criteria for clinical advancement and enhancing the potency and longevity of the immune response.

PfCP-2.9 (AMA1 and MSP1$_{19}$ chimera)

PfCP-2.9 is a chimeric molecule expressed in *P. pastoris* that includes the C-terminal region of AMA1 (domain III) and MSP1$_{19}$ from the 3D7 and K1 *P. falciparum* lines respectively (273). Both components of this chimeric protein are targets of inhibitory antibodies. Sera from animals immunized with PfCP-2.9 inhibited parasite growth in vitro. PfCP-2.9 formulated in ISA 720 has been evaluated in two phase 1 trials. In both trials, the vaccine was well tolerated, immunogenic and recognized proteins on the surface of parasites (274; Malkin, personal communication). The second trial evaluated an optimized formulation and immunization schedule.

GMZ2 (GLURP and MSP3 hybrid)

GMZ2 is a hybrid molecule including the C-terminus of MSP3 and amino acids 85 to 213 of glutamate-rich protein (GLURP) expressed by *Lactococcus lactis* (275). GLURP is expressed by the erythrocytic and pre-erythrocytic stages of the parasite, and is a target of protective antibodies from immune adults (276–279). Human anti-GLURP antibodies, in particular IgG1 antibodies raised to amino acids 85 to 213, have been shown to inhibit parasite growth in vitro in the presence of monocytes (185). GMZ2 adjuvanted with aluminum hydroxide is currently undergoing phase 1 trials in Germany and Gabon.

Concluding Remarks

The identification and generation of the most appropriate B- and T-cell immune responses that can curb malaria infection in an endemic setting remain major challenges for vaccine developers. The specificity of the immune response produced as a result of vaccination is important and may be altered by manipulating the formulations (antigens and adjuvants). Additionally, the characteristics of the field site (e.g., entomological inoculation rate, and seasonality of transmission) chosen for testing and the clinical outcomes may impact the results of the trial.

Blood-stage proteins have so far fallen short in the promise to induce significant protective responses (280). A blood-stage vaccine including three protein components, called Combination B (composed of MSP1, MSP2 and RESA), failed to prevent clinical disease in children in Papua New Guinea, even though there was a reduction in parasite density in one study group (255), and a leading blood-stage antigen, MSP1$_{42}$, formulated as a recombinant protein in adjuvant, failed to provide protection in Kenyan adults (Angov, personal communication). More recently, a second leading blood-stage antigen, AMA1, despite inducing high antibody titers and CMI responses, failed to induce a significant delay in the onset of parasitemia, as detected by blood smear, in a phase 2a challenge study conducted in the United States (Spring, personal communication). There was a slight delay in parasitemia of vaccinated volunteers compared with nonvaccinated volunteers when monitored by qPCR, but this was not statistically significant. It was not possible to avoid treating these volunteers once parasitemia had developed to see if the vaccine dampened clinical acuity. It could be hypothesized that these antigens are intrinsically unable to induce protective responses; in other words, that they are the wrong choices for inclusion in a vaccine. At this point, the value of blood-stage antigens for achieving anti-disease effects remains unproven.

Transmission-Blocking Malaria Vaccines

Introduction

Transmission-blocking malaria vaccines (TBMV) are based on sexual- or sporogonic-specific antigens and designed to arrest the development of sporogonic stages inside the mosquito. The specific antibodies generated in the human host are passively ingested together with parasites when mosquitoes take a blood meal and bind to the parasites in the lumen of the mosquito midgut thereby preventing progression of their sporogonic development.

Once inside the mosquito midgut, gametocytes rapidly emerge from the intracellular red blood cell environment to prepare for fertilization and are directly exposed to hostile immune components of the ingested blood. The sporogonic cycle may be the most vulnerable part of the life cycle because, at least in the case of some transmission-blocking targets, it appears to have evolved in the absence of immune pressure from the human host, and is therefore an attractive target for interventions.

Reduction or absence of the infectious mosquito reservoir will lead to reduction or eradication of malaria in the human population (281). TBMV are fundamentally different from the pre-erythrocytic and blood-stage vaccines previously discussed since they do not provide immediate protection against clinical disease but rather reduce chances to become infected. This differentiation between actual clinical disease and disease risk is due to the special characteristics of the *Plasmodium* life cycle where separate forms are responsible for disease (pathogenic asexual stages) and transmission (nonpathogenic gametocytes). Asymptomatic parasite carriers are primarily responsible for transmission since that fraction of the population is of larger magnitude than that of patients suffering from clinical disease. However, in very low endemicity areas, carriers will likely be symptomatic and the main source of transmission. The effectiveness of TBMV is determined by the degree of herd immunity induced in that part of the population responsible for transmission in a given area. The herd immunity induced by TBMV represents a variation from what is often thought of as herd immunity in than no members of the population are in fact protected against infection with the pathogen or the disease that it causes.

Similar to all malaria vaccines the public health endpoint of a TBMV is reduction of the incidence of (severe) malaria. The biological endpoint is reduction or elimination of infected mosquitoes by blocking sporogonic development.

Reliable assays are needed that are preferably close to the biological transmission-blocking effect or represent a good correlate. Pioneering work was conducted in the late eighties resulting in the development of the Standard Membrane Feeding Assay (SMFA), which is the gold standard for measurement of transmission-blocking activity in sera (282,283) (Fig. 3). In the case of *P. falciparum*, cultured gametocytes are used from a standard laboratory strain with stable gametocyte production (e.g., NF54). Because of the inability to culture *P. vivax* in the laboratory, packed cells from *P. vivax*–infected donors are used in the membrane feeding assay (284). The SMFA is believed to give a reasonable measure showing a fair correlation with natural feeding on gametocyte carriers (285). However, validation of the SMFA will depend on clinical trials showing acquired immunity. While an attractive assay because of its biological nature, interassay variation is clearly placing restrictions on the usage of the SMFA. However, when comparisons are made within experiments, the assay is appropriate for