SAFETY AND EFFICACY OF A RECOMBINANT DNA PLASMODIUM FALCIPARUM SPOROZOITE VACCINE

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Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine produced in Escherichia coli (FSV-1) was tested in doses of 10 to 800 protein in fifteen volunteers. No serious adverse reactions occurred. Antibodies that reacted with P falciparum sporozoite antigens by enzyme-linked immunosassay were developed in twelve of the volunteers. The highest antibody titres were similar to those resulting from lifelong natural exposure to sporozoite-infected mosquitoes.

Postimmunisation serum samples from a majority of volunteers mediated the circumsporozoite (CS) precipitation reaction and inhibited sporozoite invasion of hepatoma cells in vitro. Serum from the three volunteers who received 800 mcg doses reacted with the surface of sporozoites in an immunofluorescence assay. Six immunised volunteers receiving a fourth dose of FSV-1 and two non-immunised controls were challenged by bites of mosquitoes infected with cultured P falciparum gametocytes. Parasitaemia did not develop in the volunteer with the highest titre of CS antibodies, and parasitaemia was delayed in two other immunised volunteers. This study confirms that human beings can be protected by CS protein subunit vaccines and provides a framework for the further development and testing of more immunogenic sporozoite vaccines.

Introduction

CONTROL of falciparum malaria remains one of the world’s greatest health challenges. Global malaria eradication and control programmes carried out over the past 50 years have been seriously hampered by the widespread development of drug resistance by the parasite and insecticide resistance by the mosquito vector. There is, therefore, great interest in developing vaccines to prevent malaria. One of several potential vaccine targets is the sporozoite, the stage that is injected into the blood by mosquitoes and is exposed to serum antibodies for a brief time before it invades hepatocytes. Previous studies indicate...
the feasibility of immunisation with radiation-attenuated sporozoites in human beings and animals,1,3 but limited availability of purified sporozoites makes such an approach impractical for vaccine development. Sporozoite-immunised individuals show development of antibodies that are almost exclusively directed against a circumsporozoite (CS) protein which covers the parasite surface.4 The general structure of CS proteins is remarkably similar among all malaria species studied and contains multiple tandem repeats of short amino acid sequences.5 Protection against sporozoite challenge in the Plasmodium berghei rodent malaria model can be passively transferred by means of monoclonal antibodies to intact P berghei sporozoites11 or polyclonal antisera to synthetic P berghei CS repeat peptides.7 Rapid progress towards a human malaria vaccine was made by the cloning of the CS gene of P falciparum and identification of its CS repeat epitopes as targets for humoral and cellular antibodies.8 A series of recombinant DNA P falciparum CS proteins expressed in Escherichia coli6 have been identified as potential sporozoite vaccine candidates.11 We now report the results of a human phase I safety and immunogenicity study and a preliminary efficacy study with one of these alum-adsorbed recombinant proteins, designated falciparum sporozoite vaccine 1 (FSV-1).

Subjects and Methods

Volunteers were recruited under protocols approved by an institutional human use review committee. Healthy men aged 22–50 years underwent baseline physical and laboratory examination including complete blood count, serum chemistry (blood urea nitrogen, creatinine, alanine and aspartate aminotransferases, and bilirubin), antibodies to both sporozoite and blood-stage parasites of P falciparum, antibodies to human immunodeficiency virus (HIV), and routine urinalysis. Volunteers were excluded if they gave a history of malaria; had evidence of cardiac, haematological, renal, hepatic, or immunological illnesses; were taking immunosuppressive medication; or had pre-existing antibodies to blood-stage P falciparum parasites by immunofluorescent assay or to R32tet3i by enzyme-linked immunosorbent assay (ELISA). Fifteen volunteers meeting the enrolment criteria gave informed consent and entered the phase I study. Two volunteers consented to serve as non-immunised controls. A series of recombinant R32tet3i is MDNP(NANP)₅NVDP(NANP)₅NVDPtet3i, where M = methionine, D = aspartic acid, P = proline, N = asparagine, A = alanine, V = valine, and tet3i is the first 32 amino acids encoded by a tetracycline resistance gene, read out of petroleum jelly. Thiomeral was added as a preservative.

The vaccine was given intramuscularly to three volunteers for each of the five doses. Volunteers received primary immunisation at week 0 and were boosted with identical doses at weeks 4 and 8 according to an immunisation schedule that had been used successfully in preclinical studies with small animals.11 50 weeks after primary immunisation, six of these volunteers received a fourth identical dose. Volunteers were observed for immediate toxic effects for 20 min after immunisation. 24 and 48 h later they were examined for evidence of fever, local tenderness, erythema, warmth, induration, and lymphadenopathy, and were asked about complaints of headache, fever, chills, malaise, local pain, nausea, and joint pain. Before each vaccine dose, blood and urine samples were taken for full laboratory examination. Complete blood count and serum chemistry profiles were rechecked 2 days after each vaccine dose.

Serum samples were taken 1 week after the first dose, then every 2 weeks for 16 weeks, and at the time of sporozoite challenge. Previously characterised human sera from malaria-endemic regions of Indonesia (4076) and western Kenya (5095) were used as controls. Serum was separated from blood that had clotted overnight at 4°C and stored at -70°C until assay. Samples were randomly coded before testing in all assays except ELISA.

The ELISA for CS antibodies was carried out as previously described.12 As the test antigen we used R32LR-MDP(NANP)₅NVDP(NANP)₅NVDPPLR; this is a purified recombinant construct that contains only the first 2 amino acids encoded by the tetracycline resistance gene. Horseradish peroxidase conjugated to rabbit anti-human-IgG (heavy and light chains) was used to detect antibodies. Assays were run in triplicate and the mean absorbance and standard deviation were calculated for each dilution. Background values at a given dilution were determined with preimmunisation samples and defined as an optical density less than the mean plus 2 standard deviations of the week 0 serum sample’s optical density for all volunteers. All SD were less than 0.1 units.

Serum samples were assayed for IgG, IgM, and IgA antibodies to R32LR by ELISA as described above, except that peroxidase-conjugated rabbit anti-human-heavy-chain (α, γ, μ) specific secondary antibodies were used. For selected serum samples the concentrations of IgG subclasses reactive with R32LR were determined by ELISA14 with murine anti-human-subclass-specific monoclonal antibodies as second antibody followed by horseradish peroxidase conjugated to goat anti-mouse-IgG (heavy and light chains). Estimates of IgG subclass concentrations were calculated from standard curves prepared by coating wells with serial dilutions of IgG1, IgG2, IgG3, or IgG4 human myeloma proteins. IgE antibodies reactive with R32tet3i or R32LR were measured by ELISA with biotin-conjugated goat anti-human-IgE as second antibody and detected with streptavidin horseradish-peroxidase complex. Human IgE myeloma protein was used to prepare a standard curve. The limits of detection in these assays were 0.01 μg/ml for all IgG subclasses and 0.1 μg/ml for IgE.

Immunofluorescence assays were carried out as previously described.15 Salivary-gland sporozoites in ‘Medium 199’ containing bovine serum albumin were spread onto multiwell printed ELISA slide for 30 sec. Serum was added followed by 4% formaldehyde for 5 min and washing. Serum was added followed by 4% formaldehyde for 5 min and washing. Slides were incubated in serum diluted 1:20 and incubated at 37°C for 30 min. Reactions were evaluated by phase-contrast microscopy at ×400 magnification. 25 random sporozoites were examined for each serum sample and the number of CS-precipitation-positive organisms was recorded.

Serum samples were assayed for circumsporozoite precipitation activity.16 Salivary-gland sporozoites from P falciparum strain NF54 (500 to 1000 sporozoites in 5 μl medium 199), were mixed with 5 μl serum on a microscope slide, sealed under a cover slip rimmed with petroleum jelly, and incubated at 37°C for 30 min. Reactions were scored with phase-contrast microscopy at ×400 magnification. 25 random sporozoites were examined for each serum sample and the number of CS-precipitation-positive organisms was recorded.

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falciparum volunteers were treated with oral chloroquine. Flelds = 0.25 day challenged with the chlormquine-sensitive cultures of non-stimulated cells and the difference in counts per minute incorporated between stimulated and non-stimulated cells. For each antigen concentration serum samples were harvested and counted in a liquid scintillation counter. Results were expressed as the stimulation index (mean sea we hav yet orvd ro rian

of Kenya (0) were assayed by ELISA for antibodies against R32LR.

THE LANCET, JUNE 6, 1987

Fig 1—Humoral responses to FSV-1.

Mean ELISA absorbance for triplicate assays of serum diluted 1:50 for each volunteer 2 weeks after each dose of FSV-1.

Optical density at 405 nm

Week

2 4 6 8 10

10 15 µg
8 20 µg
4 30 µg
2 100 µg
0 400 µg

Fig 2—Comparison of antibodies to P. falciparum CS protein repeat epitopes after immunisation with FSV-1 or natural exposure to infected mosquitoes.

Week 10 sera from volunteer 14 receiving 800 g doses of FSV-1 (A) and sera from a hypendemic region of Indonesia (B) and a holoendemic region of Kenya (C) were assayed by ELISA for antibodies against R32LR.

(RFS2tet4 or R32LR) or culture medium in 96-well round-bottom tissue-culture plates for 7 days at 37°C in 5% carbon dioxide; 0.4 µCi tritiated thymidine was then added to each well. 7 h later, cultures were harvested and counted in a liquid scintillation counter. Results were expressed as the stimulation index (mean cpm incorporated by triplicate cultures of antigen-stimulated cells divided by the mean cpm incorporated by triplicate cultures of non-stimulated cells) and the difference in mean cpm between stimulated and non-stimulated cells. For each antigen concentration a positive lymphocyte proliferative response was defined as a stimulation index that exceeded the mean plus 2 SD of the baseline (pre-immunisation) stimulation index for all thirteen volunteers.

3 weeks after they had received a fourth dose of FSV-1, six immunised and two non-immunised control volunteers were challenged with the chloroquine-sensitive NF54 strain of P. falciparum by allowing 5 mosquitoes with a mean salivary gland infection rate of 3-3 to feed as previously described. Beginning on day 5 after mosquito feeding, daily thick blood films (200 fields = 0.25 µl blood) were examined for parasites. Infected volunteers were treated with oral chloroquine.

TABLE 1—IGG SUBCLASS RESPONSES IN FOUR VOLUNTEERS IMMUNISED WITH FSV-1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (µg)</th>
<th>Igg1</th>
<th>Igg2</th>
<th>Igg3</th>
<th>Igg4</th>
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<tr>
<td>10</td>
<td>300</td>
<td>2.10</td>
<td>0.38</td>
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<td>11</td>
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<td>&lt;0.10</td>
<td>&lt;0.10</td>
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<td>13</td>
<td>800</td>
<td>7.50</td>
<td>0.72</td>
<td>&lt;0.10</td>
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<tr>
<td>14</td>
<td>800</td>
<td>9.50</td>
<td>1.50</td>
<td>1.25</td>
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Results

The vaccine was well tolerated at all five doses. There were no episodes of fever, chills, malaise, headache, nausea, or joint pain. Minor pain associated with the injection of vaccine occurred in seven of nine volunteers receiving doses of 100 µg or greater. The injection site was slightly tender in eight of fifteen volunteers after at least one dose of vaccine, including all those receiving 300 µg or 800 µg doses. In no case did these complaints limit function or persist more than 48 h.

One volunteer who received 800 µg doses suffered repeated sneezing followed by a general urticarial eruption without associated hypotension or dyspnoea within 5 min of a third dose of FSV-1. These symptoms resolved spontaneously within 30 min and did not recur. He had CS-repeat-epitope-specific IgE (1 µg/ml) in serum obtained 4 weeks after the second dose; IgE levels were higher (10 µg/ml) 2 weeks after the third dose but were undetectable 6 weeks later. Antigen-specific IgE did not develop in any other volunteer. Prick and intradermal inoculation tests with R32tet4, R32LR, or thiomersal were negative in the allergic volunteer 3 months after the third dose and he had no toxic effects or IgE response after a fourth dose. There were no abnormalities in complete blood count, serum chemistry profiles, or urinalysis during the study.

To exclude the measurement of antibodies directed against the tet4 portion of R32tet4, a related recombinant protein containing the identical CS-repeat portion but only the first two aminocids of the tet4 tail (R32LR) was used as antigen in the ELISA. Antigen-specific IgG was detected 2 weeks after the primary immunisation and was dose-dependent between 100 µg and 800 µg R32tet4 (fig 1). Twelve of fifteen (80%) volunteers had antibody titres of 1:50 or greater. Maximum antibody responses were sustained for 2 to 3 weeks and disappeared with a half-life of about 28 days. The titre rose significantly after repeated doses in only one volunteer, who received the 800 µg dose. His antibody levels were similar to those of the highest-titre sera we have yet observed from malaria-endemic populations (fig 2). Immunoglobulin class determinations revealed IgM, IgA, and IgG antibodies to the antigen in all positive serum samples, with IgG antibodies predominating.

TABLE 2—CS PRECIPITATION (CSP) AND INHIBITION OF SPOROZITE INVASION (ISI) OF HEPG2-16A HEPATOMA CELLS ACTIVITIES IN WEEK 10 SERUM SAMPLES

<table>
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<tr>
<th>Subject</th>
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<th>2+</th>
<th>4+</th>
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<td>25</td>
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<td>0</td>
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<td>0</td>
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<tr>
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<td>30</td>
<td>21</td>
<td>4</td>
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<tr>
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<td>9</td>
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<tr>
<td>15</td>
<td>800</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>55</td>
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</table>

*On 25 sporozoites. 0 = no detectable reaction; 2+ = fine granular precipitate around sporozoite; 4+ = filamentous tail of precipitate streaming from sporozoite.
controls was T lymphocyte blastogenesis to R32LR; mean responding to R32tett2 in this assay 4 weeks after the first with immunodominant epitopes on week 4 were significantly higher (p < 0.05, Student's t test) have yet measured and exceeded those required for passive activities preventing adequate analysis of postimmunisation protection against sporozoites is unknown. Volunteers (100 between ELISA titres and the results of either (table II). Inhibition of sporozoite invasion developed in similar doses of one individual at each dose; reactions were limited to 2 + high doses of vaccine were required to elicit antibody levels ranged from 1 pg/ml to more than 1280 THE.

**Discussion**

This study confirms that human beings can be protected from sporozoite-induced malaria by immunisation with a highly purified recombinant CS protein subunit vaccine. The vaccine we used was safe and well tolerated at all five doses but was not optimally immunogenic. Each volunteer developed an immune response to the vaccine as determined by at least one assay of cellular or humoral immunity, but high doses of vaccine were required to elicit antibody levels similar to those reported for other highly purified subunit protein or protein-polysaccharide vaccines. At doses above 300 pg, antigen-specific IgG developed. The antibodies elicited were predominantly IgG1, the pattern of IgG response that is most commonly reported for protein antigens. The role of specific IgG subclass antibodies in protection against sporozoites is unknown.

The 800 pg dose of FSV-1 elicited antibody levels similar to the highest titres of naturally occurring CS antibodies we have yet measured and exceeded those required for passive protection of mice against experimental challenge with small numbers of sporozoites. Protective antibodies that react with immunodominant epitopes on CS proteins are detected by immunofluorescence assay with intact sporozoites and mediate CS precipitation and inhibition of sporozoite invasion. No specific level of these two activities is correlated with protective immunity in animals or naturally exposed human subjects. The levels that developed in our volunteers were, however, generally lower than those found in animals and human beings immunised with irradiated sporozoites or in adults living in malaria-endemic regions.

Higher and more sustained antibody titres would be needed before CS subunit vaccines could be useful. Although higher doses of FSV-1 could be used, the recombinant protein itself may need to be modified. Our data and studies of antibody responses and lymphocyte blastogenesis with R32tet2 in mice have identified both T-cell and B-cell epitopes on the repeat portion of the

**TABLE III—IMMUNOGENICITY AND EFFICACY OF A FOURTH DOSE OF FSV-1**

<table>
<thead>
<tr>
<th>Subject</th>
<th>OD* (1:50)</th>
<th>Dose (pg)</th>
<th>S1T</th>
<th>IFA (1:100)</th>
<th>Prepatent period (days)</th>
<th>Incubation period (days)</th>
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<td>1.08</td>
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<td>800</td>
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<td>30</td>
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*ELISA absorbance.

*Lymphocyte blastogenesis to R32LR; mean S1 of four non-immunised controls was 1:71. ND = not done.
molecule, and antibody levels in mice primed with these CS epitopes can be boosted by intact sporozoites.\(^\text{16}\) 80% of the recombinant CS protein in FSV-1 consists of multiply repeated units of only four aminosacids. This region of the molecule contains but a single murine T-cell epitope and so may be inefficient in eliciting boosting responses in human beings under natural exposure to sporozoites.\(^\text{20}\) The identification of additional T epitopes on the CS gene and their incorporation into new vaccines\(^\text{21}\) or the addition of non-sporozoite T epitopes by means of other carrier proteins or fusion products may enhance CS antibody production.

We recognize the fundamental role of cellular immune responses in the protection against intense sporozoite challenge afforded by immunization with irradiated sporozoites,\(^\text{22}\) but target antigens for cell-mediated immunity are unknown and may be independent of the CS protein. Antibody-mediated immunity may, however, be adequate to protect some individuals against natural mosquito-transmitted malaria. Although this preliminary study has demonstrated that human beings can be protected with a subunit CS protein vaccine and provides a framework for the further development of sporozoite vaccines, it is clear that FSV-1 as formulated is not a suitable candidate for field trials.

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EXCLUSION TESTING FOR HUNTINGTON’S DISEASE IN PREGNANCY WITH A CLOSELY LINKED DNA MARKER

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**Summary**  
55 couples where one partner was at 50% risk of Huntington’s disease (HD) were investigated with a DNA probe closely linked to HD, with a view to exclusion testing in a future pregnancy. In 3 of 9 pregnancies so far, HD was excluded in the absence of recombination. In 3 the risk was raised to around 50%, and in 2 exclusion tests were uninformative. The remaining couple changed their minds about termination of the pregnancy and the test was therefore judged inappropriate.

**Introduction**

HUNTINGTON’S disease (HD) is a progressive dominantly inherited neurodegenerative disorder. The mutation rate is low, penetrance is complete, and symptoms usually begin between age 30 and 50, so that most individuals at risk have had children before they realise they have inherited the disorder. Until lately it has not been possible to distinguish presymptomatic gene carriers from their unaffected sibs, so preventive measures have relied upon non-directive genetic counselling and a voluntary reduction in family size by those at high risk.\(^\text{3}\)

In 1983 a DNA probe (G8) was found which is localised to the short arm of chromosome 4 and assigned the locus D4S10.\(^\text{2}\) Close genetic linkage was reported between D4S10 (G8) and the locus for the HD gene in two large kindreds.\(^\text{3}\) This study has now been extended to include 52 families from various parts of the world, including our own region (South Wales); the maximum total lod score proved to be 75.3 at a recombination fraction of about 5 centimorgans (cM).\(^\text{3}\) The 95% confidence intervals for the recombination fraction were 2.4 cM and 6.5 cM, with no evidence to support multilocus heterogeneity.\(^\text{3}\)

A DNA marker tightly linked to the HD locus has two possible clinical applications: presymptomatic predictive testing for an individual at risk for the disorder (about which there has been much debate) or exclusion tests in pregnancy, where the estimated risk to the parent is not altered. We

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**W. R. BALLOU AND OTHERS: REFERENCES—continued**

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References continued at foot of next column
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### 2. SECURITY CLASSIFICATION AUTHORITY

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