INVESTIGATING INTERLEUKIN-18 INVOLVEMENT AND ITS MODULATORY EFFECTS ON MAJOR CYTOKINES RELEASE DURING MALARIA INFECTION IN MICE

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2010

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by

KARTINI HASBALLAH

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

October 2010

DEDICATION

This dissertation is dedicated to my lovely husband, Dr. H. Rusly Aly, SpTHT-KL and my sweetest & blessing daughter, Dewi Karlina Rusly, also sons Ronaldy Ferdian Rusly and Renzavaldy Rusly. I give my deepest expression of love and appreciation for the encouragement that you gave and the affection you made during this graduate program. Thank you for the support, pray and sacrifice.

ACKNOWLEDGEMENTS

In the name of Almighty Allah, who gave the strength, inspiration and patience to continue this research. Alhamdulillah, after enduring all the challenges, by the grace of Allah, this research has been accomplished.

I feel pleasure to place my sincere appreciation and gratitude to my supervisor Associate Professor Dr. Gam Lay Harn and Dr. Rusliza Basir for her persistent effort, excellent guidance, support, hospitality and invaluable advice throughout the whole course of my work and preparation of this thesis. I am also deeply grateful to their patiently reviewing and editing of this thesis. I also wish to thank my co-supervisor Dr. Amin Malik Shah Abdul Majid for his advice and suggestion in my laboratory work.

I would like to express my gratitude to Vice Cancelor of USM, the Ministry of Higher Education Malaysia for the relief fund after tsunami and library facilities. I would like to acknowledge the research grant provided by Institute of Graduate Studies, USM, which support a part of the research expenses. I also kindly thank Syiah Kuala University, Banda Aceh, Indonesia for financial support and for providing me the opportunity to undertake this study.

I would like to thank the Dean of School of Pharmaceutical Sciences, USM and the Dean of Faculty of Medicine, Syiah Kuala University, Banda Aceh, for giving me the opportunity to continue my education.

I wish to express my sincere appreciation and thanks to all staff of the School of Pharmaceutical Sciences in particular Mr. Yusof and Mr. Adnan, who always made animals available for me. Mr Roseli, who always helped me as a colleague and friend, Mrs.Yong, Mr. Hassan, Mr. Basri, Mr. Suhaimi, Mr. Chuah, Mr. Tan, Mrs. Mahfuzah,

Ms. Hani, Mr. Malek Rose, Mrs. Salida, and Mr. Ahmad Faizal for their help and kindness throughout the course of this work. I would also like to acknowledge the technical staff of the Poison Centre, USM, in allowing me to use their laboratory especially for using VersaDoc Imaging System and Quantity One 1-D Analysis software for capturing images of all gels and membranes.

Not forgetting, sincere thanks to all my friends and colleagues, my lab mates in lab 118, 202 and 115 in the School of Pharmaceutical Sciences, USM who had help and encouraged me during my project, especially my best friends Yam Mun Fei, See Kok Keat, Lau Wai Hoe, Ang Lee Fung, Mahfoud, Ram, Chin Jin Han, Lim, Vanessa, Hanim, Seng Liang, Nurul for their help and assistance in some of my experiment.

I appreciate all my Indonesian friends, especially my friend at the 'Achenese Student Club' for their encouragements and friendships. Special thanks to Jasman/Arda, Mahdani, Irin/Kamal, Nasrullah, Khadijah Rambe, Nurma, Rosidah, Izwani, Irfan, Linda, Suwarno, Fahrizal, Afrizal, Iskandarsyah Madjid, Taufiq A Gani, Fadil, Muchlisin, T. Iskandar/Ame, Faisal/Wina, Yanti/Saiful, Yeni, Yuni, Hamidah, Azizah, Syukri/Rini, Thaher/Rina, Tatat, Risna, Elly and Erna for helping me through my difficult time.

Finally, I would like to deeply thank and my sincere appreciations to my beloved parents, Drs. H. Hasballah Abu and Hj. Marhamah; brothers, the late Fauzi Rizal, Syamsul Rizal and Taufiq Iskandar; and sister, Asmanizar; my brother and sister-in-law for their prayers and moral support in Indonesia throughout my study in Malaysia. Their loves and beliefs are very meaningful to me.

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binding protein; M+AmIL-18 = Malaria + Anti-mouse IL-18 monoclonal antibody; M+rmIL-18 = Malaria + recombinant mouse IL-18. ≠ and ϕ denote the significant differences (P<0.05) between the M+NS group and M + (rmIL-18BP or AmIL-18 or rmIL-18) groups on day 1, 3 & 5 respectively after the initiation of malaria infection. One-way ANOVA method is used for analysing the data.

LIST OF ABBREVIATIONS

aa	:	amino acid
AmIL-18	:	antimouseIL-18 monoclonal antibody
ANOVA	:	analysis of variance
APS	:	ammonium persulfate
BBB	:	blood brain barrier
B cells	:	B lymphocyte cells
BSA	:	bovine serum albumin
BWF	:	black water fever
°C	:	degree Celsius
CM	:	cerebral malaria
cm	:	centimeter
CNS	:	central nervous system
CTL	:	cytotoxic T lymphocytes
DC	:	dendritic cell
DDT	:	dichlorodiphenyltrichloroethane
DIC	:	disseminated intravascular coagulation
e.g.	:	example
ELISA	:	Enzyme Linked Immunosorbent Assay
EPO	:	erythropoietin
et al	:	elsewhere or and others
Fas-L	:	Fas Ligand
g	:	gram
GM-CSF	:	granulocyte macrophage colony stimulating factor
GNP	:	Gross National Product
GPI	:	glycosyl phosphatidyl inositol

h	:	hour
HAT	:	human African trypanosomiasis
Hb	:	haemoglobin
ICAM-1	:	intercellular adhesion molecule-1
ICE	:	IL-1β converting enzyme
IFNγ	:	interferon-γ
IFNγR	:	IFNγ receptor
IgA	:	Immunoglobulin A
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
IL-	:	Interleukin
IL-18BP	:	Interleukin-18 binding protein
i.p.	:	intraperitoneal
IU	:	international unit
i.v.	:	intravenous
kDa	:	kilodalton
kg	:	kilogram
L	:	liter
LFA-1	:	lymphocyte function associated molecule-1
LPS	:	lipopolysaccharide
LTα	:	lymphotoxin-α
M	:	molar
mA	:	milliampere
mg	:	milligram
MHC	:	major histocompatibility complex
mIFNγ	:	mouseIFNγ
mIL-18	:	mouseIL-18

mIL-1α	:	mouseIL-1α
min	:	minute
mL	:	mililiter
mM	:	milimolar
mmol	:	millimol
MSP-1	:	merozoite surface protein-1
N	:	number of observation
ng	:	nanogram
NK Cells	:	Natural Killer Cells
nm	:	nanometer
NO	:	nitric oxide
P.	:	*Plasmodium*
PAGE	:	polyacrylamide gel electrophoresis
PBS	:	phosphate buffered saline
pg	:	picogram
PGE_2	:	prostaglandin E_2
PRBC	:	parasitized red blood cells
RBC	:	red blood cells
RES	:	reticuloendothelial system
rmIL-18	:	recombinant mouse Interleukin-18
rmIL-18BP	:	recombinant mouse Interleukin-18 binding protein
rpm	:	revolution per minute
S.D.	:	standard deviation
SDS	:	sodium dodecyl sulphate
SDS-PAGE	:	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
sec	:	second
S.E.M.	:	standard error of the mean

T cells	:	T lymphocytes
TEMED	:	N,N,N',N'-tetramethylethylenediamine
TGF-β	:	tumour growth factor-β
Th1	:	T helper type 1
Th2	:	T helper type 2
TMB	:	tetramethylbenzidine
TNFα	:	tumour necrosis factor-α
TNFβ	:	tumour necrosis factor-β
μg	:	microgram
μL	:	microliter
μm	:	micrometer
V	:	voltage
VCAM-1	:	vascular cell adhesion molecule-1
wet wt.	:	wet weight

MENYELIDIKI PENGLIBATAN INTERLEUKIN-18 DAN KESAN PENGUBAHSUAIANNYA TERHADAP PELEPASAN SITOKIN-SITOKIN UTAMA SEMASA JANGKITAN MALARIA DALAM MENCIT

ABSTRAK

IL-18 merupakan sitokin pro-inflamasi poten yang memainkan pelbagai peranan dalam tindakbalas imunisasi dan aktiviti inflamasi dalam banyak penyakit, tetapi penglibatannya dalam mendasari patogenesis malaria masih belum terhurai sepenuhnya. Dalam kajian ini, peranan dan penglibatan IL-18 semasa jangkitan malaria diselidiki dan penilaian awal telah dijalankan tentang kesan modulasi laluannya ke atas perkembangan jangkitan dan sitokin utama yang dirembeskan semasa jangkitan. Jangkitan *Plasmodium berghei* ANKA pada mencit ICR digunakan sebagai model malaria sepanjang kajian ini. Haiwan diinokulasi secara intravena dengan 2 X 10^7 sel darah merah berparasit (PRBC) yang diperolehi daripada mencit penderma yang dijangkiti parasit. Haiwan kawalan menerima isipadu dan pencairan (0.2 mL, i.v.) sel darah merah mencit normal (RBC) yang sama. Keputusan menunjukkan bahawa mencit malaria memperlihatkan perubahan tingkah laku sakit pada hari keempat selepas inokulasi, iaitu ketika paras parasitemia ≥ 60% dan terus meningkat sehingga parasitaemia beredar mencapai sekitar 80%. Mencit yang dijangkiti tewas kepada hiperparasitemia 5-6 hari selepas infeksi. Mencit ICR juga menunjukkan penurunan suhu badan dan berat badan yang signifikan ketika parasitemia puncak. Kepekatan IL-18 dalam plasma yang ditentukan melalui kaedah ELISA, menunjukkan peningkatan yang signifikan sepanjang jangkitan dan satu hubungan korelasi yang positif dengan perkembangan parasitemia telah diperhatikan. Oleh kerana jangkitan malaria menyebabkan ketidakfungsian pelbagai organ, tisu-tisu dari organ-

organ utama yang diketahui terpengaruh semasa jangkitan termasuk limfa, hati, otak, paru-paru dan ginjal telah diperiksa bagi pengekspresan IL-18 menggunakan kaedah SDS-PAGE dan analisa Western blot. Kajian mendapati bahawa IL-18 diekspreskan pada intensiti yang berbeza dalam limfa, hati, otak dan paru-paru. Tiada IL-18 dikesan dalam ginjal mencit semasa jangkitan. Pengeluaran IL-18 semasa jangkitan malaria dimodulasi melalui rawatan dengan rmIL-18BP, AmIL-18 dan rmIL-18. Pemberian rmIL-18BP dan AmIL-18 menghalang perkembangan parasitemia pada tahap awal infeksi. Perencatan signifikan terhadap perkembangan parasit berlaku pada hari pertama hingga ketiga selepas rawatan. Selepas rawatan, berat badan dan suhu badan mencit malaria menurun pada hari keempat hingga keenam daripada nilai awal eksperimen. Pemberian rmIL-18 menyebabkan paras parasitemia meningkat lebih pantas diikuti dengan penurunan berat badan dan suhu badan. Mencit malaria yang diberi rmIL-18 didapati mati lebih awal. Hasil kajian ini juga menunjukkan bahawa perencatan terhadap IL-18 melalui pemberian rmIL-18BP dan AmIL-18 telah menurunkan pelepasan paras IFNγ, TNFα, IL-6 dan IL-1 tetapi meningkatkan paras IL-10 secara signifikan. Sebaliknya, peningkatan pengeluaran IL-18 meningkatkan paras IFNγ, TNFα, IL-6 dan IL-1 dan menurunkan paras IL-10 secara signifikan dalam serum semasa jangkitan malaria. Kesimpulannya, hasil kajian ini mencadangkan bahawa IL-18 terlibat semasa jangkitan malaria dan memainkan peranan penting sebagai perantara terhadap ketenatan penyakit tersebut. Modulasi laluannya menghasilkan kesan yang signifikan ke atas rangkaian sitokin semasa jangkitan di mana ini mungkin mencadangkan bahawa modulasi terhadap penghasilan IL-18 semasa jangkitan boleh menjadi satu konsep terapeutik yang berpotensi bagi terapi malaria

INVESTIGATING INTERLEUKIN-18 INVOLVEMENT AND ITS MODULATORY EFFECTS ON MAJOR CYTOKINES RELEASE DURING MALARIA INFECTION IN MICE

ABSTRACT

IL-18 is a potent proinflammatory cytokine that plays multiple roles in immune responses and inflammatory activities in many disease conditions, but its involvement in the underlying pathogenesis of malaria has not been fully elucidated. In this study, the role and involvement of IL-18 during malaria infection was investigated and the impact of its pathway modulation on the course of the infection and the major cytokines released during the infection was preliminary evaluated. *Plasmodium berghei* ANKA infection in ICR mice were used as malaria model throughout the study. The animals were inoculated intravenously with 2 x 10^7 parasitized red blood cells (PRBCs) obtained from a donor mouse infected with the parasite. The control animals received an equivalent volume and dilution (0.2 mL, i.v.) of normal mouse RBC. Results demonstrated that the malarial mice showed sick behavioral changes on day 4 after inoculation when the levels of parasitaemia were ≥ 60% and then continued to increase until circulating parasitaemia reached around 80%. The infected mice succumbed to hyperparasitaemia 5-6 days after infection. ICR mice also showed significant decrease in body temperature and body weight during the peak parasitaemia. IL-18 concentrations in the plasma determined by means of ELISA, showed significant elevation throughout the infection and a positive correlation with parasitaemia development. Since malaria infection causes multi organ dysfunction, tissues from major organs known to be affected during malaria infection which include the spleen, liver, brain, lungs and kidney

were examined for IL-18 expression using SDS-PAGE and Western blot analysis. The study revealed that IL-18 was expressed in different intensity in the spleen, liver, brain and lungs. No IL-18 was detected in the kidney of mice during the infection. IL-18 production during malaria infection was modulated by treatment with rmIL-18BP, AmIL-18 and rmIL-18. Treatment with rmIL-18BP and AmIL-18 inhibited the parasitaemia development at early phase of infection. Significant inhibition on parasites development occurred on day 1 until day 3 after treatment. Body weight as well as body temperature of malarial mice decreased on day 4 until day 6 from their initial values after the treatment. rmIL-18 treatment caused the parasitaemia levels to increase rapidly followed by a decrease in body weight and body temperature. Earlier mortality was also observed in the malarial mice treated with rmIL-18. Results also showed that inhibition of IL-18 by treatment with rmIL-18BP and AmIL-18 significantly reduced the release of pro-inflammatory cytokines IFNγ, TNFα, IL-6 and IL-1α levels and on the other hand increased the level of anti-inflammatory cytokine IL-10 significantly. In contrast, augmentation of IL-18 production significantly increased the levels of the pro-inflammatory cytokines and reduced the level of the anti-inflammatory cytokine in the serum during the infection. In conclusion, the results from this study suggest that IL-18 is involved during malaria infection and it may well play a crucial role in mediating the severity of the disease. Its pathway modulation produced a significant impact on the cytokine network during the infection which may suggest that modulating IL-18 production during malaria infection could be a promising therapeutic concept for malaria therapy.

CHAPTER 1

GENERAL INTRODUCTION

1.1 The problem of malaria

1.1.1 Endemic problem

Malaria is a major public health problem that causes severe morbidity and mortality in the tropical and sub tropical countries such as Africa, South America, Eastern Europe and Asia. The disease potentially affects about half of the world's population and more than 3 billion people are at risk of being infected. It is estimated that 250 million cases led to nearly 1 million deaths from malaria annually (WHO, 2008). Young children under 5 years old and pregnant women, especially primigravidae are the highest risk groups for malaria in the endemic areas (WHO, 1999). Up to 10,000 pregnant women (Guyatt and Snow, 2001) and 200,000 newborns (Steketee *et al*., 2001) died as a consequence of malaria during pregnancy annually.

Malaria is endemic in 101 countries and is caused by a parasite, *Plasmodium*. *Plasmodium falciparum* and *P. vivax* are co-occurring in 61 countries. *P. falciparum* occurs in 28 countries, mainly in tropical Africa, and accounts as the major source of malaria in Africa which causes 83% of the total case of malaria. Whereas, *P. vivax* occurs in other 12 countries such as North Africa, Latin America and Western Asia and accounts as the major source of malaria in Australasia which causes 74% of the total case of malaria in the region (Schlagenhauf-Lawlor, 2001). As the parasite – *P. vivax* could be distributed widely and recorded to have about 70-80 million cases annually (Mendis *et al*., 2001). With the current increase of global traveling, malaria has become a threat to human health worldwide.

1.1.2 Drug resistant

There has been many research aimed to control malaria, for instance the discovery of chloroquine and other antimalarials as well as the development of insecticides to eliminate the vector. Unfortunately, the efficacy of antimalarial drugs and insecticides in controlling malaria has yet to be optimised. In addition, the parasite has developed resistance to previous found malarial drugs such as chloroquine, the spread of multidrug resistant strains to newer classes of antimalarial drugs (Newton and White, 1999), The increased resistance of the vector to insecticides are related to lack of infrastructure in endemic countries (Pinzon-Charry *et al*., 2006; Asahi, *et al*., 2005).

1.1.3 Socio economic problem

Many of the world's poorest people live in areas with high rates of malaria. Thus malaria is also called the disease of the poor and associated with social and economic hardships (WHO, 2002). The economic impact includes (a) households, (b) health systems, and (c) national economies. At the household level, malaria imposes both direct and indirect costs. Direct costs include time lost from work and medical treatment costs (including transportation and medical care). Indirect costs include loss of work efficiency and time and work reallocation within the household. For children in particular, indirect costs also include nutritional deficiencies, cognitive and educational disabilities, and physical retardation (Hutubessy *et al*., 2001). Costs to health care include treatment and medication costs. In most economies, households pay a small amount for treatment and medication, with the rest of the cost borne by the health system. These direct and indirect impacts above can collectively impede economic development and growth. It is estimated to cause a

decline in economic growth in the range of 0.25% to 1.3% of per capita gross national product (GNP) growth in tropical countries (Guerin *et al*., 2002; Sachs and Malaney, 2002).

1.2 Malaria parasite

Malaria is a parasitic infectious disease which is predominantly intracellular parasites found in blood and tissues. The disease is caused by protozoa of the genus *Plasmodium* belongs to family Plasmodiidae, phylum Apicomplexa (Menard, 2000). One hundred and twenty species of *Plasmodium* are found in the blood of mammals, reptiles and birds. Nonetheless, *Plasmodium* and *Laverania* are the subgenera that contribute to humans malaria. There are four foremost known species of malaria parasites that infect humans: *P. falciparum, P. vivax, P. malariae* and *P. ovale* which are exclusively transmitted by female mosquito of the *Anopheles* genus (Warrel and Gilles, 2002; Malaguarnera and Musumeci, 2002a; Ramasamy, 1998). Particularly, *P. falciparum* is the most virulent form of malaria and the major cause of mortality (Warrell and Gilles, 2002).

The common type of mixed infections is *P. falciparum* with *P. vivax* and is found in the subtropical area. While in tropical Africa, the more frequent mix is between *P. falciparum* and *P. malariae* or *P. ovale*. Infections by more than two species at a time are rare occasions and could be differentiated via microscopic examination of blood smears. Clinical observation has shown that the different species of malaria parasites cause different symptoms (Warrell and Gilles, 2002).

1.3 The life cycles of malaria parasite

There are two kinds of reproduction cycles of malaria parasite, which are sexual and asexual. The sexual reproduction (sporogony) occurs in insect vector – mosquito, whereas asexual reproduction occurs in the vertebrate host (pre-erythrocytic schizogony and erythrocytic schizogony) (Warrell and Gilles, 2002).

1.3.1 The life cycles of malaria parasite in human

During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host (into the peripheral circulation) by depositing saliva on the skin. The saliva contains an anticoagulant, a topical anaesthetic and a form of the sporozoite resident (Davidson and Gowda, 2001). Then the sporozoite travel through the bloodstream and invades hepatocytes (liver parenchymal cells) within 1 hour of inoculation. The sporozoite surface protein contains a domain that is homologous to thrombospondin and thus has a very high affinity to human hepatocyte receptors, serum proteins thrombospondin and properdin, and thereby infects the hepatocytes (Boutin *et al*., 2005). Following liver invasion, the parasites begin to develop and multiply asexually over a period of about one week into an exoerythrocytic schizont containing approximately 10,000-30,000 merozoites (Warrell and Gilles, 2002). In *P. vivax* and *P. ovale*, dormant hypnozoite forms can persist in the liver and cause relapses by invading the bloodstream in weeks, months or even years later. Contrariwise, there is no dormant form of *P. falciparum*. The rupture of infected liver cells releases merozoites into the bloodstream, that will invade the red blood cells (RBCs) to undergo erythrocytic schizogony, thereby initiating the erythrocytic cycle merozoite which develops into a ring trophozoite. This occurs within a parasitophorous vacuole in the RBC (Boutin *et*

al., 2005; Ramasamy, 1998). Ring trophozoites synthesize proteases to degrade haemoglobin into amino acids. These and other nutrients obtained from the host enable the parasite to grow within 15-18 hours into mature trophozoites (Ramasamy, 1998), and finally into a schizont, which gives rise to approximately 16 new merozoites (Boutin *et al*., 2005; Ramasamy, 1998). The parasite dramatically alters the physiological and biochemical processes of its host's RBC (Boutin *et al*., 2005) and adorns its surface with parasite-encoded molecules that further affect the RBCs mobility and trafficking within the body (Chowdhury and Bagasra, 2007). Following rupture of the infected erythrocytes, merozoites are released and infect new erythrocytes. To complete the life cycle in human host, some merozoites develop into male and female gametocytes within the RBCs. They will enter the mosquito's gut after it bites an infected human. Male and female gametes emerge from the infected RBCs in a process referred to as exflagellation. The gametes fertilized in the gut giving rise to ookinetes that burrow into the gut wall. The ookinete traverses the midgut epithelium to develop into an oocyst and result in the production of infected sporozoites which migrate to the salivary gland of the mosquito to continue the infection cycle (Okie, 2005; Boutin *et al.*, 2005). Figure 1.1 demonstrates the malaria parasite life cycle in the human and mosquito host (Chowdhury and Bagasra, 2007).

In *P. falciparum* infection, merozoites invade the red blood cells and reinitiate an erythrocytic cycle as of once in three days and caused malignant tertian malaria. *P. vivax* and *P. ovale* have also a 48 hours development period that causes benign tertian and ovale tertian malaria respectively. The other human malaria parasite, *P. malariae*, has a longer cycle that lasts 72 hours. It is responsible for benign quartan malaria, in which patients present symptoms every four days.

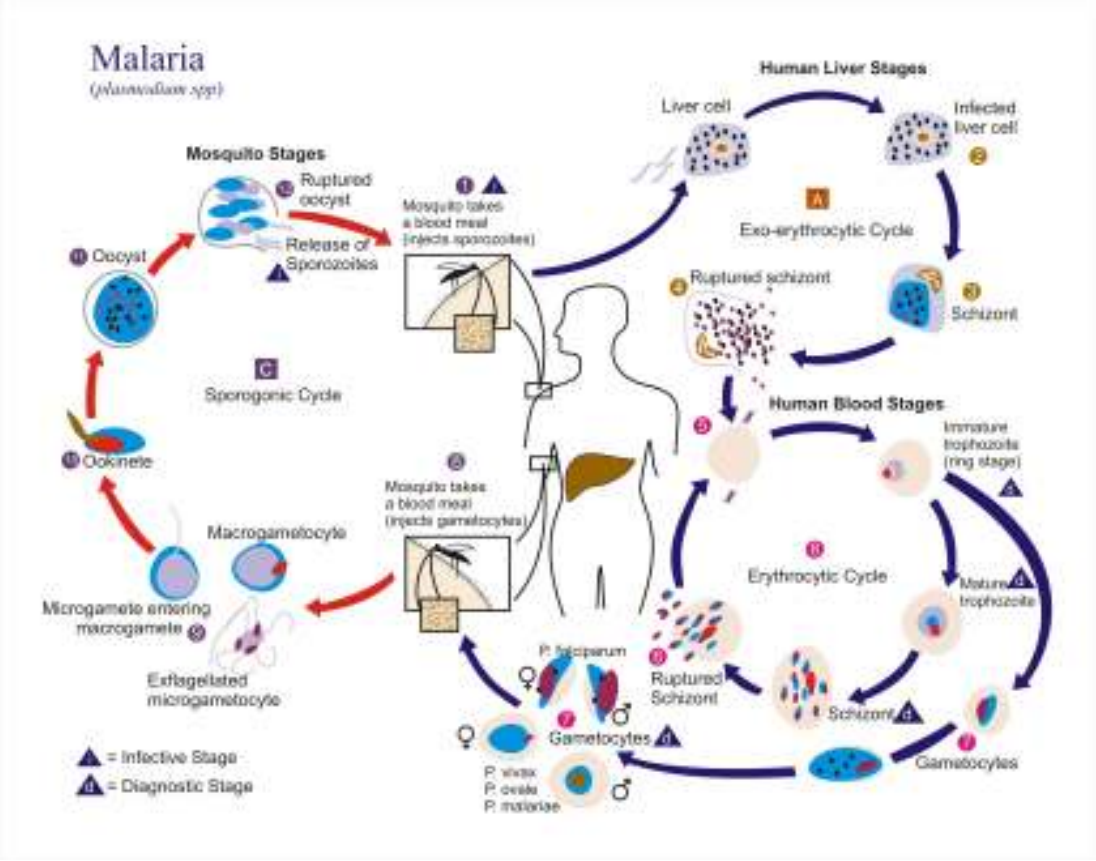

Figure 1.1: The life cycle of malaria parasite, adapted from Chowdhury and Bagasra (2007).

Parasites of any of these four species can develop simultaneously in the same patient (Warrell and Gilles, 2002).

The ability of the organism to invade erythrocytes that cause parasitaemia depends on the species of *Plasmodia*. Merozoites from species of *P. vivax* and *P. ovale* could only invade reticulocytes (immature erythrocytes) at the erythrocytic stage of the life cycle, which serves to limit the magnitude of parasitaemia in these types of malaria (Warrell, 2002; Sinden and Gilles, 2002; Powell and Grima, 2002). *P. malariae* can invade mature erythrocyte and the progress is slow in both human and mosquito and therefore leads to lower degree of parasitaemia (Sinden and Gilles, 2002; Warrell, 2002). *P. falciparum* has the ability to invade all age of RBCs and therefore causes a very high level of parasitaemia (higher than in the other species)

that rapidly direct to severe or most frequently fatal cases of malaria (Lallo *et al*., 2007; Warrell, 2002; Heddini, 2002).

1.4 Malaria symptoms

1.4.1 Fever

Fever is the most common presenting symptoms of malaria infection (Warrell and Gilles, 2002) and it is directly related to the release of blood merozoite upon the break up of the parasitized red blood cells (PRBCs) while the schizogony ends with relatively synchronous at the initial stages of infection (Ramasamy, 1998; López-Antuñano and Schmunis, 1990). Fever due to tertian malaria occurs every 48 hours in *P. falciparum, P. vivax* and *P. ovale* infection and quartan malaria occurs every 72 hours in *P. malariae* (López-Antuñano and Schmunis, 1990).

In *P. falciparum* infection, fever is accompanied by nausea, headache and chills (Ramasamy, 1998). At the time of schizont rupture, the lipid glycosyl phosphatidyl inositol (GPI) and merozoite surface protein 1 (MSP-1) are released, which are toxic and cause intermediate production of pyrogenic cytokines, such as interleukines (IL-1, IL-6, IL-8), interferon (IFNγ) and tumour necrosis factor-α (TNFα) that are released by activated macrophage (Warrell *et al*., 2002; Leon, 2002). The height and magnitude of fever is a sum of the interaction of pyrogenic cytokines and endogenous anti pyretics (Leon, 2002). Indeed, TNFα has been implicated as the major cause of malarial fever (Warrell *et al*., 2002).

In a non-immune individual infected with *vivax* malarial, the typical symptom is chill, which may last for one hour. During this period, body temperature rises sharply, reaching to ~41 ^{o}C within the first two hours after the onset of the chill, then temperature begin to fall and accompanied by profuse sweating (Karunaweera *et al.*,

1992). Researchers found that *vivax* patients had a significant and up-regulated TNFα and IL-6 levels and down-regulated of IL-10 in patients before the start of treatment (Sohail *et al*., 2007).

Temperature change is one of the responses to the biological active component of TNF. On the patients response to the differentiated level of TNF may reflect variation in their ability to neutralize and/or inactivate TNF. Nevertheless, production of cytokines in circulation is considered to be important because cytokines including TNFα are large hydrophilic peptides that would cross the blood brain barrier (BBB) in order to act on the hypothalamic thermoregulatory region that lead to the development of fever (Netea *et al*., 2000). Moreover, IL-6 contributes to the systemic action that stimulates the synthesis of acute phase proteins by hepatocytes (Abbas and Lichtman, 2005; Zetterstrom *et al*., 1998). These acute phase proteins act cohesively with TNF and IL-1 to promote fever that enhances the production of IL-6 during malaria infection (Zetterstrom *et al*., 1998). The importance of pyrogenic cytokines such as TNF, IL-1β and IL-6 in sustaining fever in malaria has also been demonstrated (Clark and Cowden, 2003). Furthermore, therapeutic trials of monoclonal anti-TNF antibodies have shown to abrogated febrile responses (Kwiatkowski *et al*., 1993).

1.4.2 Anaemia

Severe anaemia in non immune patient with an acute *P. falciparum* infection is common and associated with haematological changes related to parasitaemia (Weatherall, 1988). Haematological changes of RBCs are the principal clinical outcome of malaria infection. All other clinical manifestations are primarily due to the involvement of RBCs. Anaemia is an important cause of morbidity and

potentially leads to mortality. Other clinical manifestation was reported to have less serious health consequences (Weatherall, 1988).

Anaemia is an inevitable consequence of erythrocyte parasitization as all PRBCs are destroyed at merogony (schizont rupture). Furthermore, dyserythropoiesis enhanced splenic clearance and even blood loss that contributed towards malaria anaemia. The survival of non-parasitized erythrocytes was found reduced for several weeks after clearance of parasitaemia in patients with *falciparum* and *vivax* malarial (WHO, 2000).

The merozoites of *P. falciparum* could infect a high proportion of RBCs leading to parasitaemia that resulted in >50 % of lethality. The mechanisms of anaemia are multifactor. The principle factors are haemolysis of parasitized red cells due to rupture of the schizonts; decline in the production of erythrocytes due to depression of erythropoiesis; increased phagocytosis of the red cells due to the change in sodium metabolisms; and haemolysis of the parasitized or unparasitized red cells through immunologic mechanism (Perin *et al*., 1982).

1.4.3 Thrombocytopenia

Thrombocytopenia often occurs in most patients with *falciparum* and *vivax* malaria. Platelet survival is reduced to 2-4 days in severe *falciparum* malaria (WHO, 2000). Thrombocytopenia may be related to the sequestration of the platelets in the spleen and disseminated intravascular coagulation (DIC). Platelets may be removed from the circulation at sites of fibrin deposition (Warrell *et al*., 2002).

1.4.4 Leucopenia

In mild leucopenia, a neutrophil leucocytosis is detected in patient with severe *falciparum* malaria. Indeed, the severity of malaria correlates with the level of TNFα (Day *et al*., 1999), neutrophil activation (Chen *et al*., 2000) and haemozoin loaded in circulating monocytes and neutrophils (de Souza and Riley, 2002). TNFα is a major mediator of the acute inflammatory response that induces cellular functions in leukocytes leading to leukocytosis. This cytokine is important in reducing parasitaemia at its early production (Kremsner *et al*., 1995). However, the over expression of TNFα could increase the severity of the disease (Day *et al*., 1999). The excess of TNFα production is also important in activation of endothelial cell expression of ligands for adhesion molecules which result in the subsequent sequestration of infected erythrocytes, leukocytes and platelets thus leading to microvascular obstruction (Kremsner *et al*., 1995).

1.5 Organ dysfunction in malaria

Malaria infection in human causes dysfunction of multiorgan (Warrell, 2002), such as brain, spleen, liver, lung, kidney and gastrointestinal organs.

1.5.1 Brain

The pathological changes of the blood brain barrier (BBB) leads to human cerebral malaria (Carvalho *et al*., 2000). Cerebral malaria (CM) is one of the most serious complications of *falciparum* infection, associated with neuropathological features causing a blockade of brain microvessels due to accumulation of host cells. Predominantly, erythrocytes are invaded by the parasite followed by sequestration of leukocytes and platelets as a result from adhesion of PRBC to receptor on the endothelial surface of the microvasculature (Potter *et al*., 2006; Combes *et al*., 2006).

These events could cause brain oedema, alterations of the BBB, microhaemorrhages and necrosis of the surrounding tissue (Coltel *et al.*, 2004). On the post-mortem, specimen commonly reveals brain damage as the main factor of fatal syndrome (Combes *et al*., 2006).

1.5.2 Spleen

Splenomegaly is common in all four species of human malaria (Warrell *et al*., 2002). It is a well-established phenomenon in malaria endemic areas (Strickland *et al*., 1988; Greenwood *et al*., 1987; Bryceson *et al*., 1983; Thomas *et al*., 1981). In acute *falciparum* infections, the spleen is enlarged with either texture soft or firm, the colour varying from dark red to dark or state grey, depending on the duration of the infection and the amount of pigment (Warrell *et al*., 2002). Enlargement of the spleen may be detected a few days after the acute attack for non-immune or semi-immune individuals, which then gradually decrease after recovery (Bryceson *et al*., 1983). A huge splenomegaly with size >10 cm and high serum Immunoglobulin M (IgM) levels was reported in patients with hyperactive malaria splenomegaly (Fakunle, 1981).

In *vivax* malaria, enlargement of the spleen is more rapid and might lead to splenic rupture. Splenic cords and sinuses are massively congested with monocytes and macrophages which contain pigment, PRBCs and non infected red cells. The red pulp is expanded and filled with lymphocytes, immunoblast and plasma cells. In addition, extramedullary haemopoiesis is a common phenomenon. Moreover, focal haemorrhage and infarction are also often observed. The process where pigment malaria migrate through the splenic cords, littoral cells phagocytose pigment as well as extract the malaria parasites from PRBCs is known as pitting. In patient with

repeated attacks of malaria, lymphoid compartment of white pulp exhibits a considerable depletion. However, the red pulp still exhibits reticuloendothelial hyperplasia with thickening of the fibrous trabulae and capsule (Warrell *et al*., 2002).

1.5.3 Liver

It is known that plasmodia infection causes hepatomegaly (von Brand, 1973) but the condition tends to be more severe in *P. falciparum* infection. The major changes are in the hepatic sinusoids and lining cells with relatively little damage to hepatocytes. After the first paroxysms, the usual circumstances are enlarged liver, sequestered blood cells and sinusoidal engorgement. The liver is oedematous and its colour is either brown, grey or even black as a result of deposition of malaria pigment. In acute infection with repeated attacks on lobular accentuation will cause the accumulation of the pigment in the portal tracts, the liver possibly friable and firm.

Intrahepatocyte schizonts and merozoites were observed without inflammatory changes. In early infection, hepatic sinusoids are dilated due to congestion with Kupffer cell hyperplasia, PRBCs and fine pigment in red cells and Kupffer cells. The increase number of Kupffer cells, endothelial cells and sinusoidal macrophages that were recruited by the spleen led to phagocytosis on PRBCs and non infected erythrocyte. The filling of infected erythrocytes in the sinusoids causes the reduction of hepatic circulation which lead to splanchnic constriction (Warrell *et al*., 2002). Severe liver failure is the other common clinical complication of untreated cases (Ramasamy, 1998).

1.5.4 Lungs

Certain complications are reported on lungs in malaria infection. Pneumonia is one of fatal complication of severe *falciparum* malaria (Warrell *et al*., 2002). Besides, respiratory complications due to severe malaria are frequent phenomena, although it is increasingly recognized that tachypnoea and abnormal breathing patterns are often related to respiratory acidosis that may indicate generalized metabolic abnormalities or intracranial events rather than primary lung disease (Crawley *et al*., 1998).

Malaria infection can cause pulmonary oedema that is identified commonly by both free alveolar fluid and interstitial oedema. Morphologically, the infected lung is dark with scattered haemorrhages. Numerous alveolar and septal haemorrhages may present due to accumulation of leukocytes in alveolar vessels. The alveoli are usually found congested with pigment-laden macrophages, plasma cells, neutrophils and PRBCs. Leukocytes are seen adherent to alveolar endothelial cells. Activated macrophages and lymphocytes occur in acute pulmonary syndrome (Warrell *et al*., 2002). Acute pulmonary oedema is an infrequent but nearly fatal complication in untreated cases of acute *falciparum* malaria (Ramasamy, 1998).

1.5.5 Kidney

In severe *falciparum*, renal dysfunction is common in non-immune adult indicated by proteinuria (Fletcher and Gilles, 1988). The acute renal failure may develop, associated with anuria and acidosis as part of multisystem illness that induce haemodynamic shock and jaundice. On the acute tubular necrosis, PRBCs, host leukocytes and pigment-laden macrophages are found within glomerular capillaries and interstitial vessels. It has been reported that the density of parasite

sequestration in kidney is often less compared to other organs, despite its remarkable content of lymphocyte, besides, fibrin thrombi can be seen in some glomerular capillaries and tubular red cells casts are also observed (Warrell *et al*., 2002). In *P. malariae* infection, nephrotic syndrome oedema appears to be common (Quartan malaria nephropathy) (Playfair and Bancroft, 2004). In acute *falciparum* malaria, glomerulonephritis is also a common phenomenon and usually associated with proteinuria, with or without haematuria. The glomeruli become hypercellular with expansion of mesangial ground substance, noticeably thickened glomerular on membranes base, swollen glomeruli and obvious adhesions to Bowman's capsule (Warrell *et al*., 2002).

The passage of dark or red urine in malaria, known as black water fever (BWF) is linked to renal failure that is attributed to acute haemoglobinuria and intravascular haemolysis (WHO, 1990). The syndrome is generally seen in people with limited immunity to malaria (Bruneel *et al*., 2001). Recently, dark urine attributed to myoglobinuria in adult who had complicated by rhabdomyolysis was reported (Sinniah and Lye, 2000; De Silva *et al*., 1988). The occurrence of dark urine in children with malaria is frequent. Both haemoglobin and myoglobin are said to contribute to dark urine in children with malaria. Muscle cell injury that also contributes to dark urine is more common in children with intravascular haemolysis (Donnel *et al*., 2006).

1.5.6 Gastrointestinal organs

Gastrointestinal symptoms such as anorexia, nausea, vomiting and abdominal pain are usually associated with acute *falciparum* malaria. Sequestration and cytoadherence occur in small and large bowel, predominantly within the lamina

propriety capillaries and also in larger submucosal vessels. In many severe cases, mucosal ulceration and haemorrhage may develop. At this stage, fibrin thrombi can be found in small blood vessels (Warrell *et al*., 2002).

In the severest form of malaria, diarrhea may develop. Fatal gastrointestinal bleeding has been reported with high *P. falciparum* parasitaemia (Boonpucknavig *et al*., 1984). Multiple foci of mucosal haemorrhage were observed in stomach and small intestine (except jejunum) but less prominent in colon. Gastrointestinal dysfunction is identified by some changes of absorptive function. Apart from congestion and pigmentation as a result of high parasitaemia, the pancreas demonstrates some alteration of vascular congestion with parasitized and non-parasitized erythrocytes and the presence of pigment-laden macrophages. There are generally no major pathological alterations of the gastrointestinal tract in *vivax* and quartan malaria (Fletcher and Gilles, 1988).

1.6 Immunity to malaria

Immunity is a reaction of the body towards foreign substances, i.e. macromolecules such as proteins and polysaccharides. The immune system can be induced by cells and molecules (Abbas and Lichtman, 2005). It physiologically functions to fight against infection (Abbas and Lichtman, 2005; Bruce-Chwatt, 1985).

The role of the spleen in the immune response has been known. A study shows that removal of this organ affects profoundly the course of malaria in animal or human subjects where protection is seen with intact spleen when challenged with the asexual intra-erythrocytic forms of the parasite (Bruce-Chwatt, 1985).

1.6.1 Innate immunity

Innate immunity is also called natural or native immunity, it acts as an early line of defense against the infection. It also stimulates and modulate adaptive immune responses (Abbas and Lichtman, 2005; Carayannopoulos and Yokoyama, 2004). Innate immunity consists of cellular and biochemical defense mechanism prior to infection and poised to respond rapidly towards infections. The principal components to innate immunity involve physical and chemical barriers, such as production of epithelia and antimicrobial substances produced at epithelial surfaces. The component of innate immunity include phagocytic cells (neutrophils, macrophages) and natural killer (NK) cells, blood proteins, including members of the complement system, mediators of inflammation and proteins called cytokines that regulate and coordinate many of the activities of the cells (Abbas and Lichtman, 2005).

Innate immune system contributes to the control of acute infection by mounting protective responses against invading pathogens before the onset of T and B cell-mediated immunity. NK cells are known to be the key players in these early innate responses (Carayannopoulos and Yokoyama, 2004). The role of NK cells in immunity to malaria is investigated by administrating exogenous IL-12 in susceptible A/J mice infected with *P. chabaudi chabaudi* AS parasite. The result shows that the mice exhibit the NK cell related mechanism of immunity in early stages of the disease. The result significantly indicates that at the initial stage of infection, interferon-γ (IFNγ), TNFα and nitric oxide (NO) were detected (Stevenson *et al*., 2001).

NK cells are large, granular, cytotoxic lymphocytes that can recognize and destroy aberrant cells such as those infected by intracellular viral, bacterial, protozoa

pathogens, transformed cells and a range of stress induced molecules (Hansen *et al.*, 2007; Robbin and Brossay, 2002). NK cells can also be rapidly activated to produce high levels of pro-inflammatory cytokines, particularly IFNγ, TNFα, lymphotoxin-α (LTα), granulocytes-macrophage colony stimulating factor (GM-CSF), IL-3 and tumour growth factor-β (TGF-β) (Hansen *et al.*, 2007; Robertson, 2002). NK Cells are able to influence adaptive immunity by modulating dendritic cell (DC) function and inducing the T helper type 1 (Th1) polarization via IFNγ production (Hansen *et al.*, 2007). The invasion of pathogens, or antigens of viral, bacterial and protozoa, are captured by dendritic cells (DCs) or monocyte macrophages which then release NK cell-activating cytokines (Cooper *et al*., 2004; Ferlazzo *et al*., 2003; Lande *et al*., 2003; Sher *et al*., 2003; Biron *et al*., 1999).

Interleukin-2, IL-12, IL-15, IL-18, TNFα, and IFNα/β all contribute to activation of NK cells whereas IL-4, IL-10 and TGF-β suppress NK cells function (Colucci *et al*., 2003; Biron *et al*., 1999). Interleukin-12 is a pro-inflammatory cytokine mainly produced by macrophages and DCs is the most potent inducer of NK cell cytotoxicity and IFNγ secretion, along with IL-18, it plays a crucial role in triggering NK-mediated immune responses (Wei *et al*., 1999; Trinchieri, 1998). NK cells appear to play an important role in the early immune response to a wide variety of pathogens, including a number of protozoal infections (Korbel *et al*., 2004).

The principal innate immune response to protozoa is phagocytosis, but many of these parasites are resistant to phagocytic killing and may even replicate within macrophages. Parasites recovered from infected hosts appear to develop resistance to complement-mediated lysis (Abbas and Lichtman, 2005).

Macrophage function is reduced in malaria infection (Nielsen *et al*., 1986). It has shown that inhibition of macrophage function is due to granules of malaria

pigment known as haemozoin. The haemozoin pigments consist of insoluble polymers resulting from the ingestion of intraerythrocytic malaria parasites that are unable to catabolise haem that precipitates in the erythrocytes. However, the accumulation of pigment inside macrophages has been shown to impair macrophage activation and function (Malaguarnera and Musumeci, 2002a). Haemozoin is a key factor in malaria-associated immunosuppression; affecting both the antigen processing and immunomodulatory function of macrophages (Metzger *et al.*, 1995).

Macrophages phagocytose the PRBCs, it is intriguing that macrophages may not only ingest intact infected erythrocytes but also extract parasites from recently infected erythrocytes, leaving the erythrocytes to continue to circulate (Chotivanich *et al*., 2002; Angus *et al*., 1997). Macrophage participates in the control of the infection through both antibody dependent and independent phagocytosis, and also through the secretion of soluble factors directly or indirectly toxic to the parasite, such as IL-1, TNFα, GM-CSF, reactive nitrogen (NOI) and oxygen radicals (ROI) (Prada *et al*., 1996).

1.6.2 Adaptive immunity

Adaptive immunity is immune responses that are stimulated resulting from exposure to infectious agents, it was then developed as a response to infection and also adaption to the infection (Abbas and Lichtman, 2005). Acquisition of protection following natural parasite exposure is a slow process that may take years or decades to develop and probably never results in sterile immunity (Greenwood, 2005). There are two types of adaptive immune responses, namely humoral immunity and cell-mediated immunity. These two immune responses are mediated by different

components of antigen, both the immune systems function to eliminate different types of infection (Abbas and Lichtman, 2005).

1.6.2.1 Humoral immunity

Humoral immunity is mediated by molecules in the blood and mucosal secretions, called antibodies, which is produced by B lymphocytes cells (also called B cells). Antibodies recognize parasite antigens and consequently neutralize the infectivity of the parasite. The principal defense mechanism in humoral immunity is against intracellular microbes and their toxins by binding onto these microbes and toxins for their elimination (Abbas and Lichtman, 2005).

1.6.2.2 Cell-mediated immunity

Cell-mediated immunity, also called cellular immunity is mediated by T lymphocytes or also known as T cells. Intracellular parasites survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. The function of cell-mediated immunity is as defensive systems against infections, particularly macrophage activation by Th1 cell-derived cytokines which promotes the destruction of parasites residing in phagocytes. It also kills the infected cells and eliminates the reservoirs of infection.

Protective immunity against protozoa may be induced by the host's response through production of antibodies or lymphocytes specific for the parasite. Protective immunity to the asexual blood forms of malaria parasites is mediated by mechanism that involves both cellular and antibody (Abbas and Lichtman, 2005).

Multiple genes appear to control and protect against intracellular parasites in mice and presumably in humans. Attempts to identify these genes are ongoing in

many laboratories. Protozoa that replicate inside various host cells lyse were found to stimulate both specific antibody and cytotoxic T lymphocytes (CTL) responses. It was thought for many years that antibodies were the major protective mechanism against malaria as the vaccination against this infection was focused on generating antibodies. It is now apparent that the CTL response is an important defense mechanism against the spread of this intracellular protozoan (Abbas and Lichtman, 2005).

The three immunoglobulins: IgG, IgM and IgA have shown to exert various degree of specific activity against malaria. Studies conducted in non-immune volunteers showed that serum concentration of IgG, IgM and IgA rose shortly after beginning of parasitaemia. The levels of IgG, IgM were higher than IgA, while IgG persisted longer than the other two (Bruce-Chwatt, 1985).

The T cell could either act by promoting cell-mediated immunity against the parasite or to producing specific antibody as demonstrated by murine models (Fell and Smith, 1998). Th1 type T cell was confirmed to be responsible for controlling the primary acute parasitaemia as demonstrated in the model of malaria immunity in mice infected with *P. chabaudi* (Langhorne *et al*., 1995). The responses of T cells are expected to inhibit the parasite growth by: (1) phagocytosis of granulocytes and macrophages through the production of cytokines such as IL-12 and TNF by macrophages in response to parasite molecules that lead to macrophage activation, increased phagocytosis and production of oxygen radicals and NO; (2) Activation of γδ T cells; and (3) activation of NK cells and the production of IFNγ by these cells (Biron and Gazzinelli, 1995).

1.6.3 Passive Immunity

Immunity can also be conferred on an individual by transferring serum or lymphocytes from a specifically immunized individual. This type of immunity is also known as adoptive transfer. The recipient of such a transfer becomes immune to the particular antigen without being exposed or having responded to antigen. Therefore, this form of immunity is called passive immunity. Passive immunization is a useful method for distributing immunity rapidly without having to wait for an active immune response to develop. It is a similar mechanism as transferring of maternal antibodies to the fetus to enable the newborns to combat infections instantly (Abbas and Lichtman, 2005).

In *P. falciparum* infection, the immune mechanism is largely mediated by immunoglobulin G (IgG) (Sabchareon *et al*., 1991), the role for the antibody was demonstrated by the passive transfer of adult immune IgG to infected children (McGregor, 1964b).

1.6.4 Age and malaria

Age was reported to influence the acquisition of immunity (Marsh, 1993). For example, newborns receive passive immunity from their mother, they usually have lower chances to contact parasitaemia and even during intensive transmission and therefore children less than 6 months rarely show clinical features of severe malaria (Snow *et al*., 1997). However, they become highly susceptible thereafter (Bruce-Chwatt, 1985) when the level of antibodies declined and thus the ability to develop the effective acquired anti-parasite immunity dropped (Marsh, 1993). Due to this reason, children aged 3-5 often harbour high parasite loads and are the more susceptible group to *P. falciparum*. A significant proportion of these children died

from cerebral malaria and other severe complications (Taylor-Robinson, 2002). Older children exposed continuously to the parasite for many years will tend to accumulate an active acquired immune response that is sufficient to lessen the parasite rate and severity of the symptoms. In adulthood, clinical attacks are generally self-limiting fevers and headache, and parasitaemia initially stabilizes at a low level but later develops into serious symptoms particularly those from the non endemic areas. Acquired immunity in adults exposed to a long unbroken period of heavy exposure, is neither sterile nor permanent. In comparison semi-immune individuals often appear healthy despite harbouring parasites. Persistent immunity requires years to develop. The maintenance of this persistent immunity known as premunition depends upon a persistent subclinical infection and is lost when an individual leaves the malaria-endemic area (Taylor-Robinson, 1998).

Unlike other acute viral diseases which produce life long resistance to reinfection, malaria only elicits immunity after several years of continuous exposure, during the period of recurring infections, illness occurs. This turn of malaria immunity is only partially effective and results in milder or sometimes asymptomatic infections when harbouring low blood-borne parasitaemias. This immunity is short-lived unless it is reinforced through frequent reinfection and is therefore only acquired or semi-immune adults resident in malaria endemic areas (WHO, 1996).

1.6.5 Malaria in pregnancy

The risk of infection is highest for women when conceived a baby for the first time (primigravidae) and the risk is reduced, although not absent, in subsequent pregnancy, indicating the development of effective immune responses to these infections. Two host factors are responsible for the increased susceptibility to malaria

in pregnant women (Rogerson and Beeson, 1999; Menendez, 1995) which are changes in immunological responses that could reduce the parasite clearance (Lea and Cadler, 1997; Riley *et al.,* 1989) and changes in specific antimalarial antibodies (Nambei *et al*., 1998; Deloron *et al*., 1989). The immune mechanism during pregnancy down-regulates inflammatory type Th1 responses and shifts to a predominant Th2 response that contributes to the increased susceptibility of pregnant women towards infection. This may lead to increased morbidity and mortality (Taylor-Robinson, 2002; Krishnan *et al*., 1996; Wegmann *et al*., 1993). Studies conducted in pregnant mice with malaria showed that since Th1 mediation that is instrumental in controlling acute infection was down-regulated, switching to Th2 resulted in increased pathogenicity of the disease (Krishnan *et al*., 1996; Wegmann *et al*., 1993).

1.7 Rodent models of malaria

Host specificity demonstrated by human malaria parasites is the major constraint for the study of malaria. Furthermore, the actual causative organisms cannot be maintained in the small laboratory animals. In early 1950s, simian and avian parasites were the only models available until the surfacing of *P. berghei*, which is now used in many laboratories internationally (Cox, 1988).

In *in vivo* protective immunity studies, various models systems have been developed using parasites isolated from thicket rats in five different countries of Africa (Carlton *et al*., 2001; Taylor-Robinson, 1998). These parasites have shown to be better than simian and avian malaria models in that they are easy and cheaper to maintain. Mouse immune system is well characterized, where large-scale of dissection can be performed that is impossible to be carried out in humans or non-

human primates (Taylor-Robinson, 1998). Four species of rodent malaria, *P. berghei, P. yoelii, P. chabaudi*, and *P. vinckei* have been adapted to be grown in the laboratory (Killick-Kendrick and Peters, 1978; Carter and Diggs, 1977).

Plasmodium berghei and *P. yoelii*, like *P. vivax* and *P. ovale*, are able to invade reticulocytes, whereas *P. chabaudi* and *P. vinckei* preferentially invade mature red blood cells, as do *P. falciparum* and *P. malariae* (Cox, 1988). Like *P. falciparum*, rodent malaria parasites have 14 chromosomes, which are polymorphic both between species and between isolates of the same species (Carlton *et al*., 2001). *P. berghei* and *P. vinckei vinckei*, and some of *P. chabaudi* and *P. yoelii* strains cause lethal infection in mice, whereas *P. chabaudi adami*, *P. chabaudi chabaudi*, *P. vinckei petteri* and other isolates of *P. yoelii* cause infections in most strains of mice which resolve after the initial parasitaemia is then either eliminated completely (*P. yoelii*) or have smaller patent recrudescence for several months (*P. chabaudi chabaudi, P. vinckei petteri*) (Cox, 1988). Lethal infections are preferably used for screening of compounds with chemotherapeutic activity (Taylor-Robinson, 1995), testing of putative vaccine candidate molecules and examining of vaccine-induced immunity. Non-lethal infections serve well as models to investigate the mechanisms of acquired immunity (Taylor-Robinson, 1998).

The model parasites most commonly used are the sibling species *P. berghei* and *P. yoelii*, which share more than 90% genome identity (Kooij *et al*., 2005). Both present numerous similarities to human *P. falciparum* and *P. vivax* and are extensively used to study the biological changes of liver stage and blood stage antigens and their role in immunity and vaccine development (Carlton *et al*., 2005). In recent years, numerous studies have used *P. berghei* to understand *Plasmodium* infection of liver, blood and mosquito (Franke-Fayard *et al*., 2005; Frevert *et al*.,

2005; Frischknecht *et al*., 2004; Vanderberg and Frevert, 2004; Vlachou *et al*., 2004).

1.8 The role of cytokines in malaria infection

1.8.1 IL-18

Interleukin-18 is 18 kDa glycoprotein derived from enzymatic cleavage of a 24 kDa precursor, pro-IL-18. The enzyme involved is caspase 1 (IL-1β converting enzyme, ICE) (Ghayur *et al*., 1997; Gu *et al*., 1997). A recently cloned novel IL-18 cytokine was initially identified as a potent interferon-γ inducing factor due to its ability to induce high levels of IFNγ secretion from T, B and NK cells and activated macrophages (Dinarello, 1999b; Okamura *et al*., 1998; Yoshimoto *et al*., 1998; Okamura *et al*., 1995b; Nakamura *et al*., 1989). IFNγ is one of the best characterized cytokines and also an important cytokine that contributes to the host defense (Boehm *et al*., 1997; Billiau, 1996). It also works as a pivotal regulator of chronic inflammation in human autoimmune diseases (Nakanishi *et al*., 2001b; Dinarello, 2000; Nakamura *et al*., 1989).

Interleukin-18 is type 1 pro-inflammatory cytokine produced by different cell types including Kupffer cells, activated macrophages (Okamura *et al*, 1995a), osteoblasts, keratinocytes, and intestinal epithelial cells (Dinarello, 1999b; Stoll *et al*., 1998; Stoll *et al*., 1997; Gu *et al*., 1997). It shares structural similarities with the IL-1 family of cytokines. Yamamura *et al*. (2001) reported that articular chondrocytes and synoviocytes are able to synthesize IL-18. IL-18 has also been detected in a wide variety of tissues that also secrete IL-1β, these tissues are adrenal cortex, anterior and posterior pituitary lobe (Nagai *et al*., 2006; Nagai *et al*., 2005; Conti *et al*., 1997), cerebellum, hippocampus, hypothalamus, cerebral cortex, medial