transfer of T lymphocyte–enriched immune splenocytes recognizing TRAP protected naive mice (159), while in the second, in vivo depletion of $CD4^+$ T cells eliminated vaccine-induced protection and in vivo treatment with anti-IFN-γ reversed vaccine-induced activity against infected hepatocytes (160).

As mentioned above, immunization of humans with a recombinant protein formulation of TRAP failed to protect (113). However, given the protection observed with the PySSP2 in the murine model and the ability of immune responses targeting the PfTRAP to reduce or block sporozoite infection of hepatocytes, it is believed that presentation of the TRAP molecule in a context that induces high antibody titers coupled to potent $CD8^+$ T cells should constitute an effective vaccine. Indeed, studies of TRAP delivered as a heterologous regimens of DNA and recombinant poxviruses demonstrated protection against experimental sporozoite challenge in nonhuman primates (161) and in humans (162,163). Disappointingly, however, these prime-boost genetic vaccines based on TRAP failed to protect in two field trials (152,153) (see chap. 70).

Liver-stage antigens (LSA1 and LSA3). Substantial epidemiological evidence has suggested that individuals who were naturally protected from malaria recognized determinants expressed on the protein LSA1 (164). LSA1 as expressed in native form by *P. falciparum* is a large 230-kDa MW protein having conserved N- and C-terminal regions flanking a region containing approximately 86 repeats of 17 amino acids each. An LSA1-based protein vaccine, LSA-NRC, containing the entire amino and carboxy-terminal regions of LSA1 flanking two repeats (165) was emulsified in either AS01B or AS02A adjuvant and administered to volunteers in a combined phase 1/2a trial. Despite the induction of high LSA1-specific antibody and IFN-γ producing T-cell responses, none of the vaccinees were protected or showed any significant delay in onset of parasitemia following sporozoite challenge (Cummings, manuscript in preparation).

LSA3 is a pre-erythrocytic antigen found in infected liver cells but also in erythrocytic stages. It is an antigen that contains highly conserved regions as well as highly variable regions. Long synthetic peptides and recombinant proteins have been made representing the conserved regions, and in limited small trials have protected nonhuman primates, including chimpanzees (166) and *Aotus* monkeys (167) against *P. falciparum* sporozoite challenges. A recombinant protein construct is currently being tested in Nijmegen, the Netherlands in a phase 1 conditional phase 2a study (Sauerwein, personal communication).

Concluding Remarks

The only malaria vaccine that has demonstrated anti-disease effects without necessarily preventing parasitemia is based on a single pre-erythrocytic stage antigen, the CSP. This vaccine, RTS,S, protected 40% to 50% of malaria-naive vaccine recipients tested in the United States against experimental sporozoite challenge (114). While it has less effect on parasitemia in the field, remarkably, it has significantly reduced the frequency of clinical disease for a period of at least 18 months in African children, even though most of these children nevertheless did become parasitemic (168,169) The mechanism for this protective effect is unclear, but may relate to the killing in the liver of more virulent parasite strains (with less virulent strains still getting through), or to a general reduction in the number of parasites entering the blood following liver-stage development. In the latter case, it is argued, a smaller blood inoculum from the liver would prolong the time required to the onset of the clinical syndrome, thereby allowing the immune system a greater opportunity to respond effectively. The achievement of clinical protection by a ''leaky'' pre-erythrocytic stage vaccine was not anticipated, and suggests the need to redefine the classic anti-disease vaccine paradigm.

The positive findings associated with RTS,S have emphasized the potential impact of pre-erythrocytic stage antigens on clinical disease. The rationale for a pre-erythrocytic stage approach is strengthened by the finding that reduction in liver-stage burden by other mechanisms, such as insecticide-treated bed nets, also favorably impacts malaria-related morbidity (170,171). It makes sense to attack the early stages of infection, when parasites are present in small numbers (<100), compared with the blood stages, which, if not checked on release from the liver, number in the hundreds of billions.

Blood-Stage Malaria Vaccines

Introduction

Following release from the liver, merozoites invade red blood cells where multiple cycles of infection result in high-level parasitemia and the many pathological manifestations associated with the disease. Interventions that significantly reduce parasitemia would alleviate morbidity and ultimately reduce mortality in infected individuals. Over time, people living in endemic areas develop natural immunity to *P. falciparum* as a result of repeated infection, mediated in part by blood-stage parasite-specific antibodies (172) that reduce parasite multiplication rates. Thus, parasite proteins expressed during blood-stage infection have been proposed to be good candidates for inclusion in a vaccine. Candidate antigens for blood-stage vaccine development have been chosen on the basis of such indicators as location on the surface of merozoites or infected erythrocytes, correlation of antibody levels with protection in the field and/or demonstrated protection in animal models.

The purpose of an asexual blood-stage vaccine is to elicit immune responses that either destroy the parasite in the blood stream or inhibit the parasite from infecting red blood cells. The net effect is to reduce or prevent the burden of parasites and hence decrease the incidence, severity, or the complications of disease. The initial vaccination could act to prime the immune system for subsequent boosting on exposure to infection or repeat immunization, or it could boost already present, yet weak, natural immunity in young children. The enhancement of naturally induced immune responses could be maintained by subsequent natural infections. Thus, the goal of blood-stage vaccines is not to provide sterile protection against primary infection or disease in malaria-naïve individuals (e.g., travelers), but rather to slow parasite multiplication, thereby limiting morbidity, severe disease, and death in residents of malaria-endemic areas, primarily young children and infants. As yet, no blood-stage vaccine has been developed that has achieved any of these favorable outcomes when tested in the field.

Development of Blood-Stage Malaria Vaccines

The degree of protective immunity in humans has been shown to parallel the level of antibody against asexual blood-stage antigens (173–175), and these levels increase with age. However, the specificity and level of antibody that must be induced to confer protection against clinical disease in malaria is unknown. Immunological correlates will only be obtained