Malaria and the Immune System in Humans

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Introduction

Malaria infection gives rise to host responses which are regulated by both the innate and adaptive immune system as well as by environmental factors. Acquired immunity is both species- and stage-specific. It is rarely sterile, but rather associated with low-grade parasitemia and episodes of clinical disease throughout life [1, 2]. In endemic areas, children born to immune mothers are protected against disease during their first half year of life by maternal antibodies. This passive immunity is followed by 1 or 2 years of increased susceptibility before acquisition of active immunity [1]. In general, acquisition of active immunity to malaria is slow and requires repeated parasite exposure to be maintained. Genetic variability of both the human host and the parasite, parasite-induced immunosuppression and other reasons account for this instability [3].

In this chapter we will discuss the immune regulation of malaria blood-stage infection in humans, focusing on *Plasmodium falciparum*, the most widely spread and dangerous of the human parasites.

Innate Immunity

Innate mechanisms of parasite growth inhibition by the human host are probably the reason for the low parasitemias seen in acute *P. falciparum* infection [3]. The humoral and cellular mechanisms of this ‘nonspecific’ defense are poorly defined. Recent studies in nonparasitic systems have demonstrated that a family
of germ line encoded receptor proteins are important for innate host defense in both invertebrates and vertebrates. In mammals, activation of macrophages through such ‘toll-like receptors’ leads to the induction of effector genes whose products control and execute this innate defense in a large variety of bacterial and viral systems [4]. Although not as yet as extensively investigated for parasitic infections, it is likely that this system is of equal importance for the innate defense against malaria.

Malaria infection gives rise to strongly elevated blood concentrations of non-malaria-specific immunoglobulin [5], but the importance of the underlying polyclonal B-cell activation for innate immunity is not known. This is also true for the CD4+ T cells from malaria-naïve donors responding by in vitro proliferation and cytokine production upon exposure to malaria antigens [6]. In contrast, neutrophils, mononuclear phagocytes and natural killer (NK) cells appear to play a role in innate immunity seen early in malaria infections. In particular, NK cells have been shown to increase in numbers and to be able to lyse *Plasmodium falciparum*-infected erythrocytes in vitro [7]. However, NK cells are also potent producers of cytokines such as interferon-γ (IFNγ) and this capacity, leading to parasiticidal macrophage activation, may be of greater importance for innate malaria immunity than their potential to lyse infected host erythrocytes [8].

Related cell types probably playing a role in innate malaria immunity are the NKT cells which in mice carry both the NK1.1 surface marker and αβ T-cell receptors (TCR) [9]. These cells are potent inhibitors of liver-stage parasite replication in mouse malaria systems in vitro [10]. Furthermore, NK1.1+ CD4+ murine T cells have also recently been reported to regulate IgG antibody responses to glycosylphosphatidyl inositol-anchored *P. falciparum* protein, a response which may be important for a rapid, specific but major histocompatibility complex (MHC) unrestricted parasite control [11]. Human NKT cells express TCR homologous with those of murine NKT cells as well as other NK cell markers. Both murine and human NKT cells are activated via their invariant TCR when confronted with lipid antigen in association with the MHC class I like CD1 molecules [12]. This activation does not require immunization and may, therefore, be important for regulating innate malaria immunity.

T cells bearing the γδ TCR are also strongly expanded during the early phases of malaria infection and may contribute to innate parasite control [13]. In support of this, γδ T cells but not αβ T cells from malaria-naïve donors inhibit parasite replication in vitro [14, 15]. This difference might be related to differences in antigen recognition by the two types of TCR or, alternatively, to the presence on γδ T cells of NK receptors [16, 17], the non-antigen-specific ligation of which results in rapid secretion of proinflammatory cytokines. For further details regarding γδ T cells see the section on cell-mediated immunity below.
Humoral Immunity

In residents of endemic areas, malaria infection induces strong humoral immune responses, involving production of predominately IgM and IgG but also of other immunoglobulin isotypes. While a large proportion of this immunoglobulin is non-malaria-specific, reflecting polyclonal B-cell activation, up to 5% or more represent species- as well as stage-specific antibodies reacting with a wide variety of parasite antigens. Passive transfer of IgG from immune donors already suggested long ago that antibodies may be protective [7, 18] by reducing parasitemia and clinical disease. These early studies also established that some of the important antigens inducing such protective responses were shared by *P. falciparum* parasites worldwide regardless of geographical origin [19].

Some Important Malaria Antigens

Of major importance for the development of humoral immunity to the malaria blood stages are the parasite antigens expressed on the surface of infected erythrocytes. The predominant antigens involved are members of highly variant families. This variability enables the parasites to evade the immune response and, therefore, constitutes an important virulence factor [20]. In line with this, antibody-mediated inhibition of merozoite invasion of erythrocytes is less effective with parasites from the antibody donor than with those from other donors [21]. Similarly, culture of parasites in the presence of anti-malarial antibodies reduces their susceptibility to antibody-mediated growth inhibition as compared to that of those previously cultured without antibodies [22].

The predominant variant parasite antigens on the surface of *P. falciparum*-infected erythrocytes are encoded by the multi-gene family var [23, 24]. The gene products, called *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1), are highly variant polypeptides of 200–350 kD [25] equipped with several binding sites mediating adhesion of infected erythrocytes to the vascular endothelium of capillaries and post-capillary venules [26, 27]. This ‘cytoadherence’ in small peripheral vessels is believed to protect the parasites from being destroyed in the spleen. Although the var genes occur in 40–50 copies/haploid genome, only one gene product is expressed in infected erythrocytes containing late-stage parasites [28, 29]. Another multi-gene family encoding parasite antigens on the erythrocyte surface are the rif genes, occurring in at least 200 copies, mostly located subtelomERICally on several parasite chromosomes [30, 31]. The rifins have an accessory role in the binding of uninfected to infected erythrocytes giving rise to rosetting [32; for further references and discussion of these variant parasite antigens see, 20, 33, 34].

Many additional parasite encoded molecules in infected erythrocytes display a high degree of antigenic diversity, reflecting expression of allelic genes or of
alternative genes belonging to multi-gene families [20, 33]. Candidate antigens for the induction of protective antibodies may be located in apical organelles or on the surface of merozoites as well as on the surface of infected erythrocytes. Important examples are merozoite surface proteins (MSP)-1–5 [35]. The most thoroughly investigated of these is MSP-1, containing both a conserved C-terminal amino acid sequence (19 kD) which is carried over by the parasite when it invades uninfected erythrocytes and antigenically variable sequences which are released [20, 33].

**Antibodies**

Malaria infection induces both polyclonal and specific immunoglobulin production. Although antibodies of different isotypes may have protective functions, IgG is most important in this respect. In protected individuals, cytphilic antibodies of IgG1 and IgG3 isotype have frequently been found to prevail [36, 37]. The ratio of IgG1 to IgG3 antibodies appears to be highest in subjects whose antibodies are also most efficient in parasite neutralization in vitro, supporting the functional relevance of these findings [38]. Significant elevations of IgG3 antibodies in certain populations and associated with disease episodes have been reported [39, 40]. However, elevated concentrations of IgG2 antibodies may also be associated with decreased risk of *P. falciparum* infection: this has been seen in certain individuals whose monocytes carry a special allelic variant of a Fcγ receptor (RIIA) having the capacity to bind this normally not cytphilic immunoglobulin subclass [41].

Malaria infections of both humans and experimental animals are also associated with elevations in total IgE and IgE anti-malarial antibodies [42, 43]. Induction of this immunoglobulin isotype reflects a switch of regulatory T cell activities from Th1 to Th2 due to repeated exposure of the immune system to the parasites. However, IgE elevation is also under genetic control as demonstrated by comparison of mono- and dizygotic twins from malaria endemic areas [44]. IgE elevation appears to be associated with malaria pathogenesis as the blood concentrations of this isotype are significantly higher in patients with cerebral or other forms of severe disease than in those with uncomplicated malaria [42, 45]. A pathogenic effect of IgE is probably due to local overproduction in microvessels of tumor necrosis factor (TNF) and nitric oxide (NO) caused by IgE-containing immune complexes. Such complexes may induce and cross-link CD23, the low-affinity receptor for IgE on monocytes and perhaps endothelial cells, resulting in their activation [46]. However, these results do not exclude that IgE antibodies also may be protective.

**Antibody-Dependent Protection**

Antibodies may protect against malaria by a variety of mechanisms. Thus, they may inhibit merozoite invasion of erythrocytes [47] and intra-erythrocytic
growth or enhance clearance of infected erythrocytes from the circulation by binding to their surface, thereby preventing sequestration in small vessels and promoting elimination by the spleen [48, 49]. In particular, opsonization of infected erythrocytes significantly increases their susceptibility to phagocytosis, cytotoxicity and parasite inhibition by various effector cells such as neutrophils and monocytes/macrophages [50, 51]. Interaction of opsonized erythrocytes with these effector cells induces release of factors such as TNF which may cause tissue lesions but which are also toxic for the parasites [52].

Obviously, antigenic diversity and variation of the parasites will greatly affect the protective efficiency of antibodies [20]. Thus, exposure of the immune system to an infecting parasite gives rise to variant-specific anti-PfEMP-1 antibodies which will inhibit cytoadherence and reduce the risk of renewed infection by parasites expressing the same PfEMP-1 as the originally infecting one [53]. However, the presence of such antibodies will also contribute to the selection of different variants against which these antibodies do not protect [54, 55]. Similarly, natural infection also induces strain-specific antibodies against the highly variable rifins [32]. However, the possible protective function of anti-rifin antibodies remains to be established.

**Cell-Mediated Immunity**

Cell-mediated immune responses induced by malaria infection may protect against both pre-erythrocytic and erythrocytic parasite stages [56].

*CD4+ and CD8+ T Cells*

Of these major T-cell subpopulations, CD4+ T cells are essential for immune protection against asexual blood stages in both murine and human malaria systems. For CD8+ T cells which have important effector functions in pre-erythrocytic immunity [57] and which contribute to protection against severe malaria [58, 59], this role is less clear. It has been proposed that CD8+ T cells may regulate immunosuppression in acute malaria and downmodulate inflammatory responses [60]. In any event, as human erythrocytes do not express MHC antigens, lysis of infected erythrocytes by CD8+ cytotoxic T lymphocytes has no role in the defense against blood-stage parasites.

In contrast to the CD8+ T cells, the regulatory and effector functions of CD4+ T cells are well established for both experimental and human malaria. For experimental malaria, evidence for this is based on adoptive transfer of protection by such cells and on increased susceptibility to infection of CD4+ T-cell-depleted mice [for references and discussion see, Langhorne et al., pp 204–228; 3]. For *P. falciparum* malaria in humans, the existence of
functionally different CD4+ T cells in naturally exposed donors has also been established experimentally. These cells respond to malaria antigen by in vitro proliferation and/or secretion of cytokines, e.g. IFNγ or IL4 [61]. In general, these in vitro responses are poorly correlated with protection [3, 60]. Nevertheless, in vitro stimulation of CD4+ T cells from malaria-exposed donors may result in the production of IL4 in concordance with the serum concentrations of antibodies specific for the antigens used for lymphocyte stimulation [62, 63]. Furthermore, enhanced IFNγ production and proliferation have been reported for T cells from donors recovering from a malaria attack [64].

**γδ T Cells**

Cells expressing the γδ TCR normally represent less than 5% of all T cells in the peripheral blood of healthy adults. The TCR of approximately 75% of these cells is made up of Vγ9 and Vδ2 chains while a minor fraction expresses Vδ1 with no preferential Vγ association [65]. In healthy West Africans, the frequency of γδ T cells in the blood is about twice that of Caucasians, mainly due to an increase in the Vδ1 subset [66]. In vitro stimulation with *P. falciparum* extracts of blood mononuclear cells not previously exposed to malaria also results in γδ T-cell activation, with a majority of the responding cells expressing Vγ9/Vδ2 [67, 68] and a minority Vδ1 [69]. Activated γδ T cells but not αβ T cells from malaria-naïve donors inhibit parasite replication in erythrocytes in vitro, supporting their protective function and, in particular, their role in innate defense against the malaria parasites [14, 15]. γδ T-cell activation is associated with IL-2 receptor (IL2R) signaling, initiated by cytokines such as II-2, IL-4 and IL-15 [70, 71]. Malaria antigen-activated γδ T cells produce primarily but not exclusively pro-inflammatory cytokines [15], suggesting that protection against the parasites by these cells involves both regulatory and cytotoxic functions. However, it should be emphasized that these cellular activities also may be implicated in malaria pathogenesis [for references see, 3, 15].

Antigens from plasmodial schizonts potently stimulate γδ T cells [72, 73]. These cells recognize certain antigens conventionally in association with MHC class-I or II molecules [68, 71]. However, γδ T cells also recognize non-peptide antigens, with no need of MHC presentation [74]. These activating ligands are relatively small (molecular weight <500 kD) and mostly contain phosphoesters [75]. Such phosphoantigens were first described for *Mycobacterium tuberculosis* and also for *P. falciparum* [72, 76]. These ligands bind directly and specifically to the γδ TCR.

The antigens seen by Vδ1 T cells are less well known although it has been reported that intra-epithelial Vδ1 T cells may react with the stress-induced proteins MICA and MICB [77], suggesting that they might recognize epithelial cells damaged by infection [75].
The Cytokine Network

Protective anti-malarial immunity reflects cellular activities such as antibody production, phagocytosis, cellular cytotoxicity and parasite inhibition exerted by lymphocytes, neutrophils and mononuclear phagocytes. However, some of these cellular activities may also cause tissue damage and the course of a malaria infection is highly dependent on the balance between the cytokines secreted by the various cells when activated [60]. In any event, proinflammatory cytokines such as IFNγ, IL-1, IL-6 and others may be protective by inducing parasite killing by monocytes/macrophages and neutrophils [60, 78]. IL-12, produced by mononuclear phagocytes and other cells, contributes to protection against pre-erythrocytic and blood infection by initiating a Th1 anti-malaria response in mice as well as in monkeys [79, 80]. In contrast, anti-inflammatory cytokines such as IL-10 counteract the production and possible cytopathic effects of the proinflammatory cytokines [81, 82]. Recent studies of human *P. falciparum* malaria emphasize the importance of the balance between pro- and anti-inflammatory cytokines. Thus, elevated IL-6/IL-10 ratios in plasma due to relative IL-10 deficiencies predict a fatal outcome of severe malaria [83]. Moreover, anemic children from certain holoendemic areas have lower IL-10/TNF ratios than those with uncomplicated disease, suggesting that IL-10 may inhibit induction of anemia by TNF [84]. Malaria-induced IL-10 has also been found to predict resistance to *P. falciparum* infection, supporting the balancing role of anti-inflammatory cytokines [85].

A cytokine which has a central role for both protection and malaria pathogenesis is TNF. TNF does not kill parasites directly but exerts protection by activating the anti-parasitic effects of the various leukocytic effector cells [for references see, 86]. With regard to pathogenesis, TNF levels are positively correlated with disease severity as well as with malaria fever [87–90]. The primary source of this TNF are monocytes/macrophages activated by various parasite products [60]. However, as described in a previous section, IgE containing immune complexes also contribute to local overproduction of TNF in severe malaria [46]. Variation in the amounts of TNF produced by these cells has a genetic basis and is decisive for the outcome of an infection. Thus a single nucleotide polymorphism in the TNF-promoter region –308 is associated with elevated TNF production and an increased risk of cerebral *P. falciparum* malaria [91–93]. In contrast, children with low plasma levels of TNF due to a single nucleotide polymorphism at the TNF promoter allele –238A are susceptible to severe malarial anemia [94]. The mechanisms governing the underlying regulations may involve altered gene transcription due to changes in transcription factor binding to the corresponding TNF promoter region [91].

Nitric Oxide

As discussed above, anti-malarial antibodies may control blood-stage parasites both on their own and in cooperation with different effector cells [48, 49, 52].

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