

MALARIA

Prime and target immunization protects against liver-stage malaria in mice

Anita Gola^{1,2}, Daniel Silman^{1,3}, Adam A. Walters¹, Saranya Sridhar^{1,3}, Stefan Uderhardt², Ahmed M. Salman^{1,4}, Benedict R. Halbroth¹, Duncan Bellamy¹, Georgina Bowyer¹, Jonathan Powlson¹, Megan Baker^{1,3}, Navin Venkatraman^{1,3}, Ian Poulton^{1,3}, Eleanor Berrie⁵, Rachel Roberts^{1,3}, Alison M. Lawrie^{1,3}, Brian Angus⁶, Shahid M. Khan⁴, Chris J. Janse⁴, Katie J. Ewer¹, Ronald N. Germain², Alexandra J. Spencer¹, Adrian V. S. Hill^{1*}

Despite recent advances in treatment and vector control, malaria is still a leading cause of death, emphasizing the need for an effective vaccine. The malaria life cycle can be subdivided into three stages: the invasion and growth within liver hepatocytes (pre-erythrocytic stage), the blood stage (erythrocytic stage), and, finally, the sexual stage (occurring within the mosquito vector). Antigen (Ag)-specific CD8⁺ T cells are effectively induced by heterologous prime-boost viral vector immunization and known to correlate with liver-stage protection. However, liver-stage malaria vaccines have struggled to generate and maintain the high numbers of *Plasmodium*-specific circulating T cells necessary to confer sterile protection. We describe an alternative “prime and target” vaccination strategy aimed specifically at inducing high numbers of tissue-resident memory T cells present in the liver at the time of hepatic infection. This approach bypasses the need for very high numbers of circulating T cells and markedly increases the efficacy of subunit immunization against liver-stage malaria with clinically relevant Ags and clinically tested viral vectors in murine challenge models. Translation to clinical use has begun, with encouraging results from a pilot safety and feasibility trial of intravenous chimpanzee adenovirus vaccination in humans. This work highlights the value of a prime-target approach for immunization against malaria and suggests that this strategy may represent a more general approach for prophylaxis or immunotherapy of other liver infections and diseases.

INTRODUCTION

Malaria, caused by *Plasmodium* species, is an important disease causing about 500,000 deaths annually, with particularly high morbidity and mortality in sub-Saharan Africa (1). With growing anti-malarial drug and insecticide resistance, an effective vaccination regimen is needed urgently (2). A variety of different subunit vaccine strategies have been investigated: RTS,S/AS01, a protein-in-adjuvant formulation inducing antibodies against circumsporozoite protein (CSP), completed phase 3 field trials with partial efficacy but is still years from licensure (3).

An alternative approach to inducing protection against pre-erythrocytic malaria has been to generate cellular immunity directed against infected hepatocytes. Seminal studies in mice demonstrated the ability of interferon- γ (IFN- γ)-producing CD8⁺ T cells to protect against liver-stage malaria (4, 5). To this end, substantial effort has been invested in optimizing a viral vector heterologous prime-boost strategy (known to be particularly effective at inducing T cell responses) able to generate high frequencies of antigen (Ag)-specific CD8⁺ T cells (6, 7). A leading approach comprises a chimpanzee adenovirus (ChAd) viral vector prime, subsequently boosted with modified vaccinia Ankara (MVA) expressing the same Ag [such as thrombospondin-related adhesion protein (TRAP)], resulting in high numbers of CD8⁺ T cells in circulation with partial clinical efficacy

in both malaria-naïve and preexposed individuals (8–11). The induced CD8⁺ T cell numbers correlate with efficacy in controlled human malaria infection challenges, suggesting that increased numbers of CD8⁺ T cells would be required for higher protection.

Vaccines aimed at inducing cell-mediated immunity against malaria have been hindered by challenging spatiotemporal factors pertaining to parasite biology and liver microanatomy. First, with only peripheral vaccination regimens, it is difficult to generate and maintain the high numbers of *Plasmodium*-specific T cells necessary to confer protection (12). Second, it has been difficult to direct a robust cell-mediated immune response to the liver to enable parasite clearance in the short time window of liver-stage malaria (5 to 7 days for *Plasmodium falciparum* infections in humans and 2 days in *Plasmodium berghei* infections in mice) (13–16). In recent years, tissue-resident memory (T_{RM}) T cells have been identified (17–21) and shown to act as sentinels against invading pathogens under certain settings (22, 23). CXCR6⁺ CD8⁺ T cells [enriched after radiation-attenuated sporozoite (spz) vaccination], when lacking CXCR6 expression, result in both reduced numbers of liver memory T cells and protection during spz challenges (24, 25). Furthermore, intravenous (i.v.), but not intramuscular (i.m.), administration of irradiated, cryopreserved spz generated high frequencies of hepatic T cells in nonhuman primates (26).

T_{RM} has been generated experimentally by recruiting effector CD8⁺ T cells into tissues by administration of monoclonal antibodies (mAbs) fused to pathogenic epitopes (27), chemokines (28, 29), inflammatory agents (30), and inactivated pathogens (31). Although this work was in progress, the use of peptide Ag conjugated to mAb specific for splenic CD8⁺ dendritic cells, followed by intravenous injection of recombinant adeno-associated virus vector, was shown to generate high frequencies of liver T_{RM}, mediating sterile protection

¹Jenner Institute, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK. ²Lymphocyte Biology Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. ³Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ, UK. ⁴Department of Parasitology, Leiden University Medical Center, 2333 ZA Leiden, Netherlands. ⁵Clinical Biomanufacturing Facility, University of Oxford, Churchill Hospital, Oxford OX3 7JT, UK. ⁶Nuffield Department of Medicine, University of Oxford, Oxford, UK. *Corresponding author. Email: adrian.hill@ndm.ox.ac.uk

against liver-stage malaria (32). Our approach here focused on identifying a highly translatable and deployable vaccine strategy that can first induce abundant circulating T cells against full-length Ag in diverse human populations and subsequently increase liver CD8⁺ T cells expressing T_{RM} markers by targeting the second dose of immunogen to the liver. Targeting CD8⁺ T cells to the liver was achieved with either poly(lactic-co-glycolic acid) (PLGA) protein-loaded nanoparticles (Nps) or a recombinant viral vector. This approach generates durable liver Ag-specific CD8⁺ T cells for up to 6 months and markedly increases the efficacy of subunit immunization against liver-stage malaria with clinically relevant vaccines. Clinical development of this prime-target approach in humans has begun with a small phase 1 trial, in which healthy volunteers have received intravenous vaccination of a ChAd-based vaccine with encouraging safety and immunogenicity data.

RESULTS

Prime and target regimen results in sterile protection upon *P. berghei* challenge

In an effort to direct cell-mediated immune responses to the liver, we initially used nanoparticles. Consistent with previous data (33), after intravenous administration, PLGA-Nps containing ovalbumin (OVA) conjugated to allophycocyanin (APC) (Np.APC-OVA) were found to be concentrated within hepatic tissue and taken up by Kupffer cells (fig. S1, A and B, and movie S1). In contrast, intramuscular injection of nanoparticle did not show liver-specific concentration (fig. S1B). Adoptively transferred naïve OVA-specific OTI, but not naïve polyclonal, CD8⁺ T cells clustered and increased CD69 expression at sites of Np.APC-OVA uptake by Kupffer cells, indicating that the associated OVA Ag had been processed and presented by major histocompatibility complex class I (MHC-I) molecules (fig. S1C) (34).

To evaluate the ability of nanoparticles to promote localization of primed effector memory CD8⁺ T cells (T_{EM}) to the liver and mediate protection against liver-stage malaria, mice were primed intramuscularly with human adenovirus-5 expressing OVA (Ad.OVA-i.m.), and 2 weeks later, Np.OVA was administered intravenously (Ad.OVA-i.m./Np.OVA-i.v.; fig. S2A). Protection was assessed by challenging vaccinated mice with two different transgenic (Tg) *P. berghei* spzs expressing OVA (OVA-spz) (35). Vaccine efficacy was determined as the time to reach the end point of 1% blood parasitemia. Strikingly, after Ad.OVA-i.m./Np.OVA-i.v. vaccination, 100% of mice displayed sterile protection in both challenge models, whereas Ad.OVA-i.m. or Ad.OVA-i.m./Np.OVA-i.m. failed to protect the animals (Fig. 1, A and B, and fig. S2B). Subsequent flow cytometry analysis showed that Ad.OVA-i.m./Np.OVA-i.v., but not Ad.OVA-i.m./Np.OVA-i.m. or Ad.OVA-i.m., immunization produced a 10-fold increase in frequency and total number of OVA-specific [SIINFEKL-pentamer (Pen⁺) binding] CD8⁺ T cells in the liver, as well as a modest increase in CD4⁺ T cells, with no changes observed in the spleen (Fig. 1, C to H, and fig. S2C). In contrast, intramuscular administration of Np (Ad.OVA-i.m./Np.OVA-i.m.) increased the Pen⁺ CD8⁺ T cell frequency in the draining lymph node (dLN) but not in the liver or spleen. These data suggest that concentrating the primed CD8⁺ T cells in the liver, the site of initial malaria infection, may play an important role in mediating protection. To investigate whether a nanoparticle intravenous boosting increased Ag-specific T cells within the liver and/or in peripheral secondary lymphoid sites, we administered FTY720 to limit egress of activated T cells from lymphoid sites (36)

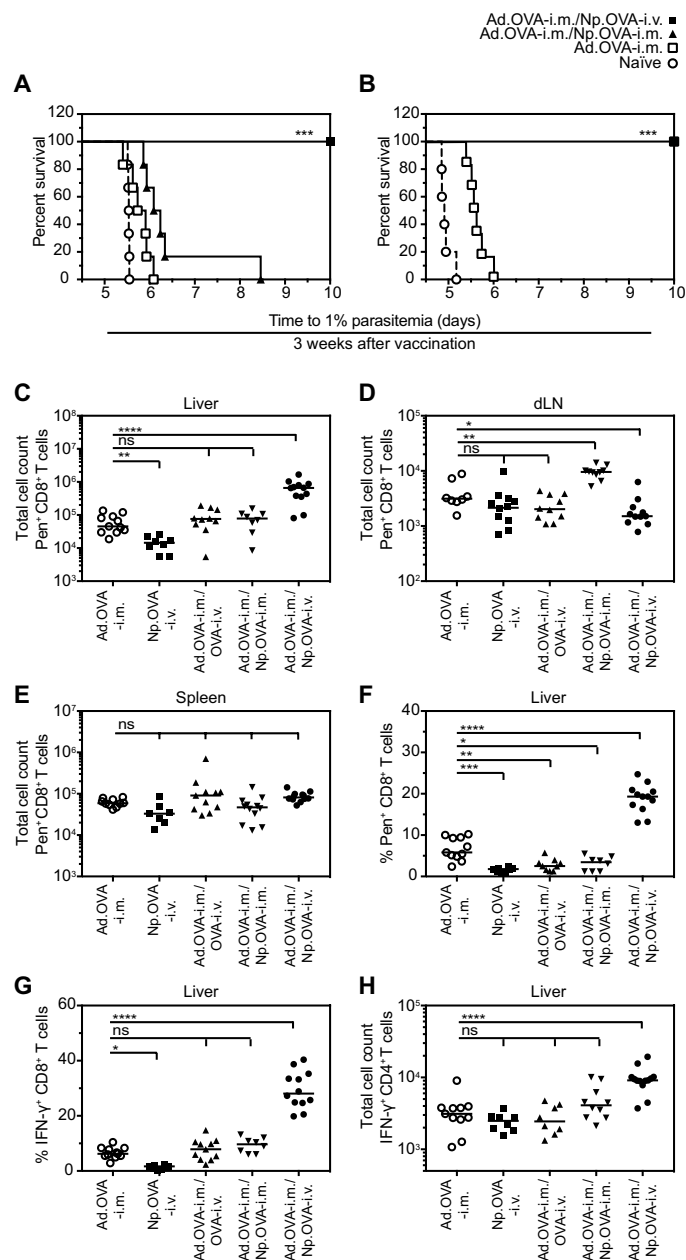


Fig. 1. Prime and target immunization protects mice against challenge with Tg OVA-spz. C57BL/6 mice ($n = 6$ per group) were primed with Ad.OVA-i.m., followed by Np.OVA-i.v. (Ad.OVA-i.m./Np.OVA-i.v.), Np.OVA-i.m. (Ad.OVA-i.m./Np.OVA-i.m.), or no Np.OVA (Ad.OVA-i.m.). Mice were challenged with (A) OVA::Hep17 *P. berghei* spz or (B) OVA::mCherry_{Pbhsp70} *P. berghei* spz. Data shown are representative of two to three independent experiments (log-rank Mantel-Cox test). C57BL/6 mice were primed with either Ad.OVA-i.m. or Np.OVA-i.v. Ad.OVA-i.m.-primed animals either were subsequently vaccinated with OVA protein (Ad.OVA-i.m./OVA-i.v.), Np.OVA-i.m. (Ad.OVA-i.m./Np.OVA-i.m.), or Np.OVA-i.v. (Ad.OVA-i.m./Np.OVA-i.v.) or were left untreated (Ad.OVA-i.m.). Three weeks after Np or Ad.OVA-i.m. vaccination, animals were examined by flow cytometry for (C) to (E) total Pen⁺ CD8⁺ T cells in the liver, dLN, and spleen; frequency of (F) Pen⁺ and (G) IFN-γ⁺ CD8⁺ T cells of total CD8⁺ T cells in the liver; and (H) total cell count of IFN-γ⁺ CD4⁺ T cells in the liver. Data shown are pooled from two to four independent experiments ($n = 7$ to 12 per group). Median shown. Data were analyzed with a linear mixed model (LMM), with experiment and mouse as random effects and vaccination and boost as fixed effects. **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. ns, not significant.

and pulsed 5-bromo-2'-deoxyuridine to track cell proliferation. These data suggested that both in situ proliferation in secondary lymphoid tissues and a migration of Pen⁺ CD8⁺ T cells to the liver were occurring (fig. S3). Last, we attempted to improve immunogenicity of prime-target vaccination by conjugating nanoparticle to adjuvants previously shown to increase T cell responses (37). However, addition of adjuvants [Resiquimod (R848) and monophosphoryl lipid A] only marginally increased liver Ag-specific T cells (fig. S4). Prime-target vaccination involving Np.OVA-i.v. alone generates particularly high numbers of Ag-specific CD8⁺ T cells in the liver tissue and results in sterile protection from spz challenge.

CD8⁺ T cells cluster around infected hepatocytes, resulting in effector responses

To study the cellular mechanisms underlying this protective response, we conducted multiparameter confocal imaging. Mice were immunized with either Ad.OVA-i.m./Np.OVA-i.v. or Ad.OVA-i.m. only and, 3 weeks later, challenged with OVA-spz or non-Tg spz, with livers harvested 12 hours later. Livers from Ad.OVA-i.m./Np.OVA-i.v.-vaccinated animals, but not Ad.OVA-i.m.-vaccinated animals, showed OVA-spz surrounded by large clusters of Kupffer cells (F4/80⁺) and polyclonal CD8⁺ T cells producing IFN- γ and granzyme B (GrzB) (Fig. 2, A to C, and fig. S5A). In addition, some of the clustered cells were Ki67⁺, indicating recent cell proliferation (Fig. 2, B and D) (38). In contrast, mice given only Ad.OVA-i.m. showed no cell accumulation or effector staining around infected hepatocytes, suggesting that, at 12 hours after infection, few cells were actively recruited (Fig. 2, A and B, and fig. S5A). As previously observed in the liver (39–41), these T cell clusters were only present in animals challenged with spz-OVA parasites, whereas smaller size clusters were found in animals challenged with spz not expressing OVA. The low number of lymphocytes present around non-Tg spz suggests that large cluster formation requires cognate Ag recognition. Immunizing animals with an irrelevant encapsulated protein (Np.BSA-i.v.) failed to confer protection against OVA-spz challenge (fig. S5B), further supporting the hypothesis that the cluster formation and effector response observed are associated with protective immunity.

To identify the cell population critical for mediating liver-stage protection, cell depletion was performed after prime-target immunization (Ad.OVA-i.m./Np.OVA-i.v.). Depletion of CD8⁺ T cells before OVA-spz challenge completely ablated vaccine efficacy (Fig. 2F and fig. S6). Furthermore, protection was found to scale in a dose-dependent manner, with higher numbers of liver Ag-specific CD8⁺ T cells correlating with increased protection (fig. S7). CD4⁺ T cells, although also present in large numbers around infected hepatocytes (Fig. 2, B and C), were largely dispensable for protection against OVA-spz challenge (Fig. 2F). Together, the imaging and depletion studies further confirmed a critical role for CD8⁺ T cells in mediating protection against liver-stage malaria.

CD8⁺ T cells expressing markers of tissue residency are found to be sufficient at mediating protection

Recent data suggest that an important contribution to local immunity is provided by T_{RM} (28, 32, 42). We postulated that protection from OVA-spz challenge by prime-target immunization could be the result of such CD8⁺ liver-resident T cells, rather than the protection due to acute recruitment of effector memory cells from lymphoid sites. To examine whether T_{RM} cells were present in the liver after vaccination with Ad.OVA-i.m./Np.OVA-i.v. or Ad.OVA-i.m., we used quantitative histocytometric analysis (43) of liver tissue

5 weeks after administration of Np.OVA-i.v. or Ad.OVA-i.m. only. After prime-target immunization, an increase in T_{RM} (CXCR6⁺ CD69⁺ CD44⁺) (32) and T_{EM} (CXCR6⁻ CD69⁻ CD44⁺) was observed predominantly in the liver sinusoids [as previously noted (20, 44, 45)] compared to Ad.OVA-i.m.-vaccinated animals (fig. S8 and Fig. 3, A and B). Not previously described was an increase in small clusters containing T_{EM}, T_{RM}, CD4⁺ T cells, and F4/80 MHC-II⁺ Kupffer cells in the tissue parenchyma of Ad.OVA-i.m./Np.OVA-i.v.-vaccinated animals, appearing similar to memory lymphocyte clusters (Fig. 3, A, C, and D) (42). In addition to T_{RM} marker expression, after isolation and transfer to naïve recipient hosts, liver CD69⁺ CD8⁺ T cells preferentially homed back to the liver, even when transferring bulk liver lymphocytes (fig. S9). To determine the durability of prime-target vaccine, mice were immunized, lymphocytes were isolated at different time intervals after vaccination, and a population of liver-localized Ag-specific CD8⁺ T cells, expressing markers of tissue residency, could be detected for at least 36 days after Ad.OVA-i.m./Np.OVA-i.v. immunization (Fig. 3, E and F, and fig. S10, A and B). Protection in vaccinated animals was durable with 100% sterile efficacy at 2 months and 70% at 6 months after vaccination (Fig. 3G and fig. S10C).

Although many of the T cells found in the liver 5 weeks after nanoparticle boost were T_{RM}, to further assess whether immunity resulted from T_{EM} drawn into the liver upon OVA-spz infection or whether liver T_{RM} were sufficient to mediate protection, mice were challenged under two conditions. First, vaccinated animals were administered FTY720 (fig. S11A) and splenectomized (removing the remaining splenic red pulp T effector pool) (46) (Fig. 3H). Second, FTY720 alone or in combination with anti-CD8–depleting antibodies was administered (fig. S11B). In either experimental setting, T_{RM} were found to be sufficient for protection in liver-stage malaria. In summary, these data suggest that prime-target vaccination with Np.OVA-i.v. induces a high frequency of T_{RM} that is stably maintained over time and sufficient for protection against liver-stage malaria.

Viral vectors were found to be an effective alternative targeting approach to PLGA nanoparticles

Although nanoparticles are being examined in many preclinical vaccine studies, nonreplicating viral vectors have been used intramuscularly safely in many thousands of humans in a wide variety of vaccines against several infectious diseases, generating impressive T cell immunogenicity (47). Hence, we examined whether a similar protection to nanoparticles could be obtained with a recombinant viral vector to target. Clinically tested human and chimpanzee adenoviral and MVA vectors, known to induce CD8⁺ T cell responses in humans (8), were evaluated. Bioluminescent imaging revealed that adenoviral, as well as MVA, vectors were highly liver-tropic after intravenous administration (Fig. 4, A and B) and thus delivered Ag to hepatic tissue. Vaccine regimens involving combinations of Ad.OVA-i.m./Ad.OVA-i.v. or Ad.OVA-i.m./MVA.OVA-i.v. were able to generate substantial numbers of Ag-specific CD8⁺ T cells in the liver (Fig. 4C). A high percentage of these cells also produced IFN- γ and expressed markers of tissue residency (fig. S12, A and B). Vaccine efficacy was assessed after challenge with OVA-spz. Similar to prime-target vaccination with Np.OVA-i.v., the protective response mediated by prime-target vaccination with viral intravenous boost was found to be dose-dependent and Ag-specific (fig. S12, C and D). As expected, boosting with Ad.OVA-i.m. or MVA.OVA-i.m. vectors failed to induce strong protection. However, boosting with Ad.OVA-i.v. or MVA.OVA-i.v. resulted in 80% sterile protection for 2 months after vaccination (Fig. 4D and fig. S12E).

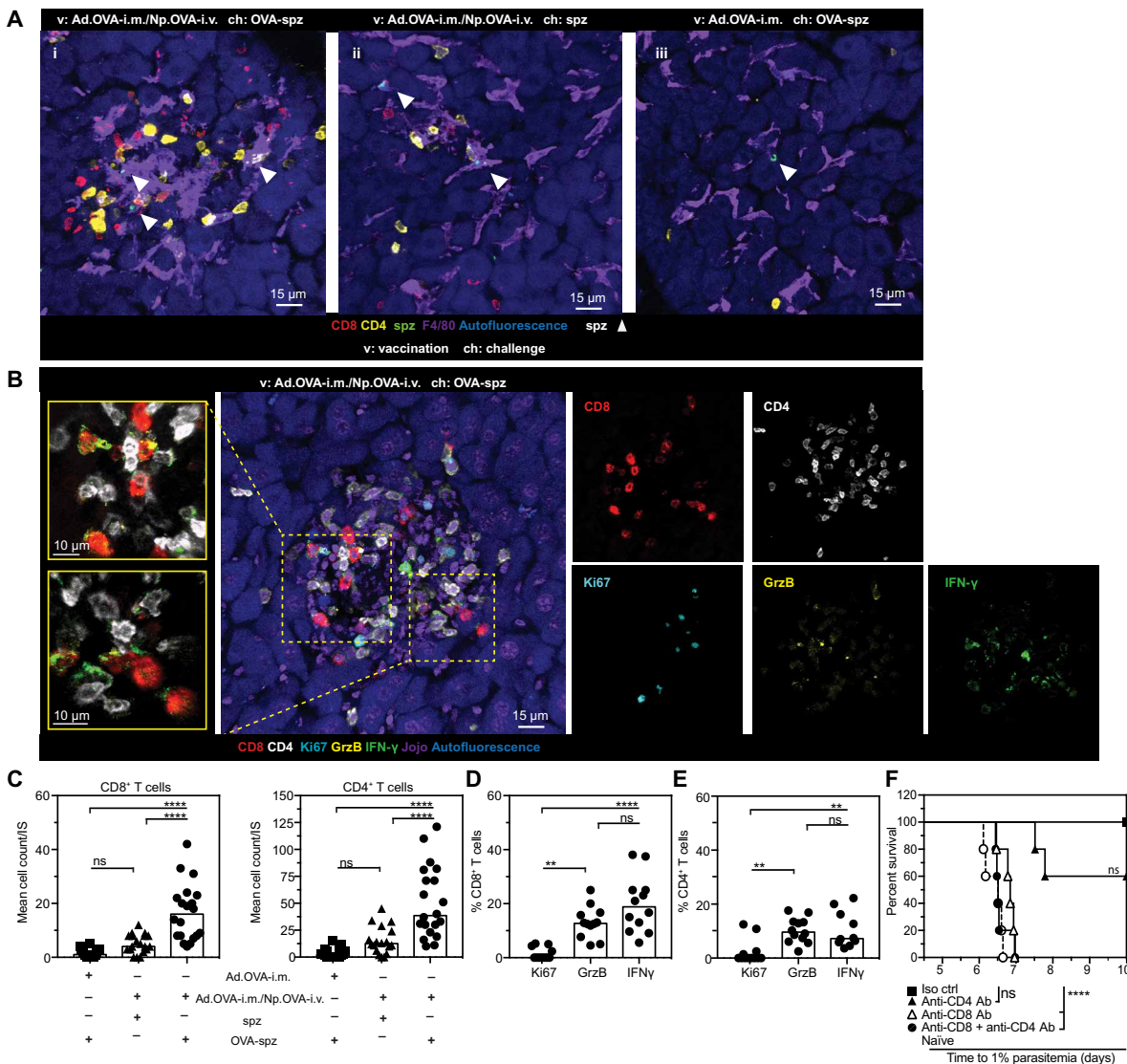


Fig. 2. Localization and quantification of immune response to spz challenge. Immunofluorescence (IF) images depicting spz in the liver 12 hours after challenge (ch:) with an intravenous injection of 5×10^4 OVA::mCherry_{Pbbsp70} *P. berghei* spz (OVA-spz) or mCherry_{Pbbsp70} (spz). Mice had been previously vaccinated (v:) with Ad.OVA-i.m./Np.OVA-i.v. or Ad.OVA-i.m. only and challenged 3 weeks after Np.OVA-i.v. injection. **(A)** IF images of liver sections [maximum intensity projection (MIP) of a 20- μ m z-stack] stained for CSP protein, CD8, CD4, and F4/80; arrowheads denote spz. **(B)** IF image depicting a liver section from Ad.OVA-i.m./Np.OVA-i.v. challenged with OVA-spz. Section stained for CD8, CD4, Ki67, GrzB, IFN- γ , and Jojo (nucleus). Right: Single-color panels. **(C)** Quantification of IF images: Mean cell count per infectious site (IS) of CD8⁺ and CD4⁺ T cells (equal numbers of IS imaged per condition). In situ frequency of **(D)** CD8⁺ and **(E)** CD4⁺ T cells expressing Ki67, GrzB, or IFN- γ from mice vaccinated with Ad.OVA-i.m./Np.OVA-i.v. and challenged with OVA-spz. IF images are representative of two independent experiments, for a total of $n = 4$ animals per condition (two liver sections imaged per animal). Median shown from all pooled data. Data were analyzed with LMM, with experiment and liver section as random effects and vaccination and spz as fixed effects. **(F)** C57BL/6 mice ($n = 5$ per group) were vaccinated with Ad.OVA-i.m./Np.OVA-i.v. Three weeks after Np.OVA-i.v. vaccination, mice were administered anti-CD4, anti-CD8–depleting antibodies (Abs), or a combination of both antibodies. Alternatively, immunoglobulin G2b isotype control (Iso ctrl) antibody was administered. Mice were subsequently challenged with OVA::Hep17 *P. berghei* spz. Data shown are representative of two independent experiments (log-rank Mantel-Cox test). **** $P < 0.0001$, ** $P < 0.01$.

Sterile protection with clinically relevant Ags is achieved in mice

To investigate the protective efficacy of a prime and target strategy with clinically relevant Ags, we immunized mice with three *P. falciparum* liver-stage Ags—TRAP (*Pf*TRAP), liver-stage Ag 1 (*Pf*LSA1), and liver stage–associated protein 2 (*Pf*LSAP2)—all now in clinical development. Mice were also immunized with the immunodominant *P. berghei* Pb9 epitope (48) from CSP, present in the clinically tested

multiple epitope (ME)–TRAP construct (8, 11). Each Ag was individually expressed in the ChAd63 vector administered intramuscularly. Two weeks later, mice were vaccinated with either MVA-i.m. or ChAd63-i.v. expressing the same cognate Ag, and animals were challenged 3 weeks later with wild-type (WT) or Tg spz expressing the *P. falciparum* Ag in *P. berghei* (49, 50). Vaccination with a recombinant ChAd63-i.m./ChAd63-i.v. regimen expressing all these *P. falciparum* Ags singly conferred 100% sterile protection against

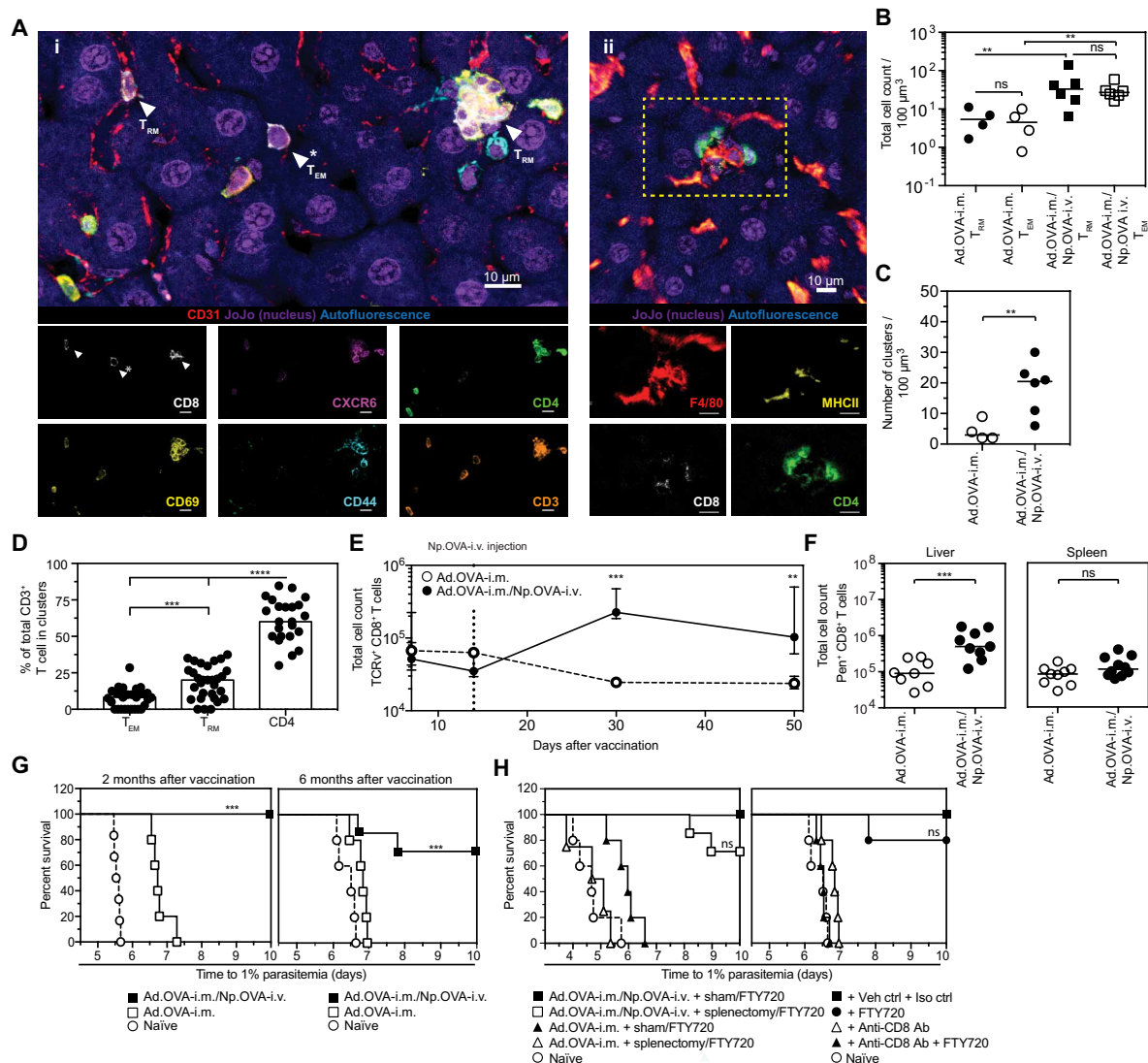


Fig. 3. Prime and target vaccination induces liver T_{RM} stably maintained in the tissue and sufficient in mediating protection. (A) Representative IF images depicting liver tissue of mice vaccinated with Ad.OVA-i.m./Np.OVA-i.v. 5 weeks after Np.OVA-i.v. vaccination. (i) CD8, CXCR6, CD4, CD69, CD44, CD3, CD31, and JoJo (nucleus). (ii) F4/80, MHC-II, CD8, CD4, and JoJo. Bottom: Single-color panels. Arrowhead denotes examples of T_{RM} ($CD8^+ CXCR6^+ CD69^+ CD44^+$ T cell), whereas asterisk-arrowhead denotes T_{EM} ($CD8^+ CXCR6^- CD69^- CD44^+$ T cell) (MIP of a 20- μm z-stack). (B) Quantification of IF images: Total cell count/100 μm^3 of T_{RM} or T_{EM} in animals vaccinated with Ad.OVA-i.m. or Ad.OVA-i.m./Np.OVA-i.v. Data were analyzed with LMM, with experiment and tissue slice as random effects and cell type and vaccination as fixed effects. (C) Total number of clusters/100 μm^3 in animals vaccinated with Ad.OVA-i.m. or Ad.OVA-i.m./Np.OVA-i.v. Data were analyzed with LMM, with experiment and cluster as random effects and vaccination as fixed effect. (D) Frequency of T_{EM} , T_{RM} , and CD4⁺ T cells of total CD3⁺ cells in clusters from mice vaccinated with Ad.OVA-i.m./Np.OVA-i.v. Data were analyzed with LMM, with experiment and cluster as random effects and vaccination as fixed effect. (A to D) Quantification of IF images is from two independent experiments for a total of $n = 4$ to 6 animals per condition (two liver sections imaged per animal); median shown. (E) C57BL/6 mice were vaccinated with Ad.OVA-i.m./Np.OVA-i.v. or Ad.OVA-i.m. only. Total number of TCRV⁺ ($V\alpha_2/V\beta_5$) liver CD8⁺ T cells over time as determined by flow cytometry. Median shown from two pooled experiments. Data were analyzed with LMM, with experiment as random effect and vaccination and time as fixed effects. (F) At day 36 after Np.OVA-i.v. administration, total Pen⁺ T cells were determined in the liver and spleen. Data shown are pooled from two independent experiments, each symbol representative of an individual mouse ($n = 8$ to 9 per group). Data were analyzed with LMM, with experiment and mouse as random effects and vaccination as fixed effect. (G) C57BL/6 mice ($n = 6$ per group) were vaccinated with Ad.OVA-i.m./Np.OVA-i.v. or Ad.OVA-i.m. Mice were challenged with OVA::Hep17 *P. berghei* spz 2 or 6 months after Np.OVA-i.v. vaccination. (H) C57BL/6 mice ($n = 6$ per group) were vaccinated with Ad.OVA-i.m./Np.OVA-i.v. Three weeks after Np.OVA administration, mice were either splenectomized or sham-operated, and FTY720 was administered. Alternatively, mice were treated with FTY720- and/or anti-CD8-depleting antibodies. Mice were subsequently challenged with OVA::Hep17 *P. berghei* spz. Data shown are representative of two independent experiments (log-rank Mantel-Cox test). **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$.

the Ag-matched Tg spz (Fig. 5, A to D). Targeting with Np.Pb9-i.v. or ChAd63.ME-TRAP-i.v. resulted in equivalent levels of protection against WT *P. berghei*. Collectively, these results demonstrated that effective protection through prime-target immunization could be achieved preclinically with clinical-stage viral vectors. To further

assess the translatable potential of viral vector prime-target immunization against other hepatic diseases, high numbers of liver Ag-specific T cells specific to hepatitis B surface Ag (HBsAg) could also be successfully induced (fig. S13), indicating the potential flexibility of this platform.

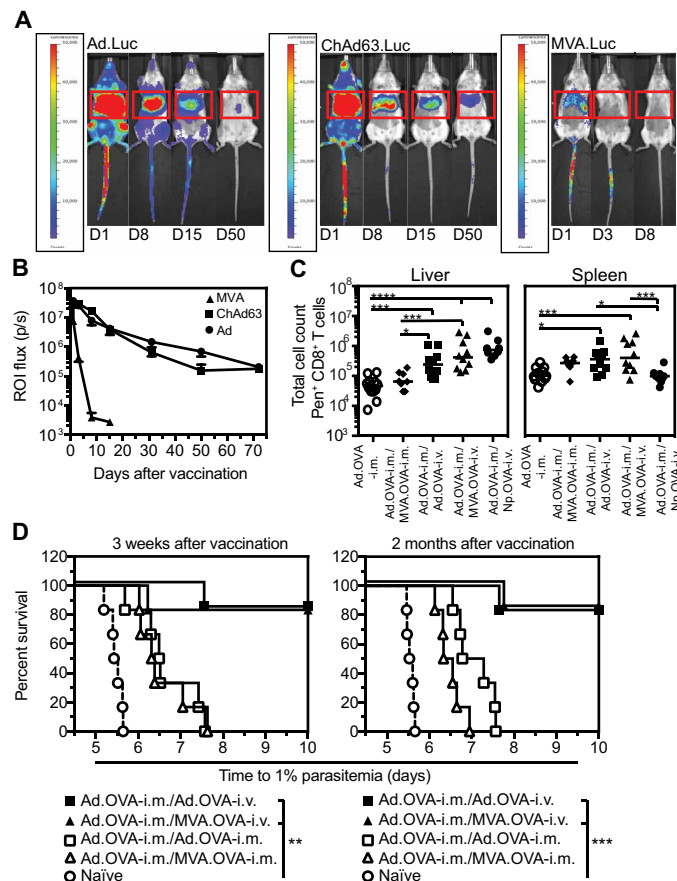


Fig. 4. Viral vectors as targeting agents induce strong protective immunity against challenge with Tg OVA-spz. BALB/c mice ($n = 5$ per group) were vaccinated intravenously with Ad, ChAd63, or MVA expressing luciferase (Luc) and imaged at different days (D) after vaccination. (A) Bioluminescence image indicating the location of luciferase. In red, region of interest (ROI) is shown, with (B) ROI flux analysis over time. (C) C57BL/6 mice were all primed with Ad.OVA-i.m. and followed, 2 weeks later, by MVA.OVA-i.m. (Ad.OVA-i.m./MVA.OVA-i.m.), Ad.OVA-i.v. (Ad.OVA-i.m./Ad.OVA-i.v.), MVA.OVA-i.v. (Ad.OVA-i.m./MVA.OVA-i.v.), and Np.OVA-i.v. (Ad.OVA-i.m./Np.OVA-i.v.) or were left untreated (Ad.OVA-i.m.). Total Pen⁺ T cells in the liver or spleen. Data shown are pooled from two independent experiments, each symbol representative of individual mouse ($n = 8$ to 12 per group). Median shown. Data were analyzed with LMM model, with experiment and mouse as random variables and vaccination and boost as fixed variables. All statistical comparisons were made with only statistically significant comparisons shown. (D) C57BL/6 mice ($n = 6$ per group) were vaccinated with Ad.OVA-i.m./Ad.OVA-i.m., Ad.OVA-i.m./MVA.OVA-i.m., Ad.OVA-i.m./Ad.OVA-i.v., or Ad.OVA-i.m./MVA.OVA-i.v. Mice were challenged 3 weeks after final immunization or 2 months after final immunization with OVA:Hep17 *P. berghei* spz. Data shown are representative of two independent experiments (log-rank Mantel-Cox test). **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

A phase 1 clinical trial involving intravenous administration of viral vectors showed a positive safety profile in humans

After the completion of satisfactory toxicology testing, we performed a small phase 1 clinical trial with the primary objective of assessing the safety and tolerability of intravenous administration of ChAd63. ME-TRAP in healthy human volunteers. This dose escalation trial involved three subjects per dose group [group 1, 5×10^8 viral particles (vp); group 2, 5×10^9 vp; group 3, 5×10^{10} vp], with 90 days of follow up. After vaccination, adverse events (AEs) local to the injection site were rare, and systemic AEs were more frequent but still

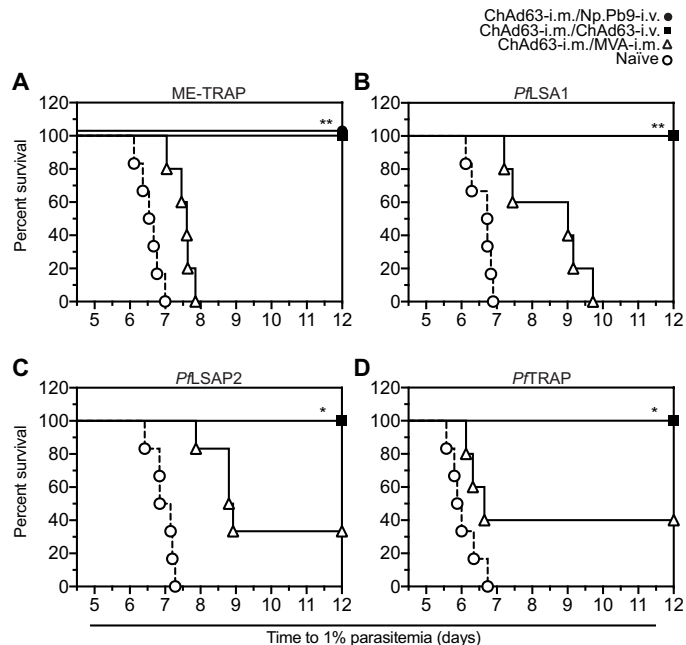


Fig. 5. Prime and target approach sterily protects mice against challenge with Tg spz expressing prominent clinical candidate *P. falciparum* vaccine Ags. BALB/c mice ($n = 6$ per group) were primed intramuscularly with ChAd63 viral vectors expressing the following Ags: (A) ME-TRAP, (B) PflSA1, or (C) PflSAP2. Mice were subsequently vaccinated with either MVA-i.m. (ChAd63-i.m./MVA-i.m.) or ChAd63-i.v. (ChAd63-i.m./ChAd63-i.v.), each expressing the respective cognate Ag. For the ME-TRAP-vaccinated group (A), an additional group of BALB/c mice ($n = 6$ per group) received ChAd63.ME-TRAP-i.m., followed by Np.Pb9-i.v. (ChAd63-i.m./Np.Pb9-i.v.). (D) CD-1 mice ($n = 6$ per group) were primed with ChAd63 viral vectors expressing PfTRAP-i.m. Mice received a subsequent immunization of either MVA-i.m. or ChAd63-i.v. vector, each expressing PfTRAP Ag. Protective efficacy of immunization was determined after challenge with (A) WT or (B to D) Tg *P. berghei* spz expressing the cognate *P. falciparum* Ag. Data shown are representative of two independent experiments. Statistical comparison (log-rank Mantel-Cox test) between ChAd63-i.m./ChAd63-i.v. and ChAd63-i.m./MVA-i.m. vaccinations. * $P < 0.05$, *** $P < 0.01$.

mild to moderate (Fig. 6A). A single severe AE (fever) was seen in one group 3 volunteer who incidentally developed clinically evident mumps (confirmed serologically) 4 to 5 days after immunization—infection almost certainly having occurred before immunization. Close monitoring of laboratory parameters was followed to assess for any derangement of liver function and disseminated intravascular coagulation (fig. S14A) (51–53). No transaminitis was observed; three volunteers had a mild transient rise in d-dimer within normal limits. Overall, ChAd63.ME-TRAP-i.v. was well tolerated, comparing favorably with previously reported intramuscular administrations (54).

After ChAd63.ME-TRAP-i.v. administration, ex vivo IFN- γ ELISPOT responses peaked at day 14 after immunization and appeared to be maintained up until day 42 after vaccination (Fig. 6B). Although low vaccine doses resulted in marginal responses, group 3 responders showed higher magnitudes (Fig. 6C), at least comparable to a previous clinical trial where the same dose of ChAd63.ME-TRAP was administered intramuscularly (54). The nonresponder in group 3 was the subject found to be incubating mumps at the time of immunization. Mumps virus has been reported to potentially interfere with innate immunity by inhibiting tumor necrosis factor- α (TNF α)–mediated apoptosis and nuclear factor κ B activation (55). The frequency of T_{EM} cells detected in peripheral blood by flow cytometry was increased

Downloaded from <http://stm.sciencemag.org/> by guest on December 2, 2020

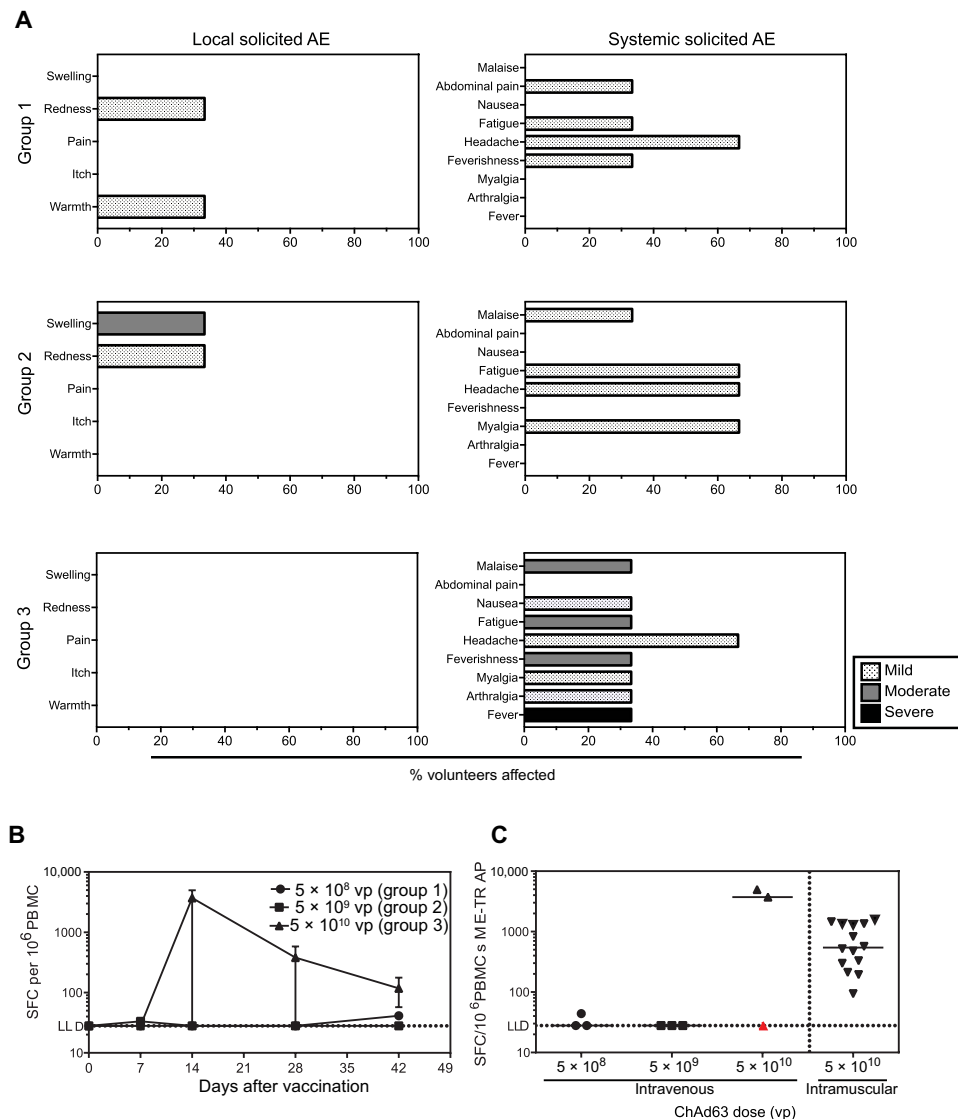


Fig. 6. Safety and immunogenicity data after ChAd63.ME-TRAP-i.v. vaccination in healthy human volunteers. (A) After vaccination with ChAd63.ME-TRAP by intravenous peripheral cannula, safety was assessed by active and passive collection of local and systemic AEs. The percentage of volunteers with local (left) and systemic (right) AEs are shown for all tested dose groups: group 1, 5 × 10⁸ vp; group 2, 5 × 10⁹ vp; group 3, 5 × 10¹⁰ vp (all n = 3). (B) Median with interquartile range of ex vivo IFN-γ enzyme-linked immunospot (ELISPOT) responses to ME-TRAP peptides for each dose group from volunteers' peripheral blood mononuclear cells (PBMCs) over time, measured in spot-forming cells (SFCs) per 10⁶ PBMCs. (C) Individual ELISPOT responses for each participant at day 14 after immunization, compared with data from a previous study with the same vaccine construct and dose, administered intramuscularly (54). Notably, a subject in group 3 (marked in red) was incubating mumps at time of immunization.

14 days after vaccination by either route (fig. S14B), and expression of IFN-γ or TNFα on CD8⁺ T cells did not differ by vaccination route (fig. S14C). These initial safety and immunogenicity data support future clinical testing of the efficacy of prime-target regimens in humans.

DISCUSSION

We describe the use of a prime and target immunization approach that provides a pre-erythrocytic vaccine strategy mediating sterile protection via CD8⁺ T cells. This vaccination regimen, requiring just a single viral vector, primes endogenous CD8⁺ T cells in peripheral tissue

and subsequently targets them to the liver by expressing suitable amounts of Ag in this organ. This results in a 10-fold increase of Ag-specific CD8⁺ T cells localized in the liver. After OVA-spz challenge, mice showed 100% sterile protection at 2 months after vaccination and 70% when challenged at 6 months. Hence, prime and target vaccination appears to generate sufficient Ag-specific CD8⁺ T cells to mediate relatively durable protection in mice, a crucial feature for translatable vaccines.

With multiparameter imaging techniques, polyclonal CD8⁺ T cells were found to cluster in large numbers around infected hepatocytes when cognate Ag was present, producing IFN-γ and GrzB, as previously observed (39–41). Despite the high frequencies of CD4⁺ T cells around infected hepatocytes, our depletion studies confirmed a known paradigm in the literature that CD8⁺ T cells are essential for mediating protection (56). Furthermore, sterile protection was found to scale in a dose-dependent manner with higher levels of Ag-specific CD8⁺ T cells in the liver, correlating with increased protection.

After careful in situ phenotypic characterization, Ag-specific CD8⁺ T cells were found to express markers of T_{RM} (CXCR6⁺ CD69⁺). In agreement with previous work (25, 32), the number of liver T_{RM} CD8⁺ T cells correlated with protection in the context of liver-stage malaria, clearly showing that this subset of cells is necessary to mediate protection. Here, we wanted to address whether T_{RM} alone were sufficient in mediating protection to spz challenge or whether they acted as sentinels, recruiting other CD8⁺ T cells from the circulation or dLN. To address this, we used a combination of chemical (FTY720) and surgical (splenectomy) methods to inhibit or remove T effector CD8⁺ lymphocytes from the circulation. Notably, T_{RM} were found to be sufficient in mediating protection against

liver-stage malaria, suggesting that a resident Ag-specific T_{RM} population was indeed effective at eliminating infected hepatocytes. Hence, together with previous work (25, 26, 32), liver T_{RM} appear both necessary and sufficient to mediate protection against liver-stage malaria. It should be noted, however, that we cannot completely exclude the contribution of circulating Ag-specific CD8⁺ T cells during a natural liver-stage infection. In the case of vaccination regimens such as ChAd63-i.m./ChAd63-i.v. that result in high numbers of spleen T_{EM}, circulating Ag-specific CD8⁺ T cells may also play a role in mediating protection. Vaccine regimens that induce high numbers of T_{RM} and circulating T_{EM} might show improved efficacy in human clinical trials.

In addition to nanoparticles, intravenous administration of leading clinical recombinant viral vectors, ChAd and MVA, both sterilely protected 80% of mice 2 months after vaccination. Moreover, complete sterile protection with the leading *P. falciparum* pre-erythrocytic Ags PfTRAP, PflSA1, and PflSAP2 expressed in a ChAd vector was also observed. The efficacy and immunogenicity of these viral vector and Ag combinations have so far only been assessed in mice, and further work will be required for evaluation in humans.

A range of experimental immunization strategies have been reported to induce Ag-specific T_{RM} : Ag-coupled to mAb (27), chemokine administration (28, 29), inflammatory agents (30), or inactivated pathogens (31). However, unlike other vaccine strategies with substantial translational challenges, nonreplicating viral vectors are excellent targeting agents, with clinically tested ChAd offering a particularly suitable clinical option with only one recombinant adenoviral vector required and no change in immunogen necessary, significantly simplifying biomanufacturing. Furthermore, because viral vectors are able to express any Ag of interest, prime-target vaccination is an adaptable vaccine platform potentially protective against other hepatic diseases.

To assess the likely safety and feasibility of incorporating intravenous viral-vectored vaccination in a deployable prime-target regimen, a small phase 1 study was conducted, testing safety and immunogenicity of a single ChAd63.ME-TRAP-i.v. dose. The intravenous administration of ChAd63.ME-TRAP was well tolerated with no severe vaccine-related AEs. Close monitoring of laboratory parameters revealed no indication of hepatocellular damage or significant inflammation previously seen with intravenous administration of some Ads in the setting of oncolytic virotherapy with much higher doses (51–53). This represents a significant step in bringing prime-target vaccination into clinical testing. Furthermore, the immunogenicity of a single intravenous vaccine dose as assayed by a standard *ex vivo* ELISPOTs appears encouraging. Together, this prime-target approach substantially improves current Ag-based liver-stage malaria vaccines, providing a potent and flexible immunization platform.

MATERIALS AND METHODS

Study design

The study aimed at developing a translatable malaria vaccine strategy that resulted in sterile protection in a *P. berghei* spz challenge model of the disease. Accordingly, experiments were performed to test various vaccination models. The *n* for these experiments was determined by performing an *a priori* power analysis, used in estimating sufficient sample sizes to achieve adequate power. No randomization or blinding was performed; mice were excluded from experiments only when tail intravenous injections were not performed successfully, determined at the time of vaccination. The end point of 1% parasitemia was chosen as a humane standard widely used in the literature. After 1% parasitemia, malaria symptoms in mice only progress in severity, inevitability leading to death, with animals unable to recover from the infection. After challenge experiments, flow cytometry and immunohistochemistry (IHC) experiments were performed to investigate the mechanism of protection. The hypothesis that vaccine regimens would affect sterile protection was specified in advance, and an LMM was performed, which takes into account variation among mice, experiments, or tissue slices (in the context of IHC) as potential sources of random variation in addition to fixed effects (explanatory variables). Most experiments were performed

with the model Ag OVA; however, clinical malaria vaccine candidates were also tested with this platform and found to mediate sterile protection. Primary data are located in table S1. A small phase 1 clinical trial was performed to assess the safety and tolerability of intravenous administration of ChAd63 ME-TRAP in healthy malaria-naïve volunteers.

Experimental animals and immunizations

All animal work was approved by the University of Oxford Animal Care and Ethical Review Committee and performed under license 30/2889 or P9804B4F1 or approved by the National Institute of Allergy and Infectious Diseases (NIAID) Animal Care and Use Committee [National Institutes of Health (NIH)] under the LSB-4E animal license. Male and female C57BL/6 CD45.2 and CD45.1, BALB/c, and ICR (CD-1) mice (ENVIGO); B6.SJL-Cd45a(Ly5a)/Nai and C57BL/6Nai-[Tg]TCR OTI-[KO]RAG1 mice (Taconic Biosciences) (57); and ubiquitin-tdTomato Tg mice [gift from R. Tsien (University of California San Diego)], of at least 6 weeks of age were used. Mice were immunized intramuscularly into the musculus tibialis with a dose of 1×10^8 infectious units (iu) for Ad or 1×10^6 plaque-forming units (pfu) for MVA. For tail vein intravenous administration, the following doses were administered: 1×10^9 iu for Ad vectors or 1×10^6 pfu for MVA vectors. Description of viral vectors used was found in the Supplementary Materials.

Intracellular cytokine staining assay

Intracellular cytokine staining was performed, as previously described (58); see the Supplementary Materials for greater detail. In short, liver lymphocytes were isolated after *in situ* phosphate-buffered saline perfusion of the liver via the portal vein and stimulated for 6 hours at 37°C with appropriate peptides at a final concentration of 1 µg/ml (OVA peptides used were SIINFELK and/or ISQAVHAAHAEINEAGR and HBsAg H2-L^d-dominant peptide used was IPQSLDSWWTSL; ProImmune) and GolgiPlug (1 µg/ml; BD Biosciences) or left unstimulated in complete media and GolgiPlug for control.

Cells were subsequently surface-stained on ice (see the Supplementary Materials for detailed antibody list), and samples were acquired using an LSR II flow cytometer (BD Biosciences) or LSR-Fortessa (BD Biosciences) and analyzed with FlowJo version 9 or version 10.1 (Tree Star Inc.). Lymphocytes were gated on live, size, and singlet and subsequently gated on CD8⁺ T cells.

Methodological information regarding clinical trial (NCT03084289)

See data file S1 for details on clinical trial VAC064 (NCT03084289).

Statistical analysis

Prism version 5.0c (GraphPad) and RStudio were used for all analyses. Median values with interquartile range are shown in all graphs, unless otherwise stated. Data were tested for normality and homoscedasticity, and where appropriate, data were log-transformed. For these reasons, all experiments showing total cell counts were log-transformed. When data were pooled from multiple experiments, a multilevel analysis with an LMM was performed in R. Hence, lme4 was used to perform a linear mixed effects analysis of the relationship between measured vaccine output (dependent variable) and vaccination (independent variable) (59). Fixed effects used were determined to be the variable/treatment under examination, whereas

random effects were variability caused by experiment-to-experiment variation (specified for each figure in the figure legends). A *P* value was then obtained by performing a likelihood of ratio test. This was done by comparing the full model and a null model (lacking the fixed variable of interest) with an analysis of variance (ANOVA). If significance was found from running an LMM, then a one-way or two-way ANOVA was performed, with multiple comparisons corrected with a Bonferroni's post hoc test. Survival in challenge experiments is presented using Kaplan-Meier curves, and significance is tested with the log-rank (Mantel-Cox) test.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/10/460/eaap9128/DC1

Materials and Methods

Fig. S1. Intravenous nanoparticle localization and OTI activation in liver tissue after intravenous and intramuscular Np.APC-OVA administration.

Fig. S2. Schematic representation of prime and target vaccination regimen, efficacy, and immunogenicity.

Fig. S3. Total Pen⁺ CD8⁺ T cell numbers in the liver are not affected by FTY720 administration.

Fig. S4. T cell recruitment to the liver is dependent in part on the dose of PLGA-OVA administered.

Fig. S5. A prime and target approach protects mice against challenge with Tg *P. berghei* OVA-spz only when mice are vaccinated against OVA.

Fig. S6. Flow plots showing representative lymphocyte depletion after administration of depleting antibodies.

Fig. S7. Higher numbers of liver Pen⁺ CD8⁺ T cells correlated with greater protection after spz challenge.

Fig. S8. Histocytometric analysis of liver sections.

Fig. S9. Liver Pen⁺ CD8⁺ T cells preferentially home back to the liver in recipient mice.

Fig. S10. Gating strategy showing T_{RM} phenotype of Pen⁺ T cells in the liver after nanoparticle administration and long-term efficacy data.

Fig. S11. Flow plots showing representative lymphocyte depletion and inhibition of egress from lymphatics after administration of depleting antibody and FTY720.

Fig. S12. A prime and target approach with viral vectors as targeting agents shows similar immunization profile to PLGA nanoparticle targeting.

Fig. S13. A prime and target approach with viral vectors generates high numbers of HBsAg CD8⁺ T cells in the liver.

Fig. S14. Safety and human immune responses to immunization with ChAd63.ME-TRAP-i.v.

Movie S1. Video showing the internalization of Np.APC-OVA within Kupffer cells.

Table S1. Primary data file.

Data file S1. Methodological information regarding clinical trial (NCT03084289).

REFERENCES AND NOTES

- S. Bhatt, D. J. Weiss, E. Cameron, D. Bisanzio, B. Mappin, U. Dalrymple, K. E. Battle, C. L. Moyes, A. Henry, P. A. Eckhoff, E. A. Wenger, O. Briët, M. A. Penny, T. A. Smith, A. Bennett, J. Yukich, T. P. Eisele, J. T. Griffin, C. A. Fergus, M. Lynch, F. Lindgren, J. M. Cohen, C. L. Murray, D. L. Smith, S. I. Hay, R. E. Cibulskis, P. W. Gething, The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* **526**, 207–211 (2015).
- Global. WHO declares emergency against AIDS, TB, malaria. *AIDS Policy Law* **21**, 5 (2006).
- TR5, S Clinical Trials Partnership, S. T. Agnandji, B. Lell, S. S. Soulanoudjingar, J. F. Fernandes, J. F. Fernandes, B. P. Abossolo, C. Conzelmann, B. G. Methogo, Y. Doucka, A. Flamen, B. Mordmüller, S. Issifou, P. G. Kremsner, J. Sacarlal, P. Aide, M. Lanaspá, J. J. Aponte, A. Nhamuave, D. Quelhas, Q. Bassat, S. Mandjate, E. Macete, P. Alonso, S. Abdulla, N. Salim, O. Juma, M. Shomari, K. Shubis, F. Machera, A. S. Hamad, R. Minja, A. Mtoro, A. Sykes, S. Ahmed, A. M. Urassa, A. M. Ali, G. Mwangoka, M. Tanner, H. Tinto, U. D'Alessandro, H. Sorgho, I. Valea, M. C. Tahita, W. Kaboré, S. Ouédraogo, Y. Sandrine, R. T. Guiguemdé, J. B. Ouédraogo, M. J. Hamel, S. Kariuki, C. Odero, M. Onoko, K. Otieno, N. Awino, J. Omoto, J. Williamson, V. Muturi-Kioi, K. F. Laserson, L. Slutsker, W. Otieno, L. Otieno, O. Nekoye, S. Gondi, A. Otieno, B. Ogutu, R. Wasuna, V. Owira, D. Jones, A. A. Onyango, P. Njuguna, R. Chilengi, P. Akoo, C. Kerubo, J. Gitaka, C. Maingi, T. Lang, A. Olotu, B. Tsopa, P. Bejon, N. Peshu, K. Marsh, S. Owusu-Agyei, K. P. Asante, K. Osei-Kwakye, O. Boahen, S. Ayamba, K. Kayan, R. Owusu-Ofori, D. Dosoo, I. Asante, G. Adjei, G. Adjei, D. Chandramohan, B. Greenwood, J. Lusingu, S. Gesase, A. Malabeja, O. Abdul, H. Kilavo, C. Mahende, E. Liheluka, M. Lemnge, T. Theander, C. Drakeley, D. Ansong, T. Agbenyega, S. Adjei, H. O. Boateng, T. Rettig, J. Bawa, J. Sylverken, D. Sambian, A. Agyekum, L. Owusu, F. Martinson, I. Hoffman, T. Mvalo, P. Kamthunzi, R. Nkomo, A. Msika, A. Jumbe, N. Chome, D. Nyakuipa, J. Chintedza, W. R. Ballou, M. Bruls, J. Cohen, Y. Guerra, E. Jongert, D. Lapiere, A. Leach, M. Lievens, O. Ofori-Anyinam, J. Vekemans, T. Carter, D. Leboulleux, C. Loucq, A. Radford, B. Savarese, D. Schellenberg, M. Sillman, P. Vansadia, First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N. Engl. J. Med.* **365**, 1863–1875 (2011).
- L. Schofield, J. Villaquiran, A. Ferreira, H. Schellekens, R. Nussenzweig, V. Nussenzweig, γ Interferon, CD8⁺ T cells and antibodies required for immunity to malaria sporozoites. *Nature* **330**, 664–666 (1987).
- W. R. Weiss, M. Sedegah, R. L. Beaudoin, L. H. Miller, M. F. Good, CD8⁺ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 573–576 (1988).
- A. V. Hill, A. Reyes-Sandoval, G. O'Hara, K. Ewer, A. Lawrie, A. Goodman, A. Nicosia, A. Folgori, S. Colloca, R. Cortese, S. C. Gilbert, S. J. Draper, Prime-boost vectored malaria vaccines: Progress and prospects. *Hum. Vaccin.* **6**, 78–83 (2010).
- S. Li, M. Rodrigues, D. Rodriguez, J. R. Rodriguez, M. Esteban, P. Palese, R. S. Nussenzweig, F. Zavala, Priming with recombinant influenza virus followed by administration of recombinant vaccinia virus induces CD8⁺ T-cell-mediated protective immunity against malaria. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5214–5218 (1993).
- K. J. Ewer, G. A. O'Hara, C. J. Duncan, K. A. Collins, S. H. Sheehy, A. Reyes-Sandoval, A. L. Goodman, N. J. Edwards, S. C. Elias, F. D. Halstead, R. J. Longley, R. Rowland, I. D. Poulton, S. J. Draper, A. M. Blagborough, E. Berrie, S. Moyle, N. Williams, L. Siani, A. Folgori, S. Colloca, R. E. Sinden, A. M. Lawrie, R. Cortese, S. C. Gilbert, A. Nicosia, A. V. Hill, Protective CD8⁺ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat. Commun.* **4**, 2836 (2013).
- A. Reyes-Sandoval, T. Berthoud, N. Alder, L. Siani, S. C. Gilbert, A. Nicosia, S. Colloca, R. Cortese, A. V. Hill, Prime-boost immunization with adenoviral and modified vaccinia virus Ankara vectors enhances the durability and polyfunctionality of protective malaria CD8⁺ T-cell responses. *Infect. Immun.* **78**, 145–153 (2010).
- S. Capone, A. Reyes-Sandoval, M. Naddeo, L. Siani, V. Ammendola, C. S. Rollier, A. Nicosia, S. Colloca, R. Cortese, A. Folgori, A. V. Hill, Immune responses against a liver-stage malaria antigen induced by simian adenoviral vector AdCh63 and MVA prime-boost immunisation in non-human primates. *Vaccine* **29**, 256–265 (2010).
- C. Ogwang, D. Kimani, N. J. Edwards, R. Roberts, J. Mwacharo, G. Bowyer, C. Bliss, S. H. Hodgson, P. Njuguna, N. K. Viebig, A. Nicosia, E. Gitau, S. Douglas, J. Illingworth, K. Marsh, A. Lawrie, E. B. Imoukhuede, K. Ewer, B. C. Urban, A. V. S. Hill, P. Bejon; MVVC Group, Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against *Plasmodium falciparum* infection in Kenyan adults. *Sci. Transl. Med.* **7**, 286re285 (2015).
- S. Dunachie, A. V. S. Hill, H. A. Fletcher, Profiling the host response to malaria vaccination and malaria challenge. *Vaccine* **33**, 5316–5320 (2015).
- I. A. Cockburn, Y.-C. Chen, M. G. Overstreet, J. R. Lees, N. van Rooijen, D. L. Farber, F. Zavala, Prolonged antigen presentation is required for optimal CD8⁺ T cell responses against malaria liver stage parasites. *PLOS Pathog.* **6**, e1000877 (2010).
- I. N. Crispe, M. Giannandrea, I. Klein, B. John, B. Sampson, S. Wuensch, Cellular and molecular mechanisms of liver tolerance. *Immunol. Rev.* **213**, 101–118 (2006).
- U. Protzer, M. K. Maini, P. A. Knolle, Living in the liver: Hepatic infections. *Nat. Rev. Immunol.* **12**, 201–213 (2012).
- S. S. Tay, Y. C. Wong, D. M. McDonald, N. A. W. Wood, B. Roediger, F. Sierro, C. McGuffog, I. E. Alexander, G. A. Bishop, J. R. Gamble, W. Weninger, G. W. McCaughan, P. Bertolino, D. G. Bowen, Antigen expression level threshold tunes the fate of CD8 T cells during primary hepatic immune responses. *Proc. Natl. Acad. Sci. U.S.A.* **111**, E2540–E2549 (2014).
- T. Gebhardt, L. M. Wakim, L. Eidsmo, P. C. Reading, W. R. Heath, F. R. Carbone, Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat. Immunol.* **10**, 524–530 (2009).
- D. Masopust, D. Choo, V. Vezy, E. J. Wherry, J. Duraiswamy, R. Akondy, J. Wang, K. A. Casey, D. L. Barber, K. S. Kawamura, K. A. Fraser, R. J. Webby, V. Brinkmann, E. C. Butcher, K. A. Newell, R. Ahmed, Dynamic T cell migration program provides resident memory within intestinal epithelium. *J. Exp. Med.* **207**, 553–564 (2010).
- D. Masopust, V. Vezy, E. J. Wherry, D. L. Barber, R. Ahmed, Cutting edge: Gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J. Immunol.* **176**, 2079–2083 (2006).
- E. M. Steinert, J. M. Schenkel, K. A. Fraser, L. K. Beura, L. S. Manlove, B. Z. Igyártó, P. J. Southern, D. Masopust, Quantifying memory CD8 T cells reveals regionalization of immunosurveillance. *Cell* **161**, 737–749 (2015).
- L. M. Wakim, N. Gupta, J. D. Mintern, J. A. Villadangos, Enhanced survival of lung tissue-resident memory CD8⁺ T cells during infection with influenza virus due to selective expression of IFITM3. *Nat. Immunol.* **14**, 238–245 (2013).
- T. Gebhardt, P. G. Whitney, A. Zaid, L. K. Mackay, A. G. Brooks, W. R. Heath, F. R. Carbone, S. N. Mueller, Different patterns of peripheral migration by memory CD4⁺ and CD8⁺ T cells. *Nature* **477**, 216–219 (2011).

23. J. M. Schenkel, K. A. Fraser, L. K. Beura, K. E. Pauken, V. Veys, D. Masopust, Resident memory CD8 T cells trigger protective innate and adaptive immune responses. *Science* **346**, 98–101 (2014).
24. S.-W. Tse, I. A. Cockburn, H. Zhang, A. L. Scott, F. Zavala, Unique transcriptional profile of liver-resident memory CD8⁺ T cells induced by immunization with malaria sporozoites. *Genes Immun.* **14**, 302–309 (2013).
25. S.-W. Tse, A. J. Radtke, D. A. Espinosa, I. A. Cockburn, F. Zavala, The chemokine receptor CXCR6 is required for the maintenance of liver memory CD8⁺ T cells specific for infectious pathogens. *J. Infect. Dis.* **210**, 1508–1516 (2014).
26. J. E. Epstein, K. Tewari, K. E. Lyke, B. K. L. Sim, P. F. Billingsley, M. B. Laurens, A. Gunasekera, S. Chakravarty, E. R. James, M. Sedegah, A. Richman, S. Velmurugan, S. Reyes, M. Li, K. Tucker, A. Ahumada, A. J. Ruben, T. Li, R. Stafford, A. G. Eappen, C. Tamminga, J. W. Bennett, C. F. Ockenhouse, J. R. Murphy, J. Komisar, N. Thomas, M. Loyevsky, A. Birkett, C. V. Plowe, C. Loucq, R. Edelman, T. L. Richie, R. A. Seder, S. L. Hoffman, Live attenuated malaria vaccine designed to protect through hepatic CD8⁺ T cell immunity. *Science* **334**, 475–480 (2011).
27. L. M. Wakim, J. Smith, I. Caminschi, M. H. Lahoud, J. A. Villadangos, Antibody-targeted vaccination to lung dendritic cells generates tissue-resident memory CD8 T cells that are highly protective against influenza virus infection. *Mucosal Immunol.* **8**, 1060–1071 (2015).
28. H. Shin, A. Iwasaki, A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature* **491**, 463–467 (2012).
29. J. E. Kohlmeier, S. C. Miller, J. Smith, B. Lu, C. Gerard, T. Cookenham, A. D. Roberts, D. L. Woodland, The chemokine receptor CCR5 plays a key role in the early memory CD8⁺ T cell response to respiratory virus infections. *Immunity* **29**, 101–113 (2008).
30. L. K. Mackay, A. T. Stock, J. Z. Ma, C. M. Jones, S. J. Kent, S. N. Mueller, W. R. Heath, F. R. Carbone, T. Gebhardt, Long-lived epithelial immunity by tissue-resident memory T (T_{RM}) cells in the absence of persisting local antigen presentation. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7037–7042 (2012).
31. G. Stary, A. Olive, A. F. Radovic-Moreno, D. Gondek, D. Alvarez, P. A. Basto, M. Perro, V. D. Vrbnac, A. M. Tager, J. Shi, J. A. Yethon, O. C. Farokhzad, R. Langer, M. N. Starnbach, U. H. von Andrian, A mucosal vaccine against *Chlamydia trachomatis* generates two waves of protective memory T cells. *Science* **348**, aaa8205 (2015).
32. D. Fernandez-Ruiz, W. Y. Ng, L. E. Holz, J. Z. Ma, A. Zaid, Y. C. Wong, L. S. Lau, V. Mollard, A. Cozjensen, N. Collins, J. Li, G. M. Davey, Y. Kato, S. Devi, R. Skandari, M. Pauley, J. H. Manton, D. I. Godfrey, A. Braun, S. S. Tay, P. S. Tan, D. G. Bowen, F. Koch-Nolte, B. Rissiek, F. R. Carbone, B. S. Crabb, M. Lahoud, I. A. Cockburn, S. N. Mueller, P. Bertolino, G. I. McFadden, I. Caminschi, W. R. Heath, Liver-resident memory CD8⁺ T cells form a front-line defense against malaria liver-stage infection. *Immunity* **45**, 889–902 (2016).
33. A. K. Mohammad, J. J. Reineke, Quantitative detection of PLGA nanoparticle degradation in tissues following intravenous administration. *Mol. Pharm.* **10**, 2183–2189 (2013).
34. S. Stoll, J. Delon, T. M. Brotz, R. N. Germain, Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science* **296**, 1873–1876 (2002).
35. J.-W. Lin, T. N. Shaw, T. Annoura, A. Fougère, P. Bouchier, S. Chevalley-Maurel, H. Kroeze, B. Franke-Fayard, C. J. Janse, K. N. Couper, S. M. Khan, The subcellular location of ovalbumin in *Plasmodium berghei* blood stages influences the magnitude of T-cell responses. *Infect. Immun.* **82**, 4654–4665 (2014).
36. S. Mandala, R. Hajdu, J. Bergstrom, E. Quackenbush, J. Xie, J. Milligan, R. Thornton, G.-J. Shei, D. Card, C. Keohane, M. Rosenbach, J. Hale, C. L. Lynch, K. Rupperecht, W. Parsons, H. Rosen, Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **296**, 346–349 (2002).
37. S. P. Kasturi, I. Skountzou, R. A. Albrecht, D. Koutsonanos, T. Hua, H. I. Nakaya, R. Ravindran, S. Stewart, M. Alam, M. Kwissa, F. Villinger, N. Murthy, J. Steel, J. Jacob, R. J. Hogan, A. Garcia-Sastre, R. Compans, B. Pulendran, Programming the magnitude and persistence of antibody responses with innate immunity. *Nature* **470**, 543–547 (2011).
38. S. Bruno, Z. Darzynkiewicz, Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. *Cell Prolif.* **25**, 31–40 (1992).
39. I. A. Cockburn, R. Amino, R. K. Kelemen, S. C. Kuo, S.-W. Tse, A. Radtke, L. Mac-Daniel, V. V. Ganusov, F. Zavala, R. Ménard, In vivo imaging of CD8⁺ T cell-mediated elimination of malaria liver stages. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 9090–9095 (2013).
40. K. Kimura, D. Kimura, Y. Matsushima, M. Miyakoda, K. Honma, M. Yuda, K. Yui, CD8⁺ T cells specific for a malaria cytoplasmic antigen form clusters around infected hepatocytes and are protective at the liver stage of infection. *Infect. Immun.* **81**, 3825–3834 (2013).
41. S. L. Hoffman, D. Isenbarger, G. W. Long, M. Sedegah, A. Szarfman, L. Waters, M. R. Hollingdale, P. H. van der Meide, D. S. Finbloom, W. R. Ballou, Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. *Science* **244**, 1078–1081 (1989).
42. N. Iijima, A. Iwasaki, T cell memory. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. *Science* **346**, 93–98 (2014).
43. M. Y. Gerner, W. Kastenmuller, I. Ifrim, J. Kabat, R. N. Germain, Histo-cytometry: A method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. *Immunity* **37**, 364–376 (2012).
44. H. A. McNamara, Y. Cai, M. V. Wagle, Y. Sontani, C. M. Roots, L. A. Miosge, J. H. O'Connor, H. J. Sutton, V. V. Ganusov, W. R. Heath, P. Bertolino, C. G. Goodnow, I. A. Parish, A. Enders, I. A. Cockburn, Up-regulation of LFA-1 allows liver-resident memory T cells to patrol and remain in the hepatic sinusoids. *Sci. Immunol.* **2**, eaaj1996 (2017).
45. M. Cabrera, L. L. Pewe, J. T. Harty, U. Frevert, In vivo CD8⁺ T cell dynamics in the liver of *Plasmodium yoelii* immunized and infected mice. *PLOS ONE* **8**, e70842 (2013).
46. T. I. Arnon, J. G. Cyster, Blood, sphingosine-1-phosphate and lymphocyte migration dynamics in the spleen. *Curr. Top. Microbiol. Immunol.* **378**, 107–128 (2014).
47. K. J. Ewer, T. Lambe, C. S. Rollier, A. J. Spencer, A. V. Hill, L. Dorrell, Viral vectors as vaccine platforms: From immunogenicity to impact. *Curr. Opin. Immunol.* **41**, 47–54 (2016).
48. P. Romero, J. L. Maryanski, G. Corradin, R. S. Nussenzweig, V. Nussenzweig, F. Zavala, Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria. *Nature* **341**, 323–326 (1989).
49. R. J. Longley, A. M. Salman, M. G. Cottingham, K. Ewer, C. J. Janse, S. M. Khan, A. J. Spencer, A. V. S. Hill, Comparative assessment of vaccine vectors encoding ten malaria antigens identifies two protective liver-stage candidates. *Sci. Rep.* **5**, 11820 (2015).
50. A. M. Salman, C. M. Mogollon, J.-W. Lin, F. J. A. van Pul, C. J. Janse, S. M. Khan, Generation of transgenic rodent malaria parasites expressing human malaria parasite proteins. *Methods Mol. Biol.* **1325**, 257–286 (2015).
51. J. Nemunaitis, C. Cunningham, A. Buchanan, A. Blackburn, G. Edelman, P. Maples, G. Netto, A. Tong, B. Randlev, S. Olson, D. Kirn, Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: Safety, feasibility and biological activity. *Gene Ther.* **8**, 746–759 (2001).
52. T. Reid, E. Galanis, J. Abbruzzese, D. Sze, L. M. Wein, J. Andrews, B. Randlev, C. Heise, M. Uprichard, M. Hatfield, L. Rome, J. Rubin, D. Kirn, Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): Phase II viral, immunologic, and clinical endpoints. *Cancer Res.* **62**, 6070–6079 (2002).
53. E. J. Small, M. A. Carducci, J. M. Burke, R. Rodriguez, L. Fong, L. van Ummersen, D. C. Yu, J. Aimi, D. Ando, P. Working, D. Kirn, G. Wilding, A phase I trial of intravenous CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer. *Mol. Ther.* **14**, 107–117 (2006).
54. S. H. Hodgson, K. J. Ewer, C. M. Bliss, N. J. Edwards, T. Rampling, N. A. Anagnostou, E. de Barra, T. Havelock, G. Bowyer, I. D. Poulton, S. de Cassan, R. Longley, J. J. Illingworth, A. D. Douglas, P. B. Mange, K. A. Collins, R. Roberts, S. Gerry, E. Berrie, S. Moyle, S. Colloca, R. Cortese, R. E. Sinden, S. C. Gilbert, P. Bejon, A. M. Lawrie, A. Nicosia, S. N. Faust, A. V. S. Hill, Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naïve individuals. *J. Infect. Dis.* **211**, 1076–1086 (2015).
55. S. Franz, P. Rennert, M. Woznik, J. Grützke, A. Lüdde, E. M. Arriero Pais, T. Finsterbusch, H. Geyer, A. Mankertz, N. Friedrich, Mumps virus SH protein inhibits NF- κ B activation by interacting with tumor necrosis factor receptor 1, interleukin-1 receptor 1, and toll-like receptor 3 complexes. *J. Virol.* **91**, e01037-17 (2017).
56. N. W. Schmidt, N. S. Butler, V. P. Badovinac, J. T. Harty, Extreme CD8 T cell requirements for anti-malarial liver-stage immunity following immunization with radiation attenuated sporozoites. *PLoS Pathog.* **6**, e1000998 (2010).
57. K. A. Hogquist, S. C. Jameson, W. R. Heath, J. L. Howard, M. J. Bevan, F. R. Carbone, T cell receptor antagonist peptides induce positive selection. *Cell* **76**, 17–27 (1994).
58. A. J. Spencer, M. G. Cottingham, J. A. Jenks, R. J. Longley, S. Capone, S. Colloca, A. Folgori, R. Cortese, A. Nicosia, M. Bregu, A. V. S. Hill, Enhanced vaccine-induced CD8⁺ T cell responses to malaria antigen ME-TRAP by fusion to MHC class II invariant chain. *PLOS ONE* **9**, e100538 (2014).
59. D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).

Acknowledgments: We thank M. Ulaszewska and A. Fyfe for technical help; V. Clark and H. Gray for animal husbandry; A. Nicosia, S. Colloca, and R. Cortese (ReiThera, Rome) for supplying ChAd vectors; and J. Furze, A. Worth, A. Turner, and the Jenner Institute vector core for assistance. We thank C. Mair for assistance with processing clinical trial samples, T. Prustel for help in statistical analyses, and A. J. Radtke for scientific input and critical manuscript reading. **Funding:** A.G. is funded by the Wellcome Trust and by the Intramural Program of NIAID (NIH). B.R.H. is funded from the European Union Seventh Framework Programme FP7/2012-2106 under grant agreement 316655 (VACTRAIN). A.V.S.H. is a Wellcome Trust and National Institute of Health Research (NIHR) senior investigator. This work was in part funded by a Wellcome Trust Senior Investigator award (to A.V.S.H.) and a Wellcome Trust Enhancement award (to A.V.S.H.) for the clinical trial and also was supported in part by the Intramural Research Program of NIAID (NIH) (to R.N.G. and S.U.). The clinical trial was supported in part by funding from the UK NIHR Oxford Biomedical Research Centre. **Author contributions:** A.G. designed, conducted, and analyzed the experiments and drafted the manuscript. A.A.W. developed the PLGA-Np and designed and interpreted the experiments.

B.R.H. and A.M.S. conducted the challenge experiments and in vivo luminescence imaging. A.M.S., S.M.K., and C.J.J. provided the Tg spz. S.U. performed the splenectomies. D.S. designed, conducted, and drafted the clinical study with assistance from S.S., N.V., E.B., and B.A. I.P. and M.B. were lead clinical nurses. R.R. and A.M.L. managed the ethical and regulatory affairs in relation to NCT03084289. K.J.E. designed and analyzed the clinical data. J.P., G.B., and D.B. performed the human immunology assays. R.N.G. helped with imaging, data interpretation, and writing of the manuscript. A.J.S. and A.V.S.H. designed the experiments and interpreted data. All authors contributed and approved the manuscript. **Competing interests:** A.V.S.H., A.G., A.A.W., and A.M.S. are inventors on a patent application PCT/GB2017/051009 submitted by the Oxford University Innovation Limited that covers prime and target vaccination with viral vectors. **Data and materials availability:** All data associated with the study are present in the paper or the Supplementary Materials.

Submitted 8 September 2017
Resubmitted 8 March 2018
Accepted 20 August 2018
Published 26 September 2018
10.1126/scitranslmed.aap9128

Citation: A. Gola, D. Silman, A. A. Walters, S. Sridhar, S. Uderhardt, A. M. Salman, B. R. Halbroth, D. Bellamy, G. Bowyer, J. Powlson, M. Baker, N. Venkatraman, I. Poulton, E. Berrie, R. Roberts, A. M. Lawrie, B. Angus, S. M. Khan, C. J. Janse, K. J. Ewer, R. N. Germain, A. J. Spencer, A. V. S. Hill, Prime and target immunization protects against liver-stage malaria in mice. *Sci. Transl. Med.* **10**, eaap9128 (2018).

Prime and target immunization protects against liver-stage malaria in mice

Anita Gola, Daniel Silman, Adam A. Walters, Saranya Sridhar, Stefan Uderhardt, Ahmed M. Salman, Benedict R. Halbroth, Duncan Bellamy, Georgina Bowyer, Jonathan Powlson, Megan Baker, Navin Venkatraman, Ian Poulton, Eleanor Berrie, Rachel Roberts, Alison M. Lawrie, Brian Angus, Shahid M. Khan, Chris J. Janse, Katie J. Ewer, Ronald N. Germain, Alexandra J. Spencer and Adrian V. S. Hill

Sci Transl Med **10**, eaap9128.
DOI: 10.1126/scitranslmed.aap9128

A malaria vaccine to harness hepatic immunity

Plasmodium, the parasitic agent of malaria, has a fairly complicated life cycle in different organs of the host. Malaria vaccines can be designed to disrupt a particular parasitic stage. Gola *et al.* are interested in developing a malaria vaccine that protects against liver-stage malaria. To do so, they used nanoparticles or viral vectors. The vaccines induced tissue-resident memory CD8⁺ T cells in mice, which conferred sterilizing protection against sporozoite challenge. One of the viral vector vaccine candidates was tested in healthy human volunteers and was found to be safe. The investigators are hopeful that this vaccine can induce protective liver-resident T cells in humans.

ARTICLE TOOLS

<http://stm.sciencemag.org/content/10/460/eaap9128>

SUPPLEMENTARY MATERIALS

<http://stm.sciencemag.org/content/suppl/2018/09/24/10.460.eaap9128.DC1>

RELATED CONTENT

<http://stm.sciencemag.org/content/scitransmed/9/395/eaag2490.full>
<http://stm.sciencemag.org/content/scitransmed/9/371/eaad9099.full>
<http://stm.sciencemag.org/content/scitransmed/9/387/eaad9735.full>
<http://stm.sciencemag.org/content/scitransmed/9/393/eaal2094.full>
<http://science.sciencemag.org/content/sci/362/6419/1112.full>
<http://science.sciencemag.org/content/sci/362/6419/eaat9446.full>
<http://stm.sciencemag.org/content/scitransmed/11/474/eaau1458.full>
<http://stm.sciencemag.org/content/scitransmed/11/495/eaav3963.full>

REFERENCES

This article cites 59 articles, 20 of which you can access for free
<http://stm.sciencemag.org/content/10/460/eaap9128#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science Translational Medicine (ISSN 1946-6242) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science Translational Medicine* is a registered trademark of AAAS.

Copyright © 2018 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works