

Review

Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine

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Summary

Annually, malaria causes >300 million clinical cases and 1 million deaths, is responsible for the loss of >1% of gross domestic product (GDP) in Africa and is a serious concern for travelers. An effective vaccine could have a dramatic impact on the disease. For 20 years, scientists have tried to develop modern, recombinant ‘subunit’ malaria vaccines. This has been difficult. In fact, there is only one recombinant protein vaccine on the market for any disease, and no vaccines based on synthetic peptides, recombinant viruses, recombinant bacteria or DNA plasmids. Most vaccines are based on attenuated or inactivated whole pathogens or material derived directly from the infectious agent. It is in that context that our recent report summarizing the protection of humans with attenuated *Plasmodium falciparum* (*Pf*) sporozoites produced at four different sites over 25 years is important. In studies utilizing live mosquitoes as the vaccine delivery mechanism, there was complete protection against malaria in 93% of volunteers (13/14) and 94% of challenges (33/35). Sanaria’s goal is to develop and commercialize a non-replicating, metabolically active *Pf* sporozoite vaccine.

Three practical questions must be addressed before manufacturing for clinical trials: (1) can one administer the vaccine by a route that is clinically practical; (2) can one produce adequate quantities of sporozoites; and (3) can sporozoites be produced with the physical characteristics that meet the regulatory, potency and safety requirements of regulatory authorities? Once these questions have been answered, Sanaria will demonstrate that the vaccine protects >90% of human recipients against experimental challenge with *Pf* sporozoites, can be produced with an efficiency that makes it economically feasible, and protects >90% of African infants and children from infection, and thus from severe morbidity and mortality. By producing a vaccine for travelers, Sanaria will provide the infrastructure, regulatory foundation and funds necessary to speed licensure, manufacturing and deployment of the vaccine for the infants and children who need it most.

Key words: malaria vaccine, immunization, *Plasmodium falciparum*, radiation-attenuated sporozoite, irradiated sporozoite.

Introduction

An effective vaccine against *Plasmodium falciparum* malaria remains one of the great challenges of medicine. Despite over one hundred years of effort, hundreds of millions of dollars in research, lifelong sacrifice from dedicated physicians and scientists and many promising experimental vaccines, there is no marketed vaccine to alleviate one of the great infectious scourges of humanity. A generation ago, public health initiatives employing chloroquine, DDT and vector control programs seemed poised to consign falciparum malaria to insignificance as a worldwide menace. The lack of an effective vaccine complicated these efforts, but sustainable control seemed imminent.

The promise of impending success was short lived and the reasons for failure were multi-factorial. The parasites grew increasingly resistant to highly effective and affordable anti-

malarial medications, vector control measures lapsed, and transmigration, war and economic disruption became increasingly more common in endemic areas of the developing world. As a result, falciparum malaria has resurged, annually placing 2.5 billion humans at risk, causing 300–900 million infections and killing 1–3 million people. Of the many social, economic, environmental and political problems that afflict the developing world, falciparum malaria is increasingly seen as both a root cause and cruel result of these inequities and is a singular impediment to solving these complex problems. Controlling falciparum malaria in the developing world may be possible without an effective vaccine. In practice, given social, political and economic realities, we believe that a vaccine may be an essential component of a sustainable control program and will be required for a global eradication campaign.

It is in this context that the modern period of malaria vaccine development has been particularly frustrating. Since the early 1980s, breathtaking technological advances in molecular biology and medical science have occurred. These advances accelerated the identification of stage-specific *P. falciparum* proteins and epitopes and host immune mechanisms and responses. This knowledge was translated into a range of novel vaccine candidates (Richie and Saul, 2000; Long and Hoffman, 2002). In one sense, this modern period has been the golden age of malaria vaccine research and human testing. However, in spite of the herculean efforts of malaria researchers, the majority of these vaccines has failed to provide any protective immunity in humans – with only one demonstrating reproducible short-term protection against infection in 40–70% of recipients (Stoute et al., 1998; Kester et al., 2001; Bojang et al., 2001).

Given enough time and resources, these vaccine strategies, or others yet to be developed, may ultimately lead to a robust vaccine. However, at a recent Keystone meeting, ‘Malaria’s Challenge: From Infants to Genomics to Vaccines’ (Long and Hoffman, 2002), the attendees were polled as to when they thought a malaria vaccine might be ‘launched’ as a commercial product. Many in the room indicated that they thought the first vaccine would not be launched until 2016–2025. The leader of GlaxoSmithKline (GSK)’s efforts to develop a recombinant *P. falciparum* circumsporozoite protein (PfCSP) vaccine voiced the most optimism. It was indicated that, if all went well, this single protein vaccine could be ‘launched’ in 7–8 years (2009–2010). Given that GSK and the US Army have been working on a recombinant protein vaccine since the 1984 cloning of the PfCSP (Dame et al., 1984) and that many malariologists express concern as to whether a single protein vaccine will be adequate to sustainably control malaria, this time line of >25 years for development of a single protein vaccine places a chillingly realistic perspective on the possibilities for developing vaccines that will truly reduce the burden of this disease.

Protective immunity after immunization with radiation-attenuated sporozoites

In 1967, Nussenzweig reported that immunizing mice with radiation-attenuated *Plasmodium berghei* sporozoites protected them against challenge with fully infectious sporozoites (Nussenzweig et al., 1967). These rodent studies provided the impetus for human studies, and, during the 1970s, Clyde, Rieckmann and colleagues established that immunizing human volunteers with the bites of irradiated mosquitoes carrying *P. falciparum* sporozoites in their salivary glands could protect volunteers against challenge with fully infectious *P. falciparum* sporozoites (Clyde et al., 1973a,b, 1975; Rieckman et al., 1974, 1979; McCarthy and Clyde, 1977; Clyde, 1990; Rieckmann, 1990). These studies demonstrated that a malaria vaccine offering sterile protective immunity was possible. However, the only way to produce sporozoites at that time was to infect a volunteer with *P. falciparum*, treat the volunteer with doses of chloroquine to suppress but not

eliminate the parasite, allow gametocytes to develop and then feed mosquitoes on the volunteer. Even if one could produce sporozoites in adequate numbers by this method, it was considered clinically, technically and logistically impractical to immunize large numbers of individuals with an irradiated sporozoite vaccine, in large part because the sporozoites had to be delivered alive, either by the bite of infected mosquitoes or by intravenous injection, as was done with mice.

Potential solutions to these problems, although not necessarily recognized at the time as being related to developing an attenuated sporozoite vaccine, were being reported. In 1975, a method for culturing *P. falciparum* *in vitro* was reported (Trager and Jensen, 1976; Haynes et al., 1976), followed in 1982 by a method for producing gametocytes from these cultures (Campbell et al., 1982). In 1986, it was reported that humans could be infected by the sporozoites produced in mosquitoes that had fed on these *in vitro* cultures (Chulay et al., 1986). There was therefore a way to produce sporozoites without the difficulties of *in vivo* production of gametocytes in humans.

However, by that time, several promising developments launched the modern era of malaria subunit vaccine development. A monoclonal antibody against the major surface protein of sporozoites, the circumsporozoite protein (CSP), had been produced and shown to protect mice in passive transfer experiments (Yoshida et al., 1980). Additionally, the gene encoding the PfCSP protein had been cloned and sequenced (Dame et al., 1984). Coincidentally, the first purified recombinant protein vaccine, the hepatitis B surface antigen vaccine, was developed and marketed (Hilleman, 1987). The weight of evidence and trends in vaccine science seemed to offer malaria researchers a roadmap to quickly develop a human malaria vaccine. Returning to an attenuated whole-parasite vaccine seemed unnecessary and dated, and all subsequent efforts focused on the promise of subunit vaccines.

In 1987, when the first recombinant protein (Ballou et al., 1987) and synthetic peptide (Herrington et al., 1987) vaccines did not prove to be as protective as expected, instead of considering the development of an attenuated sporozoite vaccine, scientists focused on understanding the immune mechanisms responsible for protective immunity, and the antigenic targets of these protective immune responses, and developing subunit vaccines and vaccine delivery systems that induced such protection. Much of this basic work was carried out in the *P. berghei* and *P. yoelii* rodent model systems. This rodent malaria work provided important insights into irradiated sporozoite vaccine-induced protection and led to the development of a number of candidate vaccines (Nussenzweig and Nussenzweig, 1989; Hoffman et al., 1996; Hoffman and Miller, 1996). Importantly, in stark contrast to subunit vaccine formulations, the protective results of rodent and human irradiated sporozoite studies have been strikingly concordant.

In 1989, after a number of disappointing clinical trials of subunit PfCSP vaccines, immunization of volunteers with gamma radiation-attenuated *Pf* sporozoites was begun at the Naval Medical Research Center and Walter Reed Army

Institute of Research. The goal of this research was to better delineate the clinical characteristics and requirements that led to protecting humans with the irradiated sporozoite vaccine, to assess the protective immune responses elicited in humans and to identify the antigens and epitopes on those proteins that elicited immune responses in humans. Preliminary clinical results and extensive immunological assay results from these studies were published (Egan et al., 1993; Malik et al., 1991; Wizel et al., 1995a,b; Krzych et al., 1995; Doolan et al., 1997, 2000). These immunological studies, combined with those of others on this subject (Herrington et al., 1991; Edelman et al., 1993; Nardin et al., 1989, 1990; Nardin, 1990; Moreno et al., 1991, 1993), increased our understanding of the immunological responses in humans immunized with radiation-attenuated *P. falciparum* sporozoites.

Foundation for current plans

We recently reported the results of the first 10 years' clinical experience with these immunizations and challenges and combined our results with all the published clinical reports of immunizing humans with irradiated *Plasmodium* sporozoites (Hoffman et al., 2002) from the University of Maryland (1970s, late 1980s and early 1990s) and the Rush–Presbyterian–St Luke's Medical Center in Chicago and the Naval Medical Research Institute in the 1970s (Hoffman et al., 2002; Clyde et al., 1973a,b, 1975; Rieckman et al., 1974, 1979; McCarthy and Clyde, 1977; Clyde, 1990; Rieckmann, 1990). A number of observations arose from our analysis.

1. There was a dose response in regard to protective immunity among volunteers challenged by the bite of 5–14 infected mosquitoes

Thirteen of 14 volunteers (93%) immunized by the bites of >1000 infected, irradiated mosquitoes were protected against developing blood-stage *P. falciparum* infection when challenged within 10 weeks of their last primary immunization; there were 35 challenges of these volunteers and there was complete protection against development of blood-stage infection in 33 of the 35 challenges (94%).

Four of 10 volunteers (40%) immunized by the bite of >200 and <1000 infected, irradiated mosquitoes were protected against developing blood-stage *P. falciparum* infection when challenged within 10 weeks of their last primary immunization, a significantly lower level of protective immunity than among volunteers immunized with >1000 infective bites ($P=0.0088$; Fisher's exact test, two-tailed); there were 15 challenges of these volunteers and there was complete protection against development of blood-stage infection in five of the 15 challenges (33%), a significantly lower level of protective immunity than among volunteers immunized with >1000 infective bites ($P=0.000015$; Fisher's exact test, two-tailed).

2. Protective immunity lasted for at least 42 weeks (10.5 months)

Five of six of the above 14 volunteers when challenged

23–42 weeks (23, 36, 39, 41 and 42 weeks) after their last primary or secondary immunization were protected against experimental challenge. Except for a single challenge of one volunteer five years after their last immunization (not protected), there were no other challenges assessing longevity of protective immunity.

3. Protection was not strain specific

Four volunteers were challenged with isolates of *P. falciparum* that were different from the isolates with which they were immunized. The four volunteers were completely protected in seven of seven such challenges with different isolates of *P. falciparum*.

Thus, protection was achieved in >90% of challenge experiments after >1000 mosquito bites, lasted for at least 10.5 months and was not *P. falciparum* isolate (strain) specific.

Back to the future

A 'subunit' vaccine demonstrating this level of protective efficacy in human subjects would be recognized as a major breakthrough. Although we routinely observed that protection resulted from our experimental irradiated sporozoite vaccine, the sheer power of attenuated sporozoites remained unrecognized until after we completed the careful analysis necessary to publish the report (Hoffman et al., 2002). Prior to this, we agreed with the conventional wisdom that a radiation-attenuated sporozoite vaccine was clinically impractical. However, this sentiment was formed 25 years ago and was based upon the limitations of science and technology at that time. We elected to challenge these assumptions and initiated a feasibility study to determine whether the irradiated sporozoite vaccine could be extended from the laboratory and into clinical practice.

We concluded that there were three critical questions that had to be answered before moving into cGMP manufacturing and clinical trials:

- (1) Can one administer the radiation-attenuated sporozoites by a route that is practical for a vaccine?
- (2) Can one produce adequate quantities of sporozoites?
- (3) Can one produce sporozoites that have the physical characteristics to allow them to meet regulatory, potency and safety requirements to be a vaccine?

Can one administer the vaccine by a route that is clinically practical?

In the past, humans have been immunized by exposure to irradiated *Anopheles* mosquitoes infected with *P. falciparum* or *P. vivax* sporozoites. Generally, mice have been immunized by intravenous administration of *P. berghei* or *P. yoelii* sporozoites. It is not practical to consider large-scale immunization by the bite of infected mosquitoes, and there are no vaccines used as public health measures that are administered intravenously. Essentially, all vaccines are administered intradermal, subcutaneous or intramuscular

routes. Work is in progress to demonstrate that this is feasible for irradiated sporozoites.

Can one produce adequate quantities of sporozoites?

It requires the bites of 1000 irradiated infected mosquitoes to consistently protect humans (Hoffman et al., 2002). Most entomologists believe that each mosquito injects only 10–20 sporozoites but may inject up to 100 sporozoites (Beier et al., 1991). Therefore, the bites of 1000 infected mosquitoes inoculating 10–100 sporozoites per mosquito bite = 10^4 – 10^5 sporozoites delivered for a full immunization regimen. Most laboratories produce 2×10^4 *P. falciparum* sporozoites per mosquito. We and others are now working on producing many more sporozoites per mosquito. If one could produce $>10^5$ *P. falciparum* sporozoites per mosquito, a single infected mosquito could provide all the sporozoites needed for a full human immunization regimen.

Can sporozoites be produced with the physical characteristics that meet regulatory, potency and safety requirements to be a licensed vaccine?

A process for manufacturing a vaccine in mosquitoes that meets regulatory requirements will require development and optimization of: (1) a process that yields aseptic, purified, potent sporozoites; (2) a process for optimal radiation attenuation of cryopreserved sporozoites and (3) validated lot release assays that indicate that aseptic, purified, optimally attenuated and cryopreserved sporozoites are potent and adequately attenuated (safe). Sanaria is working on developing and optimizing all three.

Factors that will speed licensing a malaria vaccine that prevents infection

Non-immune individuals can be immunized and experimentally challenged (Church et al., 1997; Hoffman, 1997; Hoffman et al., 2002). Within 2 weeks of parasite challenge, one can safely know whether a vaccine is effective. Malaria transmission is so intense in Africa that one can anticipate that in a group of non-immune controls in a vaccine trial, 90% will develop *Pf* parasitemia within 10–14 days after exposure and 50–80% of indigenous infants, young children and adults will become re-infected within 10 weeks of being treated (Hoffman et al., 1987; Beier et al., 1994; Owusu-Agyei et al., 2001; Baird et al., 2002; Hale and Hoffman, 2003). These individuals can be rapidly diagnosed and safely treated. Therefore, one can relatively easily and rapidly, in comparison with virtually all other vaccines, assess the protective efficacy of a malaria vaccine that prevents infection, thereby performing the studies in support of a Biologics License Application (BLA) for a vaccine to ‘prevent’ malaria with a series of easily designed and executed, short studies. Thus, our strategy for developing, testing, launching and deploying a vaccine to reduce *Pf*-associated severe morbidity and mortality in infants and young children in Africa and other endemic areas includes rapid licensure for a developed world population. This will facilitate the large pivotal field studies required to prove

safety and efficacy in infants because we will be utilizing a vaccine that has already been approved by regulatory agencies. Furthermore, building the manufacturing, packaging and distribution capabilities for a vaccine will provide the foundation for building the much larger capabilities required for a vaccine to be used in the 27 million babies born annually in sub-Saharan Africa and reduce cost.

Rationale for why an irradiated sporozoite vaccine will reduce malaria-associated severe disease and mortality in infants and children in Africa

The erythrocytic stage of *Pf* causes all disease pathology. If one prevents erythrocytic stage infection in $>90\%$ of recipients, one will prevent severe disease and death in those individuals. We know that in non-immune individuals the vaccine protects for at least 42 weeks without intercurrent exposure. We expect that the immunity induced by this vaccine will be continuously boosted by natural exposure to sporozoites. Adults in highly malarious areas continue to become infected, develop gametocytes and transmit parasites to mosquitoes. Because the first goal for a vaccine will be to reduce severe disease and mortality, we anticipate that the vaccine will be primarily given to infants in the expanded program for immunization. Considering the basic case reproduction rate (R_0) in sub-Saharan Africa, transmission of malaria will continue for years after introduction of the vaccine. This means that even if the irradiated sporozoite vaccine-induced immunity eliminates infections in infants and children, this immunity will be boosted (maintained) on a daily basis by exposure to sporozoites that develop in mosquitoes that have fed on adolescents and adults.

Conclusion

We know that immunization with radiation-attenuated *P. falciparum* sporozoites provides sterile protective immunity in $>90\%$ of immunized individuals for at least 10.5 months against multiple isolates of *Pf* from throughout the world. Given the immediate need for an effective *Pf* malaria vaccine, we believe that an attenuated sporozoite vaccine should be produced and tested for safety and protective efficacy as soon as possible. Although technically and scientifically challenging, such an approach has an enormous advantage over other approaches. The clinical hypothesis has already been proven. Exposure of humans to radiation-attenuated *P. falciparum* sporozoites produced at multiple different sites (Bethesda, Rockville, Baltimore and Washington, DC) elicits a high level of reproducible protective immunity in human volunteers at multiple locations (Chicago, Baltimore, Bethesda and Washington, DC). Much remains to be accomplished, but the path forward is clear. We are encouraged by the support of so many recognized experts in a wide spectrum of disciplines who have offered constructive advice and assistance to this nascent endeavor.

The opinions and assertions herein are the private ones of the authors and are not to be construed as official or reflecting the views of the US Navy or the Department of Defense.

References

- Baird, J. K., Owusu, A. S., Utz, G. C., Koram, K., Barcus, M. J., Jones, T. R., Fryauff, D. J., Binka, F. N., Hoffman, S. L. and Nkrumah, F. N. (2002). Seasonal malaria attack rates in infants and young children in northern Ghana. *Am. J. Trop. Med. Hyg.* **66**, 280-286.
- Ballou, W. R., Hoffman, S. L., Sherwood, J. A., Hollingdale, M. R., Neva, F. A., Hockmeyer, W. T. and Gordon, D. M. (1987). Safety and efficacy of a recombinant DNA *Plasmodium falciparum* sporozoite vaccine. *Lancet* **1**, 1277-1281.
- Beier, J. C., Davis, J. R., Vaughan, J. A., Noden, B. H. and Beier, M. S. (1991). Quantitation of *Plasmodium falciparum* sporozoites transmitted in vitro by experimentally infected *Anopheles gambiae* and *Anopheles stephensi*. *Am. J. Trop. Med. Hyg.* **44**, 564-570.
- Beier, J. C., Oster, C. N., Onyango, F. K., Bales, J. D., Sherwood, J. A., Perkins, P. V., Chumo, D. K., Koech, D. V., Whitmore, R. E., Roberts, C. R. et al. (1994). *Plasmodium falciparum* incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am. J. Trop. Med. Hyg.* **50**, 529-536.
- Bojang, K. A., Milligan, P. J., Pinder, M., Vigneron, L., Allouche, A., Kester, K. E., Ballou, W. R., Conway, D. J. and Reece, W. H. (2001). Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet* **358**, 1927-1934.
- Campbell, C. C., Collins, W. E., Nguyen-Dinh, P., Barber, A. and Broderick, J. R. (1982). *Plasmodium falciparum* gametocytes from culture in vitro develop to sporozoites that are infectious to primates. *Science* **217**, 1048-1050.
- Chulay, J. D., Schneider, I., Cosgriff, T. M., Hoffman, S. L., Ballou, W. R., Ouakry, I. A., Carter, R., Trosper, J. H. and Hockmeyer, W. T. (1986). Malaria transmitted to humans by mosquitoes infected from cultured *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* **35**, 66-68.
- Church, L. W., Le, T. P., Bryan, J. P., Gordon, D. M., Edelman, R., Fries, L., Davis, J. R., Herrington, D. A., Clyde, D. F., Shmuklarsky, M. J. et al. (1997). Clinical manifestations of *Plasmodium falciparum* malaria experimentally induced by mosquito challenge. *J. Infect. Dis.* **175**, 915-920.
- Clyde, D. F. (1990). Immunity to falciparum and vivax malaria induced by irradiated sporozoites: a review of the University of Maryland studies, 1971-75. *Bull. WHO* **68**, 9-12.
- Clyde, D. F., McCarthy, V. C., Miller, R. M. and Hornick, R. B. (1973b). Specificity of protection of man immunized against sporozoite-induced falciparum malaria. *Am. J. Med. Sci.* **266**, 398-401.
- Clyde, D. F., McCarthy, V. C., Miller, R. M. and Woodward, W. E. (1975). Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. *Am. J. Trop. Med. Hyg.* **24**, 397-401.
- Clyde, D. F., Most, H., McCarthy, V. C. and Vanderberg, J. P. (1973a). Immunization of man against sporozoite-induced falciparum malaria. *Am. J. Med. Sci.* **266**, 169-177.
- Dame, J. B., Williams, J. L., McCutchan, T. F., Weber, J. L., Wirtz, R. A., Hockmeyer, W. T., Maloy, W. L., Haynes, J. D., Schneider, I., Roberts, D. et al. (1984). Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. *Science* **225**, 593-599.
- Doolan, D. L., Hoffman, S. L., Southwood, S., Wentworth, P. A., Sidney, J., Chestnut, R. W., Keogh, E., Apella, E., Nutman, T. B., Lal, A. A. et al. (1997). Degenerate cytotoxic T cell epitopes from P. falciparum restricted by HLA-A and HLA-B supertypes alleles. *Immunity* **7**, 97-112.
- Doolan, D. L., Southwood, S., Chesnut, R., Appella, E., Gomez, E., Richards, A., Higashimoto, Y. I., Maewal, A., Sidney, J., Gramzinski, R. A. et al. (2000). HLA-DR-promiscuous T cell epitopes from *Plasmodium falciparum* pre-erythrocytic-stage antigens restricted by multiple HLA class II alleles. *J. Immunol.* **165**, 1123-1137.
- Edelman, R., Hoffman, S. L., Davis, J. R., Beier, M., Sztein, M. B., Losonsky, G., Herrington, D. A., Eddy, H. A., Hollingdale, M. R., Gordon, D. M. et al. (1993). Long-term persistence of sterile immunity in a volunteer immunized with X-irradiated *Plasmodium falciparum* sporozoites. *J. Infect. Dis.* **168**, 1066-1070.
- Egan, J. E., Hoffman, S. L., Haynes, J. D., Sadoff, J. C., Schneider, I., Grau, G. E., Hollingdale, M. R., Ballou, W. R. and Gordon, D. M. (1993). Humoral immune responses in volunteers immunized with irradiated *Plasmodium falciparum* sporozoites. *Am. J. Trop. Med. Hyg.* **49**, 166-173.
- Hale, B. R. and Hoffman, S. L. (2003). A randomized, double-blind, placebo-controlled dose ranging trial of tafenaquine for weekly prophylaxis against *Plasmodium falciparum*. *Clin. Infect. Dis.* **36**, 541-549.
- Haynes, J. D., Diggs, C. L., Hines, F. A. and Desjardins, R. E. (1976). Culture of human malaria parasites *Plasmodium falciparum*. *Nature* **263**, 767-769.
- Herrington, D. A., Clyde, D. F., Losonsky, G., Cortesia, M., Murphy, J. R., Davis, J., Bager, S. and Felix, A. M. (1987). Safety and immunogenicity in man of a synthetic peptide malaria vaccine against *Plasmodium falciparum* sporozoites. *Nature* **328**, 257-259.
- Herrington, D., Davis, J., Nardin, E., Beier, M., Cortese, J., Eddy, H., Losonsky, G., Hollingdale, M., Sztein, M., Levine, M. et al. (1991). Successful immunization of humans with irradiated sporozoites: humoral and cellular responses of the protected individuals. *Am. J. Trop. Med. Hyg.* **45**, 539-547.
- Hilleman, M. R. (1987). Yeast recombinant hepatitis B vaccine. *Infection* **15**, 3-7.
- Hoffman, S. L. (1997). Experimental challenge of volunteers with malaria. *Ann. Intern. Med.* **127**, 233-235.
- Hoffman, S. L. and Miller, L. H. (1996). Perspectives on malaria vaccine development. In *Malaria Vaccine Development: A Multi-Immune Response Approach* (ed. S. L. Hoffman), pp. 1-13. Washington, DC: ASM Press.
- Hoffman, S. L., Franke, E. D., Hollingdale, M. R. and Druilhe, P. (1996). Attacking the infected hepatocyte. In *Malaria Vaccine Development: A Multi-Immune Response Approach* (ed. S. L. Hoffman), pp. 35-75. Washington, DC: ASM Press.
- Hoffman, S. L., Goh, L. M., Luke, T. C., Schneider, I., Le, T. P., Doolan, D. L., Sacci, J., de la Vega, P., Dowler, M., Paul, C. et al. (2002). Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. *J. Infect. Dis.* **185**, 1155-1164.
- Hoffman, S. L., Oster, C. N., Plowe, C. V., Woollett, G. R., Beier, J. C., Chulay, J. D., Wirtz, R. A., Hollingdale, M. R. and Mugambi, M. (1987). Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. *Science* **237**, 639-642.
- Kester, K. E., McKinney, D. A., Tornieporth, N., Ockenhouse, C. F., Heppner, D. G., Hall, T., Krzych, U., Delchambre, M., Voss, G., Dowler, M. G. et al. (2001). Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J. Infect. Dis.* **183**, 640-647.
- Krzych, U., Lyon, J. A., Jareed, T., Schneider, I., Hollingdale, M. R., Gordon, D. M. and Ballou, W. R. (1995). T lymphocytes from volunteers immunized with irradiated *Plasmodium falciparum* sporozoites recognize liver and blood stage malaria antigens. *J. Immunol.* **155**, 4072-4077.
- Long, C. A. and Hoffman, S. L. (2002). Parasitology: malaria - from infants to genomics to vaccines. *Science* **297**, 345-347.
- Malik, A., Egan, J. E., Houghten, R. A., Sadoff, J. C. and Hoffman, S. L. (1991). Human cytotoxic T lymphocytes against the *Plasmodium falciparum* circumsporozoite protein. *Proc. Natl. Acad. Sci. USA* **88**, 3300-3304.
- McCarthy, V. C. and Clyde, D. F. (1977). *Plasmodium vivax*: correlation of circumsporozoite precipitation (CSP) reaction with sporozoite-induced protective immunity in man. *Exp. Parasitol.* **41**, 167-171.
- Moreno, A., Clavijo, P., Edelman, R., Davis, J., Sztein, M., Herrington, D. and Nardin, E. (1991). Cytotoxic CD4+ T cells from a sporozoite-immunized volunteer recognize the *Plasmodium falciparum* CS protein. *Int. Immunol.* **3**, 997-1003.
- Moreno, A., Clavijo, P., Edelman, R., Davis, J., Sztein, M., Sinigaglia, F. and Nardin, E. (1993). CD4+ T cell clones obtained from *Plasmodium falciparum* sporozoite-immunized volunteers recognize polymorphic sequences of the circumsporozoite protein. *J. Immunol.* **151**, 489-499.
- Nardin, E. H. (1990). T cell responses in a sporozoite-immunized human volunteer and a chimpanzee. *Immunol. Lett.* **25**, 43-48.
- Nardin, E. H., Herrington, D. A., Davis, J., Levine, M., Stuber, D., Takacs, B., Caspers, P., Barr, P., Altszuler, R., Clavijo, P. et al. (1989). Conserved repetitive epitope recognized by CD4+ clones from a malaria-immunized volunteer. *Science* **246**, 1603-1606.
- Nardin, E. H., Nussenweig, R. S., Altszuler, R., Herrington, D., Levine, M., Murphy, J., Davis, J., Bathurst, I., Barr, P., Romero, P. et al. (1990). Cellular and humoral immune responses to a recombinant P. falciparum CS protein in sporozoite-immunized rodents and human volunteers. *Bull. WHO* **68**, 85-87.
- Nussenweig, R. S., Vanderberg, J., Most, H. and Orton, C. (1967). Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. *Nature* **216**, 160-162.

- Nussenzweig, V. and Nussenzweig, R. S. (1989). Rationale for the development of an engineered sporozoite malaria vaccine. *Adv. Immunol.* **45**, 283-334.
- Owusu-Agyei, S., Koram, K. A., Baird, J. K., Utz, G. C., Binka, F. N., Nkrumah, F. K., Fryauff, D. J. and Hoffman, S. L. (2001). Incidence of symptomatic and asymptomatic *Plasmodium falciparum* infection following curative therapy in adult residents of northern Ghana. *Am. J. Trop. Med. Hyg.* **65**, 197-203.
- Richie, T. L. and Saul, A. (2000). Progress and challenges for malaria vaccines. *Nature* **415**, 694-701.
- Rieckmann, K. H. (1990). Human immunization with attenuated sporozoites. *Bull WHO* **68**, 13-16.
- Rieckmann, K. H., Beaudoin, R. L., Cassells, J. S. and Sell, D. W. (1979). Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. *Bull WHO* **57**, 261-265.
- Rieckmann, K. H., Carson, P. E., Beaudoin, R. L., Cassells, J. S. and Sell, K. W. (1974). Sporozoite induced immunity in man against an Ethiopian strain of *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* **68**, 258-259.
- Stoute, J. A., Kester, K. E., Krzych, U., Welde, B. T., Hall, T., White, K., Glenn, G., Ockenhouse, C. F., Garcon, N., Schwenk, R. et al. (1998). Long-term efficacy and immune responses following immunization with the RTS,S malaria vaccine. *J. Infect. Dis.* **178**, 1139-1144.
- Trager, W. and Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science* **193**, 673-675.
- Wizel, B., Houghten, R. A., Parker, K., Coligan, J. E., Church, P., Gordon, D. M., Ballou, W. R. and Hoffman, S. L. (1995a). Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the *Plasmodium falciparum* surface sporozoite protein 2. *J. Exp. Med.* **182**, 1435-1445.
- Wizel, B., Houghten, R., Church, P., Tine, J. A., Lanar, D. E., Gordon, D. M., Ballou, W. R., Sette, A. and Hoffman, S. L. (1995b). HLA-A2-restricted cytotoxic T lymphocyte responses to multiple *Plasmodium falciparum* sporozoite surface protein 2 epitopes in sporozoite-immunized volunteers. *J. Immunol.* **155**, 766-775.
- Yoshida, N., Nussenzweig, R. S., Potocnjak, P., Nussenzweig, V. and Aikawa, M. (1980). Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. *Science* **207**, 71-73.

