Duffy Blood Group System and the malaria adaptation process in humans

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Malaria is an acute infectious disease caused by the protozoa of the genus Plasmodium. The antigens of the Duffy Blood Group System, in addition to incompatibilities in transfusions and hemolytic disease of the newborn, are of great interest in medicine due to their association with the invasion of red blood cells by the parasite Plasmodium vivax. For invasions to occur an interaction between the parasites and antigens of the Duffy Blood Group System is necessary. In Caucasians six antigens are produced by the Duffy locus (Fya, Fyb, F3, F4, F5 and F6). It has been observed that Fy(a-b-) individuals are resistant to Plasmodium knowlesi and P. vivax infection, because the invasion requires at least one of these antigens. The P. vivax Duffy Binding Protein (PvDBP) is functionally important in the invasion process of these parasites in Duffy / DARC positive humans. The proteins or fractions may be considered, therefore, an important and potential inoculum to be used in immunization against malaria.

Keywords:*Duffy Blood Group System; Malaria; Plasmodium vivax; Chemokines; Antigens, protozoan; Protozoan proteins*

Introduction

Malaria is an acute infectious disease caused by protozoa of the genus *Plasmodium*. The etiologic agents in Brazil are the species *Plasmodium vivax*, *Plasmodium falciparum* and *Plasmodium malariae*. The disease is transmitted through the bite of female *Anopheles mosquitoes*, even though in certain situations other mechanisms are possible. [1]

The antigens of the Duffy Blood Group System, in addition to involvement in transfusion incompatibility and hemolytic disease of the newborn, are of great interest in medicine because of their association with the invasion of red blood cells (RBCs) by the parasite *P. vivax*. Additionally they are receptors for the chemokine family involved in the regulation of inflammatory processes.[2-5]

The finding that Fy(a-b-) erythrocytes are resistant to infection by *Plasmodium knowlesi* and *P. vivax* validated the proposition that the absence of these antigens represents an example of natural selection especially in areas where *P. vivax* is widespread. This resistance appears to have significantly influenced the distribution of Duffy system phenotypes in areas where malaria is endemic.[5-7]

This article discusses the prospect of a vaccine against malaria and also makes a brief review of the parasite and its interaction with Fy^a and Fy^b antigens as resistance and susceptibility factors to malaria, particularly that caused by *P. vivax*.

The terminology used in this article, to refer to antigens and antibodies to the Duffy Blood Group System was recommended by the International Society of Blood Transfusion (ISBT).[8]

Malaria

General

Malaria, also known as paludism, palustre fever and ague is one of the most important parasitic diseases among populations of tropical and subtropical countries of the world. The etiologic agent, *Plasmodium sp*., was first identified in 1880 by Charles Alphonse Laveran. Malaria is caused by infestation of different species of protozoa of the genus *Plasmodium*: *P. vivax*, is responsible for the benign tertian disease, *P. falciparum*, the malignant tertian disease, *P. malariae*, the benign quartan disease, and *P. ovale*, also causes benign tertian disease, but only exists in Africa.[9]

Malaria caused by *P. falciparum* is more severe because it causes microvascular impairment, which makes it pathophysiologically different from the malaria caused by *P. vivax* that under certain conditions may have less severe clinical symptoms, as the

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immune response is more of the inflammatory and hemolytic type.[10]

The most characteristic clinical signs and symptoms of malaria are fever, chills, sweating and anemia, as well as general symptoms such as headaches, myalgia, malaise and weakness and in severe cases, visceral involvement (splenomegaly and hepatomegaly).[9]

Epidemiology

The overall socioeconomic impact of Malaria is extensive, as this disease is still a major public health problem in tropical and subtropical areas of the world.[1] *P. vivax* accounts for less than half of all malaria cases in Latin America, Oceania and Asia[11] and it is calculated that from 70 to 80 million clinical cases caused by *P. vivax* occur annually worldwide.[12]

In Brazil, there has been a decrease in the annual parasite incidence in the states of the Brazilian Amazon region from 32 per 1,000 inhabitants in 1999 to 13 per 1,000 inhabitants in 2008. Mortality was also reduced from 3 deaths per 10,000 cases in 1999 to 1.5 in 10,000 cases in 2008, and the proportion of hospital admissions dropped from 3.3% in 1999 to 1.3% in 2008. In this same period, there was a considerable change in the transmission dynamics of malaria with concentrations of cases in specific municipalities. The number of municipalities at high risk, i.e. an incidence of 50 cases or more per 1,000 inhabitants, was reduced from 160 to 67 municipalities. Over 80% of reported cases outside the Amazon were imported from other states in endemic regions, from Africa or from Paraguay. Sporadic autochthonic cases occurred in restricted focal areas. It is important to mention the municipals located on the shores of the lake of the Itaipu hydroelectric plant, areas covered by Atlantic Forest in the states of Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo and Bahia, the central-west states of Goiás and Mato Grosso do Sul and the northeastern state of Piauí.[13]

Children are the most affected with between one or two million childhood deaths each year. Often, infant mortality and morbidity are related to delay in establishing the correct diagnosis because of few clinical features.[14]

In school children and adolescents, the clinical symptoms resemble those seen in adults, whereas in infants and preschool children, the clinical characteristics of the disease are often atypical and the triad of fever, chills and headache may not be presented. Ventura et al. evaluated the clinical presentation of malaria in 100 children and adolescents and found that the combination of these three symptoms occurred in only 13.6% of cases.[15]

Transmission

Transmission occurs through the bite of female *Anopheles mosquitoes* that feed on human blood that is infected with gametocytes. This promotes the fertilization process producing zygotes that become invasive and grow and divide thus producing thousands of invasive sporozoites. These migrate through the body and invade the salivary glands of female mosquitoes. Females, when they feed again, inoculate sporozoites in the blood of man, which rapidly migrate into hepatocytes and transform into trophozoites in the liver where they mature and divide to form thousands of merozoites. The liver cells rupture releasing merozoites into the circulation, thereby initiating the blood cycle. In this phase, symptoms do not appear in the host. In the blood cycle, the merozoites develop into trophozoites forming schizonts. These, when ripe, lead to the rupture of RBCs releasing merozoites that can re-infect other RBCs. This stage of the cycle is associated with clinical symptoms. After a period of asexual replication, some merozoites differentiate into gametocytes and become infective to mosquitoes.[16]

The transmission dynamics is variable among human groups belonging to different epidemiological strata, because of the interaction of risk factors, including biological, ecological, economic, sociocultural and public health. The combination of these factors establishes different transmission rates for the disease.[1]

Diagnosis

Routine laboratory diagnosis uses light microscopy of thick blood smears, with the identification of parasites in peripheral blood. Improvement of microscopy and the introduction of biological stains that identify the species, developmental stage, viability and quantification of parasites, have made this test simple, rapid and satisfactory in terms of its sensitivity and specificity.[17] However, the low sensitivity of the technique to diagnose patients with low parasitemia (common in asymptomatic carriers of malaria) and mixed infections is under discussion.[18]

Since 1990, several studies have mentioned the application of polymerase chain reaction (PCR) in the molecular diagnosis of malaria for epidemiological surveys, in screening of infected donors in blood banks, in the identification of asymptomatic carriers of malaria and in the monitoring therapeutic response.[19] Despite several experiments showing that PCR is more sensitive than microscopy and its use by some authors as the gold standard in diagnosis, the employment of this technique under current conditions requires more evidence, especially due to its high cost and the sophisticated infrastructure needed for implementation.[20]

New rapid methods that detect antigenic components of *Plasmodium*, including immunochromatographic assays, are being developed. Performed on nitrocellulose strips containing monoclonal antibodies against specific antigens of the parasite, the sensitivity of the test is above 95% and comparable to thick smears with parasitemia of more than 100 parasites/μL. Although the tests available today specifically differentiate *P. falciparum* and other species, they are not able to diagnose mixed malaria. Because of their convenience and simplicity, they are useful for screening and even diagnostic confirmation, especially in situations where the

processing of a thick blood smears is complicated, such as in remote areas with difficult access to healthcare services and in regions with low incidence.[13]

Treatment

Treatment is by a combination of chloroquine (blood schizonticide) and primaquine, the drug of choice to eliminate hypnozoites. Because of its oxidizing character, primaquine can induce severe hemolytic anemia, even at the conventional dose of 0.25 mg/kg/day, but only in patients with glucose-6-phosphate dehydrogenase deficiency (G6PD).[21]

The regimen using primaquine adopted by the National Health Foundation for the rapid treatment of malaria due to *P. vivax* infection was over fourteen days. Although effective, this extended regimen can explain the high number of cases of noncompliance and thus the appearance of relapse.[22]

Duffy blood group antigens

Brief history

In 1950 Cutbush, Mollison and Parkin[23] discovered anti-Fy^a, by detecting an agglutinin in the serum of a multitransfused hemophiliac patient that was not recognized as a blood group antigen. This antibody was named anti-Fy^a, after the patient in question, Mr. Duffy; the antibody reacted with 64.9% of 205 blood samples from unrelated English individuals. The following year, Ikin et al.[24] described the anti-Fy^b antibody that defined the antithetical pair to Fy^a. In 1955, Sanger, Race, and Jack[25] observed that the Fy(a-b-) phenotype was rare in Caucasians but was the most common in African-Americans and probably represented a product of a silent allele, *FY.*

Biochemistry

The Duffy blood group antigen was identified on the surface of RBCs eliminating toxic excesses of circulating chemokines, called DARC.[26] The determinant is a glycoprotein that traverses the membrane seven times and includes an extracellular epitope, N-terminal domain that mediates RBC invasion by merozoites of *P. vivax.*[27] The *FY* gene has two exons (*FY*^{A} and *FY*^{B}) that are encoded by the codominant *FYA* and *FYB* alleles, located on chromosome 1.[28,29]

FYA and *FYB* alleles differ by a single base substitution at nucleotide 125. Such an amino acid substitution in the amino-terminal domain of the protein is sufficient to define the two antithetical antigens. This variation leads to the identification of the Fy(a+b-), Fy(a-b+), Fy(a-b-) and Fy(a+b+) phenotypes (Table 1).[30]

Table 1 - Constitution of the phenotypes

	Fy(a+b+)	Fy(a+b-)	Fy(a-b+)	Fy(a-b-)
Antigen	Fy^a and Fy^b	Fy^a	Fy^b	-
Antibody	-	Anti-Fy^b	Anti-Fy^a	Anti-Fy^a and Anti-Fy^b

Modified from Parasol N et al.[30]

FYA and *FYB* alleles differ by a mutation in an important transcription complementary *DNA* (cDNA) that encodes glycine (*FYA*) or aspartic acid (*FYB*) at position 42 of the most important proteins encoded by exon 2. The molecular mechanism that gives rise to the null Duffy phenotype [Fy (a-b-)] has been classically associated with a mutation in the Graphic Alignment Tool for Comparative Sequence Analysis (GATA)-box of the DARC silent promoter gene encoding the Duffy antigen system in RBCs of these individuals, resulting in the *FYB* allele.[31-33]

It was demonstrated that Caucasian and African descent blood donors in southeastern Brazil were serologically Fy(b-) with the majority of African descents presenting *FYB* with the GATA box single nucleotide polymorphism (SNP), while the majority of Fy(b-) type Caucasians presented *FYB* and 265 thymine/298 adenine SNPs.[34] Fy(a-b-) RBCs are common in black African ethnic groups but rare in many other populations.[35] In blacks it was shown to be due to a mutation at position -33 (T>C) in the promoter region of the *FYB* gene, the GATA-box.[31,36] This change leads to a disruption in erythroid transcription factor GATA-1, resulting in an absence of the Fy^b antigen expression only in RBCs without changing the expression of this protein in other tissues. As a result, these individuals may develop anti-Fy^a, but not anti-Fy^b antibodies.[37] Most individuals of West African and 68% of African-Americans do not express Fy^a or Fy^b in their RBCs, which results in resistance to infection by *P. vivax* because RBCs with the Fy(a-b-) phenotype cannot be invaded by this parasite.[25,31]

Epidemiology

The phenotype frequencies found in studies of blood donors in São Paulo for antigens of the Duffy blood group system were: the Fy(a+b-) phenotype in 19.8% of Caucasians and in 14.0% of Blacks; the Fy(a+b+) phenotype in 41.4% of Caucasians and in 1.6% of Blacks; the Fy(a-b+) phenotype in 37.8% of Caucasians and in 17.5% of Blacks and the Fy(a-b-) phenotype in 1.1% of Caucasians and in 66.9% Blacks (this phenotype is considered to be a marker for Black individuals). (Figure 1).[38]

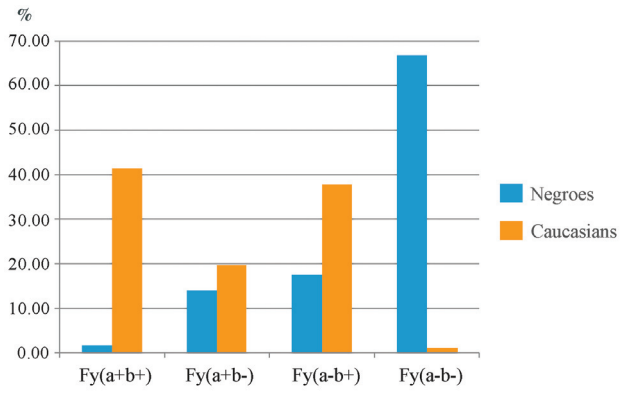

Figure 1 – Phenotypes found for the Duffy blood group system in blood donors of São Paulo. (Modified from Novaretti MC et al.[38])

The Fy^a antigen is common among the Chinese, Japanese and Melanesians, but uncommon among Black Africans.[39,40] However, the Fy^b antigen is more frequent in the Caucasian population than in Asians and Blacks and the Fy(a-b-) phenotype is extremely rare outside the black population.[41,42]

Relationship between *Plasmodium vivax* and Duffy system antigens

History

The discussion about the role of the RBC antigens of the Duffy system began in 1975 when Miller et al.[41] suggested that the Fy^a or Fy^b antigenic determinants could be receptors for *P. knowlesi* and that resistance to the invasion of this merozoite in Blacks was due to the Fy(a-b-) phenotype. The strength of Fy(a-b-) against invasion by *P. knowlesi* merozoites was demonstrated *in vitro* and Barnwell et al.[43] confirmed this with *P. vivax* suggesting that there was a natural selection of individuals with the *FY* gene who do not produce Fy^a and Fy^b.

Epidemiology

P. falciparum uses a series of receptors on the surface of human RBCs to invade them, while *P. vivax* and *P. knowlesi* depend on an interaction with the Fy^a or Fy^b antigens of the Duffy Blood Group System.[5,41,44,45] In Africa, where the Fy(a-b-) phenotype has achieved stability in different ethnic groups, the transmission of *P. vivax* is uncommon.[46,47] In the Papua New Guinea population, carriers of the Fy(a-b+) or Fy(a+b-) express half the level of Duffy antigens on red blood cells compared to wild type homozygotes and exhibit reduced susceptibility to blood stage infection by *P. vivax*.[33,48] These observations suggest that total or partial restriction to access Duffy antigen reduces the ability of the parasite to invade new red blood cells and thus this might inhibit parasitemia by *P. vivax*.[49]

P. vivax Duffy binding protein (PvDBP) is functionally important in the invasion process of parasites in human Duffy/DARC positive RBCs, and it has recently been demonstrated that regions where malaria transmission rates are low or medium, such as in inhabitants of the Brazilian Amazon, this protein is naturally immunogenic.[50] In these populations, the proportion of DBP Immunoglobulin G (IgG) increased with exposure to malaria, reaching a peak in individuals with prolonged exposure such as in residents of endemic areas (> 15 years). This observation was later confirmed in the Rondonia population, an malaria endemic area where IgG1 and IgG3 DBP-specific antibodies were identified.[51]

The populations of various ethnic regions of Thailand and Indonesia were studied, and there was a high incidence of the *FYA* allele (> 0.9) and the presence of a new phenotype, $Fy(a+^{weak}b-)$, while these data were similar to those found in previous phenotypic studies conducted in Southeast Asia and Australasia. It is striking that $Fy(a+^{weak}b-)$ individuals had *FYA*/*FYA* genotypes while Fy(a-b-) individuals had *FYA*/*FYA* or *FYA*/*FYB* genotypes as seen by Polymerase Chain Reaction-Restriction Fragment Lenght Polymorphism (PCR-RFLP) using BanI and the Sty enzymes; the genetic mechanisms that cause this phenotype have not been determined. The implications of the presence of these phenotypes are still unknown but it has been suggested that in malaria endemic regions, there are defense mechanisms against *P. vivax* infection distinct from those in African descendents.[40]

Field observations in West Africa and Ethiopia have established strong correlations between the absence or low endemicity for *P. vivax* and the high prevalence of negative *FYA* and *FYB* alleles.[47,52] In these populations in which there is an African gene influence, four main phenotypes, Fy(a+b-), Fy(a-b+), Fy(a+b+) and Fy(a-b-), have been identified.[53] Thus, the Duffy blood group system is considered one of the most interesting chromosomal loci to assess the impact of natural selection in different geographic regions.[54,55]

The Brazilian population has a great diversity in its ethnic composition as a result of the hybridization of numerous indigenous populations and immigrants from Europe, Africa and Asia. Immigration was not uniform across the country.[56,57] The differential distribution of Duffy antigenic determinants among ethnic groups is a characteristic feature of this blood group system. So this has been used as a marker for ethnic composition, as well as an indicator of population trends.[58]

Mechanism of action

In regards to merozoite invasion of RBCs, Aikawa et al.[59] and Miller et al.[60] observed by electron microscopy that the apical portion of the merozoite makes the first contact with the RBC creating a small depression in the membrane. This area begins to thicken and binds with the membrane of the merozoite which then enters the red blood cell by invagination. After the *Plasmodium* enters, this access closes. This binding is crucial for parasite invasion. The presence of one member of a large family of proteins bound to RBCs, referred to as Duffy Binding Protein (DBP), is required to mediate with the DARC as this antigen was identified as a chemokine receptor.[2]

This critical binding occurs in the cysteine-rich domain between the amino acids at positions 291 and 460 of region II.[61] According to several observations, region II of the DBP (DBPII) can be a critical target for protective immunity of the host. Firstly, certain DBPII are highly polymorphic and appear to be maintained by immune selection.[62-64] Secondly, antibodies against DBPII found in populations of endemic areas for *P. vivax* inhibit binding of COS-7 cells expressing the DBPII ligand to DARC-positive RBCs.[65,66] Thirdly, the high antibody levels against region II of *P. knowlesi* a protein, a molecule that is 70% homologous to *P. vivax* DBP, also mediates infections of human DARC-dependent RBCs

and may inhibit invasion of human RBCs by *P. knowlesi*.[67]

P. vivax DBP is a 140-kDa protein that belongs to a family homologous to Duffy DBL-EBP, located inside organelles of merozoites of *Plasmodium*.[68] The similarity between DBL-EBPs is most prominent in two conserved cysteine-rich domains in regions II and VI. The binding functional domains of DBL-EBP lie in region II, which for *P. vivax* is DBP 330-amino acid and the critical residues of map linking a central region of 170- amino acid including cysteines 5-8.[61,69]

The gene encoding the *P. vivax* DBPII is highly polymorphic and this diversity varies geographically from region to region.[70,71] This polymorphism is consistent with a high pressure on DBP selection and suggests that an allelic variation functions as an immune evasion mechanism.[72,73]

DBP is likely to be exposed during the invasion, therefore, it can become accessible to antibodies. Currently, data on humoral immune response to DBP in the human population is limited, and studies have been mainly restricted to areas where malaria is highly endemic.[65,74,75]

Research on cytokines and their receptors converged in the investigation of the antigens of the Duffy Blood Group System, showing an important physiological role for the alleles of this glycoprotein.[76] Horuk et al. showed in a study of multispecific chemokine receptors in human RBCs, that interleukin (IL)-8 minimally binds to Fy(a-b-) RBCs, that monoclonal antibodies against Duffy antigens block the binding of IL-8 to positive Duffy RBC antigens and that IL-8 also prevented the binding and invasion of RBCs by *P. knowlesi*. This led to several lines of evidence suggesting that Duffy blood group antigens are receptors for chemokines.[2]

It was also observed that if a mutation occurred in the DARC promoter region of the gene in erythroid precursor cells, this would prevent its expression in RBCs and complete resistance to infection by *P. vivax* would occur.[5,31]

Immune response

It is believed that merozoite invasions seize organelle protein until contact of merozoites with the target RBC presumably as a mechanism to reduce the exposure of DBP to immune inhibition.[77] At present, the data available on the humoral immune response to DBP in human populations show that anti-DBP antibodies increase with exposure to *P. vivax* and that immune response, which includes antibody activity, blocks adhesion of DBPII to its receptor on RBCs.[50,65,74,78] The same antibodies that block DBPII-DARC also interact inhibiting RBC invasion by *P. vivax*[49] which is the concept that proves that the *P. vivax* Duffy Binding Protein antibody (anti-PvDBP) can inhibit invasion of merozoites. It is notable, that children living in endemic areas of *P. vivax* develop inhibitory anti-DBP antibodies that seem to confer protection against the blood infection stage.[79]

Over 90% of sub-Saharan Africans do not express DARC, and as a result, *P. vivax* has limited distribution in this region of Africa. Therefore, the BPD protein represents one of the most promising antigens among the candidates to be used in the production of vaccines against malaria, but little is known about the immune response against this molecule. However, host immune responses to *P. vivax* DBP appear to play a key role in acquired immunity to P. vivax, due to their essential role in the invasion of RBCs by merozoites. This acquired immunity is probably partly due to the production of anti-BPD antibodies that block it binding to receptors in red blood cells. In support of this model, an increase in the proportion of bonds and antibodies that recognize recombinant Duffy Binding Protein (rDBP) was observed as the age advanced, concomitant with a reduction in prevalence and intensity of *P. vivax* infection.[80]

To better define potentially protective epitopes in the critical link of DBPII, at least four dominant linear epitopes were identified, two of which correspond to the aforementioned T cell epitopes.[80] The accumulation of these antibodies against linear epitopes is closely correlated with the correct recognition of folded rDBPII antibodies.[66] This suggests that antibodies directed to some of these linear epitopes also recognize native DBP expressed on the surface of merozoites.[75]

With current knowledge, the innate resistance to malaria infections in humans has been attributed to the polymorphisms of blood groups.[81]

Vaccine

The attempt to control malaria through the use of a vaccine has been encouraged for years and today it is a controversial and widely discussed topic. The development of a vaccine is of great importance for infection control, since resistance to drugs used in therapy is increasing around the world as is the emergence of the vector's resistance to insecticides.[82]

Several antigens of asexual blood forms of *P. vivax* are considered potential components to include in a vaccine for species-specific malaria.[83] Among these, the three best characterized from the immune viewpoint are the merozoite surface protein 1 (MSP-1),[84] DBP [66,85] and apical membrane antigen 1 (AMA-1).[86]

MSP-1 is the best characterized protein in the blood stage of *Plasmodium.* This antigen is an integral membrane protein that appears to be linked to the merozoite surface through Glycosylphosphatidylinositol anchors (GPI) and is synthesized as a high molecular weight precursor (180 to 230 kDa) during the schizogony stage. After being processed, the MSP-1 protein generates smaller fragments of 19 kDa and 42 kDa which remain on the surface of the parasite; these are essential for merozoite invasion.[87]

Several studies have shown that the *P. vivax* MSP-1 protein (PvMSP-1) is highly immunogenic in different regions of the world including Korea,[88] Sri Lanka,[89] Colombia[90] and Brazil.[91-94] Additionally, studies in BALB/c[90,93] and nonhuman primate immunization[90,95,96] performed with

PvMSP-1 showed the presence of high levels of IgG antibodies and anti-PvMSP-1. In addition, nonhuman primates immunized with this protein showed a partial protective immunity when tested with blood forms of the parasite.[90,95,96]

Recently, PvMSP-1 has been tested in preclinical and clinical studies, however, additional studies are needed to identify the main fragment of this protein that is capable of inducing a protective response.[97]

Despite the PvMSP-1 antigen being considered an important candidate for the inoculation of the vaccine against the asexual blood stage of the parasite,[84,96,98,99] other studies have demonstrated that this protein shows polymorphisms which certainly could compromise its potential for this purpose.[89,100,101] Other proteins, also associated with the merozoite surface such as MSP-3, MSP-4, MSP-5 and MSP-9, have been identified and characterized as potentially useful for vaccines[102-105] while some of these have already been produced for studies as recombinant proteins.[106,107]

DBP represents one of the most promising vaccine candidate antigens against *P. vivax* infection.[78] This is probably exposed on the surface of merozoites during invasion of RBCs, which allows its binding to the receptor and, importantly from the standpoint of a vaccine, makes it accessible to antibodies in the serum. Although having great importance as a candidate protein, there are some limitations that have hindered its study such as its low abundance in the parasite, low immunogenicity, as well as the limitations for the *in vitro* cultivation of *P. vivax*.[74,75,108,109] With the spread of drug resistance and with cases of *P. vivax* infection increasing, more emphasis has been placed on developing a vaccine against the malaria caused by *P. vivax*.[46] Residents of a specific endemic area develop acquired immunity to *P. vivax* over several years. The gradual acquisition of immunity probably occurs because of the many antigenically different strains of the parasite and/or weakly immunogenic epitopes recognized by the host immune system.[110]

Although this molecule represents a good candidate to be used in vaccine production, polymorphisms may be a serious difficulty, and therefore probably conserved regions within the protein should be used or multiple allelic forms of the antigen should be incorporated. Understanding the nature and origin of this polymorphism in Plasmodium antigens is crucial for vaccine development.[72]

One of the goals to be achieved will be to determine how the genetic diversity of parasites is related to vaccine immunity, and thus the DBP antigen of *P. vivax* is a leading candidate. To attain this goal, it is important to assess levels of genetic diversity of DBP within and between populations in different geographic regions and assess how this genetic diversity is generated and maintained. Many factors can contribute to the genetic diversity of the population with malaria, especially mutations, intragenic recombination (determined by the multiplicity of infection and transmission intensity), natural selection, gene flow between different regions and population size.[72]

The AMA-1 protein is a member of a family of molecules expressed in micronemes of *Plasmodium merozoites*. This protein is 83 kDa in *P. falciparum* and 66 kDa in the other species and plays an important role in the invasion of RBCs in the asexual blood stage. In addition, the AMA-1 protein is also expressed in sporozoites, and seems to be involved in the invasion of hepatocytes.[111] However, despite the importance of this protein as a vaccine candidate antigen, few studies have been conducted on its immune response. [97]

In experimental models using nonhuman primates, the AMA-1 protein showed high immunogenicity.[86] Subsequently, immuno-epidemiological studies conducted in Korea[112] and Sri Lanka[89] showed that individuals with positive stool examinations for *P. vivax* developed antibodies against AMA-1. Similarly, in Brazil, the AMA-1 protein was highly immunogenic in individuals from endemic areas during natural infections and antibody levels increased depending on exposure to malaria.[113,114]

Many studies have shown that the genetic diversity of AMA-1 is variable between different regions of the world. In highly endemic areas such as Papua New Guinea,[115] a high polymorphism diversity was observed among different isolates of the region, however in low endemic regions such as Korea,[116] there was a low polymorphism diversity. In Brazil, the MSP-1 protein has been shown to be highly immunogenic in natural infections of individuals originating from six different endemic malaria regions[113,114] with limited polymorphism diversity[113] as observed in Korea.

In the last decade, research to produce a vaccine received much investment.[117] RTS,S, the most advanced vaccine in development, will cost $500 million in its next phase.[118] In August 2006, the organization Malaria Vaccine Technology Roadmap stipulated that its goal was to develop a vaccine by 2015, which offered 50% protection against the severe form of infection for at least one year. Today, however, only one may achieve this goal, the RTS,S (project of the Glaxo Smith Kline pharmaceutical industry in conjunction with the Research Institute Walter Reed Army).[117] The challenge of an effective vaccine against malaria is to develop immunity against the etiologic agent, as the infection occurs again and again and evolves to a chronic infection. Moreover, existing knowledge about the complex host-parasite interaction is still a major limitation in the development of research.[82]

New perspectives for malaria vaccination arise with the use of monoclonal antibodies (AcM). AcM are attractive tools to assist in the identification of epitopes and mechanisms. These antibodies show specificity to antigens in the asexual blood stage of the parasite and also showed *in vitro* and *in vivo* neutralizing capacity. Most specific AcM result from hybridoma technology in mice, which makes their use restrictive both for *in vitro* investigations and for studies employing mice infected with common rodents species of

Plasmodium. To meet this deficiency, a recent alternative is the development of recombinant human antibodies employing the use of genomic libraries, thus avoiding the need for hybridomas or vaccines. This technology enables the creation of monoclonal IgG antibodies specific to human *Plasmodium* and recent work has demonstrated the effectiveness of this methodology in *in vitro* and *in vivo* models. This new approach also offers advantages, allowing "molecular adjustments" to the specific antigens and functional characteristics of antibodies produced in this way.[82,119,120]

Conclusion

There have been significant advances in recent years in studies on the relationship between human infestation by pathogens of malaria antigens and the Duffy blood group system. However much remains to be discovered and better understood as, for example, the mechanisms that allow interaction between *Plasmodium* and the Fy^a and Fy^b antigens. The intense polymorphism and different alleles of the immuno-dominant region that produce potential candidate antigens for use as inocula for the vaccine, emphasize the need to better understand the relationship between genetic and humoral immunity. Studies show that PvDBP is equally or more promising than other protein candidates for vaccine antigens; it is naturally immunogenic in different populations of the Brazilian Amazon, the antibody response is proportional to the length of individual exposure to the parasite in areas of malaria transmission. A major challenge to vaccine development is the specific technical difficulties to cultivate *in vitro* in a continuous and efficient process, although today, with a new perspective in using monoclonal antibodies, the limitation is not to be able to extrapolate the results to *in vitro* to *in vivo* models. We believe that with the development of new molecular and immunological technology, the challenges can be overcome in a relatively short time thus allowing the production of human vaccine against malaria, in order thus to prevent an endemic disease that affects millions of people in various regions of the world. We emphasize that an additional difficulty in Brazil is the limited resources available for research in this area.

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