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Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa[1]

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Abstract

BACKGROUND—The efficacy of BCG may be enhanced by heterologous vaccination strategies that boost the BCG-primed immune response. One leading booster vaccine, MVA85A, has shown promising safety and immunogenicity in UK human trials. We investigated the safety and immunogenicity of MVA85A in mycobacteria-exposed, but *Mycobacterium tuberculosis*-uninfected, healthy adults from a TB-endemic region of South Africa.

METHODS—Twenty-four adults were vaccinated with MVA85A. All subjects were followed up for one year for adverse events and for immunological assessment.

RESULTS—MVA85A vaccination was well tolerated and induced potent T cell responses, measured by IFN-γ ELISPOT assay, which exceeded pre-vaccination levels up to 364 days after vaccination. BCG-specific $CD4^+$ T cells boosted by MVA85A comprised of multiple populations expressing combinations of IFN-γ, TNF-α, IL-2 and IL-17, as measured by polychromatic flow cytometry. IFN-γ expressing and polyfunctional IFN-γ^+TNF-α^+IL-2^+ $CD4^+$ T cells were boosted during the peak BCG-specific response 7 days post-vaccination.

CONCLUSION—The excellent safety profile and quantitative and qualitative immunogenicity data strongly support further trials to assess the efficacy of MVA85A as a boosting vaccine in TB endemic countries.

Keywords

Vaccination; tuberculosis; T cells; MVA85A; South Africa

[1]**Competing interests:** AH and HM are named inventors on a composition of matter patent for MVA85A filed by the University of Oxford.

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Introduction

Tuberculosis (TB) kills about two million people annually and one third of the world's population is latently infected with *Mycobacterium tuberculosis* (*M.tb*) [1]. The increasing spread of HIV and the emergence of multi-drug and extensively resistant strains of *M.tb* (MDR-TB and XDR-TB) make the need for improved TB control even more urgent [2]. The only vaccine currently available, *Mycobacterium bovis* bacille Calmette-Guerin *(BCG)*, is largely ineffective at protecting against adult pulmonary disease in endemic areas [3]. However, as BCG does confer consistent and reliable protection against miliary TB and TB meningitis in infants [4, 5], and against leprosy [6], it may be unethical and impractical to test and deploy a vaccine aimed at replacing BCG. Furthermore, an immunisation strategy that includes BCG is attractive because most populations in need of a new TB vaccine have already been immunised with BCG.

To design a more effective vaccine, an understanding of protective immunity to *M.tb* is required. Our knowledge of this immunity remains incomplete. It is well recognised that T helper type 1 (Th1) cytokines such as interferon-γ (IFN-γ) [7-9] and tumour necrosis factor-α (TNF-α) [10-12] are essential for primary resistance to *M.tb*. Interleukin-12 (IL-12), linked to the development of Th1 cells, is also involved in immunity to *M.tb* in humans[13, 14] and animals [15-17]. IL-2 may also be important as it is required for secondary expansion of memory T cells [18] and thus in vaccine-induced generation of long-lived immunity. Other cytokines undoubtedly contribute to protective immunity. IL-17 mediates a host of inflammatory disorders [19-22] but has been proposed as a protective cytokine. In the mouse Th17 memory cells induced by a novel TB vaccine initiated recruitment of Th1 cells to the lung by upregulating the chemokines CXCL9, CXCL10 and CXCL11[23].

Recent evidence from non-TB models suggests that qualitative characteristics of the immune response may also be important [24, 25]. The presence of so-called “polyfunctional” T cells, which express multipe effector cytokines, has been associated with more effective control of intracellular infections [25]. Murine BCG-induced polyfunctional $CD4^+$ T cells have also been linked with reduced bacterial burden after aerosol *M.tb* challenge, suggesting that these cells may be beneficial in mycobacterial immunity [25]. To investigate polyfunctional T cells in the context of MVA85A vaccination, Beveridge *et al.* showed that polyfunctional Ag85A-specific CD4 T cells with the capacity to express IFN-γ, TNF-α, IL-2 and MIP-1β are present after vaccination with MVA85A in BCG-vaccinated adults from the UK [26].

MVA85A is one of the leading new TB vaccines in clinical development today. MVA85A is a recombinant strain of modified vaccinia Ankara expressing antigen 85A from *M.tb* [27]. It is safe, immunogenic and efficacious in laboratory animals, including mice, guinea pigs and macaques [28, 29] [Verreck F et al. unpublished]. The safety and immunogenicity of the BCG prime-MVA85A boost vaccination strategy is currently being evaluated in a series of Phase I and II studies in the UK, The Gambia and South Africa [30, 31]. The main immunological outcome in these trials is the *ex vivo* IFN-γ ELISPOT assay. In the UK, MVA85A was found to be safe, well tolerated and highly immunogenic [32]. This series of Phase I studies was replicated in The Gambia, where the results were equally promising (Brookes R et al, manuscript submitted). Our aim was to test the safety and immunogenicity of MVA85A in the Western Cape Province of South Africa, a setting with a very high TB prevalence. South African trials would complement the UK and Gambian trials, as the population is likely to be genetically distinct. Also, although the levels of exposure to environmental mycobacteria in the South African setting are unknown, exposure and the species of mycobacteria are likely to be different to the UK. This is suggested by studies from Malawi, where BCG-naïve individuals have higher responses to non-tuberculous mycobacteria than in the UK [33, 34]. These factors may be important predictors of both

safety and immunogenicity of the vaccine. The prevalence of *M.tb* infection and disease is also considerably higher in South Africa than in The Gambia.

Methods

Study Design

This is an open label Phase I safety and immunogenicity study. The aim was to enroll 24 participants, vaccinate with MVA85A, and follow them over the course of 12 months. This sample size was judged sufficient to determine the character and magnitude of the outcome measures, especially serious and severe adverse events. Written informed consent was obtained from all participants and the protocol and subsequent amendments were approved by the Medicines Control Council of South Africa and the Research Ethics Committees of the Universities of Cape Town and Oxford. The trial was conducted according to International Conference on Harmonisation/Good Clinical Practice (ICH-GCP) guidelines and was externally monitored by an independent contract research organisation. This trial was registered on a clinical trials database: ClinicalTrials.gov ID NCT00460590; URL http://clinicaltrials.gov/ct/show/NCT00460590?order=1.

Recruitment and enrolment

Healthy adult volunteers aged 18 to 50 years were recruited from the general population of Worcester, 110km from Cape Town in the Western Cape province of South Africa. Only volunteers with no evidence of *M.tb* infection, defined as a negative early secretory antigenic target 6 (ESAT-6)/culture filtrate protein 10 (CFP-10) ELISPOT test and a Mantoux test less than 15mm, were enrolled. This exclusion was because of concerns about inducing Koch-like reactions in individuals previously infected with *M.tb*. A normal chest radiograph was also required, with no evidence of active or past TB disease; together with a negative human immunodeficiency virus (HIV) ELISA test. Each enrolled subject was vaccinated with a single dose of 5×10^7 pfu MVA85A, administered intradermally.

Follow up and safety evaluation

Participants were evaluated on days 2, 7, 14, 28, 56, 84, 168 and 365 post-vaccination. Blood for safety evaluation, which included biochemistry and haematology tests, was collected on days 7 and 84. Diary cards were given to participants to monitor possible adverse events during the first seven days after vaccination.

IFN-γ ELISPOT assays

Blood for immunogenicity tests was collected 7-14 days pre-vaccination, and on days 7, 14, 28, 56, 84, 168 and 365 post-vaccination. The *ex-vivo* IFN-γ ELISPOT assay was used as the primary immunological endpoint and performed as described [32]. Antigens included recombinant Ag85A protein (10μg/mL), pooled 15-mer peptides spanning the Ag85A protein (66, overlapping by 10 amino acids, 2μg/mL each), 7 pools of 9-10 Ag85A peptides, live BCG (strain SSI, 1.2×10^6 CFU) and *M.tb* PPD (20μg/mL). Peptide pools spanning the *M.tb*-specific antigens ESAT-6 and CFP-10 (15-mers, overlapping by 10 (10μg/mL each) were also included. Medium alone served as negative control. Varidase (Streptokinase, 250U/mL; Streptodornase, 62.5 U/mL) and phytohemagglutinin (PHA, 10μg/mL) served as positive controls. Plates were incubated for 18h at 37°C and developed according to the manufacturer's protocol (Mabtech). Assays were performed in duplicate and all results were averaged. The cut-off for positive responses was 17 SFC per million PBMC. When responses were too high to count, a maximum cut-off of 500 spots/well or 1667 SFC per million PBMC was used. For the 7 peptide pool wells, the results were summed across all peptide pools for each time point (referred to as summed peptide pool). This will count

twice a T cell that responds to any of the 10-mer overlap regions that occur in two pools with adjacent peptides, as each pool contains non-overlapping peptides.

Whole blood intracellular cytokine staining

Whole blood intracellular cytokine staining was performed as previously described [35]. Briefly, 1mL heparinized whole blood was incubated immediately after collection with antigens in the presence of anti-CD28 and anti-CD49d Abs (BD Biosciences). After 7 hours, Brefeldin A (Sigma-Aldrich) was added and samples were incubated for a further five hours. BCG from the vaccine vial (1.2×10^6 CFU/mL) was used as antigen. No antigen (co-stimulant Abs only) was used as negative control and Staphylococcal enterotoxin B (Sigma-Aldrich) as positive control. RBC were lysed and white cells fixed using FACSlysing Solution (BD Biosciences) before cryopreservation. Cells were thawed in batch and stained with surface marker antibodies. For intracellular staining cells were permeabilized with BD Perm/Wash buffer before staining. Antibodies were as follows: CD3-Pacific Blue (UCTH1), CD4-PerCPCy5.5 (SK3), CD8-APC (SK1), IFN-γ-AlexaFluor700 (K3), IL-2-FITC (5344.111) and TNF-α-PECy7 (MAb11; all from BD Biosciences) and IL-17-PE (eBio64CAP17, eBiosciences).

At least 1 million cells were acquired on an LSR II flow cytometer (BD Biosciences). Cell doublets were excluded using forward scatter (FSC)–area versus FSC-height parameters. Unstained cells and single stained mouse κ beads were used to calculate compensations for every run. Data analysis was performed with FACSDiva software (BD Biosciences). The cut-off for positive cytokine responses was 0.01% of gated cells.

Data analysis

Statistical tests were performed with Prism 4.03 (GraphPad). Non-parametric tests were used throughout. Multiple comparisons were performed with Kruskal-Wallis tests, with Dunn's Multiple Comparison Test.

Results

Participants

One hundred and nineteen subjects were screened between September 2005 and June 2006 and 24 were recruited for vaccination. Demographic details of these individuals are shown in Table 1. The reasons for exclusions are given in Table 2.

Safety of MVA85A

There were no clinically significant changes in any of the monitored blood biochemistry or haematology parameters for any of the subjects vaccinated with MVA85A (data not shown).

One hundred and eighty six adverse events were recorded in total (Table 3a). There was one serious adverse event (SAE), an elective hysterectomy, which was unrelated to vaccination.

Local adverse reactions—Twenty three (96%) vaccinees reported swelling at the vaccination site by day 2 post vaccination, and fifteen (63%) still experienced swelling on day 6. By day 3, twelve (50%) reported scaling and itching. Twelve vaccinees reported warmth at the injection site by day 2, but none by day 7. Three (13%) reported limitation of arm movement between days 2 and 4 post vaccination. Pain was maximal on day 2, with nine (37.5%) reporting painful arms, tailing off to none by day 7. Redness was maximal on day 2, with 17 (71%) vaccinees recording a reaction larger than 15mm and 5 (20%) recording 40mm or more. Swelling at the vaccination site was maximal on day 2 with 11 vaccinees reporting 10mm or more. By day 6 the swelling had subsided to less than 5mm in

15 (62.5%) of the vaccinees. Half of the vaccinees reported scaling at the vaccination site by day 5 and all by day 7. (Table 3b).

Systemic reactions—Systemic symptoms peaked on day 1 post vaccination and are shown in Table 3b. All systemic symptoms had disappeared by day 7 and the majority by day 3 post vaccination.

Immunogenicity of MVA85A

The kinetics and magnitude of the antigen-specific T cell response to MVA85A vaccination were assessed by IFN-γ ELISPOT assay. Vaccination induced potent immune responses detectable with all specific antigens and peaking 7 days post-vaccination (Figure 1). The frequency of Ag85A-specific IFN-γ-producing cells at day 7 was significantly higher than at baseline (summed Ag85A peptide pools, $p < 0.0001$; single Ag85A peptide pool, $p < 0.0001$; rAg85A protein, $p < 0.0001$; Figure 1a and b). At baseline, 19 and 18 out of 24 volunteers had positive responses to BCG and PPD, respectively. These responses were boosted by vaccination as IFN-γ^+ cells specific for both antigens were increased significantly 7 days post-vaccination (PPD, $p = 0.0002$ and BCG: Figure 1c). No differences in pre- or post-MVA85A vaccination immune responses were observed in vaccinees who were BCG-primed and those who were BCG-naïve (data not shown).

Longitudinal follow-up showed that the vaccine-induced responses were maintained at levels above the baseline response. T cell responses one year (364 days) after vaccination were significantly higher than baseline counts for the single Ag85A peptide pool ($p = 0.0001$), summed peptide pools ($p = 0.0005$) and BCG ($p = 0.0358$) (Fig 1D and E and data not shown). Responses to rAg85A protein and PPD at day 364 post-vaccination were not higher than at baseline.

Although responses to the single Ag85A peptide pool were markedly lower than summed responses to the 7 Ag85A peptide pools, there was a high degree of correlation between these measures (Figure 1f). Similarly, BCG-specific T cell responses were lower than PPD-specific responses but these measures also correlated (Figure 1g). Importantly, single Ag85A peptide pool-specific responses also correlated with BCG-specific ($r = 0.391$, $p < 0.0001$) and PPD-specific ($r = 0.529$, $p < 0.0001$) responses (data not shown).

MVA85A vaccination boosts the BCG-specific T cell-associated cytokine response

We characterised the qualitative effect of MVA85A vaccination on the BCG-specific response by measuring intracellular expression of IFN-γ, TNF-α, IL-2 and IL-17 by $CD4^+$ and $CD8^+$ T cells (Figure 2 and 3). As seen by IFN-γ ELISPOT assay, cytokine-producing BCG-specific T cells were detected before MVA85A vaccination in most subjects. The frequency of $CD4^+$ T cells producing any cytokine combination was boosted after MVA85A vaccination ($p = 0.013$), but this had returned to baseline levels six months after vaccination. This was also seen for polyfunctional cells, which express IFN-γ, TNF-α and IL-2 simultaneously ($p = 0.0061$), and when the frequencies of IFN-γ, IL-2 or TNF-α-expressing $CD4^+$ T cells were assessed independently ($p = 0.005$, $p = 0.001$, $p = 0.002$, respectively, Figure 2c). However, despite detecting a distinct BCG-specific IL-17^+ $CD4^+$ T cell subset, vaccination did not significantly boost this population (Figure 2c). We also studied the expression of these cytokines in combination. At all vaccination timepoints, the BCG-specific $CD4^+$ T cell response was complex and comprised numerous subsets of distinct cytokine-producing cells (Figure 2d). The dominant subset at all timepoints expressed exclusively IFN-γ, while polyfunctional cells and Th17 cells, also made up major subsets. The frequency of polyfunctional $CD4^+$ T cells was boosted by vaccination (Figure 2b). However, the proportion of polyfunctional cells out of the total cytokine response, was

significantly lower 7 days post-vaccination than before or 168 days after vaccination (Figure 2d). This relative decline in proportion of polyfunctional cells resulted from the marked increase in cells expressing IFN-γ only during the early post-vaccination response. Although cells expressing TNF-α and IFN-γ, or IL-2 and IFN-γ, comprised less dominant subsets, the relative proportions of these subsets were also boosted at day 7 ($p < 0.01$ and $p < 0.05$, respectively). Single positive IL-2^+ and TNF-α^+ cells were relatively infrequent and not significantly boosted. Similar to the polyfunctional subset, the proportion of the BCG-specific Th17 cells also fell immediately after vaccination (Figure 2d).

The BCG-specific $CD8^+$ T cell response was also characterised (Figure 3) and was strongly dominated by IFN-γ-producing cells at all vaccination timepoints. IFN-γ^+ T cells comprised more than 80% of the total $CD8^+$ response. In contrast to $CD4^+$ T cells, the frequencies of total cytokine$^+$ or IFN-γ^+ $CD8^+$ T cells were not boosted 7 days post-vaccination (Figure 3b-c). The proportion of IFN-γ^+ cells out of the total $CD8^+$ T cell response was also not significantly different after vaccination.

Discussion

We show that MVA85A is safe and immunogenic in South African adults. Our results expand on previously reported qualitative characteristics of the MVA85A-induced immune responses [26]. In that UK-based study MVA85A vaccination induced long-lasting, polyfunctional Ag85A-specific $CD4^+$ T cells with a relatively immature phenotype. We did not observe differences in immune responses between BCG-vaccinated and BCG-naïve individuals. This is different to the UK studies, where MVA85A vaccination induced significantly higher responses in BCG-primed compared with BCG-naïve individuals [32]. In our cohort all subjects had detectable BCG-specific T cell responses before vaccination. This was most likely the result of BCG vaccination, in the BCG–vaccinated individuals, or of environmental mycobacterial exposure, as *M.tb* infection was an exclusion criterion. Pre-vaccination, BCG-specific T cells produced either one, or multiple cytokines. We show that the polyfunctional BCG-specific $CD4^+$ T cell population (IFN-γ^+, IL-2^+ and TNF-α^+) is boosted by MVA85A vaccination. We hypothesize that polyfunctional cells may be a better predictor of vaccine efficacy than single-cytokine readout assays. Polyfunctional T cells, and not IFN-γ^+ cells, have been associated with protection against murine *Leishmania major* infection [25] and with control of viremia during non-progressive human HIV infection [36].

The $CD4^+$ T cell cytokine response 7 days after MVA85A vaccination was strongly dominated by IFN-γ production compared with a more diverse response pre-vaccination and on day 168 post-vaccination. Therefore the proportion of polyfunctional $CD4^+$ T cells out of the total cytokine$^+$ cell subset was lower at day 7 than before vaccination. The day 7 bias of predominant IFN-γ production is consistent with functional characteristics of highly activated effector cells, typically observed following vaccination or during chronic viral infections [37, 38]. In contrast, pre-vaccination and on day 168 post-vaccination more cells produced IL-2, thought to be characteristic of a long-lived, central memory T cell population. A vaccine-induced switch from IL-2^+central memory cells to IFN-γ^+ effector cells was reported after tetanus toxoid boost [39]. The latter study revealed a switch to central memory cells later which was similar in our trial.

The cytokine expression profiles of BCG-specific $CD4^+$ T cells in our study differed from those observed in MVA85A-boosted Ag85A-specific $CD4^+$ T cells in the UK study [26]. In the latter, the peak post-vaccination response was dominated by polyfunctional cells. This may reflect differences in antigen-specificity. The UK study characterised $CD4^+$ T cells specific for a peptide pool, whereas we focused on T cells specific for the more complex *M. bovis* BCG. BCG requires antigen presenting cell processing, and many additional antigenic

targets not boosted by MVA85A vaccination are presented and recognized. Compared with other antigens, BCG may induce differential expression of TNF-α and IL-2, despite IFN-γ production being very similar [40]. In the latter study the BCG-specific response was dominated by single IFN-γ^+ cells, whereas polyfunctional cells dominated the PPD-specific response. The immune inhibitory effect of live BCG and its attenuating role in the expression of mature MHC class II on macrophages may also play a role in differential T cell cytokine expression [41]. This effect may have contributed to the ELISPOT data showing lower BCG-specific responses than PPD-specific responses and highlights the importance of characterising immune responses in detail using multiple antigens.

The observed differences in MVA85A-vaccinated persons between the UK and South Africa may also reflect a difference in exposure to environmental mycobacteria. This is suggested by data comparing the immunogenicity of BCG in Malawi and the UK, which show that BCG-naïve people in the southern African country have higher levels of pre-vaccination anti-mycobacterial immunity than in the UK [33, 34]. The BCG-specific responses detected in our study before MVA85A vaccination in the BCG-naïve individuals support this. Finally, differences in specimen and assay systems may contribute to the different profiles; Beveridge et al. stimulated PBMC with peptides in a 6-hour assay [26], while we analysed whole blood stimulated with BCG for 12 hours.

Since $CD4^+$ T cells producing Th1 cytokines were boosted, the finding that IL-17-producing BCG-specific cells were not boosted after vaccination was surprising. We propose that cytokine expression by BCG-specific Th17 cells was suppressed by the dominant Th1 response, as Th17 lineage formation in humans is inhibited by IFN-γ [21, 42, 43]. Production of IL-17 by differentiated, mycobacteria-specific T cells is also inhibited by IFN-γ [40].

BCG-specific $CD8^+$ T cells were not boosted in our intracellular cytokine analysis. This is consistent with results from a UK trial, in which the BCG-specific $CD8^+$ response did not significantly increase after MVA85A vaccination (Beveridge and Hughes et al., unpublished data). However, in the same trial, the Ag85A-specific $CD8^+$ T cell response was significantly boosted, whether whole blood was stimulated with Ag85A peptides or with recombinant Ag85A protein.

The safety data obtained here were very similar to those obtained in the UK and The Gambia in previous Phase I clinical trials as is the safety profile of MVA85A across the three different trial sites [44](Brookes R et al., manuscript submitted). There were no serious or severe vaccine related adverse events. Minor local and systemic reactions were common in the first week, but overall the vaccine was well tolerated and appears safe. This profile is consistent with that seen with other recombinant MVAs evaluated in clinical trials [45, 46].

Of those screened, 68% and 29% had a positive ESAT-6 or CFP-10 ELISPOT test or a positive Mantoux test, respectively. This occurred despite the fact that those with known TB contacts or previous TB exposure or disease were excluded from screening. This illustrates the difficulty of recruiting TB-naïve individuals in high-incidence areas such as the Western Cape: most of the population has been exposed. A trial of MVA85A in *M.tb* infected adults is also currently ongoing at the SATVI site. *M.tb*-infected people were excluded in the first trial to avoid the possibility of inducing a Koch reaction.

The MVA85A vaccine was found to be safe and highly immunogenic in this population of TB naïve, HIV uninfected, otherwise healthy adults in a very high TB prevalence area. MVA85A was shown to induce potent, polyfunctional T cell responses as a boost vaccine. These data are very encouraging and justify the further clinical evaluation of the protective

efficacy of these immune responses in a proof-of-concept efficacy trial in infants in this population.

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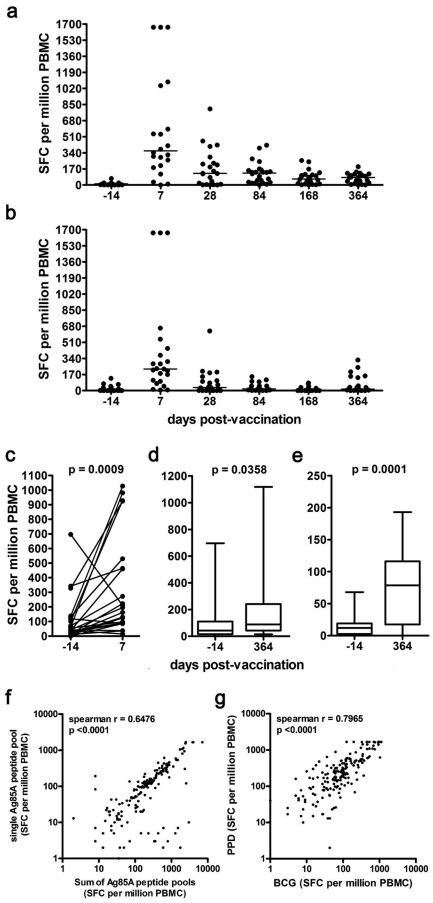

Figure 1.

Vaccine-induced IFN-γ ELISPOT responses in 24 adult volunteers. (**a**) Single Ag85A peptide pool responses. (**b**) Recombinant Ag85A protein responses. Horizontal lines represent the medians. (**c**) BCG-specific responses at baseline (day -14) and 7 days after vaccination. (**d**) Comparison of BCG-specific responses at baseline and one year (day 364) after MVA85A vaccination. (**e**) Comparison of single Ag85A peptide pool responses at baseline and one year after vaccination. Medians are represented by horizontal lines, inter-quartile range by the boxes and minimum and maximum values are represented by the whiskers. Differences were calculated with the Mann-Whitney test. (**f**) Correlation analysis between the single Ag85A peptide pool responses and the summed Ag85A peptide pool responses from all timepoints. (**g**) Correlation analysis between PPD-specific responses and BCG-specific responses from all timepoints.

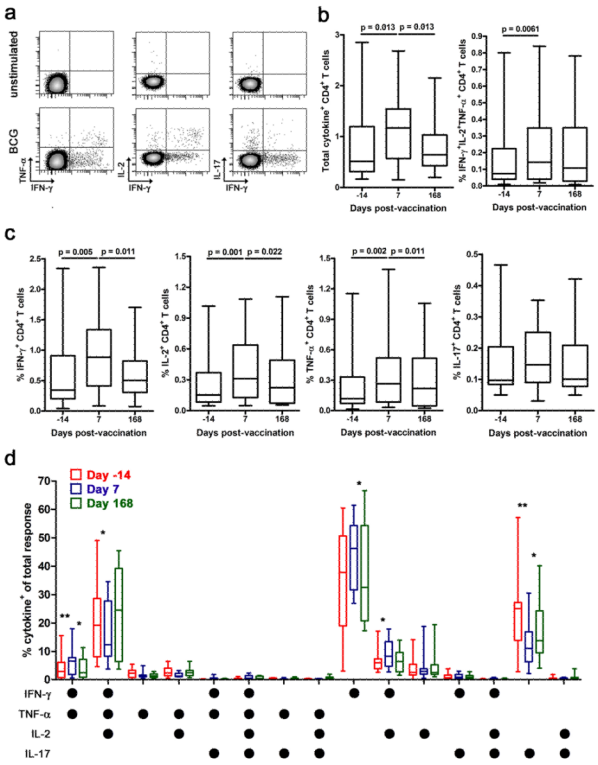

Figure 2.

Analysis of cytokine production by BCG-specific $CD4^+$ T cells. Production of IFN-γ, TNF-α, IL-2 and IL-17 by $CD3^+$ $CD4^+$ T lymphocytes was assessed by polychromatic flow cytometry. (**a**) Representative flow cytometry plots showing cytokine expression by $CD4^+$ T cells in unstimulated or BCG-stimulated whole blood 7 days after vaccination. (**b**) Frequency of total cytokine-producing BCG-specific $CD4^+$ T cells and polyfunctional (IFN-γ^+, TNF-α^+ and IL-2^+) $CD4^+$ T cells in 13 vaccinees. The median is represented by the horizontal line, the interquartile range by the box and the range by the whiskers. For each timepoint, in each individual, background values (unstimulated) were subtracted. (**c**) Frequencies of BCG-specific $CD4^+$ T cells producing IFN-γ, IL-2, TNF-α or IL-17 in 13 vaccinees. (**d**) Functional composition of the BCG-specific $CD4^+$ T cell response, expressed as a proportion of cytokine$^+$ cells out of the total response (as shown in section **b**). Three vaccination timepoints are shown for each combination of expressed cytokines. For comparisons the Wilcoxon signed rank test was used. Only significant differences are shown. *p< 0.05, **P<0.01, ***p<0.001.

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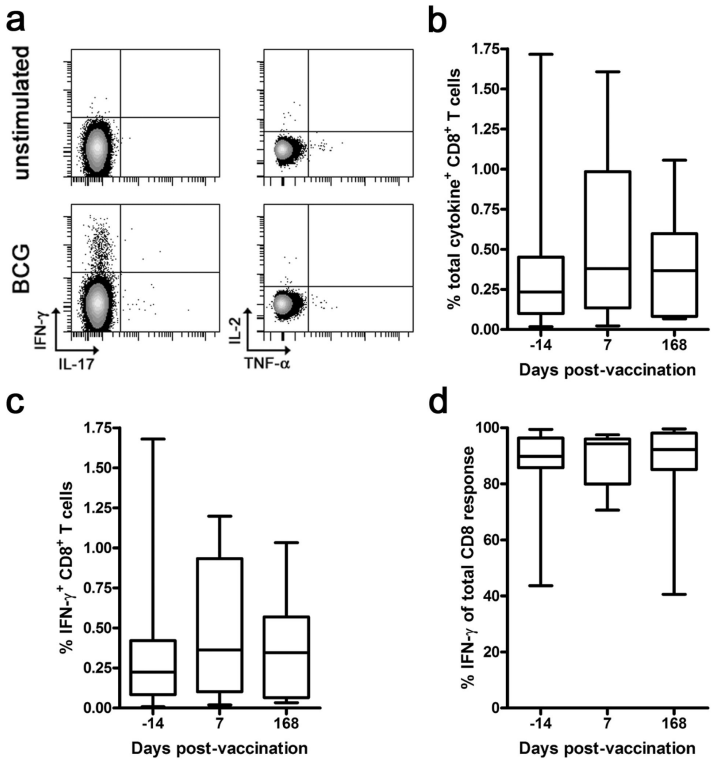

Figure 3.

Analysis of cytokine production by BCG-specific $CD8^+$ T cells. Production of IFN-γ, TNF-α, IL-2 and IL-17 by $CD3^+$ $CD8^+$ T lymphocytes was assessed by polychromatic flow cytometry. (**a**) Respresentative flow cytometry plots showing cytokine expression by $CD8^+$ T cells in unstimulated or BCG-stimulated whole blood 7 days after vaccination. (**b**) Frequency of total cytokine-producing BCG-specific $CD8^+$ T cells in 13 vaccinees. The median is represented by the horizontal line, the interquartile range by the box and the range by the whiskers. For each timepoint, in each individual, background values (unstimulated) were subtracted. (**c**) Frequency of IFN-γ-producing BCG-specific $CD8^+$ T cells in 13 vaccinees. (**d**) BCG-specific IFN-γ-producing $CD8^+$ T cell response, expressed as a proportion of cytokine$^+$ cells out of the total response (as shown in section **b**). For comparisons the Wilcoxon signed rank test was used. None of the differences were significant.

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Table 1

Demographic details of enrolled participants (n = 24).

Male, n (%)		8 (33.3%)
Median age in years (min-max)		35.5 (20.7-48.7)
Occupation class, n (%)	Administrative	11 (46%)
	Labour	5 (21%)
	Technical	4 (17%)
	Professional	2 (8%)
	Unemployed	2 (8%)
Race, n (%)	Black	5 (20.8%)
	Coloured	10 (41.7%)
	White	9 (37.5%)
Median body mass index in kg/m^2 (min-max)		27.6 (21.8-39.2)
BCG status (based on scar and history), n (%)	Vaccinated	12 (50%)
	Not vaccinated	12 (50%)

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Table 2

Reasons for Exclusion

Total screened, n	**119**
Excluded, n (%)	95 (80%)
ESAT6 or CFP10 positive by ELISPOT, n (% of screened)	81 (68%)
Mantoux test positive, n (% of screened)	34 (29%)
HIV ELISA test positive, n (% of screened)	10 (8%)
Withdrew voluntarily, n (% of screened)	3 (3%)
Excluded for other reasons, n (% of screened) (breastfeeding 3, evidence of hepatitis B virus infection 2, raised blood pressure 1, elevated liver enzymes 1, allergy to egg 1, did not return for visit 3, other medical reasons 2)	13 (11%)

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Table 3a

Recorded adverse events, n = 186.

	n (%)
Mild	173 (93%)
Moderate	12 (6.5%)
Definitely vaccine related	103 (55.4%)
Probable vaccine related	62 (33.3%)
Possible vaccine related	9 (4.8%)
Not vaccine related	12 (6.5%)

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Table 3b

Self-reported local and systemic side-effects during the first 7 days after MVA85A vaccination. Adverse events reported on at least one day during the period are shown.

Local Adverse event	n (%)	Systemic Adverse event	n (%)
Redness	24 (100%)	Feeling unwell	8 (33%)
Swelling	23 (96%)	Tiredness	4 (17%)
Pruritis	12 (50%)	Feeling feverish	11 (46%)
Pain	9 (38%)	Documented fever >37.5°C	1 (4%)
		Arthralgia	5 (21%)
		Headache	11 (46%)
		Myalgia	3 (13%)
		Nausea	2 (9%)