher Ag85A-specific CD4+ T cell polyfunctional responses compared to intramuscular vaccination, in particular, IFNγ + IL17+ and IFNγ + IL2+ co-expression subsets, with higher frequencies (4.9-fold) of Ag85A-specific CD4 + IFNγ + IL2 + TNF+ T cells in the aerosol group compared to the intramuscular group (Fig. 4a and Appendix Table 3). Overall BAL CD8+ T cell responses showed less differences between groups (Fig. 4b). The cytokine co-expression profiles of Ag85A-CD4+ T cells in the aerosol group participants were different from the intramuscular group (Fig. 4c). This was primarily due to the aerosol group having a larger proportion of three cytokine co-expression profile dominated by IFNγ + IL2 + TNF + Th1 CD4+ T cells as well as by the presence of IFNγ + IL2 + IL17 + TNF+ T cells, a subset unique to aerosol vaccination, absent in the intramuscular group. In

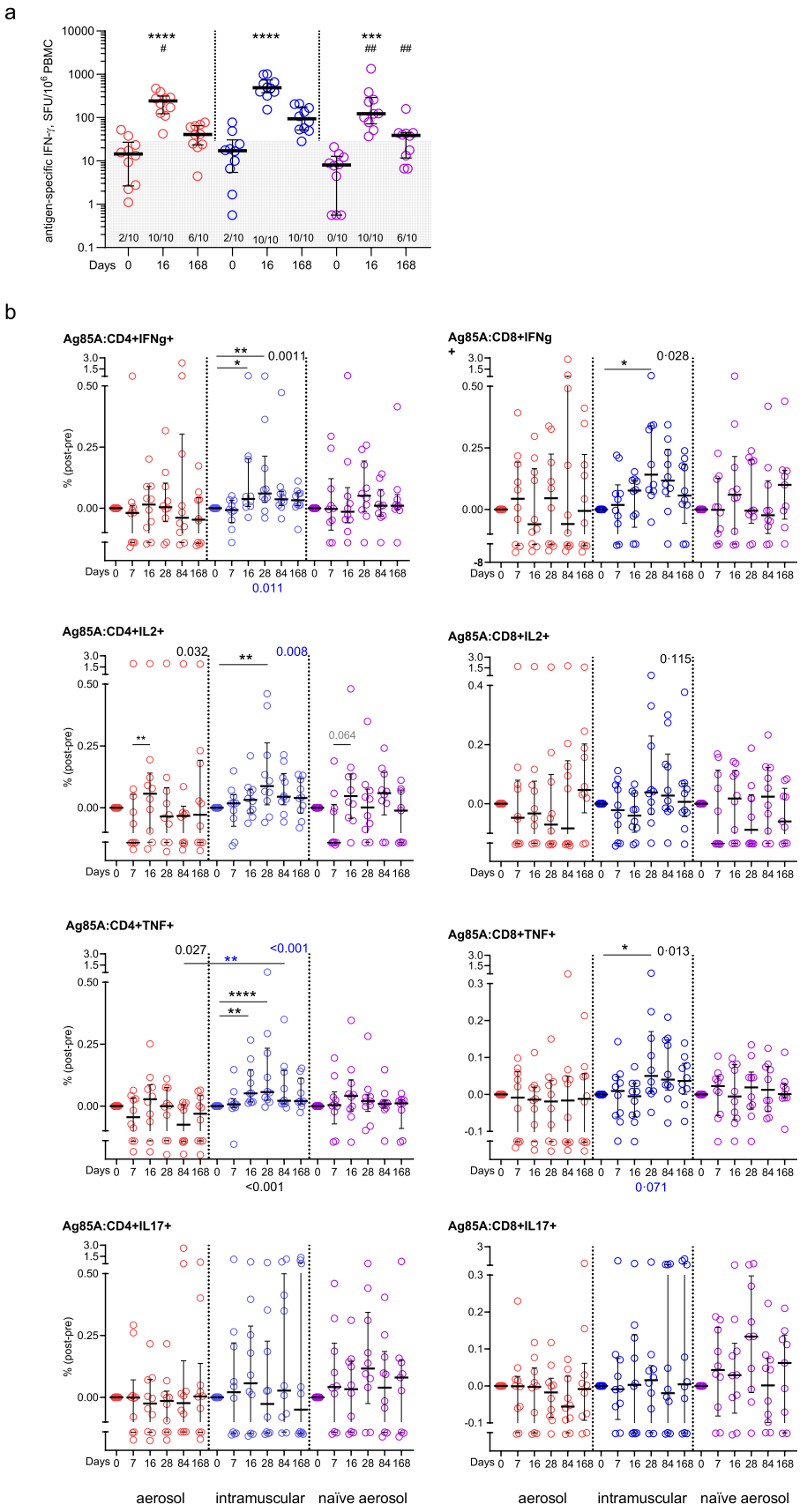

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Fig. 3. Systemic cell mediated responses to Ag85A. (a) IFNγ ELISPOT responses. Analysis by cytometry was performed using blood taken at days 0, 16 and 168 in all volunteers from all groups (n = 10 per group). Frequencies of Ag85A-specific cells are expressed as SFU per million PBMC. Bars show IQR. Intra-group comparison of responses to D0 were performed using Friedman test with Dunn's post-test, for which p values are indicated; $^{*}p < 0.05$; $^{***}p < 0.001$; $^{****}p < 0.0001$. At each time point, inter-group comparison with the intramuscular group were performed using Kruskal-Wallis test, with Dunn's post-test, for which p values are indicated; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$. Numbers of responders, defined by a response above the baseline indicated in grey, on total number of subjects per group are indicated above x axis. (b) CD4 and CD8 T cell responses in ICS. Analysis by cytometry was performed using blood taken at days 0, 7, 16, 28, 84 and 168 in all volunteers from all groups (n = 10 per group). Ag85A-specific CD4 (left panels) and CD8 (right panels) T cells expressing IFNγ, IL2, TNFα, or IL17 (top to bottom panels) were measured by intracellular staining assay after overnight stimulation of PBMC. Results shown are change from baseline, after subtraction of cytokine production in the unstimulated control, with median and quartiles. The Friedman test was performed to indicate difference from baseline within a group and a significant *p*-value indicated above group, * indicate the post-test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. Kruskal-Wallis test was performed to indicate difference between groups and indicated when $p < 0.05$ on x-axis. Wilcoxon test was performed for CD4 + IL2+ responses at D0vs7, D7vs16. Aerosol = BCG-vaccinated aerosol; intramuscular = BCG-vaccinated i.m.; naïve aerosol = BCG-naïve aerosol; dose: 1×10^{10} vp ChAdOx1-85A. Ag85A, antigen 85A; ICS, intracellular staining; PBMC, peripheral blood mononuclear cells.

addition, Ag85A-specific CD4+ T cells were predominantly composed of Tem, representative of tissue-resident subsets (Appendix Fig. 13, p. 26).

Early after vaccination, transient peripheral leukopenia observed in all groups at D1 was maintained at D7 in the aerosol group (Appendix Fig. 14a, p. 27). Moreover, the kinetics of various subsets of Ag85A-specific peripheral CD4+ T cells differ according to the vaccination group (Appendix Fig. 14b). Correlating peripheral and BAL Ag85A-specific responses, we found that, only in the aerosol group, leukopenia at D1 correlated with decreased peripheral Ag85-specific CD4+ IL17+ and with increased non-specific memory and Ag85A-specific CD4+ T cells in BAL. In aerosol group, peripheral and lung IL17 responses were inversely correlated (Appendix Fig. 14c, p. 27).

Intramuscular, but not inhaled ChAdOx1-85A induced systemic humoral responses

At baseline, there was no difference between all groups in serum titres of anti- Ag85A, PPD and ChAdOx1 serum antibodies ($p > 0.05$) (Fig. 5). The AUC of mycobacterial-specific IgG responses showed increasing titres of Ag85A-IgG in the intramuscular group and decreasing PPD-IgG titres in the aerosol group (KW, $p < 0.0001$ and $p = 0.0358$, respectively). A specific IgG response to Ag85A was detected at D16 and peaked at D28 in the intramuscular group (Friedman, $p < 0.0001$), while there was no response after aerosol administration. A late increase in anti-Ag85A IgM levels was observed in the BCG-naïve aerosol group, (Friedman, $p = 0.0043$). While anti-PPD- IgG titres did not change in the intramuscular and BCG-naïve aerosol groups, titres declined approximately 10 % with time in the aerosol group (Friedman, $p = 0.0288$). Anti-ChAdOx1 IgG measured at D28 were significantly increased in the intramuscular group compared to baseline, (13 % increase, Wilcoxon, $p = 0.002$) while no change from baseline could be detected in the aerosol groups. In dose-escalating groups, AUC analysis of Ag85A, PPD and ChAdOx1-specific antibody serum titres did not show a change from baseline. A tendency of a decrease anti-PPD IgG was observed with the high dose (Appendix Fig. 15, p. 28). In the lungs, no difference between groups or between time points was observed (Appendix Fig. 16, p. 29).

Discussion

This phase I clinical trial has demonstrated that aerosol vaccination with ChAdOx1-85A is well-tolerated and induces specific mucosal and systemic immune responses.

The systemic reactogenicity to ChAdOx1-85A was mild after both vaccination routes. Lung function was preserved during follow-up. The same level of respiratory AEs occurred after aerosol ChAdOx1-85A and saline, as previously observed with MVA85A.[12] This suggests that the aerosol procedure induces mild and transient adverse events, not the vaccine itself. Alternatively, irrespective of the vaccination route, boosting with ChAdOx1–85A induces mild inflammation in all tissues, particularly in the mucosa. The observed dysgeusia was not experienced after aerosol with MVA85A,[11,12] and was thus not solicited in this study. However, it is a common transient AEs observed after drug nebulization.[16–18]

Of note, with regards to immunogenicity, we observed a compartmentalisation of the immune responses with aerosol ChAdOx1-85A inducing higher lung cellular responses, particularly IFNγ/IL17+ T cells, than intramuscular ChAdOx1-85A, which inversely induced higher systemic cellular and humoral responses. Boosting BCG with ChAdOx1-85A via aerosol was a potent route to induce higher magnitudes of mucosal Ag85A-specific T cell responses and comparable systemic Ag85A specific IFNγ T cell responses compared to intramuscular immunisation. These outcomes are consistent with other studies boosting BCG with Ag85A using viral vectors (eg, MVA85A, AdHu5Ag85A).[11,12,19] We observed a trend for increased expression of IFNγ, IL2, TNF and IL17 expressing mucosal CD4+ T cells after aerosol vaccination; cytokines which all play a key role in control of mycobacterial infection.[20–23] Additionally, aerosol vaccination induced a predominantly IFNγ co-expressing IL17 polyfunctional mucosal CD4+ T cell response. The presence of such IFNγ + IL17+ polyfunctional CD4 T cell-specific subsets in the lung was associated with protection in NHP models of BCG vaccination and *M. tuberculosis* infection.[7,24]

Intramuscular ChAdOx1-85A induced Ag85A specific mono- and polyfunctional peripheral Th1 CD4+ T cells that peaked at D28 post-vaccination. Although the induction of peripheral antigen-specific polyfunctional T cells after vaccination has not been established as a correlate of protection against tuberculosis disease,[25] these multifunctional cells have been shown to play a role in protection against intracellular pathogens.[26,27]

Our data suggest that aerosol vaccination of BCG-primed individuals promotes the trafficking of antigen-specific memory T cells to the lung, which might explain the relatively weak peripheral response observed in the aerosol group compared to that observed in the intramuscular group. The overall shape of the response in the aerosol group may be the result of a 'classical' bell shape response, as after intramuscular vaccination, and prolonged depletion of T cells, from the periphery likely homing to the lung (Appendix Figs. 14, 17, pp. 27 and 30). The peripheral PPD-specific CD4+ T cell response to aerosol AdHu5Ag85A had the same profile.[19] This peripheral depletion may include Ag85A- and PPD-specific IL2, TNFα and IFNγ producing T cells, particularly Ag85A-specific CD4 + IL2+ Tcm and Ag85A-specific CD8 + IL2+ and TNF+ Tem (Appendix Figs. 7 and 8, pp. 17–20).

Comparing aerosol vaccination of BCG-vaccinated and BCG-naïve groups two weeks post aerosol vaccination, lung Ag85A specific T cell cytokine responses were similar between these groups, irrespective of BCG vaccination status. Since we did not analyse lung responses at later time points, we cannot comment on whether prior BCG vaccination affects the longevity of lung T cell responses as was done in the AdHu5Ag85A study,[19] but in which comparisons were limited since 50 % of late bronchoscopies were not performed. In the periphery, the capacity of PPD-specific CD8+ central memory T cells

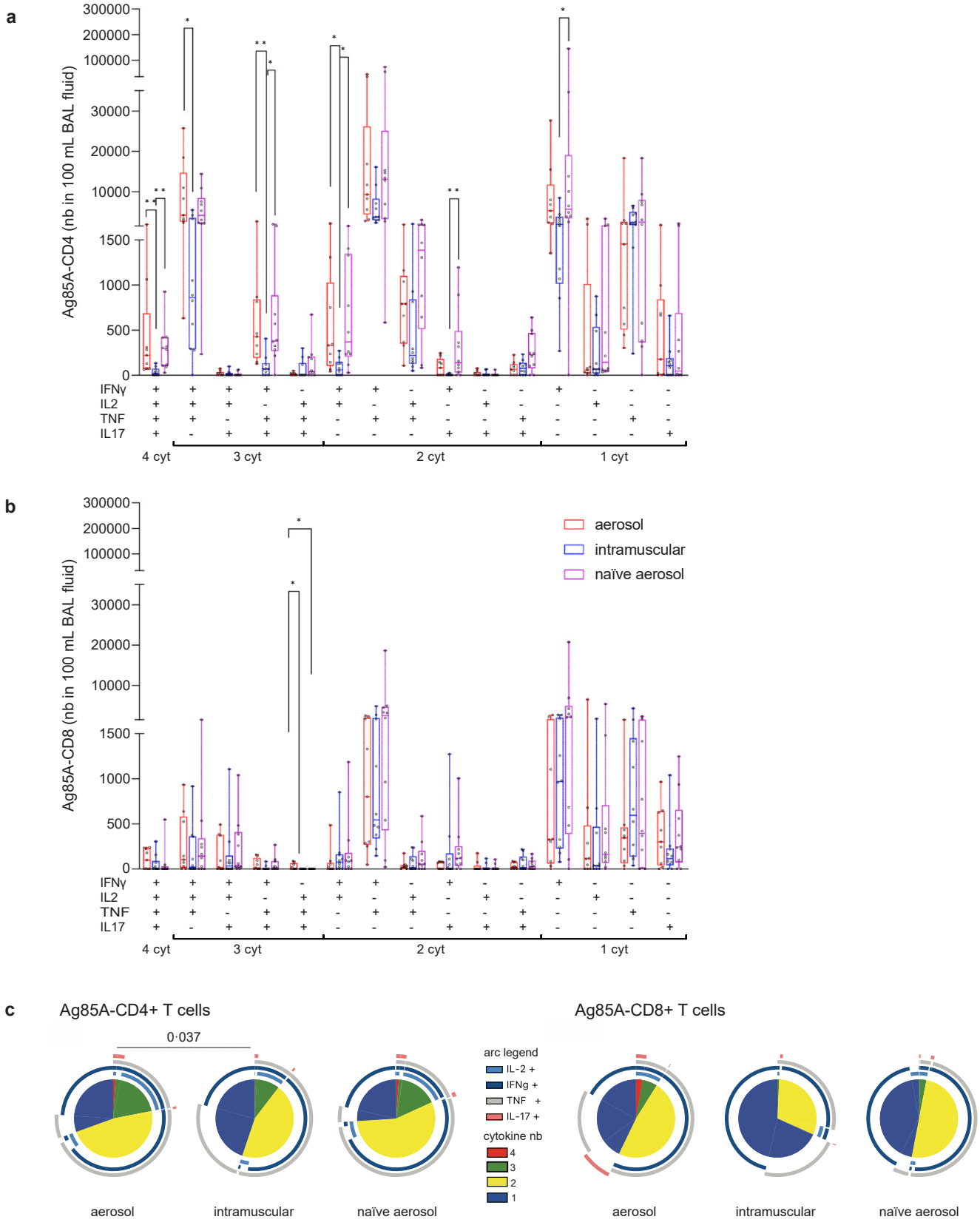

Fig. 4. BAL Ag85A-specific CD4+ and CD8+ T cell cytokine responses. Ag85a-specific CD4+ and CD8+ T cell responses in the BAL at day 16 post vaccination in all groups. (a and b) Expression and co-expression patterns of IFNγ, IL2, TNFα and IL17 were measured by intracellular staining assay after overnight stimulation of BAL cells. Data is shown as the number of cytokine producing CD4+ (a) and CD8+ (b) T cells per 100 mL BAL fluid. The horizontal lines represent medians, the boxes and whiskers represent IQR and full range. The Kruskal-Wallis test was performed to indicate difference between groups, * indicate the post test, *$p < 0.05$, **$p < 0.01$. (c) Pies represent the total Ag85a-specific T cells expressing any cytokine, slices show the relative proportion of cells expressing 1 (blue), or co-expressing 2 (yellow), 3 (green) or 4 (red) cytokines, identified by the external arcs IL2 (light blue), IFNγ (dark blue), TNFα (grey), and IL17 (pink). Results shown are after subtraction of cytokine production in the unstimulated control. Aerosol = BCG-vaccinated, aerosol, n = 9; intramuscular = BCG = vaccinated, i.m., n = 10; naïve aerosol = BCG-naïve, aerosol, n = 10; dose: 1×10^{10} vp ChAdOx1-85A. Ag85A, antigen 85 A. BAL, bronchoalveolar lavage. IFNγ, interferon-gamma. IL, interleukin. TNF, tumour necrosis factor.

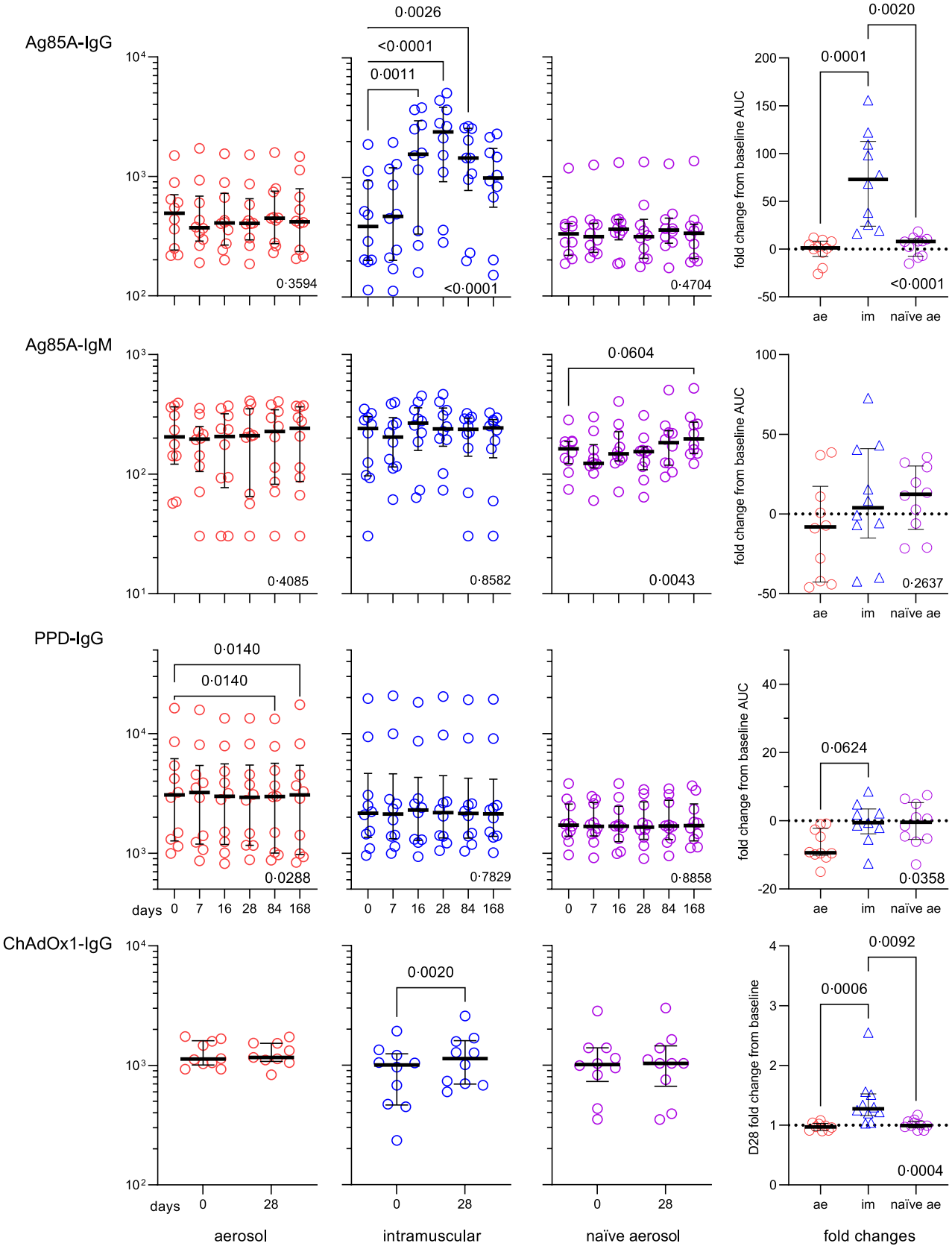

Fig. 5. Kinetics of serum humoral responses to ChAdOx1-85A. Ag85A-specific IgG and IgM, PPD- and ChAdOx1-specific IgG (top to bottom panels) were measured by ELISA in sera from volunteers from all groups, pre and post vaccination. Results are shown as individual titres (left panels, ng/mL) and fold change from baseline (AUC of log (post/pre) Ig titres, right panels) with median and quartiles. For titres, Friedman and < 0.1 post-test p value from intra-group comparisons with D0 and, for fold changes, Kruskal-Wallis and < 0.1 post-test p value from inter-group comparisons are shown. Aerosol = BCG-vaccinated aerosol; intramuscular = BCG-vaccinated i.m.; naïve aerosol = BCG-naïve aerosol; dose: 1×10^{10} vp ChAdOx1-85A; n = 10 per group.

to produce any cytokine was significantly higher in aerosol BCG-vaccinated participants compared to BCG-naïve participants at six months post-vaccination.

The role of antibodies in protection against tuberculosis disease is unclear. However, more recently, Lu et al. highlighted that those participants with latent *M. tuberculosis* infection who are considered to have been able to establish immune protective mechanisms against the disease had higher levels of PPD-specific functional antibodies (enhanced macrophage killing of intracellular *M. tuberculosis*) compared to patients with tuberculosis disease.[28] Our data show that only intramuscular boosting with ChAdOx1-85A induces peripheral Ag85A-specific IgG for up to three months. In the M72/AS01e efficacy trial, participants that received the vaccine had anti-M72 IgG antibodies by two months post vaccination that lasted up to 36 months.[5]

Our study has caveats and limitations. First, the choice of the highest dose of vaccine was based solely on safety parameters during the dose-escalation phase. It would have been of interest to consider local and peripheral immunogenicity since an aerosol dose of 5×10^9 vp was at least equivalent to 1×10^{10} vp. In this regard, in the AdHu5Ag85A study, a high aerosol dose was shown to be counterproductive to induce a lung response.[19] Second, we only performed a single bronchoscopy two weeks after vaccination, impairing a comparison with baseline samples or the detection of persistence of the local response in later samples. However, considering the potential AEs induced by bronchoscopy itself, performing this procedure a few days before aerosol vaccination could have interfered with the local response. Third, mediastinal lymph node aspiration was not performed in a sufficient number of participants to allow for robust intergroup comparisons.

Taken together, in this phase 1 clinical trial, we described the first aerosol administration of the candidate tuberculosis vaccine ChAdOx1-85A that was safe and immunogenic both systemically and in the respiratory mucosa. Mucosal delivery of a vaccine to the lung to protect against a respiratory disease such as tuberculosis may improve immunogenicity and efficacy, as demonstrated by NHP challenge models. These findings are relevant for other respiratory pathogens, including respiratory viruses such as SARS-CoV-2. The vector utilised in this study, ChAdOx1, is the same vector used in the Oxford AstraZeneca COVID-19 vaccine.[29] The establishment of mycobacterial-specific tissue-resident memory cells in the lung that will be activated at the site of infection could be superior to circulating memory cells in playing a key role in disease prevention. Small experimental medicine clinical trials such as that presented here are important to establish proof of concept before candidate vaccines progress to large and expensive efficacy trials.

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Author contributions

HM, FS, RA, and IS conceived the study design. OK, FS, and LD recruited participants and collected participant safety data. AL and LN performed bronchoscopy and EBUS. RA collected and analysed immunology data. RA, FS, OK, HM, IS, and MC contributed to the interpretation of results. RA wrote the first draft of article. AFM provided clinical operations and regulatory support, as well as project management. VS and FF were responsible for database development and management. All authors had full access to the data, and reviewed, revised, and approved the manuscript before submission.

All authors contributed to the 4 ICMJE criteria.

Data availability

Individual data that underlie the results reported in this work will be available upon request, after coding. The full study protocol is available as Supplemental Material.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106205.

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