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Review Article

Malaria: role of antibodies in protection and pathogenesis: an overview

Tyagi P, Biswas S

National Institute of Malaria Research , Indian Council of Medical Research, 22-Sham Nath Marg. Delhi 110054, *India*

Abstract

The research scenario for malaria has improved in the last three decades to understand the epidemiology and host immune responses to plasmodial infection. Due to the augmented episodes of resistance development against the commonly used antimalarials in plasmodium parasites, especially in *Plasmodium falciparum*, neutralization of infection through effective vaccine(s) remains the feasible alternative in malaria control. In this direction, lot of attention was paid towards the identification of stage specific malaria antigens targeted by host's immune system. Preparation of synthetic or recombinant peptides and evaluation of their immunogenecity in naturally occurring antibody response were also given much importance, as these studies could help in finding potential candidates for future malaria vaccine(s). Attention was also paid on the pathogenic consequences of antibody formation in malaria infection as polyclonal activation of B cells, which is a very prominent feature in malaria infection. Formation of circulating immune complexes in chronic malaria infection was also viewed as pathogenic parameter of severe malaria. The present survey focuses mainly on protective and pathogenic aspects of malaria antibodies (eliciting against various stage specific antigens), and future research plan in antibody-mediated immune response.

Keywords: malaria; *Plasmodium falciparum*; *P. vivax, antigen*; antibobody; immune complex

INTRODUCTION

People living in malaria endemic areas, acquire clinical immunity gradually after repeated infections in an age dependent manner [1,2]. This immunity is however not sterile, a low-grade parasitemic condition (premunition) persists for prolonged period. Small children (6 months - 14years), non-immune adults (travelers visiting endemic areas) and pregnant women living even in endemic areas however don't acquire clinical immunity: they are always at high risk to contract malaria[3].

The malaria causing parasites, i. e, *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malarae* in human follow a very complex life cycle involving distinctly different developmental stages with large numbers of antigenic proteins[4]. The effector mechanism of protective immunity in the inhabitants residing in stable, unstable and epidemic prone malaria areas is not clearly known. Naturally exposed individuals however shown to elicit both humoral as well as cell mediated immune responses [4-7]. Important protective role for innate resistance in malaria has also been documented. Development of innate resistance in population living in intense transmission zone was postulated to be acquired in the form of altered genetic setup after prolonged interactive exposure to malaria parasites through natural selection pressure. Protective role for innate immune system such as monocytes/macrophases and complement mediated effector limbs have also been ascribed in malaria [8].

Antibodies have primarily been shown as the main immune effectors against the erythrocytic stages

Correspondence to: Sukla Biswas, PhD, Deputy Director, National Institute of Malaria Research (ICMR) 22 Sham Nath Marg, Delhi 110 054, India suklabiswas@ yahoo. com
Tel: +91-11-23928804 Fax: +91-11-23946150

of plasmodium (the major causative agents for the development of acute clinical symptoms) [9]. In addition, antibodies against the antigens expressed in liver stage, on the surface of erythrocytes and in sexual stage have also been demonstrated to play role as immune effectors, indicating pivotal role of antibodies in evoking antimalarial immune response[4, 10-12].

A detailed knowledge of antibody response is thus valuable in malaria vaccine development and control. Identification of reliable and reproducible immune correlates of protection against malaria and also recognition of the pathogenic potential of the antibody mediated reaction is very important in the development of effective vaccine as well as evaluation of efficacy of chemothereuptic agents. The present review is therefore aimed at to understand the protective as well as pathogenic immune mechanism of antimalarial antibodies in human.

ANTIMALARIAL ANTIBODY

In antibody mediated immune mechanisms, elimination of pathogens/antigens from the blood circulation and extravascular sites is executed through specifically formed antibodies produced against invading parasites/antigens[13]. Antibodies first bind with these antigens, according to their degree of affinity/avidity and form antigen-antibody complexes (immune complexes). Antibodies present in immune complex (IC) acquire some new biological functions, such as activation of complement system and deposition of activated components of complement on ICs. That leads to the opsonization of ICs, which are further taken up by phagocytic system, then *via* production of reactive oxygen species (ROS) and oxidative stress are cleared from the body.

Antibodies are a group of glycoproteins called immunoglobulins (Igs). They are present in serum and tissue fluids and are produced by activated B lymphocytes called ´plasma cells´ in response to the non-self antigens and antigens of infected organisms or pathogens [13]. Depending upon the immunochemical nature of antigen, i. e. T cell independent or T cell dependent antigens, B cells produce antibody either independently or take T cell help in producing antibodies. The basic structure of Igs is composed of four-chain polypeptide molecule, two light chains, and two heavy chains, linked together by disulphide bonds. Five distinct classes of Ig (ie, IgG, IgM, IgA, IgE and IgD) and some subclasses known as isotypes (ie, four isotypes of IgG: IgG1, IgG2, IgG3 and IgG4 and two isotypes of IgA: IgA1 and IgA2) have been recognized in higher mammals[13]. Different classes of antibodies execute different types of functions depending upon their anatomical sites of production in the body and immunochemical nature of eliciting antigens. For example, IgG is mainly present in blood circulation and also in extra vascular sites and constitutes major antibody of secondry immune response: IgA type of antibodies is present in blood in low percentage, and mainly present in seromucous secretions such as saliva, colostrum, milk, tracheobronchial and genito-urinary-secretions: IgM is largely confined to intravascular pool and is the predominant early antibody response during an infection: IgD is present in large quantities on the membrane of many circulating B-lymphocytes and probably play role in lymphocyte differentiation: IgE is mainly present on the surface membrane of basophils and mast cells and play role in active immunity to helminthic infections and immediate type of hypersensitivity [13].

In malaria infection, vast amount of antibody related research work has been published in the last three decades highlighting their detection methods. For example: antibody responses against various stage specific antigens, such as sporozoites, (the preerythrocytic or liver stage), merozoites, ring stage, schizonts, (the asexual erythrocytic stages), gametocytes (the sexual stage) and *P. falciparum* antigens expressed on the infected erythrocytes (IRBC), could be detected by immunofluorescence, enzyme linked immunoabsorbant assay (ELISA), immunoblotting and flow cytometry methods. The stage-specific antibodies help in evaluation of immunogenecity and characterization of antigenic molecules, mapping of their B-cell/T-cell epitopes for the development of effective vaccine, preparation of recombinant/chimeric protein containing *P. vivax*/*P. falciparum* vaccine candidates and their role in protective immune response [10-12].

Protective role

The life cycle of malaria parasite and involvement of various stages in development of immunity

are shown in Figure 1. The protective role of naturally acquired antibodies in malaria immunity has been demonstrated since the early sixties[14]. For example, 1) Passive transfer of Igs from malaria immune individuals to *P. falciparum* infected patients and consequent induction of antiparasitic effect and reduction of parasitemia and improvement in clinical condition of acutely infected children after the administration of purified IgG[9,14]; 2) Induction of protective response in Aotus monkeys challenged with *P. falciparum* with human IgG from immune individuals[15]; 3) Inhibition of parasite development by antimalarial cytophilic antibodies in Saimiri monkeys and human [16]. All these indicate protective role played by antimalarial antibodies. Moreover inhibitory effects of antimalarial IgG (collected from diverse geographical areas, as East and West Africa, Papua New Guinea, Thailand, Vietnam and China) on parasite growth also indicate important protective role played by antibodies[1]. Inspite of all these observations, the actual mechanism of antibody-mediated protection is still not clear. It has been opined that antibody mediated immune protection in malaria, may include several pathways and actions[17]. For example: 1) Inhibition of intrahepatocytic development of sporozoites by antisporozoite antibodies[18]; 2) Blocking of parasite invasion of erythrocytes[5]; 3) Antibody mediated agglutination of merozoites, thereby preventing reinvasion of erythrocytes[5]; 4) Antibody dependent cytotoxicity of parasitized erythrocytes[16]; 5) Blocking of the activity of parasite toxins ie, antibodies against parasite antigens inducing TNF-α production in *P. vivax* and *P. falciparum* infection have shown to block TNF-α production[19-21]; 6) Inhibition of merozoite dispersal after schizont rupture through opsonization and phagocytosis and thereby prevention of sequestration of these cells to capillary endothelium and resulting into enhanced removal via spleen and other tissues of reticuloendothelial system[22]; 7) Killing of free merozoites through antibody mediated release of soluble mediators by monocytes (Igs from clinically immune African adults were shown to induce human monocytes to release TNF alpha and subsequent inhibition of parasite growth via the antibody dependent cellular inhibition (ADCI) process[16,23]; 8) Binding with exflagellating gametes in the midgut of vector mosquitoes and inhibition of fertilization and oocyst development [24]; 9) Inhibition of sequestration of parasite laden RBCs at the placental sites in pregnant women and protection in multigravid women in endemic areas[25,26]. Transplacental transfer of IgG from the mother to the foetus was shown to be at least in part responsible for the resistance to malaria in newborns. Recent evidences showed an association between antibodies recognising antigens on the infected erythrocytes in placenta and protection against maternal malaria [26]. A study conducted in infants (*P. falciparum* hyperendemic area of Western Kenya) highlighted antibody responses to circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1) and merozoite surface protein-2 (MSP-2) repeat epitopes in assessing transplacental transfer of maternal antibodies and their role in protection [27].

The results of these studies on correlation between antibody titres and protection from *P. falciparum* infection showed: 1) Age-related responses (90% individuals (10-30 years) had anti-sporozoite and 70-80% individuals (31-40 years) had anti-merozoite/anti- IRBC antibodies) [28]; 2) Parasite stage related protective responses(antibodies against blood stage antigens of *P. falciparum* correlated with negative blood smear) [28].

ANTIBODIES AGAINST DIFFERENT STAGES

Liver stage

Antibodies induced against irradiated sporozoites were shown to enhance sporozoite clearance, induce phagocytosis by macrophages and inhibit sporozoite penetration into hepatocytes[29]. Antisporozoite antibodies were shown to inhibit intrahepatocytic development of sporozoites[18]. Anti-CSP antibody response measured in non-immune individuals (infected with *P. falciparum* malaria) showed 36.4% seropositivity during the first 7 days after the onset of symptoms and decreased steadily thereafter[30]. Antibodies to sporozoite and liver stage antigen (SALSA), sporozoite surface protein (STARP) and liver stage antigen-3 (LSA-3) were shown strong inhibitory activity in vitro. Presence of high levels of anti-CSP, anti-LSA-1, and anti-thrombospondin related adhesive protein (TRAP) IgG class of antibodies was correleted with decreased risk of infection. High levels of IgG to CSP, LSA-1 and TRAP were thus shown to be good immune correlates of protection a-

gainst *P. falciparum* infection. Anti-TRAP antibodies (evoked against a conserved motif peptide sequence of *P. falciparum* TRAP) were shown to inhibit merozoite invasion in vitro[31-32]. Antibody response to CSP measured in low endemic area showed low prevalence rate[33].

Blood stage

Antibody response against the merozoites, the invasive stage of asexual blood stage was shown to play an important role with regard to parasite neutralization. Immune response against three main categories of merozoite associated antigens, such as 1) proteins anchored on the surface membrane (MSP-1, MSP-2, MSP-4); 2) soluble antigens associated at merozoite surface (MSP-3, GLURP, SERA, ABRA and S-antigen); 3) antigen present in the apical organelles of the merozoite (rhoptry antigens, RAP-1 RAP-2, RAP-3 apical merozoite antigen-1 (AMA-1), microneme antigen (EBA-175), dense granule antigens (Pf155/RESA, RIMA) were shown to exert protective effect by blocking erythrocyte invasion process. Antibodies were shown to form immune clusters of merozoites by entering into leaky erythrocyte membrane at the time of schizont burst and preventing their dispersal. Antibodies produced against antigens on the surface of merozoites such as MSP-1, MSP-2, MSP-4 (anchored in the merozoite plasma membrane by a glycosylphosphatidylinositol, GPI moiety) shown to be protective[4]. Antibody prevalence to MSP-1 showed no correlation apparently with clinical immunity. However, high antibody levels to MSP-119 (epitope for inhibitory antibody) have been shown to be associted with protection from clinical malaria and severe parasitemia. Short lived IgG and IgM peaks of anti MSP-119 antibodies were observed in infants in their first year of life, and protection against parasitemia and febrile illness was also observed in infants[4, 34-36]. Anti-MSP-2 antibody response has been shown to be directed against its both polymorphic and conserved regions. However, antibody response to conserved region of MSP-2 generally develops after prolonged exposure to malaria and was shown to be associated with reduced anemia and less febrile condition[4, 37]. Individuals with *P. falciparum* infection showed increase in IgG3 antibody response against MSP-2 and schizont stage. Anti-MSP-3 IgG1 and IgG3 isotypes developed against *P. falciparum* showed anti parasitic properties in an antibody dependent cellular inhibition assay[38, 39].

The presence of high IgM antibody levels against haemozoin among the complicated *P. falciparum* patients showed an inhibitory effect on the production of monokines TNF-α and interleukin-1β suggesting that the anti-haemozoin IgM antibodies may have an anti-disease effect[40]. In non-immune *P. falciparum* malaria patients, antibody responses to merozoite stage antigens were observed in 89.1% individuals during the period between days 8 and 90 after onset of symptoms and decreased steadily thereafter[30]. Antibodies directed against serine repeat antigen (SERA) and, acidic basic repeat antigen (ABRA) were shown to form immune clusters resulting into inhibition of merozoite dispersal in parasite cultures. This was shown to be mediated via antibodies inhibiting the proteolytic activity of SERA and ABRA antigens. Clinical protection was achieved in children living in hyperendemic malaria areas in Gabon and Cameroon by elevated levels of antibodies measured against schizont extract, merozoite surface antigen-2 (MSA-2) and RAP-1 peptides [4]. Antibody responses to high and low protein complexes of apical organelle of merozoites were shown to be protective[4]. IgG class of antibodies to RAP-1 were found to be associated with decreased level of parasitemia in Tanzanian children[41]. Protective role of antibodies to AMA-1 has also been demonstrated. IgG response to AMA-1, MSP-119 in Gambian childern was found to be associated with recovery from chloroquine-resistant *P. falciparum* infection[42]. Association between HLA class II alleles and levels of antibodies to RAP-1 and RAP-2 has also been demonstrated. This indicates that HLA influences antibody responses to vaccine candidates. Antibodies developed in rabbits against erythrocyte binding antigen (EBA-175) were shown to block binding of EBA-175 to erythrocytes, thus inhibit merozoite invasion[43]. In individuals living in malaria endemic areas, the prevalence of naturally acquired antibodies found to be relatively low, only in 30% adults. Antibody response against the antigens of dense granules such as Pf155/RESA was shown to be involved in parasite neutralization[44]. IgG response to

P. falciparum (young trophozoites and mature schizont stage antigens) measured in an endemic area of Indonesia showed correlation with level of endemicity. Anti-RESA antibody was shown to be more pronounced in the aparasitemic group comparaed to the parasitemic group, whereas anti- schizont antibody was more pronounced in the parasitemic group. Antibody responses to *P. vivax* reticulocyte binding protein 1 and 2 (PvRBP1 and RBP2) and Duffy binding protein (PvDBP) were shown to be correlated with malaria exposure in terms of previous infection and time period spent in endemic area and age of inhabitants45. Naturally acquired antibodies to *P. vivax* PvDBP-RII could inhibit in vitro binding of merozoites to RBC[46]. Several studies have shown elicitation of anti-GPI antibodies in people living in endemic areas in age dependent manner[47,48]. Anti-GPI IgG3 antibodies were found to be short lived and low level of IgG1 were also reported [49]. Antibodies to *P. vivax* antigen, pv200 were measurable in the sera after transmission to evaluate persistence of antibody in a rural community of Brazil exposed to *P. vivax* outbreak (transmission intrupted by chemotherapy). Sera showed anti pv200 IgG positivity in 47% after 7 years and the levels of antibodies were shown to decrease with time in all positive subjects[50]. Clinical immunity was suggested to be correlated with qualitative change in age dependent switch from non-cytophilic IgG2 to cytophilic IgG1 and IgG3 subclasses, as cytophilic antibodies can bind with human monocytes and macrophages via Fc-γ R1 and RII receptors, and this leads to opsonization and phagocytosis and antibody dependent cellular inhibition reactions[16,51].

Individuals experienced with malaria showed a wide profile of antimalarial antibodies. Patients with acute *P. falciparum* malaria during an epidemic outbreak were found to contain high levels of IgM and low levels of IgG type of antibodies[52]. Childern (3-12years) with acute malaria infection showed elevated levels of IgM and low levels of IgG antibodies[53]. Clinically immune adult Africans were shown to contain cytophilic IgG antibodies, while non-immune patients were shown to contain high levels of non-cytophilic (IgG2) antibodies during their primary malaria attacks. Sera with high antibody titres to parasite antigens were shown to contain IgM and IgG1, IgG4 subclasses whereas sera with low titre were shown to contain IgM, IgG1 and IgG3 subclasses[14]. The cytophilic antibodies IgG1 and IgG3 were shown their predominance in protected subjects, while noncytophilic IgG2 and IgM classes or overall low levels of antibodies were shown in unprotected children[51,54]. Besides these, high levels of antimalarial IgA and IgE class of antibodies were also reported in individuals with repeated attacks of malaria in an age dependent manner [55,56].

Variant surface antigens on infected RBCs

Antibodies to *P. falciparum* antigens expressed on the surface of IRBCs, such as *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), sequestrin, Pf332 have been attributed to exert several protective actions[4, 57]. Antibody mediated opsonization of IRBC and thereafter phagocytosis by monocytes/macrophages, complement dependent lysis of IRBCs, blocking of cytoadherence of IRBCs to endothelial cells and clearance via spleen. PfEMP-1 is a high molecular weight 200-400 kDa polymorphic polypeptide of multigene var family, and prominently involving in endothelial adherence is considered as the principal target of anti-variant surface antigens (VSA) antibodies. The antibody response against the PfEMP-1 was shown to agglutinate IRBCs in a variant specific manner. Building up repertoir of anti-PfEMP-1 antibodies, following the multiple episodes of clinical malaria in children have been shown to correlate with protection against parasite variants. Study carried out in Gabonese adults and children showed prevalence of IgG3 antibody (cytophilic) response to Pf EMP-1 and age related increase in IgG2 isotype (non cytophilic) to the anti-VSA[58]. Antibodies to rifin proteins (a second polymorphic parasite derived family of antigens that are inserted into the infected erythrocyte membrane) were also found in elevated levels in exposed populations [59]. A correlation was found between transmission intensity and levels of VSA specific IgG antibodies in individuals of African countries [60]. Antibodies to PfEMP-1, rifin were found to be absent or infrequent at the time of malaria attack but were found to enhance and remain in circulation after the treatment. However the profile of antibody to variant surface antigen ex-

pressed by the heterologus parasite isolates showed no such consistent pattern [61].

In pregnant women, IRBCs bind with chondrotin sulfate-A (CSA), the hyaluronic acid receptors of placenta through PfEMP-1 antigen resulting into heavy sequestration in placental space. In multigravid women residing in endemic areas, antibody response against PFEMP1 was shown to exert protective effect[25]. Study conducted in Sudan showed age dependent increase in antibody levels. Affinity purified human and rabbit anti-Pf332 antibodies were shown to exert inhibitory effect on the growth of *P. falciparum in vitro*. Inhibitory effect was shown to be on intraerythrocytic development of parasite and was increased on addition of normal human monocytes indicating Pf332 antigen as target of opsonizing antibodies. However in another study, immunization of non-immune monkey (to malaria) with combination of recombinant fragment of Pf332 and other recombinant *P. falciparum* antigen showed protective effect in the absence of opsonizing antibodies [4]. Antibodies to the PfAARP1 (aspargine and aspartate rich protein-1), another giant protein (700kDa) present in the membrane of IRBCs was shown to be opsonizing in nature. Antibody to the infected erythrocyte surface antigen develops after symptomatic malaria infection was shown to be associated with protection against infection in children and adults [62].

Sexual stage

Studies on the natural immune responses to the sexual stages of malaria parasites have revealed that antibodies against the sexual stages i. e. gametocytes and gametes are readily evoked by natural course of infection [63]. Anti-gametocyte antibodies were shown to suppress infectivity at high concentration, whereas at low concentration enhance the development of the parasite in the mosquito. The potential targets of anti-sexual stage immunity can be grouped in two parts: 1) Antigens present on the gametocytes and 2) antigens present on the zygotes/ookinetes (mosquito stages). The sexual stages are known to be pathologically inert to host. Antibody responses against the *P. falciparum* protein doublet of 48/45 and Pf230 expressed on the gametocytes have been demonstrated[10]. Antibodies to *P. falciparum* gametocyte antigen Pf 48/45 were demonstrated in Papua New Guinea (recognizing epitopes I, IIa, III and IV) population [12]. Antibodies to transmission blocking vaccine candidates Pv25 and Pv28 (sexual stages) prevent further development of gametocyte stage into zygote/ookinetes in mosquito vector, thus inhibiting oocyst development [64]. Monoclonal antibodies to a Pfs2400 (gametocyte specific antigen) were found to reduce infectivity of gametocytes. Naturally occurring anti-Pfs2400 antibodies were found to be short lived and mostly appeared in those individuals who had multiple malaria episodes and were gametocytemic at the time of blood collection[65]. Anti Pfs230 antibodies were shown to initiate antibody dependent complement mediated lysis [66,67]. Antibodies to Pfs48/45 were shown to block fertilization. Monoclonal antibodies to Pfs27/25 (a highly abundant antigen, expressed throughout gametocytogenesis) and cross-reacting with denatured and reduced Pfs230 and Pfs48/45 have shown to reduce parasite infectivity of mosquitoes[68]. Polyclonal antiserum to Pfs25 was found to interfere with zygote development but monoclonal antibodies to the same protein were shown to inhibit the ookinete development. Fusion protein of Pfs25 and Pfs28 has been considered as vaccine candidate due to its induction in tranmission blocking activity by high antibody titre.

PATHOGENIC ROLE

The pathogenic implications of antimalaria antibodies revealed through research studies, which include polyclonal activation of B cells in malaria[69]. The exact cause of polyclonal activation in malaria is not known. The presence of extensive repetitive regions in the most antigenic protein of malaria parasite, with T cell-independent characteristics may cause induction in polyclonal antitibody response [70]. Figure 2 describes immune mechanisms (both innate and acquired) and formation of immune complexes in antibody excess. Many studies have identified the presence of B-cell epitopes in the repeats of CSP, LSA-1 and MSP-2 of *P. falciparum*, which causes polyclonal activation of B cells without any participation of T helper cells. Whatsoever the reason, the polyclonal activation of B-cell has been implicated with immunopathology in malaria, as it involves in several pathological phenomena, such as, production of polyclonal antibodies , autoantibodies , formation of

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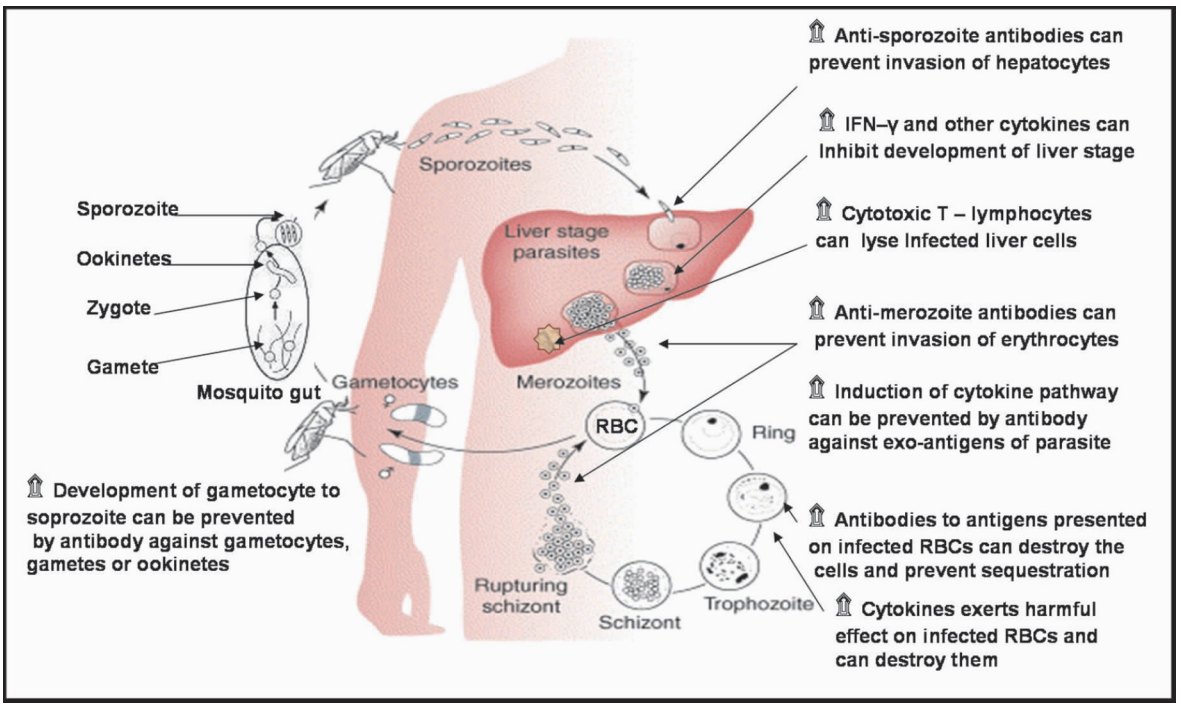

Figure 1 Life cycle of malaria parasite
Website: www. fda. gov/cber/blood/malana071206sk5. gif(in human)
http: //gsbs. utmb. edu/microbook/images/fig833. JPG(in mosquito gut)

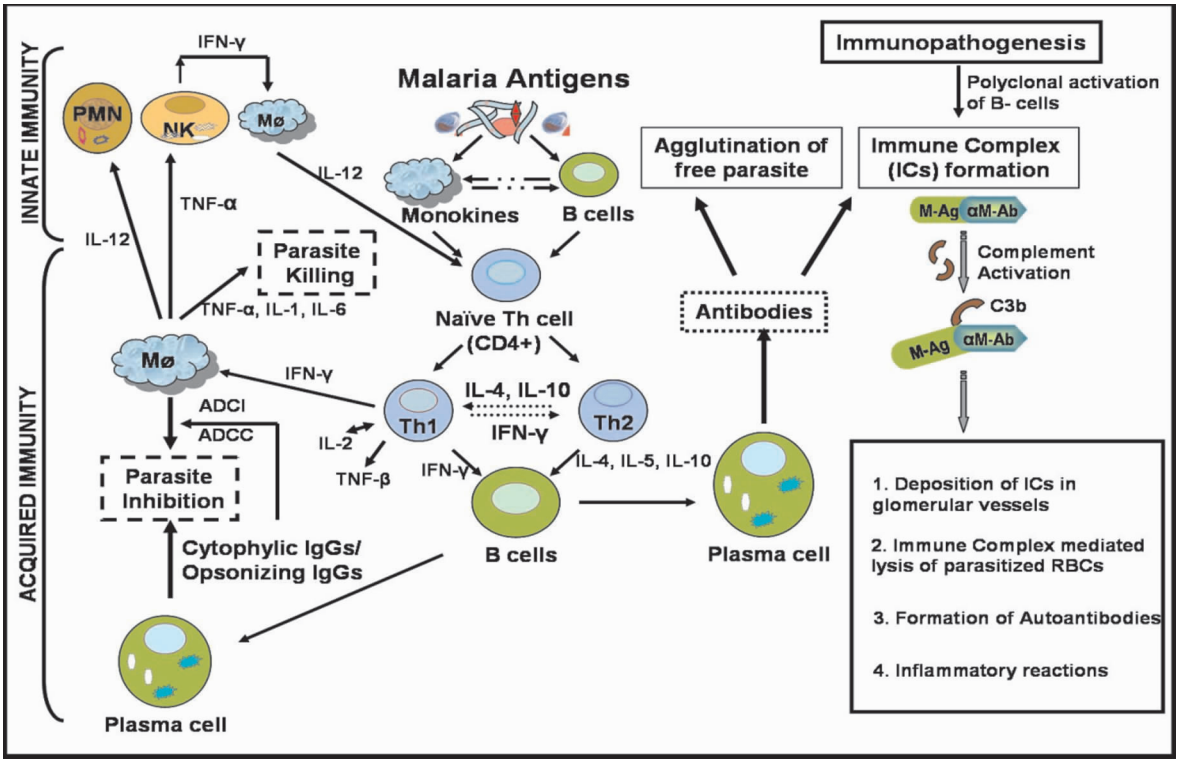

Figure 2 Malaria Immune Mechanisms

immune complexes (ICs), circulating immune complexes (CICs) and anaemia[71]. Presence of CICs and deposition of ICs in tissues has been shown to be a pathogenic process mediated via type III hypersensitivity. Presence of elevated levels of CICs have extensively been studied and demonstrated in malaria[72,73]. Individuals residing in malaria endemic area of India were shown to contain elevated levels of antiplasmodial circulating immune complexes containing both IgM and IgG antibodies [74]. Immune complexes were shown to cause tissues injury by triggering inflammatory reactions in malaria. Histological findings in infected children showed thickening of capillary wall and segmental glomerular sclerosis leading to glomerular damage and secondry tubular atrophy in chronic kidney lesions (characteristic of quartan malaria). Deposition of Igs and complement in the walls of glomerular vessels were detected in the vast majority of cases and presence of *P. malariae* antigen was also observed [72, 75]. Activation of complement system has also been observed in malaria [76]. Observation of reduced levels of C3 and C4 components of complement system in individuals residing in endemic area has been shown to indicate complement activation via classical pathway. Immunoglobulin complex deposition in placentas in *P. falciparium* infected pregnant women in Malawi and Papua New Guinea have been demonstrated[73]. In placental studies, high deposition of ICs in Papua New Guinean women and IgE type of antibodies were observed in the fetal blood vessels. However all placentas with deposition of IgE in fetal blood vessels showed no sequestration of parasite in intervillous spaces. Children with severe malaria associated anemia and cerebral malaria were found to contain significantly higher levels of immune complexes. An inverse relationship was observed between hemoglobin levels and immune complex levels in patients with severe anemia[77,78]. Children who developed cerebral malaria were found to contain high levels of antimalarial antibodies, indicating that cerebral malaria may be an immune-mediated disorder. Involvement of ICs in the pathogenesis of cerebral malaria is mainly observed in experimental animals, in human cerebral cases, low levels of complement were also reported, and was viewed that CICs may damage cerebral vessels[79,80]. The exact mechanism of ICs mediated injury is not known however the sequence of steps in initiation of clinical lesions has been reviewed recently[81]. Role of antibody response in the pathological features of placental malaria is not clearly known, however pathological changes in parasitized microvilli such as cyto-trophoblast focal syncytiotrophoblastic necrosis, loss of syncytial microvilli and irregular thickening of trophoblastic membrane, mononuclear cells containing pigment, increased amount of C1q, C4, C3, C9 (complement components), fibrinoid fibrinogen and plasmin indirectly point towards the role of depositing complement components and immunoglobulins. Presence of antimalaria IgE antibodies in malaria infection is an indication of pathogenic role as IgE containing immune comlexes were found to induce IL-4 production in human basophils and thought to affect Th-1/Th-2 lymphocyte balance in malaria[82].

Production of autoantibodies against normal erythrocytes and their role in pathogenesis of hemolysis and anemia has been reported in malaria[17,21]. Presence of antibodies to components of the central nervous system (CNS) during malaria infection has also been documented[83]. Antibodies to the voltage-gated calcium channels have been shown to increase with the severity of malaria infection in Kenyan children. Formation of autoantibodies to nuclear, double stranded DNA, histone, cytoplasmic neutrophil, myeloperoxidase, proteinase-3 and lactoferrin have been observed in *P. falciparum* as well as *P. vivax* infection[84].

Presence of anaemia is a very common feature of malaria infection. Whether this is due to depressed rate of erythropoiesis or due to the immunologically mediated hemolysis is not clearly known. However, based on certain observations it is believed that anaemia may be caused by the destruction of infected erythrocytes during schizont rupture, due to removal of uninfected erythrocytes and due to suppression of bone marrow, since supply of new red cells is also impaired[85]. It is known that the erythrocyte life span is reduced in malaria patients[86]. Cause of erythrocyte destruction has been ascribed to various factors, such as schizont rupture, hypersplenism, and loss of antioxidant function of erythrocytes, complemented mediated lysis or opsonization for phagocytosis due to surface Ig binding. Role of complement activation and erythrophagocytosis in the pathogenesis of anaemia in falciparum malaria in African childern has been documented[87]. Malaria antigens are present on the surface of parasitized eryth-

rocytes and also soluble antigens of parasite present in plasma are absorbed onto the surface of erythrocytes and these can react with antibodies. In addition to this, there is also a possibility that immune complexes formed outside the cells can adhere to the surface of red cells. Antimalarial therapy may increase the supply of antigens from dying parasites and augment the formation of IC. Such situation can further perpetuate hemolytic activities.

The role of spleen in malaria immunity is very important[88]. Enlargement of spleen during malaria (splenomegaly) has been used as a measure of malaria endemicity. It has been opined that the primary immune response against blood stage parasites may occur in the spleen. While serving as a mechanical filter for removal of immunologically modified parasitzed or unparasitized erythrocytes, it also serves as a primary site of erythropoiesis. During the malaria infection the number of antibody producing B-cells in spleen is increased: recruitment and activation of T cells is also increases. The pathogenesis of splenomegaly is also thought to be antibody-mediated disorder. The exact mechanism of splenomegaly is not known. However elevated level of IgM class of antibodies was observed. Roles of genetic factor (s) and impaired suppressor T-cell functions were also suggested. Impaired T cell function results into polyclonal B-cell expansion, thus causing over production of IgM and that may be the major cause of spleen malfunction in chronic malaria in children. Studies conducted in Nigeria and Uganda in Africa and in Papua New Guinea confirmed the polyclonal type of IgM antibody production in splenomegaley. However malaria antigen-specific antibodies represented only small proportion of IgM, the remaining part contains different autoantibodies with high prevalence of rheumatoid factor type of antiglobulins and cryoglobulins[89].

It is documented that during malaria infection, initially low affinity antibodies are produced. The exact reason for this is not known, but it has been proposed that the immunodominant repeat regions present in plasmodium antigens are responsible for producing T-cell independent immune response including low affinity and non-neutralizing antibodies, thus inhibiting the process of protective immune response maturation in malaria. Hypothetically it may be believed that these low affinity antibodies may lead to the formation of low affinity CICs, as thorough clearance of these antibodies through reticuloendothelial system may not be possible due to poor avidity binding properties. Besides this, in chronic malaria situation with persistent infections, formation of immune complexes can take place, leading to deposition of complexes in the tissues, which may further cause the hypersensitivity type III reactions.

LOOKING AHEAD

Inspite of availability of so much research data, many aspects of antimalarial antibodies are still need to be investigated. For instance, the onset of acute malaria infection evokes production of IgM class of antibody in non-immune individuals living in the epidemic prone area and suffered from epidemic outbreak is not clear. Moreover the parasitic antigens, which evoke the production of IgM type antibodies and their role as either protective in nature or pathogenic (inducing inflammatory reactions), are not known. Whether the same IgM producing B-cell clone further differentiates into IgG producing cells by the immunoglobulin class switch process (switching of IgM to IgG class or sub classes IgG1, IgG2, IgG3 and IgG4) or separate B cell clones are generated (primed by separate antigen sets) for maturation of immune response is also not known.

Protective nature of antibodies may be hampered by competition between non-specific and specific moieties for binding to antigenic epitopes, thereby causing reduction in the efficacy of antibodies. How to increase the duration of short-lived antibodies, which are thought to be protective to reinfection? The stage-specific antigens, which may be the important mediators of antiparasitic responses with protective property, need to be identified in both *P. vivax* and *P. falciparum*[90]. Further studies are needed for demonstrating longevity of antibody secreting B cells against malaria infection, as shown in *P. falciparum* malaria, the persistence of antibodies even 8 years after first exposure. From the vaccine point of view: 1) The antigens, responsible for significant protective antibody production, are very important for further investigation as they exhibit high immunogenic potential and are recognized by immune system at a very short exposure time. The dose of parasite inoculum (antigen) and relative link between age of human host and antibody elicitation pattern are crucial factors and need to be investigated to understand

antibody mediated immune response; 2) In malaria infection, so far no data is available regarding parasite antigens, which induce cytophilic type of antibodies for effective neutralization of parasites. Therefore, study needs to be done to characterize parasitic antigens eliciting cytophilic type of antibodies in the light of efficacious vaccine development; ; 3) The parasite stage-specific antigens, which cause polyclonal activation of B cells and produce antibobodies of several classes and form immune complexes, which in turn cause pathogenic reactions, such as hemolytic aenemia and related complications need thorough investigation[91]. Attention also needs to be focused on parasitic antigens expressed on infected RBC; 4) It is important to know that how host immune response, especially antibodies against asexual blood stages can influence process of gametocytogenesis, as host immunity to rising asexual parasitemia has been correlated with both increased and decreased gametocytaemia. In addition, lymphocytes and serum taken from *P. falciparum*-infected Gambian children increased gametocyte production both independently and in an additive manner[92]; 5) Further studies in various endemic situations are required to understand the association between production of IgE type of antimalarial antibodies and induction of tumour necrosis factor alpha in malaria[93]; 6) The effect of immune responses, mediated by either mixed population of plasmodia species or by other concurrent infections, on the antibody production in malaria infected patients need to be studied, as malaria endemic areas are also infested with several devastating diseases, such as waterborne disease, tuberculosis, AIDS and helminthic infections[94]. Infection with HIV has been shown to increase the risk of malaria infection in pregnant women. Interference of HIV in elicitation of antibody response against variant surface antigen of *P. falciparum* increases susceptibility to malaria[95]; 7) Furthermore, there is no report available in declining of B-cell response and antibody production in malnourished/undernourished individuals, rather elevated levels of antibody production was observed in malnourished individuals[96]. Whether, prevalence of antibodies in individuals in malnourished or starved condition is protective or pathogenic, remains to be investigated. Such studies will be meaningful in relation to community, who are poverty-stricken or live in impoverish condition.

Further study is required on the formation of CICs in antigen/antibody excess, elevated levels of CICs in individuals residing in malaria endemic areas and their implication in disease like malaria. Whether ICs play any role in immunosuppression as observed in mycobacterial infection[97]. Why CICs (including IgE containing IC) persist in circulation for prolonged periods and how it can be prevented? Whether the immune complex neutralization capacity of complement system (especialy through alternative pathways) in malaria is impaired? Whether elicitations of non-cytophilic anti plasmodial antibodies contribute in formation of high amount of CIC? Focussed attention on all above points would help in categorize the role of antibodies for the benefit of host or parasite.

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