Review

CellPress

Complement activation in malaria: friend or foe?

Sergei Biryukov[1] and José A. Stoute[1,2]

[1] Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA
[2] Department of Medicine, Division of Infectious Diseases and Epidemiology, Pennsylvania State University College of Medicine, 500 University Drive, MC H036, Hershey, PA 17033, USA

Complement is activated during malaria infection, but there is little evidence that it benefits the host. On the contrary, growing evidence points to the central role of complement activation in the pathogenesis of complicated malaria. Recent evidence suggests a critical role for C5a and the membrane attack complex in the pathogenesis of cerebral malaria, and for C5a in the pathogenesis of placental malaria. In addition, erythrocytes of children with severe malarial anemia have increased deposition of C3b and decreased capacity to regulate complement activation, that probably increase their susceptibility to destruction by liver and splenic macrophages. These observations justify further investigation of the role of complement in malaria and the testing of complement inhibitors as adjunctive treatment for severe malaria.

The malaria problem

Malaria remains one of the world's most important infectious diseases and is responsible for over 1 million deaths each year [1]. Five apicomplexa protozoan species of the genus *Plasmodium* infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malaria*, and *Plasmodium knowlesi*. Of the five plasmodium species, *P. falciparum* is the deadliest, accounting for most of the malaria-related deaths, the majority of which occur in children under 5 years of age and primigravid women due to complications from severe malaria. The life cycle of the parasite is complex and begins with transmission to humans via the bite of infected female *Anopheles* mosquitoes during a blood meal [2]. Sporozoites injected by the mosquito travel to the liver where they multiply asexually into merozoites (see Glossary) inside hepatocytes. Then, 10–14 days later, the infected hepatocytes lyze releasing thousands of merozoites, which are now free to invade erythrocytes. Inside erythrocytes, merozoites once again divide asexually causing lysis of the cell 24–72 h later depending on the species. This erythrocytic cycle is responsible for all the complications of malaria. The innate immune response of the host to the parasite, partly consisting of complement activation and production of proinflammatory cytokines, is activated to control the infection. However, this innate response may be largely responsible for the severe complications associated with malarial infection. The role of complement in these complications has recently taken center stage [3]. Although complement activation is intended to be harmful to the parasite, it may be even more harmful to the host during malaria infection. Here we make the case that there is an urgent need to develop a greater understanding of the role of complement activation in the pathogenesis of complicated malaria, with the goal of developing potential interventions that could curb the morbidity and mortality of this condition. Unless stated otherwise the studies reviewed refer to *P. falciparum*.

Corresponding author: Stoute, J.A. (jstoute@psu.edu).
Keywords: malaria; complement; *Plasmodium falciparum*; cerebral malaria; placental malaria; severe malarial anemia.

1471-4914/$ – see front matter
 http://dx.doi.org/10.1016/j.molmed.2014.01.001

The complement system

The complement system is composed of soluble proteins circulating in extracellular fluids, notably the blood, as well as membrane-associated cell surface proteins that play a major role in host defense and inflammation. The classical (CP), mannose-binding lectin (MBLP), and alternative (AP) pathways serve as the three major complement activation routes (Figure 1). Upon activation of complement by pathogens, enzymatic reactions drive the sequential cleavage cascade of inactive zymogenic precursors that generate effector fragments. The main effector fragment (C3b) plays a role in opsonization that leads to phagocytosis of invading pathogens. Other fragments known as anaphylatoxins (C5a, C4a, and C3a) activate neutrophils and macrophages. The end result is lysis through the formation of the membrane attack complex (MAC) or terminal complement complex (TCC), composed of C5b-9 [4].

Complement activation and malaria

Several studies have shown that the complement cascade is activated during malaria infection. Studies in both humans and animal models show evidence of extensive complement activation leading to reductions in serum C1, C2, C3, C4, and C1q levels [5–11], or increased TCC (sC5b-9) [12] during malarial paroxysms. When merozoites egress from erythrocytes they are exposed to plasma and, in theory, complement activation could take place on the merozoite surface by any pathway. Two recent studies suggested that merozoites escape complement

Glossary

Alternative pathway of complement (AP): the alternative pathway of complement is activated by the hydrolysis of C3b or can be initiated by any C3b molecule that binds to factor B. Factor B is in turn activated by factor D leading to a convertase that activates more C3 molecules.
Anaphylatoxins: soluble polypeptides such as C3a, C4a, and C5a that are generated after proteolytic cleavage of C3, C4, and C5 during complement activation and function in leukocyte activation and chemotaxis, and increase vascular permeability and adhesion.
Cerebral malaria (CM): a severe encephalopathy characterized by coma and the presence of *P. falciparum*-infected erythrocytes in the peripheral circulation and the absence of other explanatory conditions.
Classical pathway (CP): the pathway by which complement is activated by the interaction of antigen-bound antibodies with C1q.
Complement hemolytic activity 50 (CH50): a screening test that reflects the integrity of the complement pathway. It is defined as the dilution of serum required to lyze 50% of sensitized sheep erythrocytes. It most commonly addresses the activity of the classical pathway but can be adapted to the AP or MBLP.
Complement receptor 1 (CR1/CD35): CR1 is a complement regulator found on the surface of many cells including leukocytes and erythrocytes. It serves several functions including binding of C3b or C4b-opsonized immune complexes, acceleration of the decay of C3 and C5 convertases, and cofactor activity in the factor I-mediated breakdown of C3b and C4b.
Decay accelerating factor (DAF/CD55): DAF is another membrane-bound complement regulator found on erythrocytes and leukocytes. As its name indicates, it accelerates the decay of C3 and C5 convertases.
Hyperparasitemia: parasitemia well above the level normally seen in humans. In mice, it is not uncommon to see parasitemias of 30% or higher.
Innate immune response: this is the part of the immune response that is not learned or acquired through exposure. Unlike the adaptive immune response, the innate immune response does not require immunological memory. In addition to complement activation via the MBLP and AP, innate immunity also involves the interaction of phagocytic cells (such as macrophages, dendritic cells, and neutrophils) with bacteria via pattern recognition receptors, which stimulate direct killing of the pathogen and production of cytokines and inflammatory mediators.
Intercellular adhesion molecule 1 (ICAM-1): an adhesion molecule expressed by endothelial cells. It allows the binding of white cells to the endothelium. During malaria infection, late stage trophozoites can bind to ICAM-1 causing sequestration of parasitized erythrocytes.
Interferon-gamma (IFN-γ): a proinflammatory cytokine that can activate macrophages and induce death of intracellular organisms.
Intervillous space: the space between placental villous capillaries perfused with maternal blood where exchange of oxygen and nutrients occurs.
Malarial paroxysms: acute clinical episodes of high fever accompanied by chills and rigors that recur at intervals depending on the species of malaria parasite. For instance, in *P. falciparum* infections the intervals are 48 h.
Mannose-binding lectin pathway (MBLP): the pathway of complement activation initiated by the recognition of mannose residues by mannose-binding lectin.
MBL-associated serine proteases (MASPs): these proteases (MASP1, MASP2, and MASP3) are embedded in the structure of MBL and a conformational change leads to their activation. They, in turn, cleave C4 and C2 leading to the formation of the C4b2b C3 convertase.
Merozoites: single-celled asexual erythrocytic stage of the parasite that is initially generated in the liver but then invades and multiplies cyclically in erythrocytes producing the clinical symptoms and pathology associated with infection.
Opsonization: a process of pathogen recognition and coating by antibody and complement components which enhances phagocytic uptake.
Placental malaria (PM): the finding of *P. falciparum*-infected erythrocytes in the intervillous spaces of the placenta.
***Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1):** a variant surface protein expressed on the surface of erythrocytes containing *P. falciparum* late stage forms (trophozoites). It binds to ICAM-1 and other endothelial receptors to allow sequestration of infected erythrocytes.
Polymorphisms: sequence variations in the sequence of genes within a population.
Severe malarial anemia (SMA): a hemoglobin concentration of ≤50 g/l (or a hematocrit of less than 15%) in the presence of *P. falciparum*-infected erythrocytes.
Terminal complement complex or membrane attack complex (TCC/MAC): a molecule formed by the sequential activation of C5–C9 forming C5b-9, which has the ability to insert into membranes, forming a pore that leads to cell lysis.
Transmission intensity: an assessment of the risk of infection. It is often measured as the entomological inoculation rate or the mean number of infectious mosquito bites per individual per unit of time.
Tumor necrosis factor-alpha (TNF-α): a cytokine that induces inflammation by binding to its receptor on macrophages and T cells. It activates these cells and induces production of other inflammatory cytokines such as interleukin-8 (IL-8) and IFN-γ.
Vascular endothelial growth factor (VEGF): an angiogenic growth factor that promotes the growth of endothelial cells.
Vascular endothelial growth factor receptor 1 or Fms-like tyrosine kinase-1 (VEGFR-1/Flt-1): the receptor for VEGF, which is expressed on the surface of endothelial cells. The soluble form, sFlt-1, is inhibitory.

activation in non-immune serum [13,14]. Other studies have explored the contribution of immune serum to merozoite phagocytosis but the role of complement in this process was not explored [15]. Additional studies are needed to determine the role of complement in immunity against merozoites (Box 1).

The most likely mechanism of complement activation during malaria is via the classical pathway by formation of antigen–antibody immune complexes (ICs) containing merozoites or circulating antigen [16–18]. However, there is both direct and indirect evidence that other pathways are activated. *In vitro* evidence suggests that the food vacuole of *P. falciparum* may activate the AP [13,14]. The MBLP may also be activated during malaria infection because MBL has been reported to bind to parasite proteins [19,20]. Other evidence of the role of the MBLP in malaria comes from genetic studies. Three polymorphisms of the MBL gene (*MBL2*) exon 1 that result in non-synonymous substitutions have been linked to decreased serum levels of MBL ('B' G54D, 'C' G57E, and 'D' R52C) [21]. Of these alleles, the *MBL2 C* allele is found almost exclusively in Africans, suggesting that it may provide a survival advantage within that population [22,23]. Several studies have investigated the association of these and other *MBL2* polymorphisms with parasitemia and severe disease. Whereas some studies have shown associations [24–27], others have not [20,28,29]. The lack of consistent results among these studies is probably due to differences in experimental design, population demographics such as age and gender, differences in transmission intensity, and statistical rigor. For example, Garred *et al.* [20] reported lack of association between low MBL expressors and severe malaria in a case–control study of children in Accra, Ghana, where transmission is seasonal, whereas Holmberg *et al.* [25] reported an association with severe malaria in older children in Northern Ghana where transmission is hyperendemic. Nevertheless, these results also suggest that the role of MBL in protection against *P. falciparum* malaria is complex.

Animal studies do not provide clear evidence that complement is critical in protection against malaria. Studies in Rhesus monkeys infected with *Plasmodium coatneyi* and treated with cobra venom factor (CVF) to deplete complement showed no significant change in parasitemia [30]. Likewise, $C3^{-/-}$ mice infected with the *Plasmodium berghei* Antwerpen–Kasapa (ANKA) strain showed no increase in parasitemia compared with wild type mice [31]. *Plasmodium chabaudi*-infected mice deficient in C1q exhibited little increase in parasitemia, and only during reinfection was a significant impairment in the control of parasitemia observed [32]. By contrast, complement deficiency in rats infected with *P. berghei* resulted in increased parasitemia and mortality [33]. Mice deficient in the complement receptor 3 (CR3, CD11b/CD18, MAC-1)