Published in final edited form as:

Trends Parasitol. 2020 February ; 36(2): 91–97. doi:10.1016/j.pt.2019.12.002.

Autoimmune Anemia in Malaria

Juan Rivera-Correa[#], Ana Rodriguez^{*}

New York University School of Medicine

Abstract

Severe anemia is a major cause of death by malaria. The loss of uninfected erythrocytes is an important contributor to malarial anemia; however, the mechanisms underlying this pathology are not well understood. Malaria-induced anemia is related to autoimmune antibodies against the membrane lipid phosphatidylserine (PS). In mice, these antibodies induce the clearance of uninfected erythrocytes after binding to PS exposed in their membrane. In human malaria patients, there is a strong correlation between anemia and anti-PS antibodies. During malaria, anti-PS antibodies are produced by atypical B cells, whose levels correlate with the development of anemia in patients. Autoimmune responses, which are documented frequently in different infections, contribute to the pathogenesis of malaria by inducing the clearance of uninfected erythrocytes.

Keywords

Plasmodium falciparum, *Plasmodium vivax*; autoimmunity; atypical B cells; autoantibodies; anti-phosphatidylserine antibodies

Malaria-induced anemia is a major pathology

Malaria is a major cause of anemia in large areas of the world where this disease is endemic. After a period of several years with high success in global malaria control, progress has stalled for the past three years and malaria remains highly prevalent in tropical areas (219 million cases worldwide) where it causes approximately 450,000 deaths yearly [1]. Severe anemia is a major cause of death by malaria. It appears frequently in malaria patients, but it is young children who bear the brunt of this complication with most cases presenting under the age of five [2]. Even after successful anti-parasitic treatment, hematocrit levels typically continue to decrease for 6 days before recovery. This period is critical for patients, who may die if anemia is not controlled. The only effective treatment for malarial anemia, after anti-parasitic drugs have been administered, is blood transfusion; however, the availability of this procedure is very limited in most malaria endemic areas [2].

^{*}Correspondence: ana.rodriguez@nyumc.org (A. Rodriguez).

[#]Current affiliation: Hospital for Special Surgery, Weill-Cornell Medicine

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Malaria is caused by infection with *Plasmodium*, a eukaryotic parasite that infects erythrocytes. Infection with the most prevalent species, *P. falciparum* and *P. vivax*, can induce severe anemia in patients. Although it appears obvious that a pathogen infecting erythrocytes may induce anemia, this is not a major factor for human malaria, since the level of parasitemia is usually low and it was calculated that about 8 uninfected erythrocytes are cleared in addition to each parasitized erythrocyte in *P. falciparum* [3, 4] and 34 in *P. vivax* [5] infections. Malarial anemia has been attributed mostly to dyserythropoiesis in the bone marrow and loss of uninfected erythrocytes in the circulation, however the mechanisms underlying these processes are not well understood [2]. Different mechanisms have been associated with the loss of uninfected erythrocytes during malaria, including loss of complement regulatory proteins [6] and the dysregulation of the heme-hemopexin axis [7].

Malaria induces strong autoimmune antibody responses

Autoimmunity during and after an infection is an extensively reported phenomenon, but little is known about the mechanisms underlying infection-related autoimmune responses and their role in pathogenesis. Malaria has been associated with the development of autoimmunity in patients and mice models inducing the generation of anti-self antibodies against a variety of antigens, such as erythrocytes cytoskeletal [8] and membrane [9] proteins, enzymes [10], sugar moieties [11], DNA [12] [13, 14], and phospholipids [15, 16].

An autoimmune component in malaria anemia had been suspected early on, since elevated levels of anti-erythrocyte antibodies were found in patients with severe anemia [17] and there are numerous reports of autoimmune hemolytic anemia in *P. falciparum* [18, 19] and *P. vivax* [9, 20] malaria patients. Recently, anti-self antibodies against two surface erythrocyte proteins, band-3 and spectrin, have been identified in *P. vivax* patients and their levels correlated to anemia, suggesting that these antibodies may contribute to the elimination of uninfected erythrocytes during malaria [21].

Anti-self antibodies against phosphatidylserine promote anemia in malaria

Studies in mice infected with rodent species of *Plasmodium*, which are used as experimental models for human malaria, showed that *Plasmodium* infection induces the generation of anti-self antibodies with different specificities, including autoantibodies recognizing specifically the membrane lipid phosphatidylserine (PS) (see Glossary) [15]. PS is normally not exposed in the surface of cells, but it is flipped from the inner leaflet to the outer leaflet in apoptotic cells [22]. Exposure of PS in erythrocytes has been observed in mice [15, 23] and in human patients [24] with malaria, although the mechanism inducing this phenomenon is not clear yet. The uninfected erythrocytes exposing PS during infection are mostly newly generated erythrocytes (reticulocytes), which is unusual, since PS exposure is typically found in aged erythrocytes [25], but may be a result of inflammatory or oxidative stress induced during infection.

Binding of autoimmune anti-PS antibodies to uninfected erythrocytes in mice with malaria resulted in accelerated clearance of erythrocytes and anemia, indicating that autoimmunity contributes to anemia in malaria infected mice [15]. Studies in malaria patients have

observed increased in PS exposure in erythrocytes from patients with severe malaria anemia compared to patients with uncomplicated malaria [24], suggesting an important role of erythrocyte PS exposure in human malaria-induced anemia.

Importantly, a strong correlation of anti-PS antibodies and anemia has been observed in different patient cohorts [13] [15] [26] [27], underscoring the important role autoimmunity in malaria-induced anemia. The study of the levels of anti-PS antibodies in children with severe malaria caused by *P. falciparum* infection in Uganda revealed a strong correlation with anemia [13], suggesting an important role for anti-PS antibodies in promoting malaria-induced anemia. No relation was found between different antibodies recognizing Plasmodium antigens and anemia, pointing to the specificity of anti-PS antibodies. Interestingly, a strong correlation between autoimmune anti-DNA antibodies and anemia was also observed in this cohort. Since there are high levels of circulating DNA during malaria [28] and free DNA is known to bind to erythrocytes in the circulation [29], it is possible that anti-DNA antibodies also contribute to anemia through the binding to erythrocyte-associated DNA in patients.

A different study found anti-PS antibodies in patients infected with *P. falciparum*, *P. vivax*, *P. malariae* and *P. knowlesi*, and an inverse correlation between hemoglobin levels and anti-PS antibodies in malaria caused by *P. vivax* and *P. falciparum* infections [26], suggesting that the observed effect in anemia may be common to human *Plasmodium* infections.

Some malaria patients develop a syndrome called post-malarial anemia, where hemoglobin levels drop after treatment and effective clearance of the parasite. Post-malarial anemia is observed more frequently after *P. falciparum* cases treated with artesunate [30] and correlates with levels of the parasite protein HRP2 in plasma [31]. In patients with post-malarial anemia, the levels of anti-PS antibodies increased for three weeks after effective artesunate treatment and correlated inversely with hemoglobin levels, indicating that anti-PS antibodies may also be generated after parasite clearance and may be responsible for delayed erythrocyte losses [15].

Cellular autoimmune responses leading to anemia in malaria

Malarial anemia is highly influenced by the host immune response in multiple aspects. Many different immune cells can have different roles in promoting anemia such as professional phagocytes (Monocytes/ Macrophages and Dendritic cells), T-cells and B-cells [32, 33].

Professional phagocytes, such as Monocytes, Macrophages and Dendritic cells, have various roles in promoting malarial anemia both with direct (phagocytosis) and indirect (cytokine secretion) mechanisms. Phagocytes, specifically macrophages, have an important role in erythropoiesis, as well as in the clearance of age/damaged erythrocytes [34], being essential players in determining their life span. In physiological states, only aged or altered erythrocytes are removed from circulation, mainly in the spleen and liver by professional hemophagocytes [35]. Some of the known erythrocyte alterations that could lead to their clearance during physiological conditions include: band 3 aggregation, membrane

alterations, changes in cell volume, density, and deformability [36]. Malaria induces a decrease in deformability in all erythrocytes, infected and uninfected, which correlates inversely with hemoglobin levels, suggesting that loss of erythrocytes deformability is a contributor to anemia [2]. Autoantibodies may contribute to the loss of erythrocytes deformability, since it was observed that purified autoantibodies from *P. vivax* anemic patients decreased membrane fluidity in uninfected erythrocytes *in vitro* [37]. It is possible that autoimmune antibodies not only promote phagocytosis by opsonization, but also by decreasing erythrocyte deformability.

During highly inflammatory states, such as malaria, phagocytes can display an unusual highly activated state, clinically called macrophage activation syndrome (MAS) that will lead to massive clearance of erythrocytes from circulation resulting in anemia [38]. A recent report has implicated chronic TLR-7 and TLR-9 activation of monocytes in causing the development of inflammatory hemophagocytes and resulting in increased phagocytosis of uninfected erythrocytes during malaria in mice [39]. Additionally, phagocytes can release many different cytokines that can actively suppress erythropoiesis, such as Type I interferon, and could even lead to exposure of PS [40, 41] on healthy erythrocytes resulting in their premature clearance during infection. Lastly, many phagocytes express high levels of PS receptors and Fc receptors that would either directly recognize PS on erythrocytes or with the help of anti-self anti-PS antibodies could lead to their premature clearance during malarial anemia. Although phagocytes do have a homeostatic role in erythrocyte turnover, hyper activation of this system during malarial infection by cytokines and autoantibodies is a major contributor to anemia.

Another cell type that has been attributed a role during malarial anemia is cytotoxic CD8⁺ T-cells. These adaptive immune cells normally recognize pathogen infected-cells, with mechanisms such as recognition of MHC class I molecules, and release cytotoxic and lytic granules that will lead to the eventual death and clearance of the infected cell having key protective roles during liver stage malaria [42]. Since erythrocytes do not express MHC class I, lysis of infected erythrocytes by cytotoxic CD8⁺ T-cells has traditionally not been considered a mechanism of immune protection against blood-stage malaria. However, recent findings show that infected reticulocytes, that are the host cell of *P. vivax* infections, still retain surface expression of MHC class I molecules and are vulnerable to cytotoxic CD8⁺ T-cells [43].

Studies in mice infected with *P. yoelii* have detailed how CD8⁺ cytotoxic T-cells can directly promote PS exposure on infected erythroblasts through direct interaction involving Fas-FasL [44], which leave erythrocytes more susceptible to phagocytosis, enhancing clearance by macrophages [45]. Since a percentage of uninfected erythroblasts were found to express Fas during *P. yoelii* infection, activated CD8⁺ T cells expressing FasL might induce erythroblast clearance contributing to anemia [44]. Additionally, both CD4⁺ T helper and CD8⁺ T-cells have been proposed to have an indirect role in promoting malarial anemia through secretion of cytokines such as IFN γ , IL-21 and IL-17 [46]. These cytokines can suppress erythropoiesis [33, 47], stimulate phagocyte activation [25, 47] and promote autoimmune B-cell expansion [48].

Lastly, and perhaps more relevant to autoimmune anemia, B-cells play a major role in promoting malarial anemia in both mice and humans. B-cells are the only immune cell that can produce antibodies, hence having an essential role in the protective anti-parasite response. On the other hand, B-cells also are major producers of pathogenic autoantibodies that have been highly linked with malarial anemia in mice [16, 49], and also in patient studies [9, 13, 15, 26, 37]. Particular atypical B-cells that expressed the transcription factor T-bet and the integrin CD11c have been implied as major secretors of anti-PS antibodies that can directly promote malarial anemia in mice [16]. In this animal model, inflammatory cytokines, such as IFN γ in addition to parasite DNA, induced stimulation of TLR9 on B-cells leading to the differentiation of these atypical T-bet⁺ CD11⁺ autoimmune B-cells (Figure 1, Key Figure). Similar mechanisms have been described in mice models of autoimmunity where these autoimmune B-cells are designated as major producers of pathogenic anti-nuclear antibodies [48, 50]. With the undeniable similarities between malaria and autoimmune syndromes it is highly likely that shared autoimmune triggers play a role in both diseases.

In humans, a similar if not analogous, B-cell population has been described to expand in individuals from malaria-endemic areas designated atypical memory B-cells (AtMBCs) [51, 52]. These cells are also characterized by the expression T-bet, CD11c and FcRL5 [53–56], which have also been confirmed to be markers of these atypical B cells in mice [57]. AtMBCs studied in the context of malaria-endemic settings and have been characterized by reduced B-cell receptor (BCR) activity and minimal antibody secretion [55, 58]. Additionally, these cells have been correlated with increased malaria-exposure and have the capacity to secrete anti-parasite antibodies [53, 57–60]. Using a parasite antigen-specific BCR transgenic mice, AtMBCs with the same phenotype were shown to be short activated cells that do not form part of the anti-parasite memory response during malaria infection [57]. However, the study of natural, polyclonal endogenous B cells in wild type mice found that AtMBCs are fully functional, form stable memory and respond robustly to recall [53].

A recent study on first-time *P. falciparum*-infected European travelers show that levels of AtMBCs, malarial anemia development and plasma levels of anti-PS antibodies correlate with each other during infection [27]. Additionally, *in-vitro* parasite-induced AtMBCs were able to secrete anti-PS antibodies. On the other hand, Classical memory B-cells were found to correlate negatively with anti-PS antibodies and anemia development suggesting a dichotomous relationship between these two B-cell memory populations in malarial anemia. Since there were no significant correlations between anti-PS IgG antibodies with other B-cell subsets (naïve, immature or plasma cells), or of other antibodies (anti-DNA, anti-parasite PfEBA) with anemia or with AtMBCs, these results suggest specificity and support the hypothesis that AtMBCs secrete anti-PS IgG antibodies contributing to malarial anemia in *P. falciparum*-infected patients.

Concluding remarks

Malarial anemia of mice and humans is a multi-factorial syndrome that has posed puzzling questions about its etiology and efficient treatment. Autoimmunity has been correlated to and mechanistically linked with malaria anemia mainly through anti-PS antibodies. The

contribution of autoantibodies, particularly anti-PS antibodies, initially described in mice has been expanded to human (*P. falciparum* and *P. vivax*) infection. Central players mediating this autoimmune malarial anemia are the AtMBCs observed in both mice and humans. The undeniable similarity between AtMBCs differentiated during malaria and during different autoimmune disorders already suggest a link between autoimmunity and malaria pathology [48]. It is possible that the role of other immune cells in malarial anemia, such as phagocytes, may be related to these atypical B-cells through the production of autoantibodies that recognize uninfected erythrocytes. For example, high levels of anti-PS antibodies can enhance malarial anemia by both direct lysis of erythrocytes [27] or opsonization by phagocytes [15]. Although phagocytes can directly recognize stressed erythrocytes exposing PS, their phagocytosis would be inhibited by expression of “do not eat me” signals such as CD47 [22], which are high in young uninfected erythrocytes (reticulocytes) during malaria infection [15]. The presence of high levels of anti-PS antibodies would shift the balance towards enhanced clearance of these healthy erythrocytes bypassing the inhibitory signal, resulting in premature removal of uninfected erythrocytes from the circulation and aggravating anemia [15]. Additionally, anti-PS antibodies would preferentially target reticulocytes because they expose PS during their development [61]. Lastly, eliminating specifically AtMBCs (by B-cell specific deficiencies in IFN γ or T-bet) in a rodent malaria model, led to reduced plasma anti-PS antibody levels, but to enhanced anti-parasite MSP1 antibodies [62]. This association further suggests that the humoral autoimmune response has a key pathological role during malaria and may limit protective immune responses.

Although this autoimmune targeting of PS on erythrocytes has a clear pathological role during malarial anemia, the possibility of having a beneficial effect in combating malarial infection remains in question [49, 63] (see Outstanding Questions). Since both uninfected and infected erythrocytes expose high levels of PS, there is a possibility that these anti-PS antibodies could aid in the clearance of parasite-infected cells. In accordance, another set of autoantibodies, which have specificity against DNA have been implicated in possibly aiding in pathogen clearance during malaria [14] and other infections [64]. Additionally, a recent study has hypothesized that transient autoimmune targeting of erythrocytes could be advantageous for the host by limiting parasite reservoirs and reducing the numbers of their target host cell [65]. Evolutionarily, a strong potent autoimmune system can be advantageous for antigenically complex infections such as malaria, suggesting a divergent role for autoimmunity in promoting both protection and bystander pathology. Nevertheless, humoral autoimmunity seems to have a key pathological role in promoting anemia during malaria, particularly mediated by secretion of anti-PS antibodies that are produced by AtMBCs. Understanding of the basic mechanisms underlying malaria-induced anemia may allow the development of therapeutics to this pathology, which currently can only rely on blood transfusion. Blocking of anti-self antibodies generated during infection, such as the use of annexin V to cover exposed PS on erythrocytes, may prevent premature clearance by phagocytes during malaria.

Glossary

Atypical memory B-cell (AtMBC)

B cells that are characterized by the expression of inhibitory markers (T-bet, CD11c and FcRL5) and are found in some autoimmune disorders and chronic infections

B-cell receptor (BCR)

An immunoglobulin with a transmembrane domain that is exposed in the surface of B cells and has the same antigen specificity as all secreted antibodies from this cell

Phosphatidylserine (PS)

A membrane phospholipid found normally in the inner leaflet of the cell membrane. PS is exposed in the outer leaflet during apoptosis and in response to cell stress

References

1. WHO. World malaria report 2018. 2018.
2. White NJ. Anaemia and malaria. *Malar J* 2018;17(1):371 Epub 2018/10/21. doi: 10.1186/s12936-018-2509-9. [PubMed: 30340592]
3. Jakeman GN, Saul A, Hogarth WL, Collins WE. Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology*. 1999;119 (Pt 2):127–33. [PubMed: 10466119]
4. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkijaroen L, ter Kuile F, et al. Factors contributing to anemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg*. 2001;65(5):614–22. Epub 2001/11/22. doi: 10.4269/ajtmh.2001.65.614. [PubMed: 11716124]
5. Collins WE, Jeffery GM, Roberts JM. A retrospective examination of anemia during infection of humans with *Plasmodium vivax*. *Am J Trop Med Hyg*. 2003;68(4):410–2. Epub 2003/07/24. [PubMed: 12875288]
6. Oyong DA, Kenangalem E, Poespoprodjo JR, Beeson JG, Anstey NM, Price RN, et al. Loss of complement regulatory proteins on uninfected erythrocytes in vivax and falciparum malaria anemia. *JCI Insight*. 2018;3(22). Epub 2018/11/16. doi: 10.1172/jci.insight.124854.
7. Elphinstone RE, Conroy AL, Hawkes M, Hermann L, Namasopo S, Warren HS, et al. Alterations in Systemic Extracellular Heme and Hemopexin Are Associated With Adverse Clinical Outcomes in Ugandan Children With Severe Malaria. *J Infect Dis*. 2016;214(8):1268–75. Epub 2016/08/16. doi: 10.1093/infdis/jiw357. [PubMed: 27515862]
8. Berzins K, Wahlgren M, Perlmann P. Studies on the specificity of anti-erythrocyte antibodies in the serum of patients with malaria. *Clin Exp Immunol*. 1983;54(2):313–8. Epub 1983/11/01. [PubMed: 6652962]
9. Mourao LC, Baptista RP, de Almeida ZB, Grynberg P, Pucci MM, Castro-Gomes T, et al. Anti-band 3 and anti-spectrin antibodies are increased in *Plasmodium vivax* infection and are associated with anemia. *Sci Rep*. 2018;8(1):8762 Epub 2018/06/10. doi: 10.1038/s41598-018-27109-6. [PubMed: 29884876]
10. Ritter K, Kuhlencord A, Thomssen R, Bommer W. Prolonged haemolytic anaemia in malaria and autoantibodies against triosephosphate isomerase. *Lancet*. 1993;342(8883):1333–4. Epub 1993/11/27. doi: 10.1016/0140-6736(93)92248-r. [PubMed: 7901638]
11. Ravindran B, Satapathy AK, Das MK. Naturally-occurring anti-alpha-galactosyl antibodies in human *Plasmodium falciparum* infections--a possible role for autoantibodies in malaria. *Immunol Lett*. 1988;19(2):137–41. Epub 1988/10/01. doi: 10.1016/0165-2478(88)90133-2. [PubMed: 3069703]
12. Hirako IC, Gallego-Marin C, Ataide MA, Andrade WA, Gravina H, Rocha BC, et al. DNA-Containing Immunocomplexes Promote Inflammasome Assembly and Release of Pyrogenic Cytokines by CD14+ CD16+ CD64high CD32low Inflammatory Monocytes from Malaria Patients. *MBio*. 2015;6(6):e01605–15. doi: 10.1128/mBio.01605-15. [PubMed: 26578679]
13. Rivera-Correa J, Conroy AL, Opoka RO, Batte A, Namazzi R, Ouma B, et al. Autoantibody levels are associated with acute kidney injury, anemia and post-discharge morbidity and mortality in

- Ugandan children with severe malaria. *Sci Rep.* 2019;9(1):14940 Epub 2019/10/19. doi: 10.1038/s41598-019-51426-z. [PubMed: 31624288]
14. Wozencraft AO, Lloyd CM, Staines NA, Griffiths VJ. Role of DNA-binding antibodies in kidney pathology associated with murine malaria infections. *Infection and immunity.* 1990;58(7):2156–64. Epub 1990/07/01. [PubMed: 2365456]
 15. Fernandez-Arias C, Rivera-Correa J, Gallego-Delgado J, Rudlaff R, Fernandez C, Roussel C, et al. Anti-Self Phosphatidylserine Antibodies Recognize Uninfected Erythrocytes Promoting Malarial Anemia. *Cell Host Microbe.* 2016;19(2):194–203. Epub 2016/02/13. doi: 10.1016/j.chom.2016.01.009. [PubMed: 26867178]
 16. Rivera-Correa J, Guthmiller JJ, Vijay R, Fernandez-Arias C, Pardo-Ruge MA, Gonzalez S, et al. Plasmodium DNA-mediated TLR9 activation of T-bet(+) B cells contributes to autoimmune anaemia during malaria. *Nat Commun.* 2017;8(1):1282 Epub 2017/11/05. doi: 10.1038/s41467-017-01476-6. [PubMed: 29101363]
 17. Rosenberg EB, Strickland GT, Yang SL, Whalen GE. IgM antibodies to red cells and autoimmune anemia in patients with malaria. *Am J Trop Med Hyg.* 1973;22(2):146–52. Epub 1973/03/01. doi: 10.4269/ajtmh.1973.22.146. [PubMed: 4631762]
 18. Arndt PA, Garratty G, Maranto LS, Wohl H. Immune hemolytic anemia associated with mefloquine. *Transfusion.* 1997;37(11–12):1220–1. Epub 1998/01/14. doi: 10.1046/j.1537-2995.1997.37111298088058.x. [PubMed: 9426652]
 19. Raffray L, Receveur MC, Beguet M, Lauroua P, Pistone T, Malvy D. Severe delayed autoimmune haemolytic anaemia following artesunate administration in severe malaria: a case report. *Malar J.* 2014;13:398 Epub 2014/10/13. doi: 10.1186/1475-2875-13-398. [PubMed: 25306236]
 20. Ghosh A, Sharma S, Choudhury J. Autoimmune Hemolytic Anemia in Plasmodium vivax Malaria. *Indian J Pediatr.* 2017;84(6):483–4. Epub 2017/03/16. doi: 10.1007/s12098-017-2327-z. [PubMed: 28290054]
 21. Mourao LC, Baptista RP, de Almeida ZB, Grynberg P, Pucci MM, Castro-Gomes T, et al. Anti-band 3 and anti-spectrin antibodies are increased in Plasmodium vivax infection and are associated with anemia. *Sci Rep.* 2018;8(1):8762 Epub 2018/06/10. doi: 10.1038/s41598-018-27109-6. [PubMed: 29884876]
 22. Nagata S Apoptosis and Clearance of Apoptotic Cells. *Annu Rev Immunol.* 2018;36:489–517. Epub 2018/02/06. doi: 10.1146/annurev-immunol-042617-053010. [PubMed: 29400998]
 23. Totino PR, Magalhaes AD, Silva LA, Banic DM, Daniel-Ribeiro CT, Ferreira-da-Cruz Mde F. Apoptosis of non-parasitized red blood cells in malaria: a putative mechanism involved in the pathogenesis of anaemia. *Malar J.* 2010;9:350 Epub 2010/12/04. doi: 10.1186/1475-2875-9-350. [PubMed: 21126362]
 24. Fendel R, Brandts C, Rudat A, Kreidenweiss A, Steur C, Appelmann I, et al. Hemolysis is associated with low reticulocyte production index and predicts blood transfusion in severe malarial anemia. *PLoS One.* 2010;5(4):e10038 Epub 2010/04/14. doi: 10.1371/journal.pone.0010038. [PubMed: 20386613]
 25. Buttari B, Profumo E, Rigano R. Crosstalk between red blood cells and the immune system and its impact on atherosclerosis. *Biomed Res Int.* 2015;2015:616834 Epub 2015/02/28. doi: 10.1155/2015/616834. [PubMed: 25722984]
 26. Barber BE, Grigg MJ, Piera K, Amante FH, William T, Boyle MJ, et al. Anti-phosphatidylserine IgM and IgG antibodies are higher in vivax than falciparum malaria, and associated with early anemia in both species. *J Infect Dis.* 2019 Epub 2019/06/30. doi: 10.1093/infdis/jiz334.
 27. Rivera-Correa J, Mackroth M, Jacobs T, Schulze zur Wiesch J, Rolling T, Rodriguez A. Atypical memory B-cells are associated with Plasmodium falciparum anemia through anti-phosphatidylserine antibodies. *Elife.* 2019. doi: 10.7554/eLife.48309.
 28. Gal S, Fidler C, Turner S, Lo YM, Roberts DJ, Wainscoat JS. Detection of Plasmodium falciparum DNA in plasma. *Ann N Y Acad Sci.* 2001;945:234–8. Epub 2001/11/16. doi: 10.1111/j.1749-6632.2001.tb03891.x. [PubMed: 11708485]
 29. Hotz MJ, Qing D, Shashaty MGS, Zhang P, Faust H, Sondheimer N, et al. Red Blood Cells Homeostatically Bind Mitochondrial DNA through TLR9 to Maintain Quiescence and to Prevent

- Lung Injury. *Am J Respir Crit Care Med*. 2018;197(4):470–80. Epub 2017/10/21. doi: 10.1164/rccm.201706-1161OC. [PubMed: 29053005]
30. Rolling T, Wichmann D, Schmiedel S, Burchard GD, Kluge S, Cramer JP. Artesunate versus quinine in the treatment of severe imported malaria: comparative analysis of adverse events focussing on delayed haemolysis. *Malar J*. 2013;12:241 Epub 2013/07/17. doi: 10.1186/1475-2875-12-241. [PubMed: 23855745]
 31. Ndour PA, Larreche S, Mouri O, Argy N, Gay F, Roussel C, et al. Measuring the Plasmodium falciparum HRP2 protein in blood from artesunate-treated malaria patients predicts post-artesunate delayed hemolysis. *Sci Transl Med*. 2017;9(397). doi: 10.1126/scitranslmed.aaf9377.
 32. Ghosh K Pathogenesis of anemia in malaria: a concise review. *Parasitol Res*. 2007;101(6):1463–9. Epub 2007/09/18. doi: 10.1007/s00436-007-0742-1. [PubMed: 17874326]
 33. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, Ong'echa JM. Severe malarial anemia: innate immunity and pathogenesis. *International journal of biological sciences*. 2011;7(9):1427–42. Epub 2011/11/24. [PubMed: 22110393]
 34. de Back DZ, Kostova EB, van Kraaij M, van den Berg TK, van Bruggen R. Of macrophages and red blood cells; a complex love story. *Front Physiol*. 2014;5:9 Epub 2014/02/14. doi: 10.3389/fphys.2014.00009. [PubMed: 24523696]
 35. de Back DZ, Kostova EB, van Kraaij M, van den Berg TK, van Bruggen R. Of macrophages and red blood cells; a complex love story. *Front Physiol*. 2014;5:9 Epub 2014/02/14. doi: 10.3389/fphys.2014.00009. [PubMed: 24523696]
 36. Lutz HU, Bogdanova A. Mechanisms tagging senescent red blood cells for clearance in healthy humans. *Front Physiol*. 2013;4:387 Epub 2014/01/09. doi: 10.3389/fphys.2013.00387. [PubMed: 24399969]
 37. Mourao LC, Roma PM, Sultane Aboobacar Jda S, Medeiros CM, de Almeida ZB, Fontes CJ, et al. Anti-erythrocyte antibodies may contribute to anaemia in Plasmodium vivax malaria by decreasing red blood cell deformability and increasing erythrophagocytosis. *Malar J*. 2016;15(1):397. doi: 10.1186/s12936-016-1449-5. [PubMed: 27488382]
 38. Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. *Curr Opin Rheumatol*. 2010;22(5):561–6. Epub 2010/06/03. doi: 10.1097/01.bor.0000381996.69261.71. [PubMed: 20517154]
 39. Akilesh HM, Buechler MB, Duggan JM, Hahn WO, Matta B, Sun X, et al. Chronic TLR7 and TLR9 signaling drives anemia via differentiation of specialized hemophagocytes. *Science*. 2019;363(6423). Epub 2019/01/12. doi: 10.1126/science.aao5213.
 40. Lang E, Lang F. Triggers, inhibitors, mechanisms, and significance of eryptosis: the suicidal erythrocyte death. *Biomed Res Int*. 2015;2015:513518 Epub 2015/03/31. doi: 10.1155/2015/513518. [PubMed: 25821808]
 41. Ohyagi H, Onai N, Sato T, Yotsumoto S, Liu J, Akiba H, et al. Monocyte-derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses. *Immunity*. 2013;39(3):584–98. Epub 2013/09/17. doi: 10.1016/j.immuni.2013.06.019. [PubMed: 24035363]
 42. Holz LE, Fernandez-Ruiz D, Heath WR. Protective immunity to liver-stage malaria. *Clin Transl Immunology*. 2016;5(10):e105 Epub 2016/11/22. doi: 10.1038/cti.2016.60. [PubMed: 27867517]
 43. Junqueira C, Barbosa CRR, Costa PAC, Teixeira-Carvalho A, Castro G, Sen Santana S, et al. Cytotoxic CD8(+) T cells recognize and kill Plasmodium vivax-infected reticulocytes. *Nat Med*. 2018;24(9):1330–6. doi: 10.1038/s41591-018-0117-4. [PubMed: 30038217]
 44. Imai T, Ishida H, Suzue K, Taniguchi T, Okada H, Shimokawa C, et al. Cytotoxic activities of CD8(+) T cells collaborate with macrophages to protect against blood-stage murine malaria. *Elife*. 2015;4. doi: 10.7554/eLife.04232.
 45. Safeukui I, Gomez ND, Adelani AA, Burte F, Afolabi NK, Akondy R, et al. Malaria induces anemia through CD8+ T cell-dependent parasite clearance and erythrocyte removal in the spleen. *MBio*. 2015;6(1). Epub 2015/01/22. doi: 10.1128/mBio.02493-14.
 46. Raballah E, Kempaiah P, Karim Z, Orinda GO, Otieno MF, Perkins DJ, et al. CD4 T-cell expression of IFN-gamma and IL-17 in pediatric malarial anemia. *PLoS One*. 2017;12(4):e0175864 Epub 2017/04/21. doi: 10.1371/journal.pone.0175864. [PubMed: 28426727]

47. Morceau F, Dicato M, Diederich M. Pro-inflammatory cytokine-mediated anemia: regarding molecular mechanisms of erythropoiesis. *Mediators Inflamm.* 2009;2009:405016 Epub 2009/01/01. doi: 10.1155/2009/405016. [PubMed: 20204172]
48. Phalke S, Marrack P. Age (autoimmunity) associated B cells (ABCs) and their relatives. *Curr Opin Immunol.* 2018;55:75–80. Epub 2018/11/06. doi: 10.1016/j.coi.2018.09.007. [PubMed: 30388513]
49. Rivera-Correa JR A. A Role for Autoimmunity in the Immune Response against Malaria In: Rodriguez A, Mota M, editors. *Malaria - Immune Response to Infection and Vaccination*: Springer; 2016.
50. Rubtsova K, Rubtsov AV, Thurman JM, Mennona JM, Kappler JW, Marrack P. B cells expressing the transcription factor T-bet drive lupus-like autoimmunity. *J Clin Invest.* 2017;127(4):1392–404. Epub 2017/02/28. doi: 10.1172/JCI91250. [PubMed: 28240602]
51. Portugal S, Pierce SK, Crompton PD. Young lives lost as B cells falter: what we are learning about antibody responses in malaria. *Journal of immunology.* 2013;190(7):3039–46. Epub 2013/03/26. doi: 10.4049/jimmunol.1203067.
52. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, et al. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J Immunol.* 2009;183(3):2176–82. Epub 2009/07/14. doi: 10.4049/jimmunol.0901297. [PubMed: 19592645]
53. Kim CC, Baccarella AM, Bayat A, Pepper M, Fontana MF. FCRL5(+) Memory B Cells Exhibit Robust Recall Responses. *Cell Rep.* 2019;27(5):1446–60 e4. Epub 2019/05/03. doi: 10.1016/j.celrep.2019.04.019. [PubMed: 31042472]
54. Obeng-Adjei N, Portugal S, Holla P, Li S, Sohn H, Ambegaonkar A, et al. Malaria-induced interferon-gamma drives the expansion of Tbethi atypical memory B cells. *PLoS Pathog.* 2017;13(9):e1006576 Epub 2017/09/28. doi: 10.1371/journal.ppat.1006576. [PubMed: 28953967]
55. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, Li S, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. *Elife.* 2015;4 Epub 2015/05/09. doi: 10.7554/eLife.07218.
56. Sullivan RT, Kim CC, Fontana MF, Feeney ME, Jagannathan P, Boyle MJ, et al. FCRL5 Delineates Functionally Impaired Memory B Cells Associated with Plasmodium falciparum Exposure. *PLoS pathogens.* 2015;11(5):e1004894 Epub 2015/05/21. doi: 10.1371/journal.ppat.1004894. [PubMed: 25993340]
57. Perez-Mazliah D, Gardner PJ, Schweighoffer E, McLaughlin S, Hosking C, Tumwine I, et al. Plasmodium-specific atypical memory B cells are short-lived activated B cells. *Elife.* 2018;7 Epub 2018/11/06. doi: 10.7554/eLife.39800.
58. Portugal S, Obeng-Adjei N, Moir S, Crompton PD, Pierce SK. Atypical memory B cells in human chronic infectious diseases: An interim report. *Cell Immunol.* 2017;321:18–25. Epub 2017/07/25. doi: 10.1016/j.cellimm.2017.07.003. [PubMed: 28735813]
59. Muellenbeck MF, Ueberheide B, Amulic B, Epp A, Fenyo D, Busse CE, et al. Atypical and classical memory B cells produce Plasmodium falciparum neutralizing antibodies. *J Exp Med.* 2013;210(2):389–99. Epub 2013/01/16. doi: 10.1084/jem.20121970. [PubMed: 23319701]
60. Sundling C, Ronnberg C, Yman V, Asghar M, Jahnmatz P, Lakshmikanth T, et al. B cell profiling in malaria reveals expansion and remodelling of CD11c+ B cell subsets. *JCI Insight.* 2019;5 Epub 2019/04/03. doi: 10.1172/jci.insight.126492.
61. Mankelov TJ, Griffiths RE, Trompeter S, Flatt JF, Cogan NM, Massey EJ, et al. Autophagic vesicles on mature human reticulocytes explain phosphatidylserine-positive red cells in sickle cell disease. *Blood.* 2015;126(15):1831–4. Epub 2015/08/16. doi: 10.1182/blood-2015-04-637702. [PubMed: 26276668]
62. Guthmiller JJ, Graham AC, Zander RA, Pope RL, Butler NS. Cutting Edge: IL-10 Is Essential for the Generation of Germinal Center B Cell Responses and Anti-Plasmodium Humoral Immunity. *J Immunol.* 2017;198(2):617–22. doi: 10.4049/jimmunol.1601762. [PubMed: 27940658]
63. Rivera-Correa J, Rodriguez A. Divergent Roles of Antiself Antibodies during Infection. *Trends Immunol.* 2018;39(7):515–22. Epub 2018/05/05. doi: 10.1016/j.it.2018.04.003. [PubMed: 29724608]

64. Karnell JL, Kumar V, Wang J, Wang S, Voynova E, Ettinger R. Role of CD11c(+) T-bet(+) B cells in human health and disease. *Cell Immunol.* 2017;321:40–5. Epub 2017/08/02. doi: 10.1016/j.cellimm.2017.05.008. [PubMed: 28756897]
65. Wale N, Jones MJ, Sim DG, Read AF, King AA. The contribution of host cell-directed vs. parasite-directed immunity to the disease and dynamics of malaria infections. *Proc Natl Acad Sci U S A.* 2019;116(44):22386–92. Epub 2019/10/17. doi: 10.1073/pnas.1908147116. [PubMed: 31615885]

Highlights

- Autoimmune antibody responses against phosphatidylserine (PS) generated during malaria contribute to anemia.
- Anti-PS antibodies recognize uninfected erythrocytes during malaria and facilitate their clearance through opsonization or hemolysis.
- Similarly to some autoimmune disorders, during malaria, atypical B cells are activated through inflammatory signals, such as IFN γ and the activation of TLR-9 by parasite DNA.
- Levels of atypical memory B cells correlate with anti-PS antibodies and anemia in malaria patients.
- AtMBCs are able to secrete antibodies against parasite and autoimmune antigens.

Outstanding Questions

- Since published reports indicate that adults in malaria endemic areas present high levels of AtMBCs that have reduced antibody production, is the production of anti-PS antibodies different in first versus subsequent malaria infections?
- Is there a mechanistic relation between the increase of AtMBCs and the decrease of classical memory B cells during malaria?
- Is there a beneficial role for anti-PS antibodies during malaria?
- With the availability of PS-specific binding proteins, such as Annexin V, can targeting of anti-PS antibodies ameliorate human malarial anemia?

Figure 1, Key Figure. Proposed model for differentiation of atypical B cells secreting anti-PS antibodies and their roles in erythrocyte clearance during malaria.

During malaria, naïve B cells would encounter phosphatidylserine (PS), parasite DNA and IFN- γ which result in the activation of the B-cell receptor (BCR), TLR-9 and IFN- γ receptor, respectively. Activation of these receptors results in the differentiation of atypical B cells producing anti-PS antibodies. These antibodies bind uninfected erythrocytes that expose PS in their surface during malaria and facilitate their clearance by complement-mediated lysis or opsonization, promoting anemia in malaria patients.