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A Review of Immune Responses to Plasmodium vivax Duffy Binding Protein from a 15-Year Follow-up Study in an Area of Unstable Malaria Transmission in the Amazon

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Abstract

Plasmodium vivax, the second most common human malaria parasite, remains a major public health concern, and no vaccine has yet been approved. Nevertheless, a recent clinical trial provided evidence of partial protection by a vaccine targeting Duffy binding protein region II (DBPII), a ligand that mediates reticulocyte invasion through its interaction with the human atypical chemokine receptor 1 (ACKR1), also known as the Duffy antigen receptor for chemokines (DARC). This success supports the DBPII/DARC interaction as a validated and promising target for blood-stage vaccines against *P. vivax*. Amid progress in the development of *P. vivax* blood-stage vaccines, we are investigating immune responses to DBPII in individuals exposed to different levels of malaria transmission in Brazil. Here, we reviewed key advances in DBP research and analyzed data from a 15-year open cohort of *P. vivax*-exposed individuals in the Brazilian Amazon, providing new insights into immune features associated with persistent DBPII antibody responses and protection against malaria.

Background

The effectiveness of most current malaria prevention tools remains insufficient against *Plasmodium vivax*, a parasite that continues to be highly prevalent in Southeast Asia and South America [1] and whose public health significance is increasingly recognized in Africa [2]. A major challenge for *Plasmodium vivax* control and elimination lies in its biological complexity. First, eliminating the dormant liver stage of *P. vivax*, which is responsible for multiple relapses, remains difficult because the efficacy of the only available anti-relapse therapy is affected by human genetic polymorphisms [3–6]. Second, subpatent *P. vivax* reservoirs cannot be detected by conventional malaria diagnostic methods, including cryptic erythrocytic infections that are sequestered in the bone marrow and spleen (revised by [7, 8]). Consequently, subpatent infections are not taken into account in global burden estimates [9], although contributing to recurrent and chronic infections [10]. Additional biological challenges include the ability to infect mosquitoes before the onset of clinical symptoms (revised by [11]) and emerging antimalarial drug resistance [12]. Consequently, it is not unexpected that the proportion of malaria caused by *P. vivax* has been increasing in co-endemic regions where intensive malaria-control activities have successfully reduced the incidence of *P. falciparum* [13].

Developing a safe and effective vaccine against vivax malaria could assist efforts to eliminate *P. vivax*. Despite potential obstacles associated with the development of *P. vivax* vaccines -- which include a complex life cycle, the absence of long-term cell culture systems and restricted animal models -- significant progress has been made in the last few years (revised by [10, 14–16]). Notably, a substantial number of *P. vivax* vaccine trials have been registered in recent years (ClinicalTrials.gov; https://clinicaltrials.gov, *P. vivax* vaccine). Of interesting, modelling studies predict that a combination of vaccines, even with modest efficacy against different parasite stages, can provide additive or synergistic reductions in *P. vivax* transmission [17].

Aiming to contribute to current efforts towards *P. vivax* vaccine development, we have concentrated on naturally acquired immunity to the Duffy binding protein (DBP), a blood-stage invasion ligand that plays a critical role in the *P. vivax* blood-stage infection [18–20]. Briefly, the DBP is a microneme protein that belongs to the Duffy binding-like erythrocyte-binding protein (DBL-EBP) family, and the DBL domain of DBP (region II, DBPII) binds to the human atypical chemokine receptor 1 (ACKR1), also known as the Duffy antigen receptor for chemokines (DARC), which is located on the surface of red blood cells [21–24]. The importance of this interaction is underscored by the finding that individuals with a mutation in the DARC promoter are highly resistant to *P. vivax* infection [25]. DBPII and DARC form a 2:2 complex through receptor-mediated dimerization, in which an N-terminal helix from each DARC monomer binds at the interface between two DBL domains [26, 27]. The DBP/DARC-binding interface was recently expanded to include a sulfated tyrosine binding site adjacent to the primary DARC binding site [28] **(**Fig. 1**).** Therefore, a vaccine that elicits antibodies that bind the DBPII/DARC or DBPII dimer interfaces would be expected to block parasite invasion (revised by [16]).

A historical overview of key research findings on DBP is presented in Fig. 2 [18, 22–27, 29–53]. In this review, we compiled data from a long-term follow-up study of the DBP in the Brazilian Amazon area, where *P. vivax* is responsible for almost two thirds of all cases [1].

DBPII Antibodies in Primary P. vivax Infection: Short-Lived and Strain-Specific

Few studies have investigated DBP antibody responses during primary *P. vivax* infection in healthy adults, with limited data largely derived from controlled human malaria infection (CHMI) studies involving DBPII-based vaccines [52]. To investigate whether a first *P. vivax* exposure was able to induce DBPII antibodies, and if this response could be boosted by relapses/recrudescence, we took advantage of a small outbreak of vivax malaria, located in a non-malarious area of Brazil [36]. Although DBP was recognized initially by only 20% of primary infected individuals, a new episode of blood-stage infection increased the proportion of IgG responders to 80%. Despite the occurrence of recurrent infections, most of these individuals lost their anti-DBP antibody response within 6 months of follow-up. A similar pattern of response was detected in serology using a recombinant protein representing the ligand domain of the DBP (cysteine-rich region II, DBPII) whose sequences were homologous of the *P. vivax* outbreak isolate [54]. An important finding of the *P. vivax* outbreak study was the discovery of how parasite genetic diversity relates to naturally acquired human antibodies for inhibition of DBPII–DARC binding (BIAbs). In vitro assays with mammalian cells expressing homologous or heterologous DBPII sequences from the outbreak isolate, allowed us to undoubtedly demonstrate that BIAbs activity is biased towards a specific allele [36], as suggested previously using rabbit immune sera [35]. This first description of DBPII strain specificity in humans with primary infection was later confirmed in children from Papua New Guinea (PNG) [37]. More recently, we demonstrated that a primary *P. vivax* infection leads to long-term DBPII-specific memory B cells (MBCs) [55]. Specifically, using an ELISPOT assay, we demonstrated that individuals exposed to a *P. vivax* outbreak more than 10 years earlier harbored MBCs that produced

antibodies with specific activity against DBPII (Sal-1) as well as the engineered DBPII immunogen, DEKnull-2. Further studies of isolated DBPII-specific MBCs from individuals exposed to *P. vivax* outbreaks are needed to confirm these findings.

A second putative erythrocyte binding protein (EBP2, also named EBP/DBP2) was identified, and this protein harbors all the features of the DBL-EBP family, including a Duffy-binding-like domain (Region II, EBP2-II) [56]. We demonstrated that EBP2-II is antigenically distinct from DBPII and could not be functionally inhibited by anti-DBPII antibodies [57]. Considering the relevance of this novel DBL member, the EBP2 antibody response profile was retrospectively investigated in primary/recurrent samples from the *P. vivax* outbreak. The results showed that a primary *P. vivax* infection was not sufficient to induce significant IgM/IgG antibodies to EBP2-II and contrary to anti-DBPII antibody response, EBP2-II antibodies were not boosted by relapses or recrudescences [54]. After sequencing the full DBL domain of the EBP2 outbreak isolate, it was possible to conclude that polymorphisms in the *ebp2* gene were unlikely to explain the absence of anti-EBP2 booster after a brief *P. vivax* exposure. In accordance, our results showed that the DBL domain of EBP2 was much less polymorphic than that of DBPII, with a single haplotype as highly prevalent worldwide [58]. In this context, it is possible to speculate that the absence of booster could be associated with restricted host cell binding specificity of EBP2 which preferentially binds to an immature reticulocyte subpopulation that is generally restricted to the bone marrow (DARC-positive $CD71^{high}$) [57]. Considering *P. vivax* bone marrow niche environment [7, 59, 60], antibodies against EBP2-II may require a much more prolonged exposure to peripheral blood stages to be detected. According, EBP2-II was highly recognized by long-term malaria-exposed individuals, with higher levels of IgG associated with multiple vivax malaria episodes in the Amazon area [54, 61]. Altogether, the outbreak study showed EBP2-II, compared with DBPII, was poorly immunogenic among individuals who experienced their first blood-stage *P. vivax* infection. However, EBP2 was highly immunogenic in long-term malaria exposed individuals, reinforcing its potential to compose a multiple blood-stage *P. vivax* vaccine [61].

DBPII Antibodies after Long-Term P. vivax Exposure: Strain-Specific and Strain-Transcending Responses

Malaria and Population Background in the Study Setting

In Latin America, most of malaria burden is found within the Amazon Basin, which includes nine countries and territories in South America [62]. In this region, three countries, the Bolivarian Republic of Venezuela, Brazil and Colombia accounted for more than 75% of all cases in the region [1], with most of the cases due to *P. vivax* (~ 70% in 2024). In Brazil, the economic burden of malaria is concentrated in the Brazilian Amazon, a malaria-endemic region that accounts for more than 99% of malaria cases, which are predominantly caused by *P. vivax* [62, 63]. *P. vivax* infections are more likely to be subpatent and remain asymptomatic as malaria transmission decreases [64].

In a small rural community in the Brazilian Amazon, the Rio Pardo settlement, we have conducted a 15-year longitudinal study of DBP-specific immune responses [61]. Malaria transmission in the study area is considered hypo- to mesoendemic [65, 66]. Most residents are native to the Amazon region, contributing to a relatively high Amerindian genomic ancestry (~ 40%) despite the highly admixed Brazilian population [67]. In this area, the distribution of DARC genotypes was consistent with the heterogeneous ethnic origins of the Amazon population, with a predominance of non-silent DARC alleles, with *FY*A* > *FY*B* [68]. As most individuals were DARC-positive, the *FY*A* allele was associated with a reduced risk of clinical malaria, whereas the *FY*B* allele was associated with an increased susceptibility, as previously demonstrated by others [41].

Strain-specific and Strain-transcending antibodies in the Amazon area

In the study area, we demonstrated that, within the same population with DBPII strain-specific immunity, a small proportion of individuals exhibited stable, strain-transcending DBPII inhibitory responses [46, 68, 69]. This finding appears to be common across different endemic settings and populations [37, 70]. The reason for this may lie in the fact that, while naturally acquired DBPII antibodies tend to be biased toward strain-specific responses [36], protective neutralizing IgG antibodies target epitopes that overlap with conserved residues essential for DARC binding and DBP dimerization [47, 48, 71, 72].

Using a DBPII-based multiplex assay to assess strain-transcending antibodies, we demonstrated that antibody breadth, defined as reactivity to multiple DBPII variants, rather than the magnitude of anti-DBPII antibodies, correlated with the BIAb response [73]. Considering that broadly neutralizing antibodies are associated with reduced risk of *P. vivax* clinical malaria [40, 74, 75], DBPII inhibitory responses should be tracked in all DBPII-based vaccine strategies.

To focus protective immune responses on conserved epitopes, structure-based design strategies have been employed to develop surface-engineered DBP immunogens [43, 44, 76]. In the first generation of engineered DBP immunogens (named DEKnull), all B-cell epitopes, except for the mutated polymorphic DEK motif, were conserved with the original native DBP sequence from which it was derived [76]. While the DEKnull vaccine confirmed the potential to target strain-transcending immunity, its low immunogenicity led to additional surface engineering of the next generation immunogen, DEKnull-2 [45]. In the study area, the same authors demonstrated that conserved functional epitopes on the newly engineered DEKnull-2 antigen are targeted by naturally acquired blocking inhibitory antibodies (BIAbs), contributing to long-term *P. vivax* protective immune responses. Considering the kinetics of IgM/IgG antibodies over time, IgG responses to the epitope-conserved DEKnull-2 remained stable and associated with a broadly neutralizing BIAb response, while DBP variant-specific IgG antibodies waned during low transmission [77]. In contrast, IgM responses tended to be strain-specific and were not associated with the presence or absence of broadly neutralizing IgG antibodies.

More recently, increased DBP gene copy number has been linked to parasite evasion of anti-DBP humoral immunity [78], with multi-copy parasites being more frequent among asymptomatic than symptomatic infections [79]. In this context, we observed a low but appreciable frequency of gene amplification in members of the erythrocyte-binding-like protein family, including DBP and EBP2, in *P. vivax* isolates from the Amazon rainforest, where DARC negativity is rare [80]. These findings support the current view that *P. vivax* parasites carrying multiple copies of the DBP gene represent a globally widespread phenomenon [81–85], and this should be considered when designing DBPII-based vaccines.

Host genetics influences DBPII-Specific Antibody Responses

In the open cohort study, it was demonstrated that host DARC polymorphisms may influence the naturally acquired inhibitory anti-DBPII immunity [68]. Of note, the naturally acquired DBPII specific binding inhibitory antibodies (BIAbs) tended to be more frequent in heterozygous individuals carrying a DARC-silent allele (*FY*BES*). No clear explanation exists for the higher inhibitory antibody levels in individuals with a single versus two DARC-positive alleles, as DARC has not been shown to downregulate humoral responses to *P. vivax* [86]. Although these in vitro assays have advanced our understanding of DBPII–DARC interactions, long-term *P. vivax* culture could provide further insights into the role of DARC genotypes in invasion and immune responses.

Given the high rate of non-responsiveness to DBPII among *P. vivax*-exposed individuals, we explored whether DBPII responsiveness was linked to HLA class II. The results showed that individuals carrying the *DRB1*13:01* allele were more likely to develop and sustain a DBPII-specific IgG response, whereas those with the *DRB1*14:02-DQA1*05:03-DQB1*03:01* haplotype were persistent non-responders [46]. HLA class II gene polymorphisms also influenced the BIAbs response, with three alleles (*DRB1*07:01, DQA1*02:01* and *DQB1*02:02*) comprising a single haplotype linked with the presence and persistence of the BIAbs response. Notably, these alleles exhibited strong linkage disequilibrium, indicating they are co-inherited more frequently than expected by chance. Despite limited data on the association between HLA alleles and *P. vivax* antibody responses [87], systematic reviews and meta-analyses have identified associations between HLA alleles linked to higher (e.g., *DRB1*13:01*) or poorer DBPII antibody responses (e.g., the DRB1*14 allelic group) and vaccination responses to microbial infections, including hepatitis B, influenza, serogroup C meningococcus, measles, and rubella [88–90]. Although responsiveness to *P. vivax* antigens is likely multifactorial and influenced by different genetic factors [91], our findings suggest that DBPII peptide-binding affinities to HLA class II molecules may guide strategies to modulate helper T-cell activation, which could be relevant for the development of DBPII-based vaccines.

Coordinated Cytokine Signatures of persistent Anti-DBPII responders

Despite the key role of antibodies in malaria immunity, mechanisms sustaining long-lasting DBPII responses remain unclear [74, 92]. In this open cohort study, *P. vivax*-exposed individuals with long-term strain-transcending DBPII responses -- defined here as persistent responders -- were characterized by

exhibiting IgG responses to multiple *P. vivax* blood-stage antigens, with prominent reactivity to surface antigens (such as MSP1-19 and MSP5), the apical antigen (AMA1), and invasion-related antigens, including EBP2 and RBP2a [93]. This broadly reactive antibody profile remained consistent throughout the cohort, even in the absence of frequent boosting from reinfection.

To further describe the profile of persistent responders, cytokine responses were measured in supernatants of PBMC cultures stimulated with DBPII-based antigens. Persistent responders showed an inverse pattern of pro-inflammatory cytokine responses, with increased IFN-γ associated with lower TNF and IL-6 levels [93]. This pattern may reflect a balance between protective immunity and immunopathology, as IL-6 and TNF are commonly associated with malaria infection and disease severity [94–96]. Of note, DBPII-persistent responders presented high levels of both IL-4 and IFN-γ as compared with non-responder individuals, consistent with previous studies indicating that these cytokines are strong predictors of the protective immunity induced by malaria vaccines [97, 98]. Further studies should investigate the complex nature of cellular interactions required for long-term DBPII protective immune responses.

Conclusions

In our long-term open cohort study conducted in an area of low and intermittent malaria transmission, we demonstrated that, beyond strain-specific DBPII immunity, a proportion of individuals were DBPII-persistent responders, exhibiting stable, strain-transcending DBPII inhibitory responses. While these findings have been reported by others, our cohort study provides evidence that DBPII-persistent responders also maintained stable and broad antibody responses against diverse *P. vivax* blood-stage proteins, even in the absence of frequent reinfection-induced boosting. These targets included proteins with distinct parasite localizations and functions, such as merozoite surface proteins, exported or parasite-associated proteins, apical organelle proteins, and members of different protein families involved in erythrocyte invasion. Furthermore, DBPII-persistent responders exhibited a distinct immunological profile characterized by regulation and expansion of multiple functional cytokine pathways. Factors associated with parasite genetics, including gene amplification, and host genetics appear to influence this profile of response. Taken together, further studies should investigate the complex cellular interactions underlying long-term DBPII protective immune responses and determine whether *P. vivax*-experienced IgG^+ B cells require continuous activation to maintain these responses or whether they reflect bona fide immunological memory.

Declarations

Author Contribution

FSK: Conceptualization, Data curation, Writing – original draft, Writing – review and editing, Supervision. MFAN: Writing – review and editing, Visualization, and figure preparation. SLMN: Writing – review and editing, Visualization, and figure preparation. GMF: Writing – review and editing, Visualization, and figure

preparation. LHC: Writing – original draft and revision, Conceptualization, Supervision, Resources, and Data curation. All authors reviewed and approved the final version of the manuscript.

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References

1. World malaria report. 2025 [https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2025]
2. Golassa L, Amenga-Etego L, Lo E, Amambua-Ngwa A. The biology of unconventional invasion of Duffy-negative reticulocytes by Plasmodium vivax and its implication in malaria epidemiology and public health. Malar J. 2020;19:299.
3. Alving AS, Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. Science. 1956;124:484–5.
4. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. Lancet. 2008;371:64–74.
5. Pybus BS, Sousa JC, Jin X, Ferguson JA, Christian RE, Barnhart R, Vuong C, Sciotti RJ, Reichard GA, Kozar MP, et al. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, anti-malarial drug primaquine. Malar J. 2012;11:259.
6. Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, Deye G, Melendez V, Ockenhouse CF. Primaquine failure and cytochrome P-450 2D6 in Plasmodium vivax malaria. N Engl J Med. 2013;369:1381–2.
7. Fernandez-Becerra C, Aparici-Herraiz I, Del Portillo HA. Cryptic erythrocytic infections in Plasmodium vivax, another challenge to its elimination. Parasitol Int. 2022;87:102527.
8. Khan N, Daily JP. Update on pathogenesis, management, and control of Plasmodium vivax. Curr Opin Infect Dis. 2022;35:404–9.
9. Battle KE, Lucas TCD, Nguyen M, Howes RE, Nandi AK, Twohig KA, Pfeffer DA, Cameron E, Rao PC, Casey D, et al. Mapping the global endemicity and clinical burden of Plasmodium vivax, 2000-17: a spatial and temporal modelling study. Lancet. 2019;394:332–43.

10. Anstey NM, Tham WH, Shanks GD, Poespoprodjo JR, Russell BM, Kho S. The biology and pathogenesis of vivax malaria. Trends Parasitol. 2024;40:573–90.
11. Adams JH, Mueller I. The Biology of Plasmodium vivax. Cold Spring Harb Perspect Med 2017, 7.
12. Ferreira MU, Nobrega de Sousa T, Rangel GW, Johansen IC, Corder RM, Ladeia-Andrade S, Gil JP. Monitoring Plasmodium vivax resistance to antimalarials: Persisting challenges and future directions. Int J Parasitol Drugs Drug Resist. 2020;15:9–24.
13. Price RN, Commons RJ, Battle KE, Thriemer K, Mendis K. Plasmodium vivax in the Era of the Shrinking P. falciparum Map. Trends Parasitol. 2020;36:560–70.
14. De SL, Ntumngia FB, Nicholas J, Adams JH. Progress towards the development of a P. vivax vaccine. Expert Rev Vaccines 2021:1–16.
15. da Veiga GTS, Moriggi MR, Vettorazzi JF, Muller-Santos M, Albrecht L. Plasmodium vivax vaccine: What is the best way to go? Front Immunol. 2022;13:910236.
16. Dickey TH, Tolia NH. Designing an effective malaria vaccine targeting Plasmodium vivax Duffy-binding protein. Trends Parasitol. 2023;39:850–8.
17. White M, Chitnis CE. Potential role of vaccines in elimination of Plasmodium vivax. Parasitol Int. 2022;90:102592.
18. Chitnis CE, Chaudhuri A, Horuk R, Pogo AO, Miller LH. The domain on the Duffy blood group antigen for binding Plasmodium vivax and P. knowlesi malarial parasites to erythrocytes. J Exp Med. 1996;184:1531–6.
19. Adams JH, Sim BK, Dolan SA, Fang X, Kaslow DC, Miller LH. A family of erythrocyte binding proteins of malaria parasites. Proc Natl Acad Sci U S A. 1992;89:7085–9.
20. Barnwell JW, Galinski MR. Plasmodium vivax: a glimpse into the unique and shared biology of the merozoite. Ann Trop Med Parasitol. 1995;89:113–20.
21. Haynes JD, Dalton JP, Klotz FW, McGinniss MH, Hadley TJ, Hudson DE, Miller LH. Receptor-like specificity of a Plasmodium knowlesi malarial protein that binds to Duffy antigen ligands on erythrocytes. J Exp Med. 1988;167:1873–81.
22. Wertheimer SP, Barnwell JW. Plasmodium vivax interaction with the human Duffy blood group glycoprotein: identification of a parasite receptor-like protein. Exp Parasitol. 1989;69:340–50.
23. Adams JH, Hudson DE, Torii M, Ward GE, Wellems TE, Aikawa M, Miller LH. The Duffy receptor family of Plasmodium knowlesi is located within the micronemes of invasive malaria merozoites. Cell. 1990;63:141–53.
24. Fang XD, Kaslow DC, Adams JH, Miller LH. Cloning of the Plasmodium vivax Duffy receptor. Mol Biochem Parasitol. 1991;44:125–32.
25. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med. 1976;295:302–4.
26. Batchelor JD, Zahm JA, Tolia NH. Dimerization of Plasmodium vivax DBP is induced upon receptor binding and drives recognition of DARC. Nat Struct Mol Biol. 2011;18:908–14.

27. Batchelor JD, Malpede BM, Omattage NS, DeKoster GT, Henzler-Wildman KA, Tolia NH. Red blood cell invasion by Plasmodium vivax: structural basis for DBP engagement of DARC. PLoS Pathog. 2014;10:e1003869.
28. Moskovitz R, Pholcharee T, DonVito SM, Guloglu B, Lowe E, Mohring F, Moon RW, Higgins MK. Structural basis for DARC binding in reticulocyte invasion by Plasmodium vivax. Nat Commun. 2023;14:3637.
29. Barnwell JW, Nichols ME, Rubinstein P. In vitro evaluation of the role of the Duffy blood group in erythrocyte invasion by Plasmodium vivax. J Exp Med. 1989;169:1795–802.
30. Chitnis CE, Miller LH. Identification of the erythrocyte binding domains of Plasmodium vivax and Plasmodium knowlesi proteins involved in erythrocyte invasion. J Exp Med. 1994;180:497–506.
31. Tsuboi T, Kappe SH, al-Yaman F, Prickett MD, Alpers M, Adams JH. Natural variation within the principal adhesion domain of the Plasmodium vivax duffy binding protein. Infect Immun. 1994;62:5581–6.
32. Ampudia E, Patarroyo MA, Patarroyo ME, Murillo LA. Genetic polymorphism of the Duffy receptor binding domain of Plasmodium vivax in Colombian wild isolates. Mol Biochem Parasitol. 1996;78:269–72.
33. Ranjan A, Chitnis CE. Mapping regions containing binding residues within functional domains of Plasmodium vivax and Plasmodium knowlesi erythrocyte-binding proteins. Proc Natl Acad Sci U S A. 1999;96:14067–72.
34. Xainli J, Adams JH, King CL. The erythrocyte binding motif of plasmodium vivax duffy binding protein is highly polymorphic and functionally conserved in isolates from Papua New Guinea. Mol Biochem Parasitol. 2000;111:253–60.
35. VanBuskirk KM, Cole-Tobian JL, Baisor M, Sevova ES, Bockarie M, King CL, Adams JH. Antigenic drift in the ligand domain of Plasmodium vivax duffy binding protein confers resistance to inhibitory antibodies. J Infect Dis. 2004;190:1556–62.
36. Ceravolo IP, Sanchez BA, Sousa TN, Guerra BM, Soares IS, Braga EM, McHenry AM, Adams JH, Brito CF, Carvalho LH. Naturally acquired inhibitory antibodies to Plasmodium vivax Duffy binding protein are short-lived and allele-specific following a single malaria infection. Clin Exp Immunol. 2009;156:502–10.
37. Cole-Tobian JL, Michon P, Biasor M, Richards JS, Beeson JG, Mueller I, King CL. Strain-specific duffy binding protein antibodies correlate with protection against infection with homologous compared to heterologous plasmodium vivax strains in Papua New Guinean children. Infect Immun. 2009;77:4009–17.
38. Ryan JR, Stoute JA, Amon J, Dunton RF, Mtalib R, Koros J, Owour B, Luckhart S, Wirtz RA, Barnwell JW, Rosenberg R. Evidence for transmission of Plasmodium vivax among a duffy antigen negative population in Western Kenya. Am J Trop Med Hyg. 2006;75:575–81.
39. Cavasini CE, Mattos LC, Couto AA, Bonini-Domingos CR, Valencia SH, Neiras WC, Alves RT, Rossit AR, Castilho L, Machado RL. Plasmodium vivax infection among Duffy antigen-negative individuals

from the Brazilian Amazon region: an exception? Trans R Soc Trop Med Hyg. 2007;101:1042–4.

40. King CL, Michon P, Shakri AR, Marcotty A, Stanisic D, Zimmerman PA, Cole-Tobian JL, Mueller I, Chitnis CE. Naturally acquired Duffy-binding protein-specific binding inhibitory antibodies confer protection from blood-stage Plasmodium vivax infection. Proc Natl Acad Sci U S A. 2008;105:8363–8.
41. King CL, Adams JH, Xianli J, Grimberg BT, McHenry AM, Greenberg LJ, Siddiqui A, Howes RE, da Silva-Nunes M, Ferreira MU, Zimmerman PA. Fy(a)/Fy(b) antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to Plasmodium vivax malaria. Proc Natl Acad Sci U S A. 2011;108:20113–8.
42. Menard D, Chan ER, Benedet C, Ratsimbasoa A, Kim S, Chim P, Do C, Witkowski B, Durand R, Thellier M, et al. Whole genome sequencing of field isolates reveals a common duplication of the Duffy binding protein gene in Malagasy Plasmodium vivax strains. PLoS Negl Trop Dis. 2013;7:e2489.
43. Ntumngia FB, Adams JH. Design and immunogenicity of a novel synthetic antigen based on the ligand domain of the Plasmodium vivax duffy binding protein. Clin Vaccine Immunol. 2012;19:30–6.
44. Ntumngia FB, Barnes SJ, McHenry AM, George MT, Schloegel J, Adams JH. Immunogenicity of a synthetic vaccine based on Plasmodium vivax Duffy binding protein region II. Clin Vaccine Immunol. 2014;21:1215–23.
45. Ntumngia FB, Pires CV, Barnes SJ, George MT, Thomson-Luque R, Kano FS, Alves JRS, Urusova D, Pereira DB, Tolia NH, et al. An engineered vaccine of the Plasmodium vivax Duffy binding protein enhances induction of broadly neutralizing antibodies. Sci Rep. 2017;7:13779.
46. Kano FS, Souza-Silva FA, Torres LM, Lima BA, Sousa TN, Alves JR, Rocha RS, Fontes CJ, Sanchez BA, Adams JH, et al. The Presence, Persistence and Functional Properties of Plasmodium vivax Duffy Binding Protein II Antibodies Are Influenced by HLA Class II Allelic Variants. PLoS Negl Trop Dis. 2016;10:e0005177.
47. Carias LL, Dechavanne S, Nicolete VC, Sreng S, Suon S, Amaratunga C, Fairhurst RM, Dechavanne C, Barnes S, Witkowski B, et al. Identification and Characterization of Functional Human Monoclonal Antibodies to Plasmodium vivax Duffy-Binding Protein. J Immunol. 2019;202:2648–60.
48. Urusova D, Carias L, Huang Y, Nicolete VC, Popovici J, Roesch C, Salinas ND, Dechavanne S, Witkowski B, Ferreira MU, et al. Structural basis for neutralization of Plasmodium vivax by naturally acquired human antibodies that target DBP. Nat Microbiol. 2019;4:1486–96.
49. Rawlinson TA, Barber NM, Mohring F, Cho JS, Kosaisavee V, Gerard SF, Alanine DGW, Labbe GM, Elias SC, Silk SE, et al. Structural basis for inhibition of Plasmodium vivax invasion by a broadly neutralizing vaccine-induced human antibody. Nat Microbiol. 2019;4:1497–507.
50. Bouyssou I, El Hoss S, Doderer-Lang C, Schoenhals M, Rasoloharimanana LT, Vigan-Womas I, Ratsimbasoa A, Abate A, Golassa L, Mabilotte S, et al. Unveiling P. vivax invasion pathways in Duffy-negative individuals. Cell Host Microbe. 2023;31:2080–92. e2085.
51. Dechavanne C, Dechavanne S, Bosch J, Metral S, Redinger KR, Watson QD, Ratsimbasoa AC, Roeper B, Krishnan S, Fong R et al. Duffy antigen is expressed during erythropoiesis in Duffy-negative

individuals. *Cell Host Microbe* 2023, 31:2093–2106 e2097.

52. Hou MM, Barrett JR, Themistocleous Y, Rawlinson TA, Diouf A, Martinez FJ, Nielsen CM, Lias AM, King LDW, Edwards NJ, et al. Vaccination with Plasmodium vivax Duffy-binding protein inhibits parasite growth during controlled human malaria infection. Sci Transl Med. 2023;15:eadf1782.
53. Martinez FJ, White M, Guillotte-Blisnick M, Huon C, Boucharlat A, Agou F, England P, Popovici J, Hou MM, Silk SE, et al. PvDBPII elicits multiple antibody-mediated mechanisms that reduce growth in a Plasmodium vivax challenge trial. NPJ Vaccines. 2024;9:10.
54. Lima BAS, Fernandes GM, Torres LM, Pires CV, Alves JRS, Moreira-Nascimento SL, Nascimento MFA, Afonso SL, Costa HL, Ceravolo IP, et al. Antibody response to a new member of the DBL family (EBP2) after a brief Plasmodium vivax exposure. PLoS Negl Trop Dis. 2022;16:e0010493.
55. Lima BAS. Antígenos de Plasmodium vivax envolvidos na invasão de reticulócitos: avaliação da resposta imune naturalmente adquirida após única exposição. Fiocruz, PPG Ciências da Saúde; 2022.
56. Hester J, Chan ER, Menard D, Mercereau-Puijalon O, Barnwell J, Zimmerman PA, Serre D. De novo assembly of a field isolate genome reveals novel Plasmodium vivax erythrocyte invasion genes. PLoS Negl Trop Dis. 2013;7:e2569.
57. Ntumngia FB, Thomson-Luque R, Torres Lde M, Gunalan K, Carvalho LH, Adams JH. A Novel Erythrocyte Binding Protein of Plasmodium vivax Suggests an Alternate Invasion Pathway into Duffy-Positive Reticulocytes. mBio 2016, 7.
58. Fernandes GM, Rodrigues-Mattos GH, Torres LM, Guedes KS, Fontes CJF, Ntumngia FB, Adams JH, Brito CFA, Kano FS, de Sousa TN, Carvalho LH. Natural genetic diversity of the DBL domain of a novel member of the Plasmodium vivax erythrocyte binding-like proteins (EBP2) in the Amazon rainforest. Infect Genet Evol. 2024;123:105628.
59. Silva-Filho JL, Lacerda MVG, Recker M, Wassmer SC, Marti M, Costa FTM. Plasmodium vivax in Hematopoietic Niches: Hidden and Dangerous. Trends Parasitol. 2020;36:447–58.
60. Angrisano F, Robinson LJ. Plasmodium vivax - How hidden reservoirs hinder global malaria elimination. Parasitol Int. 2022;87:102526.
61. Fernandes GM, Moreira-Nascimento MFA, Torres S, Abreu LM, Silva BG, ;Ntumngia CFOD, Adams FB, Fontes JH, Kano CJF, Carvalho FS. L.H.: Magnitude and longevity of antibody responses to key Plasmodium vivax Erythrocyte Binding–Like proteins in an amazonian cohort: a long-term follow-up study. BMC Infect Dis 2026, 18 August 2026.
62. Ferreira MU, Gamboa D, Torres K, Rodriguez-Ferrucci H, Soto-Calle VE, Pardo K, Fontoura PS, Tomko SS, Gazzinelli RT, Conn JE, et al. Evidence-Based Malaria Control and Elimination in the Amazon: Input from the International Center of Excellence in Malaria Research Network in Peru and Brazil. Am J Trop Med Hyg. 2022;107:160–7.
63. Andrade MV, Noronha K, Guedes GR, Carvalho LR, Bracarense H, Juliao NA, Souza A, Diniz BC, Silva VA, Motta-Santos AS, et al. Economic burden of malaria in the Brazilian Amazon from a societal perspective. PLOS Glob Public Health. 2026;6:e0006061.

64. Fontoura PS, Macedo EG, Calil PR, Corder RM, Rodrigues PT, Tonini J, Esquivel FD, Ladeia WA, Fernandes ARJ, Johansen IC, et al. Changing Clinical Epidemiology of Plasmodium vivax Malaria as Transmission Decreases: Population-Based Prospective Panel Survey in the Brazilian Amazon. J Infect Dis. 2024;229:947–58.
65. Kano FS, Sanchez BA, Sousa TN, Tang ML, Saliba J, Oliveira FM, Nogueira PA, Goncalves AQ, Fontes CJ, Soares IS, et al. Plasmodium vivax Duffy binding protein: baseline antibody responses and parasite polymorphisms in a well-consolidated settlement of the Amazon Region. Trop Med Int Health. 2012;17:989–1000.
66. Guimaraes LFF, Rodrigues BA, Dias MHF, Barcelos MG, Nascimento MFA, Moreira-Nascimento SL, Afonso SL, Abreu BGS, Middeldorp JM, Ntumngia FB, et al. Antibody response to Plasmodium vivax in the context of Epstein-Barr virus (EBV) co-infection: A 14-year follow-up study in the Amazon rainforest. PLoS ONE. 2025;20:e0311704.
67. Kano FS, de Souza AM, de Menezes Torres L, Costa MA, Souza-Silva FA, Sanchez BAM, Fontes CJF, Soares IS, de Brito CFA, Carvalho LH, Sousa TN. Susceptibility to Plasmodium vivax malaria associated with DARC (Duffy antigen) polymorphisms is influenced by the time of exposure to malaria. Sci Rep. 2018;8:13851.
68. Souza-Silva FA, Torres LM, Santos-Alves JR, Tang ML, Sanchez BA, Sousa TN, Fontes CJ, Nogueira PA, Rocha RS, Brito CF, et al. Duffy antigen receptor for chemokine (DARC) polymorphisms and its involvement in acquisition of inhibitory anti-duffy binding protein II (DBPII) immunity. PLoS ONE. 2014;9:e93782.
69. Ceravolo IP, Souza-Silva FA, Fontes CJ, Braga EM, Madureira AP, Krettli AU, Souza JM, Brito CF, Adams JH, Carvalho LH. Inhibitory properties of the antibody response to Plasmodium vivax Duffy binding protein in an area with unstable malaria transmission. Scand J Immunol. 2008;67:270–8.
70. Xainli J, Cole-Tobian JL, Baisor M, Kastens W, Bockarie M, Yazdani SS, Chitnis CE, Adams JH, King CL. Epitope-specific humoral immunity to Plasmodium vivax Duffy binding protein. Infect Immun. 2003;71:2508–15.
71. Chootong P, Ntumngia FB, VanBuskirk KM, Xainli J, Cole-Tobian JL, Campbell CO, Fraser TS, King CL, Adams JH. Mapping epitopes of the Plasmodium vivax Duffy binding protein with naturally acquired inhibitory antibodies. Infect Immun. 2010;78:1089–95.
72. George MT, Schloegel JL, Ntumngia FB, Barnes SJ, King CL, Casey JL, Foley M, Adams JH. Identification of an Immunogenic Broadly Inhibitory Surface Epitope of the Plasmodium vivax Duffy Binding Protein Ligand Domain. *mSphere* 2019, 4.
73. Alves JRS, de Araujo FF, Pires CV, Teixeira-Carvalho A, Lima BAS, Torres LM, Ntumngia FB, Adams JH, Kano FS, Carvalho LH. Multiplexed Microsphere-Based Flow Cytometric Assay to Assess Strain Transcending Antibodies to Plasmodium vivax Duffy Binding Protein II Reveals an Efficient Tool to Identify Binding-Inhibitory Antibody Responders. Front Immunol. 2021;12:704653.
74. Nicolete VC, Frischmann S, Barbosa S, King CL, Ferreira MU. Naturally Acquired Binding-Inhibitory Antibodies to Plasmodium vivax Duffy Binding Protein and Clinical Immunity to Malaria in Rural

Amazonians. J Infect Dis. 2016;214:1539–46.

75. He WQ, Shakri AR, Bhardwaj R, Franca CT, Stanisic DI, Healer J, Kiniboro B, Robinson LJ, Guillotte-Blisnick M, Huon C, et al. Antibody responses to Plasmodium vivax Duffy binding and Erythrocyte binding proteins predict risk of infection and are associated with protection from clinical Malaria. PLoS Negl Trop Dis. 2019;13:e0006987.
76. Chen E, Salinas ND, Ntumngia FB, Adams JH, Tolia NH. Structural analysis of the synthetic Duffy Binding Protein (DBP) antigen DEKnull relevant for Plasmodium vivax malaria vaccine design. PLoS Negl Trop Dis. 2015;9:e0003644.
77. Medeiros CMP, Moreira EUM, Pires CV, Torres LM, Guimaraes LFF, Alves JRS, Lima BAS, Fontes CJF, Costa HL, Brito CFA, et al. Dynamics of IgM and IgG responses to the next generation of engineered Duffy binding protein II immunogen: Strain-specific and strain-transcending immune responses over a nine-year period. PLoS ONE. 2020;15:e0232786.
78. Popovici J, Roesch C, Carias LL, Khim N, Kim S, Vantaux A, Mueller I, Chitnis CE, King CL, Witkowski B. Amplification of Duffy binding protein-encoding gene allows Plasmodium vivax to evade host anti-DBP humoral immunity. Nat Commun. 2020;11:953.
79. Baldor L, Seng D, Heng S, Khim N, Hor S, Thin S, Salinas N, Tolia N, Chitnis C, Mueller I et al. PvDBP gene amplification is associated with functional immune evasion by Plasmodium vivax in vivo. Res Sq 2026.
80. Guedes KS, Fernandes GM, Sanchez BAM, Ntumngia FB, Adams JH, Kano FS, Fontes CJF, de Sousa TN, Carvalho LH. The pattern of gene amplification of members of the Plasmodium vivax erythrocyte binding-like proteins family across the Amazon rainforest. Malar J. 2025;25:6.
81. Gunalan K, Lo E, Hostetler JB, Yewhalaw D, Mu J, Neafsey DE, Yan G, Miller LH. Role of Plasmodium vivax Duffy-binding protein 1 in invasion of Duffy-null Africans. Proc Natl Acad Sci U S A. 2016;113:6271–6.
82. Pearson RD, Amato R, Auburn S, Miotto O, Almagro-Garcia J, Amaratunga C, Suon S, Mao S, Noviyanti R, Trimarsanto H, et al. Genomic analysis of local variation and recent evolution in Plasmodium vivax. Nat Genet. 2016;48:959–64.
83. Shaikh R, Kanjaksha G, Gorakshakar A. Duffy Binding Protein Ligand (PvDBP) gene duplication in Indian P. Vivax Malaria isolates: implication for malaria research. Curr Genet. 2024;70:18.
84. Pestana K, Ford A, Rama R, Abagero B, Kepple D, Tomida J, Popovici J, Yewhalaw D, Lo E. Copy Number Variations of Plasmodium vivax DBP1, EBP/DBP2, and RBP2b in Ethiopians Who Are Duffy Positive and Duffy Negative. J Infect Dis. 2024;230:1004–12.
85. Yengo BN, Ruszin V, Dieng CC, Kipayko C, Mdluli N, Ayukenchengamba B, Bisong EC, Sona DT, Nematchoua Weyou Z, Sumbele I et al. Digital PCR reveals high PvDBP1 but not PvEBP/DBP2 and PvRBP2b copies in Plasmodium vivax from Duffy-negative Individuals in Central Africa. J Infect Dis 2025.
86. Maestre A, Muskus C, Duque V, Agudelo O, Liu P, Takagi A, Ntumngia FB, Adams JH, Sim KL, Hoffman SL, et al. Acquired antibody responses against Plasmodium vivax infection vary with host

genotype for duffy antigen receptor for chemokines (DARC). PLoS ONE. 2010;5:e11437.

87. Storti-Melo LM, da Costa DR, Souza-Neiras WC, Cassiano GC, Couto VS, Povoa MM, Soares Ida S, de Carvalho LH, Arevalo-Herrera M, Herrera S, et al. Influence of HLA-DRB-1 alleles on the production of antibody against CSP, MSP-1, AMA-1, and DBP in Brazilian individuals naturally infected with Plasmodium vivax. Acta Trop. 2012;121:152–5.
88. Li ZK, Nie JJ, Li J, Zhuang H. The effect of HLA on immunological response to hepatitis B vaccine in healthy people: a meta-analysis. Vaccine. 2013;31:4355–61.
89. Posteraro B, Pastorino R, Di Giannantonio P, Ianuale C, Amore R, Ricciardi W, Boccia S. The link between genetic variation and variability in vaccine responses: systematic review and meta-analyses. Vaccine. 2014;32:1661–9.
90. Caillat-Zucman S, Gimenez JJ, Wambergue F, Albouze G, Lebkiri B, Naret C, Moynot A, Jungers P, Bach JF. Distinct HLA class II alleles determine antibody response to vaccination with hepatitis B surface antigen. Kidney Int. 1998;53:1626–30.
91. De SL, May S, Shah K, Slawinski M, Changrob S, Xu S, Barnes SJ, Chootong P, Ntumngia FB, Adams JH. Variable immunogenicity of a vivax malaria blood-stage vaccine candidate. Vaccine. 2021;39:2668–75.
92. Changrob S, McHenry AM, Nyunt MH, Sattabongkot J, Han ET, Adams JH, Chootong P. Persistence of Long-lived Memory B Cells specific to Duffy Binding Protein in individuals exposed to Plasmodium vivax. Sci Rep. 2018;8:8347.
93. Moreira-Nascimento SLN, Fernandes MFA, Afonso GM, Gunderman SL, Rodrigues VHG, Lopes BA, Fabbri SCP, Lacerda C, Almeida MVG, Longley GG, Mueller RJ, Antonelli I, Ntumngia LRV, Adams FB, Teixeira-Carvalho JH, Carvalho A, Kano LH. F. S.: Cytokine signatures associated with persistent antibody responses to multiple Plasmodium vivax blood-stage antigens. Front Immunol 2026, 17.
94. Andrade BB, Reis-Filho A, Souza-Neto SM, Clarencio J, Camargo LM, Barral A, Barral-Netto M. Severe Plasmodium vivax malaria exhibits marked inflammatory imbalance. Malar J. 2010;9:13.
95. Barber BE, William T, Grigg MJ, Parameswaran U, Piera KA, Price RN, Yeo TW, Anstey NM. Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. PLoS Pathog. 2015;11:e1004558.
96. Wilairatana P, Mala W, Milanez GJ, Masangkay FR, Kotepui KU, Kotepui M. Increased interleukin-6 levels associated with malaria infection and disease severity: a systematic review and meta-analysis. Sci Rep. 2022;12:5982.
97. Carvalho LH, Sano G, Hafalla JC, Morrot A, Curotto de Lafaille MA, Zavala F. IL-4-secreting CD4 + T cells are crucial to the development of CD8 + T-cell responses against malaria liver stages. Nat Med. 2002;8:166–70.
98. Robinson TM, Bolton JS, Duncan EH, Bergmann-Leitner ES, Mura M. IFN-gamma and IL-4 correlate with protection induced by an attenuated malaria vaccine. NPJ Vaccines. 2025;10:268.

Figures

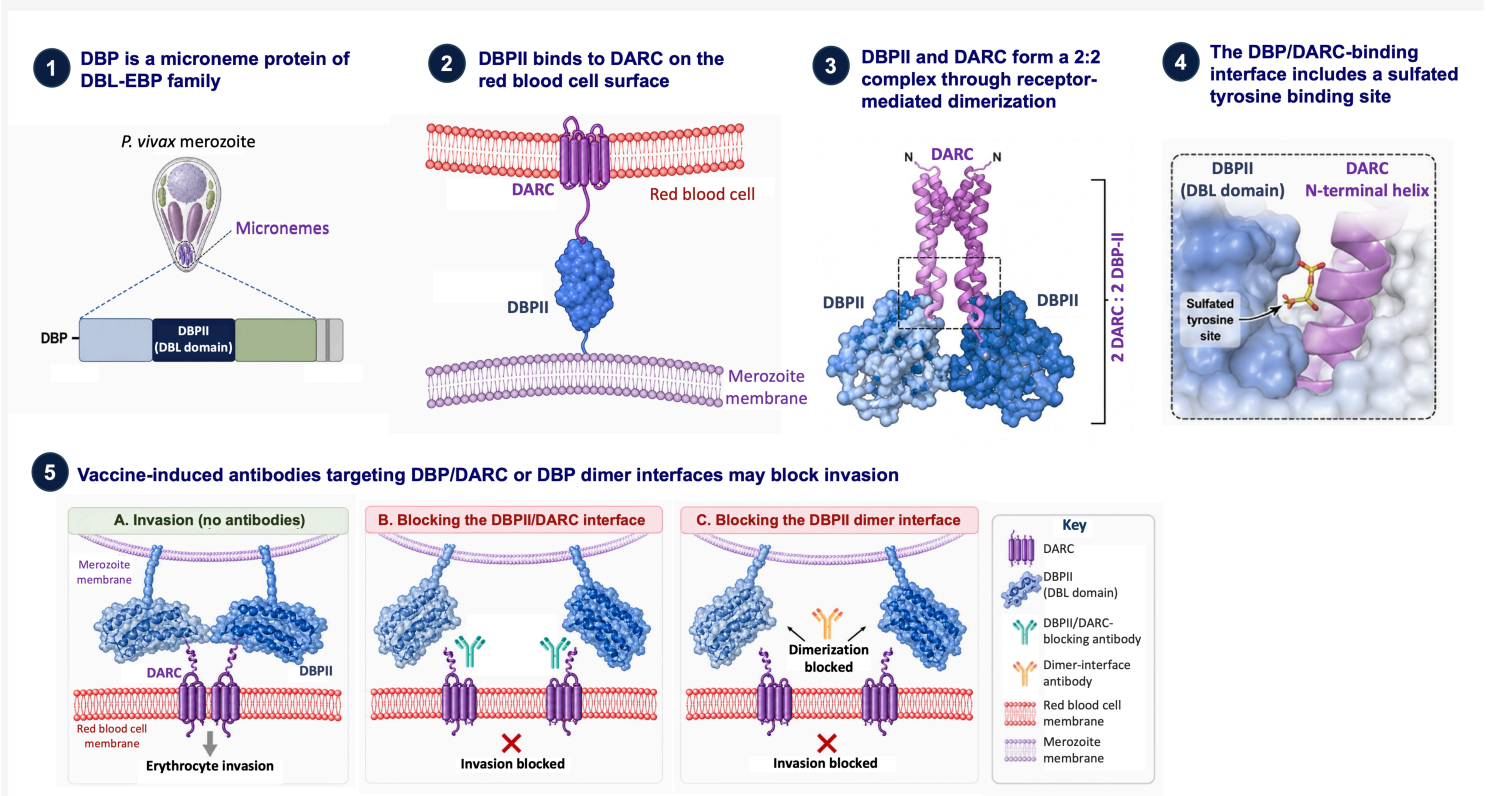


Figure 1

Interaction between *Plasmodium vivax* Duffy binding protein (DBP) and the Duffy antigen receptor for chemokines (DARC) and key targets of vaccine-induced inhibitory antibodies. (1) DBP is a microneme protein of the Duffy binding-like erythrocyte-binding protein (DBL-EBP) family and, region II (DBPII) contains the DBL domain responsible for receptor binding [19, 23, 30]. **(2)** DBPII binds to DARC on the red blood cell surface (reticulocytes) [21-24]. **(3)** Receptor binding promotes DBPII dimerization and formation of a 2:2 DBPII–DARC complex [26, 27]. **(4)** The DBPII/DARC-binding interface includes a sulfated tyrosine-binding site adjacent to the primary DARC-binding region [28]. **(5)** Antibodies targeting functionally relevant regions of DBPII may interfere with erythrocyte invasion through distinct mechanisms [15, 16]. **(A)** In the absence of inhibitory antibodies, DBPII binds to DARC and undergoes receptor-mediated dimerization, allowing invasion.**(B)** Antibodies targeting the DBPII/DARC-binding interface may prevent receptor engagement. **(C)** Antibodies targeting the DBPII dimer interface may inhibit receptor-mediated dimerization and subsequent complex formation.Illustration was created with assistance from ChatGPT (OpenAI) and Gemini (Google) and reviewed by the authors for scientific accuracy.

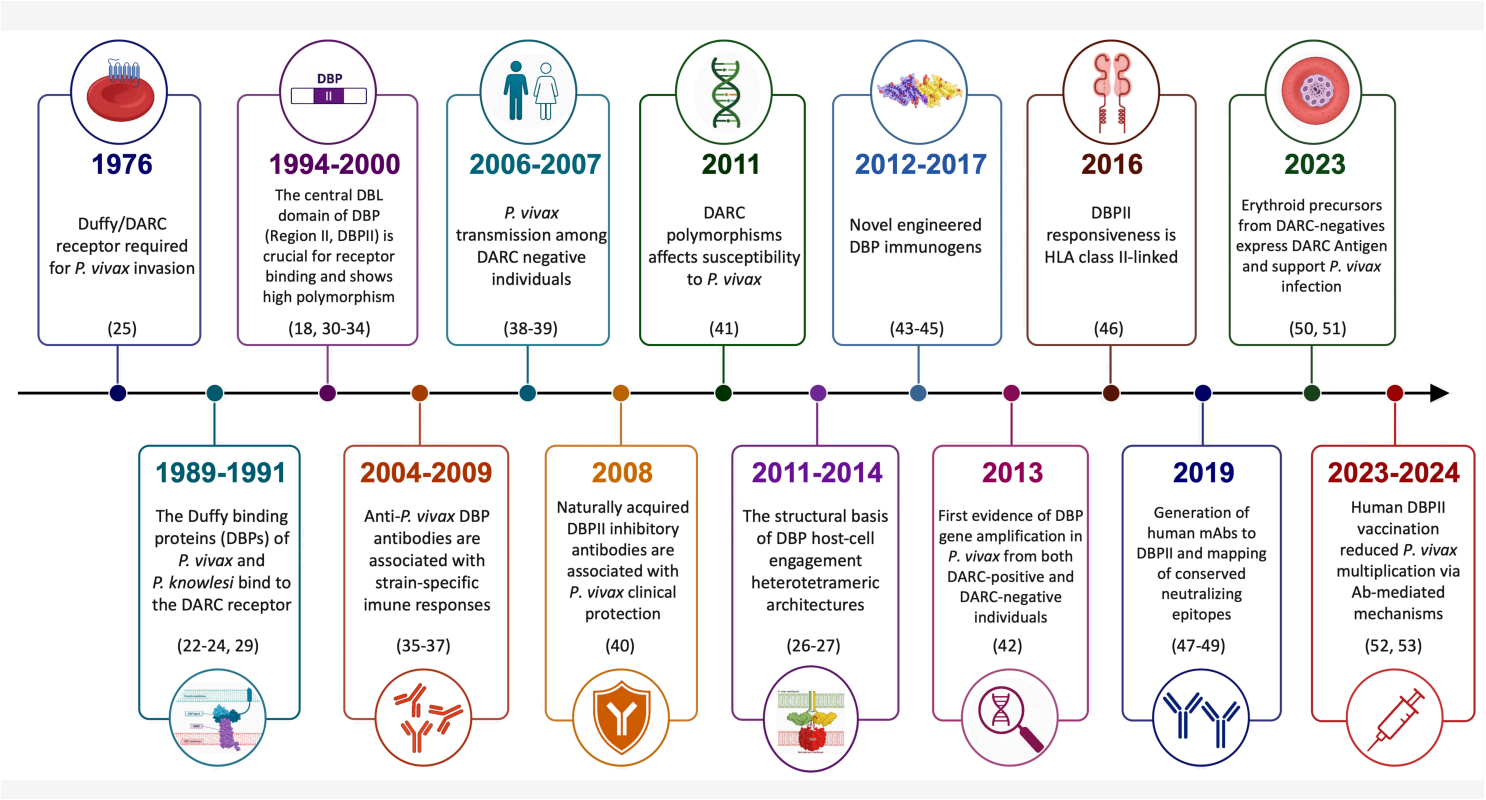


Figure 2

Milestones in *Plasmodium vivax*Duffy binding protein (DBP) research. Key discoveries and advances are shown chronologically, including the identification and characterization of the DBP–DARC interaction, DBP genetic diversity and naturally acquired immunity, structural determinants of receptor binding, engineered DBP immunogens, and recent advances in DBP-based vaccination. Numbers in parentheses correspond to the references. Illustration was created with assistance from ChatGPT (OpenAI) and Gemini (Google) and reviewed by the authors for scientific accuracy.