Dose-Finding Study of the Novel Tuberculosis Vaccine, MVA85A, in Healthy BCG-Vaccinated Infants

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(See the editorial commentary by Dockrell, on pages 1708–9.)

Background. BCG, the only licensed tuberculosis vaccine, affords poor protection against lung tuberculosis in infants and children. A new tuberculosis vaccine, which may enhance the BCG-induced immune response, is urgently needed. We assessed the safety of and characterized the T cell response induced by 3 doses of the candidate vaccine, MVA85A, in BCG-vaccinated infants from a setting where tuberculosis is endemic.

Methods. Infants aged 5–12 months were vaccinated intradermally with either $2.5 \times 10^7$, $5 \times 10^7$, or $10 \times 10^7$ plaque-forming units of MVA85A, or placebo. Adverse events were documented, and T-cell responses were assessed by interferon γ (IFN-γ) enzyme-linked immunospot assay and intracellular cytokine staining.

Results. The 3 MVA85A doses were well tolerated, and no vaccine-related serious adverse events were recorded. MVA85A induced potent, durable T-cell responses, which exceeded prevaccination responses up to 168 d after vaccination. No dose-related differences in response magnitude were observed. Multiple CD4 T cell subsets were induced; polyfunctional CD4 T cells co-expressing T-helper cell 1 cytokines with or without granulocyte-macrophage colony-stimulating factor predominated. IFN-γ-expressing CD8 T cells, which peaked later than CD4 T cells, were also detectable.

Conclusions. MVA85A was safe and induced robust, polyfunctional, durable CD4 and CD8 T-cell responses in infants. These data support efficacy evaluation of MVA85A to prevent tuberculosis in infancy.

Clinical Trials Registration. NCT00679159.
with BCG vaccination at birth [4]. CD4 and CD8 T cell expression of IFN-γ [5–7] and tumor necrosis factor α (TNF-α) [8–10], key Th1 cytokines, are thought to be critical for effective immunity against tuberculosis [11, 12]. Little is known about the effect of age at vaccination with new tuberculosis vaccines on the induced T-cell response.

Among 9 million annual tuberculosis cases worldwide, 1 million are estimated to occur in children <15 years of age [13]. The greatest risk for developing tuberculosis disease after exposure to Mycobacterium tuberculosis is in those aged <2 years [14, 15]. To prevent disease at this early age, a preventative vaccine would have to be given before 1 year of age. BCG vaccination at birth is routine in settings where tuberculosis is endemic. BCG confers protection against severe forms of tuberculosis in infants, such as miliary tuberculosis and tuberculosis meningitis [16, 17]. However, efficacy in protecting against adult and childhood pulmonary disease is variable and mostly poor [18]. A more efficacious vaccine is urgently needed. Twelve new tuberculosis vaccines are currently in clinical testing in human trials [19]. MVA85A, a recombinant strain of modified vaccinia Ankara expressing antigen 85A (Ag85A) from M. tuberculosis [20], is the first new tuberculosis vaccine to be tested in children and infants. This vaccine, designed to enhance the BCG-induced immune response, has an extensive and promising safety and immunogenicity record in adults from different settings [21–24]. The vaccine was also well tolerated in children aged 2–7 years from a tuberculosis endemic setting in South Africa [25] and induced robust and durable T-cell responses.

We investigated the safety and characterized the induced immune response of 3 different doses of MVA85A in healthy, BCG-vaccinated infants from a region where tuberculosis is endemic. We also compared these T-cell responses to those induced by MVA85A at older ages.

MATERIALS AND METHODS

Study Design, Enrollment, and Vaccination
We conducted an open-label, phase 2a safety, immunogenicity, and dose-finding study in healthy infants. Written, informed consent was obtained from parents or legal guardians. The protocol and amendments were approved by the Medicines Control Council of South Africa and the research ethics committees of the University of Cape Town and University of Oxford. The trial was conducted according to International Conference on Harmonization—Good Clinical Practice guidelines and was externally monitored by an independent contract research organization. The trial was registered at ClinicalTrials.gov (ID NCT00679159).

Healthy, M. tuberculosis–uninfected infants who were not exposed to human immunodeficiency virus type 1 (HIV-1) were recruited from the general population of Worcester, 110 km from Cape Town, South Africa. The aim was to enroll 144 infants into 3 consecutive vaccine dose groups of 48, who would be systematically allocated at a 3:1 ratio to receive either MVA85A (groups 1–3) or placebo, the pneumococcal 7-valent conjugate vaccine, Prevenar (Wyeth). Infants allocated to MVA85A group 1 received $2.5 \times 10^7$ plaque-forming units (PFUs; volume, 35 μL), those in group 2 received $5 \times 10^7$ PFUs (70 μL), and those in group 3 received $1 \times 10^8$ PFUs (135 μL). MVA85A, contract manufactured at Impfstoffwerk Dessau-Tornau (Biologika), was administered intradermally over the deltoid region of the arm contralateral to where BCG was administered. All participants had received BCG within 3 d of birth, according to the standard South African expanded program of immunization schedule. Prevenar was administered intramuscularly. Safety data for the first 7 d after vaccination from group 1 was reviewed by a data safety monitoring board before commencing enrollment into group 2. Similarly, review of group 2 safety data preceded enrollment into group 3. After the visit on day 168 after vaccination, MVA85A-vaccinated infants also received Prevenar.

Follow-up and Safety Evaluation
Following vaccination, all infants were evaluated on-site for at least 60 min and returned on days 2, 7, 28, 84, and 168 for additional evaluation. Blood samples for safety evaluation, which included biochemistry and hematology tests, were collected on days 7 and 84. Parents or guardians used diary cards to monitor local and systemic adverse events (AEs) during the first 7 d. AEs were assessed for causality and vaccine relatedness, and classified as not related, possibly related, probably related, or definitely related. The severity was classified with the National Institute of Allergy and Infectious Diseases Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events of December 2004 (http://rsc.tech-res.com/safetyandpharmacovigilance/) as mild, moderate, severe, or life-threatening. A serious adverse event (SAE) was defined as one which, regardless of severity, resulted in death, a life-threatening event, hospitalization or prolongation thereof, or a persistent or significant disability.

T Cell Assays
Blood samples were collected for immunogenicity tests 7–14 d before vaccination (0 or baseline) and on days 7, 28, 84, and 168 after vaccination. The ex vivo IFN-γ enzyme-linked immunospot (ELISPOT) assay was the primary immunological endpoint and was performed as described elsewhere [25]. Whole blood intracellular cytokine staining was performed as described elsewhere [25], before vaccination and on days 28 and 168 after vaccination, on samples from 18 randomly selected infants from the MVA85A groups and 12 infants who received Prevenar. Additional information is available online as supplementary data.
Data Analysis
Statistical tests were performed using Stata software (version 11; StataCorp) and Prism software (version 4.03; GraphPad). Distributions of T-cell frequency data were skewed, but log transformations did not result in symmetry. Thus, linear regression-type models with underlying assumptions of normality could not be used. As an alternative, the area under the curve (AUC) was calculated for each individual, using the trapezoidal rule (Winnonlin Professional software, version 5.2; Pharsight). Median AUC values for each group were compared using Kruskal-Wallis and Mann-Whitney tests. To account for the multiple testing, the Bonferroni adjustment was applied. A P value of <.0167 (.05 divided by 3) was considered to be significant when comparing the 3 vaccine doses.

RESULTS
Participants
A total of 261 infants were screened and 144 were found to be eligible for inclusion. Demographic characteristics are shown in Table 1. Infants in group 3 (1 × 10⁸ PFUs of MVA85A) were significantly younger than infants in groups 1 and 2. Infants in group 3 also had a lower weight-for-age z score, compared with those in group 1 (Table 1).

Safety Profile of MVA85A Doses
Of the 108 infants who received the intradermal MVA85A, 106 experienced at least 1 AE at the injection site, compared with 6 infants who received the intramuscular Prevenar (P < .0001). The symptoms in MVA85A recipients included desquamation (scaling), pain, redness, and swelling, and were of mild intensity (Table 2). Although many AEs appeared to be more frequent in the high-dose group, desquamation was the only injection-site symptom that significantly increased with greater vaccine dose (Table 2). A similar number of infants in each group, including Prevenar recipients, experienced at least 1 systemic event in the first 7 d.

Systemic AEs peaked in frequency on day 2 and were mostly resolved by day 28. More than 90% were mild in nature, and they occurred equally in MVA85A- and Prevenar-vaccinated infants. Elevated levels of liver enzymes were noted in infants receiving MVA85A and Prevenar, with increasing frequency at higher MVA85A doses (Table 2). In all participants except 1 Prevenar recipient, these elevated enzyme levels had resolved by day 28. All increases in liver enzyme levels for infants in groups 1 and 2 were classified as mild AEs. One increased alkaline phosphatase level and 4 increased alanine transaminase (ALT) levels among infants in group 3 were classified as moderate AEs, whereas 1 increased ALT level in a Prevenar recipient was rated as severe.

In addition to the severe AE in the Prevenar recipient, 2 other AEs were severe. An increased white cell count concurrently occurred with the increased ALT level in the same Prevenar recipient during an episode of gastroenteritis. The other severe AE was a case of gastroenteritis in group 3, which required hospitalization. Although this episode occurred within 7 d of vaccination, it was judged to be unrelated to the vaccine because the concomitant respiratory tract infection suggested a viral origin.

We recorded 11 SAEs (serious AEs; note that these are different from severe AEs), all requiring hospitalization, in total: 1 in group 1, 3 in group 2, 6 in group 3, and 1 in the Prevenar group. These SAEs included gastroenteritis, pneumonia, viral meningitis, bacterial meningitis, and lower respiratory tract infection. SAEs were recorded for 6 months after vaccination and appeared to occur evenly distributed throughout this period. None were considered to be vaccine related.

Three infants (all in group 1) converted to an early secretory antigenic target 6/culture filtrate protein 10 (ESAT-6/CFP-10)–positive ELISPOT response during follow-up, suggestive of M. tuberculosis infection. These infants were referred to the local

Table 1. Demographic Characteristics of Enrolled Infants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1, 2.5 × 10⁷ PFUs (n = 36)</th>
<th>Group 2, 5 × 10⁷ PFUs (n = 36)</th>
<th>Group 3, 1 × 10⁸ PFUs (n = 36)</th>
<th>Prevenar, placebo (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, no. (%)</td>
<td>15 (42)</td>
<td>17 (47)</td>
<td>14 (39)</td>
<td>22 (62)</td>
</tr>
<tr>
<td>Median age, d (range)</td>
<td>270.5 (206–370)</td>
<td>278.5 (208–364)</td>
<td>188 (155–359)</td>
<td>252.5 (160–363)</td>
</tr>
<tr>
<td>Ethnic origin, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black African</td>
<td>11 (30.6)</td>
<td>11 (30.6)</td>
<td>3 (8.3)</td>
<td>12 (33.3)</td>
</tr>
<tr>
<td>Mixed race</td>
<td>25 (69.4)</td>
<td>25 (69.4)</td>
<td>33 (91.7)</td>
<td>24 (66.7)</td>
</tr>
<tr>
<td>Median weight-for-age z score (range)</td>
<td>.44 (−1.7 to 2.98)¹⁰</td>
<td>−.145 (−2.18 to 3.1)</td>
<td>−.4 (−2.52 to 1.64)</td>
<td>.06 (−2.71 to 3.32)</td>
</tr>
</tbody>
</table>

**NOTE.** PFU, plaque-forming unit.

¹⁰ Group 3 vs group 1, P < .001; group 3 vs group 2, P < .001.

¹¹ Weight-for-age z scores were calculated using Anthro software (version 3.0.1; World Health Organization).

¹² Group 1 vs group 3, P = .0057 (Mann-Whitney test). No other significant differences were observed between the groups.
Local and Systemic Differences were Observed between the Groups.

Differences were observed between the groups. No detectable responses were observed in the Prevenar group (Figure 1C). No CD8 cytokine expression was detected in Prevenar recipients (Figure 2H); AUC values in the MVA85A groups significantly exceeded those in the Prevenar group, compared with the low-dose and high-dose groups (Figure 2G). CD8 T cell expression of TNF-α peaked 28 d after vaccination in most infants who received 2.5 × 10⁷ or 1 × 10⁸ PFUs and on day 168 in infants who received 5 × 10⁷ PFUs. AUC analysis supported the observation of a different CD8 response kinetic in the mid-dose group, compared with the low-dose and high-dose groups (Figure 2G). No CD8 cytokine expression was detected in Prevenar recipients (Figure 2H); AUC values in the MVA85A groups significantly exceeded those in the Prevenar group (Figure 2G). CD8 T cell expression of TNF-α, IL-2, GM-CSF, or IL-17 was not detectable (data not shown).

MVA85A Induces Multiple Polyfunctional CD4 T-Cell Subsets

Next, we assessed qualitative characteristics of Ag85A-specific CD4 T cells and observed a complex pattern of cytokine co-expression. Polyfunctional CD4 T cells, co-expressing IFN-γ, TNF-α, IL-2, and GM-CSF, comprised the predominant subset, whereas IFN-γ⁺TNF-α⁺IL-2⁺CD4 T cells were also prominent (Figure 3A). MVA85A dose did not affect frequencies of total cytokine-positive CD4 T cells (Figure 3B) or of polyfunctional subsets (Figure 3C and 3D).

A novel population of polyfunctional CD4 T cells, that co-expressed all 5 cytokines, was also induced at very low frequencies in all MVA85A dose groups (Figure 3A and 3E). These polyfunctional Th1/Th17 subsets peaked 28 d after vaccination and were long-lived, as frequencies at 168 d still exceeded pre-vaccination levels. This kinetic was different from CD4 T cells expressing IL-17 in any combination (total IL-17 response) (Figure 2E), which peaked on day 168 after vaccination in most infants. Again, MVA85A dose did not affect frequencies of these specific T cells (comparison of AUC values; data not shown).

Overall, ~50% of Ag85A-specific CD4 T cells expressed ≥3 cytokines at 28 and 168 d after vaccination (Figure 3F). No

### Table 2. Local and Systemic Adverse Events Reported on at Least 1 d During the First 7 d After MVA85A Vaccination

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Group 1, 2.5 × 10⁷ PFUs (n = 36)</th>
<th>Group 2, 5 × 10⁷ PFUs (n = 36)</th>
<th>Group 3, 1 × 10⁸ PFUs (n = 36)</th>
<th>Prevenar, placebo (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness</td>
<td>34 (94.4) 36 (100) 36 (100) 7 (19.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swelling</td>
<td>34 (94.4) 36 (100) 36 (100) 5 (13.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritis</td>
<td>0 (0)</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pain</td>
<td>3 (8.3)</td>
<td>5 (13.9)</td>
<td>8 (22.2)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Warmth</td>
<td>2 (5.6)</td>
<td>2 (5.6)</td>
<td>3 (8.3)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Desquamation</td>
<td>8 (22.2)</td>
<td>16 (44.4)</td>
<td>23 (63.9)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaise</td>
<td>1 (2.8)</td>
<td>1 (2.8)</td>
<td>4 (11.1)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>1 (2.8)</td>
<td>1 (2.8)</td>
<td>4 (11.1)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Tactile fever</td>
<td>4 (11.1)</td>
<td>7 (19.4)</td>
<td>7 (19.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Documented fever</td>
<td>3 (8.3)</td>
<td>4 (11.1)</td>
<td>6 (16.7)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (5.6)</td>
<td>0 (0)</td>
<td>4 (11.1)</td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Elevated liver enzyme</td>
<td>1 (2.8)</td>
<td>3 (8.3)</td>
<td>9 (25)</td>
<td>4 (11.1)</td>
</tr>
<tr>
<td>AST</td>
<td>0 (0)</td>
<td>1 (2.8)</td>
<td>3 (8.3)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>ALT</td>
<td>1 (2.8)</td>
<td>1 (2.8)</td>
<td>6 (16.7)</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>ALP</td>
<td>0 (0)</td>
<td>1 (2.8)</td>
<td>3 (8.3)</td>
<td>2 (5.6)</td>
</tr>
</tbody>
</table>

**NOTE.** ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; PFU, plaque-forming unit.

- Group 1 vs group 2, P = .0279.
- Group 1 vs group 3, P = .0014 (Fisher exact test). No other significant differences were observed between the groups.
- Temperature of >37.5°C.
- Group 1 vs group 3, P = .063 (Fisher exact test). No other significant differences were observed between the groups.
- Some infants had elevated levels of >1 liver enzyme.

health service for clinical assessment and possible therapy. No marked changes in specific T cell responses were observed upon *M. tuberculosis* infection, and these infants were not excluded from analysis.

MVA85A Induces Potent T Cell Responses

We assessed the kinetics and magnitude of the Ag85A-specific T cell response to 3 MVA85A doses with an IFN-γ ELISPOT assay. Specific IFN-γ responses before vaccination were absent or very low and increased significantly after MVA85A vaccination (Figure 1A). This response peaked 7 d after vaccination and was still higher than the baseline level 168 d after vaccination, for all doses. There were no differences in magnitude or kinetics of response between the different MVA85A doses (Figure 1B). No detectable responses were observed in the Prevenar group (Figure 1C).

MVA85A Induces Complex CD4 T-Cell Subsets

To characterize the MVA85A-induced response, we measured T cell expression of the Th1 cytokines IFN-γ, TNF-α, and interleukin 2 (IL-2), as well as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 17 (IL-17). The gating strategy and representative flow cytometry plots are shown in Supplementary Figure 1. CD4 T cells expressing Th1 cytokines and/or GM-CSF increased markedly following vaccination and peaked on day 28 (Figure 2A–2E). Frequencies of these Ag85A-specific cells still exceeded baseline frequencies at 168 d after vaccination. IL-17-expressing CD4 T cells, by contrast, appeared to have different kinetics. These T cells peaked on day 168 in most infants, regardless of MVA85A dose (Figure 2E). The magnitudes and kinetics of CD4 T cell subsets expressing the 5 cytokines did not differ among infants who received different MVA85A doses. Ag85A-specific CD4 T cells were not detectable in Prevenar recipients (Figure 2A–2E).

MVA85A Induces IFN-γ-Expressing CD8 T Cells

We also assessed cytokine expression by Ag85A-specific CD8 T cells. IFN-γ-expressing CD8 T cell frequencies after vaccination exceeded prevaccination levels in all MVA85A dose groups (Figure 2F), although the responses were low. The CD8 response peaked 28 d after vaccination in most infants who received 2.5 × 10⁷ or 1 × 10⁸ PFUs and on day 168 in infants who received 5 × 10⁷ PFUs. AUC analysis supported the observation of a different CD8 response kinetic in the mid-dose group, compared with the low-dose and high-dose groups (Figure 2G). No CD8 cytokine expression was detected in Prevenar recipients (Figure 2H); AUC values in the MVA85A groups significantly exceeded those in the Prevenar group (Figure 2G). CD8 T cell expression of TNF-α, IL-2, GM-CSF, or IL-17 was not detectable (data not shown).
marked differences in the proportions of polyfunctional Ag85A-specific CD4 T cells were observed between dose groups.

**Different T Cell Responses in Infants and Older Individuals**

To investigate the role of age on immunity relevant to tuberculosis vaccination, we compared Ag85A-specific T cell responses in adults [24], adolescents, children [25], and infants before and after vaccination with $5 \times 10^7$ PFUs of MVA85A. These adults, adolescents, and children were enrolled previously at the same clinical trial site as the infants, and T cell responses were measured using identical assays in the same laboratory.

We observed higher prevaccination T cell responses to Ag85A in adults and adolescents, compared with those in children. Infants had the lowest prevaccination Ag85A-specific T cell response (Figure 4A). Ag85A-specific T cell responses 7 d after vaccination were also lower in infants than in adults and adolescents (Figure 4B). Finally, we compared the increase in T cell response 7 d after vaccination, relative to the prevaccination response, and observed significantly greater responsiveness in children and infants, compared with adults and adolescents (Figure 4C).

**DISCUSSION**

We assessed 3 doses of MVA85A in infants, a major target population for novel vaccination against tuberculosis. Four major findings emerged: (1) MVA85A was safe; (2) the vaccine induced robust, long-lived, and predominantly polyfunctional CD4 T cell responses that peaked 1 month after vaccination and low frequencies of IFN-γ-expressing CD8 T cells that peaked later than the CD4 response; (3) different MVA85A doses did not markedly affect safety or immunogenicity; and (4) a number of characteristics of the MVA85A-induced response were unique
Figure 2. Ag85A-specific CD4 and CD8 T cell cytokine expression in whole blood samples from infants measured by flow cytometry. A–E, Frequencies of cytokine-expressing CD4 T cells after whole blood samples from infants were incubated with an Ag85A peptide pool (n = 18 infants in each MVA85A group; n = 12 infants in the Prevenar group). A, Total interferon γ-positive (IFN-γ⁺) CD4 T cells for each infant group before (day 0) and after MVA85A or Prevenar vaccination. B–E, Total frequencies of CD4 T cells expressing tumor necrosis factor α (TNF-α), interleukin 2 (IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin 17 (IL-17), respectively. For each T cell population, the response on day 0 and that on day 168 were compared using a Wilcoxon signed rank test (shown P values). Area under the curve (AUC) values for any of these T cell populations in the 3 MVA85A
to infants. The latter finding included induction of detectable CD8 T cells (not readily detectable in older individuals), a different pattern and kinetic of IL-17–expressing CD4 T cells, and lower frequencies of pre- and postvaccination Ag85A-specific cells in infants, compared with older individuals.

No MVA85A-related serious AEs were recorded. The increased frequency of desquamation in infants who received higher vaccine doses may reflect a greater degree of inflammation induced by the vector.

The unsolicited local AE profile closely resembled data from MVA85A trials at our vaccine trial site in adults [24], adolescents, and children [25]. However, systemic and solicited AEs were less frequent in infants, most likely because events were reported by a third party, in most cases parents. Elevated levels of liver enzymes were recorded after MVA85A vaccination. Although 4 Prevenar recipients also had elevated levels of liver enzymes, greater frequencies of this AE appeared to be associated with higher MVA85A doses. A confounder in this observation may be the lower age of infants in group 3, who received the highest MVA85A dose. The cause for these elevated liver enzyme levels is not clear; investigations for infection with hepatitis B or C virus were negative. Cases of isolated elevated alkaline phosphatase level may be of bony origin and reflect bone growth. Notably, a number of infants had elevated liver enzyme levels during screening and were subsequently not enrolled into the study.

These first safety data in infants complement the good safety profile of MVA85A in healthy adults [24], adolescents, and children [25] from the same region, as well as adults from the United Kingdom [26] and the Gambia [23]. Similarly good safety data have been reported for other recombinant modified vaccinia Ankara viruses in clinical trials [27, 28].

Figure 2. continued.

dose groups were not significantly different (using the adjusted \( P \) value of < .0167). F, Frequencies of Ag85A-specific CD8 T cells expressing IFN-\( \gamma \) for each infant group before (day 0) and after MVA85A vaccination (\( n = 18 \) infants for each vaccine dose). CD8 T cell expression of GM-CSF, IL-2, IL-17, or TNF-\( \alpha \) was not detected above background levels. G, AUC comparisons of IFN-\( \gamma \)- CD8 T cell frequencies between the 3 MVA85A dose groups (\( n = 18 \) infants in each group) and the Prevenar group (\( n = 12 \) infants). The overall effect was calculated using the Kruskal-Wallis test. H, Frequencies of Ag85A-specific CD8 T cells expressing IFN-\( \gamma \) before (day 0) and after Prevenar vaccination (\( n = 12 \) infants). For panels \( F \) and \( H \), prevaccination and postvaccination responses were evaluated with the Kruskal-Wallis test, followed by the Wilcoxon signed rank test. PFU, plaque-forming units.
MVA85A vaccination induced robust, mostly polyfunctional CD4 T cells in infants. This response was also highly durable; magnitudes exceeded prevaccination levels up to 168 d after vaccination. GM-CSF co-expression with Th1 cytokines by specific CD4 T cells has been described after MVA85A vaccination [25] and in natural *M. tuberculosis* infection or Figure 3. Cytokine co-expression of CD4 T cells in whole blood samples from infants measured by flow cytometry. A, Patterns of single or combined expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interleukin 2 (IL-2), interleukin 17 (IL-17), and/or tumor necrosis factor α (TNF-α) by CD4 T cells after whole blood samples from infants in group 2 (5 × 10⁷ plaque-forming units [PFUs] of MVA85A; *n* = 18 infants) were incubated with an Ag85A peptide pool. The median frequency for each cytokine-expressing cell subset is represented by the horizontal line, the interquartile range by the box, and the range by the whiskers. For each pattern, for each individual, background values (unstimulated) were subtracted. Each box plot represents a particular time point before or after vaccination, for the indicated cytokine pattern. B, Frequencies of total cytokine-positive CD4 T cells from infants who received 2.5 × 10⁷ PFUs (left plot), 5 × 10⁷ PFUs (middle plot), and 1 × 10⁸ PFUs (right plot) of MVA85A. C, Frequencies of polyfunctional CD4 T cells co-expressing IFN-γ, TNF-α, IL-2, and GM-CSF. D, Frequencies of polyfunctional CD4 T cells co-expressing IFN-γ, TNF-α, IL-2, and GM-CSF, irrespective of GM-CSF expression. E, Frequencies of polyfunctional CD4 T cells co-expressing IFN-γ, TNF-α, IL-2, GM-CSF, and IL-17. F, Pie charts representing the mean proportions of cells co-expressing cytokines, among all CD4 T cells expressing cytokines, after whole blood stimulation with Ag85A peptide pool. From left to right, day 28 and day 168 postvaccination samples from infants who received 2.5 × 10⁷ PFUs, samples at the same time points from infants who received 5 × 10⁷ PFUs, and samples at the same time points from infants who received 1 × 10⁸ PFUs. For each T cell population, the responses on days 0 and 168 were compared using a Wilcoxon signed rank test (shown *P* values). Area under the curve values for any of these T-cell populations in the 3 MVA85A dose groups were not significantly different (using the adjusted *P* value of <.0167).
tuberculosis disease [29]. This cytokine may play a role in immunity to *M. tuberculosis*, since GM-CSF–expressing T cells have been identified in granulomatous tissue from *M. tuberculosis*–infected individuals [30].

Polyfunctional T cells have been hypothesized to be a good predictor of vaccine efficacy, because polyfunctional Th1 cells, and not T cells expressing IFN-γ alone, were associated with protection against intracellular *Leishmania major* infection in mice [31]. Novel murine tuberculosis vaccination studies also suggest that polyfunctional CD4 T cells may correlate with protection against tuberculosis [32]. In HIV-infected individuals, the elevated risk for developing tuberculosis may be associated with impaired mycobacteria-specific polyfunctional Th1 responses in the lungs [33]. However, the scenario in humans appears more complicated: we recently showed that the frequency of BCG-induced polyfunctional CD4 T cells does not correlate with protection against tuberculosis in infants [34]. Tuberculin-specific T cell responses in adult patients with tuberculosis were also more polyfunctional than responses from healthy, household contacts [35]. Polyfunctional T cells may therefore best be described as a surrogate of vaccine take, until new data on correlates of protection become available from efficacy trials [36].

MVA85A also induced IL-17–expressing Ag85A-specific CD4 T cells, as described elsewhere for adolescents and children [25]. These IL-17⁺ cells were mostly distinct from Th1 cells in infants, whereas most IL-17–expressing T cells in older individuals co-expressed Th1 cytokines. This response also followed a different kinetic from that of Th1 cells, as frequencies of IL-17–expressing cells 168 d after vaccination exceeded those at 28 d in most infants. A delay in the peak IL-17 response, relative to IFN-γ–expressing T cells, was also reported in MVA85A-vaccinated adults [37]. These authors proposed that the robust Th1 response to MVA85A may suppress, and thus delay, the Th17 response [37]. Our finding that MVA85A-induced CD4 T cells co-express IL-17 with Th1 cytokines does not support this [25]. In the mouse, where IL-17–expressing memory CD4 T cells may play a role in protective immunity against tuberculosis, the kinetics of Th17 and Th1 cells were not different [38]. Further investigation is required to understand this better.

Figure 3. continued.
MVA85A vaccination of infants also induced low frequencies of IFN-γ–expressing CD8 T cells. Like Th17 cells, these cells peaked 168 d after vaccination. The CD8 T cell response in infants contrasts with results from children and adolescents from the same site, in whom CD8 responses were not detected [25]. However, Ag85A–specific CD8 T cell responses were observed in MVA85A trials conducted in the Gambia [23] and the United Kingdom [22, 39], using other assay systems. It is not known why this discrepancy in detection of CD8 responses is seen. We previously proposed that a higher MVA85A dose may boost greater CD8 responses [25], as increased CD4 and CD8 responses were described with increasing doses of MVA vaccines [27, 40, 41]. However, our data on infants are not consistent with this: no dose effect was observed on the magnitude or character of the CD4 T cell response. Furthermore, infants in the mid-dose group had greater magnitudes of Ag85A–specific CD8 T cells, compared with those in the high-dose group. We cannot rule out the possibility that the younger age and lower weight-for-age z score of infants in the high-dose group may have been confounders. However, we detected no significant associations between response magnitude and age or ethnicity (data not shown). Such an age effect was observed by Kagina et al [3], who observed greater specific T cell responses in infants vaccinated with BCG at 10 weeks of age, compared with infants who received BCG at birth. Dose-finding studies of MVA85A in UK adults [37] and Gambian infants (M.O. Ota et al, unpublished data) showed higher Th1 cell responses in recipients of higher doses of MVA85A. Given our data that vaccine dose did not markedly affect T cell responses, and the acceptable safety profile at all doses, it was decided to use the highest dose, at $1 \times 10^8$ PFUs, in efficacy trials.

Our data suggest that determinants of vaccine take may vary at different ages and in different populations. Strikingly different response rates and T cell magnitudes have been reported after BCG vaccination in populations from Malawi and the United Kingdom [42, 43]. These findings highlight the importance of testing candidate vaccines in different populations.

An interesting finding was the greater magnitude in prevaccination responses to Ag85A in adults and adolescents, compared with that in infants. This was unexpected because infants received BCG at birth, 4–6 months before the prevaccination response was measured. Adolescents and adults were enrolled decades after receiving BCG. We propose that the Ag85A–specific response reflects BCG-induced T cells in infancy and, perhaps, early childhood, whereas in adolescents and adults it reflects exposure to environmental mycobacteria and/or M. tuberculosis. This may also account for the differences observed in IL-17/Th1 cytokine co-expression and CD8 T cell responses to MVA85A in infants and older persons. These data imply that all new vaccines should be specifically tested in populations of different ages.

In conclusion, MVA85A was safe and highly immunogenic in tuberculosis-naive, HIV-uninfected, BCG-vaccinated infants. These data support studies to evaluate the efficacy of this vaccine to prevent tuberculosis in infancy.

Figure 4. Age-related differences in Ag85A–specific T cell responses before and after MVA85A vaccination, with interferon γ (IFN-γ) enzyme-linked immunospot (ELISPOT) responses to Ag85A peptide pool in adults (median age, 35.5 years; range, 20.7–48.7 years; n = 24) [24], adolescents (median age, 14.4 years; range, 13.3–15.0 years; n = 12) [25], children (median age, 4.3 years; range, 1.4–7.7 years; n = 24) [25], and infants (n = 36). Horizontal lines represent the median. Differences were evaluated with the Mann-Whitney test. Only significant differences are shown. A, Ag85A–specific responses before MVA85A vaccination. B, Responses 7 d after vaccination with $5 \times 10^7$ plaque-forming units of MVA85A. C, Relative increase in IFN-γ ELISPOT response upon vaccination, calculated as the fold increase between the day 7 postvaccination response and the prevaccination response to Ag85A. PBMC, peripheral blood mononuclear cells; SFC, spot-forming cells.

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**Supplementary Data**

Supplementary data are available at http://jid.oxfordjournals.org online.

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**References**