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PARASITOLOGY (M BELEN CASSERA, SECTION EDITOR)

Insights on Rosetting Phenomenon in *Plasmodium vivax* Malaria

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Abstract

Purpose of Review Information about rosettes in *Plasmodium vivax* infection is scarce. However, the understanding of this phenomenon is important for elucidating the pathobiology of *Plasmodium* spp. This review summarizes the advances in the knowledge of rosetting phenomenon in *P. vivax* malaria.

Recent Findings In vitro and ex vivo studies seek to shed light on some aspects of rosetting in vivax malaria. The major efforts are to determine the purpose of this phenomenon and the elements involved in rosetting. Recent data reveal a receptor and suggest that specific components are involved in rosetting. Moreover, there is strong evidence supporting the role of rosettes as an immune evasion strategy.

Summary Although there are many unknown aspects behind rosetting, recent findings have contributed to elucidating rosette formation mechanisms and have clarified its role and biological hallmarks. These findings reinforce that rosetting is important and understanding the underlying biology may help develop new strategies for malaria control.

Keywords Rosetting · Rosette formation · *Plasmodium vivax* · *Malaria*

Introduction

Malaria is the most important and widespread tropical disease. Over 200 million new malaria cases are reported globally every year [1]. It is caused by the protozoan parasites *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* that normally infect apes. In addition to *P. knowlesi*, there are other *Plasmodium* species that infect non-human primates and can be transmitted to humans, such as *P. brasilianum* [2], *P. cynomolgi* [3], and recently identified *P. simium* [4].

Plasmodium vivax is the most prevalent species outside of sub-Saharan Africa [5]. Globally, 53% of *P. vivax infections* occur in the Southeast Asia region. Furthermore, this species is the most prevalent in Latin America responsible for 75% of malaria cases [1]. While *P. vivax* infection has lower mortality rates than that of *P. falciparum*, the impact of *P. vivax* on global public health remains profound. Moreover, severe cases including cerebral and placental malaria caused by *P. vivax* begun to be reported in recent years [6–13].

In falciparum malaria, the severity of cerebral and placental malaria is in part attributed to the adhesive potential of the infected red blood cells [14, 15]. Cytoadherence is primarily observed in adhesion of infected red blood cell (iRBC) to endothelial cells [16–18], placental tissues [19–22], and to uninfected red blood cells (uRBCs) [23, 24]. Although cytoadhesion is better described in *P. falciparum* infections, it also occurs with *P. vivax*. In the past decade, indication of this phenomenon has been observed in the spleen, lung, and endothelial cells [25–28]. The adhesion of a *P. vivax* iRBC to uRBCs, forming rosettes, has also been observed in several studies [29••, 30••, 31•, 32].

Rosette phenomenon is very complex, and many of the components involved are known in falciparum malaria [33]. The interactions between infected and uninfected red blood cells are best understood in falciparum malaria, owing to a well-established in vitro culture [34]. However, there is not

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an efficient long-term in vitro culture method for *P. vivax*. Cryopreserved field isolates, once thawed, present a lower frequency of rosetting compared to fresh ones [29••, 35]. As rosetting has been linked to pathogenesis, its understanding and functional significance in vivax malaria are essential. In this article, we discuss the recent advances in understanding the importance of this phenomenon to vivax malaria pathogenesis.

General Rosetting Characteristics in *Plasmodium* Species

Rosetting occurs when two or more uRBCs bind to a central parasitized red blood cell [14]. The first reports of rosette formation were published in the 1980s in *P. fragile* [14], *P. chabaudii* [36], and *P. falciparum* [37, 38]. Some years later, rosette formation was reported in three other human-infecting species: *P. ovale* [39], *P. malarie* [40], and *P. vivax* [32].

As seen for *P. falciparum*, *P. vivax* rosettes are also formed by a RBC infected with late parasite stages (mature trophozoites or schizonts) [29••, 32] or gametocytes [29••] and uRBCs. However, for *P. vivax*, this interaction occurs almost exclusively between iRBC and normocytes (mature uRBCs) [29••]. The adhesion between iRBC and uRBC cells is strong in both *P. falciparum* and *P. vivax*, resulting in similar stability even by high physiological shear flow [35, 41]. The attachment of at least one uRBC to an iRBC reduces the membrane deformability of the *P. vivax*-parasitized cell; however, the molecular basis involved in this process is still not elucidated [35].

Rosetting has been implicated in the pathogenesis of severe falciparum malaria [42–44]. This phenomenon is thought to contribute to the vascular occlusion in individuals infected with *P. falciparum* [45], which can lead to complications such as cerebral malaria. Indeed, some studies show the association between rosetting and cerebral malaria [44, 46]. In addition, high levels of rosette formation are associated with increased severity in African children infected with *P. falciparum* [42, 44, 47]. In keeping with the association with high pathogenicity, rosettes are seldom seen in isolates from uncomplicated falciparum malaria [42, 44, 45].

While cases of severe vivax malaria are less frequent than of falciparum malaria, rosetting is a common phenomenon observed more frequently in *P. vivax* [30••, 48] than *P. falciparum* isolates [29••]. Rosetting in vivax infections were correlated with anemia in pregnant women, as decreased hemoglobin and hematocrit were associated with higher levels of rosettes [31•]. However, no clear correlation with severity has yet been observed.

There is growing evidence that the ABO blood group influences host susceptibility to severe disease in *P. falciparum* infection. Studies showed that blood group O conferred protection against severe disease compared with non-O blood groups (A, B, or AB) [49–51]. *P. falciparum* binds to molecules on the surface of uRBCs for rosette formation, and the involvement of ABO antigens in this phenomenon is well studied. In vitro experiments showed that rosettes are preferentially formed in the presence of non-O blood groups [32, 52–54]. The same pattern was visualized in *P. falciparum* field isolates, where individuals with high levels of rosetting were from non-O blood groups [42, 50, 55]. However, in infections caused by *P. vivax*, rosettes are formed independently of human blood groups A, B, and O [29••, 48]. At this time, there are no published studies directed to understanding this disparity.

The Role of Rosette Formation during *Plasmodium vivax* Infection

Rosetting has been described for four parasite species infecting humans and in several species that infect other hosts. Hence, rosettes likely have an important biological function in the parasite–host relationship. However, their role remains unclear. In addition, most of data available thus far are based on *P. falciparum* studies.

There are two major hypotheses to explain the functions of this formation, suggesting their contribution to the growth and survival of the parasite during infection. The first hypothesis is that rosettes could facilitate merozoite invasion into erythrocytes ensuring parasite survival and replication [56]. However, this postulate is controversial and unlikely. Although there is evidence of a positive correlation between rosetting and parasitemia in both *P. falciparum* [57] and *P. vivax* [30••], this was not observed in other studies [29••, 58, 59].

The hypothesis of "facilitating merozoite invasion" could be supported in vivax malaria if the uRBCs forming rosettes were predominantly composed of reticulocytes, since *P. vivax* invades exclusively young erythrocytes. However, there is a preference for normocytes instead of reticulocytes in rosette formation, indicating that rosetting is unlikely to help merozoite invasion in *P. vivax* infection [29••]. Moreover, Lee and colleagues defend that this is an improbable role, since *P. vivax* and *P. falciparum* start to form rosettes at early trophozoite stage, long before merozoite expulsion and re-invasion. Finally, the observation of rosetting in gametocytes reinforces even more that this hypothesis is improbable [29••].

The second postulated role is that rosettes act as an immune evasion mechanism when uRBCs mask the parasitized RBC and prevent its clearance by the host immune system [52]. Recent studies have attempted to elucidate this potential escape mechanism. When leukocytes were added to *P. vivax* and *P. falciparum* field isolates, the rosetting rates increased. Next, it was visualized that iRBCs stimulate monocytes to produce insulin growth factor binding protein

7 (IGFBP7) and other molecules. In the presence of IGFBP7, the rate of rosetting increased while the number of iRBC phagocytosed by monocytes decreased, suggesting that rosettes protect iRBCs from the immune system [60••]. In another recent work, transcriptional analyses showed that the phagocytosis pathway may be affected by rosette formation [30••]. In this study, it was also found that a higher phagocytosis index by both the THP-1 cell line and human peripheral blood mononuclear cells (PBMC) was higher when rosettes were mechanically disrupted when compared to intact rosettes [30••]. Together, these data support the role of rosetting as an evasion mechanism in vivax malaria.

Parasite Ligands and Erythrocytic Receptors Involved in Rosette Formation

Three families of variant surface antigen families are known as the most important ligands mediating rosetting in *P. falciparum* infection: *P. falciparum*-infected erythrocyte membrane protein 1 (PfEMP1) [61], repetitive interspersed family (RIFIN) [62], and subtelomeric variant open reading frame protein (STEVOR) [63] (Fig. 1a).

PfEMP1 is the most well-characterized antigen related to rosette formation and is capable of binding to multiple host cell receptors including complement receptor 1 (CR1) [64], heparan sulfate (HS), and the blood groups A and B trisaccharides [45, 54, 65]. The molecular interaction between PfEMP1 and blood group A trisaccharide is the best understood [54]. Interestingly, there is a preference for group A over B, with formation of larger and stronger rosettes as a result of the interaction between PfEMP1 and the A trisaccharide [33, 54, 57]. Likewise, RIFINs are likely binding to uRBCs using the glycophorin A receptor and blood group A antigens [62]. Finally, STEVOR specifically binds to glycophorin C and mediates the rosette formation [63]. Besides the receptors mentioned, the surface antigen CD36 can also act as a receptor for iRBCs in falciparum malaria [66].

Recently, a second type of rosette has been described and named type II rosettes, where the interaction between iRBC and uRBCs does not occur directly but mediated by IGFBP7 and other serum factors, which acts as a bridge between ligand of iRBC and HS expressed by uRBCs. PfEMP1 is probably the main ligand involved in this process in *P. falciparum* [60••].

Ligands and receptors involved in rosette formation are not well described in non-falciparum malaria (Fig. 1b). In addition, extrapolation of the knowledge about *P. falciparum* receptors and ligands is not possible. *P. vivax* does not express PfEMP1 orthologues and the erythrocytic receptors involved in rosetting of *P. falciparum*, which interact with PfEMP1, such as the ABO blood group and CR1, does not seem to be involved in *P. vivax* rosetting [29••, 64]. Thus far, glycophorin C is the only well-described receptor for *P. vivax* rosette formation [29••].

The type II rosettes also occur in vivax malaria since iRBCs interact with IGFBP7. However, there is still no evidence for which protein(s) might mediate *P. vivax* interactions since this parasite does not express PfEMP1. Lee and colleagues suggested that many ligands are involved in rosetting, whereas the one interacting with IGFBP7 is very sensitive to trypsin [60••]. The receptor HS also seems to be important to type II rosette formation. According to the authors, parts of the receptor need to be present on the membrane of uRBCs for IGFBP7 to stimulate the formation of the rosettes [60••]. Moreover, as observed for *P. falciparum* [53, 67], *P. vivax* rosettes are sensitive to heparin [48].

One of the family proteins that might mediate the rosetting phenomenon in vivax malaria is the tryptophan-rich antigens (PvTRAgs). These proteins, synthetized by *Pv-fam-a* family, are highly expressed in *P. vivax* and present the capacity to interact with a restricted number of erythrocyte antigens, notably chymotrypsin-sensitive erythrocyte receptor [68, 69]. Some TRAgs expressed in early stages of the parasite life cycle (ring or trophozoite) may be able to recognize distinct receptors on the membrane of uRBC, contributing to rosette formation [69, 70]. Additionally, other antigens are related to this phenomenon. The VIR proteins, members of the biggest family of variant genes in *P. vivax*, have been studied for their potential to adhere to endothelial receptors such as ICAM-1 and CSA [25, 71], especially in subfamily C [26]. Therefore, it is speculated that these antigens may have a role in the adhesion to uRBCs.

Rosette-Stimulating Factors

The process of rosette formation does not only depend on receptors and ligands but also on serum components that stimulate adhesion. Several *P. falciparum* isolates are depending on plasma components to form rosettes [72]. The same seems to happen in *P. vivax* rosettes. When rosettes were evaluated in presence of Albumax instead of plasma, this formation was not observed [30••]. Moreover, rosettes are enhanced in presence of autologous immune plasma, indicating that some plasma factor(s) might afford rosette formation in vivax malaria [30••].

Scholander and collegues (1996) stated that IgM and possibly IgG were essential for the stable adhesion in rosettes of *P. falciparum* [73]. At the time, the exact role of these molecules was not clear. In the context of IgM, there are iRBCs that bind to natural antibodies, also called “non-immune” IgM from the human serum. However, some *P. falciparum* isolates do not bind to natural IgM [74, 75]. It is also known that different parasite isolates expressing distinct PfEMP1 variants are capable of binding similar sites on the IgM, preferably at Fc domains [74]. However, despite some studies suggesting

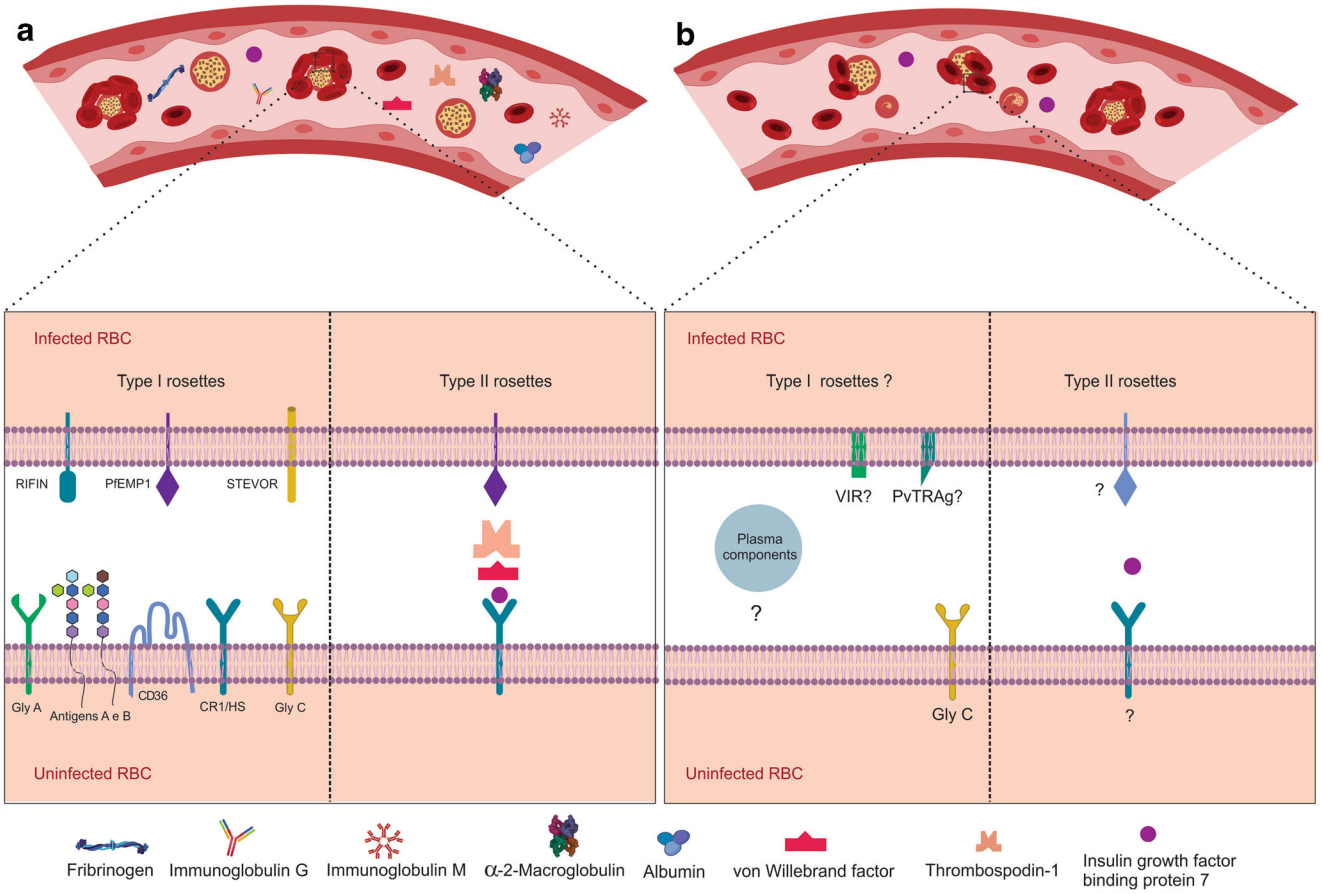

Fig. 1 Schematic illustrating the rosetting phenomenon in malaria. Rosetting is a very complex phenomenon and several plasma components are involved. Expressed ligands on the surface of the infected RBC interact with receptors expressed on the uninfected RBC, mediating rosette formation during *P. falciparum* (**a**) and *P. vivax* (**b**) infections. Question marks represent plasma components, receptors, and ligands that are not well described. *PfEMP1 Plasmodium falciparum* erythrocyte membrane protein 1, *RIFIN* repetitive interspersed repeats, *STEVOR* subtelomeric variable open reading frame, *RBC* red blood cell, *CR1*, complement receptor 1, *HS* Heparan sulfate, *CD36* cluster of differentiation 36, *Gly A* Glycophorin A, *Gly C* Glycophorin C

that IgG could also be involved in *P. falciparum* rosetting [73, 76, 77], the role of IgG in rosettes is still controversial [57, 74]. In vivax malaria, a positive correlation between total IgM but not IgG and rosetting was observed; however, it is still not known the meaning of this connection [30••].

As immunoglobulins cannot induce rosette formation just by themselves, it is speculated that other serum components play a role in rosetting [72, 73]. High levels of adhesive molecules such as IgM, fibrinogen, and albumin may form agglutinates called rouleaux. According to Treutiger and colleagues, intense episodes of rouleaux might assist adhesion [74]. Furthermore, it was recently described that type II rosettes require, in addition to IGFBP7, two serum components—von Willebrand factor (VWF) and thrombospondin-1 (TSP-1)—to mediate rosetting in *P. falciparum* [60••]. In addition, other serum factors such as complement factor D (CFD) and periostin (OSF2) seem to play less of a role in rosette stimulation than IGFBP7 in both *P. falciparum* and *P. vivax* infections [60••].

Information about the nature of other serum factors in rosetting is still vague in vivax malaria but is certain that plasma contain important components to promote rosetting. Future studies would provide more details on how plasma factors are related to *P. vivax* rosetting phenomenon.

Conclusions

Rosette formation is an important aspect in the pathobiology of malaria. Although studies of *P. falciparum* help in formulating hypotheses for *P. vivax*, rosettes are clearly distinct in each species. In recent years, some studies have shown the relevance of this process to vivax malaria pathology. However, there are still many unresolved questions, such as which is (are) the parasite ligand(s) for *P. vivax* rosettes and what is the importance of plasma factors for rosetting formation in vivax malaria. The rosetting phenomenon in vivax malaria is a potentially important future area of study that will

help in understanding the pathogenesis and contribute to the design of new strategies for malaria control.

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Compliance of Ethical Standards

Conflict of Interest Najara C. Bittencourt, Letícia P. Bertolla, and Letusa Albrecht declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. WHO. World malaria report 2019. Geneva2019. p. 232.
2. Lalremruata A, Magris M, Vivas-Martínez S, Koehler M, Esen M, Kempaiah P, et al. Natural infection of Plasmodium brasilianum in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. EBioMedicine. 2015;2(9):1186–92. https://doi.org/10.1016/j.ebiom.2015.07.033.
3. Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. First case of a naturally acquired human infection with Plasmodium cynomolgi. Malar J. 2014;13:68. https://doi.org/10.1186/1475-2875-13-68.
4. Brasil P, Zalis MG, de Pina-Costa A, Siqueira AM, Júnior CB, Silva S, et al. Outbreak of human malaria caused by Plasmodium simium in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. Lancet Glob Health. 2017;5(10):e1038–e46. https://doi.org/10.1016/S2214-109X(17)30333-9.
5. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK, et al. Global epidemiology of Plasmodium vivax. Am J Trop Med Hyg. 2016;95(6 Suppl):15–34. https://doi.org/10.4269/ajtmh.16-0141.
6. Costa FT, Lopes SC, Albrecht L, Ataide R, Siqueira AM, Souza RM, et al. On the pathogenesis of Plasmodium vivax malaria: perspectives from the Brazilian field. Int J Parasitol. 2012;42(12):1099–105. **S0020-7519(12)00221-4 [pii]**. https://doi.org/10.1016/j.ijpara.2012.08.007.
7. Alexandre MA, Ferreira CO, Siqueira AM, Magalhães BL, Mourão MP, Lacerda MV, et al. Severe Plasmodium vivax malaria. Brazilian Amazon Emerg Infect Dis. 2010;16(10):1611–4. https://doi.org/10.3201/eid1610.100685.
8. Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, et al. Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med. 2008;5(6):e127. https://doi.org/10.1371/journal.pmed.0050127.
9. Aashish A, Manigandan G. Complicated vivax malaria, an often underestimated condition - case Report. J Fam Community Med. 2015;22(3):180–2. https://doi.org/10.4103/2230-8229.163040.
10. Rahimi BA, Thakkinstian A, White NJ, Sirivichayakul C, Dondorp AM, Chokejindachai W. Severe vivax malaria: a systematic review and meta-analysis of clinical studies since 1900. Malar J. 2014;13:481. https://doi.org/10.1186/1475-2875-13-481.
11. Chaikitgosiyakul S, Rijken MJ, Muehlenbachs A, Lee SJ, Chaisri U, Viriyavejakul P, et al. A morphometric and histological study of placental malaria shows significant changes to villous architecture in both Plasmodium falciparum and Plasmodium vivax infection. Malar J. 2014;13:4. https://doi.org/10.1186/1475-2875-13-4.
12. Naing C, Whittaker MA, Nyunt Wai V, Mak JW. Is Plasmodium vivax malaria a severe malaria?: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2014;8(8):e3071. https://doi.org/10.1371/journal.pntd.0003071.
13. Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-resistant Plasmodium vivax associated with severe and fatal malaria: a prospective study in Papua. Indonesia PLoS Med. 2008;5(6):e128. https://doi.org/10.1371/journal.pmed.0050128.
14. David PH, Handunnetti SM, Leech JH, Gamage P, Mendis KN. Rosetting: a new cytoadherence property of malaria-infected erythrocytes. Am J Trop Med Hyg. 1988;38(2):289–97. https://doi.org/10.4269/ajtmh.1988.38.289.
15. MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, Warrell DA. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. Am J Pathol. 1985;119(3):385–401.
16. Chattopadhyay R, Taneja T, Chakrabarti K, Pillai CR, Chitnis CE. Molecular analysis of the cytoadherence phenotype of a Plasmodium falciparum field isolate that binds intercellular adhesion molecule-1. Mol Biochem Parasitol. 2004;133(2):255–65. https://doi.org/10.1016/j.molbiopara.2003.08.014.
17. Claessens A, Adams Y, Ghumra A, Lindergard G, Buchan CC, Andisi C, et al. A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells. Proc Natl Acad Sci U S A. 2012;109(26):E1772–81. https://doi.org/10.1073/pnas.1120461109.
18. Sampath S, Brazier AJ, Avril M, Bernabeu M, Vigdorovich V, Mascarenhas A, et al. Plasmodium falciparum adhesion domains linked to severe malaria differ in blockade of endothelial protein C receptor. Cell Microbiol. 2015;17(12):1868–82. https://doi.org/10.1111/cmi.12478.
19. Reeder JC, Cowman AF, Davern KM, Beeson JG, Thompson JK, Rogerson SJ, et al. The adhesion of Plasmodium falciparum-infected erythrocytes to chondroitin sulfate A is mediated by P. falciparum erythrocyte membrane protein 1. Proc Natl Acad Sci U S A. 1999;96(9):5198–202. https://doi.org/10.1073/pnas.96.9.5198.
20. Buffet PA, Gamain B, Scheidig C, Baruch D, Smith JD, Hernandez-Rivas R, et al. Plasmodium falciparum domain mediating adhesion to chondroitin sulfate A: a receptor for human placental infection. Proc Natl Acad Sci U S A. 1999;96(22):12743–8. https://doi.org/10.1073/pnas.96.22.12743.
21. Trimnell AR, Kraemer SM, Mukherjee S, Phippard DJ, Janes JH, Flamoe E, et al. Global genetic diversity and evolution of var genes associated with placental and severe childhood malaria. Mol Biochem Parasitol. 2006;148(2):169–80. https://doi.org/10.1016/j.molbiopara.2006.03.012.
22. Higgins MK. The structure of a chondroitin sulfate-binding domain important in placental malaria. J Biol Chem. 2008;283(32):21842–6. https://doi.org/10.1074/jbc.C800086200.
23. Fernandez V, Treutiger CJ, Nash GB, Wahlgren M. Multiple adhesive phenotypes linked to rosetting binding of erythrocytes in Plasmodium falciparum malaria. Infect Immun. 1998;66(6):2969–75. https://doi.org/10.1128/IAI.66.6.2969-2975.1998.
24. Ribacke U, Moll K, Albrecht L, Ahmed Ismail H, Normark J, Flaberg E, et al. Improved in vitro culture of Plasmodium falciparum permits establishment of clinical isolates with preserved

multiplication, invasion and rosetting phenotypes. PLoS One. 2013;8(7):e69781. https://doi.org/10.1371/journal.pone.0069781.
25. Carvalho BO, Lopes SC, Nogueira PA, Orlandi PP, Bargieri DY, Blanco YC, et al. On the cytoadhesion of Plasmodium vivax-infected erythrocytes. J Infect Dis. 2010;202(4):638–47. https://doi.org/10.1086/654815.
26. Fernandez-Becerra C, Bernabeu M, Castellanos A, Correa BR, Obadia T, Ramirez M, et al. Spleen-dependent genes encode antigens associated with cytoadhesion and clinical protection. Proc Natl Acad Sci U S A. 2020;117(23):13056–65. https://doi.org/10.1073/pnas.1920596117.
27. Lacerda MV, Fragoso SC, Alecrim MG, Alexandre MA, Magalhães BM, Siqueira AM, et al. Postmortem characterization of patients with clinical diagnosis of Plasmodium vivax malaria: to what extent does this parasite kill? Clin Infect Dis. 2012;55(8):e67–74. https://doi.org/10.1093/cid/cis615.
28. Lopes SC, Albrecht L, Carvalho BO, Siqueira AM, Thomson-Luque R, Nogueira PA, et al. Paucity of Plasmodium vivax mature schizonts in peripheral blood is associated with their increased cytoadhesive potential. J Infect Dis. 2014;209(9):1403–7. https://doi.org/10.1093/infdis/jiu018.
29.•• Lee WC, Malleret B, Lau YL, Mauduit M, Fong MY, Cho JS, et al. Glycophorin C (CD236R) mediates vivax malaria parasite rosetting to normocytes. Blood. 2014;123(18):e100–9. https://doi.org/10.1182/blood-2013-12-541698. **This article identified glycophorin C as a receptor at non-infected red blood cells for *P. vivax* rosettes.**
30.•• Albrecht L, Lopes SCP, da Silva ABIE, Barbosa V, Almeida RP, Siqueira AM, et al. Rosettes integrity protects Plasmodium vivax of being phagocytized. Sci Rep. 2020;10(1):16706. https://doi.org/10.1038/s41598-020-73713-w. **This article showed that *P. vivax* parasites forming a rosette are less likely phagocyted.**
31.• Marín-Menéndez A, Bardají A, Martínez-Espinosa FE, Bôtto-Menezes C, Lacerda MV, Ortiz J, et al. Rosetting in Plasmodium vivax: a cytoadhesion phenotype associated with anaemia. PLoS Negl Trop Dis. 2013;7(4):e2155. https://doi.org/10.1371/journal.pntd.0002155. **This article associated *P. vivax* rosettes with anemia in pregnant women.**
32. Udomsanpetch R, Thanikkul K, Pukrittayakamee S, White NJ. Rosette formation by Plasmodium vivax. Trans R Soc Trop Med Hyg. 1995;89(6):635–7. https://doi.org/10.1016/0035-9203(95)90422-0.
33. McQuaid F, Rowe JA. Rosetting revisited: a critical look at the evidence for host erythrocyte receptors in. Parasitology. 2020;147(1):1–11. https://doi.org/10.1017/S0031182019001288.
34. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193(4254):673–5. https://doi.org/10.1126/science.781840.
35. Zhang R, Lee WC, Lau YL, Albrecht L, Lopes SC, Costa FT, et al. Rheopathologic consequence of Plasmodium vivax rosette formation. PLoS Negl Trop Dis. 2016;10(8):e0004912. https://doi.org/10.1371/journal.pntd.0004912.
36. Wahlgren M. Antigens and antibodies involved in humoral immunity to *Plasmodium falciparum*. **Karolinska Institutet**; 1986.
37. Udomsangpetch R, Wåhlin B, Carlson J, Berzins K, Torii M, Aikawa M, et al. Plasmodium falciparum-infected erythrocytes form spontaneous erythrocyte rosettes. J Exp Med. 1989;169(5):1835–40. https://doi.org/10.1084/jem.169.5.1835.
38. Handunnetti SM, David PH, Perera KL, Mendis KN. Uninfected erythrocytes form "rosettes" around Plasmodium falciparum infected erythrocytes. Am J Trop Med Hyg. 1989;40(2):115–8. https://doi.org/10.4269/ajtmh.1989.40.115.
39. Angus BJ, Thanikkul K, Silamut K, White NJ, Udomsangpetch R. Short report: rosette formation in Plasmodium ovale infection. Am J Trop Med Hyg. 1996;55(5):560–1. https://doi.org/10.4269/ajtmh.1996.55.560.
40. Lowe BS, Mosobo M, Bull PC. All four species of human malaria parasites form rosettes. Trans R Soc Trop Med Hyg. 1998;92(5):526. https://doi.org/10.1016/s0035-9203(98)90901-4.
41. Nash GB, Cooke BM, Carlson J, Wahlgren M. Rheological properties of rosettes formed by red blood cells parasitized by Plasmodium falciparum. Br J Haematol. 1992;82(4):757–63. https://doi.org/10.1111/j.1365-2141.1992.tb06955.x.
42. Rowe A, Obeiro J, Newbold CI, Marsh K. Plasmodium falciparum rosetting is associated with malaria severity in Kenya. Infect Immun. 1995;63(6):2323–6. https://doi.org/10.1128/IAI.63.6.2323-2326.1995.
43. Mayor A, Hafiz A, Bassat Q, Rovira-Vallbona E, Sanz S, Machevo S, et al. Association of severe malaria outcomes with platelet-mediated clumping and adhesion to a novel host receptor. PLoS One. 2011;6(4):e19422. https://doi.org/10.1371/journal.pone.0019422.
44. Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM, Wahlgren M. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. Lancet. 1990;336(8729):1457–60. https://doi.org/10.1016/0140-6736(90)93174-n.
45. Chen Q, Schlichtherle M, Wahlgren M. Molecular aspects of severe malaria. Clin Microbiol Rev. 2000;13(3):439–50. https://doi.org/10.1128/cmr.13.3.439-450.2000.
46. O'Donnell RA, Saul A, Cowman AF, Crabb BS. Functional conservation of the malaria vaccine antigen MSP-119across distantly related Plasmodium species. Nat Med. 2000;6(1):91–5. https://doi.org/10.1038/71595.
47. Doumbo OK, Thera MA, Koné AK, Raza A, Tempest LJ, Lyke KE, et al. High levels of Plasmodium falciparum rosetting in all clinical forms of severe malaria in African children. Am J Trop Med Hyg. 2009;81(6):987–93. https://doi.org/10.4269/ajtmh.2009.09-0406.
48. Chotivanich KT, Pukrittayakamee S, Simpson JA, White NJ, Udomsangpetch R. Characteristics of Plasmodium vivax-infected erythrocyte rosettes. Am J Trop Med Hyg. 1998;59(1):73–6. https://doi.org/10.4269/ajtmh.1998.59.73.
49. Pathirana SL, Alles HK, Bandara S, Phone-Kyaw M, Perera MK, Wickremasinghe AR, et al. ABO-blood-group types and protection against severe, Plasmodium falciparum malaria. Ann Trop Med Parasitol. 2005;99(2):119–24. https://doi.org/10.1179/136485905X19946.
50. Rowe JA, Handel IG, Thera MA, Deans AM, Lyke KE, Koné A, et al. Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced rosetting. Proc Natl Acad Sci U S A. 2007;104(44):17471–6. https://doi.org/10.1073/pnas.0705390104.
51. Deepa AVA, Rameshkumar K, Ross C. ABO blood groups and malaria related clinical outcome. J Vector Borne Dis. 2011;48(1):7–11.
52. Moll K, Palmkvist M, Ch'ng J, Kiwuwa MS, Wahlgren M. Evasion of immunity to Plasmodium falciparum: rosettes of blood group A impair recognition of PfEMP1. PLoS One. 2015;10(12):e0145120. https://doi.org/10.1371/journal.pone.0145120.
53. Carlson J, Wahlgren M. Plasmodium falciparum erythrocyte rosetting is mediated by promiscuous lectin-like interactions. J Exp Med. 1992;176(5):1311–7. https://doi.org/10.1084/jem.176.5.1311.
54. Vigan-Womas I, Guillotte M, Juillerat A, Hessel A, Raynal B, England P, et al. Structural basis for the ABO blood-group dependence of Plasmodium falciparum rosetting. PLoS Pathog. 2012;8(7):e1002781. https://doi.org/10.1371/journal.ppat.1002781.
55. Barragan A, Kremsner PG, Wahlgren M, Carlson J. Blood group A antigen is a coreceptor in Plasmodium falciparum rosetting. Infect

Immun. 2000;68(5):2971–5. https://doi.org/10.1128/iai.68.5.2971-2975.2000.
56. Wahlgren M, Carlson J, Udomsangpetch R, Perlmann P. Why do Plasmodium falciparumm-infected erythrocytes form spontaneous erythrocyte rosettes? Parasitol Today. 1989;5(6):183–5. https://doi.org/10.1016/0169-4758(89)90141-5.
57. Rowe JA, Obiero J, Marsh K, Raza A. Short report: positive correlation between rosetting and parasitemia in Plasmodium falciparum clinical isolates. Am J Trop Med Hyg. 2002;66(5):458–60. https://doi.org/10.4269/ajtmh.2002.66.458.
58. Clough B, Atilola FA, Pasvoi G. The role of rosetting in the multiplication of Plasmodium falciparum: rosette formation neither enhances nor targets parasite invasion into uninfected red cells. Br J Haematol. 1998;100(1):99–104. https://doi.org/10.1046/j.1365-2141.1998.00534.x.
59. Deans AM, Rowe JA. Plasmodium falciparum: rosettes do not protect merozoites from invasion-inhibitory antibodies. Exp Parasitol. 2006;112(4):269–73. https://doi.org/10.1016/j.exppara.2005.11.007.
60.•• Lee WC, Russell B, Sobota RM, Ghaffar K, Howland SW, Wong ZX, et al. -infected erythrocytes induce secretion of IGFBP7 to form type II rosettes and escape phagocytosis. Elife. 2020;9. doi: https://doi.org/10.7554/eLife.51546. **This article identified IGFBP7 as an important component for type II rosettes.**
61. Chen Q, Barragan A, Fernandez V, Sundström A, Schlichtherle M, Sahlén A, et al. Identification of Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) as the rosetting ligand of the malaria parasite P. falciparum. J Exp Med. 1998;187(1):15–23. https://doi.org/10.1084/jem.187.1.15.
62. Goel S, Palmkvist M, Moll K, Joannin N, Lara P, Akhouri RR, et al. RIFINs are adhesins implicated in severe Plasmodium falciparum malaria. Nat Med. 2015;21(4):314–7. https://doi.org/10.1038/nm.3812.
63. Niang M, Bei AK, Madnani KG, Pelly S, Dankwa S, Kanjee U, et al. STEVOR is a Plasmodium falciparum erythrocyte binding protein that mediates merozoite invasion and rosetting. Cell Host Microbe. 2014;16(1):81–93. https://doi.org/10.1016/j.chom.2014.06.004.
64. Rowe JA, Moulds JM, Newbold CI, Miller LH. P. falciparum rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. Nature. 1997;388(6639):292–5. https://doi.org/10.1038/40888.
65. Normark J, Nilsson D, Ribacke U, Winter G, Moll K, Wheelock CE, et al. PfEMP1-DBL1alpha amino acid motifs in severe disease states of Plasmodium falciparum malaria. Proc Natl Acad Sci U S A. 2007;104(40):15835–40. https://doi.org/10.1073/pnas.0610485104.
66. Handunnetti SM, van Schravendijk MR, Hasler T, Barnwell JW, Greenwalt DE, Howard RJ. Involvement of CD36 on erythrocytes as a rosetting receptor for Plasmodium falciparum-infected erythrocytes. Blood. 1992;80(8):2097–104.
67. Rowe A, Berendt AR, Marsh K, Newbold CI. Plasmodium falciparum: a family of sulphated glycoconjugates disrupts erythrocyte rosettes. Exp Parasitol. 1994;79(4):506–16. https://doi.org/10.1006/expr.1994.1111.
68. Alam MS, Zeeshan M, Rathore S, Sharma YD. Multiple Plasmodium vivax proteins of Pv-fam-a family interact with human erythrocyte receptor band 3 and have a role in red cell invasion. Biochem Biophys Res Commun. 2016;478(3):1211–6. https://doi.org/10.1016/j.bbrc.2016.08.096.
69. Zeeshan M, Tyagi RK, Tyagi K, Alam MS, Sharma YD. Host-parasite interaction: selective Pv-fam-a family proteins of Plasmodium vivax bind to a restricted number of human erythrocyte receptors. J Infect Dis. 2015;211(7):1111–20. https://doi.org/10.1093/infdis/jiu558.
70. Tyagi K, Gupta D, Saini E, Choudhary S, Jamwal A, Alam MS, et al. Recognition of human erythrocyte receptors by the tryptophan-rich antigens of monkey malaria parasite Plasmodium knowlesi. PLoS One. 2015;10(9):e0138691. https://doi.org/10.1371/journal.pone.0138691.
71. Bernabeu M, Lopez FJ, Ferrer M, Martin-Jaular L, Razaname A, Corradin G, et al. Functional analysis of Plasmodium vivax VIR proteins reveals different subcellular localizations and cytoadherence to the ICAM-1 endothelial receptor. Cell Microbiol. 2012;14(3):386–400. https://doi.org/10.1111/j.1462-5822.2011.01726.x.
72. Treutiger CJ, Scholander C, Carlson J, McAdam KP, Raynes JG, Falksveden L, et al. Rouleaux-forming serum proteins are involved in the rosetting of Plasmodium falciparum-infected erythrocytes. Exp Parasitol. 1999;93(4):215–24. https://doi.org/10.1006/expr.1999.4454.
73. Scholander C, Treutiger CJ, Hultenby K, Wahlgren M. Novel fibrillar structure confers adhesive property to malaria-infected erythrocytes. Nat Med. 1996;2(2):204–8. https://doi.org/10.1038/nm0296-204.
74. Ghumra A, Semblat JP, McIntosh RS, Raza A, Rasmussen IB, Braathen R, et al. Identification of residues in the Cmu4 domain of polymeric IgM essential for interaction with Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1). J Immunol. 2008;181(3):1988–2000. https://doi.org/10.4049/jimmunol.181.3.1988.
75. Ghumra A, Semblat JP, Ataide R, Kifude C, Adams Y, Claessens A, et al. Induction of strain-transcending antibodies against Group A PfEMP1 surface antigens from virulent malaria parasites. PLoS Pathog. 2012;8(4):e1002665. https://doi.org/10.1371/journal.ppat.1002665.
76. Rasti N, Namusoke F, Chêne A, Chen Q, Staalsoe T, Klinkert MQ, et al. Nonimmune immunoglobulin binding and multiple adhesion characterize Plasmodium falciparum-infected erythrocytes of placental origin. Proc Natl Acad Sci U S A. 2006;103(37):13795–800. https://doi.org/10.1073/pnas.0601519103.
77. Flick K, Scholander C, Chen Q, Fernandez V, Pouvelle B, Gysin J, et al. Role of nonimmune IgG bound to PfEMP1 in placental malaria. Science. 2001;293(5537):2098–100. https://doi.org/10.1126/science.1062891.