Phase 1 Evaluation of 3 Highly Immunogenic Prime-Boost Regimens, Including a 12-Month Reboosting Vaccination, for Malaria Vaccination in Gambian Men

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Successful vaccination against intracellular pathogens, including liver-stage *Plasmodium falciparum*, will require induction of strong antigen-specific T lymphocyte responses. The multiple epitope (ME)–thrombospondin-related adhesion protein (TRAP) construct includes CD8+ and CD4+ T cell epitopes from pre-erythrocytic *P. falciparum* antigens fused in-frame to the entire pre-erythrocytic antigen TRAP. Three carriers for this construct—plasmid DNA and 2 recombinant nonreplicating poxviruses (modified vaccinia virus Ankara [MVA] and fowlpox strain 9 [FP9])—were administered at 3-week intervals in a heterologous prime-boost combination to 29 Gambian men aged 18–45 years. Doses of DNA ME-TRAP, MVA ME-TRAP, and FP9 ME-TRAP were 2 mg and and plaque-forming units, respectively. DNA ME-TRAP was injected intramuscularly; MVA ME-TRAP and FP9 ME-TRAP were injected intradermally. There were no clinically relevant laboratory abnormalities and no severe or serious adverse events related to vaccination. DNA/MVA and FP9/MVA regimens were the most potent inducers of circulating effector T cells seen to date in sub-Saharan Africa. Twelve months after the final vaccination, a single booster vaccination expanded the effector T cell pool to a similar or higher magnitude than that after the primary vaccinations. These results highlight optimized combination regimens with general relevance to the development of vaccines targeting intracellular pathogens.

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primate models, the use of prime-boost vaccination (usually with DNA/modified vaccinia Ankara [MVA]) has been highly immunogenic for induction of CD4+ and CD8+ T cells against hepatitis B virus (S. McConkey and A.V.S.H., unpublished data), tuberculosis [22], HIV [23, 24], ebola virus (DNA/adenovirus) [25], and melanoma. DNA/MVA polyprotein vaccinations controlled a mucosal challenge of a highly pathogenic simian immunodeficiency virus/HIV chimera and prevented AIDS in a macaque model. In that study, challenge occurred 7 months after the final vaccination [26].

In a previous phase 1 study in Gambia, priming with 2 intramuscular (im) injections of 1 mg of DNA ME-TRAP, followed by boosting with 1 intradermal (id) injection of $3 \times 10^7$ pfu of MVA ME-TRAP, all at 3-week intervals, produced strong CD4+ by boosting with 1 intradermal (id) injection of 3 tramuscular (im) injections of 1 mg of DNA ME-TRAP, followed 7 months after the final vaccination [26].

AIDS in a macaque model. In that study, challenge occurred simian immunodeficiency virus/HIV chimera and prevented adenovirus) [25], and melanoma. DNA/MVA polyprotein vaccines established data), tuberculosis [22], HIV [23, 24], ebola virus (DNA/MVA polyprotein vaccines controlled a mucosal challenge of a highly pathogenic simian immunodeficiency virus/HIV chimera and prevented AIDS in a macaque model. In that study, challenge occurred 7 months after the final vaccination [26].

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Fowlpox virus is an avipoxvirus that causes disease in chickens but not in mammals. Recombinant, attenuated fowlpox virus has been used as a rabies vaccine in mammals, in which it shows good expression of recombinant protein [28]. FP9 was derived from wild-type fowlpox virus by 400 passages in tissue culture, leading to marked attenuation and loss of pathogenicity in chickens by all routes of administration, to the extent of avirulence in 1-day-old chicks [29]. Like all avipoxviruses, FP9 and FP9 ME-TRAP cannot replicate in mammalian cells. Attenuated strains of fowlpox virus (e.g., TROVAC) and of another avipoxvirus, canarypox (i.e., ALVAC), have been used safely as recombinant viral vaccines in many human clinical trials [30, 31] and have been demonstrated to be nonvirulent in a variety of immunosuppressed animals and in immunosuppressed humans. The excellent profile of MVA as a candidate viral vector for human use has been discussed elsewhere [32].

We conducted a phase 1 study of Gambian men to assess the following issues: safety and immunogenicity of FP9 ME-TRAP priming, followed by MVA ME-TRAP boosting; safety and immunogenicity of increased doses of DNA ME-TRAP and MVA ME-TRAP; possible equivalence of 2 and 3 priming DNA ME-TRAP vaccinations for subsequent MVA ME-TRAP boosting; and safety and immunogenicity of a booster MVA ME-TRAP vaccination 12 months after the final vaccination.

SUBJECTS, MATERIALS, AND METHODS

Study setting and subjects. Volunteers were recruited from the town of Farafenni in Gambia. The volunteers in this study are thought to have been subject to an entomological inoculation rate of 1–30 bites/year [33]. The study occurred during the dry season, when the background rate of adverse events in adults is low. After meetings with national and local government representatives and community leaders and after a general meeting in Farafenni, information sheets were disseminated. Individual discussions were held between field assistants who were proficient in local languages and potential volunteers. Written, informed consent was then sought by a study physician. After giving consent, volunteers underwent clinical evaluation, including a full medical history and clinical examination, and were screened for hematological (complete blood count), renal (plasma creatinine level), and hepatic (plasma alanine aminotransferase [ALT] level) dysfunction and for HIV-1 and HIV-2 by use of ELISA. Thirty-eight men were enrolled; 5 men were excluded (1 with elevated ALT levels, 2 with elevated creatinine levels, and 2 with low packed cell volume), 4 men did not return for vaccination after screening, and 29 semi-immune men (18–45 years old) were enrolled. Written, informed consent was obtained from all volunteers. The study was monitored throughout by an independent safety monitor and was performed in accordance with the Declaration of Helsinki principles for conduct of clinical trials and with the local rules and regulations of the Medical Research Council unit in Gambia. Approval was obtained from the Joint Gambian Government/Medical Research Council Ethics Committee and the Oxfordshire Research Ethics Committee.

Vaccines. The 3 study vaccines were DNA ME-TRAP, FP9 ME-TRAP, and MVA ME-TRAP. The individual epitopes making up the ME string are described in detail elsewhere [20]. The strain of TRAP included in the vaccine construct is T9/H11003. The clinical vaccines were manufactured according to good manufacturing practice by contract manufacturers (DNA ME-TRAP, Qiagen; MVA ME-TRAP and FP9 ME-TRAP, IDT). DNA ME-TRAP was administered as 2 mg in 2 mL (i.e., 1 im injection of 1 mL each into each deltoid muscle). MVA ME-TRAP was administered as $1.5 \times 10^8$ pfu in 400 µL (i.e., 2 id injections of 100 µL each into the skin overlying each deltoid muscle). FP9 ME-TRAP was administered as $10^8$ pfu in 500 µL (i.e., 2 id injections of 125 µL each into the skin overlying each deltoid muscle). The cold chain was maintained and monitored until vaccine administration.

Study design. This was an open-label phase 1 trial. Men were allocated to groups alternately in order of enrollment. The initial vaccinations were administered at 3-week intervals. The doses and routes were as follows: $1 \times 10^7$ pfu of FP9 ME-TRAP id, 2 mg of DNA ME-TRAP im, and $1.5 \times 10^8$ pfu of MVA ME-TRAP id. Sixteen men received FP9 ME-TRAP at weeks 0 and 3, followed by MVA ME-TRAP at week 6 (FFM group); 8 men received DNA ME-TRAP at weeks 0, 3, and 6, followed by MVA ME-TRAP at week 9 (DDDM group); and 5 men received DNA ME-TRAP at weeks 0 and 3, followed by MVA...
ME-TRAP at week 6 (DDM group). Men were observed for at least 1 h after vaccination. Follow-up visits occurred on days 1, 2, 7, and 21 for nonfinal vaccinations and on days 1, 2, 7, 28, and 56 for final vaccinations.

**Laboratory analysis.** Thirty milliliters of venous blood was obtained from each man at screening, 7 days after the penultimate vaccination, and 7, 28, and 56 days after the final vaccination. Complete blood counts and analyses of plasma ALT and creatinine levels were performed on site, according to the standard operating procedures of the unit. Ex vivo ELISPOTs and safety assays were performed at screening and at 7 and 28 days after vaccination, as before.

**RESULTS**

**Safety and reactogenicity.** Twenty-six of 29 men completed the initial study protocol. The median ages in the 3 groups were similar. Two men migrated from the study area during the study period. One man withdrew his consent; he had suffered no moderate, severe, or serious adverse event. There were no laboratory abnormalities attributable to the vaccines. The adverse events recorded were all mild or moderate (table 1).

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>DNA (n = 34)</th>
<th>FP9 dose 1 (n = 16)</th>
<th>FP9 dose 2 (n = 16)</th>
<th>MVA after DNA (n = 12)</th>
<th>MVA after FP9 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>1 (2.9)</td>
<td>5 (31.3)</td>
<td>5 (31.3)</td>
<td>3 (25)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Malaise</td>
<td>1 (2.9)</td>
<td>7 (43.8)</td>
<td>4 (25)</td>
<td>3 (25)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>0</td>
<td>1 (6.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Local</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>1 (2.9)</td>
<td>6 (37.5)</td>
<td>1 (6.3)</td>
<td>7 (58.3)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Itching</td>
<td>1 (2.9)</td>
<td>5 (31.25)</td>
<td>6 (37.5)</td>
<td>2 (16.7)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Blister</td>
<td>0</td>
<td>6 (37.5)</td>
<td>1 (6.3)</td>
<td>3 (25)</td>
<td>2 (14.3)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of men who experienced the adverse event. Information on adverse events was solicited over the course of 7 days after vaccination. All adverse events were mild or moderate.
men from all 3 groups was 8.6 sfc/10⁶ PBMCs (range, 2.5–80 sfc/10⁶, PBMCs). There was a small, statistically insignificant increase after 2 or 3 vaccinations with DNA ME-TRAP to 12.5 and 11.3 sfc/10⁶ PBMCs, respectively. A single MVA booster vaccination after 2 vaccinations with DNA ME-TRAP induced a very large increase in effector T cell frequency (GM, 211.7 sfc/10⁶ PBMCs; figure 1A; \( P = .001 \), for increase above frequencies after vaccination with DNA ME-TRAP at weeks 0 and 3). A single MVA booster vaccination after 3 vaccinations with DNA ME-TRAP induced a GM T cell frequency of 331.0 sfc/10⁶ PBMCs (figure 1B; \( P = .008 \), for increase above frequencies after vaccination with DNA DNA ME-TRAP at weeks 0 and 3 [not statistically significantly different from the DDM group]).

Four weeks after the final MVA ME-TRAP vaccination, GM effector T cell frequencies were 142.0 and 119.1 sfc/10⁶ PBMCs (42.9% and 56.2% of the peak value) for the DDM and DDDM groups, respectively (figure 1A and 1B); 8 weeks after the final vaccination, GM effector T cell frequencies were 113.8 and 98.3 sfc/10⁶ PBMCs (34.4% and 46.4% of the peak value) for the DDM and DDDM groups, respectively. Merging the DDM and DDDM groups to evaluate the decay during the plateau phase from 4 weeks to 8 weeks after the final vaccination, the frequency at 8 weeks was 80.7% of that at 4 weeks (GM, 106.5 vs. 132.0 sfc/10⁶ PBMCs).

Efficient priming and boosting with 2 recombinant viruses, FP9 ME-TRAP and MVA ME-TRAP. GM effector T cell frequencies in this group increased after 2 vaccinations with FP9 ME-TRAP, to 46.7 sfc/10⁶ PBMCs (\( P = .001 \), for increase above baseline). After a single MVA ME-TRAP booster vaccination, GM effector T cell frequencies increased to 156.3 sfc/10⁶ PBMCs (\( P = .005 \), for increase above frequencies after vaccination with FP9 DNA ME-TRAP at weeks 0 and 3; figure 1C). At 4 and 8 weeks after the MVA vaccination, effector T cell frequencies were 74.8 and 87.6 sfc/10⁶ PBMCs (47.8% and 56.1% of the peak value). Because of venesection limitations in the study population, no comparison was available between immunogenicity of the first and second FP9 ME-TRAP vaccinations. The induced frequencies in the DNA/MVA-vaccinated men (DDM and DDDM groups merged) were not statistically significantly higher than those induced after FP9/MVA vaccination (\( P = .39 \)).

Effector T cells cross-reactive for 3D7 TRAP induced by T9/96 TRAP prime-boost vaccination. To assess the ability of induced T cells to recognize a nonvaccine strain of TRAP, cells were assayed with peptides encoding the 3D7 sequence for TRAP, a strain with a West African origin. It has 6% aa difference from T9/96, whose sequence was derived from a Thai isolate. At the peak time point after MVA boosting and merging the 3 groups, the GM 3D7 TRAP–specific effector T cell frequency was 198.1 sfc/10⁶ PBMCs, compared with 187.4 sfc/10⁶ PBMCs for T9/96 TRAP (figure 1A–1C).

Breadth of the ME-TRAP T cell response. Responses were induced to all pools. In individuals there was often 1 immunodominant response to a particular peptide pool with \( \geqslant 1 \) subdominant responses to other peptide pools, although some individuals showed a uniformly strong response across the whole antigen (figure 2).

Induction of effector T cells specific for epitopes processed from the polyepitope string. Responses to epitopes from the polyepitope string, tested as a pool, were present in many men, but at lower frequencies than those to peptides spanning the entire 3D7 and T9/96 TRAP strains. Ten of the 26 men with
analyzable responses both before vaccination and at the peak time point had positive induced responses to the ME string (defined as $P < .05$ for comparison between prevaccination and peak values by binomial distribution in both duplicates). The highest induced response to the ME string was a GM of 106.2 sfc/10$^6$ PBMCs.

Safety and immunogenicity of 12-month booster vaccination with MVA ME-TRAP. The interval between administration of MVA ME-TRAP as the final vaccination of the initial study and MVA ME-TRAP booster vaccination was 12–13 months for the 19 men. The GM frequency of T cells measured by the ex vivo ELISPOT assay just before the booster vaccination was 22.6 and 17.9 sfc/10$^6$ PBMCs in the DNA/MVA and FP9/MVA groups, respectively (≈3-fold higher than the prevaccination baseline frequencies; $P = .11$). Seven days after the booster vaccination, GM frequencies had increased to 411.8 and 283.4 sfc/10$^6$ PBMCs, compared with 241.7 and 144.5 sfc/10$^6$ PBMCs, respectively, at the initial peak for these individuals only from the 2 groups (figure 3). Twenty-eight days after the booster vaccination, GM frequencies decreased to 165.6 and 103.4 sfc/10$^6$ PBMCs, higher than the frequencies at 28 days after the final vaccination in the same individuals 1 year earlier.

DISCUSSION

It has been unclear whether it is possible to induce higher levels of immune responses through vaccination than occurs through natural infection. The requirement for such supranatural immunogenicity is a key one for effective liver-stage malaria subunit vaccination. T cell responses in semi-immune adults from sub-Saharan Africa in contrasting epidemiological settings are low [34, 35]. The GM effector T cell frequency to T9/96 TRAP in 52 unvaccinated Gambian adults screened as part of the present study and others was 6.2 sfc/10$^6$ PBMCs. Therefore, the most immunogenic regimen in the present study induced responses >50-fold higher than a lifetime of exposure to natural infection. The breadth and cross-reactivity of the induced immune response offer an encouraging indication that strain-transcending protection should be possible with DNA-based vaccination regimens. In an earlier phase 1 Gambian study that used lower doses of DNA and MVA, the distribution of T cell subsets tended toward CD4$^+$ T cells. ELISPOT assays conducted at the peak time point in the present study indicate that this pattern is unchanged (data not shown). The DNA-based prime-boost approach is not designed to induce substantial antibody responses, and, as expected, in previous UK and Gambian studies, antibody induction has been modest [15, 27]. Antibody ELISAs were not conducted in the present study.

A single 12-month MVA booster vaccination expanded the effector T cell pool to a magnitude as high as or higher than that after the initial vaccinations. This result confirms that a long-lasting immunological memory state is conferred by heterologous DNA–based vaccination in humans. Our previous data indicated that human anti-MVA immunity precludes immunogenic second MVA vaccinations at 3–8 weeks [27], but, interestingly, this effect appears to wane sufficiently by 12 months to allow effective reboosting. Further characterization of vector immunity to MVA and other highly immunogenic candidate viral vectors is desirable. Periodic booster vaccinations may be necessary and effective for maintenance of protective T cell re-

![Figure 2](https://academic.oup.com/jid/article-abstract/189/12/8587/858780)
responses against malaria and other pathogens. Although none of the 3 vaccination groups in the present study received \(1.5 \times 10^3\) pfu of MVA ME-TRAP without prior priming with either DNA or FP9, there was a clear difference in immunogenicity after reboosting in the groups who previously had received DNA and FP9 priming. Furthermore, our UK studies clearly demonstrate that heterologous prime-boost vaccination is necessary for maximal T cell immunogenicity [15].

DNA has previously been administered to humans at doses of up to 2.5 mg [36]; the safety of 2-mg doses of DNA ME-TRAP is, therefore, not surprising. We have previously reported the safety of \(3 \times 10^6\) pfu of MVA [32]. Here, we have shown that a 5-fold increase in dose presents few safety issues in malaria-exposed adults. The small blisters seen in a minority of men would need to be assessed carefully in a pediatric population. The safety and reactogenicity of \(1 \times 10^9\) pfu of FP9 ME-TRAP are acceptable, with a marked decrease in reactogenicity with the second dose, probably a result of induced immunity to the viral vector.

The collaboration between the University of Oxford in the United Kingdom and the Medical Research Council laboratories in Gambia has facilitated the conduct of a series of phase 1 studies in the United Kingdom and Gambia. Within an appropriately short time frame, by using small group sizes, we have assessed the following parameters for adult vaccination: dose, route, interval, number of priming vaccinations, and choice of priming agent. The lack of statistical significance in the difference between 2 and 3 DNA priming vaccinations indicates that there is unlikely to be a substantial benefit to the use of 3 priming vaccinations. Results from several previous phase 1 studies from our group have shown that a second vaccination with MVA does not improve immunogenicity over a single vaccination with MVA [27]. This finding highlights a regimen of 2 priming vaccinations followed by a single booster vaccination. Although we have used a 3-week interval, it is likely that little or no immunogenicity would be lost by extending the interval to 4 weeks, which would allow vaccination in the expanded program of immunization schedule.

The results of the present study confirm that the increasing body of promising preclinical data with DNA/MVA and FP9/MVA vaccines translates into human immunity with a malaria construct in an endemic setting—a translation that has not held for several other approaches that were promising in preclinical studies. The results of the present study provide a strong rationale for further clinical evaluation of both DNA/MVA and FP9/MVA for diseases caused by intracellular pathogens. They also provide hope that a contribution toward effective vaccination against liver-stage malaria, HIV, and tuberculosis can be developed with heterologous DNA–based prime-boost combinations.
References


