INTRODUCTION
1.1 Historical Review:

Malaria is a communicable disease caused by sporozoan parasites of the genus Plasmodium. Human malaria is probably as old as mankind. As legend or history makes us believe, prehistoric man in the old world was victimized by malaria. Historically, the description in Edwin Smith's Surgical Papyrus, 1600 B.C. (Breasted, 1930), associated malaria with contaminated air. In Papyrus Ebers, 1550 B.C. (Garnham, 1966), the symptoms of malaria initially described were: fever, rigour and splenomegaly. An account of malaria as it occurred in Egypt was later given by Halawani and Shawarby (1957).

Hippocrates, in 400 B.C. (Boyd, 1949), gave the first accurate clinical description of malaria. He had for the first time mentioned about the classic triad of chill, fever and sweating. He further analysed the characteristic periodicity of various forms of malaria and the associated splenomegaly with the endemicity of malaria and its topographical aspects. Many Roman historians and writers have mentioned about certain fevers, especially those which affected human populations in the vicinity of marshes (Boyd, 1949). Celsus, in the first century A.D., gave a rather precise description of a febrile disease from which falciparum, vivax and quartan malaria could be easily identified as separate entities. Malaria fevers were also known in ancient China, India and Arabia.
Early attempts to prevent what appears to have been malaria are also contained in Edwin Smith's Surgical Papyrus (Breasted, 1930). During the middle of the seventeenth century, came the first account of the clinical treatment of malaria with the bark of a Peruvian tree, Cinchona. The bark was employed in local Indian medicine as a febrifuge, although its use was quite limited (Jarcho, 1964). Polletiet and Caventon in 1920, for the first time, succeeded in extracting two alkaloids which they named quinine and cinchonine (Scott, 1939), of the two quinine was found to exert a better antimalarial effect.

But more important events in the history of malaria research took place only towards the end of the nineteenth century. In 1880, Laveran first saw and described malaria parasite in the blood cells of man. Romanowsky (1890, 1891) in Russia developed a new method of staining malaria parasites in blood films. The development of staining techniques, along with later improvements in microscope designing made the study of plasmodia much simpler. Gerhardt (1884) showed the induction of malaria in healthy persons through inoculation of blood from malaria patients. These studies and those on the morphological aspects of blood schizogony by Marchiafava and Celli (1884), and ultimately the observation of fertilization of a macrogamete by a microgamete (Mac Callum, 1897) finally dispelled the myth of a miasmatic origin of malaria as also it helped in discarding other rather adventurous hypotheses of the various far-floated
causes of the disease.

Infact, the way in which malaria was transmitted from man to man long remained a mystery, although the association between mosquitoes and malaria and the existence of a particular mechanism of transmission were long suspected. Pfeiffer, in 1892, suggested that malaria parasites passed through an exogenous cycle in the body of a blood-sucking insect. Manson (1894) discovered that mosquitoes were arthropod hosts for malaria parasites. Ross (1897) described gametocyte maturation of \( P. falciparum \) in Culex and Aedes and the production of oocysts in anopheline mosquitoes.

Bignami (1899) succeeded in infecting a healthy volunteer with \( P. falciparum \) through mosquito bites and elucidated the sporogony cycle of \( P. falciparum \) and \( P. vivax \) in anopheline mosquitoes (Bastianelli and Bignami, 1899).

1.2 HUMAN MALARIA STRAINS:

Over one hundred different species of Plasmodia are already known to cause malaria in a wide range of vertebrates, each of them exhibiting a narrowly defined host specificity. Out of these, four species; namely \( P. falciparum, P. vivax, P. malariae \) and \( P. ovale \) are naturally infective for man. Amongst the four human species, \( P. falciparum \) is the most lethal form of parasite causing malignant tertian malaria in man. Amongst the many other species
of Plasmodia, *P. knowlesi* is reported to cause natural human infection in Malaysia (Chin et al., 1965). Similarly, a doubtful human infection with *P. simium* is reported from Brazil (Deane et al., 1966). Some accidental laboratory infections are also traced to have been caused by *P. cynomolgi* (Coatney, 1979).

1.3 RODENT MALARIA:

Till the later part of 1940, malaria research was mainly carried out using avian malaria and to a lesser extent with simian malaria parasites. In 1948, malaria parasites were discovered in small rodents; thicket rats in Congo, and these parasites were found to be infective in mice and rats. Since the first isolation and description of *P. berghei*, malaria parasites from infected murine rodents have been collected from several widely separated regions of tropical Africa (Vincke and Lips, 1948). Various species and sub-species of rodent malarial exhibit different degrees of virulence in unnatural hosts. The virulence also greatly depends on the age and strain of the host. The mortality rate due to *P. berghei* infection varies with the strain of the host. Four species of rodent malaria parasites are now well recognized, namely; *P. berghei*, *P. yoelii*, *P. vinckei* and *P. chabaudi*. 
1.4 SIMIAN MALARIA:

Fourteen species or sub-species of Plasmodium occur in monkeys, and many of them have proved of immense value in malaria research during the past 60 years. Of these two (P. cynomolgi and P. simium) are tertian, two (P. inui and P. brasilianum) are quartan, one (P. knowlesi) is uniquely quartidian, and one (P. coatneyi) is tertian but with doubtful affinities.

Simian malarial parasite, P. knowlesi, is remarkable for its unique quartidian periodicity in the blood, and also for its almost 100% lethality in Macaca mulatta. The severity of infection in its natural host such as M. fascicularis is of a lower grade. A comparison of responses to P. knowlesi by the natural simian host (M. fascicularis) and an unnatural but highly susceptible host (M. mulatta) shows that the former synthesises protective antibody of broad serological specificity within 2 weeks of infection and gives rise to only mild chronic parasitaemia, while the latter shows no protective antibody and the infection often leads to a rapidly fatal malaria (Cohen et al. 1977).

1.5 HOST-PARASITE RELATIONSHIP:

Several recent studies have shown that host factors may modulate the expression of parasite antigens during malaria infection. These factors include several parasite-host interactions such as
immunity, genetic factors, age, nutritional status of the host, exposure to the mosquito bites, etc. The susceptibility of the host is usually greatest in the young animals. The primary requirement for blood stage infectivity is the presence of a specific red cell receptor for merozoite attachment (Butcher et al. 1973, Miller et al., 1973). In case of *P. knowlesi* and *P. vivax* this receptor is associated with the Duffy blood group determinants (Mason et al., 1977). The presence or absence of the immune system, or the organs responsible for it, affects the course of infection and the susceptibility of the host as well. For example, absence of spleen can convert a resistant host into a susceptible one, or low grade parasitaemia can be converted into a fulminating high grade parasitaemia (Garnham, 1970). Absence of p-aminobenzoic acid in the host diet renders it more resistant to infection. The haemoglobin constitution of the red cell may influence intracellular growth of malaria parasites. The inhibitory effect of sickle cell haemoglobin in *P. falciparum* infection has also been observed (Allison, 1954).

1.6 PATHOLOGY

Even before the discovery of the causative agent of malaria, the presence of a characteristic brownish pigment in the spleen, liver, and brain was noted by most pathologists who conducted necropsies on people who had died of this affliction. In 1847, Meckel pointed out that the brown colouration of these organs was caused
by the accumulation of pigment removed from the blood.

Pathological changes in malaria result primarily from the infection of erythrocytes by Plasmodium and from the host's response. To understand these changes three factors contributing to the development of pathological lesions in various organs must be considered: (1) parasitaemia, (2) destruction of damaged erythrocytes and (3) the host's defence response against the infection, including phagocytosis and the development of immunity. Malarial parasites invading erythrocytes initiate the pathological process, and the consequences of this infection influence the host's other tissues and organs. Destruction of host red blood cells occurs not only when plasmodia cause rupturing of the erythrocytes at the end of schizogony but also through phagocytosis of infected and un-infected erythrocytes.

1.6.1 SPLEEN PATHOLOGY

The spleen is the organ which shows the earliest changes in individuals with malaria. Spleen enlargement is a well-known physical sign of infection and its enlargement rate in human populations was used for the evaluation of malaria prevalence within a region (Boyd, 1949). Changes in spleen size in experimental non-human primates with malaria were evaluated by Coggeshall (1937) who demonstrated that Macaca rhesus infected with P. knowlesi and dying in 3 to 7 days after infection showed a 57% average increase in organ size. If infection lasted longer because of treatment,
the increase was 91%. Jervis et al (1972) working with *P. falciparum* infected Aotus found the spleen of infected monkeys to be larger and heavier than those of uninfected control monkeys.

The anaemia seen in malarial infections of humans and animals can sometimes be profound, and extramedullary erythropoiesis is often seen in the spleen. Singer (1954) found that after seven days of *P. berghei* infection, the chief activity of the spleen of mice was erythropoiesis. The tropical splenomegaly syndrome (Pitney, 1968) may be a most important consequence of malaria.

1.6.2 LIVER

Hepatomegaly is another common sign of malaria infection in humans, though variable in experimental animals. Jervis et al. (1972) found that in *P. falciparum* infected Aotus, neither the wet nor dry weight of liver was significantly altered. However, the wet weight in experimental animals with *P. berghei* parasites was found increased while the dry weight remained unchanged, indicating that increase was only due to oedema (Jervis et al., 1968). *P. cathemerium* produces a marked liver enlargement in canaries, especially during the crisis and shortly thereafter (Taliaferro and Mulligan, 1937).

In light microscopy, many workers have demonstrated profound changes in the reticuloendothelial system. It is generally
accepted that the endothelial cell lining the liver sinusoids are phagocytic in nature and that they can transform into Kupffer cells (Aikawa et al., 1968). These transformed endothelial cells divide rapidly to increase the number of macrophages. In P. falciparum of humans and experimental monkeys (Jervis et al., 1972; Gutierrez et al., 1976), large number of parasitized red cells have been seen attached to the endothelial cells.

Electronmicroscopy has shown that the Kupffer cells are vacuolated and contain electron-dense cytoplasmic bodies with large phagolysosomes containing infected erythrocytes and malarial pigment particles (Aikawa and Antonovych, 1964). No apparent digestion of the pigment particles is reported but digestion of parasitized red blood cells and acid phosphatase activity has been demonstrated in the phagolysosomes (Aikawa et al., 1968). Ultrastructural liver lesions in natural infections of P. falciparum and P. vivax in man have been respectively reported by Rosen et al. (1967) and Brito et al. (1969).

1.6.3 RENAL PATHOLOGY

Rhesus monkeys (M. mulatta) infected with P. knowlesi excrete large quantities of haemoglobin, become oliguric, and develop acute renal failure (Rosen et al., 1968a). They develop a condition resembling human black water fever. The rapid intravascular haemolysis is reflected by a progressive haematocrit decrease and a fall
in total serum proteins. Light microscopy reveals haemoglobin granules in the proximal tubules (Rosen et al., 1968b, Boonpucknavig et al., 1973). Suzuki (1974) was able to produce an immune complex disease in mice infected with *P. berghei*. Similarly, George et al. (1976) detected glomerular deposits of IgG and IgM.

1.7 CEREBRAL MALARIA

In the symptomatology and pathogenesis of malarial fevers of man, no other phenomenon appears more ominous than that of cerebral malaria. Its onset always carries grave implications and in most cases the condition is progressive and death is the outcome, unless prompt and adequate treatment is initiated. The syndrome is associated with *P. falciparum* and the most fatal cases resulting from an infection with this species show significant brain involvement. Epidemiologically, incidence of cerebral malaria varies, depending on the endemic areas or the patient populations concerned (Tharavanij et al., 1984). Pathogenicity of the parasites, as well as host factors such as age, sex, genetic background, nutritional conditions, and degree of immunity to the parasites are most likely involved in the development of a full range of pathological changes (Wright, 1968; WHO, 1975; Finley, et al., 1982; Tapchaisri et al., 1985).

Since cerebral malaria is the leading cause of mortality in *P. falciparum* infection, the elucidation of its underlying mechanism(s)
has been the subject of countless investigations by numerous workers. Some workers have maintained that there is a cerebral component in virtually all cases of falciparum malaria and that there is a common underlying mechanism varying in degree rather than kind (Desowitz, 1987). A recent World Health Organization consultative group has restricted the definition of cerebral malaria to being 'severe and complicated; frequently of sudden onset with a convulsion followed by persisting unconsciousness' (WHO, 1986). They note that malarial fever per se can produce convulsions and impairment of consciousness, particular in children, but this is different from deep, unarousable coma that is in their more narrow definition 'cerebral malaria'.

Brain damage caused by *P. falciparum* infection in humans is the most significant feature of cerebral malaria, often leading to a potentially fatal outcome. Some other clinical and histopathological manifestations of cerebral malaria include paralysis, ataxia, a comatose state, plugging of the capillaries and brain haemorrhages. The phenomenon of cerebral malaria has been extensively studied since the end of the last century, notably by Marchiafava and Bignami (1892). The first anatomical and pathological description of brain capillary occlusion, adhesion of infected red cells to the walls of the capillaries/small vessels, swelling of the vessel endothelium and other pathological changes occurring in them were first described by Marchiafava and Bignami (1900). Such studies mostly pertained
to clinical description of the involvement of the central nervous system, and the morbid anatomy of fatal falciparum cases. However, the pathogenesis is somewhat poorly understood with none of the earlier hypotheses (Yoeli, 1976) being successful in fully explaining the initiating circumstances and the sequence of events. A number of investigations had been undertaken to study the pathology of acute and fatal falciparum malaria. Most (1940) investigated cerebral malaria in a syringe-induced epidemic of *P. falciparum* in drug addicts. Rigdon and Fletcher (1946) studied different lesions of the brain associated with malaria. Kean and Smith (1944) summarised their investigation of 100 autopsy cases due to aestivoautumnal malaria. Spitz (1961) further contributed in this direction by elucidating the pathological processes.

In order to thoroughly investigate the pathogenesis and the genetic and immunological aspects of the disease, it is necessary to develop a reliable laboratory model for the study of cerebral malaria. The need for these studies is as pressing today as it was in the past, particularly in view of the high mortality from the disease. Various rodent and simian models have been investigated and proposed for studying this syndrome. Wright (1968) described cerebral malaria in golden hamsters (*Mesocricetus auratus*) infected with *P. berghei*. Williams (1970) demonstrated cerebral lesions in hamsters using the N strain of *P. berghei*. Mercado (1973) described cerebral haemorrhages in rats infected with *P. berghei*. Yoeli
and Hargreaves (1974) proposed *P. berghei yoelii* 17 x - mice model for studying cerebral malaria. Mackey et al. (1980) reported severe cerebral lesions in A/J and BALB/C inbred mouse strains using *P. berghei* ANKA infection. Rest (1982) tested *P. berghei* ANKA strain for its usefulness as a model for studying cerebral malaria in several inbred mouse strains. He reported that A or A/J strains were most suitable for studying cerebral malaria. Finely et al. (1983) are of the opinion that the ANKA strain of *P. berghei* in BALB/C mice produces cerebral malaria with lesions similar to those in humans with *P. falciparum*. In these mice, a relatively low parasitaemia is accompanied by cerebral haemorrhage and oedema. Polder et al. (1983) who made a detailed study of cerebral lesions occurring in *P. berghei* (K 173 strain) infections of Swiss mice claimed it as a congruent model of human cerebral malaria. Recently, Kamiyama et al. (1987) have investigated cerebral malaria-like symptoms in *P. berghei* strain NK 65 - WM/Ms rat model. Thumwood (1988) has reported breakdown of the blood-brain barrier in A/J and CBA/H mice infected with *P. berghei* ANKA. In recent years, however, an endeavor has been made to use simian malaria models for studying typical pathology, especially of the tissue alterations characteristic of cerebral malaria (Miller et al., 1971). Maegraith and Desowitz (1967) closely studied *P. falciparum* infection in man and comparable infections of *P. knowlesi* in monkeys. The development of *P. falciparum - Aotus trivirgatus* model has been reported by Gutierrez et al. (1976).
Various pathogenic mechanisms have been suggested to be the cause of cerebral malaria. Maegraith and Fletcher (1972) suggested cerebral vascular stasis and oedema as being secondary to leakage from cerebral capillaries. Other investigators suggested sequestration of parasitized erythrocytes in capillaries (Rest, 1983), margination and attachment of endothelial surfaces of parasitized erythrocytes (Udeiya at al., 1981) and decreased deformability of infected red cells as possible causes of capillary obstruction (Miller et al., 1972). Immune-electronmicroscopy of cerebral malaria in golden hamsters suggested that thymus-dependent humoral response is involved in the pathogenesis of cerebral malaria (Rest and Wright, 1979). Poongpontaph et al. (1985), in electronmicroscopic studies of human brain in cerebral malaria demonstrated the adhesion of parasitized erythrocytes to the endothelium of cerebral vessels via their surface knobs. The endothelial vesicular membranes are invariably involved in the mechanism of adhesion which leads to vascular obstruction and disturbance of microcirculation in the brain resulting in hypoxia. Earlier study by Maegraith (1974) has shown a significant dysfunction of the endothelium of brain vessels, accompanied with other local and general pathological changes. Another study cites the development of severe and diffuse disturbance of cerebral function as the most striking feature of cerebral malaria (Harinasuta et al., 1982).
The so-called 'Bangkok School', which has contributed significantly to the recent studies on malaria pathophysiology, now favours the 'mechanical theory' to account for the underlying mechanism of cerebral malaria (Desowitz, 1987). They suggest that patchy, focal aggregations of parasitized erythrocytes impede (but do not obstruct) cerebral blood flow. According to their theory, the cerebral hypoxia results in the impairment of glucose metabolism and lactate accumulation. Hypoglycaemia has been reported to be of common occurrence in cerebral malaria (White et al., 1983) and lactate levels are abnormally high in the CSF (White et al., 1985). Quinine, which is now given in most cases of cerebral malaria, in its side effect aids in the release of insulin (Henquin et al., 1975). The resultant hyperinsulinaemia compounds the hypoglycaemia and lactic acidosis. White et al. (1985) are of the opinion that the CSF lactate level is often predictive of the severity of clinical state.

In *P. falciparum* infection of man, there is frequently some evidence of mental disturbances, including delirium, motor aphasia, various types of psychosis, confusion and depression. Coma is a common event, later. Maegraith (1966) has suggested that in view of the rapid reversibility of severe signs such as coma following therapy, one basic contributor to the development of such stages may be reversible impedance of the blood flow through the brain arising from 'stasis' rather than from 'blocking' of vessels with parasites or due to thrombosis.
1.8 BIOCHEMICAL CHANGES

During any pathological stress, the metabolism of the host is severely altered. In the same way malarial infection also exerts considerable metabolic alteration in different organs of the host. Ever since the discovery of the malarial parasite in human blood by Laveran in 1880, extensive research on various aspects of malaria has been carried out. But even today comparatively little is known about the sequential changes that exactly take place in different biochemical parameters of the host during the course of a malarial infection.

Fletcher and Maegraith (1972) have found that metabolic activities of the host, as well as the parasite, are altered significantly during the course of malarial infection. Carter (1973) reported considerable variations in different carbohydrate metabolizing enzymes viz. lactate dehydrogenase, succinate dehydrogenase and malate dehydrogenase during P. berghei and P. vinckei infection. Hypoglycaemia in malarial infections has also been occasionally described (Maegraith, 1948). Fulton (1939) had examined the problem in P. knowlesi malaria in rhesus monkeys and reported hypoglycaemia and depleted liver glycogen in the late stages of the infection. However, White et al., (1983) reported that hypoglycaemia was not an uncommon complication of severe malaria. White (1986) has also shown that hypoglycaemia is frequently associated with severe malaria and
high parasitaemia. In the aetiology of hypoglycaemia in severe malaria, the possibility of monokines acting as significant mediators cannot, however, be ruled out (Clark et al, 1981). Clark and his co-workers have produced experimental evidence which according to them indicates a central role for these monokines. In malarial infection of rodents, they have shown a blocking of the induction of enzymes required for gluconeogenesis, a function normally promoted by glucocorticoids.

A common histological feature of the liver tissue in malaria is that of the so-called 'fatty degeneration' in addition to centrilobular necrosis. Riley and Maegraith (1962) who biochemically examined this aspect found a total increase in the liver fats from 10 to 25% over control values. The findings by gas chromatography of the fatty acid contents of these lipids supported the above observations, besides showing a significant increase in unsaturated fatty acids such as oleic and linolenic acids along with corresponding decrease in the saturated palmitic and stearic acids. A similar pattern was observed both in monkeys and mice. In electron microscopy, simple lipid deposition in the form of droplets in close proximity to the damaged mitochondria was a common finding in liver samples from infected animals (Fletcher, 1964). Gutierrez et al. (1976) have demonstrated increase in lipid contents of liver in Aotus monkeys infected with *P. falciparum*, while glycogen content was found decreased to a very large extent. Rajvir et al. (1981) have reported that
with high parasitaemia there is a marked increase in total lipids in plasma of monkeys during *P. knowlesi* infection. Saxena et al. (1981) have found increase in the values of serum transaminases viz. SGPT, SGOT, serum lactic acid, total liver lipids and lipid peroxide during *P. berghei* infection. Angus et al. (1971b) showed that plasma concentrations of non-esterified fatty acids increased three-fold during *P. knowlesi* infection in monkeys. Ononghu and Onyeneke (1983) have reported increase in plasma triglycerides, phospholipids and cholesterol in human *P. falciparum* malaria in Nigeria.

The role of adenosine and its breakdown products may have a greater significance in the pathogenic processes in acute malaria, when considered in the light of the ischaemia/reperfusion concept of tissue damage arising from free radical activity (Granger et al., 1981).

In recent years, however scientists are engaged in assessing the possible role of highly reactive reduction products of oxygen generally termed free radicals in the pathogenesis of malaria and other parasitic infections. The killing of extracellular and intracellular pathogens by phagocytes is due in part to the production of oxygen radicals, namely superoxide, hydrogen peroxide, hydroxyl radicals and possibly singlet molecule oxygen (Locksley & Klebanoff, 1983). Oxygen radicals are generated inside leukocytes, enabling them to
kill phagocytosed microorganisms (Babior et al., 1973). Unfortunately
for the host, these leukocytes can also secrete \( \cdot O_2^- \), along with other medi­
ators, from their outer membrane into the surroundings (Natham
and Root, 1977). This indiscriminate and self-inflicted process contribu­
tes to the tissue damage from inflammation (Freeman and Crapo,
1982), having the capability of causing tissue damage in parasite
induced diseases. In addition to oxygen radicals, certain products
of radical induced lipid peroxidation including a series of aldehydes
may also be toxic to invading organisms (Gutteridge et al., 1974)
and host cells (Morel et al., 1983). Being much more stable then
free radicals, these toxic compounds can cause injury at some distance
from the site of radical generation.

With a steady rise in parasitaemia, the various tissues
of the host are damaged to a varying degree. A close relationship
exists between cellular injury peroxidation of membrane lipids and
oxidative damage to the cells (Sharma and Krishnamurti 1976).
Saxena et al. (1981) have shown that liver, kidney and spleen produce
higher amounts of lipid peroxides due to increased susceptibility
of these tissues to oxidative damage under the stress of a malarial
infection. Chauhan et al. (1981) have also reported increase in
the level of lipid peroxides in various tissues of mice infected with
P. berghei. Recently, Clark et al. (1987) have observed increased
level of lipid peroxide in human liver during P. falciparum infection.
Mahdi and Ahmad (1989) have reported an increase in the rate of lipid peroxidation in *P. berghei*-infected mice brain.

Superoxide dismutase (SOD) activity has been studied in various parasitic microorganisms (Docampo and Moreno, 1984). This enzyme contributes to the survival of parasites by scavenging oxygen intermediates that are produced during immune or chemotherapeutic attacks. Suthipark et al. (1982) reported increased level of SOD activity in infected versus uninfected erythrocytes during malarial infection, while Fairfield et al. (1986) demonstrated a decreased SOD activity per cell count in *P. berghei* infected erythrocytes. Sharma et al. (1979) have reported depressed SOD activity in *P. berghei* infected *Mastomys natalensis*. Kulkarni et al. (1984) have found similar results in *P. berghei* infected mouse. Recently, Mahdi et al. (1988d) have also reported depletion of SOD activity in *P. berghei* infected *M. natalensis* brain.

1.9 IMMUNITY

It has been recognised that malarial infection generates detectable humoral responses in humans. Sotiriades (1917) reported clinical improvement in a malarious patient following administration of 10 ml of 'immune serum'. Pewny (1918) used immunoprecipitation while Thomson (1919) employed complement fixation tests to demonstrate the presence of malaria antibodies in serum. Later, the successful transfer of malaria immunity in human subjects through transfusion...
of gamma globulin (IgG fraction) from West African immune adults
(Edozien et al., 1962) focussed attention on the protective nature
of at least some malarial antibodies there by implying that humoral
mechanisms constituted a major effect or component of human
immunoresponsiveness (Mc Gregor, 1987).

More recent evidences have indicated that cellular mechanisms
also play an important role in host defence. For example, mice
that are B cell-deficient can mount effective protective responses
that are quite independent of antibody mediated mechanisms (Grun
and Weidanz, 1983). It is widely accepted now that host responses
to plasmodial infections are complex and involve close collaboration
between macrophages and T & B cells. The mediation by the above
lymphoid tissues results in the production of specific and non-specific
humoral factors which help in restricting parasite growth and replication
(Taylor and Siddiqui, 1982, Allison and Engui, 1983; WHO, 1984;
Perlmann, 1986). However T cells appear to play a central role,
although some malarial antigens also seem to stimulate B cells
to produce antibodies directly. But an efficient and persistent
B cell response still seems to be T cell controlled. Helper T cells
are probably important in antibody production and in the induction
of suppressor T cells for modulation of immune responses. The
T cells, on appropriate antigenic stimulation, secrete regulatory
lymphokines. One such lymphokine, interleukin 2 (IL2), stimulates
the clonal expression of T cells, while another, interferon (INF)
gamma, is a powerful macrophage activator and may itself possess the capacity to kill intracellular parasites (Ferreira et al., 1986).

Several intrinsic parasite factors confound the development of effective host immunity. The first is the prevalence of antigenically diverse parasite populations in endemic areas. This is certainly true for *P. falciparum* in its asexual, erythrocytic phase (Mc Bride, et al., 1984). A second important factor is the survival of antigenically variant forms of parasites in an immune host (Brown and Brown, 1965). A third factor is the capacity of some species of parasite, notably *P. falciparum*, to sequester in the deep vasculature of the host, and its ability to avoid passing through organs such as spleen at the time of their development cycle when they are immunologically vulnerable to cell mediated actions (Miller, 1969). Lastly, the release of soluble antigens at the time of erythrocytic schizogony helps in effectively binding the circulating antibodies before they could combine with the parasite or with parasitized cells (Mc Gregor, 1987).

Acquired resistance to malaria shows a high degree of species and stage specificities. The malaria cycle presents a variety of potential targets for the action of such protective (target) antibodies. There are three extracellular stages of development, each presenting itself transiently in the circulation, and appearing vulnerable to the immune attack. In addition, the red cells which
contain maturing parasites express plasmodial antigens on their membranes and as such become more accessible to surface labelling (Dean and Cohen, 1979).

1.10 VACCINES

Malaria, although infrequent in the developed world, occurs in hundreds and millions of people each year in the tropical countries and is a potential threat to hundreds and millions of others (Miller et al., 1986). Despite remarkable success of the intensive efforts made against malaria, the disease remains a major health problem of the tropical and subtropical countries. The current resurgence of the disease in Africa, Asia and Central America is increasingly a source of great alarm. In our own country, hundreds of people suffer and die of this dreaded parasite disease.

For a brief period in early 1970s it appeared that malaria might soon be brought under control. Extensive spraying of DDT greatly helped in reducing anopheline mosquito population, while effective chemotherapeutic measures were equally successful in controlling the disease. A few novel drugs such as Chloroquine and Mefloquine were successfully used for treating the patients. But ten years later with the resurgence of malaria, the situation appeared entirely different.
Logistically, the tools and methods so far available for malaria control did not prove adequate enough to keep pace with the increasing complexity of the disease over the past three decades. For instance, the chlorinated hydrocarbon insecticides such as DDT are proving ineffective against anopheline mosquitoes due to a newly acquired phenomenon, the insecticidal resistance. Then a more alarming situation had arisen due to resistance of the parasite *P. falciparum* to various anti-malarial drugs. Currently, these factors constitute the most important threat towards our efforts of achieving an effective control of the disease. Chloroquine resistant cases have been detected in various regions of the world, including India.

The failure of conventional measures to control/eradicate malaria from tropical and subtropical areas of the world has ultimately led many scientists to search for a possible malaria vaccine. Following development of cultural techniques for *in vitro* growth of erythrocytic stages of *P. falciparum* by Trager and Jenson (1976), and with the advent of hybridoma technology following Kohler and Milstein's work in 1975 for producing monoclonal antibodies, the goal of developing a malaria vaccine seemed nearer than ever before (Anonymous, 1984).

The malaria parasite undergoes progressive transformation during the course of its life cycle in which process the parasite generates an enormous number of antigens. Some of these antigens
stimulate protective immune responses of the host, while the others are immunologically, nonentity or insalubrious (Bruce-Chwatt, 1986). For the production of protective malaria vaccine, the use of antigens which effectively stimulate the protective response of the host against a homologous parasite species is imperative. Several strategies have been devised for the complex task of selecting antigens that may be important for vaccine production.

Much of the preliminary work has been done in several plasmodial species including *P. falciparum* and, hopefully, a protective vaccine against human malaria will soon be developed before long. There are numerous candidate vaccines which are currently under investigation. Presently there are three candidate vaccines, the merozoite vaccine, the sporozoite vaccine and a gametocyte vaccine. Recently, S-antigens of *P. falciparum* have also been successfully employed for vaccination experiments (Ristic et al., 1985).

1.10.1 SPOROZOITE VACCINE

Although sporozoites remain in body circulation only for a brief period of time before entering hepatocytes, but vaccination of rodents, monkeys and humans with attenuated, X-irradiated sporozoites has provided complete protection against malaria (Miller, et al., 1986). The main protective antigens of sporozoite are concentrated on its surface. When fully mature, the sporozoite within
the salivary gland of a mosquito is enveloped by a proteinaceous layer, the circumsporozoite (cs) protein. The circumsporozoite proteins (CSP) of several species of animal and human plasmodia react with a corresponding monoclonal antibody and have been designated by their plasmodial species and the antigen's relative molecular weight. The circumsporozoite molecule apparently plays an important part in the penetration of sporozoites into the liver cells. The corresponding monoclonal antibodies have been shown to inhibit their entry into the liver cells (Nussenzweig and Neussenzweig, 1984). The most promising achievement with regard to the development of a sporozoite vaccine was gene cloning of P. knowlesi antigens in Escherichia coli. This was done by extracting the messenger RNA encoding circumsporozoite protein from infected mosquitoes through conversion (by the enzyme reverse transcriptase) into DNA. The fragment of cDNA was inserted into bacterial plasmids which were then introduced into E. coli. The bacterial clones excreted peptides binding to the monoclonal antibodies against circumsporozoite protein of P. knowlesi (Sharma and Godson, 1985). Moreover, the peptide corresponding to the relevant sequence of nucleotides of P. knowlesi was synthesised and coupled to a protein carrier with an adjuvant and inserted into animals. These inoculations induced the synthesis of antipeptide antibodies which neutralised infective sporozoites.

The CS proteins of different plasmodial species possess structural similarities, yet retain the differences which make each
species antigenically unique. Each CS protein possesses an immunodominant epitope which is repeated several times within the molecule. For *P. falciparum*, the epitope is situated in an area composed of some 37 tandemly repeated peptides comprising four amino acids (Dame et al., 1984). A 12-amino acid peptide comprising three repeats of the tetrapeptide has been shown to contain the immunodominant epitope, and the antibodies raised against a synthetic peptide were found to react with the surface of sporozoites for neutralising their infectivity (Zavala et al., 1985b). Since this epitope has been detected on *P. falciparum* sporozoites isolated from different geographical areas of the world, it could conceivably constitute an effective vaccine against *falciparum* malaria (Zavala et al., 1985a). Also for *P. vivax*, the dominant epitope is located in an area of tandemly repeating peptides. In this case, the peptide comprising nine amino acids is repeated some 19 times (Arnot et al., 1985). Circumsporozoite proteins are immunogenic in man and animals and when sporozoites are in immune serum, the homologous antibodies crosslink with sporozoite surface antigens to give rise to a characteristic circumsporozoite precipitation (CSP) reaction (Vanderberg et al., 1969).

1.10.2 ASEXUAL BLOOD STAGES

The merozoite vaccine or asexual erythrocyte vaccine inducing immunity against blood forms of the parasite would act as a curative measure. An effective merozoite vaccine will overcome the
problem of drug resistance and will also provide rapid cure. The main target of this vaccine would be free circulating merozoites in the blood. Immunization using blood stage antigens derived from avian, rodent, simian and human malaria parasites can induce impressive protection in the appropriate host. However, the degree of protection varies, depending on the nature of the antigen used and the form in which it is administered (Taylor and Siddiqui, 1982). The major merozoite surface antigen of *P. falciparum* is a 195 kilodalton (kD) protein which is synthesised during schizogony and deposited on the surface of the intracellular parasite (Holder et al., 1985). Immunization studies in monkeys have shown this large molecular weight antigen protectively immunogenic (Perrin et al., 1984). However, this molecule exhibits considerable antigenic diversity (Mc Bride et al., 1985) within endemic regions, and it remains to be seen whether it also possess other shared immunogenic epitopes that may confer protection following challenge with various strains.

The earliest attempts to immunize rhesus monkeys (*Macaca mulatta*) against *P. knowlesi* were nearly all unsuccessful (Eaton and Coggeshall, 1939; Short and Menon, 1940). In subsequent studies the monkeys which were immunized sub-cutaneously with two doses of formalin treated *P. knowlesi* infected blood (emulsified with FCA) became extremely resistant to otherwise lethal challenge of *P. knowlesi* (Freund et al., 1945, 1948). Simpson et al. (1974) reported the studies carried out on vaccination of rhesus monkeys
against *P. knowlesi* using fractions of schizont antigen prepared on sucrose density gradient. Cabrera al al. (1977) carried out long term vaccination studies of rhesus monkeys with French Press antigen (FPC). Rieckmann et al. (1979) compared the efficacy of three non-viable blood stage antigens (Schizont, FPC, merozoites) of *P. knowlesi* which induced protective immunity in rhesus monkeys. Khanna (1988) demonstrated considerable protection of rhesus monkeys against *P. knowlesi* challenge following immunization with whole antigen in combination with an immunomodulator the MDP.

Using a *P. falciparum* mature schizont vaccine or extracellular merozoites emulsified in FCA, Siddiqui (1977) obtained 100% protection of immunized owl monkeys following challenge with a homologous strain of *P. falciparum*. This study was the first one to demonstrate an effective, successful immunization of an experimental animal against *P. falciparum*. Recently, Siddiqui et al. (1987) have demonstrated protection of Aotus monkeys against malaria using merozoite surface and rhoptry polypeptides, and as such establishing their candidacy for a human malaria vaccine.

The possibility of using S-antigens of *P. falciparum* for vaccine development is yet another exciting area of current research interest. Ristic et al. (1985) have shown that S-antigens of *P. falciparum* induce significant protective immunity in squirrel monkeys against a virulent challenge by *P. falciparum*. James at al. (1985)
demonstrated that cation exchange-purified *P. falciparum* exoantigens induce significant homologous protection with a moderate degree of heterologous strain immunity against clinical malaria caused by a virulent challenge exposure with *P. falciparum*. Shamansky et al. (1985) have purified two 83 and 100 k Da S-antigens and have proposed that these may be good candidates for potential host protective antigens in malarial immunoprophylactic regimens. Delplace et al. (1985) have identified a 50 Kd protein as a major exoantigen, which gives a strong immunoprecipitation reaction against a highly strong inhibitory human immune serum. Bhatia et al. (1987) have demonstrated the presence of epitopes on the schizont protein P 126. This is a precursor of the major culture supernatant antigen of *P. falciparum* and as such may be a potential candidate for an antimalaria vaccine. Mahdi et al. (1988a) have demonstrated the usability of the culture derived S-antigens in seroepidemiological surveys.

1.11 ADJUVANTS

A proper choice of the adjuvant is an important consideration in the development of a parasite vaccine. After all, the ultimate aim of any vaccine research is to immunize and protect man. Therefore the search for an immunologically acceptable adjuvant is very important for the development of an effective and functional human malaria vaccine.
So far, numerous adjuvants have been used in combination with malarial antigens for immunization purposes.

Freund et al. (1948) was the first to demonstrate the importance of using Freund's Complete Adjuvant (FCA), in combination with parasite antigen material, for achieving protective immunity. Subsequent vaccination studies in avian, rodent and simian malaria further confirmed the above findings (Cohen et al., 1977). Many early investigators working with *P. knowlesi* antigen used FCA in their vaccination studies (Freund et al., 1948, Targett and Fulton, 1965). The FCA was an essential requirement for effective immunization of owl monkeys against *P. falciparum* (Siddiqui, 1977; Taylor and Siddiqui, 1979).

Various microbial products, in particular their polysaccharidic components, possess the property of enhancing non-specific host resistance if given prior to the infection. For example, Beta-1,3 glucan, isolated from *Saccharomyces cerevisies*, Lentinan from *Lentinus edodes* and Schizophyllum from *Schizophyllum commune* have all been widely studied for their adjuvant action (Song and DiLuzio 1979). The ability of glucan to modify resistance and enhance effectiveness of vaccines for microparasitic diseases was studied. Williams et al. (1978) studied the protective effect of glucan in experimentally induced candidiasis. In this study, pretreatment with glucan resulted in 80% survival in contrast to the 80% mortality in control mice. Glucan
has been widely used for prevention or therapy of toxoplasmosis (Nguyen and Stadtsbaeder, 1980), experimental leprosy (Delville and Jacques, 1980), malaria (Gillett et al., 1978) and leishmaniasis (Cook et al., 1982). When glucan was used as an adjuvant for immunization against *P. berghei* (Holbrook et al., 1981b, Kumar and Ahmad 1985), *Entamoeba histolytica* (Ahmad et al. 1980, Sharma et al. 1984b) and *Leishmania donovani* (Obaid et al., 1989) it was found that it elicits a very specific protective immune response.

Bloch (1960) isolated a well defined mycobacterial glycolipid, Trehalose-6, 6'-dimycolate (TDM). The TDM in oil emulsion when injected intraperitoneally has been shown to protect mice against challenge by *Salmonella typhi* and *Salmonella typhimurium* (Yarkoni and Bekierkunst 1976). In vivo experiments have shown the TDM suspensions in saline can protect mice against infections by *Klebsiella pneumoniae* and *Listeria monocytogenes* (Parant et al., 1977), *Babesia microti* (Clark, 1979) and *Plasmodium berghei* (Kumar et al., 1984).

Another adjuvant, muramyl dipeptide (MDP), synthesised from whole *Tubercle bacilli* has been shown to replace FCA for enhancing the immune response of an animal against a particular antigen following its injection with mineral oil (Kotani et al., 1975). Siddiqui et al., (1978c) have used MDP in mineral oil, or peanut oil as an effective adjuvant. The above investigators were successful in vaccinating the owl monkeys against *P. falciparum*. Later, many
derivatives of MDP have also been used as adjuvants. It has been demonstrated that stearoyl-MDP (6-0-stearoyl-N-acetyl muramyl-L-alanyl-D-isoglutamine) could easily be used as a replacement for effective immunization of owl monkeys against \textit{P. falciparum} infection (Siddiqui et al. 1978b, 1979). In these studies stearoyl-MDP was used with carrier liposomes (Cholesterol plus lecithin). Kotani et al. (1977) have demonstrated the absence of any side effect with stearoyl-MDP adjuvant in guinea pigs against influenza vaccine. In recent years, a series of 6-0-acyl derivatives of MDP, B30-MDP (6-0-tetra-decylhexadecanoyl)-N-acetyl muramyl-L-alanyl-D-isoglutamine) and BH48-MDP (6-0-3-hydroxy-2-docoyl-hexacosanoyl)-MDP were examined for their immunopotentiating activity, particularly for stimulating the primary immune response of guinea pigs to ovalbumin. Both of these derivatives were found to stimulate the humoral and cellular immune responses (Tsujimoto et al., 1986). Khanna (1988) have demonstrated considerable protection of rhesus monkeys against malarial infection following immunization with \textit{P. knowlesi} whole antigen in combination with MDP.