The Immunologic Complexity of Growing Up with Malaria—Is Scientific Understanding Coming of Age?

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In the current issue of *Clinical and Vaccine Immunology*, Mandala et al. report changes in lymphocyte populations in children with uncomplicated malaria, severe malarial anemia, and cerebral malaria compared to controls (W. L. Mandala et al., Clin Vaccine Immunol 23:95–103, 2016, http://dx.doi.org/10.1128/CVI.00564-15). This commentary discusses the importance of understanding both helpful and detrimental aspects of the antimalarial immune response that are critical to malaria vaccine development and considers how these responses may relate to antimalarial vaccine safety and efficacy.

Malaria infection stimulates the human immune system to generate both "good" immune responses that protect against disease manifestations and "bad" responses that are either misdirected or even pathogenic. The article by Mandala et al. (1) in the current issue of *Clinical and Vaccine Immunology* that discusses lymphocyte disturbances in different forms of malaria adds to our understanding of both immune overresponsiveness that presumably contributes to severe forms of malaria and the competent immune responses that result in semi-immunity accumulated over time. Teasing apart the seemingly complex immune hyperstimulation from a more efficient, directed immune response has potential public health implications for malaria prevention strategies. Understanding both the beneficial and deleterious aspects of malaria immunity is especially significant now that the RTS,S vaccine is being considered for deployment, with the WHO's Strategic Advisory Group of Experts on Immunization and Malaria Policy Advisory Committee recommending large pilot studies to determine how vaccine can best be delivered (2). These implementation studies will likely include assessments of immune correlates of protection that seek to confirm the recent association of anti-circumsporozoite protein antibody levels to protection (3) and perhaps will also evaluate additional potential vaccine-induced immune responses that are true correlates of protection. Elucidation of a true vaccine-induced correlate of protection would be a major advance for malaria vaccine development.

In 2013, malaria caused 198 million clinical illnesses and 584,000 deaths, with 80% in children <5 years of age living in sub-Saharan Africa (4). Children and malaria-naive adults are at higher risk for severe malaria manifestations, including severe malarial anemia and cerebral malaria. In a single transmission setting, children at a younger age are at an increased risk for severe malarial anemia compared to older children, who are at risk for cerebral malaria (5). When comparing different settings, as transmission intensity increases, the age of highest risk for severe malarial anemia and cerebral malaria decreases (5–7). The risk of malaria death is greatest in those with severe malaria, for whom a malaria vaccine would have the highest public health impact. An effective malaria vaccine would also work toward the recently established Sustainable Development Goal 3 to ensure healthy lives and promote wellbeing for all (8).

The high incidence of deaths from severe malaria in sub-Saharan Africa may be due to a combination of immune hyperstimulation and/or lack of an organized immune response to malaria infection (9). Different risk factors appear to play a role in the development of severe forms of malaria, including severe malarial anemia and cerebral malaria. For severe malarial anemia, risk factors include altered immune responses, poor nutritional status, impaired erythropoiesis, splenomegaly, and the presence of intestinal parasites (10, 11). For cerebral malaria, risk increases after presumed initial exposure to malaria and with HIV infection (12). As the immune response to malaria may contribute to either severe malarial anemia or cerebral malaria, it makes sense to study these distinct clinical entities separately as Mandala and colleagues (1) have done. To date, many studies assess immune responses in all forms of severe malaria in aggregate compared to community controls. Such studies may not elicit critical differences in the immune response that may contribute to distinct forms of severe malaria through different pathogenic mechanisms. If these differences in immune response are characterized for each form of severe malaria, this would advance understanding of the immunologic background against which each form develops.

Apart from the distinct clinical phenotype for each form of clinical malaria, little is understood about the pathogenic mechanisms that *Plasmodium* infections employ to cause disease. As the framework of contributions from different factors contributing to severe malaria forms is built, a more complete picture of each pathological pathway is established. Strategies for intervention and prevention can then be better informed and more carefully targeted. While some risk factors cannot be modified, such as age, others can potentially be exploited, such as a hyperimmune response potentially treated with immunomodulatory drugs to limit subsequent direct destructive and bystander effects. At the same time, deficiencies in an organized immune response against the malaria parasite can be assessed and supplemented using vaccines

to target these deficiencies. An interesting study published in the current issue of *Clinical and Vaccine Immunology* highlights the reduced functionality of antibodies in early childhood (31). Similar analyses of immune deficiencies would also illuminate potential immune correlates of protection that would preclude costly preliminary field trials of candidate malaria vaccines and significantly advance malaria vaccine development. Most of these interventions would be directed toward children in sub-Saharan Africa, the group at highest risk for severe malaria and death. At the same time, malaria-naive adults and persons with little malaria exposure may also benefit from interventions that target common pathways in disease pathogenesis.

The gaps in our understanding of background immunologic responses to clinical forms of malaria are essential to explore so that we can better characterize protective immunity and use vaccines to induce this protective immunity. Studies of the antimalarial immune response point toward a complex cascade of events involving antibody production, cytokine release, regulatory and T cell effector stimulation, and neutrophil and monocyte activation (13–16). Interplay of humoral and cell-mediated immune arms appears to be involved, but the lack of our ability to distinguish an effective immune response from an ineffective one has hampered efforts to establish a correlate of protection against malaria. The work of Dent et al. in this issue (31) describes the development of IgG antibody to preerythrocytic and blood-stage antigens over the first 24 to 36 months of life, while antibodies to variant surface antigens and functional antibody responses remain low. If immune mechanisms that lead to less efficient antibody responses and to malaria complications via induction of an ineffective antimalarial response are well characterized, these effects can be compared to an effective antimalarial response seen in malaria-experienced adults. The unwanted hyperstimulation of the immune system that contributes to malaria complications can become a potential target for treatment and possibly chemoprevention of complicated malaria. These ineffective mechanisms can be avoided during malaria vaccine design, so that deleterious effects of severe malarial anemia and cerebral malaria are not increased in vaccinees. At the same time, the effective antimalarial immune response seen in adults can be compared to the ineffective ones seen in severe malaria illnesses, so that vaccine candidates can be designed to emulate the effective antimalarial immune response.

Previous studies of lymphocyte populations in African children with clinical malaria have not routinely analyzed expanded lymphocyte subsets in different clinical forms of severe malaria as separate entities (17–21). When they do, these studies have not also accounted for age-related decreases in lymphocyte counts that have been documented to occur in Africans (22–24). The relatively small number of participants included in these studies, though understandable due to the infrequency of severe malaria, also makes comparisons between groups challenging. This limits interpretation and potentially masks important distinctions between clinical forms of severe malaria such as severe malarial anemia and cerebral malaria. The analysis by Mandala et al. (1) provides a welcome step toward redressing this imbalance and attaining a more holistic overview of antimalarial immunity. This study has overcome limitations common to these types of studies, including difficulties in collecting large quantities of blood from relatively large numbers of infants and small children over multiple visits, logistical and technical challenges of peripheral blood mononuclear cell (PBMC) collection and processing at field sites,

analysis limitations in developing countries with limited resources, and ethics committees that are reluctant to release samples for transport outside country borders despite a paucity of infrastructure and expertise for complex immunologic analyses in areas where malaria is endemic.

The study by Mandala et al. (1) builds on previous work by the same researchers that found decreasing lymphocyte counts with age in healthy Malawians (23). This appreciation and documentation of age-related changes in lymphocyte count in the same population allowed them to carefully plan the current study to explore the lymphocyte changes that occur in severe and uncomplicated malaria independently of age-related changes. Mandala et al. (1) used clearly defined case definitions to distinguish three different forms of clinical malaria with no overlap for a clean analysis of changes in lymphocytes. Due to the relatively high prevalence of pediatric HIV in the study population and the lymphocyte dysregulation that occurs with HIV infection, researchers screened all potential study participants and excluded those who tested positive. Their results confirmed what other researchers have documented—that children with severe malaria were generally older than children with severe malarial anemia and that panlymphopenia affecting all three main lymphocyte subsets and T cell subsets was experienced by children with both cerebral malaria and uncomplicated malaria but not by children with severe malarial anemia. Lymphocyte counts returned to normal during convalescence, and the authors speculated that this provides evidence that lymphopenia is transient and does not predispose patients to subsequent cerebral malaria.

Interestingly, the range of lymphocyte counts in children with severe malarial anemia during illness and convalescence was much broader than those in other groups, supporting the idea that severe malarial anemia is likely multifactorial, influenced by other factors in addition to immunologic dysregulation. The multifactorial nature of severe malarial anemia has been documented (10, 11), highlighting the need for a multidisciplinary approach for prevention, potentially including nutrition, intestinal parasite clearance, and basic malaria preventive measures. If children who are predisposed to develop severe malarial anemia can be identified through risk stratification analyses, they can be targeted for such interventions, and potentially malaria vaccination.

Most intriguingly, panlymphopenia was most exaggerated in cerebral malaria and not in severe malarial anemia. The authors explain that this may not be expected for cerebral malaria, where high levels of proinflammatory cytokines secreted by T cells are presumed to drive the pathophysiology of brain edema, but they postulate that this expanding population of lymphocytes may be sequestered. The finding of exaggerated lymphopenia in cerebral malaria patients has been demonstrated in other studies of African children (20). This theory is also consistent with an experimental model of cerebral malaria where increased numbers of lymphocytes accumulate in postcapillary venules and restrict venous blood flow and possibly increase intracranial pressure (25). The risk of brainstem herniation and death is therefore increased through this mechanism. Experimental models also show that, compared to hyperparasitemia, cerebral malaria is associated with enhanced leukocyte recruitment to postcapillary venules in the cortical microvasculature, which is consistent with the finding in the current study that leukopenia is enhanced compared to that in uncomplicated malaria (25). Sequestration of lymphocytes to postcapillary venules potentially explains the decrease in total

lymphocyte counts seen during acute disease and the rapid return to baseline during convalescence.

Mandala et al. (1) demonstrated lymphocyte activation as evidenced by CD69 expression in all three forms of clinical malaria and enhanced activation in cerebral malaria. At the same time, CD69 expression was unexpectedly high in innate NK and γδ T cells, which is contrary to the premise that cerebral malaria results from immune priming during previous exposure. The authors propose that this is explained by increased priming from adaptive T cells that trigger an increased response in innate T cells, leading to elevated cytokine levels demonstrated to occur in cerebral malaria. The authors further propose that the increased CD69 expression may interact with secondary lymphoid tissue to inhibit lymphocyte transit as an explanation for the decreased total lymphocytes seen in cerebral malaria and uncomplicated malaria, and yet, this is not entirely consistent with the enhanced CD69 expression and normal total lymphocyte population seen in severe malarial anemia. A potential explanation is the relative immaturity of CD69 receptors in secondary lymphoid tissue of younger children with severe malarial anemia.

The novel result that B cells do not return to normal levels during convalescence in cerebral malaria patients compared to other forms of clinical malaria caused the authors to postulate that patients with cerebral malaria are at increased risk of clinical malaria during their recovery period. This finding, if confirmed, would call for posttreatment chemoprophylaxis and/or other malaria prevention measures for the time period at risk, including consideration for targeted vaccination strategies if B cell abnormalities are documented to persist for long periods.

A vaccination strategy to implement RTS,S vaccination is being considered for African children pending results of implementation studies. Results of extended efficacy of RTS,S in the phase 3 study demonstrate overall efficacy against severe malaria in the 4-dose group (26), an encouraging sign that antimalarial vaccination can accomplish protection and be considered a preventive strategy in the near future. Next-generation antimalarial vaccines, including live-attenuated whole-organism sporozoite vaccines (27), may also become available in the next few years to provide enhanced protection against infection, including severe disease. At the same time, pilot trials of RTS,S and studies of other candidate malaria vaccines should include preplanned analyses of severe malaria risk stratified by clinical manifestation, including severe malarial anemia and cerebral malaria. This will ensure that the potential risk of increased severe malaria complications associated with immune priming from vaccination and subsequent hyperstimulation of the immune response is considered and that unintended sequelae are detected early in the vaccine development process.

As malaria vaccine development leaves its childhood behind, growing pains remain, but some lessons can be learned from other vaccines that survived a troubled adolescence—like the rotavirus vaccine. After the unanticipated effect of intussusception was documented in 15 children who received RotaShield, the CDC recommended suspension of its use in 1999 (28). Next-generation vaccines were not licensed for use until 2006 (29), and only after phase 3 clinical trials that enrolled over 63,000 infants documented no increased risk of this relatively rare complication (30). During the interim, 80% of U.S. children contracted rotavirus by age 5, and rotavirus accounted for over 200,000 emergency room visits and 50,000 hospitalizations in the United States annually

(29). Targeted deployment of a partially effective malaria vaccine and development of next-generation vaccines should not be delayed until we have fully elucidated countervailing helpful and harmful immune responses, but understanding these responses and how they relate to vaccine safety and efficacy should be an integral part of developing and improving malaria vaccines.

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