The Journal of Immunology

Young Lives Lost as B Cells Falter: What We Are Learning About Antibody Responses in Malaria

Silvia Portugal, Susan K. Pierce and Peter D. Crompton

J Immunol 2013; 190:3039-3046; ;
doi: 10.4049/jimmunol.1203067
http://www.jimmunol.org/content/190/7/3039

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Brief Reviews

THE JOURNAL OF IMMUNOLOGY

Young Lives Lost as B Cells Falter: What We Are Learning About Antibody Responses in Malaria

Silvia Portugal, Susan K. Pierce, and Peter D. Crompton

Plasmodium falciparum malaria remains a major public health threat for which there is no licensed vaccine. Abs play a key role in malaria immunity, but Ab-mediated protection is only acquired after years of repeated infections, leaving children in endemic areas vulnerable to severe malaria and death. Many _P. falciparum_ Ags are extraordinarily diverse and clonally variant, which likely contribute to the inefficient acquisition of protective Abs. However, mounting evidence suggests that there is more to the story and that infection-induced dysregulation of B cell function also plays a role. We herein review progress toward understanding the B cell biology of _P. falciparum_ infection, focusing on what has been learned from population-based studies in malaria-endemic areas. We suggest ways in which advances in immunology and genomics-based technology can further improve our understanding of the B cell response in malaria and perhaps illuminate new pathways to the development of effective vaccines. _The Journal of Immunology_, 2013, 190: 3039–3046.

Malaria is caused by mosquito-borne parasites of the genus *Plasmodium*. Of the five *Plasmodium* species that infect humans, *P. falciparum* is the deadliest, each year causing ~225 million cases of malaria and nearly one million deaths, with most being among African children and pregnant women (http://www.who.int/malaria/world_malaria_report_2011/en/) (1). Optimism that a first-generation malaria vaccine, RTS,S, may soon be licensed has been tempered by the interim results of an ongoing phase 3 clinical trial in Africa that indicated that the vaccine confers short-lived protection from malaria in only ~30% of infants (2). Clearly, the ongoing effort to develop a highly effective vaccine would benefit from a more detailed understanding of malaria immunity.

P. falciparum has a complex life cycle (Fig. 1) (3–22) in which only the blood stage of infection is associated with disease, typically an undifferentiated febrile illness that in a minority of cases progresses to severe disease and death (23). Epidemiologic studies in areas where individuals are repeatedly infected with *P. falciparum* show that immunity to severe, life-threatening malaria is usually acquired early in childhood, whereas immunity to uncomplicated febrile malaria is not reliably acquired until early adulthood (3), and once acquired appears to wane (24), at least partially (25), in the absence of ongoing *P. falciparum* exposure. Despite decades of exposure, sterile immunity to infection develops rarely if at all (3), as adults often carry blood-stage parasites without symptoms. In this review we focus our attention on naturally acquired immunity to the blood stage of *P. falciparum* infection where B cells are known to play a critical role in protection.

The central role of Abs in naturally acquired malaria immunity was shown by early experiments in which the transfer of purified IgG from malaria-immune West African adults to children with malaria in West Africa (8), East Africa (26), or Thailand (27) led to rapid and profound reductions in parasite numbers in the blood and resolution of fever. More than 50 y after understanding that Abs mediate malaria immunity, it remains a major challenge to determine which of the ~5400 predicted *P. falciparum* proteins (28) elicit protective Abs and the mechanisms by which these Abs protect (described in Fig. 1) (29). Indeed, without this information there are currently no unambiguous immune correlates of protection from malaria. It also remains incompletely understood why the acquisition of Ab-mediated immunity is so slow to develop (30, 31). The gradual acquisition of protective Abs has been proposed to be due in large part to the extensive genetic diversity of many *P. falciparum* Ags (17) as well as the parasite's ability to clonally vary the Ags it expresses on the surface of infected RBCs (iRBCs) (32). The reasoning goes that it may take years living in an endemic area for an individual to be exposed to a sufficient number of parasite clones to generate protective Abs. However, the data supporting this view are not ironclad. We herein review studies that point toward an alternative but non–mutually exclusive explanation for the gradual acquisition of Ab immunity to malaria; that is, that *P. falciparum* subverts B cell responses, making the acquisition of protective Abs inefficient, particularly as compared with responses to other pathogens in which long-lived Ab-mediated protection is acquired after a single or few exposures.

The Ab response to malaria

How clone-specific are Abs that protect against malaria? The proposal that the slow acquisition of protective Abs in individuals

Laboratory of Immunogenetics, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852

Received for publication December 14, 2012. Accepted for publication January 24, 2013.

S.P., S.K.P., and P.D.C. are supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Address correspondence and reprint requests to Peter D. Crompton, National Institute of Allergy and Infectious Diseases, 12441 Parklawn Drive, Twinbrook 2, Rockville, MD 20852. E-mail address: pcrompton@niaid.nih.gov

Abbreviations used in this article: iRBC, infected RBC; LLPC, long-lived plasma cell; MBC, memory B cell; OAS, original antigenic sin; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1203067

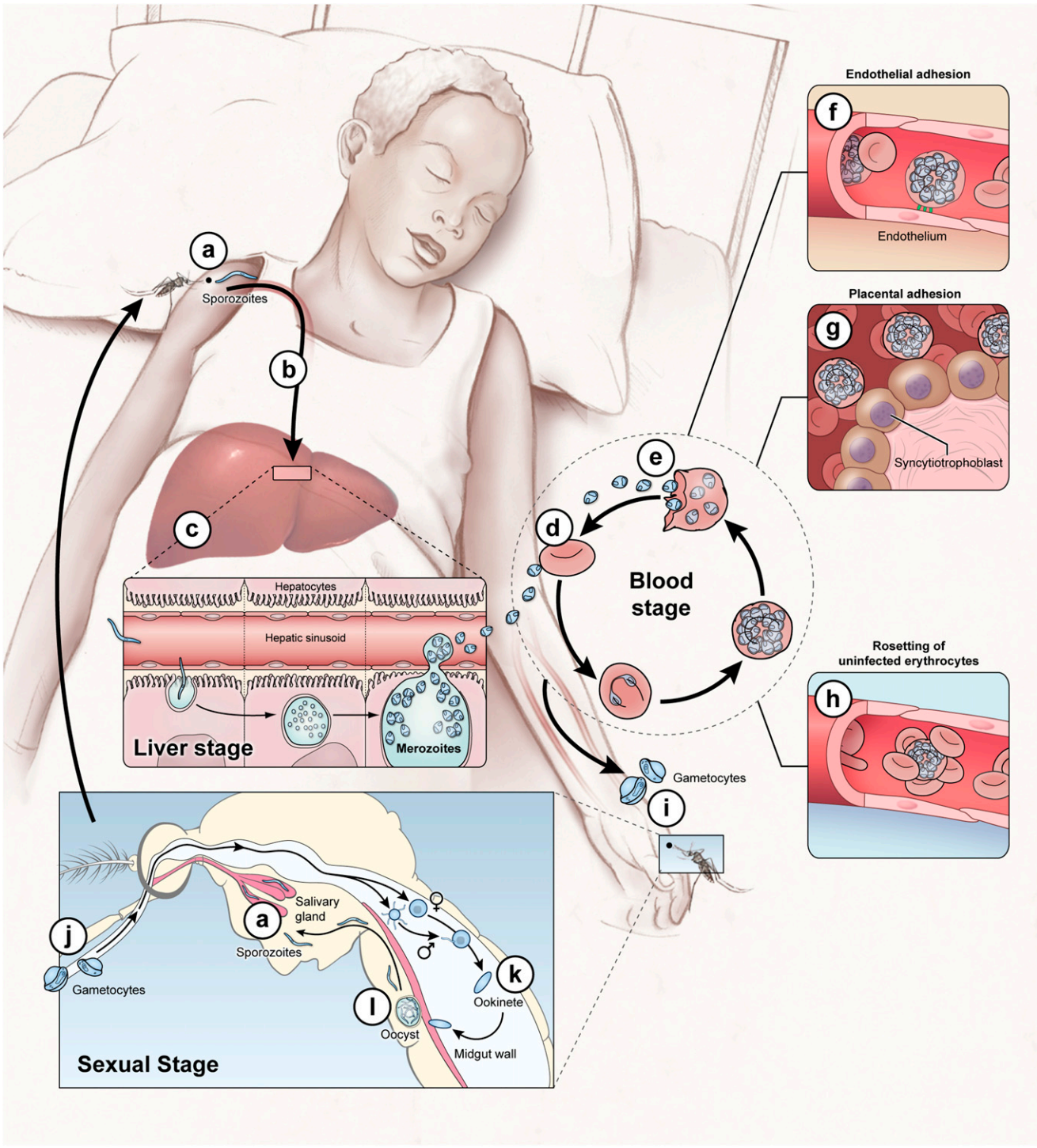

FIGURE 1. The *P. falciparum* life cycle: no shortage of Ab targets. The *P. falciparum* life cycle in humans includes the asymptomatic liver stage; the blood stage, which causes disease; and the sexual gametocyte blood stage, which infects mosquitoes that transmit the parasite. Infection begins when *Anopheles* mosquitoes inject sporozoites into the skin and blood (a) which migrate to the liver and invade a small number of hepatocytes (b). Each sporozoite gives rise to thousands of asexual parasites called merozoites (c). About 1 wk after hepatocyte invasion, merozoites exit the liver into the bloodstream and begin a 48-h cycle (d) of RBC invasion, replication, RBC rupture, and merozoite release (e). Once inside RBCs, the parasite exports variant surface Ags such as PfEMP1 to the RBC surface. Variant surface Ags mediate binding of iRBCs to the microvascular endothelium of various organs (f) and placental tissue (g), allowing parasites to avoid splenic clearance and promoting the inflammation and circulatory obstruction associated with clinical syndromes such as cerebral malaria (coma) and pregnancy-associated malaria. Variant surface Ag–mediated rosetting (binding of iRBCs to RBCs) may also contribute to disease (h). A small number of blood-stage parasites differentiate into sexual gametocytes (i), which are taken up by mosquitoes (j) where they differentiate into gametes that fuse to form a motile zygote, the ookinete (k), where meiosis occurs. The ookinete crosses the midgut wall and forms an oocyst (l) that develops into sporozoites that enter the mosquito salivary gland to complete the life cycle (a). Abs that sterilely protect by neutralizing sporozoites (a) and/or blocking hepatocyte invasion (b) are rarely if ever acquired through natural infection (3). Abs induced by the RTS,S vaccine target the circumsporozoite protein on the sporozoite surface (4) and correlate with sterile protection in malaria-naive adults (5); however, in African children, RTS,S generally protects against disease and not infection, and the correlation between Abs and protection is less clear (6, 7). Abs are a key component of naturally acquired blood-stage immunity (8), but the Ag targets and mechanisms of protection are incompletely understood and likely multifaceted. Abs may contribute to protection by clearing merozoites (9) and iRBCs (e) through opsonization (10) or complement-mediated lysis (11), by (*Figure legend continues*)

living in malaria-endemic areas is due to the time required to be exposed to a large number of *P. falciparum* clones predicts that Abs to *P. falciparum* variant Ags should be highly clone specific. A major target of protective Abs in malaria appears to be *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the surface of iRBCs (33). PfEMP1s are encoded by the highly polymorphic *var* multigene family (~60 genes/genome), and their expression is clonally variant (32). PfEMP1s mediate adhesion of iRBCs to microvascular endothelium, allowing the parasite to sequester in capillaries of various organs and avoid destruction in the spleen (Fig. 1) (23). Additionally, sequestration of iRBCs in organs such as the brain and placenta has been linked to the pathogenesis of clinical syndromes such as cerebral malaria and pregnancy-associated malaria (Fig. 1) (34). Several studies support the hypothesis that repeated infections are required to elicit a protective repertoire of PfEMP1-specific Abs (33, 35–40). However, other studies suggest that there is a limited subset of common PfEMP1s Ags that are important targets of immunity (41–44) or that protective Abs target cross-reactive or conserved epitopes on PfEMP1s (45–50). The observation that purified IgG from immune West African adults was therapeutic in children not only in West Africa but in East Africa (26) and Thailand as well (27) suggests a significant degree of Ab cross-reactivity to genetically diverse *P. falciparum* parasites (51). Taken together these observations raise the possibility that the gradual acquisition of protective Abs may not simply be a reflection of the time required for an individual to be exposed to all the parasite clones circulating in a given endemic area.

How long-lived is the Ab response to P. falciparum*?* It is well established that long-lived protective Ab responses, which are induced by many pathogens and vaccines after one or a few exposures (52), depend on the generation of high-affinity memory B cells (MBCs) and long-lived plasma cells (LLPCs) (53–55). LLPCs reside in the bone marrow where they constitutively secrete Abs and provide a critical first line of defense against reinfection, whereas MBCs mediate recall Ab responses after re-exposure to their Ag by rapidly proliferating and differentiating into plasma cells.

Although there is significant heterogeneity in the magnitude, quality, and longevity of Ab responses following infection or vaccination, in general, Ab responses are long-lived. For instance, estimates of half-lives of IgG responses range from 11 y for tetanus vaccines to >300 y for measles vaccines (52, 55). In sharp contrast, the half-lives of *P. falciparum*–specific Ab responses generally appear to be much shorter, particularly in young children. For example, in a 12-wk study of Kenyan children following acute malaria, the half-lives of IgG responses specific for five merozoite surface Ags (56) were estimated to be 9.8 d for IgG1 and 6.1 d for IgG3. In additional longitudinal studies, *P. falciparum*–specific Ab responses have been reported to decline to undetectable or nearly undetectable levels within 3–9 mo of documented malaria episodes in children (57–65). It is of interest that Ab responses to conserved (or semiconserved) *P. falciparum* Ags (59) or conserved regions of polymorphic Ags (62), to which individuals presumably were repeatedly exposed, were similarly short-lived. Taken together, these studies in children indicate that *P. falciparum* infection does not reliably induce a stable pool of LLPCs. Short-lived *P. falciparum*–specific IgG responses have also been observed in older children and adults (59, 62, 66). In contrast, there are studies that point toward more stable IgG responses with increasing age in areas of stable *P. falciparum* transmission (57, 61, 63, 67, 68); however, it is important to note that in such areas it is difficult to separate the effects of age and cumulative *P. falciparum* exposure on malaria immunity. Data from transmigrant studies in Indonesia suggest that adults may acquire clinical immunity to malaria more rapidly than do children (69, 70), but this bears further investigation as the risk of clinical malaria was similar among previously unexposed children and adults during a malaria epidemic in Madagascar (25).

How might these differences in the estimates of the duration of Ab responses be reconciled? It is possible that studies that reported short-lived *P. falciparum*–specific Ab responses were biased by the small number of Ags examined. Indeed, the studies cited above measured IgG responses specific for one or a few Ags, often blood-stage merozoite surface Ags being developed as vaccine candidates (15) that together represent <0.5% of the predicted *P. falciparum* proteome (28). However, a recent study that employed protein microarray technology (71) to assess the IgG response to ~25% of the *P. falciparum* proteome, including proteins expressed at all stages of the parasite life cycle, showed that children exposed to intense seasonal *P. falciparum* transmission generate IgG responses to hundreds of *P. falciparum* Ags each transmission season. However, these declined rapidly during the 6-mo dry season, a period of little to no *P. falciparum* transmission (Fig. 2). These data suggest a preferential induction of short-lived plasma cells during *P. falciparum* infection that is not restricted to a subset of intrinsically less immunogenic proteins.

Even though Ab responses to *P. falciparum* Ags in children are short-lived, ultimately by adulthood stable Ab levels are acquired. The study cited above showed that with each year of *P. falciparum* exposure, the level of IgG persisting through the dry season gradually increased until young adulthood when IgG levels were maintained at high levels (Fig. 2). One simple explanation for the gradual buildup of *P. falciparum*–specific IgGs with age is the incremental accumulation of LLPCs with each year of exposure. How important for protection are long-lived Ab responses? An age-adjusted analysis in the same study showed that the overall level of *P. falciparum*–specific IgG present before but not after the malaria season predicted protection from malaria (71).

inhibiting merozoite invasion of RBCs (d) (12), and/or by blocking adhesion of iRBCs to vascular endothelium (f) (13). Nonneutralizing Abs may contribute to protection through Ab-dependent monocyte- (14) or NK cell–mediated cytotoxicity. Blood-stage vaccines have focused primarily on generating Abs to merozoite proteins but with limited success (15, 16), probably due to Ag polymorphism (16, 17) and redundant RBC invasion pathways (18). Of note, the conserved merozoite protein PfRh5 appears to be essential for RBC invasion (19) and may be susceptible to vaccine-inducible cross-strain neutralizing Ab (20). Protection from pregnancy-associated malaria correlates with IgG specific for VAR2CSA, a conserved protein that mediates adherence to placental tissue (g) (21). Vaccine-induced Abs targeting Ags on gametocytes and gametes that are ingested with the blood meal (i–l) could prevent parasite development in the mosquito and block or reduce transmission (22).

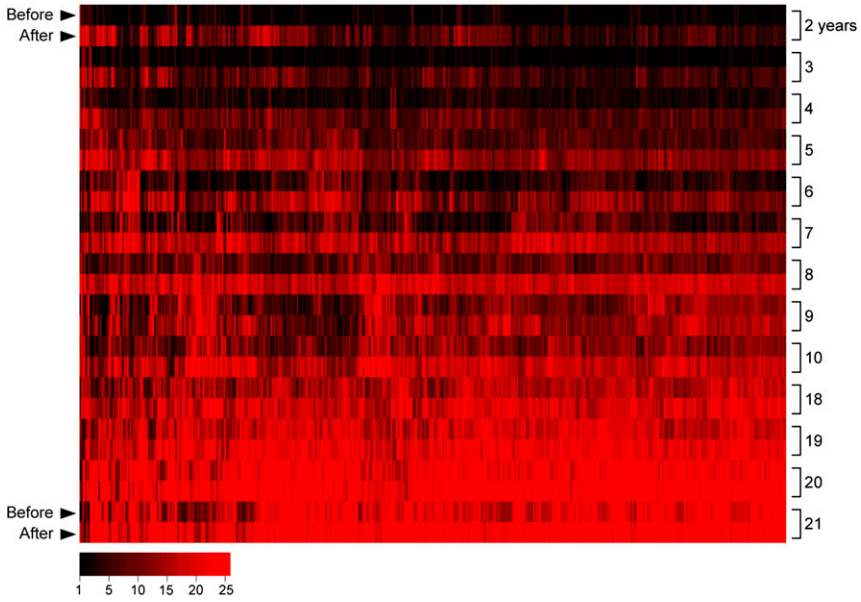

FIGURE 2. The *P. falciparum*-specific IgG response is broad but short-lived in children. Shown is IgG reactivity (red) specific for 491 *P. falciparum* proteins (columns) in plasma collected from children (n = 157) and adults (n = 37) before and after an intense 6-mo malaria season (see Ref. 71 for detailed methods and results). The average IgG level for each protein is stratified by 1-y age groups from 2 to 21 y, and within each age group from before to after the malaria season (rows). A broad *P. falciparum*–specific IgG response is present after the 6-mo malaria season in children as young as 2 y; however, the IgG response in young children is short-lived based on IgG levels at the end of the 6-mo dry season, a period of little to no *P. falciparum* transmission. With increasing age, IgG levels at the end of the dry season gradually increase until plateauing in adulthood.

Taken together, these data suggest that the gradual acquisition of clinical immunity to malaria may reflect the need for repeated infections to "fill" the *P. falciparum*–specific LLPC compartment to the point where steady-state Ab levels exceed a protective threshold. Conversely, MBC-mediated Ab boosting in response to acute infection, a process that generally peaks 6–10 d after Ag re-exposure, may not be rapid enough to prevent the onset of symptoms, which can occur as early as 3 d after blood-stage infection begins (72). Similar models have been postulated for other pathogens with short incubation periods such as *Haemophilus influenzae* type b (73), in which the infection progresses to a symptomatic threshold more rapidly than MBC-mediated Ab boosting, such that protection depends on already circulating Abs. In this light, it is likely that an effective blood-stage malaria vaccine will need to establish a critical level of circulating IgG produced by a stable pool of LLPCs, rather than depend on MBC-mediated Ab boosting. This may be especially important in areas of sporadic *P. falciparum* transmission where boosting of Ab responses does not occur through regular natural exposure. The same principle likely applies to vaccines targeting sporozoites that are in the bloodstream and exposed to Abs only transiently before invading hepatocytes.

The continued use of high-throughput Ab profiling methods (74) in various epidemiological settings in conjunction with systems biology approaches (75) to describe the contribution of T cells and innate immunity will improve our understanding of the factors that influence the specificity, longevity, and quality of Ab responses to malaria. A more detailed understanding of the mechanisms that ultimately confer protection against malaria and the reasons for their inefficient acquisition could help advance malaria vaccine development.

The MBC response to malaria

It was initially speculated that *P. falciparum* infection might not generate MBCs based on anecdotal evidence of waning malaria immunity in the absence of *P. falciparum* exposure, as well as inconsistent Ab boosting upon reinfection (56), even to conserved Ags (59). However, several recent studies have now shown that *P. falciparum*–specific MBCs can indeed be generated in response to natural infection, albeit inefficiently. For example, a longitudinal study in Mali, where there is an annual 6-mo malaria season, found that the frequency of MBCs specific for two blood-stage Ags, AMA-1 and MSP-1, increased incrementally with each malaria season, from childhood to adulthood (60). Moreover, MBCs once acquired appear to be long-lived. For example, a recent study in Kenya reported that *P. falciparum*–specific MBCs were found to persist longer than Abs in the absence of ongoing *P. falciparum* exposure (76). However, the Mali study showed that despite exposure to ~60 infective mosquito bites per month at the peak of the malaria season, only ~50% of adults had detectable *P. falciparum*–specific MBCs (60). A low prevalence (~30–50%) of *P. falciparum*–specific MBCs has also been reported in adults in studies conducted in The Gambia (77), Kenya (78), and Thailand (68), even in individuals with documented *Plasmodium* infection within 6 y (68). The findings for *P. falciparum*–induced MBCs are in sharp contrast to studies that show, for example, that smallpox vaccine–specific MBCs are generated and persist in nearly all vaccinees for ≥50 y in the absence of Ag re-exposure (79). Thus, it appears that *P. falciparum* infection can generate long-lived MBCs but less efficiently, at least compared with vaccines for other pathogens. Although it might be predicted that the prevalence and frequencies of *P. falciparum*–specific MBCs would be higher in areas of intense versus sporadic *P. falciparum* transmission, studies to date suggest that this is not the case (60, 68, 77, 80), raising the possibility that chronic or persistent *P. falciparum* exposure may be detrimental to MBC generation.

What might underlie the inefficient acquisition of P. falciparum*–specific LLPCs and MBCs?* Several mechanisms have been proposed to explain the relatively inefficient acquisition of *P. falciparum*–specific LLPCs and MBCs (Table I) (81–91). Because of limited space we comment on only a few possibilities for which there are recent data.

The relative inefficiency in acquiring *P. falciparum*–specific LLPCs and MBCs may be related to dysregulated B cell Ag-

driven differentiation, particularly in the setting of chronic *P. falciparum* exposure. Alterations in B cell function are well described in the context of chronic infections such as visceral leishmaniasis (92), HIV (93), and hepatitis C virus (94). For example, HIV (95) and hepatitis C virus (96) viremia are associated with an expansion of a subset of B cells identified by the cell surface markers $CD19^+CD20^+CD21^-CD27^-CD10^-$ and Fc receptor–like-4^+ (97). In HIV-infected individuals, Moir et al. (95) showed that these cells were hyporesponsive and suggested that these hyporesponsive, or "exhausted" MBCs contribute to the humoral deficiencies observed in HIV-infected individuals.

Remarkably, an increase in a phenotypically similar subset of B cells has been observed in *P. falciparum*–exposed children and adults (84). In the context of malaria, this B cell subset has been referred to as "atypical" rather than exhausted because the function of these B cells and whether they are beneficial or detrimental in malaria remain unclear. An expansion of atypical MBCs has also been observed in malaria-endemic Peru (98), The Gambia (77), Mali (99), Kenya (100), and Gabon (101), suggesting that *P. falciparum*–associated atypical MBC expansion is generalizable to genetically and geographically disparate populations, and that the degree of atypical MBC expansion correlates with *P. falciparum* transmission intensity (98). A direct causal relationship between *P. falciparum* infection and atypical MBC expansion remains to be established; however, differential expansion of atypical MBCs has recently been observed in age-matched children living under similar conditions in rural Kenya with the exception of *P. falciparum* exposure (100), suggesting that *P. falciparum* infection per se may drive atypical MBC expansion. Intriguingly, recent work by Muellenbeck et al. (101) showed at the single-cell level that *P. falciparum* exposure is associated with the acquisition of atypical MBCs that produce broadly neutralizing Abs specific for *P. falciparum* blood-stage Ags, and furthermore that atypical MBCs, unlike classical MBCs, show signs of active Ab secretion, although spontaneous Ab secretion was not noted in other studies (84, 95). Compared to classical MBCs, atypical MBCs also differed in their IgG gene repertoire, suggesting that they develop from different precursors (101).

P. falciparum infections could also dysregulate helper T cell responses. Interestingly, a recent study showed that PD-1, a marker of T cell exhaustion, is upregulated on $CD4^+$ T cells in children following *P. falciparum* infection (86). Although the functional significance of this observation in human remains to be determined, Butler et al. (86) reported that *Plasmodium yoelii* infection in mice induces phenotypic and functional $CD4^+$ T cell exhaustion, and that in vivo blockade of the PD-1 ligand PD-L1 and the inhibitory receptor LAG-3 restored $CD4^+$ T cell function, amplified the number of T follicular helper and germinal center B cells and plasmablasts, increased Ab levels, and accelerated the clearance of blood-stage parasites. To understand the functional link between putative *P. falciparum*–specific $CD4^+$ T cell exhaustion and Ab responses in humans will require tools that allow for the detection of *P. falciparum*–specific T cells, including MHC tetramer technology that has proved to be effective in identifying pathogen-specific T cells by flow cytometry.

Table I. Potential factors contributing to the inefficient acquisition of *P. falciparum*-specific protective Abs

Factor	Reference
Clonal Ag variation	(32)
Ag genetic diversity	(17)
Germinal center disruption	(81)
Lack of Ab avidity maturation	(82)
Marginal zone B cell induction	(83)
B cell exhaustion	(84)
Increased immature transitional B cells	(85)
$CD4^+$ T cell exhaustion	(86)
Polyclonal B cell activation	(87, 88)
BAFF/BAFF-R dysregulation	(89)
TLR tolerance	(90)
Self-reactive Abs/anergy	(91)
Clonal imprinting/original antigenic sin (OAS)	(63)

Whether malaria-associated B cell dysregulation is driven by direct interactions between *P. falciparum* products and B cells or indirectly through systemic immune activation is unclear. To our knowledge the only published example of a direct *P. falciparum*/B cell interaction comes from in vitro studies that implicate the cysteine-rich interdomain regions 1α of PfEMP1 as a T cell–independent polyclonal B cell activator and Ig binding protein (87). The discovery of direct molecular interactions between *P. falciparum* and the human immune system has been accelerated by systematic protein interaction screening approaches (102). For example, a yeast two-hybrid approach identified an interaction between *P. falciparum* merozoite surface protein 1 and the proinflammatory protein S100P (103), and an avidity-based extracellular interaction screen identified basigin (CD147) as a host receptor essential for RBC invasion via the merozoite protein PfRh5 (19). Intriguingly, basigin is also expressed on B and T cells, as well as dendritic cells, monocytes, and macrophages, raising the possibility that basigin/PfRh5 interactions may modulate host responses during *P. falciparum* infection.

It is also possible that systemic mediators of B cell differentiation and survival (53), such as BAFF, APRIL, IL-4, and IL-21, are modulated during *P. falciparum* infection. A recent study of Kenyan children (89) reported that acute malaria is associated with elevated plasma levels of BAFF and transient downregulation of BAFF receptor expression on B cells. Interestingly, however, higher levels of BAFF-R expression on B cells were associated with more durable *P. falciparum*–specific IgG responses, suggesting that dysregulated BAFF-R expression may contribute to inefficient *P. falciparum*–specific LLPC responses. It has also been suggested (56) that binding of iRBCs to bone marrow stromal cells (104) may disrupt PC survival signals in bone marrow niches.

Chronic exposure to pathogen-associated molecular patterns could possibly result in tolerance of pattern recognition receptors expressed on B cells and dendritic cells, which play a critical role in enhancing B cell responses (105, 106). Although few *P. falciparum*–derived pathogen-associated molecular patterns have been identified to date (107–110), recent clinical trials demonstrate that the TLR9 agonist CpG markedly enhances the IgG and MBC response to *P. falciparum* blood-stage vaccine candidates in malaria-naive adults (111), but not in adults chronically exposed to *P. falciparum* in endemic areas (90). Further studies are needed to define how *P. falciparum* exposure modulates the function of pattern recognition receptors and how this might influence homologous and heterologous LLPC and MBC responses.

It is unclear whether Ab responses in the course of repeated *P. falciparum* infections are predominantly derived from newly recruited naive B cells or cross-reactive MBC clones generated during prior infections (63) (i.e., clonal imprinting or OAS). It useful to consider the case of influenza, which similar to *P. falciparum*, repeatedly infects much of the world's population and exhibits extensive strain diversity. Work by Wrammert et al. (112) suggests that plasmablasts producing broadly neutralizing Abs induced by pandemic H1N1 influenza are predominantly derived from activated MBCs specific for epitopes conserved in several influenza strains, consistent with OAS. Similar analyses of variable gene sequences from plasmablasts induced by human experimental (72) and natural *P. falciparum* infection, particularly serial infections with genetically distinct *P. falciparum* parasites, could define the degree to which OAS operates in malaria and how it relates to malaria susceptibility.

Conclusions

Although there appears to be more questions than answers as to how malaria immunity is acquired, it is nonetheless an exciting time in malaria research. Malaria is one of the few infectious diseases in which relatively small longitudinal cohort studies of natural infection are feasible. For example, in areas of intense seasonal *P. falciparum* transmission, where nearly all individuals are predictably exposed to *P. falciparum* each year, it is possible to analyze the human immune response before, during, and after *P. falciparum* infection and to prospectively study immune correlates of protection as well as the immunomodulatory effects of infection. Malaria is also one of the only infectious diseases for which experimental human infections are approved. These unique study populations coupled with the powerful tools made available through extraordinary advances in immunology and systems biology ought to provide a wealth of information on the impact of *P. falciparum* infections on the human immune system and on the acquisition of malaria immunity.

Disclosures

The authors have no financial conflicts of interest.

References

1. Murray, C. J., L. C. Rosenfeld, S. S. Lim, K. G. Andrews, K. J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano, and A. D. Lopez. 2012. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* 379: 413–431.
2. The RTS,S Clinical Trials Partnership. 2012. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N. Engl. J. Med.* 367: 2284–2295.
3. Marsh, K., and S. Kinyanjui. 2006. Immune effector mechanisms in malaria. *Parasite Immunol.* 28: 51–60.
4. Regules, J. A., J. F. Cummings, and C. F. Ockenhouse. 2011. The RTS,S vaccine candidate for malaria. *Expert Rev. Vaccines* 10: 589–599.
5. Kester, K. E., J. F. Cummings, O. Ofori-Anyinam, C. F. Ockenhouse, U. Krzych, P. Moris, R. Schwenk, R. A. Nielsen, Z. Debebe, E. Pinelis, et al; RTS,S Vaccine Evaluation Group. 2009. Randomized, double-blind, phase 2a trial of *falciparum* malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. *J. Infect. Dis.* 200: 337–346.
6. Alonso, P. L., J. Sacarlal, J. J. Aponte, A. Leach, E. Macete, J. Milman, I. Mandomando, B. Spiessens, C. Guinovart, M. Espasa, et al. 2004. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. *Lancet* 364: 1411–1420.
7. Bejon, P., J. Lusingu, A. Olotu, A. Leach, M. Lievens, J. Vekemans, S. Mshamu, T. Lang, J. Gould, M. C. Dubois, et al. 2008. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. *N. Engl. J. Med.* 359: 2521–2532.
8. Cohen, S., I. A. McGREGOR, and S. Carrington. 1961. Gamma-globulin and acquired immunity to human malaria. *Nature* 192: 733–737.
9. Fowkes, F. J., J. S. Richards, J. A. Simpson, and J. G. Beeson. 2010. The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: a systematic review and meta-analysis. *PLoS Med.* 7: e1000218.
10. Druilhe, P., and S. Khusmith. 1987. Epidemiological correlation between levels of antibodies promoting merozoite phagocytosis of *Plasmodium falciparum* and malaria-immune status. *Infect. Immun.* 55: 888–891.
11. Stanley, H. A., J. T. Mayes, N. R. Cooper, and R. T. Reese. 1984. Complement activation by the surface of *Plasmodium falciparum* infected erythrocytes. *Mol. Immunol.* 21: 145–150.
12. Crompton, P. D., K. Miura, B. Traore, K. Kayentao, A. Ongoiba, G. Weiss, S. Doumbo, D. Doumtabe, Y. Kone, C. Y. Huang, et al. 2010. In vitro growth inhibitory activity and malaria risk in a cohort study in Mali. *Infect. Immun.* 78: 737–745.
13. Claessens, A., Y. Adams, A. Ghumra, G. Lindergard, C. C. Buchan, C. Andisi, P. C. Bull, S. Mok, A. P. Gupta, C. W. Wang, et al. 2012. A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells. *Proc. Natl. Acad. Sci. USA* 109: E1772–E1781.
14. Bouharoun-Tayoun, H., P. Attanath, A. Sabchareon, T. Chongsuphajaisiddhi, and P. Druilhe. 1990. Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *J. Exp. Med.* 172: 1633–1641.
15. Crompton, P. D., S. K. Pierce, and L. H. Miller. 2010. Advances and challenges in malaria vaccine development. *J. Clin. Invest.* 120: 4168–4178.
16. Thera, M. A., O. K. Doumbo, D. Coulibaly, M. B. Laurens, A. Ouattara, A. K. Kone, A. B. Guindo, K. Traore, I. Traore, B. Kouriba, et al. 2011. A field trial to assess a blood-stage malaria vaccine. *N. Engl. J. Med.* 365: 1004–1013.
17. Takala, S. L., and C. V. Plowe. 2009. Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming "vaccine resistant malaria". *Parasite Immunol.* 31: 560–573.
18. Cowman, A. F., and B. S. Crabb. 2006. Invasion of red blood cells by malaria parasites. *Cell* 124: 755–766.
19. Crosnier, C., L. Y. Bustamante, S. J. Bartholdson, A. K. Bei, M. Theron, M. Uchikawa, S. Mboup, O. Ndir, D. P. Kwiatkowski, M. T. Duraisingh, et al. 2011. Basigin is a receptor essential for erythrocyte invasion by *Plasmodium falciparum. Nature* 480: 534–537.
20. Douglas, A. D., A. R. Williams, J. J. Illingworth, G. Kamuyu, S. Biswas, A. L. Goodman, D. H. Wyllie, C. Crosnier, K. Miura, G. J. Wright, et al. 2011. The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat Commun* 2: 601.
21. Rogerson, S. J., L. Hviid, P. E. Duffy, R. F. Leke, and D. W. Taylor. 2007. Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect. Dis.* 7: 105–117.
22. Carter, R. 2001. Transmission blocking malaria vaccines. *Vaccine* 19: 2309–2314.
23. Miller, L. H., D. I. Baruch, K. Marsh, and O. K. Doumbo. 2002. The pathogenic basis of malaria. *Nature* 415: 673–679.
24. Jelinek, T., C. Schulte, R. Behrens, M. P. Grobusch, J. P. Coulaud, Z. Bisoffi, A. Matteelli, J. Clerinx, M. Corachán, S. Puente, et al. 2002. Imported *Falciparum malaria* in Europe: sentinel surveillance data from the European network on surveillance of imported infectious diseases. *Clin. Infect. Dis.* 34: 572–576.
25. Deloron, P., and C. Chougnet. 1992. Is immunity to malaria really short-lived? *Parasitol. Today* 8: 375–378.
26. McGregor, I. A., S. P. Carrington, and S. Cohen. 1963. Treatment of East African *P. falciparum* malaria with West African human γ-globulin. *Trans. R. Soc. Trop. Med. Hyg.* 57: 170–175.
27. Sabchareon, A., T. Burnouf, D. Ouattara, P. Attanath, H. Bouharoun-Tayoun, P. Chantavanich, C. Foucault, T. Chongsuphajaisiddhi, and P. Druilhe. 1991. Parasitologic and clinical human response to immunoglobulin administration in *falciparum* malaria. *Am. J. Trop. Med. Hyg.* 45: 297–308.
28. Gardner, M. J., N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, et al. 2002. Genome sequence of the human malaria parasite *Plasmodium falciparum. Nature* 419: 498–511.
29. Beeson, J. G., F. H. Osier, and C. R. Engwerda. 2008. Recent insights into humoral and cellular immune responses against malaria. *Trends Parasitol.* 24: 578–584.
30. Achtman, A. H., P. C. Bull, R. Stephens, and J. Langhorne. 2005. Longevity of the immune response and memory to blood-stage malaria infection. *Curr. Top. Microbiol. Immunol.* 297: 71–102.
31. Struik, S. S., and E. M. Riley. 2004. Does malaria suffer from lack of memory? *Immunol. Rev.* 201: 268–290.
32. Scherf, A., J. J. Lopez-Rubio, and L. Riviere. 2008. Antigenic variation in *Plasmodium falciparum. Annu. Rev. Microbiol.* 62: 445–470.
33. Chan, J. A., K. B. Howell, L. Reiling, R. Ataide, C. L. Mackintosh, F. J. Fowkes, M. Petter, J. M. Chesson, C. Langer, G. M. Warimwe, et al. 2012. Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. *J. Clin. Invest.* 122: 3227–3238.
34. Beeson, J. G., and G. V. Brown. 2002. Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. *Cell. Mol. Life Sci.* 59: 258–271.
35. Cham, G. K., L. Turner, J. D. Kurtis, T. Mutabingwa, M. Fried, A. T. Jensen, T. Lavstsen, L. Hviid, P. E. Duffy, and T. G. Theander. 2010. Hierarchical, domain type-specific acquisition of antibodies to *Plasmodium falciparum* erythrocyte membrane protein 1 in Tanzanian children. *Infect. Immun.* 78: 4653–4659.
36. Bull, P. C., B. S. Lowe, M. Kortok, C. S. Molyneux, C. I. Newbold, and K. Marsh. 1998. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nat. Med.* 4: 358–360.
37. Leech, J. H., J. W. Barnwell, L. H. Miller, and R. J. Howard. 1984. Identification of a strain-specific malarial antigen exposed on the surface of *Plasmodium falciparum*-infected erythrocytes. *J. Exp. Med.* 159: 1567–1575.
38. Giha, H. A., T. Staalsoe, D. Dodoo, C. Roper, G. M. Satti, D. E. Arnot, L. Hviid, and T. G. Theander. 2000. Antibodies to variable *Plasmodium falciparum*-infected erythrocyte surface antigens are associated with protection from novel malaria infections. *Immunol. Lett.* 71: 117–126.

39. Marsh, K., L. Otoo, R. J. Hayes, D. C. Carson, and B. M. Greenwood. 1989. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. *Trans. R. Soc. Trop. Med. Hyg.* 83: 293–303.
40. Mackintosh, C. L., T. Mwangi, S. M. Kinyanjui, M. Mosobo, R. Pinches, T. N. Williams, C. I. Newbold, and K. Marsh. 2008. Failure to respond to the surface of *Plasmodium falciparum* infected erythrocytes predicts susceptibility to clinical malaria amongst African children. *Int. J. Parasitol.* 38: 1445–1454.
41. Aguiar, J. C., G. R. Albrecht, P. Cegielski, B. M. Greenwood, J. B. Jensen, G. Lallinger, A. Martinez, I. A. McGregor, J. N. Minjas, J. Neequaye, et al. 1992. Agglutination of *Plasmodium falciparum*-infected erythrocytes from east and west African isolates by human sera from distant geographic regions. *Am. J. Trop. Med. Hyg.* 47: 621–632.
42. Bull, P. C., B. S. Lowe, M. Kortok, and K. Marsh. 1999. Antibody recognition of *Plasmodium falciparum* erythrocyte surface antigens in Kenya: evidence for rare and prevalent variants. *Infect. Immun.* 67: 733–739.
43. Jensen, A. T., P. Magistrado, S. Sharp, L. Joergensen, T. Lavstsen, A. Chiucchiuini, A. Salanti, L. S. Vestergaard, J. P. Lusingu, R. Hermsen, et al. 2004. *Plasmodium falciparum* associated with severe childhood malaria preferentially expresses PfEMP1 encoded by group A *var* genes. *J. Exp. Med.* 199: 1179–1190.
44. Rottmann, M., T. Lavstsen, J. P. Mugasa, M. Kaestli, A. T. Jensen, D. Müller, T. Theander, and H. P. Beck. 2006. Differential expression of *var* gene groups is associated with morbidity caused by *Plasmodium falciparum* infection in Tanzanian children. *Infect. Immun.* 74: 3904–3911.
45. Marsh, K., and R. J. Howard. 1986. Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. *Science* 231: 150–153.
46. Bull, P. C., B. S. Lowe, N. Kaleli, F. Njuga, M. Kortok, A. Ross, F. Ndungu, R. W. Snow, and K. Marsh. 2002. *Plasmodium falciparum* infections are associated with agglutinating antibodies to parasite-infected erythrocyte surface antigens among healthy Kenyan children. *J. Infect. Dis.* 185: 1688–1691.
47. Kinyanjui, S. M., T. Mwangi, P. C. Bull, C. I. Newbold, and K. Marsh. 2004. Protection against clinical malaria by heterologous immunoglobulin G antibodies against malaria-infected erythrocyte variant surface antigens requires interaction with asymptomatic infections. *J. Infect. Dis.* 190: 1527–1533.
48. Ofori, M. F., D. Dodoo, T. Staalsoe, J. A. Kurtzhals, K. Koram, T. G. Theander, B. D. Akanmori, and L. Hviid. 2002. Malaria-induced acquisition of antibodies to *Plasmodium falciparum* variant surface antigens. *Infect. Immun.* 70: 2982–2988.
49. Giha, H. A., T. Staalsoe, D. Dodoo, I. M. Elhassan, C. Roper, G. M. Satti, D. E. Arnot, L. Hviid, and T. G. Theander. 1999. Overlapping antigenic repertoires of variant antigens expressed on the surface of erythrocytes infected by *Plasmodium falciparum. Parasitology* 119: 7–17.
50. Giha, H. A., T. Staalsoe, D. Dodoo, I. M. Elhassan, C. Roper, G. M. Satti, D. E. Arnot, T. G. Theander, and L. Hviid. 1999. Nine-year longitudinal study of antibodies to variant antigens on the surface of *Plasmodium falciparum*-infected erythrocytes. *Infect. Immun.* 67: 4092–4098.
51. Manske, M., O. Miotto, S. Campino, S. Auburn, J. Almagro-Garcia, G. Maslen, J. O'Brien, A. Djimde, O. Doumbo, I. Zongo, et al. 2012. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature* 487: 375–379.
52. Amanna, I. J., N. E. Carlson, and M. K. Slifka. 2007. Duration of humoral immunity to common viral and vaccine antigens. *N. Engl. J. Med.* 357: 1903–1915.
53. Goodnow, C. C., C. G. Vinuesa, K. L. Randall, F. Mackay, and R. Brink. 2010. Control systems and decision making for antibody production. *Nat. Immunol.* 11: 681–688.
54. Good-Jacobson, K. L., and M. J. Shlomchik. 2010. Plasticity and heterogeneity in the generation of memory B cells and long-lived plasma cells: the influence of germinal center interactions and dynamics. *J. Immunol.* 185: 3117–3125.
55. Amanna, I. J., and M. K. Slifka. 2010. Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. *Immunol. Rev.* 236: 125–138.
56. Kinyanjui, S. M., D. J. Conway, D. E. Lanar, and K. Marsh. 2007. IgG antibody responses to *Plasmodium falciparum* merozoite antigens in Kenyan children have a short half-life. *Malar. J.* 6: 82.
57. Früh, K., O. Doumbo, H. M. Müller, O. Koita, J. McBride, A. Crisanti, Y. Touré, and H. Bujard. 1991. Human antibody response to the major merozoite surface antigen of *Plasmodium falciparum* is strain specific and short-lived. *Infect. Immun.* 59: 1319–1324.
58. Müller, H. M., K. Früh, A. von Brunn, F. Esposito, S. Lombardi, A. Crisanti, and H. Bujard. 1989. Development of the human immune response against the major surface protein (gp190) of *Plasmodium falciparum. Infect. Immun.* 57: 3765–3769.
59. Fonjungo, P. N., I. M. Elhassan, D. R. Cavanagh, T. G. Theander, L. Hviid, C. Roper, D. E. Arnot, and J. S. McBride. 1999. A longitudinal study of human antibody responses to *Plasmodium falciparum* rhoptry-associated protein 1 in a region of seasonal and unstable malaria transmission. *Infect. Immun.* 67: 2975–2985.
60. Weiss, G. E., B. Traore, K. Kayentao, A. Ongoiba, S. Doumbo, D. Doumtabe, Y. Kone, S. Dia, A. Guindo, A. Traore, et al. 2010. The *Plasmodium falciparum*-specific human memory B cell compartment expands gradually with repeated malaria infections. *PLoS Pathog.* 6: e1000912.
61. Akpogheneta, O. J., N. O. Duah, K. K. Tetteh, S. Dunyo, D. E. Lanar, M. Pinder, and D. J. Conway. 2008. Duration of naturally acquired antibody responses to blood-stage *Plasmodium falciparum* is age dependent and antigen specific. *Infect. Immun.* 76: 1748–1755.
62. Cavanagh, D. R., I. M. Elhassan, C. Roper, V. J. Robinson, H. Giha, A. A. Holder, L. Hviid, T. G. Theander, D. E. Arnot, and J. S. McBride. 1998. A longitudinal study of type-specific antibody responses to *Plasmodium falciparum* merozoite surface protein-1 in an area of unstable malaria in Sudan. *J. Immunol.* 161: 347–359.
63. Taylor, R. R., A. Egan, D. McGuinness, A. Jepson, R. Adair, C. Drakely, and E. Riley. 1996. Selective recognition of malaria antigens by human serum antibodies is not genetically determined but demonstrates some features of clonal imprinting. *Int. Immunol.* 8: 905–915.
64. Ramasamy, R., K. Nagendran, and M. S. Ramasamy. 1994. Antibodies to epitopes on merozoite and sporozoite surface antigens as serologic markers of malaria transmission: studies at a site in the dry zone of Sri Lanka. *Am. J. Trop. Med. Hyg.* 50: 537–547.
65. John, C. C., J. H. Ouma, P. O. Sumba, M. R. Hollingdale, J. W. Kazura, and C. L. King. 2002. Lymphocyte proliferation and antibody responses to *Plasmodium falciparum* liver-stage antigen-1 in a highland area of Kenya with seasonal variation in malaria transmission. *Am. J. Trop. Med. Hyg.* 66: 372–378.
66. Brown, A. E., H. K. Webster, J. A. Lyon, A. W. Thomas, B. Permpanich, and M. Gross. 1991. Characterization of naturally acquired antibody responses to a recombinant fragment from the N-terminus of *Plasmodium falciparum* glycoprotein 195. *Am. J. Trop. Med. Hyg.* 45: 567–573.
67. Riley, E. M., S. Morris-Jones, M. J. Blackman, B. M. Greenwood, and A. A. Holder. 1993. A longitudinal study of naturally acquired cellular and humoral immune responses to a merozoite surface protein (MSP1) of *Plasmodium falciparum* in an area of seasonal malaria transmission. *Parasite Immunol.* 15: 513–524.
68. Wipasa, J., C. Suphavilai, L. C. Okell, J. Cook, P. H. Corran, K. Thaikla, W. Liewsaree, E. M. Riley, and J. C. Hafalla. 2010. Long-lived antibody and B cell memory responses to the human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax. PLoS Pathog.* 6: e1000770.
69. Baird, J. K., T. R. Jones, E. W. Danudirgo, B. A. Annis, M. J. Bangs, H. Basri, Purnomo, and S. Masbar. 1991. Age-dependent acquired protection against *Plasmodium falciparum* in people having two years exposure to hyperendemic malaria. *Am. J. Trop. Med. Hyg.* 45: 65–76.
70. Baird, J. K., H. Purnomo, H. Basri, M. J. Bangs, E. M. Andersen, T. R. Jones, S. Masbar, S. Harjosuwarno, B. Subianto, and P. R. Arbani. 1993. Age-specific prevalence of *Plasmodium falciparum* among six populations with limited histories of exposure to endemic malaria. *Am. J. Trop. Med. Hyg.* 49: 707–719.
71. Crompton, P. D., M. A. Kayala, B. Traore, K. Kayentao, A. Ongoiba, G. E. Weiss, D. M. Molina, C. R. Burk, M. Waisberg, A. Jasinskas, et al. 2010. A prospective analysis of the Ab response to *Plasmodium falciparum* before and after a malaria season by protein microarray. *Proc. Natl. Acad. Sci. USA* 107: 6958–6963.
72. Sauerwein, R. W., M. Roestenberg, and V. S. Moorthy. 2011. Experimental human challenge infections can accelerate clinical malaria vaccine development. *Nat. Rev. Immunol.* 11: 57–64.
73. Plotkin, S. A. 2008. Vaccines: correlates of vaccine-induced immunity. *Clin. Infect. Dis.* 47: 401–409.
74. Doolan, D. L. 2011. *Plasmodium* immunomics. *Int. J. Parasitol.* 41: 3–20.
75. Tran, T. M., B. Samal, E. Kirkness, and P. D. Crompton. 2012. Systems immunology of human malaria. *Trends Parasitol.* 28: 248–257.
76. Ndungu, F. M., A. Olotu, J. Mwacharo, M. Nyonda, J. Apfeld, L. K. Mramba, G. W. Fegan, P. Bejon, and K. Marsh. 2012. Memory B cells are a more reliable archive for historical antimalarial responses than plasma antibodies in no-longer exposed children. *Proc. Natl. Acad. Sci. USA* 109: 8247–8252.
77. Nogaro, S. I., J. C. Hafalla, B. Walther, E. J. Remarque, K. K. Tetteh, D. J. Conway, E. M. Riley, and M. Walther. 2011. The breadth, but not the magnitude, of circulating memory B cell responses to *P. falciparum* increases with age/exposure in an area of low transmission. *PLoS ONE* 6: e25582.
78. Dorfman, J. R., P. Bejon, F. M. Ndungu, J. Langhorne, M. M. Kortok, B. S. Lowe, T. W. Mwangi, T. N. Williams, and K. Marsh. 2005. B cell memory to 3 *Plasmodium falciparum* blood-stage antigens in a malaria-endemic area. *J. Infect. Dis.* 191: 1623–1630.
79. Crotty, S., P. Felgner, H. Davies, J. Glidewell, L. Villarreal, and R. Ahmed. 2003. Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* 171: 4969–4973.
80. Clark, E. H., C. J. Silva, G. E. Weiss, S. Li, C. Padilla, P. D. Crompton, J. N. Hernandez, and O. H. Branch. 2012. *Plasmodium falciparum* malaria in the Peruvian Amazon, a region of low transmission, is associated with immunologic memory. *Infect. Immun.* 80: 1583–1592.
81. Urban, B. C., T. T. Hien, N. P. Day, N. H. Phu, R. Roberts, E. Pongponratn, M. Jones, N. T. Mai, D. Bethell, G. D. Turner, et al. 2005. Fatal *Plasmodium falciparum* malaria causes specific patterns of splenic architectural disorganization. *Infect. Immun.* 73: 1986–1994.
82. Ibison, F., A. Olotu, D. M. Muema, J. Mwacharo, E. Ohuma, D. Kimani, K. Marsh, P. Bejon, and F. M. Ndungu. 2012. Lack of avidity maturation of merozoite antigen-specific antibodies with increasing exposure to *Plasmodium falciparum* amongst children and adults exposed to endemic malaria in Kenya. *PLoS ONE* 7: e52939.
83. Weill, J. C., S. Weller, and C. A. Reynaud. 2009. Human marginal zone B cells. *Annu. Rev. Immunol.* 27: 267–285.
84. Weiss, G. E., P. D. Crompton, S. Li, L. A. Walsh, S. Moir, B. Traore, K. Kayentao, A. Ongoiba, O. K. Doumbo, and S. K. Pierce. 2009. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J. Immunol.* 183: 2176–2182.
85. Asito, A. S., A. M. Moormann, C. Kiprotich, Z. W. Ng'ang'a, R. Ploutz-Snyder, and R. Rochford. 2008. Alterations on peripheral B cell subsets following an acute uncomplicated clinical malaria infection in children. *Malar. J.* 7: 238.
86. Butler, N. S., J. Moebius, L. L. Pewe, B. Traore, O. K. Doumbo, L. T. Tygrett, T. J. Waldschmidt, P. D. Crompton, and J. T. Harty. 2012. Therapeutic blockade of PD-L1 and LAG-3 rapidly clears established blood-stage *Plasmodium* infection. *Nat. Immunol.* 13: 188–195.

87. Donati, D., B. Mok, A. Chêne, H. Xu, M. Thangarajh, R. Glas, Q. Chen, M. Wahlgren, and M. T. Bejarano. 2006. Increased B cell survival and preferential activation of the memory compartment by a malaria polyclonal B cell activator. *J. Immunol.* 177: 3035–3044.
88. Abele, D. C., J. E. Tobie, G. J. Hill, P. G. Contacos, and C. B. Evans. 1965. Alterations in serum proteins and 19S antibody production during the course of induced malarial infections in man. *Am. J. Trop. Med. Hyg.* 14: 191–197.
89. Nduati, E., A. Gwela, H. Karanja, C. Mugyenyi, J. Langhorne, K. Marsh, and B. C. Urban. 2011. The plasma concentration of the B cell activating factor is increased in children with acute malaria. *J. Infect. Dis.* 204: 962–970.
90. Traore, B., Y. Koné, S. Doumbo, D. Doumtabé, A. Traoré, P. D. Crompton, M. Mircetic, C. Y. Huang, K. Kayentao, A. Dicko, et al. 2009. The TLR9 agonist CpG fails to enhance the acquisition of *Plasmodium falciparum*-specific memory B cells in semi-immune adults in Mali. *Vaccine* 27: 7299–7303.
91. Daniel-Ribeiro, C. T., and G. Zanini. 2000. Autoimmunity and malaria: what are they doing together? *Acta Trop.* 76: 205–221.
92. Sakkas, L. I., M. Boulbou, D. Kyriakou, I. Makri, C. Sinani, A. Germenis, and N. Stathakis. 2008. Immunological features of visceral leishmaniasis may mimic systemic lupus erythematosus. *Clin. Biochem.* 41: 65–68.
93. Moir, S., and A. S. Fauci. 2009. B cells in HIV infection and disease. *Nat. Rev. Immunol.* 9: 235–245.
94. Sansonno, L., F. A. Tucci, S. Sansonno, G. Lauletta, L. Troiani, and D. Sansonno. 2009. B cells and HCV: an infection model of autoimmunity. *Autoimmun. Rev.* 9: 93–94.
95. Moir, S., J. Ho, A. Malaspina, W. Wang, A. C. DiPoto, M. A. O'Shea, G. Roby, S. Kottilil, J. Arthos, M. A. Proschan, et al. 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* 205: 1797–1805.
96. Charles, E. D., R. M. Green, S. Marukian, A. H. Talal, G. V. Lake-Bakaar, I. M. Jacobson, C. M. Rice, and L. B. Dustin. 2008. Clonal expansion of immunoglobulin M^+CD27^+ B cells in HCV-associated mixed cryoglobulinemia. *Blood* 111: 1344–1356.
97. Ehrhardt, G. R., J. T. Hsu, L. Gartland, C. M. Leu, S. Zhang, R. S. Davis, and M. D. Cooper. 2005. Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. *J. Exp. Med.* 202: 783–791.
98. Weiss, G. E., E. H. Clark, S. Li, B. Traore, K. Kayentao, A. Ongoiba, J. N. Hernandez, O. K. Doumbo, S. K. Pierce, O. H. Branch, and P. D. Crompton. 2011. A positive correlation between atypical memory B cells and *Plasmodium falciparum* transmission intensity in cross-sectional studies in Peru and Mali. *PLoS ONE* 6: e15983.
99. Portugal, S., D. Doumtabe, B. Traore, L. H. Miller, M. Troye-Blomberg, O. K. Doumbo, A. Dolo, S. K. Pierce, and P. D. Crompton. 2012. B cell analysis of ethnic groups in Mali with differential susceptibility to malaria. *Malar. J.* 11: 162.
100. Illingworth, J., N. S. Butler, S. Roetynck, J. Mwacharo, S. K. Pierce, P. Bejon, P. D. Crompton, K. Marsh, and F. M. Ndungu. 2013. Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. *J. Immunol.* 190: 1038–1047
101. Muellenbeck, M. F., B. Ueberheide, B. Amulic, A. Epp, D. Fenyo, C. E. Busse, M. Esen, M. Theisen, B. Mordmüller, and H. Wardemann. 2013. Atypical and classical memory B cells produce *Plasmodium falciparum* neutralizing antibodies. *J. Exp. Med.* PubMed DOI: 10.1084/jem.20121970
102. Bushell, K. M., C. Söllner, B. Schuster-Boeckler, A. Bateman, and G. J. Wright. 2008. Large-scale screening for novel low-affinity extracellular protein interactions. *Genome Res.* 18: 622–630.
103. Waisberg, M., G. C. Cerqueira, S. B. Yager, I. M. Francischetti, J. Lu, N. Gera, P. Srinivasan, K. Miura, B. Rada, J. Lukszo, et al. 2012. *Plasmodium falciparum* merozoite surface protein 1 blocks the proinflammatory protein S100P. *Proc. Natl. Acad. Sci. USA* 109: 5429–5434.
104. Rogers, N. J., B. S. Hall, J. Obiero, G. A. Targett, and C. J. Sutherland. 2000. A model for sequestration of the transmission stages of *Plasmodium falciparum*: adhesion of gametocyte-infected erythrocytes to human bone marrow cells. *Infect. Immun.* 68: 3455–3462.
105. Manicassamy, S., and B. Pulendran. 2009. Modulation of adaptive immunity with Toll-like receptors. *Semin. Immunol.* 21: 185–193.
106. Boutlis, C. S., T. W. Yeo, and N. M. Anstey. 2006. Malaria tolerance: for whom the cell tolls? *Trends Parasitol.* 22: 371–377.
107. Shio, M. T., S. C. Eisenbarth, M. Savaria, A. F. Vinet, M. J. Bellemare, K. W. Harder, F. S. Sutterwala, D. S. Bohle, A. Descoteaux, R. A. Flavell, and M. Olivier. 2009. Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. *PLoS Pathog.* 5: e1000559.
108. Krishnegowda, G., A. M. Hajjar, J. Zhu, E. J. Douglass, S. Uematsu, S. Akira, A. S. Woods, and D. C. Gowda. 2005. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J. Biol. Chem.* 280: 8606–8616.
109. Parroche, P., F. N. Lauw, N. Goutagny, E. Latz, B. G. Monks, A. Visintin, K. A. Halmen, M. Lamphier, M. Olivier, D. C. Bartholomeu, et al. 2007. Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *Proc. Natl. Acad. Sci. USA* 104: 1919–1924.
110. Sharma, S., R. B. DeOliveira, P. Kalantari, P. Parroche, N. Goutagny, Z. Jiang, J. Chan, D. C. Bartholomeu, F. Lauw, J. P. Hall, et al. 2011. Innate immune recognition of an AT-rich stem-loop DNA motif in the *Plasmodium falciparum* genome. *Immunity* 35: 194–207.
111. Crompton, P. D., M. Mircetic, G. Weiss, A. Baughman, C. Y. Huang, D. J. Topham, J. J. Treanor, I. Sanz, F. E. Lee, A. P. Durbin, et al. 2009. The TLR9 ligand CpG promotes the acquisition of *Plasmodium falciparum*-specific memory B cells in malaria-naive individuals. *J. Immunol.* 182: 3318–3326.
112. Wrammert, J., D. Koutsonanos, G. M. Li, S. Edupuganti, J. Sui, M. Morrissey, M. McCausland, I. Skountzou, M. Hornig, W. I. Lipkin, et al. 2011. Broadly cross-reactive antibodies dominate the human B cell response against pandemic H1N1 influenza virus infection. *J. Exp. Med.* 208: 181–193.