Review Article

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IMMUNE MECHANISMS INVOLVED IN MALARIA: A REVIEW

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ABSTRACT

Despite extensive research, malaria is still very rampant and unrestrained. The complexity of Plasmodium parasite's life cycle, its intracellular nature, and its ability to evade the innate and adaptive immune responses make our efforts incompetent. Understanding the induction pathways of immune responses during malaria infection is crucial for the development of an effective vaccine. Present review explains the various aspects of immune mechanisms involved in fortification against malaria infection.

Key words: *Plasmodium*, innate, acquired, immunity, malaria

INTRODUCTION

Malaria is one of the most prevalent and devastating of all human parasitic diseases, and is closely associated with socioeconomic burden in many temperate and most tropical countries. As a result of a massive scale-up in malaria control programs by the World Health Organizations (WHO) as part of the Millennium Development Goals, the estimated incidence of malaria globally has reduced by 17% and malaria-specific mortality rates by 26% between 2000 and 2010[1]. Although this represents some progress in reducing the disease burden, malaria still remains a major global health threat and continues to cause high morbidity and mortality, especially in sub-Saharan Africa, where almost 600 million people are at risk[2]. Together, the Congo, India and Nigeria account for 40% of estimated malaria cases, and the Congo and Nigeria account for over 40% of the estimated total of malaria deaths globally in 2010[3]. Malaria is caused by a protozoan parasite of genus *Plasmodium* and is transmitted by female *Anopheles* mosquitoes. There are five species that infect humans, namely, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. *P. falciparum* is the causative agent of 90% of infections and is the target of the vaccine trials of the different initiatives and programs.

Immunity to malaria has a major role in controlling disease and pathogenesis. For malaria, partial anti-parasite immunity develops only after several years of endemic exposure. Evidences suggest that this inefficient induction of immunity is partly a result of antigenic polymorphism, poor immunogenicity of individual antigens, the ability of the parasite to interfere with the development of immune responses and to cause apoptosis of effector and memory B and T cells, and the interaction of maternal and neonatal immunity[4]. Studies of the immune responses of naive animals to malaria parasites indicate that the host response varies depending on the strain of parasite and genetic background of the host[5,6,7]. Both innate as well as adaptive immune responses play an important role in parasite suppression.

INNATE IMMUNITY

In both human infections as well as experimental malaria models, survival appears to be critically linked to the ability of the host to control blood-stage parasite replication within the first 7–14 days of infection[8]. It is noteworthy that the parasite-specific antibodies and cellular responses are basically absent during the acute stage of infection; innate immune mechanisms seem to be vital in controlling early parasite replication and decreasing the risk of advancement to severe and fatal disease.

Interferon gamma (IFN-γ) is a macrophage-activating factor involved in the innate immune response to malaria. It is mainly produced by $CD4^+$ and $CD8^+$ T lymphocytes

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in a specific immune response and by natural killer (NK) cells in a non-specific response. Early production of IFN-γ is critical since it directly mediates anti-parasitic effects and hence helps to limit progression from mild malaria to severe and life-threatening complications. Augmented release of IFN-γ stimulates monocytes/macrophage and γδ-T cells to secrete tumor necrosis factor-alpha (TNF-α), which can further promote anti-plasmodial properties through formation of toxic free radicals, such as nitric oxide[9]. Interleukin (IL)-6 and IL1-β, like TNF-α, are other inflammatory cytokines that also play a role in limiting parasite replication but are involved in induction of fever and acute phase response[10]. The ability to balance effectively the anti-parasitic and immunopathogenic effects of these cytokines is a hallmark of clinical immunity to malaria.

As a central component of the innate immune response, complement plays a critical role in neutralizing invading parasites; however, excessive activation of this system has the potential to mediate disease pathogenesis[11]. Clearance of infected erythrocytes by monocytes/macrophages is important for control of infection and in limiting excessive inflammation induced by the rupture of infected cells. Blocking complement deposition has been shown to prevent nearly 80–95% of phagocytosis of erythrocytes harboring immature (ring-stage) parasites *in vitro*[12]. Macrophages contribute in the control of the infection through both antibody-dependent and -independent phagocytosis, and secretion of soluble factors directly or indirectly toxic to the parasite, such as IL-1, TNF-α, granulocytes-macrophage colony stimulating factor (GM-CSF), reactive nitrogen and oxygen radicals[13].

CD36, a member of the class B family of scavenger receptors, was primarily expressed on dermal microvascular endothelium that supported adhesion of most natural isolates of *P. falciparum* malaria. It has been demonstrated that it performs dual function in mediating phagocytosis as well as produces cytokine responses to malaria, and helps in innate host defence to *P. chabaudi chabaudi* AS (PCCAS) malaria *in vivo*. Phagocytosis of microbial pathogens is linked to innate sensing and cytokine response mediated via cooperation between pattern recognition receptors such as scavenger receptors and toll-like receptors (TLRs)[14]. Production of IL-12 from activated macrophages is also crucial to early activation of γδ-T cells, resulting in additional production of IFN-γ[15]. γδ-T cells represent the interface between innate and adaptive immune response and together with NK cells, contribute to a rapid resolution of clinical malaria. Though, the innate effector mechanisms that actually regulate the blood stage parasitemia during acute infection are not fully understood.

ACQUIRED IMMUNITY

The acquired immunity to malaria involves activation of both humoral as well as cellular immune responses[8,16]. Dendritic cells (DCs) are supposed to play a crucial role, both as highly efficient presenters of antigen to helper T cells and in determining the balance of cell-mediated immunity and antibody-mediated immunity by steering the T cell population towards a Th1 or Th2 response[17,18]. The influence of environment, genetic background and nutritional status cannot be ruled out to explain the disparity of specific immunity.

Natural acquired immunity

Contrasting to many acute viral diseases that produce life-long resistance to reinfection, *Plasmodium* provokes immunity only after several years of continuous exposure, during which recurring infections and illness occur. Robert Koch first reported a scientific basis for naturally acquired protection against malaria. By cross-sectional studies of stained blood films, Koch inferred that protection against malaria was acquired only after heavy and uninterrupted exposure to the parasite. But it is not clear that as to how this protection comes about, and there is only little knowledge on the key determinants of protection[19]. Natural immunity against malaria develops only gradually over many years of repeated and multiple infections in endemic areas[20,21]. The identification of immune correlates of protection among the abundant non-protective host responses remains a research priority. While evasion and modulation of the host immune response clearly occurs throughout the *Plasmodium* life cycle, immune mechanisms to control blood-stage parasites are acquired and maintained by individuals living in malaria endemic areas, allowing parasite densities to be kept below the threshold for the induction of acute disease and providing protection against severe malaria pathology[22].

In human host, it appears that natural immunity is acquired only to blood stages. Conversely, naturally acquired immunity to pre-erythrocytic stages is not believed to occur and this is likely due to the small infectious load, the immunotolerant state of the liver as well as host impairment of the liver-stage infection in individuals with blood stage disease[23]. Once established, anti-malarial immunity appears to be a 'regional phenomenon', as seen in labor migrants or refugees, who lose protection against re-infection when moving to geographically separate places[24]. The concept of '*P. falciparum* diversity' postulates a rationale for the detected slow acquisition of natural immunity.

Immunity in infants

Infants seem to be relatively protected from malaria infection and its consequences for initial six months of their life. When infants become susceptible, their infection tends to be of low parasite density, asymptomatic and is cleared within a month[25]. Simister (1988)[26] reported that in humans, systematic transfer of maternal antibodies of IgG isotype occurs across the placenta. *P. falciparum* specific IgG1 and IgG3 are more reliably transferred from mother to child as compared to IgG2 and IgG4[27]. It is crucial to know about the period during which infants lose their maternally derived antibodies to malaria and instigate to acquire naturally their own immune responses against parasite antigens, so that malaria vaccines may be best administered. Duah *et al.* (2010)[28] investigated the rates of decline and acquisition of serum antibody isotypes IgG1, IgG2, IgG3, IgG4, IgM and IgA to *P. falciparum* antigens; apical membrane antigen (AMA1), merozoite surface proteins (MSP1-19, MSP2 and MSP3) in a birth cohort of 53 children living in an urban area in the Gambia, followed over the first 3 years of life (sampled at birth, 4, 9, 18 and 36 months). Antigen-specific maternally transferred antibody isotypes of all immunoglobulin G (IgG) subclasses were detected at birth and were almost totally depleted by the age of 4 months. Attainment of specific antibody isotypes to the antigens began with IgM, followed by IgG1 and IgA. Against the MSP2 antigen, IgG1 responses were observed in the children, in contrast with the maternally derived antibodies to this antigen that were mostly IgG3. This confirms that IgG subclass responses to MSP2 are strongly dependent on age or previous malaria experience, polarized towards IgG1 early in life and to IgG3 in older exposed individuals[28].

STAGE-SPECIFIC ACQUIRED IMMUNITY

Acquired immunity against the *Plasmodium* parasite is complex and stage-specific. By convention, immune responses in malaria are dichotomized into pre-erythrocytic responses (directed against sporozoites and liver-stage parasites) and erythrocytic responses (directed against merozoites and intra-erythrocytic parasites).

Pre-erythrocytic stage immunity

After their inoculation into the skin, some sporozoites get associate with Dendritic cells (DCs) in the draining lymph nodes. These cells present sporozoite antigens to naive T cells, and hence T cells get activated. Activated T cells enter the circulation and traffic to the liver, help in obliteration of the infected hepatocytes that display antigen-MHC complexes on their surface, reducing liver-stage parasite load[29].

Pre-erythrocytic immunity generally consists of cellular responses against infected hepatocytes, which inhibit intracellular parasite development through the induction of reactive nitrogen intermediates. Various antigens, specific to the liver stage, have been identified and it has been suggested that these antigens, along with those brought in with the invading sporozoites, are rapidly processed by the host cell and presented on the surface of infected hepatocytes in combination with MHC class I[30]. This presentation leads to recognition by cytotoxic T lymphocytes (CTLs) and killing of the infected cell, or stimulation of NK and $CD4^+$ T cells to produce IFN-γ. This can trigger a cascade of immune reactions and ultimately can lead to the death of intracellular parasite[30,31]. The CTLs may be directly cytolytic against malaria-infected hepatocytes by releasing perforin and granzyme or by binding to apoptosis-inducing receptors on the infected cells[32].

Plasmodium sporozoites suppress the respiratory burst and antigen presentation of Kupffer cells, which are regarded as the portal of invasion into hepatocytes. It is not known whether immune modulation of Kupffer cells can affect the liver stage. In a study, it was observed that sporozoites inoculated into wistar rats could be detected in the liver, spleen, and lungs; however, most of the sporozoites were arrested in the liver. Sporozoites were captured by Kupffer cells lined with endothelial cells in the liver sinusoid before hepatocyte invasion. Pre-treatment with TLR3 agonist poly (I:C) and TLR2 agonist BCG primarily activated the Kupffer cells, inhibiting the sporozoite development into the exoerythrocytic form, whereas, Kupffer cell antagonists dexamethasone and cyclophosphamide promoted development of the liver stage. Present data implies that sporozoite development into its exo-erythrocytic form may be associated with Kupffer cell functional status. Immune modulation of Kupffer cells could be a promising strategy to prevent *Plasmodium* infection[33].

Erythrocytic stage immunity

Merozoites that survive to the pre-erythocytic stage are responsible for the modification of infected red blood cells in terms of parasite proteins expressed on the cell surface and the concomitant immune response to the *Plasmodium* parasite, resulting in the clinical manifestations of malaria[34]. The pathogenic manifestations during a malaria crisis are due to proinflammatory cytokines released by T cells and macrophages in response to malaria parasites and their products, including glycosylphosphatidyl-inositol (GPI) moieties[35], malaria pigment[36] and *Plasmodium*-derived nitric oxide synthase (NOS)-inducing factor[37].

Humoral responses against extracellular merozoites and intraerythrocytic parasites have traditionally been considered the most important component of blood-stage immunity. An antibody binding to the surface of the merozoite, and to proteins that are externalised from the apical complex of organelles involved in erythrocyte recognition and invasion, seems to have an important role in immunity to asexual blood stages. This antibody could neutralize parasites or lead to Fc dependent mechanisms of parasite killing by macrophages[38]. T cell responses against pRBC remain less well understood, partly because erythrocytes lack MHC class I or class II presentation capacity. Nevertheless, cellular responses against pRBC have been suggested to contribute to protection in humans in the absence of antibodies[39,40]. Finally, monocyte/macrophage-mediated responses, in particular phagocytosis and antibody-dependent cellular inhibition (ADCI) also form an important component of blood-stage immunity[41].

Immunity to blood-stage *Plasmodium* parasites is critically dependent on the type 1 cytokine IFN-γ and requires coordinate and timely innate and adaptive immune responses involving dendritic cells (DC), NK cells, $CD4^+$ T helper cells, and B cells[8,41]. Moreover, a balance between pro-inflammatory and anti-inflammatory responses is essential to limit the development of life-threatening immune-mediated pathology such as CM and SMA. Although a better understanding of the mechanisms involved in protective immunity and immunopathology is emerging, still the understanding of regulatory mechanisms required to maintain the balance between beneficial and deleterious responses during blood-stage malaria infection remains limited[42].

IMMUNE EVASION BY *PLASMODIUM*

Despite the presence of various immune mechanisms, the parasite is adept at evading immunity by a variety of mechanisms, which help its survival in the host. Possible mechanisms of interference in the activation of T cells and B cells, and the generation of immunological memory by the parasite have been described by many workers[21,43]. The parasite modulates the immune mechanism either by interfering with presentation or processing and cause apoptosis of T cells and other effector cells or mutates the sequence of epitopes critical for B or T cell recognition[44]. Furthermore, because malaria is a chronic infection, it is possible that B and T cell exhaustion may contribute to the suboptimal host immunity that is inadequate to control the parasite. Data from a longitudinal study in Mali has shown that exhausted B cells comprise 20-60% of that circulating B cell pool as compared with 1–2% of the B cell pool in people from non-endemic areas[45]. Understanding the immunological and molecular mechanisms of the crosstalk between the host and parasite is a pre-requisite for the rational discovery and development of a safe, affordable, and protective anti-malaria vaccine[46].

CONCLUSIONS

Immunity contributes an essential role in controlling the disease, but partial immunity develops only after several years of endemic exposure. Innate immunity, involving complement system, macrophages and various cytokines, is vital in controlling early infection. The adaptive immunity is complex and stage-specific, and includes activation of both humoral as well as cellular immune responses. *Plasmodium* evades the immune mechanisms by interfering the activation of B and T cells, and the generation of immunological memory. Overall, a better understanding of the immunopathology and immunoregulatory pathways involved both in experimental malaria models as well as in individuals is essential for the development of an effective vaccine so that this fatal disease can be controlled.

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