Chapter 1: Review of literature
1.1. Malaria

Malaria is one of the dreadful diseases caused by protozoan of genus *Plasmodium*. It is a global health problem, especially in developing and poor countries. The parasite is transmitted by *Anopheles* mosquito vector. There are 5 species of *Plasmodium* which cause disease in humans, viz., *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale curtisi*, *P. ovale walliker* and *P. knowlesi*.

1.1.1. Malaria epidemiology

Human malaria is endemic in tropical and subtropical regions of the world. Even though improved measures against malaria have led to reduced malaria infection rate by 40% and eradication from 17 countries, it still affected 216 million individuals worldwide and caused 4,45,000 deaths in the recent years (Fig 1-1) (WHO 2016). The 90% transmission with 91% mortality is seen in African continent, especially in children of age < 5 years. This life-threatening disease kills a child every 2 minutes (WHO 2017) (1,2). Of all the species, *falciparum* has highest incidence rate globally and causes 99% mortality due to malaria. *Vivax* holds the second position in prevalence and it has attributed to around 40% of infections in southern and south-east Asia, islands of the western Pacific and South America (World Health Organization. 2017. World Malaria Report 2016).

![Malaria incidence rates, by country 2000-2015](image)

Figure 1-1. **Global malaria incidence rates from 2000-2015.** The world map representing country wise malaria transmission (adapted from world malaria report 2015).

Malaria has been a problem in India for many years. The malaria cases in India contributes to 58% of transmission in south-east Asia. The *plasmodium falciparum* (53%) and *Plasmodium vivax* (47%) are the most common species causing malaria. The transmission
is not uniform and certain areas are more prone to infection (Fig 1-2). The areas of high (1 infected/1000 individuals), low (0-1 infected/1000 individuals) and no incidence is 22%, 67% and 11% in India respectively. There are around 0.7-1.6 million cases and 400-1000 deaths every year (world malaria report 2014)(3).

Figure 1-2. Map showing high transmission areas in India from 1995-2007 (Adapted from malariasite.com)

1.1.2. Malaria lifecycle

Malaria parasite has a complex lifecycle, it shuttles between two hosts i.e. human and Anopheles mosquito (Fig 1-3). The infection initiates when mosquito carrying parasite in sporozoite form takes blood meal onto human. The vector injects 5-50 sporozoites, which move to the liver and invade the hepatocytes via lymph vessels (Sannis P, 2012). The liver stage of Plasmodium is asymptomatic. The parasites multiply asexually in hepatocytes and mature into merozoites in 5-16 days. The schizont ruptures to release thousands of merozoites into the bloodstream. These merozoites invade erythrocytes and divide asexually in a period of 48-72h depending on species of Plasmodium. The parasite invades RBC to form ring stage of the parasite. The parasite utilizes globin part of hemoglobin for nutrition and converts heme part into non-toxic heme polymer called hemozoin. The ring stage parasite matures into trophozoite and eventually into schizont. The schizont ruptures to release 16-32 merozoites, which can infect other erythrocytes and the cycle continues. Unlike liver stage, the erythrocytic stage is symptomatic (4). Some of the early ring and
trophozoite stage parasites develop into male and female gametocytes in 10-15 days. The male and female gametocytes can be taken up by anopheles mosquito, where they combine sexually to form a zygote and further development into sporozoites. The sporozoites are transferred to salivary glands of mosquito, which can infect human host. The individuals carrying gametocytes act as a carrier. Some of the parasites belonging to vivax and ovale species of Plasmodium can re-infect the hepatocytes and persist their as hypnozoites. These hypnozoites can cause relapse of malaria at any point of time.

![Plasmodium lifecycle](image)

**Figure 1-3. Plasmodium lifecycle.** The representative image shows asexual and sexual stages of the parasite in human and Anopheles vector (Adapted from sangyeon cho et al., 2012(5))

### 1.1.3. Malaria parasites

*Plasmodium* has around 126 species, which can infect different vertebrates and invertebrates. The 5 species infecting human differ in their lifecycle, symptoms and severity of malaria. The brief description of these parasites is given below.

#### 1.1.3.1. *Plasmodium vivax*

It causes (benign tertian) recurring malaria in humans and infects host by invading reticulocytes (6). Unlike *falciparum*, the occurrence of all asexual stages in bloodstream is common in *vivax* patients. This increases the rate of transmission of malaria, as gametocytes are easily available to mosquito vector and it can infect other hosts even before the clinical symptoms develop in the previous individual. The parasite can survive for years
as dormant form-hypnozoites in hepatocytes. Although *vivax* is not fatal, repeated infections can lead to a severe form of malaria leading to sickness, severe anemia, splenomegaly and death (7).

1.1.3.2. *Plasmodium malariae*

It causes benign quartan malaria with low parasitemia and long erythrocytic cycle of 72h. It is not as dangerous as *vivax* and *falciparum* malaria (8). The edema, nephrotic syndrome and renal disease are seen along with usual clinical symptoms during *malariae* infection. Even though the morbidity due to *malariae* is less, it still contributes some percentage of deaths due to *falciparum* and *vivax* (9).

1.1.3.3. *Plasmodium knowlesi*

It was first found to cause infection in long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques (10) and later in humans. The parasite has become common in infecting populations of Malaysia and south-east Asia. It has a quotidian life cycle of 24h. Usually, its infection rate is low and not fatal, but it may lead to severe malaria due to difficulty in diagnosis and the shorter erythrocytic cycle of 24h (11). The severe malaria is associated with clinical symptoms- respiratory distress, abnormal liver function including jaundice and renal failure (12).

1.1.3.4. *Plasmodium ovale*

This parasite causes tertian malaria and has a life-cycle of 49h (13). The parasite is less infectious compared to *P.falciparum* and *P.vivax* and the incident of the disease is less than 5% of malaria cases. The *P.ovale* has two subspecies i.e. *P.ovale curtisi* and *P.ovale wallikeri* (14).

1.1.3.5. *Plasmodium falciparum*

Of all the species, *falciparum* causes the most severe form of malaria. It has 48h lifecycle and the erythrocytic stages seen in blood-stream include ring stages and gametocytes exclusively (15). The *falciparum* alters surface expression of certain proteins in infected RBC which leads to cytoadherence and sequestration of pRBC in blood vessels. The cytoadherence increases the severity of disease and plays an important role in cerebral malaria (16).
1.1.4. Clinical symptoms

The erythrocytic stages of *Plasmodium* are associated with all the clinical symptoms seen during malaria. The general symptoms of malaria include fever, chills, sweating, headaches, nausea, vomiting, body aches and general malaise. *Plasmodium falciparum* can cause different manifestations of malaria depending on the immune status of the host. The different manifestations include asymptomatic, uncomplicated, severe, placental and cerebral malaria. The asymptomatic malaria patients possess parasitemia of any density with no clinical symptoms (17). The mild malaria is most common and characterized by general clinical symptoms with no complications. The naïve individuals or children are more susceptible to severe forms of malaria including complications such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), multi-organ failure, pregnancy-associated malaria (PAM), severe anemia (SA) and cerebral malaria (CM), (18). Of them, cerebral malaria is the most dangerous and fatal (19).

*Falciparum* malaria leading to coma for more than 30 minutes after the seizure is defined as cerebral malaria by WHO (20). Cerebral malaria leads to encephalopathy due to sequestration of infected erythrocytes to the endothelial cells of capillaries and accumulation of lymphocytes in the brain (21). This results in inflammation and hemorrhage in the brain with disruption of the blood-brain barrier. The onset of coma after the seizure is immediate or steady with drowsiness, confusion, disorientation, delirium or agitation symptoms and may lead to death. The occurrence of cerebral malaria is highest in children with < 5 years of age. Even after medical care, the mortality rates in children are as high as 20% (22–25). The pathophysiology of severe malaria is complex as sequestration of parasitized erythrocyte (pRBC) does not necessitate cerebral malaria and may be asymptomatic (26). Whereas, cerebral malaria can be fatal even in the absence of pRBC sequestration (27). The susceptibility to severe malaria decreases with increasing age of individual and exposure times to parasite (28). Children above 5 years are less prone to severe malaria. However, they still may suffer from mild malaria (29).

1.1.5. Drug resistance

The *P. falciparum* parasites have developed widespread resistance to frontline anti-malarial drugs- hydroxychloroquine, chloroquine, artemisinin, quinine, mefloquine in certain regions (30–34). There are reports of the return of chloroquine-sensitive malaria strains following the withdrawal of its treatment from some malaria-endemic places (35). Over the last decade, control measures like the use of insecticide-treated bed nets to prevent
infection, vector management and combination chemotherapy to treat *Plasmodium*-infected individuals have been effective in significantly reducing malaria’s incidence and mortality. The emergence of drug-resistant parasites compelled to find new approaches to combat the disease using such as novel immunomodulators and immune interventions.

1.1.6. Vaccine and antigenic variation

During past several decades of extensive research, several vaccines have developed which had induced partial protection against malaria. The failure of vaccine can be attributed to the antigenic changes in the wild parasite populations. The ineffectiveness of vaccines could be due to parasite antigenic variation. Antigenic polymorphism is the most troublesome hurdle to overcome in the development of more effective malaria vaccines. During the life cycle of *P. falciparum*, several polymorphic antigens are exposed to the human immune system i.e., the apical membrane antigen 1 (AMA1), merozoite surface protein (MSP-1), erythrocyte binding antigen 175 (*EBA-175*) and circumsporozoite protein (CSP). These are of special interest for the development of a vaccine. Different malaria parasite populations/strains expressed various antigenic alleles of these genes have evolved overtime. These antigenic variations or an antigenic polymorphism phenomenon has an impediment in the development of an effective subunit vaccine against malaria. Only one malaria vaccine candidate (RTS.S/AS01), a multi-peptide subunit vaccine has been approved for use in malaria-endemic countries recently (36,37). The development of this vaccine may be a major breakthrough in malaria vaccine development. The vaccine has limited efficacy and searches for new effective candidates are needed (38).

1.1.7. A brief introduction to the human immune system

The immune system is the defense system against foreign pathogens and altered self-cells. The immune system consists of 2 inter-connected branches i.e. innate immunity and adaptive immunity (fig 1-4). They differ from each other in terms of their specificity and immunological memory. The innate immunity offers the first line of defense to pathogens in terms of physical barriers such as skin, mucosa, anti-bacterial peptides-defensins (39), enzymes-lysozymes (40) and phospholipase A2 (41) in saliva and tears. The other defense offered by innate immunity includes patrolling leukocytes such as monocytes and neutrophils in the blood, lymphatics and tissue-resident phagocytes in different organs, complement factors and acute phase proteins. The communication between cells is established via different small molecules including cytokines, chemokines and other soluble proteins.
Whenever, there is a pathogenic invasion, the pathogen-associated molecular pattern (PAMPs) present on non-self-entities is recognized by germ-line encoded pattern recognition receptors (PRRs) on immune-cells (42). The PRRs include different families such as toll-like receptor (TLR), C-receptor type lectin (CLR), NOD-like receptor (NLR) (43). TLR family includes receptors, which can recognize different molecules including LPS, nucleic acid etc. The CLR can recognize carbohydrates in fungal cell walls (44). All NLR family members have nucleotide-binding oligomerization domain essential for its ability to stimulate inflammasome activity (45). There are 2 more families identifying cytosolic nucleic acids i.e RIG-I-like receptor (RLR) family and the cytosolic DNA receptors which recognize RNA species (46) and double-stranded DNA (47) respectively.

The activation of PRRs triggers production of pro-inflammatory cytokines to contain the infection. The other leukocytes consist of natural killer (NK) cells, mast cells, eosinophils and basophils. The innate immunity either clears the infection or at least keeps it under control, till there is activation of specific adaptive immune response.

The innate immune cells can activate the B and T cells of the adaptive immunity. The cells of adaptive immunity can possess different receptors which are not germ-line encoded and result from somatic recombination at the V (D) J locus leading to more specific response. The recognition of PAMPs by PRRs is followed by uptake of these pathogens by phagocytes and presentation of antigen-MHC II complex on the surface of antigen presentation cells. The antigen presented can be recognized by T cells only when it is complex with MHC receptor. The T cells are divided into subtypes depending on the function they perform. The cytotoxic T (Tc) cells are CD8 positive and kill other cells via their induction of apoptosis or release of cytotoxic granules. The helper T (Th) cells can differentiate into Th1 or Th2 and play an important role in the outcome of the disease. Th1 cells are important for elimination of intra-cellular pathogens as they stimulate cell-mediated immunity and secrete inflammatory cytokines i.e. IL-2, TNF-α and IFNγ. Th2 cells are important for elimination of extra-cellular pathogens as they stimulate antibody response and secrete IL-4, IL-5 and IL-13. The regulatory T (Treg) cells inhibit the excessive immune responses via secretion of IL-10 and TGF-β. The less known γδ T-cells also possess effector functions.

In contrast to T cells, B cells can be directly activated by antigen alone. The main function of B cells is antibody production in membranal or secretory form. The antibodies possess different functions including neutralization of antigens, activation of complement cascade
and antibody-mediated cell cytotoxicity (ADCC). The B cells can function as plasma cells which secrete antibody or memory cells. The memory cells decrease the time required to elicit an adaptive response to the pre-encountered pathogen.

Figure 1-4. The innate immunity responses and its inter-connection with adaptive immunity (Adapted from Gregersen et al., 2006(48))

1.1.7.1. Mononuclear phagocytic system

This system comprises of monocytes, macrophages and dendritic cells. These cells are a part of a complex which possesses common properties such as migration to foreign antigen by sensing chemokines or cytokines, antigen phagocytosis, antigen presentation, secretion of soluble mediators influencing migration of themselves and other cells and cause cytotoxicity to transformed or damaged cells. All mononuclear phagocytes play an important role in innate and adaptive immune responses (49). These cells perform variable homeostatic functions from their role in development, angiogenesis, tissue repair and remodeling. A common monocyte and dendritic cell progenitor (MDP) can give rise to monocytes and dendritic cells. However, dendritic cells can also originate from common dendritic cell progenitor (CDP). The monocytes can differentiate into macrophages or monocyte-derived dendritic cells depending on stimulus such as inflammation (Fig 1-5.)
A certain percentage of macrophages exist which originate directly from embryonic macrophages e.g. microglial cells (51).

Monocytes constitute the topic of this thesis and hence will be discussed in details in the following section.

Figure 1-5. **Mononuclear phagocytic system.** The figure represents the differentiation of progenitors into various members of MPS system. Adapted from Chow et al., 2011(50)

1.1.7.1.1. Monocyte

These are innate immune cells originating from myelomonocytic precursors in the bone marrow. They possess the ability to build a connection between inflammatory conditions and acquired immunity. They constitute 10% of total leucocyte population (52). These cells express various receptors sensing the changes in their micro-environment. The monocytosis i.e. increase in monocyte number acts as a marker for inflammatory diseases. These cells circulate for 2-3 days in bloodstream before differentiating into tissue macrophages or monocyte-derived dendritic cells on appropriate stimulation. Monocytes play an important role in tissue homeostasis, inflammation, resolution and repair (53). The patrolling monocytes can sense pathogens via PAMPs or soluble mediators such as cytokines via specific receptors. The activation of monocytes stimulates signaling in them, which may lead to functional changes by inducing gene expression (53,54).
Though all monocytes possess common functionality, the distinct functions are associated with the 3 subsets found in circulating monocytes on the basis of CD14 and CD16 expression (Fig 1-6). The CD14 is monocyte specific marker and LPS receptor, whereas CD16 is Fc-γ receptor III. The (CD14+CD16-) are classified as classical monocytes, (CD14hi CD16+) as intermediate monocytes and (CD14dim CD16++) as non-classical monocytes (55). Each subset possesses different functions and are summarized in Table 1-1. Classical and intermediate monocytes possess inflammatory and migratory properties. The non-classical monocytes play an important role in tissue damage (56).

Figure 1-6. Human monocyte subsets. The dot plot represents classical, intermediate and non-classical monocyte subsets in a healthy individual. Classical monocytes express high levels of CD14 but no CD16, intermediate monocytes express high levels of CD14 and low CD16, while non-classical monocytes express low CD14 but high CD16. Adapted from Wong et al., 2012(57)

Table 1-1. Phenotypic and functional properties of the three monocytic subsets (Wong et al., 2012) (57)

<table>
<thead>
<tr>
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<th>Classical</th>
<th>Intermediate</th>
<th>Non-classical</th>
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<tr>
<td>Approximate proportions to total monocytes</td>
<td>85%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Surface markers expressed</td>
<td>CD62L, CCR2, CLEC4D, CLEC5A, IL13Ra1, CXCR1, CXCR2</td>
<td>CD74, HLA-DR, Tie-2 (CD202B), ENG (CD105)</td>
<td>Siglec10, CD43, SLAN (subpopulation)</td>
</tr>
<tr>
<td>Surface markers not expressed</td>
<td>CX3CR1, CD123, P2RX1, Siglec10</td>
<td>CD62L, CXCR1, CXCR2, CLEC4D, IL-13Ra1</td>
<td>CCR5, CD62L, CXCR1, CXCR2, CD163, CLEC4D, IL13Ra1</td>
</tr>
<tr>
<td>Preferential responses</td>
<td>IL-10, G-CSF, CCL2, RANTES</td>
<td>IL-6, IL-8</td>
<td>TNF-a, IL-1b, IL-6, IL-8</td>
</tr>
</tbody>
</table>
Monocytes are plastic and can polarize into 2 types i.e. inflammatory M1 type and anti-inflammatory M2 type depending upon the signals received from the altered microenvironment (58). The concept of M1 and M2 was first discovered in macrophages in 1990, where classical (M1) and alternative polarization (M2) was described in presence of IFNγ and IL-4 respectively (59). The activation or polarization of monocyte and macrophages to a specific type is associated with expression of certain set of genes and resulting differential functionality. Later, the M1 and M2 phenotypes were associated with TH1 and TH2 response respectively (60,61).

**M1 phenotype**- It is inflammatory phenotype induced by bacterial LPS and IFNγ. The cells of M1 phenotype possess anti-microbial, tumoricidal and cytotoxic properties and play an important role in activation of TH1 response (62,63). Along with LPS and IFNγ, there are several stimulators of M1 polarization including TNFα, TLR4 ligand (64), GM-CSF (65,66), radiation (67), oxidized low-density lipids (68), high salt diet (69) and certain intracellular pathogens (70). M1 polarization is essential to elicit an effective immune response against pathogens. The monocytes and macrophages producing high levels of pro-inflammatory IL-12 and low levels of anti-inflammatory cytokine IL-10 are typed as M1 (64,71). The M1 phenotype is further characterized by the production of nitrogen and oxidative intermediates which enables them with microbicidal properties. Secondly, the production of pro-inflammatory cytokines (IL-12, IL-23, TNFα, IL-1β and IL-6) and specific chemokines (CXCL9, CXCL10, CXCL11 etc.) recruit cells of TH1 immunity (Th1, Tc1 and NK cells) to the site of infection (64,72). The expression of M1 related genes is partly governed by transcription factor STAT1 (6). Even though, M1 phenotype has been associated with host defense, the excessive production of certain inflammatory cytokines can lead to auto-immune pathologies (73–76).
M2 phenotype- The term ‘alternative polarization’ was first used for IL-4 mediated inhibition of reactive oxygen species production (77) and induced expression of CD206 (59,78). The M2 phenotype is associated with tissue repair, tumor progression and fibrosis, rather than host defense (79–81). M2 phenotype is characterized by expression of different surface molecules CD206 (59), CD163 (82), CD23 (83), CD209 (84), cytokines (IL-10, TGF-β (85) and chemokines CCL17 (86), CCL18 (87). The M1 and M2 phenotype show differential L-arginine metabolism. The M2 phenotype shows high arginase 1 activity, which acts on arginine and leads to the production of polyamines (88). In contrast, M1 express inducible nitric oxide synthase (iNOS) which acts on arginine and produce nitric oxide (NO). The proline production in M2 type on arginine metabolism is involved in the formation of extra cellular matrix (ECM) and contributes to fibrosis (89). Their matrix formation is also attributed to proteins such as chitinase.

![Figure 1-7. Inducers of M1 and M2 phenotypes](image)

Figure 1-7. **Inducers of M1 and M2 phenotypes.** The figure represents induction of M1 and M2 subtypes in presence of specific signals and their important functions. Adapted from Mantovani et al.,2004(64)

Altogether, these proteins play a role in wound healing(73,90–92). In contrast to pro-inflammatory M1 type, the M2 phenotype is immunosuppressive and characterized by the production of anti-inflammatory cytokines (IL-10), low antigen presentation, increased phagocytosis ability and activation of Tregs (82,93,94). Besides, these functions M2 phenotype has a role in chronic inflammation and angiogenesis (73,95,96).
The M2 phenotype is further divided into 3 subtypes depending on the stimuli i.e. M2a, M2b and M2c. M2a subset is induced by IL-4 or IL-13, the M2b subset is induced by immune complexes and TLR agonist or IL-1R and M2c subset is induced by IL-10 or TGF-β (64). (Fig 1-7). Monocytes of M2b type differ from other M2 subsets- these cells produce large amounts of inflammatory cytokines such as IL-6, TNF-α and IL-1β along with the M2 signature of IL-10\textsuperscript{high} and IL-12\textsuperscript{low} (97). Each of these subsets, express a set of cytokine, chemokine and surface markers and perform different functions (Fig 1-8). Despite these differences, all 3 subsets promote TH2 response (64,94). M2a monocytes produce chemokines which can act as ligands for CCR3, CCR4 (86) and CCR8 (98), leading to recruitment of eosinophils, basophils and Th2 cells, and organization of type II immune response. M2b polarization is characterized by the selective production of CCL1, with the consequent recruitment of Tregs and immunoregulation. Whereas M2c polarization is characterized by CCL16 and CCL18 production and consequent recruitment of eosinophils and naive T cells, respectively (99). There is a possibility of stimulation of M2b phenotype during acute stages, the release of high amounts of IL-10 from M2b may then induce M2c subset followed by M2a, which may promote wound healing and fibrosis.

The M2 subsets are given different names by different groups such as Murray et al.,2014, has classified the different types of phenotypes depending on activating agent i.e M(IL-4), M(Ig), M(IL-10), M(glucocorticoid or GC), M(IFN-g), M(LPS) (60). Another group Mosser et al has classified as M (IC) or M (GC) induced regulatory macrophages, IL-4/IL-13 induced pro-fibrotic/wound-healing macrophages, and tumor-associated macrophages (TAMs) (64,73,100). Regulatory phenotype functions to control inflammation with an unknown exact mechanism (71). Tumor-associated macrophages thought to be of the M2 type, show tumor promoting characters such as secretion of angiogenesis promoting factors may not be exactly similar to M2 type (22, 73). The phenotype may not specifically fall into the above categories but, may show spectra related to particular phenotype.

Multiple signaling pathways including JAK/STAT, PKC/ERK, and PI3K/AKT/mTOR are involved in M2 polarization of monocytes The pathways may act individually or in combination to drive M2 polarization of monocytes (88,101).The signaling pathways, p38-MAPK and PI3K-AKT pathway are involved in IL-4-induced M2a polarization in mice.
model (102). Notch1 signaling via NF-κB, p38-MAPK and AKT pathways play a role in M2b polarization of murine lupus mouse model (103).

![Diagram](image)

**Figure 1-8. Chemokine profiles of M1 and M2 phenotypes.** The figure represents production of specific chemokines by M1 and M2 subtypes and their role in activation of TH1 and Th2 responses respectively. (Adapted from Mantovani et al., 2004(64))

Patients with filarial infection showed alternatively activated monocytes characterized by IL-10$^{hi}$, MRC1$^{hi}$, MGL$^{hi}$, CCL18$^{hi}$, ARG$^{hi}$ and NO$^{low}$. The *in vitro* stimulation of purified monocytes with filarial antigen also induced characters of alternatively activation i.e. reduced levels of IL-12, IL-18 and increased levels of IL-10 and TGF-β suggesting immune evasive property of filarial antigen (104). The individuals with alcohol abuse showed the
presence of M2b monocytes and were associated with the inability to elicit an effective immune response against bacterial pneumonia and gut bacteria-associated sepsis. The CCL1 downregulation in these cells using antisense technology reverted the M2b monocytes to quiescent stage and exhibited resistance against bacterial pneumonia (105). Similarly, the M2b monocytes were found to be present in burn patients and were associated with increased secondary bacterial infections (106). Monocyte polarization has been reported in other infectious and parasitic diseases including Leishmania and Trypanosoma (107–109). Recent studies have enhanced our understanding of the importance of phenotypic changes in monocyte and macrophage in modulating immune responses in several infectious and other diseases and highlighted their potential for development of new therapeutics.

The different cytokine, chemokine and surface markers of M1 and M2 phenotype covered in this thesis are summarized in Table 1-2.

Table 1-2. M1 and M2 monocyte properties. The functional and phenotypic properties of M1 and M2 monocytes analyzed in this thesis

<table>
<thead>
<tr>
<th>Inflammatory Environment M1</th>
<th>Anti-inflammatory Environment M2</th>
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<tbody>
<tr>
<td>IL12 high</td>
<td>IL10 high</td>
</tr>
<tr>
<td>NOS high</td>
<td>ARG high</td>
</tr>
<tr>
<td>ROS high</td>
<td>IL2 low</td>
</tr>
<tr>
<td>IL10 low</td>
<td>NOS low</td>
</tr>
<tr>
<td>ARG low</td>
<td>ROS low</td>
</tr>
<tr>
<td>CD206 low</td>
<td>CD206 high</td>
</tr>
<tr>
<td>M1</td>
<td>M2a CCL1</td>
</tr>
<tr>
<td>TH1, TC1, NK cells</td>
<td>Treg, TH2/TC2</td>
</tr>
<tr>
<td>M2b CCL1, TNF-α, IL-6, IL-1β</td>
<td>Eo, Treg, TH2/TC2</td>
</tr>
<tr>
<td>M2c CXCL13</td>
<td>B cell, follicular cells, T helper cell</td>
</tr>
</tbody>
</table>

1.1.8. Immune response to malaria.

The different stages of *Plasmodium* express a different set of proteins, which also change frequently. This affects the ability of an individual to develop immunity and result in partial and short-lived immune response against consequent infections. The complex interactions between parasite proteins and immune cells have further affected the development of efficient vaccine to date.
Natural immunity is prevalent in certain highly endemic regions of malaria. For e.g. absence of Duffy antigen is related to decreased vivax infections in Africa. Similarly, individuals with sickle cell anemia, absence of glucose-6-phosphate, thalassemia are resistant to malaria (110,111). The innate immune response triggered by acute malaria infection is less understood. The immune cells such as monocytes, NK cells and dendritic cells play an important role in innate immunity. The monocytes and NK cell number is increased during infection.

The monocytes can ingest different parasitic stages as well all the parasitic components released during schizont rupture including Hz. The phagocytosis is sometimes enhanced by opsonization. The opsonization is mediated by complement receptor CR1, Fcγ receptors and antibody. The Fcγ receptor IIIA overexpression in severe malaria and not in mild or cerebral malaria enhances phagocytosis of immunoglobulin-coated uninfected erythrocytes. Further, the crosslinking of immunoglobulins induces TNFα production (112). The antibody-mediated opsonization can also promote antibody dependent cell inhibition. The non-opsonic phagocytosis occurs via CD36 receptors. The phagocytosis of parasitic stages and components such as GPIs, micro-particles and Hz not only decrease the number of infected RBCs but also activates the monocytes. The activated monocytes lead to inflammation via secretion of cytokines (Fig 1-9) (113). Parasite DNA also possess immune-modulatory activity and stimulates type 1 interferon response via cyclic GMP-AMP synthase (cGAS) (114).
Figure 1-9. **Role of monocytes in protection against malaria.** The figure represents various mechanisms utilized by monocytes to confer protection against malaria (Adapted from Chua et al., 2013) (113)

Other than well-known receptors such as TLR9, DAI, RNA polymerase-III, the *Plasmodium* nucleic acid, infected erythrocytes and oligonucleotides can induce type 1 interferon response via STING, TBK1 and IRF3-IRF7 signaling pathway (115). NK cells possess the ability to lyse the infected erythrocytes and secrete IFNγ and IL-8, which leads to recruitment and activation of other cells. Other cells such as dendritic cells, macrophages, gamma delta T cells and NKT cells also play an important role in immune response against the parasite. NKT cells mostly inhibit liver stage parasites as seen in the murine system. GPI interaction with T-cells leads to regulation of CD4⁺ helper cells. The malaria infection is accompanied by non-specific antibody production. However, its importance in innate immunity is unknown (116, 117).

Dendritic cells sense the cytokines produced such as IFNγ or directly interacts with parasite antigens via PRRs leading to their maturation. The mature dendritic cells expressing MHC II, CD80, CD86, CD40 and adhesion molecules migrate to spleen and secrete TH1 cytokine IL-12. The IL-12 can stimulate NK cells to produce IFNγ and activation of TH1 cells. The IL-2 produced by TH1 cells can again activate the NK cells thereby, amplifying the adaptive immune response. The antigen-specific naïve CD4⁺ T cells undergo clonal expansion after receiving a signal from mature dendritic cells. The cytokines TGF-β and anti-inflammatory IL-10 can suppress innate and adaptive immune responses (118).

The parasite infection induces polyclonal and specific antibody production consisting of IgM, IgG and other isotypes. However, only a small percentage of immunoglobulins i.e 5% are species or stage-specific. Increase in IgE anti-parasitic antibodies suggesting a shift to Treg and TH1 to TH2 is seen in severe and cerebral malaria. The IgE may form immune complexes leading to excessive production of TNF and NO, which contribute to immunopathology seen in severe forms of malaria. Immunoglobulins also play an important role in protection, for e.g. they can bind to merozoites and prevent them from invading erythrocytes or opsonize them. The opsonization of merozoites or other parasitic stages increases their possibility of being phagocytosed by effector cells and release of soluble factors which may fuel inflammation and thereby enhance clearance of parasite (116).
1.1.8.1. Cytokines and chemokines in falciparum malaria

The immune-protection or pathophysiological condition during parasite infection is greatly influenced by secretion of various TH1/TH2 cytokines and chemokines. There are numerous studies suggesting the role of these soluble mediators in malaria patients residing in endemic regions.

Excessive production of cytokines and chemokines is reported in severe forms of malaria such as cerebral malaria. Previous work from our lab in Gondia district of India showed that mild malaria could be differentiated from severe and cerebral malaria by the set of cytokines secreted. The patients with mild malaria secreted IFNγ, IL-2, IL-5, IL-6 and IL-12. The severe or cerebral malaria patients secreted high levels of anti-inflammatory cytokines TGFβ, IL-10 and inflammatory cytokines TNFα and IL-1β (119). Similarly, PBMCs from malaria patients of Papua, New Guinea were stimulated with pRBC and cytokines were estimated. Along with IL-10, severe malaria showed high levels of chemokines CXCL10, CCL3 and CCL8 compared to mild malaria (120). The erythropoietin levels associated with the formation of RBC correlated with increased parasitemia and cytokines TNFα, IL-10 and chemokines CXCL10, MCP-1 in cerebral malaria (121). Increased levels of CXCL10 or IP-10 mainly produced by inflammatory monocytes and neutrophils act as markers for cerebral malaria. Low levels of CXCL10 were found to be beneficial in cerebral malaria as they increased recruitment of CXCR3+ CD4+ T helper cells to spleen and improved antibody response to the parasite (122).

Children with severe malaria have shown a distinct profile of β-Chemokines characterized by increased circulating levels of CCL3 and CCL4 and decreased CCL5. Previous Studies have shown that altered patterns of circulating β-Chemokines resulting partly from Hz-induced changes in blood mononuclear cells (123). It was found that CCL5 is decreased during severe malaria and associated with suppression of erythropoiesis and parasite-induced thrombocytopenia (124). CX3CL1 was found to be associated with cytoadherence in malaria (125). Among primigravidae, levels of CCL2, CXCL9 and CXCL13 were found significantly higher during malaria infection in both the placenta and peripheral blood. Others reported that placental CXCL9 and CXCL13 levels were also higher in placental blood from secundigravidae and multigravidae (126). The absence of CXCL-10 results in up-regulation of Foxp3 and IL-10 which may be involved in attenuating fatal murine CM (127). Elevated plasma levels of CXCL10 and CXCL4 were tightly associated with Cerebral Malaria mortality (128).
1.1.8.2. Immunosuppression in malaria

CD4+ T cells are involved in protection against pre-erythrocytic and erythrocytic stages of *Plasmodium*. Unlike CD4+ T cells, the function of CD8+ T cells involved in protection against pre-erythrocytic stages, mostly in severe malaria is not completely understood. As the erythrocytic stages lack MHC antigens, CD8+ cells are not involved in cell-mediated immunity against them. Literature suggests the involvement of CD8+ Tc cells in suppression of immune responses (116). The CD8+ suppression may be dependent on PD-1 exhaustion in prolonged infection (129). One of the reports suggests antigenic B and T cell exhaustion in prolonged parasite infection leading to immune suppression (130).

The sporozoite stage, asexual erythrocytic stages and gametocytes are reported to induce an immune response against them. The adaptive immunity is acquired mostly against erythrocytic stages, specifically merozoites. The mechanisms involved in the antigenic specificity of protective immune response is not completely elucidated (111). The development of adaptive immunity and its effectiveness depends on host immunity status, number of infections suffered, age and place of stay etc. The parasite infection in the non-immune individual leads to clinical illness and the severity of infection may increase to cause morbidity. After 2-3 infections in the same individual, the clinical illness is reduced and the possibility to develop the severe disease is also minimized. Secondly, frequent and multiple *Plasmodium* infection increases immunity and thereby low parasitemia is detected (110,111). A major hurdle in the development of effective adaptive immunity is antigenic and genetic variation resulting in poor species and strain-specific immunity. However, in high transmission and endemic regions of malaria such as sub-Saharan Africa, Indian states Orissa etc. individuals develop immunity at a very early age. The early immunity up to 6 months of age to new borns is offered by maternal antibodies. The infants are highly susceptible to infection from this age to 5 years for severe and cerebral malaria. Above this age, the individuals acquire immunity due to repeated and frequent infections(3,116). However, if the individual leaves the endemic region or is not exposed to repeated infection for a year or so, the acquired immunity against parasite is lost. The adaptive anti-plasmodial immunity does not last for long. Owing to physiological suppression of immune responses and increased Chondroitin Sulfate A receptors on the placenta, the pregnant women become more susceptible to infection and its complications (placental malaria) (110,111).

The antigenic diversity and clonal variation are not the only reasons for the parasite to cause immune evasion, but they also possess the ability to modulate immune responses and
cause immune suppression. The accumulating Hz inside phagocytes has been reported to play a major role in these suppressive responses and thereby modulation of the host immunity.

1.1.9. Parasite components

Schizont rupture is accompanied with the release of certain pyrogens and parasite associated components i.e. Hemozoin, glycophosphoinositols (GPIs), microparticles etc.

1.1.9.1. Glycophosphoinositols (GPIs)

The general structure of GPI is ethanolamine phosphate-6Manα1–2Manα1–6Manα1–4GlcN, α (1–6)- linked to the phosphatidylinositol. *Plasmodium falciparum* synthesizes 2 types of glycophosphoinositols i.e Pf α and Pf β, which are present on the surface of parasite proteins of mature stages (131). The GPIs possess the ability to stimulate signaling in different immune cells and are responsible for malaria pathogenesis (132).

1.1.9.2. Microparticles

These are extracellular vesicles budding from the plasma membrane of cells. The infected RBCs secrete 10 times more microparticles than uninfected RBCs (133). The microparticles communicate between asexual stages and direct them towards the development of sexual stages (134). These vesicles are related to malaria severity (133) as they carry hemoglobin in them and lead to anemia (135). Recently, micro-particles are reported to carry micro RNA which can downregulate PfEMP1. The down-regulation of PfEMP1 suggests the ability of micro-particles in raising an innate immune response (136).

1.1.9.3. Plasmodial DNA

The 24 mega base genome of *Plasmodium falciparum* is fully sequenced and consist of 5300 genes on 14 chromosomes (137). The microvesicles carrying plasmodial DNA can be taken up by immune cells, which then interact with specific receptors like STING and stimulate expression of type 1 IFN genes (138). The protein-DNA complex has been reported to be antigenic to various immune cells (139).

Since this thesis mostly deals with Hz and its interaction with monocytes, it is discussed in details in next section.
1.1.10. Hemozoin (Hz)

It is the insoluble crystal formed by the parasite during hemoglobin digestion in infected erythrocyte. Parasite utilizes 80% of hemoglobin as a source of energy and nutrient (140,141). During this process, parasite forms toxic heme metabolites as it lacks the enzyme heme-oxygenase essential for heme-detoxification. The alternative method of heme-detoxification involving the formation of hemozoin using heme detoxification protein (PfHDP) is utilized (142). Commonly, used antimalarials kill the parasite by inhibiting hemozoin formation as it is indispensable for survival of the parasite. The hemozoin formation in food vacuole begins with erythrocytic invasion and it is released during schizont rupture into blood-stream (143). The natural Hz (nHz), which is released along with food-vacuole during schizont rupture, contains iron-crystalline core associated with lipids, bio-active lipoperoxidation products, parasite and host proteins. The natural hemozoin is sometimes modified using enzymes and chemicals to contain only heme polymer core and lacks parasite or host lipids and proteins, also called as purified hemozoin.

1.1.11. β-hematin (sHz)

It is a synthetic analog of hemozoin (144). It consists of (FeIII-protoporphyrin-IX) dimer similar to heme polymer core of nHz (145). The sHz shows similar infrared spectrum to nHz as fingerprint vibrations around 1664 and 1211 cm⁻¹ corresponding to the C=O and C-O stretching from coordination of the propionate O atom to the Fe(III) metal center of the neighboring heme molecule (fig 1-10.). β-hematin is used as a structural analog of Hz, due to the difficulty of maintaining parasitic cultures, laborious and time-consuming procedures involved in the isolation of natural hemozoin. sHz is used to screen antimalarials that act by inhibiting Hz crystal synthesis (146).
Deepali Bobade,, Ph.D. Thesis, 2018

Figure 1-10. β-hematin (sHz) structure. The unit cell is composed of head-to-tail dimers of heme-bound through propionate O-Fe (III). The extended crystal is then formed from hydrogen bonding of the propionic acid groups of neighboring dimeric units.

Even though sHz is widely used for anti-malarial screening and for studies related to immune responses, it has different size and shape of crystals than nHz (147). Moreover, sHz lacks food vacuole associated parasite proteins and lipids leading to differential immune responses. Recently, sHz has been shown to increase anti-specific responses and used as an adjuvant in not only malaria, but also in other diseases including influenza and dog allergy models (148).

1.1.12. Hz-monocyte interactions

As described earlier, hemozoin or malarial pigment is the heme polymer released during schizont rupture. nHz has been reported to induce both suppressing and activating effects on monocytes. The ingested nHz alters several functions of monocytes such as repeated phagocytosis (149), bactericidal abilities (150), oxidative burst, MHC Class II expression, antigen presentation (151) and maturation to dendritic cells (152). Owing to Hz phagocytosis in monocytes, there is a decrease in the ability of monocyte-derived dendritic cells to mature and present antigen efficiently. Especially, there is downregulation of MHC class II, CD80 and CD83 (153). This also results in immune suppression related to T cells and increased secondary infections such as non-typhoidal Salmonella, herpes zoster virus, hepatitis B virus, Moloney leukemia virus, nematode infections and reactivation of Epstein-Barr virus. nHz may also affect the immune response against certain vaccines (154,155). Moreover, nHz has been shown to impair iNOS and thereby NO production in stimulated monocytes (156,157). In line with this, LPS stimulation of Hz-fed macrophages produced less amounts of NO than LPS control macrophages suggesting suppressive property of nHz. The same report also demonstrated the IFNγ mediated enhanced NF-κB activation (158).

Previously, natural Hz has also been reported to induce secretion of pro- and anti-inflammatory cytokines -TNF α, IL-1β, IL-1RA, IL-10, IL-8 and chemokines CCL2, CCL3, CCL4, CXCL1, CXCL2, CXCL3 and CXCL5 (159,160). The immunosuppression is also attributed to increased levels of anti-inflammatory IL-10, which can decrease TH1 cytokine IL-12 in Hz-ingested monocytes (161). The NF-κB and p38-MAPK signaling is involved in IL-1β, TNF-α and CCL3 production (162). Previously, we had shown the
ability of nHz to induce high levels of cytokine IL-10, IL-1β, TNFα in monocytes and low levels of IL-2, IL-12 and IFNγ in human PBMCs. Moreover, Hz-ingested monocytes suppressed mitogen-stimulated lymphocyte proliferation mediated via IL-10 production (160). IL-10 is also reported to be involved in immune evasion via IL-10R in various viral and other infectious diseases. Strategies aiming to manipulate IL-10/IL-10 R signaling in such diseases may have a therapeutic potential. In line with this, abrogation of IL-10/IL-10R pathway has shown antiviral potential in chronic lymphocytic choriomeningitis and may also be useful in hepatitis (163). The programmed death ligand 1 (PD-L1) abrogation together with IL-10 pathway has shown to be successful in reversing anti-pathogenic T-cell response (164).

Purified Hz, on the other hand, stimulates TLR9 signaling and induces production of cytokines -TNF-α, IL-6, IL-12p40, CCL2 in MYD88 dependent manner in murine spleen and dendritic cells (165). Some studies have suggested purified Hz acts as a carrier of parasite DNA, which activates TLR9 (166). On contrary, some studies suggest that purified Hz is not a carrier of DNA and nor the activator of TLR9 (139). One study shows that sHz coated with parasite DNA induces TLR9 activation (167).

In murine MQ cell line- B10R, NF-kB has been demonstrated to be involved in the production of chemokines- CCL2, CCL3, CCL4 and CXCL2 induced by purified Hz (168). Purified Hz- induced activation of NOD-like receptor containing pyrin domain 3 (NLRP3) inflammasome and IL-1β production is mediated through Src kinase Lyn and the tyrosine kinase Syk pathways in mice (140,141). In contrast to nHz, purified Hz has been reported to induce dendritic cell maturation by upregulating expression of CD86, CD83 and secreted IL12 (169).

The severity of malaria was found to be positively correlating with the number of Hz containing monocytes and neutrophils in various regions including Northwest Ethiopia (170). This was extrapolated to define severity in malaria patients by macroscopic visualization of leucocytes (171). The clinical symptom associated with severe malaria such as acute lung injury was affected by immune-modulatory nature of Hz. Hz induced apoptosis through the CARD9 pathway in alveolar cell and decreased expression of E-cadherin is associated with poor recovery of lung injury (172).
nHz increases matrix metalloprotease-9 (MMP9) activity via production of cytokines TNFα, IL-1β and chemokine CCL3 in human monocytes. MMP9 has a role in severe forms of malaria such as cerebral malaria, where it leads to disruption of blood-brain barrier. The 15 (S, R)-hydroxy-6, 8, 11, 13-eicosatetraenoic acid (15-HETE), a lipid peroxidation product generated by nHz on heme catalysis was shown to reproduce the effect of nHz. This also suggested 15-HETE as active component responsible for the MMP9 activity. Surprisingly, sHz did not induce MMP9 production in human monocytes (Prato et al., 2008). The activation of nHz-ingested monocytes through 15-HETE leads to long-term survival via expression of TNF-a, MMP-9, IL-1RA and HSP27 (159). In agreement with this, persistence of this malarial pigment in tissues for several months is seen, even after the infection is cured (173,174)

1.1.13. Immunomodulators
They possess the ability to modulate the immune system. They may increase (immunostimulators) or decrease (immunosuppressives) the dysregulated immune responses in certain diseases and help in its recovery. Conventionally, immunostimulators are used to augment the immune responses in infectious diseases related to immunodeficiency. Whereas immunosuppressives are given in case of transplantation of organs or auto-immune disorders e.g. lupus, allergies etc. (175). Recently, immunomodulators are gaining importance in the treatment of many infectious diseases including malaria.

Immunomodulators which can alter the properties of inflammatory M1 and anti-inflammatory M2 type in terms of IL-12/IL-10 ratio or other markers have shown therapeutic potential in diseases with altered M1/M2 ratio of monocyte and macrophages. Cerebral malaria is characterized by excessive production of cytokines such as IFN-γ, IL-12 and TNF-α, which lead to inflammatory responses detrimental to host. IL-33, a pro-TH2 cytokine has shown promise in murine model of cerebral malaria by decreasing these excessive cytokine production and induction of Tregs and M2 macrophages. Similarly, mTOR inhibitors such as rapamycin which can regulate IL-12/IL-10 ratio in Leishmania donovani infected monocytes and macrophages may be explored for therapeutic potential (176). The murine model of multiple sclerosis showed the presence of M1 macrophages leading to detrimental inflammatory responses. Fasudil showed a therapeutic effect in this model by decreasing M1 related cytokines IL-1β, TNFα and CCL8 and increasing M2-related MRC1, Arg1, IL-10 and CD14 (177). The other compounds which possess the
ability to shift M1 and M2 phenotypes may be used as drugs for different diseases targeting monocyte-macrophage polarization. Dexamethasone can induce M2 phenotype in monocytes and macrophages by increasing CD206 and CD163 expression (178). Similarly Azithromycin also showed the potential to polarize cells to M2 type (179). The miRNAs such as miR-29b, miR-125a-5p, or miR-155 can induce M1 phenotype via NF-κB activation (180).

The Drugs chloroquine and artemisinin are conventional antimalarials. However, they also possess immunomodulatory effects. These drugs will be discussed in details as chloroquine and artemisinin were used in this thesis.

1.1.13.1. Chloroquine

It is an antimalarial used for the treatment of malaria caused by different Plasmodium species i.e. falciparum, vivax, ovale and malariae. However, it is not used in endemic regions with chloroquine resistance. It exhibits anti-parasitic activity against all erythrocytic stages and even gametocytes. Although the exact mechanism is not known, one of the modes of action of chloroquine is inhibition of Hz formation. The chloroquine can directly diffuse inside infected erythrocyte and food vacuole. The alkaline nature of this drug increases pH inside the food vacuole, where Hz formation takes place. Once inside, the drug binds to free heme and cannot be released outside by diffusion. As a result, the heme polymerase cannot convert heme to Hz and accumulation of toxic heme leads to parasite death. It is known autophagy inhibitor.

Other than anti-parasitic action, chloroquine also possesses immunomodulatory properties, which has extended its application in other infectious diseases. However, this property is not much explored with respect to malaria immunity. Chloroquine possesses the ability to decrease secretion of TNFα, IFNγ and IL-6 in LPS stimulated RAW cell line suggesting their immunomodulatory property (181).

Chloroquine inhibited replication of human coronavirus in L132 infected cells. This was accompanied with p38-MAPK downregulation mediated by chloroquine (182). Chloroquine was found to be cidal for Cryptococcus neoformans via decreasing the inflammatory cytokines such as TNFα, IL-1β and IL-6. The probable mechanism was alkalization of endolysosomes (183). CHQ has also been demonstrated to act via NF-κB (181,184) pathways and decrease IL-1β, IL-6 and TNF-α in mononuclear phagocytes in different diseases (181,185). In another study, CHQ has been shown to be an effective
Review of literature

anticancer drug in mice by inhibiting tumor resistant MQ (M2 MQ) and decreasing TGF-β and IL-10 reduction. The effect was accompanied by decreased myeloid-derived suppressor cells (MDSC), Tregs and increasing CD8+ T cells in tumor milieu (186).

1.1.13.2. Artemisinin

It is derived from Chinese herb Artemisia annua. It is an effective anti-malarial against *falciparum* malaria except in endemic regions with resistance. Many derivatives of artemisinin and their combinations are used effectively for the treatment of malaria. Structurally, the artemisinins consists of 1,2,4-trioxane ring. The endoperoxide linkage present in the ring is cleaved in presence of heme and releases reactive radicals. These free radicals lead to the destruction of parasite. However, artemisinins are pleiotropic and exact mechanism involved in the anti-parasitic action is unknown.

These drugs also possess immune-modulatory activity and are used for the treatment of other infectious diseases. Artemisinin can reduce secretion of inflammatory cytokines TNFα, IL-1β and IL-6 in THP-1 cells (187). From ancient times, it was used for the treatment of fever, bacterial infections and heat stroke in east Asia. It possesses the ability to modulate the immune responses by decreasing iNOS and NF-κB activation and secretion of TNFα and IL-6 (188).

Artemisinin was found to be effective in visceral leishmaniasis. The inhibition of nitric oxide production in Leishmania-infected macrophages was restored by this immunomodulator. Moreover, the parasite killing was accompanied by increased levels of protective cytokines IL-2 and IFNγ (189). An artemisinin derivative was also found to be effective in the treatment of systemic lupus erythematosus murine model. The drug decreased the clinical symptoms and also reduced secretion of IL-6, IL-10 and IL-21 cytokines involved in the pathogenesis of disease. Interference with NF-κB, TLR4, TLR7 and TLR9 was accompanied with inhibition of antibody formation (190).

Moreover, artemisinins have been reported to decrease cytokines (190,191) through MAPK (187), NF-κB (192) and PI3K/AKT (193) pathways in monocyte and macrophages. The anti-parasitic action of artemisinin is accompanied by disruption of parasite proteins, alteration of mitochondrial functions and angiogenesis (191,194). In relation to the ability
of M2 phenotype to stimulate angiogenesis, artemisinin may show therapeutic potential in disorder related to these suppressive phenotypes by altering the release of angiogenic factors.