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Review Article

IgG opsonization of merozoites: multiple immune mechanisms for malaria vaccine development

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ABSTRACT

Global eradication of the human-infecting malaria parasite *Plasmodium falciparum*, the major cause of malaria mortality, is unlikely to be achieved without an effective vaccine. However, our limited understanding of how protective immune responses target malaria parasites in humans, and how to best elicit these immune responses through vaccination, has hampered vaccine development. The red blood cell invading stage of the parasite lifecycle (merozoite) displays antigens that are attractive vaccine candidates as they are accessible to antibodies and raise high antibody titres in naturally immune individuals. The number of merozoite antigens that elicit an immune response, and their structural and functional diversity, has led to a large number of lead antigens being pursued as vaccine candidates. Despite being seemingly spoilt for choice in terms of vaccine candidates, there is still a lack of consensus on exactly how merozoite antibodies reduce parasitemia and malaria disease. In this review we describe the various immune mechanisms that can result from IgG opsonization of merozoites, and highlight recent developments that support a role for these functional antibodies in naturally acquired and vaccine-induced immunity.

1. Introduction

A large body of evidence demonstrates the critical role of antibodies in the control of apicomplexan parasites including *Toxoplasma gondii*, *Eimeria* spp. and *Babesia* spp. (Mineo et al., 1993; Wilkowsky et al., 2003; Fu et al., 2011; Frölich et al., 2012), as well as malaria parasites of the genus *Plasmodium* that are a major global contributor to childhood morbidity and mortality (reviewed in Cowman et al., (2016)). Despite efforts to reduce the global burden of malaria, approximately 212 million cases and 429,000 deaths continue to occur annually (World Health Organization, 2016). In addition, malaria control measures are under threat due to emerging resistance to our best antimalarial drugs (artemisinin combination therapies) and insecticides (pyrethroids) (Jambou et al., 2005; Dondorp et al., 2009, 2011; Trape et al., 2011), so there remains a pressing need for an effective malaria vaccine to sustain malaria control and to progress towards eradication. The rationale for developing a malaria vaccine is based upon the immunity that develops following repeated natural exposure to *Plasmodium* leading to reduced parasite densities and protection from disease. While vaccines may not need to function in a manner analogous to naturally acquired immunity, it is essential to fully understand the mechanisms of naturally acquired immunity to inform vaccine design that could underpin global malaria elimination campaigns. This review focuses on the immune mechanisms mediated through the Fc region of IgG against opsonized merozoites, and describes recent evidence that supports their role in the development of protective immunity against *Plasmodium falciparum* and as key assays to inform vaccine development.

1.1. Antibodies and immunity to blood-stage malaria

Since erythrocytes do not express major histocompatibility complex (MHC) class I or II that would ordinarily enable recognition of infected cells by innate-like and adaptive T cell responses,

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the human immune response against blood-stage *P. falciparum* infection is thought to be largely antibody-mediated. Evidence for the key role of antibodies in protecting against the disease causing blood-stage of malaria includes the adoptive transfer of immunoglobulin from hyper-immune African adults that, when transferred to children, protected against *P. falciparum* infection and reduced the risk of severe disease (Cohen et al., 1961; McGregor, 1964). Antibodies are thought to be an important factor in controlling disease in the earliest stages of a child's life as maternally transferred antibodies are thought to contribute to reduced levels of severe malaria infections in young infants (Snow et al., 1998; Kassim et al., 2000; Zhou et al., 2002), although definitive evidence supporting this conclusion from sero-epidemiological studies is currently lacking (reviewed in Dobbs and Dent, (2016)).

Antibodies directed against variant antigens on the surface of the parasitised erythrocyte have been associated with development of protective immunity and the role of these antibodies in protecting against malaria has been reviewed elsewhere (Chan et al., 2014). Vaccine development against these highly variant antigens, often part of large multi-gene families, is a challenging task, hence there is significant interest in merozoite antigens as alternative targets for vaccine design. Proteins present on the merozoite surface or released from intracellular microneme or rhoptry organelles during invasion are known to be accessible to antibodies (Beeson et al., 2016), and antibody titres to numerous *P. falciparum* merozoite antigens are correlated with immunity (Stanisic et al., 2009; Richards et al., 2013; Rono et al., 2013; Osier et al., 2014b). However, longitudinal cohorts have yielded inconsistent associations for several antigens (Fowkes et al., 2010), and the hunt for merozoite antigens definitively targeted by naturally acquired protective antibodies continues.

1.2. Quality over quantity: the importance of antibody functionality for malaria immunity

A clear-cut picture of the importance of individual merozoite antigens for the development of protective immunity is hampered by the lack of clear distinction between functional antibodies that contribute to protection and the large proportion of antibodies induced by natural malaria exposure that are of insufficient affinity or of an incorrect isotype to elicit a functional response in vivo. This issue is in part due to the common reliance on ELISA-based serology, an assay with high sensitivity that allows the detection of antibodies with a range of affinities but does not provide a functional readout. Further complicating ELISA-based readouts, recombinant proteins and synthetic peptides need the correct protein folding and conformation to ensure that antibody titres reflect those raised against the native antigen. In addition, ELISA-based serology does not distinguish the chemical modifications such as glycosylation that can alter the functional properties of antibodies (Vidarsson et al., 2014). Protein micro-arrays represent a high-throughput variation of classical serology and dramatically increase the capacity to identify sero-dominant antigens (Crompton et al., 2010a; Trieu et al., 2011), but the same concerns about recombinant protein conformation still exist. However, identifying antigens targeted by functional antibodies that confer protection from disease continues to prove the more challenging task.

The importance of measuring antibody functionality as a correlate of protection from malaria has gained significant attention in recent years (Crabb and Beeson, 2005; Beeson et al., 2008; Chan et al., 2012; Boyle et al., 2013; Sheehy et al., 2013; Ellis et al., 2014; Moormann and Stewart, 2014; Teo et al., 2016). Most functional readouts of malaria antibodies require the use of live parasites, creating a potential limitation on assay throughput and reproducibility. Growth inhibition through direct interference with target antigen function is the most widely tested mechanism of action for quantitating functional antibodies against the merozoite. Studies have demonstrated that purified human antibodies against specific merozoite antigens are capable of inhibiting parasite growth in vitro (Egan et al., 1999; Hodder et al., 2001; Reiling et al., 2012; Chiu et al., 2015). However, the in vitro growth inhibitory activity of human sera measured against total or specific antigens is often a poor correlate of protection from disease (Marsh et al., 1989; Corran et al., 2004; Perraut et al., 2005; McCallum et al., 2008; Wilson et al., 2011). Aside from directly interfering with the function of proteins involved in invasion, antibody binding to merozoites, termed opsonization, can initiate a range of effector functions mediated through the antibody fragment crystallizable (Fc) region. Of the four IgG subclasses, IgG1 and IgG3 can strongly promote binding of serum complement components and bind with high affinity to Fc Receptors (FcR) on phagocytic cells (Vidarsson et al., 2014), and thus are termed 'cytophilic' antibodies. In individuals naturally immune to malaria, the IgG response to merozoite antigens displays a strong bias towards cytophilic IgG1 and IgG3 (Bouharoun-Tayoun and Druilhe, 1992; Oeuvray et al., 2000; Stanisic et al., 2009), which strongly suggests that antibody functions mediated through the Fc region are important for malaria immunity. Naturally acquired antibodies to several *Plasmodium vivax* antigens also display a subclass bias to IgG1 and IgG3 (Cutts et al., 2014; França et al., 2016), which suggests that IgG opsonization and phagocytic effector mechanisms may contribute to *P. vivax* immunity, although this remains to be investigated.

The Fc domain of opsonizing antibodies are thought to direct immune responses against malaria merozoites through several different mechanisms including: (i) activation of the classical complement pathway resulting in lysis of opsonized merozoites, (ii) internalization of opsonized merozoites through phagocytosis, (iii) antibody-dependent cellular inhibition (ADCI) activity that involves an unknown soluble factor, and (iv) respiratory burst leading to merozoite destruction (Fig. 1). There have been several attempts to develop vaccines that would elicit opsonizing antibodies to engage these cellular responses against merozoites, including the merozoite surface protein (MSP) 3 long-synthetic peptide (MSP3-LSP) vaccine (Sirima et al., 2011), the MSP2-C1 vaccine (McCarthy et al., 2011), and the glutamine rich protein (GLURP)-MSP3 fusion vaccine, GMZ2 (Jepsen et al., 2013). However, evidence linking merozoite opsonization and protective immunity in longitudinal cohort studies that would support such vaccine strategies have only come to light in recent years (Joos et al., 2010; Hill et al., 2013; Boyle et al., 2015; Tiendrebeogo et al., 2015).

These advances are in large part due to the development of a technique to isolate viable merozoites (Boyle et al., 2010), as well as the incorporation of reproducible fluorometric methods to measure complement activation (Boyle et al., 2015), phagocytosis of merozoites (Hill et al., 2012, 2013; Osier et al., 2014a), respiratory burst (Joos et al., 2010), or ADCI (Jogdand et al., 2012). In turn, improved assays are leading to a better understanding of the importance of opsonizing antibodies, FcRs and phagocytes in the development of malaria immunity to blood-stage malaria infection. For the time being, these IgG-FcR-mediated immune responses are being studied in isolation, limiting our understanding of how different mechanisms may work together to confer protection. In the following sections, we describe the current state of knowledge on key immune mechanisms that are elicited by merozoite opsonization with IgG, and highlight where similarities exist between those.

2. Antibody-mediated activation of the classical complement pathway against merozoites

Following schizont rupture, merozoites can be exposed to the host bloodstream for several minutes before completion of ery-

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