Chapter 2
Preventing Childhood Malaria: Strategies That Work Today and Directions for the Future

Kim C. Williamson

Introduction

Malaria, an ancient plague named "Mal Air" by the Italians for its association with swampy areas, remains the most lethal single agent for children under the age of 5: it is responsible for 8% of all deaths in this population. One million children die each year, which is equivalent to one death every 30 s approximately the same number of people that die from HIV each year. Public awareness and funding for treatment and control has trailed significantly. One of the reasons for this is that 90% of the deaths are confined to children (Johansson, Newby, Renshaw, & Wardlaw, 2007). In addition, they live in sub-Saharan African, and so they are largely invisible to the industrialized world. However, malaria can be transmitted throughout the tropics and in temperate zones, including the USA and Europe, and it is as lethal to nonimmune adults as it is to young children. As with all infectious diseases drug-resistant strains have developed posing increased risk to travelers and residents. A number of economists also suggest that endemic diseases such as malaria are one of the primary obstacles to economic development (Teklehaimanot, McCord, & Sachs, 2007). In the case of malaria, 40% of the world's population is affected.

The parasite is introduced by the bite of an infectious mosquito, invades liver cells and then replicates in red blood cells. Every 2 days a single parasite multiplies into ~16 new parasites. This ruptures the RBC, releasing the parasites which are then free to invade new RBC and start the cycle over. The material released also causes fever, the only early symptom. The problem is that children often get fevers and most will resolve in a few days. However, if the infant has malaria, the fever does not go away but it gets progressively worse. As the parasite replicates, it adheres to the cells lining the capillaries, and as the number of infected red blood cells increases, the adherent cells can occlude the vessel blocking blood flow. This can lead to organ damage, coma, and death. In pregnant women, parasites sequester in the placenta affecting fetal development.

K.C. Williamson (✉)
Department of Biology, Loyola University, 6525 N Sheridan Rd, Chicago, IL 60626, USA
e-mail: kwilli4@luc.edu

J. Garbarino and G. Sigman (eds.), *A Child's Right to a Healthy Environment*,
The Loyola University Symposium on the Human Rights of Children 1,
DOI 10.1007/978-1-4419-6791-6_2, © Springer Science+Business Media, LLC 2010

As mentioned, the primary symptom of malaria is a fever and the only way to distinguish between malaria, which could be lethal if not treated, and a self-resolving common cold is to analyze a blood sample for the presence of parasites. In other words, a visit to a health clinic is necessary, which is not a trivial task for rural families whose livelihood is subsistence farming. Even if care is free, there are significant costs associated with taking the time and using limited resources to travel to and from the clinic with a sick child. Once at the clinic the malaria test is straightforward, only if you have a microscope or other commercially available tests. The next question is whether the clinic has the appropriate antimalarial drugs available. Supply, cost, and the drug sensitivity of the parasite are all issues. Imagine investing time, energy, and resources into carrying your child to the clinic, often by foot, only to find that there are no drugs or the drugs you get do not work. This outcome also makes it much less likely that the family will take the effort to visit the clinic the next time someone has a fever.

Unfortunately, this is not a hypothetical scenario. Over the past 30 years, parasites that are resistant to chloroquine, the mainstay of antimalarial treatment since the 1950s, have spread throughout the world (Hoffman & Miller, 1996). Chloroquine was in many respects a "wonder drug." A 3-day oral dose was effective against all four human malaria parasites, *Plasmodium malariae*, *ovale*, *vivax*, and *falciparum,* the last of which causes the most mortality. Chloroquine was inexpensive to produce, relatively nontoxic to humans and safe for use by children and pregnant women. There were no other antimalarials that even came close, and so public health officials were hesitant to change their drug recommendation until 60–70% of the malaria cases were insensitive to chloroquine, and it became apparent that the death rate was rising (Bryce, Boschi-Pinto, Shibuya, & Black, 2005). The second choice, Fansidar, a combination of pyrimethamine and sulfadoxine, was more expensive and drug-resistant parasites were also known to exist.

Through the 1990s, there was an overall decline in child mortality from all causes as the United Nations started to implement the Convention on the Rights of the Child that was adopted in 1989. Article 6 of the convention states that every child has the inherent right to life and that parties shall ensure to the maximum extent possible the survival and development of the child. Article 24 expands this by recognizing the right of the child to enjoy the highest attainable standard of health; in particular, the parties shall take appropriate measures:

24.2.a. To diminish infant and child mortality
24.2.b. To ensure provision of necessary medical assistance and healthcare
24.2.d. To ensure appropriate pre-natal and post-natal healthcare for mothers
24.4. To promote and encourage international cooperation

All of these matters have been lacking for children in the case of malaria risk and disease. In spite of the overall decline in child mortality, the death rate due to malaria increased during the 1990s. This caused real concern and in 1998 the Roll Back Malaria (RBM) Partnership was begun as a collaborative effort between the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank. Since

then additional governmental and private foundations, such as the Global fund for AIDS, TB and Malaria, the Bill and Melinda Gates Foundation, and the US Presidents Initiative against Malaria, have become involved in increasing the funds available from <$ 100 million in 1998 to $1 billion in 2008. The costs are high. With the tools currently available, it is estimated that three billion dollars a year would be required to control the disease and eliminate malaria mortality (Teklehaimanot, McCord, & Sachs, 2007). The only way to eliminate the need for control strategies is to develop new reagents that can effectively eradicate malaria. Some of these exciting recent advances are discussed at the end of talk. First, the basic targets of the RBM campaign, prompt effective chemotherapy, and protective measures which include insecticide treated-bed nets are discussed.

Chemotherapy

During the 1990s, as chloroquine-resistant parasites were spreading through Africa, a compound found in Wormwood or *Artemisia annua* that had been used as a fever treatment in China for centuries was actively being developed as an oral drug (Jiang et al. 1982; Cai, 1981; Gu, Liu, & Lu, 1981). This has been successful, and several artemisinin formulations, including combinations with other drugs, are commercially available from companies based in China as well as from Novartis. The use of two distinct antimalarial drugs in Artemisinin Combination Therapy or ACT is intended to decrease the development of drug resistance, as has been done with HIV medications. In 2002, WHO officially changed its recommendation to treat malaria to ACT. Novartis has agreed to provide the drug at cost, but it still costs greater than $1 per treatment, which is ten times more expensive than chloroquine. When you take into account the 300–400 million malaria cases annually, this increase has serious economic consequences. Initially, the production could not keep up with demand, but capacity has increased, as has the availability of *Artemisia annua* (Walther & Walther, 2007). Deliveries of ACT increased from 4 to 50 million treatments between 2004 and 2006 and the cost decreased to $1 per treatment. Formulations that are easy for young children to take by masking the bitter taste are still being evaluated. Major research efforts are also being directed toward the production of synthetic analogs and bacterial production methods that could reduce costs significantly (Linares & Rodriguez, 2007; Nosten & White, 2007). These and other projects are discussed in the Future Directions Section.

Intermittent antimalarial treatment of individuals at high risk for malaria, such as pregnant women, is also being used as a prevention strategy. It has been shown to protect women from severe malarial anemia and to improve the birth weights of their babies (Shulman et al., 1999). The current recommendation endorsed by the WHO and the United States Center for Disease Control (CDC) is that, during at least two of her routine antenatal clinic visits each pregnant woman should receive a treatment dose of an effective antimalarial drug. Due to concerns about the safety of ACT during pregnancy, the drug most commonly used for Intermittent Preventive

Treatment in pregnancy (IPTp) is sulfadoxine-pyrimethamine, even though some malaria parasites are resistant. This IPT strategy is also being tested for use in children as part of the Expanded Programme on Immunization (EPI). However, concerns about increasing the development of resistant parasites coupled with the paucity of good alternative antimalarial drugs has slowed its implementation (Walther & Walther, 2007).

Prevention

Insecticide Treated Bed Nets

Evidence for the efficacy of insecticide-treated bed nets (ITNs) has been growing since the first encouraging reports from small-scale trials in Gambia in 1987 (Snow, Rowan, & Greenwood, 1987; Snow, Rowan, Lindsay, & Greenwood, 1988; D'Alessandro et al., 1995). A large-scale trial in Western Kenya demonstrated that the use of ITN reduced the incidence of child mortality (16–20%), particularly among infants <12 months old (23–26%), and clinical malaria (44%) (Phillips-Howard et al., 2003; ter Kuile et al., 2003). A review of community randomized ITN trials indicated that ITN usage reduced the incidence of uncomplicated malaria episodes by 50 and 62% in areas of stable and unstable malaria transmission, respectively (Lengeler 2000). Increasing bed net usage to 80% of all children <5 years old and pregnant women in malaria endemic areas is one of the primary RBM targets (Johansson et al., 2007). Recently, a technique has been developed to incorporate insecticide directly into the fibers that will be used to make the net. This process has increased the effective life of the nets from 6 months to 5 years. These long-lasting insecticidal nets (LLIN) can be washed and reused. However, the nets remain expensive ranging from $5 to 7 dollars each, and so subsidized distribution programs and marketing approaches are needed to achieve high coverage (Teklehaimanot et al., 2007). ITN usage increased by three- to fivefold in 17 sub-Saharan countries between 2000 and 2005 and progress continues to be made (Johansson et al., 2007).

Mosquito Control

Indoor residual spraying (IRS) is also an effective approach to reduce malaria transmission in sub-Saharan Africa. Dichloro-diphenol-trichloroethane (DDT) remains the most economical and long last-lasting effective insecticide (Sadasivaiah, Tozan, & Breman, 2007). However, it persists in the environment for a long time, and it has been reported to have adverse effects on birds, although there is no clear evidence of toxicity to humans. The use of DDT was banned in the USA in 1972, and in 2001, the Stockholm convention included it on its list of persistent organic pollutants that were targeted for elimination. A provision allowed for DDT to be used for disease

vector control when locally safe, effective, and affordable alternatives are not available. Pyrethoids, which are used in ITN, are more environmentally friendly and can be used as an alternative to DDT. However, they are more expensive and evidence of resistance has been seen which could affect the efficacy of ITN as well. The development of alternative economical insecticides remains a major need.

Public Health

Educating parents and community healthcare workers about the importance of prompt diagnosis and appropriate treatment for fevers, as well as completing the full treatment course are other key components to reduce mortality and the spread of resistant parasites (Walther & Walther, 2007). Both education and access to prompt treatment requires a good local public health system, including the coverage of rural areas where malaria is most prevalent. In coastal Kenya, only 32% of the population lives within 2 km of a government dispensary or private clinic, therefore it is a long walk to obtain treatment (Goodman et al., 2007). The WHO is increasingly encouraging home-based management; however, this also has associated risks. In Togo and again in coastal Kenya, 69–70% of young children (<5 years old) with fevers were treated with medicine obtained from a medicine-seller rather than a village health worker, even though the medicine from the health worker is cheaper. The medicine sellers are usually closer, open longer hours, less likely to be out of medicine, and perceived as friendlier, and more approachable; however, they are also less likely to distribute the correct medicine and dose. Preliminary studies indicate that training improves the distribution of the appropriate dose and medication and demonstrates the efficacy of including the medicine sellers, local faith-based organizations, and schools in the public health campaigns. Developing and maintaining these education and healthcare programs is expensive and accounts for over 50% of the cost of malaria control (Teklehaimanot et al., 2007). Large-scale sustained implementation of these techniques could eliminate deaths from malaria, but new strategies are needed to actually eradicate the disease.

Ongoing monitoring of malaria cases and the environmental conditions known to be associated with increased transmission is also needed to provide time for public heath officials to respond to outbreaks (Breman & Holloway, 2007). Such surveillance is also critical for the early identification of the development of resistant parasites or mosquitoes. Early warning of changes in the pattern of disease allows time to identify the cause and develop an effective response.

The Future

The complete genomes of the three organisms involved, humans, *Plasmodium falciparum* parasites, and *Anopheles* mosquito, have been released and are fully

accessible on the web (Gardner et al., 2002; Holt et al., 2002; Venter et al., 2001; www.genome.gov, www.plasmDB.org, agambiae.vectorbase.org). This provides key resources for investigators to identify unique genes that can be targeted for drug and/or vaccine development. High throughput techniques also allow genetic comparisons of isolates from all over the world that have a variety of characteristics. The information obtained can be used to link specific genes with specific characteristics that contribute to virulence. For example, the parasite gene that confers resistance to chloroquine was identified using this strategy (Fidock et al., 2000).

Chemotherapy Development

Several approaches have been taken to identify new drug candidates. The comparative genomic analysis described above revealed a set of genes that are more closely related to bacteria than to other higher organisms such as humans. Some of these genes are similar to those that are already known to be the target of antibiotics that have been approved for human use, including doxycycline, clindamycin, and rifampicin (Goodman, Su, & McFadden, 2007). These compounds are now being tested for efficacy against malaria. Once an effective compound is identified a series of related structures are produced to attempt to improve specificity against malaria. This type of ongoing optimization has also been used to develop new derivatives of chloroquine and artemisinin that are also effective against the parasite (Medicines for Malaria Venture, www.mmv.org). The development of high throughput assays for in vitro antimalarial activity has facilitated screening large libraries of compounds (Weisman et al., 2006).

In addition to targeting the parasite directly, therapies can be developed against the pathology caused by malaria. Many of the symptoms of severe malaria are thought to be due to red blood cell rupture during the release of the parasite and the ability of *Plasmodium falciparum* to adhere to the endothelial cells that line capillaries. It has recently been shown that intravenous administration of the amino acid, arginine, reversed the endothelial dysfunction associated with severe malaria (Yeo et al., 2007). The clinical effectiveness of this treatment is now being evaluated. Similar approaches could be taken to enhance or reverse other physiologic and immunologic responses to the parasite. Advances in understanding the genes that are involved in malaria susceptibility, as well as the development of a protective immune response could also contribute to the identification of high risk individuals and allow customized treatment (Knight, 2005; Taylor, Ferdig, Su, & Wellems, 2000)

Vaccines

Ideally, a malaria vaccine would be safe and affordable. It would be administered as part of the ongoing EPI and confer lifelong immunity against the disease.

Currently, none of the available malaria vaccine candidates meet these criteria, but they could provide partial protection against severe disease and contribute an integrated malaria control strategy. In light of this, the WHO determined that a malaria vaccine with an effectiveness of 30–50% would be justified to be licensed in light of the magnitude of malaria's morbidity and mortality (Moorthy, Reed, & Smith, 2007).

Malaria vaccines have been traditionally divided into preerythrocytic or sporozoite vaccines, blood stage or merozoite vaccines, and mosquito stage or transmission blocking vaccines depending on the stage of the parasite life cycle that is targeted. To date, over 40 malaria proteins have been identified as possible vaccine candidates (Hoffman & Miller, 1996; Girard, Reed, Friede, & Kieny, 2007). The most advanced candidate to date is a preerythrocytic vaccine that will be the primary focus of this discussion. A preerythrocytic vaccine targets the sporozoite released during the bite of an infected mosquito and blocks the development of the disease. If partially effective, it could decrease parasite burden and consequently lower the incidence of clinical disease. The other types of vaccines do not prevent the initial infection, but could protect against the expansion of the parasite population in the blood or block transmission to the mosquito and spread to another human.

The importance of sporozoites as vaccine candidates was highlighted by the finding that radiation-attenuated *Plasmodium* sporozoites induced protective immunity in immune-naïve vertebrate hosts (Mulligan, Russell, & Mohan, 1941; Nussenzweig, Vanderberg, Most, & Orton, 1967). These encouraging results were then confirmed in humans using irradiated *Plasmodium falciparum* sporozoites (Clyde, 1975; Clyde, McCarthy, Miller, & Hornick, 1973; Clyde, Most, McCarthy, & Vanderberg, 1973; Rieckmann, 1990; Rieckmann et al., 1974; Hoffman et al., 2002). Volunteers exposed to irradiated sporozoites administered via mosquito bites developed protection against subsequent challenges with infectious parasites. Protection was directly related to the number of immunizing bites (Hoffman et al., 2002). However, this vaccine approach was thought to be impractical because it requires a large number of infectious sporozoites that can only be produced in mosquitoes. The use of subunit vaccines produced using recombinant DNA techniques developed in the 1980s seemed like a much more reasonable approach to an economical malaria vaccine and were the focus of further research efforts.

The first step in developing a subunit vaccine is to identify a parasite protein that is the target of a protective immune response. Many years of research resulted in the identification of a protein on the sporozoite surface, the Circumsporozoite Protein (CSP), that is an important vaccine target (Hoffman, Franke, Holligdale, & Druilhe, 1996). This work led to the addition of a portion of the CSP protein to the hepatitis B subunit vaccine, and the combination called RTS,S, is currently the most advanced malaria vaccine. Plans are in place for a large clinical trial in the near future (Aponte et al., 2007). The encouraging RTS,S vaccine results are a major milestone in malaria vaccine development. Although the current RTS,S formulation still does not confer sterilizing immunity, the studies to date all consistently

demonstrate a 20–60% decrease in severe malaria in children under 1 year of age (Aponte et al., 2007; Alonso et al. 2004; 2005; Bojang et al., 2001; Macete et al., 2007). In conjunction with the other available malaria control measures, such as ITN and prompt ACTs treatment, this vaccine could be an important component of an integrated strategy to eliminate malaria mortality. Further investigation into the mode of action of RTS,S may also facilitate the development of other malaria vaccines.

More research is required to identify parasite components that trigger a fully protective immune response. It is clear that an immune response can be stimulated by the vaccine candidates that are currently available, but the response is not strong enough to completely block parasite development. People living in endemic areas have a similar response to natural exposure to the parasites. Multiple exposures are needed to gradually develop protection against clinical symptoms, but even after many years they are still not completely protected (Schofield & Mueller, 2006).

Clearly, there is strong selective pressure on both the parasite and the human to coexist. It could be argued that a moderate host immune response maintained by continual re-exposure to the parasite evolved to balance damage caused by the parasite and the host immune response. One of the strategies used by the parasite to evade our immune response is varying the type of proteins on its surface (Smith & Craig, 2005). To become immune, a person has to generate a response to a number of these different types of proteins. This also means that a vaccine might need to include a variety of protein types to be effective. Advances in basic immunology leading to methods to increase the immune response to parasite proteins and to promote long-lasting immunity should contribute to the efficacy of future malaria vaccines.

After 25 years of focusing primarily on subunit vaccines, there is now renewed interest in developing whole parasite vaccine strategies, such as irradiated attenuated sporozoites (Hoffman et al., 2002; Labaied et al., 2007; Mueller, Labaied, Kappe, & Matuschewski, 2005). The recent demonstration that genetically attenuated sporozoites also effectively induce sterilizing immunity in mice provides an alternative to irradiation. However, it still remains unlikely that an attenuated sporozoite vaccine will be widely distributed until an efficient in vitro system is developed to produce infectious attenuated sporozoites. Other methods that have been shown to effectively provide protection against clinical malaria, such as the use of low dose parasite inoculation, passive transfer of antibodies from immune adults to malaria patients, or vaccination with parasite-produced antigens, all raise similar cost, safety, and production concerns (Pombo et al., 2002; Cohen, Mc, & Carrington, 1961; Bouharoun-Tayoun, Attanath, Sabchareon, Chongsuphajaisiddhi, & Druilhe, 1990). Further evaluation of the protective immune responses induced by these methods and coupled with the recent identification of the set of genes expressed at each stage of the life cycle (Tarun et al., 2008; Young et al., 2005; Le Roch, Johnson, & Florens, 2004; Bozdech et al. 2003) with innovative techniques to economically produce attenuated-parasites and/or reproduce a protective immune response should lead to advances that could be applied in the field.

Mosquito Control

Historically, insect vectors have been important targets for disease control programs (Catteruccia, 2007). The success of ITN and IRS programs in decreasing malaria mortality and morbidity are recent examples. However, as with malaria chemotherapy, the development of resistance to the limited number of cost effective insecticides available is a concern and additional economical, ecologically safe alternatives are needed. Other strategies include the development of biological control measures, such as using species of bacteria, fungus or fish that specifically affect mosquitoes instead of other animals (Breman & Holloway, 2007; Schote et al., 2005; Blanford et al., 2005; Knols, Bossin, Mukabana, & Robinson, 2007; Lacey, 2007). Advances in genetic engineering have also led to the production of *Anopheles* mosquitoes that have a very limited ability to transmit parasites that cause malaria in rodents. (Ito, Ghosh, Moreira, Wimmer, & Jacobs-Lorena, 2002). Unfortunately, there was no reduction in the ability of these mosquitoes to transmit the human malaria parasite, *P. falciparum*, but similar techniques are being used to identify modifications that will block *P. falciparum* transmission (Knols et al., 2007).

Summary

The tools are currently available to effectively treat malaria, but as with many resources the problem is distribution. Individuals with malaria symptoms need access to prompt diagnosis and treatment with the appropriate drugs. Currently, both infrastructure and cost are major obstacles. The UN's efforts to promote the Rights of Children have increased global awareness of the problem and catalyzed steps to improve the situation. Public health programs through the RBM campaign and its many public and private partners are having an immediate effect, but these initial efforts have also served to reveal the magnitude of the problem. Active engagement and empowerment of the people directly affected to creatively address the problem utilizing established community networks will also be critical to sustainability. Further advances in the development of new drugs, vaccines, and mosquito control methods are needed to augment our ability to efficiently and economically eliminate malaria mortality and hopefully one day eradicate the disease completely.

Acknowledgments Kim C. Williamson received financial support from Public Health Service grants AI40592 and AI48826 from the National Institute of Allergy and Infectious Disease.

References

Alonso, P. L., Sacarlal, J., Aponte, J. J., et al. (2004). Efficacy of the rts, s/as02a vaccine against Plasmodium falciparum infection and disease in young African children: Randomised controlled trial. *Lancet, 364*, 1411–1420.

Alonso, P. L., Sacarlal, J., Aponte, J. J., et al. (2005). Duration of protection with rts, s/as02a malaria vaccine in prevention of Plasmodium falciparum disease in Mozambican children: Single-blind extended follow-up of a randomised controlled trial. *Lancet, 366*, 2012–2018.

Aponte, J. J., Aide, P., Renom, M., et al. (2007). Safety of the rts, s/as02d candidate malaria vaccine in infants living in a highly endemic area of Mozambique: A double blind randomised controlled phase I/IIb trial. *Lancet, 370*, 1543–1551.

Bojang, K. A., Milligan, P. J., Pinder, M., et al. (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.

Bouharoun-Tayoun, H., Attanath, P., Sabchareon, A., Chongsuphajaisiddhi, T., & Druilhe, P. (1990). Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *The Journal of Experimental Medicine, 172*, 1633–1641.

Bozdech, Z., Llinas, M., Pulliam, B. L., Wong, E. D., Zhu, J., & DeRisi, J. L. (2003). The transcriptome of the intraerythrocytic developmental cycle of Plasmodium falciparum. *PLoS Biology, 1*, E5.

Blanford, S., Chan, B. H., Jenkins, N., et al. (2005). Fungal pathogen reduces potential for malaria transmission. *Science, 308*, 1638–1641.

Breman, J. G., & Holloway, C. N. (2007). Malaria surveillance counts. *The American Journal of Tropical Medicine and Hygiene, 77*, 36–47.

Bryce, J., Boschi-Pinto, C., Shibuya, K., & Black, R. E. (2005). Group WHOCHER. Who estimates of the causes of death in children. *Lancet, 1*, 885–888.

Cai, X. Z. (1981). Observation of therapeutic effect of single-dose combined administration of qinghaosu, sulphomethoxine, pyrimethamine and primaquine in the treatment of chloroquine-resistant malignant malaria (author's transl). *Chung Hua Nei Ko Tsa Chih, 20*, 724–727.

Catteruccia, F. (2007). Malaria vector control in the third millennium: Progress and perspectives of molecular approaches. *Pest Management Science, 63*, 634–640.

Clyde, D. F. (1975). Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. *The American Journal of Tropical Medicine and Hygiene, 24*, 397–401.

Clyde, D. F., McCarthy, V. C., Miller, R. M., & Hornick, R. B. (1973). Specificity of protection of man immunized against sporozoite-induced falciparum malaria. *The American Journal of the Medical Sciences, 266*, 398–403.

Clyde, D. F., Most, H., McCarthy, V. C., & Vanderberg, J. P. (1973). Immunization of man against sporozoite-induced falciparum malaria. *The American Journal of the Medical Sciences, 266*, 398–403.

Cohen, S., Mc, G. I., & Carrington, S. (1961). Gamma-globulin and acquired immunity to human malaria. *Nature, 192*, 733–737.

D'Alessandro, U., Olaleye, B. O., McGuire, W., et al. (1995). Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet, 345*, 479–483.

Fidock, D. A., Nomura, T., Talley, A. K., et al. (2000). Mutations in the P. falciparum digestive vacuole transmembrane protein pfcrt and evidence for their role in chloroquine resistance. *Molecular Cell, 6*, 861–871.

Gardner, M. J., Hall, N., Fung, E., et al. (2002). Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature, 419*, 498–511.

Girard, M. P., Reed, Z. H., Friede, M., & Kieny, M. P. (2007). A review of human vaccine research and development: Malaria. *Vaccine, 13*, 1263–1276.

Goodman, C., Brieger, W., Unwin, A., Mills, A., Meek, S., & Greer, G. (2007). Medicine sellers and malaria treatment in sub-Saharan Africa: What do they do and how can their practice be improved? *The American Journal of Tropical Medicine and Hygiene, 77*, 203–218.

Goodman, C. D., Su, V., & McFadden, G. I. (2007). The effects of anti-bacterials on the malaria parasite Plasmodium falciparum. *Molecular and Biochemical Parasitology, 152*, 181–191.

Gu, H. M., Liu, M. Z., Lu, B. F., et al. (1981). Antimalarial effect and toxicity of methyl-dihydroartemisinine in animals (author's transl). *Chung Kuo Yao Li Hsueh Pao, 2*, 138–144.

Hoffman, S. L., Franke, E. D., Hollingdale, M. R., & Druilhe, P. (1996). Attacking the infected hepatocyte. In S. L. Hoffman (Ed.), *Malaria vaccine development: A multi-immune response approach* (pp. 35–75). Washington, D.C.: American Society for Microbiology.

Hoffman, S. L., Goh, L. M., Luke, T. C., et al. (2002). Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. *The Journal of Infectious Diseases, 185*, 1155–1164.

Hoffman, S. L., & Miller, L. H. (1996). *Perspectives on malaria vaccine development*. ASM Press. Washington, D.C.

Holt, R. A., Subramanian, G. M., Halpern, A., et al. (2002). The genome sequence of the malaria mosquito anopheles Gambia. *Science, 298*, 129–149.

Ito, J., Ghosh, A., Moreira, L. A., Wimmer, E. A., & Jacobs-Lorena, M. (2002). Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature, 417*, 452–455.

Jiang, J. B., Li, G. Q., Guo, X. B., Kong, Y. C., & Arnold, K. (1982). Antimalarial activity of mefloquine and qinghaosu. *Lancet, 1*, 885–888.

Johansson, E.W., Newby, H., Renshaw, M., & Wardlaw, T. (2007). Malaria & children. United Nations Children's Fund.

Knight, J. C. (2005). Regulatory polymorphisms underlying complex disease traits. *Journal of Molecular Medicine, 83*, 97–109.

Knols, B. G., Bossin, H. C., Mukabana, W. R., & Robinson, A. S. (2007). Transgenic mosquitoes and the fight against malaria: Managing technology push in a turbulent GMO world. *The American Journal of Tropical Medicine and Hygiene, 77*, 232–242.

Labaied, M., Harupa, A., Dumpit, R. F., Coppens, I., Mikolajczak, S. A., & Kappe, S. H. (2007). Plasmodium yoelii sporozoites with simultaneous deletion of p52 and p36 are completely attenuated and confer sterile immunity against infection. *Infection and Immunity, 75*, 3758–3768.

Lacey, L. A. (2007). Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control. *Journal of the American Mosquito Control Association, 23*(2 Suppl), 133–163.

Lengeler, C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database System Review* (2):CD000363.

Le Roch, K. G., Johnson, J. R., Florens, L., et al. (2004). Global analysis of transcript and protein levels across the Plasmodium falciparum life cycle. *Genome Research, 14*, 2308–2318.

Linares, G. E., & Rodriguez, J. B. (2007). Current status and progresses made in malaria chemotherapy. *Current Medicinal Chemistry, 14*, 289–314.

Macete, E., Aponte, J. J., Guinovart, C., et al. (2007). Safety and immunogenicity of the RTS, S/AS02 candidate malaria vaccine in children aged 1–4 in Mozambique. *Tropical Medicine and International Health, 12*, 37–46.

Moorthy, V., Reed, Z., & Smith, P. G. (2007). Efficacy WHOSGoMoMV. Measurement of malaria vaccine efficacy in phase III trials: Report of a WHO consultation. *Vaccine, 13*, 1263–1276.

Mueller, A. K., Labaied, M., Kappe, S. H., & Matuschewski, K. (2005). Genetically modified Plasmodium parasites as a protective experimental malaria vaccine. *Nature, 433*, 164–167.

Mulligan, H. W., Russell, P. F., & Mohan, B. N. (1941). Active immunization of fowls against Plasmodium gallinaceum by infections of killed homologous sporozoites. *Journal of Malaria Institute of India, 4*, 24–34.

Nosten, F., & White, N. J. (2007). Artemisinin-based combination treatment of falciparum malaria. *The American Journal of Tropical Medicine and Hygiene, 77*, 181–192.

Nussenzweig, R. S., Vanderberg, J., Most, H., & Orton, C. (1967). Protective immunity produced by the injection of x-irradiated sporozoites of Plasmodium berghei. *Nature, 216*, 160–162.

Phillips-Howard, P. A., Nahlen, B. L., Kolczak, M. S., et al. (2003). Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. *The American Journal of Tropical Medicine and Hygiene, 68*, 23–29.

Pombo, D. J., Lawrence, G., Hirunpetcharat, C., et al. (2002). Immunity to malaria after administration of ultra-low doses of red cells infected with Plasmodium falciparum. *Lancet, 360*, 610–617.

Rieckmann, K. H. (1990). Human immunization with attenuated sporozoites. *Bulletin of the World Health Organization, 55*, 363–365.

Rieckmann, K. H., Carson, P. E., Beaudoin, R. L., Cassells, J. S., & Sell, K. W. (1974). Letter: Sporozoite induced immunity in man against an Ethiopian strain of Plasmodium falciparum. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 76*, 812–818.

Sadasivaiah, S., Tozan, Y., & Breman, J. G. (2007). Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: How can it be used for malaria control? *The American Journal of Tropical Medicine and Hygiene, 77*, 249–263.

Schofield, L., & Mueller, I. (2006). Clinical immunity to malaria. *Current Molecular Medicine, 6*, 205–221.

Scholte, E. J., Ng'habi, K., Kihonda, J., et al. (2005). An entomopathogenic fungus for control of adult African malaria mosquitoes. *Science, 308*, 1641–1642.

Shulman, C. E., Dorman, E. K., Cutts, F., et al. (1999). Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: A randomised placebo-controlled trial. *Lancet, 353*, 632–636.

Smith, J. D., & Craig, A. G. (2005). The surface of the Plasmodium falciparum-infected erythrocyte. *Current Issues in Molecular Biology, 7*, 81–93.

Snow, R. W., Rowan, K. M., & Greenwood, B. M. (1987). A trial of permethrin-treated bed nets in the prevention of malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 76*, 812–818.

Snow, R. W., Rowan, K. M., Lindsay, S. W., & Greenwood, B. M. (1988). A trial of bed nets (mosquito nets) as a malaria control strategy in a rural area of the Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 76*, 812–818.

Tarun, A. S., Peng, X., Dumpit, R. F., et al. (2008). A combined transcriptome and proteome survey of malaria parasite liver stages. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 305–310.

Taylor, J. G., Ferdig, M. T., Su, X. Z., & Wellems, T. E. (2000). Toward quantitative genetic analysis of host and parasite traits in the manifestations of Plasmodium falciparum malaria. *Current Opinion in Genetics & Development, 10*, 314–319.

Teklehaimanot, A., McCord, G. C., & Sachs, J. D. (2007). Scaling up malaria control in Africa: An economic and epidemiological assessment. *American Journal of Tropic Medicine and Hygiene, 77*, 138–144.

ter Kuile, F. O., Terlouw, D. J., Kariuki, S. K., et al. (2003). Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *The American Journal of Tropical Medicine and Hygiene, 68*, 68–77.

Venter, J. C., Adams, M. D., Myers, E. W., et al. (2001). The sequence of the human genome. *Science, 291*, 1304–1351.

Walther, B., & Walther, M. (2007). What does it take to control malaria? *Annals of Tropic Medicine Parasitology, 86*, 207–215.

Weisman, J. L., Liou, A. P., Shelat, A. A., Cohen, F. E., Guy, R. K., & DeRisi, J. L. (2006). Searching for new antimalarial therapeutics amongst known drugs. *Chemical Biology & Drug Design, 67*, 409–416.

Yeo, T. W., Lampah, D. A., Gitawati, R., et al. (2007). Impaired nitric oxide bioavailability and l-arginine reversible endothelial dysfunction in adults with falciparum malaria. *The Journal of Experimental Medicine, 204*, 2693–2704.

Young, J. A., Fivelman, Q. L., Blair, P. L., et al. (2005). The Plasmodium falciparum sexual development transcriptome: A microarray analysis using ontology-based pattern identification. *Molecular and Biochemical Parasitology, 143*, 67–79.