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A human Phase I/IIa malaria challenge trial of a polyprotein malaria vaccine

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ABSTRACT

We examined the safety, immunogenicity and efficacy of a prime-boost vaccination regime involving two poxvirus malaria subunit vaccines, FP9-PP and MVA-PP, expressing the same polyprotein consisting of six pre-erythrocytic antigens from *Plasmodium falciparum*.

Following safety assessment of single doses, 15 volunteers received a heterologous prime-boost vaccination regime and underwent malaria sporozoite challenge. The vaccines were safe but interferon-γ ELISPOT responses were low compared to other poxvirus vectors, despite targeting multiple antigens. There was no vaccine efficacy as measured by delay in time to parasitaemia. A number of possible explanations are discussed, including the very large insert size of the polyprotein transgene.

1. Introduction

Plasmodium falciparum is responsible for an enormous worldwide burden of human disease, causing an estimated 200–500 million cases of clinical disease and 1 million deaths each year [1,2], most of this occurring in sub-Saharan Africa. Two billion people are thought to live in areas at significant risk of malaria [1]. However, it is clear from irradiated sporozoite studies in humans that it is possible to induce effective and relatively durable immunity against *P. falciparum* and that this can be strain-transcending [3]. Despite this proof of principle, there remains no currently available malaria vaccine.

A number of vaccine strategies are being explored at present, most of which focus on one or very few parasite antigens. In contrast, the poxvirus-vectored vaccines used in this study were constructed to encode the entire sequence of six separate *P. falciparum* proteins expressed at the pre-erythrocytic stage yielding a 3240 amino-acid long 'polyprotein' [4]. This strategy aimed to generate a broad cellular immune response directed against a variety of pre-erythrocytic parasite antigens, rather than a strong but narrow response. The proteins were selected using immunogenicity data from humans living in malaria endemic areas and from responses against irradiated sporozoites. This approach is supported by the fact that although the immunodominant circumsporozoite (CS) protein response plays an important role in the protective effect of irradiated sporozoite vaccination in mice, protection can still be induced when CS is removed as an immune target [5]. Protection may then be achieved with the combination of modest responses against a number of parasite proteins. A broader response could also reduce the risk of parasite immune escape and be effective against a variety of parasite strains and across varying Human Leukocyte Antigen (HLA) types. Significant humoral responses were not expected or examined for in this study.

The viral vectors fowlpox strain FP9 and modified vaccinia virus Ankara (MVA) have an excellent safety record in humans [6–8], are capable of inducing powerful T-cell responses [9,10] and have been shown to induce protection against malaria in mice [10] and in humans [7]. Both have been engineered to express the polyprotein construct (FP9-PP and MVA-PP). When evaluated in mice, FP9-PP was specifically shown to induce IFNγ-secreting T cells by ELISPOT against each of the six vaccine antigens and heterologous prime-boost vaccination induced liver-stage antigen 1 (LSA-1) tetramer

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positive CD8 T-cells that demonstrated cytotoxic activity [4]. The importance of IFNγ has been shown by its ability to inhibit development of exoerythrocytic parasite forms within hepatocytes [11].

This study examines the safety, immunogenicity and challenge efficacy of these vaccines when administered to healthy human volunteers intradermally, four weeks apart in two different prime-boost regimes.

2. Materials and methods

2.1. Volunteers and recruitment

Healthy malaria naïve adults aged 18–50 years old were recruited from April 2006 to November 2006 from the Oxford area in the UK. Screening, vaccination and all study visits except for the sporozoite challenge day itself were carried out at the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Churchill Hospital, Oxford, UK. The malaria challenge took place at the insectary of the Alexander Fleming Building, Imperial College, London, UK.

Key study exclusion criteria included: abnormal baseline haematology or biochemistry; evidence of hepatitis B, C or HIV infection; history of immunosuppressive medication or immunodeficiency; previous history of malaria; malaria chemoprophylaxis within five months (for challenge volunteers); travel to a malaria endemic region within six months; or history or evidence of a significant physical or psychiatric disorder.

2.2. Funding, ethical and regulatory approval

This study was principally funded by the European Malaria Vaccine Initiative (EMVI) now European Vaccine Initiative (EVI) and sponsorship responsibilities were shared through delegation between EMVI and the University of Oxford. The trial protocol and associated documents were reviewed and approved as two studies by the Oxfordshire National Health Service Research Ethics Committee A (OxREC A, reference numbers 04/Q1604/93 and 06/Q1604/55) and by the Medicines and Healthcare products Regulatory Agency (MHRA, EudraCT numbers 2004-002424-17 and 2006-000629-67). Recombinant vaccine use was authorised by the Genetic Modification Safety Committee (GMSC) of the Oxford Radcliffe Hospitals NHS Trust (reference number GM462.04.21).

All volunteers gave written informed consent before enrolment and the study was conducted according to the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP). External study monitoring was provided by Appledown Clinical Research.

2.3. Study design

Study groups 1–5 ($n = 3$ each) were single dose-escalation groups with the following doses: FP9-PP at 1×10^8 plaque-forming units (pfu), MVA-PP at 1×10^8 pfu, FP9-PP at 2×10^8 pfu, MVA-PP at 2×10^8 pfu and MVA-PP at 5×10^8 pfu respectively. Volunteers in groups 6 and 7 (planned $n = 10$ each) received the heterologous prime-boost vaccine regimes 'FFM' or 'MMF' respectively. 'FFM' refers to the sequence of FP9-PP/FP9-PP/MVA-PP with each vaccination one month apart. 'MMF' refers to the equivalent sequence of MVA-PP/MVA-PP/FP9-PP. Doses were 1, 1 and 2×10^8 pfu for first, second and third vaccinations for both groups 6 and 7. Control volunteers ($n = 6$) were recruited to undergo malaria challenge without vaccination to confirm the infective efficacy of the sporozoite challenge. Vaccine follow-up visits for groups 1–7 were on days 2, 7 and 28 following each vaccination with additional visits on day 90 (groups 1–5) and day 150 after first vaccination (groups 6 and 7). In addition, all challengees were seen regularly during the three weeks following challenge (see *sporozoite challenge* below) and then 35 and 150 days following challenge. Blood was collected regularly for safety assessments and immunogenicity.

2.4. Vaccines and 'polyprotein' insert

FP9-PP and MVA-PP were manufactured according to Good Manufacturing Practice (GMP) regulations by Impfstoffwerk Dessau-Tornau (IDT, Roßlau, Germany). The polyprotein vaccine insert ('L3SEPTL') has been fully described before [4]. It contains six pre-erythrocytic malaria antigens linked together in a single protein (from N to C terminus): liver stage antigen 3 (LSA3) [12], sporozoite threonine and asparagine rich protein (STARP) [13], exported protein-1 (Exp1) [14], Pfs16 [15], thrombospondin-related adhesion protein (TRAP) [16] and liver stage antigen-1 (LSA1) [17]. All except possibly Pfs16 are pre-erythrocytic antigens; LSA3, Exp1 and STARP are also expressed by blood-stage parasites and Pfs16 is also a sexual-stage antigen [4].

Vaccines were stored at the trial site at −80 °C and thawed shortly before administration. Each dose was given intradermally into the skin overlying the deltoid muscle of the upper arm. Doses were divided equally between both arms. Vaccine sites were temporarily covered with an absorbent dressing which was removed when the vaccine sites were reassessed approximately 30 min later.

2.5. Adverse events

Volunteers were asked to complete study diary cards for the first seven days after vaccination, beginning with the evening of the vaccination day. These recorded local reactions (pain, redness, swelling, itching, warmth and scaling) and systemic symptoms (oral temperature, feverishness, myalgia, arthralgia, nausea or vomiting, lethargy, headache and malaise). Temperature was measured with an oral digital thermometer (Servoprax GmbH) supplied by the investigators and redness and swelling were recorded as maximal diameters (ensuring the measurement passed through the puncture site). On each clinic attendance the investigators independently collected the same measurements. Adverse events (AEs) were recorded at each clinic visit in response to direct questioning, self-reporting on volunteer diary cards and examination of the vaccine site at each attendance by the investigators.

Severity scales used for grading are shown in Online Table A. AEs were judged as either unrelated or possibly, probably or definitely related to vaccination by the investigator, taking into account the symptoms and time since vaccination. All AEs were followed until resolution where possible. If the study ended before resolution, attempts were made to determine outcome by contacting the volunteer and/or general practitioner. The data presented here includes all AEs, even if a volunteer subsequently dropped out of the study. Where an AE stopped and restarted within 30 days of vaccination it has only been reported once in these results, but durations have been summed. AE durations have been rounded up to the nearest day.

2.6. Sporozoite challenge

Volunteers underwent *P. falciparum* sporozoite challenge at Imperial College, London two weeks after the final vaccination. They each received bites from five mosquitoes subsequently confirmed to have more than 100 sporozoites per paired salivary gland. *Anopheles stephensi* mosquitoes were infected with the chloroquine-sensitive 3D7 strain of the parasite at the Walter Reed Army Institute of Research (WRAIR), Maryland, US and reared in the laboratory as previously described [18]. Volunteers began attending clinic for malaria screening from the evening of day 6 after infection. At each visit they were questioned about possible