Malaria is a devastating disease, particularly in tropical African countries that are still developing. It kills a lot of people particularly children. This has been due to the fact that most people in the developing countries do not understand fully the biology and pathogenesis of the disease. It therefore means that more books that are comprehensive, concise and easy to understand should be written in this area. This book titled "Malariology in Tropical Africa - A Primer" fulfils this purpose. It has revealed the following: background information about malaria, types, pathogenesis, pathology, clinical findings of malaria and life cycle of the parasite, immunity and host defence against the disease, epidemiology of malaria, chemotherapy of malaria and herbal remedy, considering the resistance of the parasites to existing synthetic drugs, current global effort and initiatives geared towards the eradication of malaria, using modern approaches and finally, the development, progress and future prospects of malaria vaccine production. Judging from these impressive contents, the book is recommended to the general public, health workers, students, teachers and researchers.

Dr Bala Jeremiah David obtained his PhD in Environmental Technology at Universiti Sains Malaysia. Currently, Senior Lecturer in the Department of Microbiology, Federal University of Technology, Minna, Niger state, Nigeria.He is a member of the Nigerian Society for Microbiology, Biotechnology Society of Nigeria and American Society for Microbiology.

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Jeremiah David Bala

Malariology in Tropical Africa - A Primer

LAP LAMBERT Academic Publishing

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Malariology in Tropical Africa - A Primer

ABSTRACT

Malaria is one of the most prevalent and devastating tropical parasitic diseases. Malaria is a disease of the tropical and sub tropical zones and may also occur in temperate regions. It presents enormous health problems in Africa. The book titled "*Malariology in Tropical Africa - A Primer*" has exhaustively reviewed and elucidates concisely detailed information on current development on malariology. It has revealed current approaches to malaria control, current global programmes and initiatives geared towards malaria eradication, chemotherapy and herbal remedy for malaria, pathogenesis, pathology and clinical findings of malaria infection, epidemiology, immunity/host defenses against malaria and particularly the possibility of using an efficacious vaccine against malaria in the near future.

Keywords: Chemotherapy; Control; Epidemiology; Herbal Remedy; Immunity/Host Defence; Life Cycle ; Malariology; Pathogenesis; Tropical Africa; Vaccine.

DEDICATION

This book is dedicated to:

My lovely and beloved wife:

Wazotah Jeremiah (Treasure)

Who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve. I am truly thankful for having you in my life.

My beloved sons:

Maxwell Polycarp Jeremiah, Magnus Polycarp Jeremiah and Marvin Polycarp Jeremiah
All of you have been my best cheerleaders.

ACKNOWLEDGEMENT

I give thanks to God Almighty Who gives me life, purpose and contentment and for His everlasting grace, infinite love and unquantifiable mercy He has bestowed upon me for life and for eternity.

I wish to express my profound gratitude to Professor U.J.J. Ijah, who by his often persistence corrections, helpful suggestions, regular advice, comments, guidance, assistance and encouragement led to the birth of this book. I earnestly thank you sir for your ways of understanding, for the things you have done so thoughtfully, and for your encouragement in the hopes and plans we shared.

Especially, I need to express my gratitude and deep appreciation to all Academic and non Academic Staff of the Department of Microbiology, Federal University of Technology Minna, Nigeria. They have consistently helped me keep perspective on what is important in life and shown me how to deal with reality.

I owe profound gratitude to my amiable and beloved wife, Wazotah Jeremiah, for being there for me throughout the entire compilation of the book and for her constant source of support, encouragement, great sacrifice, patience and understanding during life's challenges. Maxwell, Magnus and Marvin deserve my wholehearted thanks as well.

I need to express my gratitude and wholehearted appreciation to my beloved mother, brothers and sisters for their unrelenting encouragement, love and ceaseless prayers.

Finally, I would like to leave the remaining space in memory of my beloved late Father:

Whose words of encouragement and push for tenacity ring in my ears.
Baba, enter thy Master's joy. God bless you Baba and keep you in His care, until we meet again.

PREFACE

Malaria is a devastating disease, particularly in tropical African countries that are still developing. It kills a lot of people particularly children. This has been due to the fact that most people in the developing countries do not understand fully the biology and pathogenesis of the disease. It therefore means that more books that are comprehensive and easy to understand should be written in this area. This book titled "A Primer on Malariology in Tropical Africa" fulfils this purpose. The book has seven chapters. Chapter one has background information about malaria. Chapter two deals with the types, pathogenesis, pathology, clinical findings of malaria and life cycle of the parasite. In Chapter three, the immunity and host defences against the disease are presented, while Chapter four presents detailed information on the epidemiology of malaria. Chapter five discusses chemotherapy of malaria, including herbal remedy. This is an interesting area considering the resistance of the parasites to existing synthetic drugs. The current global effort and initiatives have been geared towards the eradication of malaria, using modern approaches. These are discussed in Chapter six. Chapter seven presents the development, progress and future prospects of malaria vaccine production. The book also contains relevant references.

Judging from these impressive contents, the book is recommended to the general public, health workers, students, teachers, researchers and policy makers. There is no doubt that this book will enrich your knowledge in malariology and provide simple ways of tackling the problem of malaria in our society.

It is not enough to purchase a copy, but also purchase for friends, relatives, and fellow workers since the prevention and control of malaria in our society is a collective effort.

Professor U.J.J. IJAH
Department of Microbiology,
Federal University of Technology,
Minna, Niger State, Nigeria.

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CHAPTER ONE

1.0 INTRODUCTION

Malaria is an ancient scourge, as evidenced by early Chinese and Hindu writings. During the fourth century B.C. the Greeks noticed its association with exposure to swamps and began drainage projects to control the disease. The Italians gave the disease its name, Malaria, which means "bad air," in the seventeenth century because of its association with the ill smelling vapours from the swamps near Rome.

According to the oxford concise medical dictionary, malaria is referred to as ague, marsh fever, periodic fever and paludism. An infectious disease due to the presence of parasitic protozoa of the genus *Plasmodium* (*P. falciparum, P. malariae, P. ovale, or P.vivax*) within the red blood cells. The disease is transmitted by the *Anopheles* mosquito and is confined mainly to tropical and subtropical areas.

Malaria is a serious acute and chronic relapsing infection in human, characterized by periodic paroxysms of chill and fever, varying degree of anemia, splenomegaly, hepatomegaly and various syndromes resulting from involvement of individual organs. It is a disease of the tropical and sub tropical zones and may also occur in temperate regions. Its dissemination diminishes with distance from the equator.

Malaria presents enormous health problems in Africa and it is possible that over 90% of the 200 million estimated malaria infected people in the world are in Africa. Of the estimated 300 -400 million acute attacks per year, world-wide, about 80% of the cases (including deaths) occur in tropical Africa. Because of drug and insecticide resistance, malaria is now on the increase globally particularly in Africa, India, the Far East and

South America. About 35% of the world's population is estimated to be infected, with some 10 million new cases annually and perhaps two million deaths. It has been estimated that more than 300 million people are infected each year, and over one million die annually of malaria in Africa alone. There are over 150 million cases of malaria in the world each year. In tropical Africa alone, malaria is responsible each year for the deaths of about a million children under the age of 14. About 1000 cases are reported each year in the United States, divided between returning U.S. travelers and non U.S. citizens.

According to World Health Organization (WHO), 270 million people currently have malaria, and each year 1 to 2 million of these infected people die of the disease. Today there are 300 to 500 million people infected annually worldwide, with about 3 million deaths and also with a child dying of the disease every 40 seconds. More people are dying of the disease. Similarly, there are reports that there are approximately 200 million to 500 million new cases each year in the world, and the disease is the direct cause of 1 million to 2.5million deaths per year.

Throughout human history, including prehistoric times, malaria has been one of the greatest afflictions in the same ranks as influenza and tuberculosis. Even now, as the dominant protozoan disease, it threatens 40% of the world's population every year and approximately 300 million to 500 million new cases are still reported each year, with about 90% of them in Africa. The most frequent victims are children and young adults, of whom at least 2 million die annually. The total case rate in the United States is about 1000 to 2000 new cases a year, most of which occur in immigrants.

Each year, over a million people world-wide die of malaria and more than 100 million new cases are discovered, with children more particularly affected. It is estimated that in rural areas in tropical Africa, one child out of every 20 die of malaria before the age of 5.

The 110 million clinical cases of malaria reported annually, more than 90 million are in Africa, south of the Sahara. An estimated 280 million people are carriers of the malaria parasite in the region. According to a United Nations Population Division report in 1990, malaria is the only disease today, apart from acquired immunodeficiency syndrome (AIDS) that shows a significant rising tendency. The fight against malaria remains one of the biggest challenges of public health.

The economic cost of this disease is enormous and the estimated cumulative cost in 1997 for fighting malaria in Africa was $2.2 billion. Successful eradication can therefore save very large sums, for example an estimate of $20 billion from eradicating smallpox.

Malaria is one of the most prevalent and devastating tropical parasitic diseases causing great suffering and also loss of life today in the world. It is a common disease in tropical Africa caused by a parasitic protozoan of the genus *Plasmodium* transmitted in nature through the bite of an infected female anopheline mosquito called *Anopheles gambiae*. The world wide prevalence of malaria, its great impact upon the health of the population of warm climate and its tolls in mortality and morbidity have made it the outstanding single global health problem.

Human malaria is known to have contributed to the fall of the ancient Greek and Roman empires. Troops in both the U.S. Civil War and the Spanish American War were severely incapacitated by the disease. More than 25% of all hospital admissions during these wars were malaria patients. During World War II malaria epidemics severely threatened both

the Japanese and Allied forces in the Pacific. The same can be said for the military conflicts in Korea and Vietnam. Overall, no greater achievement for molecular biology could be imagined than the control of malaria, a disease that has caused untold misery throughout the world since antiquity and remains one of the world's most serious infectious diseases.

Malaria parasites belong to the subphylum *Sporozoa*, subclass-*Haemosporidiae*, family-*Plasmodiidae* and genus-*Plasmodium*. There are several species of *Plasmodium*, but among them, only four (4) are known to cause malaria with symptoms in human. These are *Plasmodium malariae, Plasmodium ovale, Plasmodium vivax and Plasmodium falciparum.*

CHAPTER TWO

2.0 TYPES OF MALARIA

Malaria in humans is caused by four species of protozoa parasites of the genus *Plasmodium*. They are:

Plasmodium malariae: *P. malariae* causes quartan malaria. It is found in tropical and sub tropical Africa regions; India and the Far East.

***Plasmodium falciparum*:** Of all the species of *Plasmodium*, *P. falciparum* is the most highly pathogenic and it causes malignant tertian (or quotidian) malaria. It is the most serious form of malaria and the most widespread, accounting for up to 80% of malaria found mainly in the hotter and more humid regions of the world; the tropical and sub tropical Africa regions, Middle East, Far East and South America.

***Plasmodium vivax*:** This species of malaria parasite of human occurs throughout most of the temperate regions. It is much less common in tropical Africa, especially in West Africa. This is because *P. vivax* can infect only red blood cells possessing surface membrane receptors related to Duffy blood group antigens (FyFy). These antigens are found rarely in Negroes, a probable reason for the infrequent occurrence of *Plasmodium vivax* malaria in West Africa. It causes benign tertian malaria and is mainly found in India, North and East Africa, South America and Far East.

***Plasmodium ovale*:** This causes benign tertian malaria or *ovale* tertian malaria similar to that of *vivax* malaria. It was described by Stephen in 1922 who saw it in the blood of a

soldier who had returned from East Africa. It is found in the tropical Africa regions, mostly common in West Africa.

Infection with *Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale* occurs throughout most of the tropical Africa. *Plasmodium vivax* infection is rare in West and Central Africa. Only three species, *Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale* are known in Nigeria. This is probably due to the mechanism of erythrocyte invasion by malaria merozoites.

Malaria is holoendemic in Nigeria with *Plasmodium falciparum* as the dominant strain. It is the most common cause of out patient visits to health facilities and is consistently reported as one of the five leading causes of death.

2.1 PATHOGENESIS, PATHOLOGY AND CLINICAL FINDINGS OF MALARIA INFECTION

Malaria is a disease to which one quarter of the world's population remains at risk with an estimated 250 million clinical cases annually. At any given time, parts of the population living in an endemic area are infected with malaria parasite; but remain asymptomatic. Only a minority develop symptomatic disease and even fewer progress to combinations of the three major manifestations of severe disease, namely, anaemia, cerebral malaria and respiratory distress.

Periodic paroxysms of malaria are closely related to events in the blood stream. An initial chill, lasting from 15 minutes to one hour, begins as a synchronously dividing generation of parasites ruptures their host red cells and escape into the blood. Nausea, vomiting and headache are common at this time.

The symptoms of the disease are caused by the merozoites, which reproduce asexually within the red blood cells of their human host. Periodically, they all break out of the cells together and when they do so, they bring on the chill and fever characteristics of the disease, including vomiting, headache, paroxysms, etc. This occurs approximately every 48 hours with *P. vivax, P. ovale, P. falciparum* and every 72 hours for *P. malariae*.

The succeeding febrile stage, lasting several hours, is characterized by a spiking fever that may reach 400C or more. During this stage, the parasites presumably invade new red cells. The third, or sweating stage, concludes the episode. The fever subsides, and the patient falls asleep and later awakes feeling relatively well. In the early stages of infection, the cycles are frequently asynchronous and the fever pattern irregular; later, paroxysms may recur at regular 48 or 72 hour intervals, although *P. falciparum* pyrexia may last 8 hours or longer and may exceed 41°C. As the disease progresses, splenomegaly and, to a lesser extent, hepatomegaly appears. A normocytic anaemia also develops, particularly in *P. falciparum* infections.

Malaria is about twelve times more common in pregnant women compared with their non-pregnant counterparts. The infection is associated with increases in spontaneous abortion, still births and low birth weights. In area of intense transmission, these adverse effects of *falciparum* malaria are largely confined to first pregnancies. Moreover, pregnant women in tropical and sub-tropical regions tend to have more severe malarial infections.

It has been reported that in-vitro, there is impaired proliferative responses of peripheral and placental lymphocytes to malaria antigens; consistent with a general decrease in cellular immunity during pregnancy.

The adverse effects of *falciparum* malaria on pregnancy have been attributed to the metabolic consequences of the infection, as well as to the immuno suppressive effects of increased corticosteroid and progesterone production.

2.2 MALARIA PARASITE LIFECYCLE

Plasmodia have a complex life cycle involving a number of life cycle stages and two hosts. The human infective stage comprises the sporozoites (approximately 1x7m), which are produced by sexual reproduction in the midgut of the anopheline mosquito (vector) and migrate to its salivary gland. When an infected *Anopheles* mosquito bites a human, sporozoites are injected into the bloodstream and are thought to enter liver parenchymal cells within 30 minutes of inoculation. In these cells the parasite differentiates into a spherical, multinucleate schizont which may contain 200040,000 uninucleate merozoites. This process of growth and development is termed exoerythrocytic schizogony. This exo-erythrocytic phase usually takes between 5 and 21 days, depending on the species of *Plasmodium*; however, in *P.vivax and P. ovale* the maturation of schizonts may be delayed for up to 1-2 years. These 'quiescent' parasites are called hypnozoites.

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host (1). Sporozoites infect liver cells (2) and mature into schizonts (3), which rupture and release merozoites (4). (Of note, in *P.vivax and P.ovale* a dormant stage (hypnozoites) can

persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony, A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony, B). Merozoites infect red blood cells (5). The ring stage trophozoites mature into schizonts, which rupture releasing merozoites (6). Some parasites differentiate into sexual erythrocytic stages (gametocytes) (7). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal (8). The parasite's multiplication in the mosquito is known as the sporogonic cycle (C). While in the mosquito's stomach, the microgametes penetrate the macrogametes, generating zygotes (9). The zygotes in turn become motile and elongated (ookinetes) (10), which invade the midgut wall of the mosquito where they develop into oocysts (11). The oocysts grow, rupture and release sporozoites (12), which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle (1). Malaria parasite life cycle is presented in Figure 1.

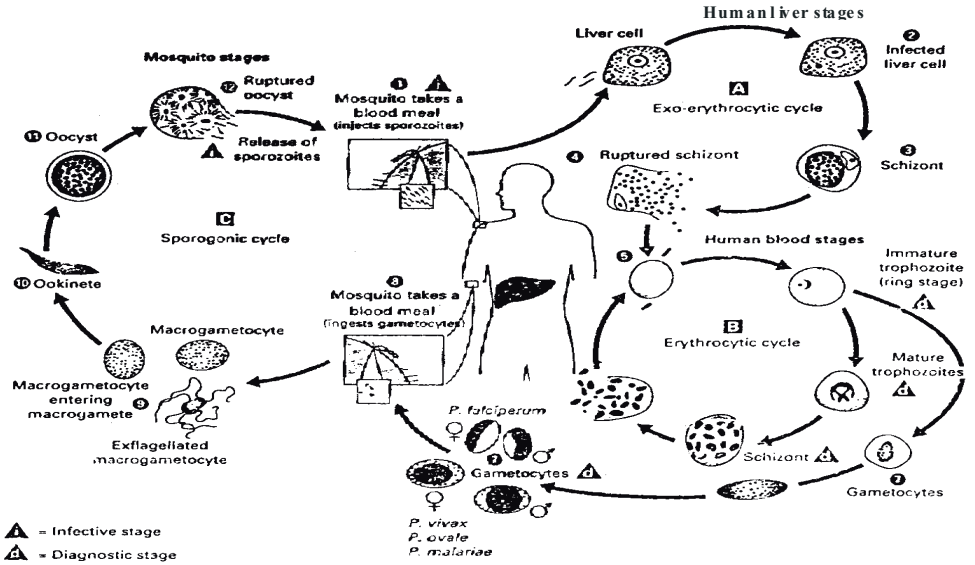

Fig. 1: Malaria Parasite Life Cycle.
Source: Denyer et al. (2004)

Clinical illness is caused by the erythrocytic stage of the parasite life cycle; no disease is associated with sporozoites, the developing liver stage of the parasite, the merozoites released from the liver, or gametocytes.

The common symptoms of malaria are due to the rupture of erythrocytes when erythrocytic schizonts mature. This release of parasite material triggers a host immune response, which in turn induces the formation of inflammatory cytokines, reactive oxygen intermediates and other cellular products. These pro-inflammatory molecules play a prominent role in pathogenesis, and are probably responsible for the fever, chills, sweats, weakness and other systemic symptoms associated with malaria. In *P. falciparum*

malaria, infected erythrocytes adhere to the endothelium of capillaries and postcapillary venules, leading to obstruction of the microcirculation and localized anoxia. The pathogenesis of anaemia appears to involve haemolysis or phagocytosis of parasitized erythrocytes and ineffective erythropoiesis.

CHAPTER THREE

3.0 IMMUNITY/HOST DEFENSES AGAINST MALARIA INFECTION

In parts of Africa where malaria is highly endemic people are infected and re-infected so frequently that they develop a degree of acquired immunity. These subjects may become asymptomatic or mild symptomatic carriers. The population develops and maintains a high degree of immune response while at the same time there is nearly, a permanent presence of very small numbers of malaria parasite in many subjects, mostly adults.

An acquired strain specific immunity has been observed that appears to depend upon the presence of a low-level parasitemia that somehow inhibits new infections or maintains the infection at a nonsymptomatic level. This so-called premonition, or concomitant immunity, is soon lost after the parasites disappear from the blood.

In endemic areas, infants are protected from infection up to the age of 6 months presumably due to the protective effect of maternal antibodies and the less favourable intracellular environment for parasite development. Thereafter, the pattern of clinical disease and development of immunity is to a large extent determined by the level of malarial transmission. In hyper-endemic areas, clinical malaria occurs mainly in children less than one year of age, and the major manifestation is anaemia. There is a rapid decline in the rate of disease after the first year of life, as antimalarial immunity is acquired. Where transmission is less intense or seasonal, exposure is insufficient to induce significant immunity. As a result, individuals of all ages are susceptible to severe

infection, but complications are especially evident in children from one to four years of age.

Natural genetically determined partial immunity to malaria occurs in some populations, notably in Africa. Therefore, susceptibility to malaria infection and disease is regulated by hereditary and acquired factors. It now seems clear that the sickle cell trait (which is the cause of sickle cell anemia) developed as a balanced polymorphism to protect against serious *P. falciparum* disease. Although individuals with hemoglobin-S (sickle cell anemia) or the sickle cell trait are as easily infected with malaria parasites as normal individuals, they rarely exhibit malaria disease because their erythrocytes have a low binding capacity for oxygen; therefore the malarial parasite which has a very active aerobic metabolism cannot grow and reproduce within these erythrocytes, as *P. falciparum* develops poorly in their erythrocytes.

In the 1960s it was demonstrated that resistance to *P. falciparum* among West Africans was associated with the presence of hemoglobin-S (Hb-S) in their erythrocytes. Hb- S differs from normal hemoglobin-A by a single amino acid, valine, in each half of the Hb molecule. Consequently these erythrocytes responsible for sickle cell disease have a low binding capacity for oxygen.

The virtual absence of *P. vivax* infections in many areas of Africa is explained by the fact that most blacks do not have Duffy blood-group antigens (FyFy), which apparently function as erythrocyte surface receptors for *P. vivax* merozoites; without the Duffy antigen, the parasites cannot invade erythrocytes. *P. ovale* frequently replaces *P. vivax* in this region.

Malaria parasites do not develop well in ovalocytes, and it has been suggested that ovalocytosis, which is quite common in some malarious areas, such as New Guinea, may reduce the incidence of malaria. Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, as well as a number of other hemoglobinopathies (including the -thalassemias and hemoglobin E), also protect against malaria infection.

Acquired immunity can also protect against malaria infection and the development of malaria disease. In malarious areas, both the prevalence and severity of malaria infections decrease with age. However, in contrast to many viral infections, multiple infections with malaria do not confer long-lasting, sterile protective immunity. Virtually all adults in malarious areas suffer repeated malaria infections. Individuals who are repeatedly exposed to malaria develop antibodies against many sporozoites, liver stage, blood stage, and sexual stage malaria antigens. It is thought that antibodies acting against sporozoites, liver stage and blood stage organisms are responsible for the decreased susceptibility to malaria infection and disease observed in adults in malarious areas, and that antibodies against the sexual stages of plasmodia may reduce malaria transmission.

Additional work also suggests that the naturally acquired immunity includes the release of cytokines that act against all stages of the parasite, and also a cytotoxic T cell response directed at liver stages of the parasite. Acquired antibody mediated immunity is apparently transferred from mother to fetus across the placenta. This passively transferred immunity is lost within 6 to 9 months, as is the immunity in adults if they leave a malarious area and are no longer exposed to plasmodia. Pregnant women, particularly primigravidas, are more susceptible to malaria infections and serious disease.

Immunity to malaria develops in stages, and in endemic areas children who survive early attacks become resistant to severe disease by about 5 years. Parasite levels fall progressively until adulthood when they are low or absent most of the time. However, 1year spent away from exposure is sufficient for most of this immunity to wane, that is, repeated boosting is needed to maintain it. The actual mechanisms seem to involve both antibody and cell-mediated immunity as presented in Table 1.

TABLE 1: IMMUNITY TO MALARIA

Stage	Mechanism
Sporozoites	Antibody
Liver stage	Cytotoxic T cells, TNF, IFN -α, IL-1
Merozoites	Antibody
Asexual erythrocyte stage	Antibody, ROI, RNI, ECP, TNF
Gametocytes	Antibody,? Cytokines
Gametes	Antibody

Source: Mims *et al* (2005)

The principal mechanisms thought to be responsible for immunity at each stage of the cycle. (IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; ROI, reactive oxygen intermediates; RNI, reactive nitrogen intermediates; ECP, eosinophil cationic proteins). Host defense against malaria is presented in Figure 2.

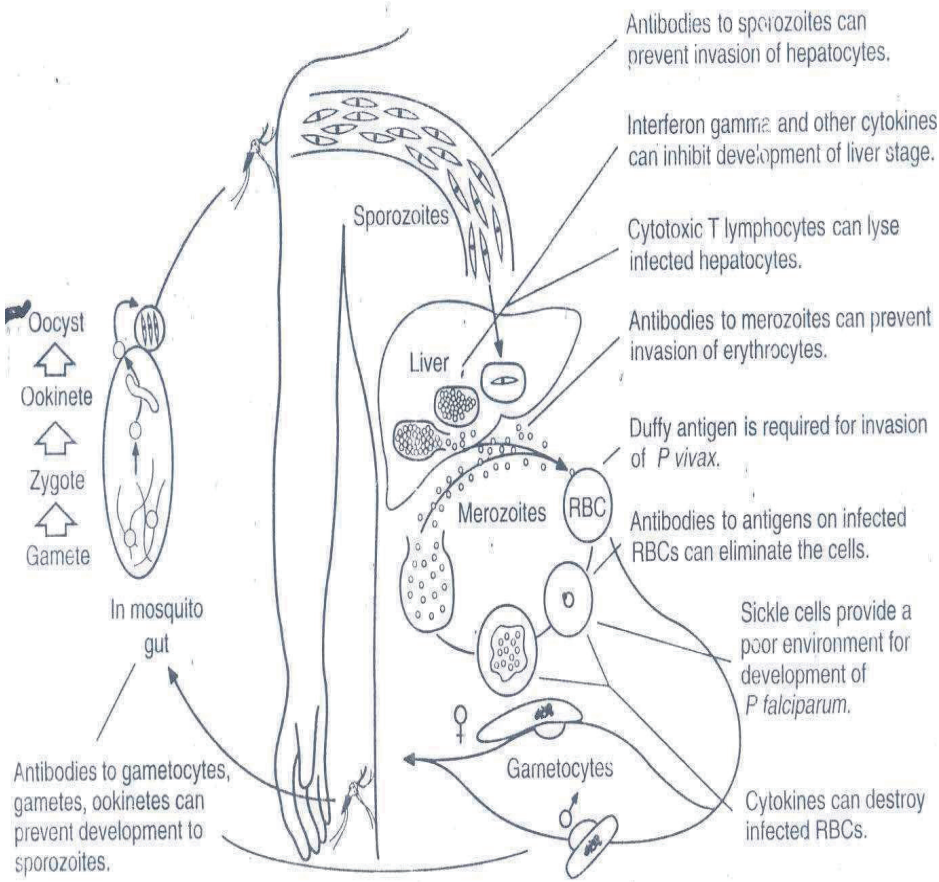

Figure 2: Host defense against malaria
Source: Baron (1996)

CHAPTER FOUR

4.0 EPIDEMIOLOGY

Malaria today is generally limited to the tropics and subtropics, although outbreaks in Turkey attest to the capacity of this disease to reappear in areas cleared of the agent. Malaria in the temperate zones is relatively uncommon, although severe epidemic outbreaks may occur when the largely non immune populations of these areas are exposed; it is usually unstable and relatively easy to control or eradicate. Tropical malaria is usually more stable, difficult to control, and far harder to eradicate. In the tropics, malaria generally disappears at altitudes above 6000 feet. *P. vivax and P. falciparum*, the most common species, are found throughout the malaria belt. *P. malariae* is also broadly distributed but considerably less common. *P. ovale* is rare except in West Africa, where it seems to replace *P. vivax*.

All forms of malaria can be transmitted transplacentally or by blood transfusion or by needles (syringes) shared among addicts when one is infected. Malaria contracted in this manner is easier to treat since it involves only red blood cells and not the liver or do not develop a liver or exoerythrocytic infection. Thus, relapse does not occur; only sporozoites from mosquitoes can infect the liver. Natural infection (other than transplacental transmission) takes place only through the bite of an infected female anopheline mosquito called *Anopheles gambiae*; the mosquito that most efficiently transmits this parasite to humans in Africa. Since suitable vectors are abundant in North America, the potential exists for the spread of malaria whenever it is introduced. Infected mosquitoes and humans constitute the reservoir for malaria.

Parasitized blood transfusion as a source of malaria infection was confirmed by Arafa in 1992 when two Saudi patients underwent cardiac surgery developed fever after being discharged from hospital. Both received blood transfusion during the operation. The cause of fever was found to be malaria parasites acquired from the transfused blood.

The disease is still widely transmitted in the tropics and subtropics. In these areas malaria transmission may be endemic, occurring predictably every year, or it may be epidemic, occurring sporadically when conditions are correct. Endemic transmission of malaria may be year-round or seasonal. In some areas of Africa, 90 to 100 percent of children less than 5 years old have malaria parasites circulating in their blood all the time. Because naturally acquired immunity develops with increasing exposure, in endemic areas malaria disease is primarily found in children. In epidemic areas, on the other hand, naturally acquired immunity falls off between epidemics, and malaria therefore affects all age groups during epidemics.

Approximately 1000 cases of malaria are reported each year in the United States in returning travelers. Of the 1016 imported cases reported in 1991, the majority were acquired in Africa (466 cases) and India (221 cases). *P. vivax* accounted for 43% of the cases and *P. falciparum* for 39%. The risk to travelers of acquiring *P. falciparum* is greatest in Africa. This is because it is the most prevalent species there. Malaria transmission is much more intense in Africa than in other parts of the world, and there is significant risk in urban areas. *Anopheles* mosquitoes capable of transmitting malaria are found in a number of areas of the United States. Local transmission may therefore occur when these mosquitoes feed upon malaria-infected individuals, generally immigrants from malaria-endemic areas. Local transmission has occurred in Southern California,

New Jersey, New York City, and Houston, Texas. Malaria may also occur when infected mosquitoes are transported into non-endemic areas, such as by airplanes or ships.

The major epidemiological breakthrough came in 1880, when French army surgeon Charles Louis Alphonse Laveran observed gametocytes in fresh blood. Five years later the Italian histologist Camillo Golgi observed the multiplication of the asexual blood forms. In the later 1890s Patrick Manson postulated that malaria was transmitted by mosquitoes. Sir Ronald Ross, a British army surgeon in the Indian Medical Service, subsequently observed developing plasmodia in the intestine of mosquitoes, supporting Manson's theory. Using birds as experimental models, Ross definitively established the major features of the life cycle of *Plasmodium* and received the Nobel Prize in 1902.

CHAPTER FIVE

5.0 CHEMO THERAPY OF MALARIA

Drug resistant malaria is common, with some 19.5 million infections in 1998. Children younger than 5 years are most at risk. Whereas in 1999 there were 475000 deaths in this age group in the developed world, there were more than 12 million deaths in the developing world, 60% of which were due to infection.

In the 20th century efforts were directed toward understanding the biochemistry and physiology of malaria, controlling the mosquito vector, and developing antimalarial drugs.

Generally, the fight against malaria is geared toward stages of the malaria parasite's life cycle; hence, there are four classes of such drugs named after each of the four stages. These are:

The Primary Tissue Schizonticides

These are drugs such as paludrine and pyrimethamine-sulfadoxine (fansidar), that blocks the first multiplication phase (pre-exoerythrocytic schizogony) in the liver.

The Secondary Tissue Schizonticides

These are anti-relapse drugs, such as primaquine which act to prevent the production of second generation liver merozoites.

The Blood Schizonticides

These are anti-malaria drugs that prevent multiplication of the first generation liver merozoites in the blood cells and could also kill them. Examples of such drugs include chloroquine, quinicrine, etc.

The Gametocides (Gametocytocidal Drugs)

These are drugs that kill the gametocytes in the blood. Examples of such drugs are primaquine and pyrimethamine-sulfadoxine (fansidar).

Drugs currently used for chemoprophylaxis are chloroquine, proguanil (Paludrine), mefloquine (Lariam), artesunate (Artequin), amodiaquine and doxycycline. On the thai-Burmese border, 100mg doxycycline has been used successfully against *falciparum* malaria in adolescent school children. Tetracycline, a slow blood schizonticide may also be used with quinine in treating drug-resistant *falciparum* malaria. Tetracycline should not be administered to children less than nine years of age or to pregnant women due to staining of immature teeth and bone. Adult women should not be on doxycycline treatment for long terms so that they do not develop *Candida vaginitis*. Treatment of acute malaria is presented in Figure 3.

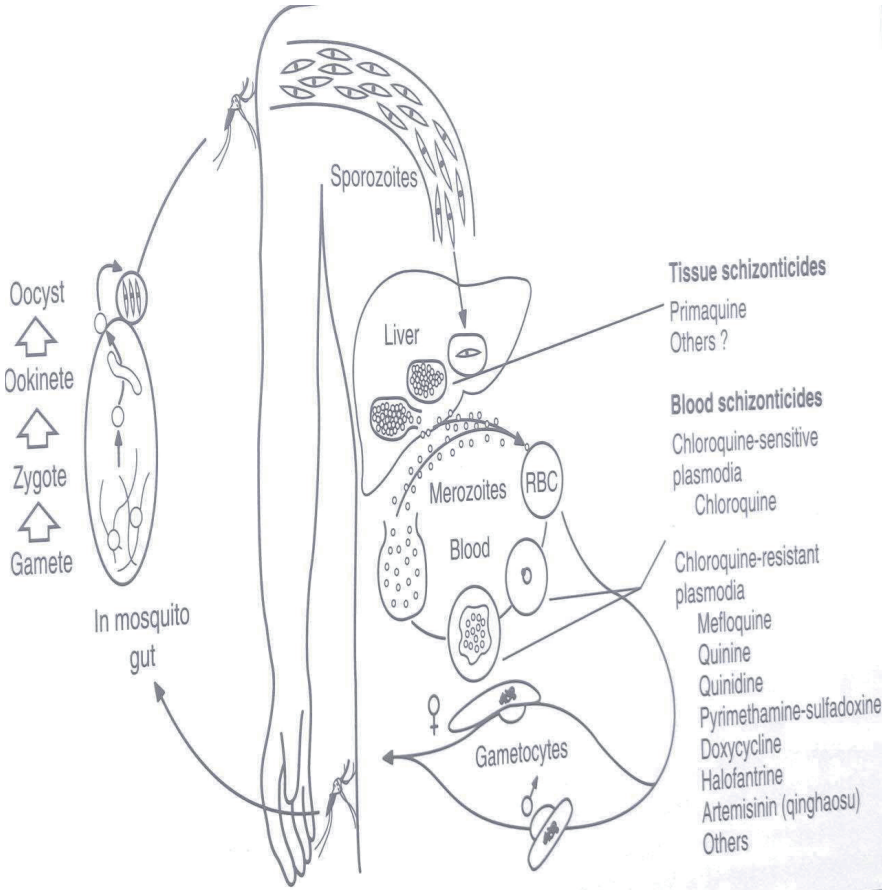

Figure 3: Treatment of acute malaria
Source: Baron (1996)

In the near future, the following drugs might be used for prophylaxis. They are, WR 238605, WR 250417, Atovaquone, Halofantrine (Halfan) and Azithromycin. It has been reported that primaquine analoque (WR238605)- a US Army primaquine was more

potent and less toxic than primaquine when tested in *P. cynomolgi* rhesus (*Macaca mulatta*) monkey model.

Another drug, WR 250417, is a new biguanide related to proguanil. It is a prodrug of an earlier compound WR 99210 and extremely active against multiple-drug-resistant *Plasmodium falciparum*. Now, it is no longer administered because it causes significant gastrointestinal intolerance.

There is also Atovaquone, originally called BW 566C80. It is an hydroxynaphthoquinone that blocks the electrone transport chain of mitochondrial respiration. Halofantrine has also been used successfully for malaria treatment, although it has no role in prophylaxis. Its variable bioavailability suggests that its prophylactic use will require a new and better absorbed formulation.

Another variation of malaria prevention that has been tried is post exposure treatment with halofantrine. It has been given to people who have been heavily exposed to malaria, but no symptoms have been found. This specialized use of halofantrine for treatment has been found successful in French soldiers returning from Africa and in Papua New Guinea Copper Miners.

In 1994 Kuschner and coworkers reported Azithromycin as a new antimicrobial agent of the azalide class, which is related to macrolide antibiotics like erythromycin. Azithromycin is known for its antimalaria activity and favourable pharmacokinetics profile which suggests it might be a useful chemoprophylactic agent.

A newer derivative, tafenoquine or primaquine, is generally effective against the exoerythrocytic stage and the *P. falciparum* gametocytes. Strains of chloroquine-resistant

P. falciparum are now common in many parts of the world, and resistant strains of *P. vivax* have appeared in some areas. Promising new medications such as a combination of atovaquone and proguanil, may help with this problem. Some patients infected with chloroquine-resistant strains respond to intravenous quinine or quinidine together with an oral medication such as tetracycline or a sulfa drug.

Quinine remains the drug of choice for life-threatening malaria. Complications of quinine treatment include massive intravascular hemolysis (blackwater fever). Other major drugs are chloroquine (to which *P. falciparum* is increasingly resistant) and the Chinese drug quinghaosu (artemisin) with primaquine for preventing relapses.

5.1 HERBAL REMEDY

Herbal remedy for malaria is important considering parasite resistance to existing drugs. Plants are the major source of most drugs against a variety of ailments. Quinine, the original antimalarial including over 35 other alkaloids are derived from the bark of *Cinchona ledgeriana*, an indigenous peruvian plant. The bark of the quinaquina (*cinchona*) tree of South America was used to treat the intermittent fevers, although it was not until the mid-19th century that quinine was identified as the active alkaloid. *Azadirachta indica* (neem) extract is used in treating malaria and other febrile illnesses. It contains nimbolides, gedunin, quereetin, rutin, etc as the bioactive components. Artemisinin, a sesquiterpene lactone is derived from the Chinese antipyretic plant, *Artemisia annua L*. and it produces the most rapid resolution of fever and parasitaemia. It is hence presently the drug of choice either alone or in combination therapy with other

antimalarials. Prevailing parasite resistivity to chloroquine and other common drugs, the presence of high morbidity and mortality attributable to malaria and the absence of relevant vaccines necessitates the investigation of local plant species for novel alternative pharmacophores.

Other pure compounds of plant origin with antimalarial activity include *Corialstonine* and *Corialstonidine*, indole alkaloids isolated from *Alstonia coriaceae*, *Crypotolepine* from *Criptolepis sanguinolenta, ancistrocladine, dioncopeltine A* and *dioncopeltine C* from species of *Ancistrocladaceae* and *Dioncophyllaceae*. A Bisbenzylsoquinaline alkaloid isolated from *Triclisia patens* has been shown to have an antimalarial to mammalian cell cytotoxicity ratio equivalent to that of chloroquine.

Isoquinoline alkaloids such as *Berberine* have antimalarial and anti-amoebic activity. *Ataphillinine* is an acridone alkaloid from the *Rutaceae* family with activity against *Plasmodium berghei* in mice. A number of limonoids such as gedunin, nimbolide and rutin with antiplasmodial activity have been isolated from the neem tree and other members of the *Meliaceae* family.

Other compounds including gossypol, a polyphenolic compound from cottonseed oil, have been shown to be active against *Plasmodium falciparum, Trypanosoma Cruzi* and *Entamoeba histolytica.*

CHAPTER SIX

6.0 CURRENT APPROACHES TO MALARIA CONTROL AND PREVENTION

Malaria control depends upon elimination of mosquito breeding places, personal protection against mosquitoes (screens, pyrethrin-treated netting, repellents), suppressive drug therapy for exposed persons, and adequate treatment of cases and carriers. Eradication requires prevention of biting contact between Anopheles mosquitoes and humans long enough to prevent transmission, with elimination of all active cases by treatment and by spontaneous cure.

Bed nets impregnated with pyrethroid insecticides, have an advantage over simple bed nets in that in the presence of a hole or somewhat defective use, mosquitoes would still not gain access or would die if they did, while the amount of insecticide needed is far less than residual spraying of houses. Additionally, the impregnated net acts as a baited trap at night, as mosquitoes are attracted to the net by the carbon dioxide from sleeping persons and may then take up a lethal dose of insecticide. In controlled trials in highly endemic areas it was found that use of impregnated bed nets substantially reduced morbidity and clinical attacks due to malaria, while having a much smaller effect on the prevalence of parasitaemia. This pattern has been found in several regions of Africa. Even in the forest zone of West Africa with perennial transmission at high level, the frequency of clinical malaria attacks in young children was reduced by 50% when impregnated bed nets were used. A national programme has been carried out in the Gambia and the method is being

widely applied following successful trials in several countries of Asia, Africa and Oceania.

The anopheline mosquitoes responsible for malaria are typically most active in the early evening and during the night. Prevention of malaria is possible when the sleeping place is protected from mosquitoes. In a tropical home, bed nets are good adjunct where screens are impracticable. Common mistakes associated with bed nets include failure to mend holes and allowing one's body to touch the net while sleeping. Thoroughly tucking in the nets lower edges is also important as it protects the sleeper from mosquitoes.

Insecticide treatment of bed nets is the most effective way of preventing mosquitoes. The nets are soaked in a permethrin solution every few months. To ensure control, stagnant water in the surrounding environment including those in containers, pits and swamps should not be found very close to the living quarters, since they are veritable breeding places for mosquitoes. Most importantly a clean environment should be maintained.

Most cases of malaria in Americans can be prevented by chemoprophylaxis and by avoiding the mosquito vector. Prophylaxis with chloroquine or mefloquine should begin 2 weeks before entering the malarious area (to ensure tolerance to the drug and to provide adequate blood levels) and should continue throughout the stay in the area and for 4 weeks after leaving. Doxycycline should be started 1 to 2 days before travelling to a malarious area and should continue throughout the stay in the area for 4 weeks after leaving. Chloroquine is the recommended chemoprophylactic for those travelling to areas where plasmodia are still chloroquine sensitive (Mexico, Central America, Haiti, The Dominican Republic, and the Middle East) and it has also been advised that after leaving malarious areas, people take primaquine to eliminate possible exoerythrocytic infection,

which if untreated could cause recurrence of the disease. Strategies for prevention of malaria are presented in Figure 4.

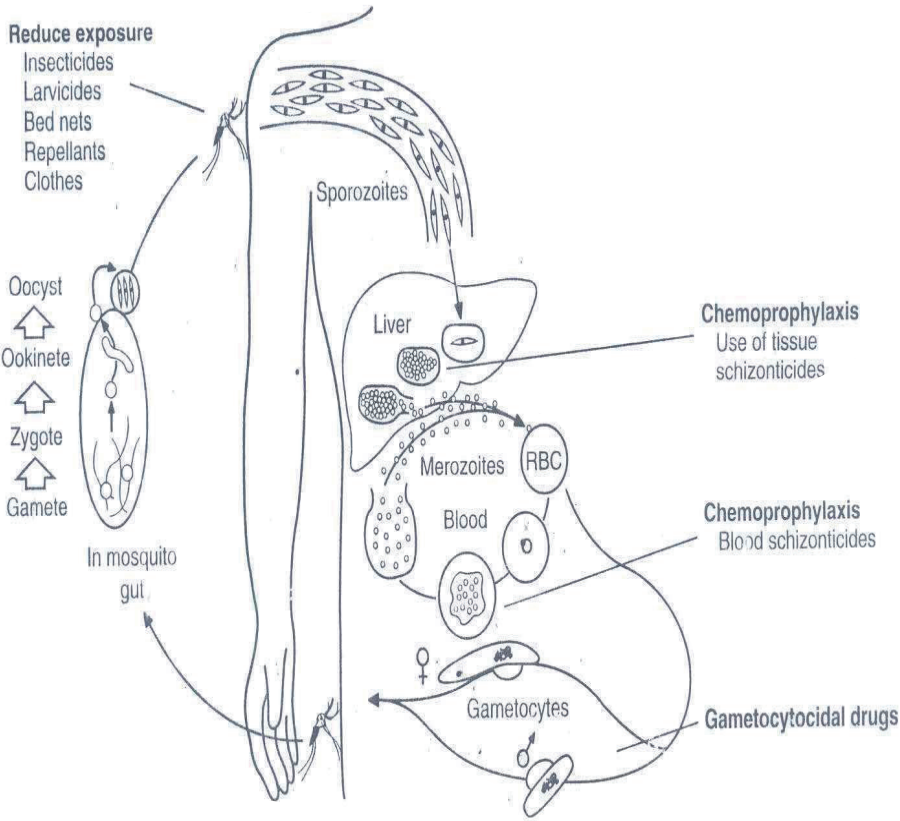

Fig. 4: Strategies for prevention of malaria
Source: Baron (1996)

6.1 CURRENT GLOBAL PROGRAMMES AND INITIATIVES GEARED TOWARDS MALARIA ERADICATION

Unfortunately, the malaria problem is likely to worsen unless effective control is achieved soon. The world population is about 6 billion, with 250 new births occurring every minute. Moreover, various computer models project the climate to warm significantly, resulting in an increase in the areas where malaria is likely to occur. The combination of increasing population, expanding areas where malaria can be easily transmitted, and the development of medication-resistant plasmodia and insecticide-resistant mosquitoes underscores the need for an effective control programme.

In 1998, a new initiative called Roll Back Malaria was begun, linking the World Health Organization (WHO), the United Nations Children Fund (UNICEF), the United Nations Development Programme (UNDP), and the World Bank in the fight against malaria. Their goal is to halve malaria deaths by the year 2010, and halve deaths again by 2015. The initial focus is on detailed mapping of malarious areas using satellite imagery and climate information, and documenting the level of malaria treatment and prevention at the village level. The aim is to organize and fund a sustained effort to improve access to medical care, strengthen local health facilities, and promote the delivery of medications and insecticide impregnated mosquito netting. Besides Roll Back Malaria, other initiatives under way are designed to spur development of new antimalarial medications and to better fund vaccine and other research. In contrast to initiatives in the past, there is now better understanding that malaria control must be part of overall economic development, since it is difficult for one to move forward without the other.

CHAPTER SEVEN

7.0 PROSPECTS OF AN EFFECTIVE MALARIA VACCINE

The search for malaria vaccine started in 1950. After the expense and demoralizing failure of malaria eradication programmes using dichlorodiphenyltrichloro ethane $(ClC_6H_4)_2$ $CH(CCl_3)$ (D.D.T) in the 1950's, no one felt the need for vaccine more keenly than the World Health Organization (WHO), but today the quest for a vaccine against malaria has reached an exciting stage.

In 1955 the World Health Organization began a worldwide malarial eradication program that finally collapsed by 1976. Among the major reasons for failure were the development of resistance to DDT by the mosquito vectors and the development of resistance to chloroquine by strains of *Plasmodium*. Scientists are exploring new approaches, such as the development of vaccines and more potent drugs. For example, in 1984 the gene encoding the sporozoite antigen was cloned, permitting the antigen to be mass produced by genetic engineering techniques. In 2002, the complete DNA sequences of *P. falciparum* and *Anopheles gambiae* (the mosquito that most efficiently transmits this parasite to humans in Africa) were determined. Together with the human genome sequence, researchers now have in hand the genetic blueprints for the parasite, its vector, and its victim. This has made possible a holistic approach to understanding how the parasite interacts with the human host, leading to new antimalarial strategies including vaccine design.

In 1971, David Clyde at the University of Maryland, School of Medicine, Baltimore, USA, repeated in human, including himself an experimental techniques using attenuated

malaria parasite previously preformed in chicken and mice. The technique was an attempt at immunization using weakened or attenuated malaria parasites which had been irradiated in the mosquito host before the insects were allowed to bite. Once in the blood stream of humans, the parasites were unable to continue their life cycle and so could not cause disease.

Earlier results on bird and mice show that they develop immunity to malaria after repeated exposure to the irradiated sporozoites. To find out if humans could develop the same immunity, Clyde in 1971 used attenuated malaria parasite previously performed in chicken and mice and found that humans could develop immunity to the natural malaria sporozoites after repeated exposure to irradiated sporozoites.

7.1 CURRENT VACCINE PRACTICE, VACCINE STRATEGY, AND VACCINE DEVELOPMENT FOR MALARIA INFECTION

Obviously, the development of vaccine is the area of greatest interest in the application of recombinant DNA technology to the control of malaria parasites. The stages in the life cycle of malaria parasite, which are potential targets of malaria parasite vaccine, are presented in Table 2.

TABLE 2: MALARIA VACCINE STRATEGIES

Stage	Vaccine Strategy
Sporozoites	Sporozoite vaccine to induce blocking antibody, already field tested in humans. Antibody produced in response to these vaccines may block sporozoite attachment.
Liver Stage	Sporozoite vaccine to induce cell-mediated immunity to liver stage, already tested in humans Subsequent development of the sporozoite in the hepatocytes will be blocked.
Merozoites	Merozoite (antigen) vaccine to induce invasion inhibitory antibody. Antibody produced by vaccination against merozoite antigen may block invasion of the red cell.
Asexual erythrocyte stage.	Asexual stage (antigen) vaccine to induce other responses to red cell stage, and against toxic products (anti-disease vaccine)
Gametocytes and Gametes	Vaccines to interrupt sexual stages-transmission blocking vaccine. Transmission blocking antibodies, bound to gametocytes may interfere with sexual reproduction in the insect vector preventing the production of new infective sporozoites and terminating the lifecycle.

Source: Mims et al. (2005)

To produce an efficacious vaccine against malaria, the recombinant DNA technique also known as gene splicing is a procedure that may be used. This is a procedure whereby segment of genetic material from one organism is transferred to another. It could also be used to integrate one DNA or part of it into another DNA which carries the foreign DNA. Using the recombinant DNA techniques in producing a potent vaccine, parasite antigen is identified, especially the immunodominant epitope that specifically stimulates protective B-cell and T-cell immune response and clone their genes.

Several vaccines are currently undergoing evaluation in clinical trials. Noteworthy is SPf66, a synthetic tripeptide vaccine which contains peptides from the organism's blood and sporozoite stages developed by Patarroyo and colleagues, has been tested in Colombia and found to be partially effective (<50%). Although initial results were promising, subsequent clinical trials have reported protective efficacies of only about 30% against first clinical episodes of *P. falciparum*. A complete prophylactic vaccine would have to be active against both sporozoites and merozoites of the target species, with an antigametocyticidic effect to curb transmission.

Scientists have been trying to perfect a vaccine against malaria for many years. The first major breakthrough was reported in 1976 from the laboratory of Dr. William Trager at the Rockefeller University, USA. Trager described a method for the continuous *in vitro* cultivation of *P. falciparum*, allowing for the production of antigens from different stages of the organism's life cycle. Later on, other scientists using genetic engineering techniques cloned the genes responsible for these antigens and identified which antigens were potential vaccine candidates.

7.2 CURRENT PROGRESS ON MALARIA VACCINES AND THE WAY FORWARD TOWARDS VACCINES DEVELOPMENT

At present, there are human trials under way with several different DNA vaccines against malaria. DNA vaccine is a more complicated genetic vaccine to emerge in recent years. A DNA vaccine elicits protective immunity against a microbial pathogen by activating both humoral and cellular immune systems. Long-lasting memory cells also are generated.

Much interest has been generated by the surprising discovery that intramuscular injection of microbial DNA itself, with a suitable promoter, can immunize laboratory animals against infection such as malaria. It is thought that the corresponding antigen is expressed and presented on muscle cells. DNA vaccines tend to promote cell-mediated immunity, but conditions have been found to encourage antibody production.

DNA vaccines are being hailed as the most promising of all of the newer approaches to immunization. The two most promising malaria vaccines currently under development are a recombinant DNA version of the sporozoite surface antigens and one that contains the parasite's DNA. DNA-based vaccines are segments of naked DNA from infectious organisms that can be introduced directly into muscle tissue. The host tissue actually expresses the DNA for a short time, producing the microbial antigens encoded by the DNA, which induces an immune responses.

A number of different vaccines are currently under development, including recombinant protein vaccines and a 15-gene naked DNA vaccine. Novel types of vaccines being actively studied also include peptide vaccines, edible vaccines, and DNA-based vaccines. Because none of these relies on whole cells, the procedures eliminate the possibility of infection with the immunizing agent, however, some of these vaccines are weakly immunogenic. Peptide vaccines are composed of key antigenic peptides from disease causing organisms. They are stable to heat and do not contain extraneous materials to cause unwanted reactions or side effects.

A credible vaccine against malaria was reported in 2005. Clinical trial data showed that Mosquirix provided partial protection against malaria for 18 to 21 months, in children 1 to 4 years of age. The vaccine reduced the number of malaria cases by 29% and the

number of severe malaria infections by 50%. Until an effective vaccine is approved for use, malaria prevention is still best attempted with the use of bed netting and insecticides.

Recently with cloning of a gene coding for surface protein of the sporozoite of *P. falciparum,* there is a hope for developing a vaccine. In human host, malarial parasite passes through several antigenically distinct phases, namely; **(i) sporozoite:** the form in which the parasite is injected with mosquito bite; sporozoites enter the liver and multiply and develop into **(ii) merozoites,** which in turn invade and multiply in red blood cells; small fraction of these merozoites in red blood cells form **(iii) gametocytes,** which may be picked up by a mosquito to start another cycle. Therefore, vaccines can be developed to control any of these phases and will be accordingly called **(i) antisporozoite vaccine,** which will prevent malaria in the vaccinated individual and also block its spread; **(ii) antimerozoite vaccine** which will protect or ameliorate the patient but will not check the spread of the disease and **(iii) antigametocyte vaccine,** which would prevent the spread without helping the patient. Of these, antisporozoite vaccine is in sight due to cloning of gene meant for **circumsporozoite (CS) protein.** This gene was obtained directly from DNA of erythrocytic form of parasite, rather than as cDNA from mRNA. This cloned gene may, in course of time, lead to the synthesis of vaccine by synthesizing CS protein by cloned gene.

The vaccines of the future may use totally new means of delivering the antigen. In edible plant vaccines, a novel approach to vaccination is to express the genes for bacterial or viral antigens in edible plant tissues. Edible vaccines are also created by transferring genes encoding key antigens from infectious agents into plants. This could be a much cheaper way of making vaccines than the existing fermentation or cell culture methods,

as the transgenic plant will make the vaccine antigen as it grows. The plant will also deliver the vaccine, as the vaccinee only has to eat some of the transgenic plant to get immunized. Potatoes, tomatoes, and tobacco plants have been used in experiments to make edible vaccines, with promising results. May be one day eating a transgenic banana will protect us against a battery of human pathogens, since the plant could be engineered to express multiple genes.

CONCLUSION AND PERSPECTIVE

Malaria represents by far the most important parasitic disease in man. The World Health Organisation has estimated that 5% of the world population is infected annually. Malaria remains as much scourge as ever, threatening primarily about two billion people who live in endemic areas and also travelers.

The current and very important problem with chemoprophylaxis is not drug resistance, but compliance with drug regimens. The expectation for new means of prevention such as malaria vaccines that will replace the need for chemoprophylaxis in the near future is yet cloudy.

The failure of vector elimination to control malaria and the emergence of drug-resistant parasites underscores the need for an effective malaria vaccine. While the world await the development of an effective malaria vaccine, preventive and control measures should depend upon elimination of mosquito breeding places, personal protection against mosquitoes by using bed nets impregnated with insecticides, mosquito repellents, suppressive drug therapy for exposed persons, and adequate treatment of cases and carriers since efforts to develop an effective malaria vaccine are under way.

With the worldwide research made possible by recombinant DNA technology, biotechnology, and genetic engineering towards the quest for an effective malaria vaccine and drug, it is hoped that the menace of malaria especially in tropical Africa would be history in the near future.

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