Review

Malaria Blood-Stage Infection and Its Control by the Immune System

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Abstract. Malaria is caused by the protozoon *Plasmodium*, transmitted to humans by *Anopheles* mosquitoes. The most dangerous of the plasmodia infecting humans is *Plasmodium falciparum*. The disease is caused by those parasite stages which multiply asexually in red blood cells. In non-immune individuals, *P. falciparum* may cause severe and life-threatening disease. Another risk group is constituted by pregnant women, particularly during their first pregnancies.

Immunity to malaria usually requires repeated exposure to the parasite to become long lasting. One reason for this is the capacity of the parasite to vary the antigens which are major targets for protective antibodies. Antibody-dependent protection is primarily mediated by cytophilic IgG antibodies activating cytotoxic and phagocytic effector functions of neutrophils and monocytes. Malaria infection also involves elevated production of IgE antibodies. However, IgE-containing immune complexes are pathogenic rather than protective by cross-linking IgE receptors (CD23) on monocytes, leading to local overproduction of TNF, a major pathogenic factor in this disease.

T cells are essential for both acquisition and regulation of malaria immunity. The major T cells controlling blood stage infections are $CD4^+$ of both the Th1 and Th2 subsets. However, T cells carrying the γδ receptor also contribute to this control. The balance between the cytokines produced by different cell types is critical for the course of infection, with IFN-γ having a key role in anti-malaria defence. Blood-stage infections are also under complex genetic control. Among the regulatory genes, those involved in elevated production of TNF are associated with increased risk of severe disease and death due to *P. falciparum* infection.

Malaria is the most widely spread and serious parasitic disease afflicting mankind, with estimated 300–500 million cases and up to 2–3 million deaths yearly (World Health Organization (WHO), 1998). In Sub-Saharan Africa, malaria is responsible for the deaths of approximately 1 million children per year, corresponding to 25% of all childhood deaths. Its morbidity is enormous, constituting the main reason for hospital admission in malaria-endemic areas, where more than 40% of the world's population live.

The most common parasite causing malaria in humans is *Plasmodium falciparum*. Although the majority of cases are relatively mild, this parasite may also cause severe and life-threatening malaria involving severe anaemia, hypoglycaemia, renal failure, respiratory distress, multiple convulsions and coma (Marsh, 1999). Cerebral malaria, one of the most severe forms of this disease, has a fatality rate varying between 11–33% (Waller et al., 1995). The risk of developing cerebral malaria is mainly confined to those who are not immune. In endemic areas this is limited to young children, immigrants and travellers from non-endemic areas. Another group at risk of developing severe malaria comprises pregnant women, where the greatest risks are for women during their first pregnancy. The reasons for this are believed to be that the placenta is a favourable site for parasite development and possibly also that natural immunity is depressed during pregnancy (Steketee et al., 1996).

During the first months of a child's life passive transfer of antibodies from the mother confers some protection (McGregor, 1984), as well as a high haemoglobin F content in the infant's erythrocytes, which retards parasite development (Marsh, 1992). However, parasite numbers soon increase and mortality in hyperendemic areas is highest during the first year of life. The pattern of severe disease such as cerebral malaria or severe anaemia is influenced by the intensity of transmission, by the seasonality of infections and by the degree of clinical immunity (Baird et al., 1991). In addition, there are data indicating that the degree of severity is influenced by host genetic factors and perhaps by virulence factors in the parasite itself (Miller et al., 1994).

The parasite

The *Plasmodium* life cycle is complicated, comprising several developmental stages in both the vertebrate host and in the insect vector. Infection of the vertebrate host is initiated by inoculation of sporozoites from the salivary glands of an infected *Anopheles* mosquito. Within

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Abbreviations: IFN – interferon, MHC – major histocompatibility complex, NBNT cells – non-B, non-T cells, NO – nitric oxide, TNF – tumour necrosis factor, RBC – red blood cells, WHO – World Health Organization.

minutes the sporozoites invade hepatocytes in the liver and multiply for a week or two when thousands of merozoites are released from the ruptured cells, ready to invade erythrocytes and initiate the erythrocytic life cycle.

After invasion of erythrocytes the parasite undergoes mitotic division and matures into a schizont containing 32 merozoites. The erythrocytes then rupture and release the merozoites into the blood stream, causing clinical symptoms such as fever and anaemia. The merozoites quickly reinvade new erythrocytes and the cycle is repeated. Some of the parasites will occasionally develop into female or male gametocytes, the stage infective to the mosquitoes. In the mosquito midgut the gametocytes will differentiate into gametes and fertilization will take place. The resulting zygotes will form oocysts and differentiate into sporozoites that finally migrate to the salivary glands of the mosquito.

Acquired immunity to *P. falciparum* malaria

In hyperendemic areas, the incidence and density of malaria parasitaemia decline with the age of the human host, a phenomenon that has long been interpreted as the result of anti-malaria immunity (Deloron et al., 1987; Warsame et al., 1997). Immunity is usually built up over a period of several years and is not long lasting (Greenwood et al., 1991). This slow acquisition has been suggested to be due to many factors including parasite and host genetic variability, the developmental stage of the invaded erythrocytes as well as induction of immunosuppression by the parasite in the host (Greenwood et al., 1971; Morges and Weidanz, 1980; Weidanz and Long, 1988; Hviid et al., 1991). Acquired immunity is practically never complete and residents of malaria-endemic areas continue to experience sporadic episodes of clinical disease throughout life, although with reduced frequency and morbidity (Marsh, 1992; Trape et al., 1994).

Malaria immunity is the result of several different immune mechanisms including non-specific inhibition of parasite growth (probably mediated by the tumour necrosis factor (TNF) and similar factors), phagocytic activity of macrophages or neutrophils stimulated by cytokines such as interferon-γ (IFN-γ), and production of specific antibodies eventually eliminating the parasites either directly or in cooperation with effector cells (Kwiatkowski, 1992).

Immune responses to the different life-cycle stages of the malaria parasite have different effects. Immunity against the pre-erythrocytic parasite forms will prevent infection or parasite development in the liver while that against the sexual stages will inhibit fertilization and parasite development in the mosquito. Immunity against the asexual blood stages will prevent parasite multiplication and growth in the blood. Importantly, as these stages are responsible for the symptoms characterizing malaria, immunity against them will also be an anti-disease immunity. Therefore, the following discussions will be focused on the different types of immune responses against the asexual blood stages of *P. falciparum.*

Humoral immunity to the asexual blood stages of *P. falciparum*

Malaria infection induces both humoral and cell-mediated immunity. In residents of malaria-endemic areas, IgM and IgG but also other immunoglobulin isotypes in the blood are elevated (Rosenberg, 1978), reflecting both polyclonal and specific B-cell activation. There is significant production of both species- and life-cycle stage-specific anti-malarial antibodies that react with a large number of antigens. There is good evidence that antibodies, particularly those of the IgG isotype, protect against the asexual blood stages. Thus, passive transfer of immune IgG to patients with acute malaria has been shown long ago to efficiently reduce parasitaemia (Cohen et al., 1961; McGregor et al., 1963; Bouharoun-Tayoun et al., 1990). Similar results have been obtained in primate (Fandeur et al., 1984; Romero, 1992) and murine (Jarra et al., 1986) malaria models.

In malaria-endemic areas, anti-malarial antibodies of the cytophilic IgG1 and IgG3 isotypes are associated with lower parasitaemias and lower risk of malaria attack. Assay of antibody-dependent parasite inhibition in the presence of monocytes shows that the IgG1 : IgG3 ratio may be highest for those whose antibodies are most inhibitory (Shi et al., 1999). Moreover, noticeably increased concentrations of IgG3 antibodies against some malaria antigens have also been found in certain *P. falciparum*-infected populations (Rzepczyk et al., 1997) and in association with malaria attacks (Aribot et al., 1996).

Antibody-mediated protection against malaria may involve several different mechanisms. Thus, anti-plasmodial antibodies may prevent invasion of erythrocytes by the merozoites (Udeinya et al., 1983). They may also contribute to the clearance of parasites from the circulation by binding to the surface of infected erythrocytes (Treutiger et al., 1992). Malaria sera have an enhanced capacity to opsonize infected erythrocytes, which are readily lysed or phagocytosed by Fcγ receptor-bearing effector cells such as monocytes/macrophages and neutrophils (Bouharoun-Tayoun et al., 1990, 1995; Kumaratilake et al., 1996). Fc-receptor binding of opsonized erythrocytes to monocytes/macrophages may also result in production of toxic factors such as TNF (Bouharoun-Tayoun et al., 1995). Recently, elevated concentrations of some IgG2 antibodies have also been seen to protect against malaria infection by binding to certain allelic forms of the monocytic Fcγ receptor RIIA (Aucan et al., 2000).

Another immunoglobulin isotype which is elevated in humans as well as in mice exposed to malaria is IgE. The elevation comprises both total IgE and anti-malarial