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A HIGHLY INFECTIOUS *PLASMODIUM YOELII* PARASITE, BEARING PLASMODIUM CIRCUMSPOROZOITE PROTEIN

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Plasmodium circumsporozoite protein (CSP) is a major surface antigen present in the sporozoite (Spz) stage of a malaria parasite. RTS,S vaccine, the most clinically advanced malaria vaccine, consists of a large portion of *Plasmodium falciparum* CSP (PfCSP). A highly infectious, recombinant rodent malaria, *Plasmodium yoelii* parasite bearing a full-length PfCSP (PfCSP/Py) was generated by double cross-over homologous recombination. This PfCSP/Py parasite produced up to 30,000 Spz in mosquito salivary glands, which is equal or even higher than the number of Spz produced by wild-type *P. yoelii* parasites. Five bites of PfCSP/Py-infected mosquitoes could induce blood infection in BALB/c mice. Our new transgenic parasite that expresses a full-length PfCSP may become a useful tool for researchers to investigate immunity against PfCSP in a mouse model.

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VECTORED PFCSP VACCINES BASED ON BACULOVIRUS DUAL EXPRESSION SYSTEM AND ADHU5 INDUCE STRONG PROTECTIVE EFFICACY AGAINST TRANSGENIC *PLASMODIUM BERGHEI*

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The baculovirus-vectored vaccine based on the "baculovirus dual expression system (BDES)" has been exploited as a novel vaccine platform for malaria. The gene encoding the human decay-accelerating factor was incorporated into the BDES malaria vaccine expressing the *Plasmodium falciparum* circumsporozoite protein (PfCSP). The newly developed BDES vaccine "BDES-sPfCSP2-Spider" resulted in complement resistance both *in vitro* and *in vivo*. To improve the vaccine efficacy, baculovirus expressing mouse interleukin-12 (mIL-12) and the adenoviral vaccine expressing PfCSP "AdHu5-sPfCSP2" were generated. Large-scale immunization studies were conducted in mice, and the protective efficacy was examined by using biting of mosquitoes infected with transgenic *P. berghei* sporozoites expressing PfCSP. After the priming immunization with AdHu5-sPfCSP2, booster immunization with BDES-sPfCSP2-Spider together with the mIL-12 vector conferred strong protective efficacy as compared to the controls (29 mice out of 44 were protected; 65%), following the high level of anti-PfCSP IgG titer. Thus, we propose that the prime-boost regimen using adenovirus and BDES offer great potential as a new malaria vaccine platform.

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MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGEN DISCOVERY USING NATURALLY ACQUIRED FUNCTIONAL ANTIBODY

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Transmission-blocking vaccines (TBV) target the *Plasmodium falciparum* parasite's sexual stages to interrupt its life cycle and would be useful for elimination efforts. We previously identified human sera from Malian adults whose purified IgG conveyed high transmission-blocking activity (TBA) by standard membrane feeding assay (SMFA) against laboratory cultured gametocytes fed to A. stephensi mosquitoes. Here, we describe the results from an iterative subtractive screening of a gametocyte stage cDNA phage display library using naturally acquired IgG with versus without TBA. A set of novel TBV candidate antigens was identified including 3 proteins with hits in four independent differential screens. The top 9 candidates are being evaluated in an animal model using DNA immunization via gold particle bombardment, and the top 3 mentioned above using protein immunization as well. Briefly, synthetic genes were cloned into pCI-SF mammalian expression vector and pET-24b(+) for *E. coli* protein expression, respectively. Mammalian cell transient transfection was used to assess expression from pCI-SF clone's prior to animal immunizations. Independently, cobalt affinity column was used for protein purification from pET-24b(+) bacterial expression. Protein and DNA immunogens are being used to immunize small animals, and functional immune responses evaluated by SMFA will be reported.

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ASSOCIATION OF SPECIFIC VAR2CSA HAPLOTYPES WITH WORSENED BIRTH OUTCOMES IN WOMEN WITH *PLASMODIUM FALCIPARUM* PLACENTAL MALARIA IN MALAWI AND BENIN

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Pregnancy associated malaria (PAM) causes adverse pregnancy and birth outcomes including low birth weight (LBW) and small for gestational age (SGA). Placental accumulation of *Plasmodium falciparum* is mediated by VAR2CSA. This protein's ID1-DBL2x region is considered a minimal binding epitope and is a promising vaccine candidate against PAM. We hypothesized that variation in the ID1-DBL2x region would be associated with differential prevalence of poor birth outcomes. Using two clinical cohorts of women with placental malaria at delivery, we deep-sequenced the 1.6kb ID1-DBL2x region in 101 placental samples in Malawi and Benin to characterize genetic diversity and identify pathogenic clades. In Malawi, we identified two genetic clades which resembled the sequences of the current vaccine candidate antigens, 3D7 & FCR3. In Benin, along with 3D7-like and FCR3-like clades, three other unique clades were detected.

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We estimated the association of specific clades with birth weight, LBW, and SGA, controlling for confounders using inverse probability weights. In our study population, the mean (SD) infant birth weight in Malawi was 2677g (540g) and 2840g (380g) in Benin. Prevalence of LBW was 19.6% (n=11) in Malawi and 13.3% (n=6) in Benin; prevalence of SGA was 16.1% (n=9) in Malawi and 24.4% (n=11) in Benin. In phylogenetic analyses, the variants present in the placentae of women delivering LBW or SGA infants clustered more readily in the 3D7-like clade in Malawi but were more evenly distributed in Benin. Compared to women infected with FCR3-like only variants, women infected with 3D7-like only variants delivered infants with lower birth weight (-267.99g; 95% CI: -466.43g - -69.55g) and higher odds of LBW (OR: 8.19; 95% CI: 1.65 - 40.57) and SGA (OR: 3.65; 95% CI: 1.00 - 13.38). These associations were attenuated in Benin, but were overall supported by country-level analyses. The results from our study provide evidence that 3D7-like genetic variants of VAR2CSA in parasites infecting the placenta are associated with worse birth outcomes including LBW and SGA. This supports the development of polyvalent vaccines that target multiple clades of VAR2CSA to combat PAM.

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EFFICACY OF PFSPZ VACCINE AGAINST HETEROLOGOUS MALARIA CHALLENGE IN MALARIA-NAÏVE ADULTS

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Sterile protection lasting 14 months against *Plasmodium falciparum* (Pf) malaria has been achieved in humans after intravenous (IV) injection of a non-replicating, cryopreserved Pf sporozoite (SPZ) vaccine (PfSPZ Vaccine). Further development is focused on identification of a dose-sparing immunization regimen that induces durable protection against heterologous Pf strains. We conducted an open-label trial of PfSPZ Vaccine, composed of attenuated, aseptic, purified cryopreserved PfSPZ, at a dose of 9.0 x105 PfSPZ administered IV 3 times at 8-week intervals to 15 healthy, malaria-naïve adults. Vaccinated and non-vaccinated control volunteers underwent controlled human malaria infection (CHMI) by exposure to mosquitoes carrying infectious PfSPZ of homologous 3D7 and heterologous 7G8 Pf strains at 19 and 33 weeks, respectively, after final immunization. Antibody and cellular immune responses were assessed. PfSPZ Vaccine was well tolerated. After CHMI with homologous PfSPZ at 19 weeks, 9/14 volunteers (64%) remained without parasitemia compared to 0/6 controls (P=0.012, Fisher's exact test, one-sided). Six non-parasitemic volunteers underwent repeat CHMI with heterologous PfSPZ at 33 weeks, and 5/6 vaccinees remained without parasitemia compared to 0/6 controls (P=0.0076). Pf-specific antibody, CD8, CD4, and γδ T cell responses were detected in all vaccinees. A 3-dose regimen of PfSPZ Vaccine conferred sterile protection for at least 33 weeks against CHMI with heterologous Pf and induced broad-based PfSPZ-specific immune responses. Ongoing studies using higher doses are evaluating protective efficacy in travelers, military personnel, and infants and adults living in endemic areas.

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IMMUNIZATION BY MOSQUITO BITE WITH RADIATION ATTENUATED SPOROZOITES (IMRAS): A PHASE 1 CLINICAL TRIAL

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Human clinical trials have demonstrated that immunization with radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) by mosquito bite is an excellent model for malaria vaccine development conferring the highest levels of sterile protection. In the study Immunization by mosquito bite with radiation-attenuated *P. falciparum* sporozoites, or IMRAS, we have applied a systems biology approach to this model to improve current understanding of immune mechanisms of protection by comparing sterilely protected with non-protected study subjects. The clinical study was designed such that approximately 50% of immunized human subjects would be protected against homologous controlled human malaria infection (CHMI) to facilitate the analysis of biomarkers and correlates of protection. Earlier studies suggested that immunization with a total of 960 bites from mosquitoes infected with PfRAS would yield 50% protective efficacy. The study was conducted with two sequential cohorts, each consisting of twelve true-immunized and four mock-immunized human subjects. Subjects in both cohorts underwent five immunization sessions every four weeks receiving approximately 200 infectious bites per immunization session. Immunization procedures were well-tolerated, and there were no vaccine-related serious adverse events. All twelve infectivity controls (six per cohort) became parasitemic and none of the mock-immunized subjects were protected. Surprisingly, despite the similar number of total infectious bites in each cohort, the percentage of subjects protected in the two cohorts was quite different. Six of the 11 (55%) true-immunized subjects in the first cohort were sterilely protected against parasitemia after primary challenge at 23-25 days post-last immunization. In the second cohort, 9 of 10 (90%) true-immunized subjects were protected after primary challenge. We will present a detailed analysis of all factors which may have impacted the discordant levels of protective efficacy in the two cohorts; such data may provide key information for the development of a highly protective malaria vaccine.