SPECIAL ISSUE ARTICLE
EDITORIAL

Naturally acquired immunity to malaria

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(*First published online 8 January 2016*)

More than 100 years ago, the German microbiologist, Robert Koch, demonstrated that individuals living in endemic areas naturally acquire immunity to symptomatic malaria. Koch based his observations on microscopically detectable parasitaemia in children, adults and transmigrants in highly endemic areas of Papua New Guinea and Indonesia where people were exposed to hundreds of infectious bites each year. By observing higher rates of parasitaemia in exposed children and transmigrants than in exposed adults he concluded that immunity develops slowly after many years of exposure but that sterile protection is never achieved. This clinical immunity (i.e. immunity against the symptoms of malaria) was described as a reduction in parasite density and prevalence of disease with age (Ewers *et al*. 1972). The variable nature of malaria epidemiology in different endemic areas underlines the importance of natural exposure in development of immunity: from unstable transmission where immunity is not achieved, characterized by frequent clinical infections in all age groups, to endemic areas where the peak of infection prevalence occurs in increasingly older age groups as transmission declines (e.g. Okiro *et al*. 2009).

A lack of understanding of the mechanisms by which natural immunity to malaria is achieved and how it is maintained has long been proclaimed as a major hurdle to the development of a malaria vaccine. In the 1950s, seminal experiments by MacGregor and Cohen confirmed that antibodies were a key component of antimalarial immunity (Cohen *et al*. 1961). In the 1960s and 70s, the first irradiated sporozoite (whole parasite) vaccine trials were conducted leading to the identification of key malaria antigens such as circumsporozoite protein (CSP) now the basis of the most developed vaccine candidate RTS,S (Clyde *et al*. 1973; Enea *et al*. 1984). In the last half century, with the identification of many additional antigens, animal model and clinical vaccine trials of so-called 'subunit vaccine' approaches have shown varying success (Schwartz *et al*. 2012). These early studies were painstakingly slow and revealed limited information. More recently, immune targets and potential vaccine candidates have been characterized at the molecular level, and large well-designed epidemiological studies have identified antigen-specific immune responses associated with protection against malaria infection and disease. The field is now armed with a growing knowledge base, which raises hopes for new approaches for the development of a broadly effective malaria vaccine.

In Volume 143, Issue 2 of *Parasitology* we present nine reviews from leading experts in host responses to malaria infections. The reviews outline current knowledge and perspectives on many critical aspects of immunity and more recent insights, such as immunity to the lesser-studied *Plasmodium vivax* and the impact of declining transmission on natural immunity. Many of the reviews provide perspectives on how this knowledge is contributing to vaccine design, and one review in particular focuses on how we can capitalize on the 'omics' era to develop novel approaches to malaria vaccines. By no means exhaustive, the reviewed topics highlight the complexity of immunity to malaria and that even after 100 years we still have much to learn.

Infants and very young children are most at risk of clinical malaria and death and therefore stand to benefit the most from a malaria vaccine. Questions have been raised as to whether children in the first few years of life respond to malaria by distinct mechanisms to that of older individuals. In their review, Dobbs and Dent describe the different phases of immunity in children in the first year of life (Dobbs and Dent, 2015). As the authors explain, immunity in this age group is broken into two distinct phases: from birth to around 6 months of age, a period when they are still protected by maternal antibodies and behavioural practices may lead to them being less exposed to mosquito bites; and

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Parasitology (2016), **143**, 125–128.
doi:10.1017/S0031182015001778

from 6 months to 1 year when they have limited immunity and thus are highly susceptible to symptomatic and severe malaria. Recent findings have also suggested that babies born to mothers who experienced malaria during pregnancy have an increased risk of malaria, although this is difficult to disentangle from other variables such as transmission and use of interventions such as long lasting insecticide treated nets and effective antimalarial therapy. A summary of studies that have investigated associations of antibody responses to different antigens is provided with a suggestion that functional antibody studies would greatly benefit the progression of these 'subunit' vaccines down the development pipeline.

Over the last decade or so, the number of malaria cases have halved worldwide, resulting in a reinvigorated goal of eradication achieved via the gradual elimination of malaria from all malaria endemic countries around the world. However, because immunity is acquired through constant exposure to the parasite, with the decrease in transmission, there are increasing concerns about declining immunity in communities and a shift towards greater susceptibility to symptomatic disease. In their review covering this topic, Fowkes *et al*. (2015) report that, rebounds of malaria infections and shifts in cases to older individuals are occurring in areas that have successfully reduced transmission. They discuss the question of how much exposure is required to achieve immunity and how long immune responses last. While it is clear that many, perhaps hundreds of exposures are required to completely protect against clinical malaria, the increasing age at which individuals develop immunity with declining transmission is further evidence that the number of exposures is critical. In areas where transmission has recently declined, this exposure may also come via an increasing proportion of low-density infections in the area. However, the ability of individuals to mount appropriate responses also appears to depend on age. Furthermore, exposures to early infections are thought to induce relatively low affinity antibodies secreted by short lived plasma cells, while subsequent infections gradually increase the pools of antigen specific effector T cells and memory B cells, which are discussed in more detail below. However, most studies on cellular responses have been conducted in animal models while studies in malaria endemic populations have been limited. In order to better understand the impact of declining transmission to immunity to malaria, it will also be important to identify immune correlates of protection such as antibodies against specific antigens and epitopes.

Koch also described that exposed individuals from an area where only *Plasmodium malariae* was found were not protected against malaria when they moved to other endemic areas. Koch posited that this was a consequence of immunity being species-specific and that vaccines would need to target different species individually (Ewers *et al*. 1972). *Plasmodium vivax* is now recognized as a major contributor to the worldwide burden of malaria. Especially outside sub-Saharan Africa malaria interventions also need to target this parasite. Longley *et al*. (2015) provide an overview of immunity to *P. vivax*. As a result of the neglect of research into this parasite until recently, there is still a limited understanding of naturally acquired immunity to *P. vivax*, but epidemiological evidence suggests such immunity is acquired more quickly than to *Plasmodium falciparum*, and that such immunity is also generated in low-transmission regions. There is evidence demonstrating the capacity for successful immunological memory responses to *P. vivax*, particularly in terms of long-lived antibodies, but further cellular immunology studies are required to determine the underlying basis of this response. There is also mounting evidence of a strong anti-inflammatory response (largely interleukin (IL)-10) during *P. vivax* infections, but whether this is protective or damaging remains to be elucidated. Recent work using high-throughput protein arrays containing hundreds of *P. vivax* proteins have demonstrated that a large proportion of the *P. vivax* proteome is immunogenic, but further work is required to identify protective targets for vaccine development. Future work should focus on the use of well-designed immuno-epidemiological studies that span a greater geographical area, multiple transmission regions and age-levels and should carefully account for past cumulative exposure to *P. vivax*.

The development of naturally acquired immunity forms a strong rationale for the development of a malaria vaccine, which will expedite the long-term goal of global malaria eradication. Both whole parasite and 'subunit' approaches incorporating one or more individual parasite surface antigens have been tested. Malaria parasites have complex lifecycles, including multiple developmental forms expressing unique surface antigens. Work in the early 80s confirmed that antigens expressed on the surface of infected erythrocytes were major targets of this antibody-mediated immunity. However, parasites from individual infections were also shown to alter the antigenic properties of the infected cell to explain the ability of *P. falciparum* to evade host immunity and establish chronic infections. It was later suggested that extensive polymorphism in these antigens amongst different strains could explain the slow development of naturally acquired immunity to malaria. Immunity to these variant antigens on the red cell surface, predominantly composed of PfEMP1 but also other subsequently described antigen families has been extensively studied by many groups. Bull and Abdi (2015) provide a detailed account of the biology of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), the

https://doi.org/10.1017/S0031182015001778 Published online by Cambridge University Press

current state of knowledge regarding how immunity against these antigens protects against malaria and prospects for development of vaccines based on these molecules. PfEMP1 plays a key role in the pathogenesis of malaria by binding to host molecules, enabling them to sequester in the tissues and proliferate while avoid clearance by the spleen. The authors explain that despite extensive diversity, there may be structurally conserved epitopes that might serve as vaccine targets.

Another developmental form of malaria that has been in the spotlight in recent years is the gametocyte. The decision of the parasite to undergo gametocytogenesis enables transmission to the mosquito vector and subsequently, to other human hosts. Stone *et al.* (2015) provide a thorough overview of evidence for natural immune responses to both the developing gametocyte inside human erythrocytes and activated gametocyte forms within the mosquito midgut. These immune responses, thought to include both humoral and cellular responses, reduce transmission by acting directly on intraerythrocytic gametocytes. Epidemiological observations demonstrate that as for asexual parasites, the density and prevalence of gametocytes decreases with age. However more sensitive techniques have now demonstrated that gametocytes are produced by the majority of infected individuals and that even low density infections can be transmitted. An obvious approach to vaccination might typically target antigens specifically expressed on the surface of the infected cell. An alternative approach aims to block transmission by targeting gametocyte proteins that are expressed well before transmission occurs. Animal models have shown that immune factors taken together with the blood meal, target the active gametocyte within the mosquito. The review covers possible mechanisms for mosquito-stage transmission blocking immunity, how it is assessed and specific immune targets of this stage including Pfs25 and Pfs28, which are transcribed (*Pfs25* and *Pfs28* transcripts indicate gametocytes in the blood), but repressed until mosquito-stage development. Immune responses however are acquired to 'pre-fertilization' antigens expressed during the human stages, namely Pfs230 and Pfs48/45, following natural exposure. While gametocyte-based approaches would not protect the vaccinated individual if administered to many people, an effective transmission blocking vaccine could drive transmission down in the larger community.

Although it is accepted that antibody responses play an important role in protection, the cellular mechanisms underlying the slow and imperfect acquisition of immunity have only recently started to be delineated. In this Special Issue, Ryg-Cornejo *et al.* (2015) reviewed our current understanding on the development of humoral immunity to natural malaria infections and highlighted some of the outstanding questions in the field. Emerging evidence has revealed that malaria infections are associated with the onset of a population of atypical memory B cells. Originally described as exhausted memory B cells implicated in humoral deficiencies associated with HIV, a phenotypically similar memory B cell subset, expressing inhibitory receptors has been detected in malaria-exposed populations. Although the functional relevance of the expansion of atypical B cells in response to malaria is unclear, recent work suggests that they have compromised activity, including reduced B cell receptor signalling, impaired cytokine production and hypo-responsiveness to stimuli that classically induce memory B cells to secrete antibodies *in vitro*. On the other hand, epidemiological evidence suggests that variable transmission levels might affect the acquisition and maintenance of memory B cells, which lends weight to the notion that frequent clinical malaria episodes could have a detrimental effect in the generation of immunologic B cell memory. In support of this concept, recent findings in field studies have shown that acute *P. falciparum* malaria induces the activation of circulating Th1 cell-like Tfh cells with limited helper capacity. Furthermore, similar findings in mouse infection models revealed that inflammatory cytokines responsible for the development of severe malaria syndromes, impair efficient Tfh cell differentiation, which results in the accumulation of a population of Tfh cell precursor that co-expresses Th1-associated molecules.

While most studies on immunity to malaria have focused on antibody responses, relatively few studies have investigated cellular responses to malaria infection. In their review, Stanisic and Good (2015) cover the major discoveries with regards to cell-mediated immunity, which invokes both innate and adaptive immunity. *Plasmodium* parasites have been shown to activate innate immune cells, including dendritic cells, monocytes/macrophages, natural killer cells and γδ T cells, which results in the production of pro-inflammatory cytokines limiting parasite growth and promoting the induction of adaptive $CD4^+$ and $CD8^+$ T cell responses. Regulation of the inflammatory response requires the production of anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)-β, the balance and timing of which are critical for determining disease outcome. Many studies in humans have focused on trying to understand the dual role that immune cells may play in the immune response to the parasite – being responsible for both control of the parasite and induction of immunopathology via production of cytokines and chemokines. Unregulated inflammatory responses can also contribute to the pathology that is associated with malaria infection. However, experimental evidence suggests that *P. falciparum* exposure may induce immunoregulatory responses that dampen excessive inflammation. Whether these immunoregulatory cells would interfere with induction of robust effector immune responses to a

https://doi.org/10.1017/S0031182015001778 Published online by Cambridge University Press

malaria vaccine is unknown. Few studies have investigated mechanisms associated with the generation and persistence of T cell memory, although a generally held perception is that *Plasmodium*-specific immunity is short lived in the absence of re-exposure, which may explain the observed short lived/unstable nature of cellular immune responses in naturally exposed individuals

The next review in the series by Scholzen and Sauerwein (2015) provides an overview of historic and recent research conducted using controlled human malaria infections (CHMIs). CHMIs are a powerful tool to assess the efficacy of drugs and/or vaccine candidates, but also to study antimalarial immune responses at well-defined time points after infection. Infection with *Plasmodium* parasites was used in the 1920s–1960s as a tool to treat syphilis, prior to the availability of antibiotics. Retrospective analysis of those patients has provided the first evidence for the development of anti-parasite and anti-disease immunity (tolerance) after a single infection. CHMI have become popular again to test drug or vaccine efficacy. Studies have demonstrated increased levels of interferon (IFN) γ shortly after the onset of blood-stage infection and prior to development of symptoms with natural killer (NK) cells and γδT-cells implicated as the source. Malaria-naive volunteers can respond to CHMI in two different manners: (i) rapid pro-inflammatory cytokine production in association with more rapid parasite control but linked with more severe clinical symptoms, or (ii) early immunosuppressive TGFβ production associated with weaker parasite control but fewer clinical symptoms. It is clear from these studies that cellular immune responses play a critical role in determining the outcome of disease and development and maintenance of immunity.

The final review in the series by de Sousa and Doolan (2015) covers the emerging field of Immunomics, which is radically changing the way vaccine candidates are selected for further development. To date, most of the vaccine candidates have been based on a few selected parasite surface antigens that are abundant and thus, are the first antigens to be discovered, such as circumsporozoite protein (CSP), merozoite surface protein (MSP1) and apical membrane antigen 1 (AMA1). These antigens are immunodominant but a high degree of polymorphism has hampered their efficacy in vaccine trials. Eukaryotic pathogens such as *Plasmodium* are complex organisms with potentially thousands of immune targets. Immunomics combines genomics, transcriptomics, proteomics and bioinformatics to identify antigens and epitopes exposed to the immune system, thus identifying novel and potentially more effective vaccine candidates. To highlight its potential as a tool to identify new malaria vaccine approaches, the review covers the advances made using Immunomics for other pathogens such as HIV, tuberculosis and schistosomiasis.

Although there remains much to be learnt about naturally acquired immunity to malaria, this collection of reviews detail the knowledge that has been gained since it was first described, recent new discoveries and questions that remain to be answered. By combining molecular and computational tools with more traditionally used approaches such as CHMI and well-designed epidemiological studies, this area of research is exploring how immunity to malaria is acquired and maintained. Insights gained from these studies will undoubtedly identify novel vaccine and therapeutic approaches to help combat this important disease.

ACKNOWLEDGEMENTS

This work was made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS.

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