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Clinical Implications

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Liver stage antigen and malaria

The symptoms of malaria are clearly described in the *Nei Ching*, the Canon of Chinese Medicine, laid down in 2700 BC. Malaria became widely recognized by the 4th century BC; Hippocrates noted the principal symptoms. In the *Susruta*, a Sansrkit medical treatise, the malarial fevers were described and attributed to the bites of certain insects. Roman writers attributed malarial diseases to the swamps. In 1880 Alphonse Laveran, a French army physician stationed in Constantine, Algeria, was engaged in the study of malaria. He noted at autopsy the constant presence of pigment granules in the liver and cerebral blood vessels. He wrote: "I discovered on the edges of the round pigmented bodies in the blood of a patient with malaria filiform elements resembling flagella, which moved about with great vivacity, displacing the neighboring red cells." Laveran painstakingly recorded his findings (Fig. 1) and presented them to the Paris *Academie des Sciences*. His findings were published but were greeted with skepticism. In the first 1892 edition of his book, *Principles and Practice of Medicine*, William Osler wrote: "We do not know how the parasite enters, or how or in what form it leaves the body; how or where it is propagated, under what outside conditions it develops, whether free or in some aquatic plant or animal." In time for the second edition, Ronald Ross, a surgeon in the Indian Medical Service, found the parasites in the stomach of a mosquito that had fed on a malarial patient. In 1898 Ross demonstrated the parasites in the salivary glands of the *Anopheles* mosquito. Ross and Laveran received the Nobel Prize in 1902 and 1907, respectively. Othmer Zeidler synthesized dichloro-diphenyl-trichloroethane in 1874 for his thesis project. Paul Müller discovered that the compound was an effective insecticide and received the Nobel Prize for this observation in 1948. By the 1950s malaria had been eradicated from Europe and the United States.

Malaria not only has been but also *still is* one of the greatest killers in history. No universal protection against the parasite has been developed. A vaccine would be the most effective. In *Journal of Molecular Medicine* García et al. [1] now report on liver stage antigen 3 (LSA-3) *Plasmodium falciparum* peptides that specifically interact with HepG2 cells, a human hepatocyte cell line. Characterization and clarification of *Plasmodium* LSA proteins is of great relevance in understanding the *Plasmodium* life cycle. The sporozoites released from the mosquito's salivary glands first circulate in the host's blood and enter hepatocytes in less than 1 h. How the sporozoites squeeze through the sinusoid lining into Disse's space to reach the hepatocytes and how they enter the cells is unclear, but the mechanism probably involves a ligand-receptor interaction. A variety of *Plasmodium* hepatic stage antigen proteins have been discovered that not only may shed light on this early process but may also be candidate antigens for vaccine development. The investigators searched for high-activity binding peptides (HABP) in the LSA-3 protein that bind to HepG2 cells. They found 16 such peptides and mapped their locations in LSA-3. Some of the HABP were located in the antigenic and immunogenic LSA-3 protein regions. They then used goat sera from animals immunized with the LSA-3 peptides. These sera recognized *P. falciparum* preerythrocyte stage proteins. The investigators showed that the VEESVAEN octapeptide motif was particularly important for reactivity.

Moorthy et al. [2] have recently reviewed malaria vaccine developments. The disease claims about 3,000,000 victims per year, about 1 death every 30 s, mostly children in sub-Saharan Africa. The first vaccine was presented in 1973. This vaccine consisted of *Plasmodium* contents from about 1000 X-irradiated mosquitoes. The vaccine was an effective but not a practical demonstration. Many candidate vaccines have come to trials, and more potential candidates warrant preclinical assessment. The focus has been on subunit vac-

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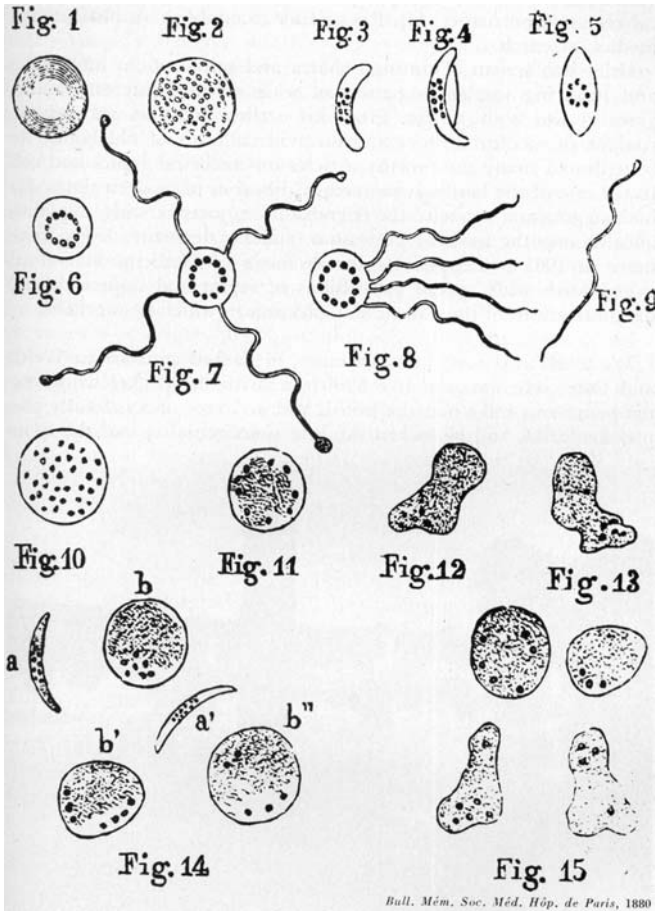

Fig. 1 Illustrations of malarial parasites in Laveran's original communication are shown (Bull Mém Soc Méd Hôp de Paris, 1880)

cines. These vaccines rely on antigens identified from the pathogen's proteomic complement, such as the LSA-3 protein for example. Such a vaccine would be a preerythrocytic vaccine, namely, sporozoites would be targeted to prevent the parasites entering the liver stage. The red blood cell stage would then never be reached by the parasite. The newest generation of subunit vaccines is DNA based. DNA sequences from *P. falciparum* are inserted into plasmid DNA molecules or attenuated DNA viruses. The DNA vaccines are taken up by host cells, the protein is expressed, and T-cell epitopes bound to HLA molecules prime naive T cells to form memory T-cell populations. Recombinant viral vaccines operate similarly but actively infect cells and express the recombinant malaria proteins before aborting infection. The *P. falciparum* genome has been sequenced, revealing about 5,300 potential antigens. Hundreds should be candidates for vaccination.

Daubersies et al. [3] identified LSA-3. They demonstrated the protein in infested mosquitoes and in liver-stage parasites. LSA-3 and a companion protein LSA-1 were used to immunize chimpanzees. The investigators found only one schizont on liver biopsy of their vaccinated chimpanzee, compared to 2,500 liver schizonts in a control chimpanzee. Further immunization and challenge experiments with LSA-3 suggested that the protein could provide protection, at least for chimpanzees. These observations gave García et al. [1] the impetus to study this protein further.

Prieur et al. [4] recently described a more comprehensive approach. They constructed a candidate vaccine containing six preerythrocytic *P. falciparum* antigens linked together to produce a 3,400 amino acid long polyprotein. The polyprotein consisted of LSA-3, the sporozoite threonine and asparigine-rich protein (STARP), the exported protein-1 (Exp1), Pfs16, thrombospondin-related adhesive protein (TRAP), and LSA-1. They called this construct L3SEPTL. The polyprotein was expressed by a plasmid DNA vaccine vector and by two attenuated poxvirus vectors. Preliminary vaccination tests in mice indicated that interferon-γ secreting T cells were produced, specific for each of the six antigens. The vaccination approach should result in multispecific T cells against *P. falciparum*. The authors suggest that polyprotein constructs in nonreplicating poxviruses will broaden the target antigen range of vaccine-induced immunity. These lines of investigation allow us to be cautiously optimistic. The end of the malaria era was heralded 50 years ago, all too soon and with far too much bravado. Fortunately, substantial advances are now being made in approaches that promise to rectify earlier false predictions.

As a final anecdote, Sir Patrick Manson, the founder of tropical

medicine, demonstrated in 1879 that filariasis (*Wuchereria bancrofti*) is transmitted by the bite of a mosquito. He was Ross' mentor and was delighted with the latter's success. As a final proof, Manson received some mosquitoes from Italy that had fed on malaria patients. Manson's son Thurburn allowed the mosquitoes to bite him and 2 weeks later came down with the disease.

Respectfully,
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