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Immuno-pathophysiological consequences in malaria: A brief review

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Abstract
Malaria, a global public health burden is estimated approximately 250 million cases reported worldwide every year with 1.5 to 2.7 million deaths annually. These deaths are primarily among children under 5 years of age and pregnant women in sub-Saharan Africa. About 94% of reported cases were recorded in the African region. 13 out of about 200 different identified protozoal species are known to be pathogenic to humans. The life cycle of the malaria parasite is a complex process comprising an *Anopheles* mosquito and a vertebrate host. Its pathophysiology is characterized by fever secondary to the rupture of erythrocytes, macrophage ingestion of merozoites, and/or the presence of antigen-presenting trophozoites in the circulation or spleen which mediates the release of tumor necrosis factor α (TNF-α). Malaria can be diagnosed through clinical observation of the signs and symptoms of the disease. Other diagnostic techniques used to diagnose malaria are the microscopic detection of parasites from blood smears and antigen-based rapid diagnostic tests. The management of malaria involves preventive and/or curative approaches. Since untreated uncomplicated malaria can progress to severe malaria. To prevent or delay the spread of anti-malarial drug resistance, WHO recommends the use of combination therapy for all episodes of malaria with at least two effective anti-malarial agents having a different mechanism of action. The Centers for Disease Control (CDC) emphasizes that there is no prophylactic agent that can prevent malaria 100%. Therefore, prophylaxis shall be augmented with the use of personal protective measures.

Keywords: Malaria, Parasite, *Plasmodium falciparum*, G6PD, Immunity

Introduction

Malaria is caused by transmission of single-celled protozoan parasites of genus *Plasmodium* from an *Anopheles* mosquito vector, to a suitable vertebrate host. Whilst there are over 200 species of *Plasmodium*, only five species have the capability to cause malarial illness in humans. *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovalae* (*P. ovalae*) *and Plasmodium malariae* (*P. malariae*) are all human specific parasites; out of which *P. falciparum* causes serious and *P. vivax, P. ovalae, P. malariae* causes milder forms of the disease [1]. More recently a fifth human species *Plasmodium knowlesi* (*P. knowlesi*) has being identified which can cause human infections from zoonotic transfer from macaques monkeys (*Macaca fascicularis*) [2]. At the present time the most dominant parasite is *P. falciparum* which accounts for 40-60% of malaria cases worldwide and >95% of all malaria deaths [3].

The transmission of malaria from the vector-mosquito was first described by Ronald Ross in 1898, at which time his discovery was described as 'important as the discovery of America'. Notwithstanding the massive strides in biology and medicine in the past 100 years, malaria still poses the greatest threat of all known parasites to human health. Despite an increased awareness of the disease, improved access to anti-malarial drugs and global economic development, more people die from malaria today than 40 years ago with an estimated incidence of infection increasing 2-3 fold over the last 35 years. Furthermore, with increasing drug resistance as well as changes in world climate, malaria is returning to areas from which it had previously been eradicated and is now entering new areas such as Eastern Europe and Central Asia [4].

Epidemiology

The 2008 World Malaria Report estimated that in 2006 there were 3.3 billion people at risk of malaria infection of whom 247 million had a reported malaria infection, resulting in nearly 1 million deaths. Those most at risk from fatal malarial infections included children under the age of 5years, pregnant women and the immunocompromised patients [5]. It has been suggested that reported Figs from the World Health Organization (WHO) could be an underestimation of the annual burden of this parasitic disease [6].

Malaria is endemic throughout the tropical areas of sub-Saharan Africa, Southeast Asia, the Pacific Islands, India and Central and South America. In 2008, a total of 109 countries were declared to be malaria endemic with 45 of these countries falling within the WHO African region. Statistically, this equates to approximately 40% of the world's population being under threat from this parasitic disease [5].

Pathophysiology and etiology

1. Historical background

The symptoms of a disease resembling malaria were first described over 4,000 years ago in the *Nei Ching* (The Canon of Medicine), the ancient Chinese medical writings edited by Emperor Huang Ti in 2700 BC [7]. Features of the disease, later named malaria, Italian for bad air, became widely recognized in 4th century BC after Hippocrates characterized the clinical symptoms and complications of this seasonal intermittent fever (In November 1880, Charles Laveran, microscopically observed the exflaggelation of a male gametocyte which led to the conclusion that the causative agent of malaria was a protozoan parasite [8]. During the next 10-20 years, four different human species of malaria parasite were identified and named by Italian and

American scientists (*P. falciparum*, *P. vivax*, *P. ovalae*, and *P. malariae*). Perhaps the greatest advancement in the study of malaria was the discovery of the role of the *Anopheles* mosquito in the transmission of malaria. On 20th August 1897 Ronald Ross found the malaria parasite within the stomach tissue of an Anopheline mosquito that had fed on a malaria-infected patient four days earlier [9].

In further malaria research Ross showed the transmission of malaria parasites between birds demonstrating that the mosquito acted as an intermediate host for the avian malaria. After feeding mosquitoes with blood from infected birds he showed that the malaria parasite developed in the mosquito stomach and later migrated to the salivary glands, allowing the mosquito to then infect other birds during subsequent blood meals. The complete sporogenic cycles of *P. falciparum*, *P. vivax and P. malariae* were soon after described by a team of Italian scientists led by Giovanni Grassi (1898-1899). Thus, the process of malaria transmission was established. By 6th century BC the symptoms of malarial fever, which were attributed to the bites of certain insects, were extensively described in the *Susruta*, a Sanskrit text. During the same time period Roman writers associated the incidence of malarial fever to swampy regions. Despite the continued characterization of disease processes, the cause of malaria was not isolated until 1880 [7-9].

2. Life cycle

The life cycle of malaria parasite has been demonstrated in Fig 1. The genus *Plasmodium* may be identified taxonomically by the presence of protozoa with two forms of asexual division, schizogony and sporogony, and a single stage of sexual division. Schizogony is the phase of parasitic asexual division and maturation occurring within the vertebrate host, while sporogony is the sexual and asexual and divisional stage occurring with a mosquito vector. In order to successfully complete the parasitic life cycle two hosts, the definitive and intermediate hosts, must be present [10].

The female *Anopheles* mosquito is the sole vector of *Plasmodium* parasites and whilst there are over 600 species of *Anopheles* mosquitoes worldwide, only 60 species have been found to transmit malaria. The schizogonic cycle is initiated when a malaria-infected mosquito takes a blood meal from a person, releasing on average 15-20, but up to 100 sporozoites, into the blood circulation from the salivary glands [1]. After entering the bloodstream the sporozoites take about 30-45 min to travel to the liver where they invade hepatocytes and begin to multiply. This cycle, referred to as the pre-erythrocytic cycle, takes approximately 6-14 days and results in the production of merozoites. Growth and division in the liver takes approximately 6, 6, 10 and 15 days for *P. falciparum*, *P. vivax*, *P. ovalae* and *P. malariae*, respectively. In *P. vivax* and *P. ovalae* infections some sporozoites appear to develop for the first 24 h at which stage they become dormant, as single-celled forms known as hypnozoites, which can remain in the hepatocytes for months to years until they are reactivated causing a relapsed malaria infection [3, 4, 11].

At the conclusion of the pre-erythrocytic cycle, the host liver cells burst releasing thousands of merozoites into the blood circulation which attach to and invade erythrocytes within 20 sec of release. This process initiates the erythrocytic cycle. Within the erythrocytes the parasites begin to again multiply resulting in erythrocyte (red blood cell) rupture 48-72 hr later, depending on parasite species. Following erythrocyte rupture, both merozoites and pyrogenic materials are released resulting in both an increase in parasite biomass and malarial symptoms of fever and anaemia. This asexual erythrocytic cycle usually continues until it is either controlled through immune responses, drug therapy or until host death. The morbidity and mortality associated with malaria are derived primarily from the erythrocytic cycle [1, 4, 11].

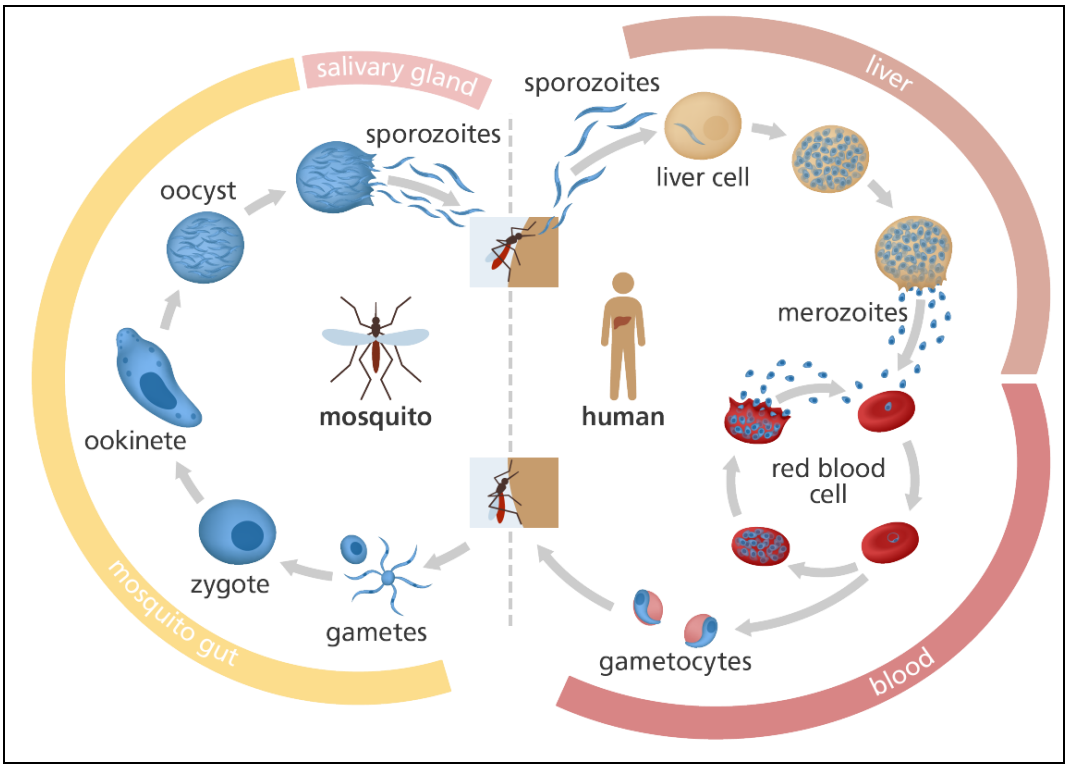

Fig 1: The life cycle of human malarial parasites.

After several erythrocytic cycles a yet unidentified trigger diverts the development of certain intra-erythrocytic merozoites into sexual forms known as gametocytes. The male and female gametocytes are taken up by a mosquito and in the mosquito midgut they mature as gametes. The sporogonic cycle is initiated through the fertilization of the gametes producing zygotes which within 24 hr matures to a motile ookinete. The ookinetes burrow through the mosquito midgut wall to encyst on the basal lamina where, within the developing oocyts, there are many miotic divisions resulting in the formation of sporozoites. When the infective oocysts rupture sporozoites are released at which time they migrate through the haemocoel to the salivary glands completing the sporogonic cycle, approximately 7 to 30 days after gametocyte ingestion (depending on the host, infective parasite species and environmental conditions [1, 4, 11].

Clinical immunity to severe non-cerebral falciparum malaria usually occurs after one or two infections, however, immunity against mild disease takes much longer to acquire. In malaria endemic regions, children born to immune mothers are protected against malaria infections, although they may be exposed to infections, for the first 6 months after birth by maternal antibodies transferred through breast milk. During this period of time the immune system in the infants compiles a repertoire of specific humoral and cellular immune responses against the infecting parasites. However, as exposure to maternal antibodies wanes, the child will have a period of 1 to 2 years of increased susceptibility to malaria infections before they are able to acquire active immunity [12-15].

3. Malaria and the immune system

Innate immunity, the immunity not associated with specific antigens, may be classified into genetically based resistance and cell mediated mechanisms. During malaria infections genetically based resistance mechanisms may influence the progression of disease through the impairment of merozoite invasion of erythrocytes, reduction in the growth of parasites within an erythrocyte, impaired liberation of merozoites from schizonts and the reduction in vitality of merozoites after they are released from the rupturing schizont [16, 17]. Cell mediated mechanisms are responsible for the phagocytosis of merozoites or parasitised erythrocytes by neutrophils, monocytes or macrophages, and through the production of cytotoxic molecules (i.e. cytokines or nitric oxide) which are produced by various immunological cells against both the free parasites or parasitised erythrocytes [13]. Whilst the innate immune system, comprising mainly dendritic cells, monocytes and macrophages, natural killer cells, and T cells, is an important defence mechanism against malaria infections it also plays a very important role in shaping the adaptive immune response to malaria [18].

In malaria endemic regions a person will often be exposed to a number of malaria infections during childhood. Surviving the malaria infection may result in the development of a state of immunity, referred to as acquired immunity, where a low level parasitaemia is maintained whilst remaining asymptomatic [15]. Acquired immunity, immunity developed in response to foreign antigens in the body, is both species- and stage- specific and results from the generation of specific antibodies to several variant antigenic proteins, most notably *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1), which are produced by trophozoite and schizont stage parasites and expressed on the surface of parasitised erythrocytes. However, as there are over 50 genes that encode PfEMP-1 molecules, acquired immunity to *P. falciparum* arises after multiple infections. Whilst acquired immunity does not prevent future re-infection with *P. falciparum*, the inflammatory response to the parasites, causes the acute symptoms, limited and mechanisms to inhibit parasite replication enhanced.

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The role of tissues in immunity to blood stage malaria parasites

The spleen participates in immune responses against many types of pathogens and it is also involved in autoimmune diseases and lymphoid malignancies [19]. Within the spleen, lymphocytes are organized as sheathes around arterioles, with the T zone located centrally (also called the per arteriolar lymphoid sheath or PALS) and the B cells distributed around the T zone in tightly packed follicles. The spleen contains an additional population of B cells in a compartment that surrounds the follicles, known as the marginal zone [20]. Antigen-presenting dendritic cells (DCs) are prevalent in marginal zones, T cell zones, and in the bridging channels between these two compartments [21]. The T zones, follicles, and marginal zones of the spleen are commonly referred to as the white pulp cords and they account for approximately half of the splenic tissue. The remainder of the spleen, termed red pulp, contains large numbers of macrophages, vascular cells, and transiting blood cells. This compartment functions in red cell and immune complex clearance and leukocyte exit. The spleen plays a central role in immunity against blood stage malaria and a significant role in mediating resistance to malaria infection [22, 23]. It is the major site of (a) elimination of parasite-infected erythrocytes via erythrophagocytosis, (b) elaboration of protective immune mechanisms, and (c) hypersensitivity reactions manifesting themselves as spleen enlargement. The spleen is greatly enlarged during malaria in experimental animals and humans, and splenomegaly is used as a measure of malaria endemicity. The precise mechanisms by which the spleen exerts its protective functions are not well understood but are likely to be complex, given the unique anatomical features and cellular composition of this organ. The primary induction of immunity to blood stage parasites may occur in the spleen, although the liver may assume this function in the absence of the spleen [24].

1. Diagnosis of malaria

Clinical diagnosis of malaria is imprecise but in many cases is the basis of therapeutic care for patients presenting with fever in malaria endemic areas, particularly if laboratory support is not available or delayed. Due to the life-threatening nature of the disease, the correct and timely diagnosis of malaria infection is critically important [25-28].

Diagnosis of malaria infection as a cause of disease is multi-factorial and includes both the presence of parasites in the blood and clinical symptoms of infection [29]. The detection of parasites on a blood film does not always indicate the cause of disease, as children who are indigenous to an endemic area may continually have low level parasitaemias, however, this does not present as symptomatic disease. Diagnosis also plays an important role in patient management and follow-up chemotherapy. Apart from

detecting parasites in the blood, laboratory testing is also imperative to detect signs of poor prognosis (i.e. haemoglobin levels, blood glucose, lactate and the presence of protein or free haemoglobin in the urine) or to guide the methods of chemotherapy [glucose-6-phosphate dehydrogenase (G6PD)]. Despite the obvious need for improvement of malaria diagnosis, this area remains the most neglected aspect of all malaria research and development. Although malaria infection has a number of distinct clinical symptoms, the overlapping of malaria symptoms with other tropical diseases (including influenza, pneumonia, viral hepatitis or typhoid) impairs its specificity. In areas of high malarial endemicity fever is most often related to malaria infection therefore, the vast majority of patients presenting with fever will be presumptively diagnosed and treated with anti-malarials [25, 26].

Light microscopic examination of Giemsa stained blood films is the most widely practiced and most appropriate diagnostic instrument for parasite detection. Examination of a correctly prepared blood film allows an inexpensive, yet definitive, diagnosis of malaria infection including speciation and quantification. Thick and thin blood films should be prepared and appropriately stained. The most commonly used stain used in the field setting is Giemsa stain [30].

2. Presumptive diagnosis

2.1 Blood examination for malaria parasites

To increase the sensitivity and specificity of malaria diagnosis, modifications to simple microscopy have been made. The quantitative buffy coat method (QBCTM, Becton-Dickinson) works by staining parasites with acridine orange stain [30]. Blood is placed in microhaematocrit tubes pre-coated in acridine orange stain which are then centrifuged. Any parasites that may be present in the blood will spin at a specific density and be easily identifiable at a predetermined position on the microhaematocrit tube. Advantages of this system are that it requires less training to operate and read the tests compared to normal Giemsa stained blood films, as well as taking less time to complete diagnosis compared to light microscopy. Under ideal conditions, the QBC system has been found to be slightly more sensitive than routine light microscopy for the diagnosis of malaria [31], however, its disadvantages of higher cost, requirement of electricity, specialized equipment and supplies as well as a decreased specificity for species identification has led to the QBC system not being routinely incorporated into diagnostic laboratories. Rapid diagnostic tests (RDTs) detect malarial antigen in small quantities of blood (5-a15 μL) by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigens that are abundant in the blood during a malaria infection [32]. A positive result on an antigen detection assay suggests infection with malaria parasites. Current antigen detection methods diagnose active malaria infections, primarily *P. falciparum*, rapidly and reliably in an easy to use immunochromatographic kit which does not need a microscope for diagnosis. The two predominant antigen testing kits commercially available today are based on the detection of the malarial antigen Histidine-Rich Protein 2 (HRP-2) or the enzyme parasite Lactate Dehydrogenase (pLDH) [33].

2.2 Detection of malarial antigen by Serology

Serological testing methods have been used for the detection of malaria infection since the early 1960s, when indirect fluorescent antibody tests (IFAT) and indirect haemagglutination assays (IHA) were described. A disadvantage of serological testing methods is that as they detect antimalarial antibodies, current and past infections cannot be differentiated and the tests have limited value in the treatment or management of malaria infections. The application of DNA or RNA hybridization via polymerase chain reaction (PCR) based probes; to malaria diagnosis has several advantages over traditional methods, although the feasibility of implementing into the field setting is limited. However, it may have a place as a research tool to monitor malaria control programs, or to perform quality control checks on microscopic diagnosis or to determine the distribution of important genes, particularly those associated with drug resistance. Recent publications have suggested that PCR is able to detect blood parasitaemias of less than 0.00002%, if performed under the best possible conditions. This level of parasitaemia is theoretically the detection of a single parasite in an entire sample, although this is rarely achieved. A detection of a parasitaemia of 0.00002%, which equates to 5 parasites per μL blood, is a detection threshold at least five times lower than that achieved by a thick blood film performed in optimal conditions (i.e. 0.0001%), assuming that an experienced scientist has spent at least 10 min examining 100 fields of view. Whilst in field conditions the sensitivity of PCR may only be comparable or slightly better than examination of a thick blood film by a trained microscopist, the specificity of PCR is generally considered to be better than microscopy, particularly if the patient has a mixed infection [25].

Anti-malarial drug

The anti-malarial drug CQ was first introduced into the clinical setting in 1945 but within 12 years CQ-resistant falciparum malaria was reported in Southeast Asia and South America [34]. By the late 1980s, the Thai-Cambodain and Thai- Myanmar borders were declared to be multidrug-resistant (MDR) areas with a prevalence of mefloquine and sulfadoxine-pyrimethamine resistant falciparum parasites [34].

Anti-malarial resistance is defined as “the ability of a parasite to survive in the presence of concentrations of drug that normally destroy parasites of the same species or prevent their multiplication” [36]. Drug resistance in malaria depends on the ability of the parasite to respond, through innate genetic diversity, to adverse conditions. It is acknowledge that the problem of anti-malarial resistance has emerged as a result of the indiscriminate use of anti-malarials leading to the development of high levels of resistance in parasites through selective pressure. The goal of malaria chemotherapy is therefore to use the anti-malarial drugs in such a way that the selection process is minimized, thus extending the therapeutic life of the drug [37, 38].

Many factors contribute to the development and spread of drug resistance including characteristics of the drug itself (dosing, drug pressure, pharmacokinetics, cross-resistance), human host factors (host immunity, maintenance of resistant parasite reservoir), parasite characteristics (genetic mutations, transmission level) and vector and environmental factors (vector affinity of parasites) [35]. Whilst both testing methods have their place in clinical assessment of parasite

resistance, it is often observed that results obtained from *in vitro* and *in vivo* testing methods are not always comparable [39]. An advantage that an *in vivo* assessment method has over *in vitro* testing is that it takes into account host factors such as immunological regulation of infection. Furthermore, pharmacokinetic data may be warranted to differentiate between true resistance and failure to achieve adequate drug concentrations, an observation that is limited to *in vivo* models. Drugs with long elimination half-lives, such as mefloquine and piperaquine, may exert substantial residual selection on new infections contracted after treatment of the primary infection when the drug persists at sub-therapeutic concentrations in the plasma. Sub-therapeutic dosing also results in an increased risk of the emergence of resistant forms because any residual parasites may proliferate [40].

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Declaration of Interest
The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

References
1. Ashley E, McGready R, Proux S, Nosten F. Malaria. Travel Med Infect Dis,2006:4(3-4):159-173.
2. Cox-Singh J, Davis TME, Lee KS, Shamsul SSG, Matusop A, Ratnam S, Rahman HA, Conway DJ, Singh B. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life-threatening. Clin Infect Dis,2008:46(2):165-171.
3. Rose SR. Malaria. In: International Travel Health Guide, 12th Edition,2001, 93-115.
4. Phillips RS. Current status of malaria and potential for control. Clin Microbiol Rev,2001:14(1):208-226.
5. World Health Organization. World Malaria Report 2008. Geneva: World Health Organization, 2008.
6. Snow RW, Gilles HM. The epidemiology of malaria. In: Warrell DA, Gilles HM, editors. Essential Malariology. 4th ed. London: Arnold, 2002, 85-106.
7. Sherman IW. A brief history of malaria and discovery of the parasite's life cycle. In: Sherman IW, editor. Malaria: Parasite Biology, Pathogenesis, and Protection. Washington DC: ASM Press, 1998.
8. Navaratnam V, Mansor SM, Mordi MN, Akbar A, Abdullah MN. Comparative pharmacokinetic study of oral and rectal formulations of artesunic acid in healthy volunteers. Eur J Clin Pharmacol,1998:54(5):411-414.
9. Bruce-Chwatt LJ. History of malaria from prehistory to eradication. In: Wernsdorfer WH, McGregor IA, editors. Malaria: Principles and Practice of Malariology. London: Churchill Livingstone, 1988.
10. Sinden RE, Gilles HM. The malaria parasites. In: Warrell DA, Gilles HM, editors. Essential Malariology. 4th ed. London: Arnold, 2002, 8-34.
11. Peters W, Robinson BL. Malaria. In: Sande MA, Zak O, editors. Handbook of Animal Models of Infection. London: Academic Press, 1999, 757-773.
12. Gupta S, Snow RW, Donnelly CA, Marsh K, Newbold C. Immunity to non-cerebral severe malaria is acquired after one or two infections. Nat Med,1999:5(3):340-343.
13. Urban BC, Ing R, Stevenson MM. Early interactions between blood-stage *Plasmodium* parasites and the immune system. In: Langhorn J, editor. Immunology and Immunopathogenesis of Malaria. Berlin: Springer-Verlag, 2005, 25-70.
14. Tanner M, Beck HP, Felger I, Smith T. The epidemiology of multiple *Plasmodium falciparum* infections. Trans R Soc Trop Med Hyg,1999:93(Suppl 1):59-64.
15. Perlmann P, Troye-Blomberg M. Malaria and the immune system in humans. In: Perlmann P, Troye-Blomberg M, editors. Malaria Immunology. Basel: Karger, 2002, 229-242.
16. Smith TG, Ayi K, Serghides L, McAllister CD, Kain KC. Innate immunity to malaria caused by *Plasmodium falciparum*. Clin Invest Med,2002:25(6):262-272.
17. McGilvray ID, Serghides L, Kapus A, Rotstein OD, Kain KC. Nonopsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*-parasitised erythrocytes: a role for CD36 in malarial clearance. Blood,2000:96(9):3231-3240.
18. Clark IA. Cell-mediated immunity in protection and pathology of malaria. Parasitol Today,1987:3(10):300-305.
19. Bowdler AJ. The Spleen. Structure, Function and Clinical Significance. London: Chapman and Hall Medical, 1990, 515.
20. Kraal G. Cells in the marginal zone of the spleen. Int Rev Cytol,1992:132:31-73.
21. Steinman RM, Pack M, Inaba K. Dendritic cells in the T-cell areas of lymphoid organs. Immunol Rev,1997:156:25-37.
22. Wyler D. Splenic functions in malaria. Lymphology,1983:16(2):121-127.
23. Yap GS, Stevenson MM. *Plasmodium chabaudi* AS: erythropoietic responses during infection in resistant and susceptible mice. Exp Parasitol,1992:75(3):340-352.
24. Dockrell HM, Desouza JB, Playfair JHL. The role of the liver in immunity to blood-stage murine malaria. Immunology,1980:41(2):421-430.
25. Hommel M. Diagnostic methods in malaria. In: Warrell DA, Gilles HM, editors. Essential Malariology. 4th ed. London: Arnold, 2002, 35-58.
26. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg,2007:77(Suppl 6):119-127.
27. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: effects on the poor and vulnerable. Lancet,2004:364(9448):1896-1898.
28. Bell D, Go R, Miguel C, Walker J, Cacal L, Saul A. Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community. Bull World Health Organ,2001:79(10):933-941.
29. World Health Organization. Malaria diagnosis: memorandum from a WHO meeting. Bull World Health Organ,1988:66(5):575-594.

30. Bloland PB. Drug resistance in malaria. Malaria Epidemiology Branch, Centers for Disease Control and Prevention, Chemblee, GA, U.S.A., 2001.
31. Lema OE, Carter JY, Nagelkerke N, Wangai MW, Kitenge P, Gikunda SM, *et al*. Comparison of five methods of malaria detection in the outpatient setting. Am J Trop Med Hyg,1999:60(2):177-182.
32. Moody A. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev,2002:15(1):66-78.
33. Lopez R, Urquiza M, Curtidor H, Caminos JE, Mora H, Puentes A, Patarroyo ME. Plasmodium falciparum: red blood cell binding studies of peptides derived from histidine-rich KAJRP-I, HRP-II and HRP-III proteins. Acta Trop,2000:75(3):349-359.
34. Wellems TE, Plowe CV. Chloroquine-resistant malaria. J Infect Dis,2001:184(6):770-776.
35. Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. Lancet Infect Dis,2002:2(4):209-218.
36. Warrell DA, Watkins WM, Winstanley PA. Treatment and prevention of malaria. In: Warrell DA, Gilles HM, editors. Essential Malariology. 4th ed. London: Arnold, 2002, 268-312.
37. White NJ. Why is it that antimalarial drug treatments do not always work? Ann Trop Med Parasitol,1998:92(4):449-458.
38. White N. Antimalarial drug resistance and combination chemotherapy. Philos Trans R Soc Lond B Biol Sci,1999:354(1384):739-749.
39. Ringwald P, Basco LK. Comparison of in vivo and in vitro tests of resistance in patients treated with chloroquine in Yaounde, Cameroon. Bull World Health Organ,1999:77(1):34-43.
40. Barnes KI, Watkins WM, White NJ. Antimalarial dosing regimens and drug resistance. Trends Parasitol,2008:24(3):127-134.