Reticulocytes: *Plasmodium vivax* target cells

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Reticulocytes represent the main invasion target for *Plasmodium vivax*, the second most prevalent parasite species around the world causing malaria in humans. In spite of these cells' importance in research into malaria, biological knowledge related to the nature of the host has been limited, given the technical difficulties present in working with them in the laboratory. Poor reticulocyte recovery from total blood, by different techniques, has hampered continuous *in vitro P. vivax* cultures being developed, thereby delaying basic investigation in this parasite species. Intense research during the last few years has led to advances being made in developing methodologies orientated towards obtaining enriched reticulocytes from differing sources, thereby providing invaluable information for developing new strategies aimed at preventing infection caused by malaria. This review describes the most recent studies related to obtaining reticulocytes and discusses approaches which could contribute towards knowledge regarding molecular interactions between target cell proteins and their main infective agent, *P. vivax.*

Introduction

Reticulocytes are erythroid cells which have not reached maturity, being characterised by presenting a reticular network formed by residual RNA (Orten, 1934). These cells represent around 1–2% of circulating human red blood cells (RBCs) and have a short life span (24 h). It has been demonstrated that varying the percentage of reticulocytes as well as defects in their messenger RNA are associated with some diseases having variable clinical relevance (Benz and Forget, 1971; Bessman, 1990; Suzuki et al., 1993).

Some parasite species belonging to the Plasmodium genera causing malaria in humans (*Plasmodium vivax*) and rodents (*Plasmodium berghei*) have shown a certain preference for invading reticulocytes (Butcher et al., 1973; Mons, 1990; Cromer et al., 2006). *P. vivax* is one such species which is characterised by being the most widely distributed throughout tropical and sub-tropical zones, causing the highest morbidity indexes on the Asiatic and American continents (Guerra et al., 2010). Obtaining a sufficient amount and concentration of reticulocytes is essential for establish-ing *in vitro P. vivax* cultures, meaning that studying them has become one of the essential research topics for groups working on malaria caused by this parasite species.

Knowledge of reticulocyte biology regarding the type of cell receptors required for invasion by the parasite is limited. Studies of the human reticulocyte transcription profile (Goh et al., 2007) and analysing murine reticulocytes' partial proteome (Prenni et al., 2012), added to using methodologies for evaluating protein–protein molecular interactions, could be of great use for carrying out future research focussed on an elucidating interaction mechanisms between this target cell and its pathogen.

Literature search

Literature included in this review was found querying the PubMed database from 1900 to date, using the following search terms: 'reticulocytes', 'reticulocyte purification', 'reticulocyte cryo-preservation', 'reticulocyte proteome', '*Plasmodium vivax in vitro* culture', '*Plasmodium vivax* vaccine' and '*Plasmodium vivax* and reticulocytes'. The literature was initially analysed according to titles and abstracts and then relevant studies were fully reviewed. The inclusion criteria were: (i) studies describing reticulocytes' biology focussing on receptor characterisation and proteomics,

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or (ii) their use in *P. vivax* research and (iii) the results were restricted to studies written in English language.

Reticulocytes and malaria

The first microscopic descriptions of reticulocytes were made by Wilhelm Erb in 1865; he demonstrated the presence of granular material in human RBC and different animal species. Twenty years later, Paul Ehrlich reported that some erythrocytes from patients suffering from anaemia, stained with methylene blue, had fine, dense, elegant blue networks; which is why they are called 'reticulated erythrocytes' (Orten, 1934). Gloria Gronowicz and her group defined the complex process of these cells maturing into erythrocytes from 1984 onwards (Gronowicz et al., 1984), thereby leading to their main characteristics being determined: from residual RNA content left behind after nuclear loss (formerly known as reticular network), the presence of ribosome, mitochondrial, lysosome and endocyte vesicular granules and so on. At clinical level, such cells' count is used for distinguishing the effectiveness of RBC production in bone marrow (Piva et al., 2010), or, on the contrary, for evaluating the type of cell disorder being presented: aplastic anaemia, haemolytic anaemia, haemoglobinopathy, β-thalassaemia or reticulocytopenia (Benz and Forget, 1971; Bessman, 1990; Suzuki et al., 1993).

Interest in studying reticulocytes among groups working on malaria has emerged as a result of research orientated towards standardising an *in vitro P. vivax* culture, due mainly to this parasite species' preference for invading immature RBC (Bass and Johns, 1912; Mons, 1990). It is well known that *in vitro* cultures require the constant addition of reticulocytes (given these cells' maturation rate, *i.e.* 24 h at 37°C) for perpetuating parasite growth; however, given these cells' reduced percentage in adult humans' blood, managing to enrich or finding a source having a high percentage of them has been one of the main research challenges. As a result of this issue, studies reporting *P. vivax* proteins' functionality and their potential as vaccine candidates are limited, compared with those described for *Plasmodium falciparum* (Patarroyo et al., 2012). As developing a vaccine against pathogenous agents involves ascertaining a particular microorganism's biology and its respective target cells (Patarroyo

et al., 2011), in the case of *P. vivax*, it is recommendable that a methodology be standardised for obtaining a good amount of reticulocytes from different biological sources, and also evaluating techniques for characterising receptor–ligand molecular interactions between such cells.

Reticulocyte sources

Different blood sources have been used in the attempt to enrich reticulocytes, including animals (primates and mice), healthy people and people suffering from blood disorders such as haemochromatosis (Golenda et al., 1997) and β-thalassaemia (Ocampo et al., 2002) and cord blood from newborns (Table 1). Different protocols have also been used for obtaining viable reticulocytes supporting parasite infection aimed at improving basic knowledge about their biology. A discontinuous *in vitro P. vivax* culture has recently been partially standardised using enriched cord blood reticulocytes (Russell et al., 2011), representing an advantage for continuing studies in this area.

Isolation from animal blood

Primates from the genera *Aotus nancymai* and BALB/c mice are frequently used as animal models for studying the pathogenesis of malaria. In attempts to standardise a culture for *P. vivax* and *Plasmodium yoelii*, these animals have been anemised by haemolytic drugs and consequently the percentage of reticulocytes in blood has increased considerably. The classical CF11 column filtration method (Sriprawat et al., 2009) has been used for eliminating leucocytes from primates' blood samples and obtaining completely pure reticulocytes (Mons et al., 1988). Even though the objective of obtaining enriched reticulocytes has been successful, these cells have shown certain fragility, given that infection could not be maintained. Mice anemised by Swardson-Olver's group had 58–72% reticulocytes which were recovered by cardiac puncture, biotinylated and enzymatically treated with trypsin and chymotrypsin. It was observed that cells pre-treated with trypsin were infected threefold more by *P. yoelii* 17X strain parasites than those pre-treated with chymotrypsin when injected in BALB/c mice, thereby suggesting that the recovered reticulocytes were viable hosts (Swardson-Olver et al., 2002). The methodology for enriching reticulocytes by inducing anaemia in animals has

Table 1 | General panorama of sources and methods for enriching reticulocytes evaluated to date

Reticulocyte source and sample collection	Enrichment method		Yield (%)	Viability in *in vitro* culture	Expenses/suitability based on source	References
Blood obtained from animals by venipuncture or by cardiac puncture	Splenectomised monkeys were treated with phenylhydrazine (100 mg/kg body weight). Leucocytes were removed by using a CF-11 column or leucocyte filters		ND	No viability	Less expensive but reticulocyte source very difficult to obtain. Useful for research in *P. vivax*	Mons et al. (1988)
	Mice were treated with phenylhydrazine (10–15 mg/kg body weight)		>30	Not tested	Less expensive and a good reticulocyte source to study mice malarias	Gronowicz et al. (1984)
	Mice were treated with 1.5 mg of phenylhydrazine		58–72			Swardson-Olver et al. (2002)
	Mice were treated with an anaemia-inducing strain of Friend leukaemia virus (FVA). Reticulocytes were separated by using 1–2% deionised bovine albumin gradient		>95			Koury et al. (2005)
	Mice were injected intraperitoneally with 2 ml of normal saline and then 700 µl of blood were removed by retro-orbital puncture. A two-step isotonic Percoll gradient with density of 1.058 and 1.096 mg/ml was used for reticulocyte recovery		35–50			Liu et al. (2010)
	Mice were treated with 1.5 mg of phenylhydrazine		ND			Wooden et al. (2011)
			50			Prenni et al. (2012)
Peripheral blood of people obtained by venipuncture	Blood extracted from healthy people was treated to enrich reticulocytes and/or remove leucocytes	62% Percoll gradient	ND	Yes	Not expensive but it is a poor reticulocyte source. Useful for research in *P. vivax*	Barnwell et al. (1989)
		Magnetic beads loaded with anti-human transferrin receptor antibodies	15–42	Not tested		Brun et al. (1990)
		Percoll/renografin-60 gradient	8–15 and rarely 25	No viability		Lanners (1992)
		CF-11 column or Plasmodipur filter	ND	Not tested		Sriprawat et al. (2009)
	Blood extracted from people having haemochromatosis or β-thalassaemia, respectively, was treated to enrich reticulocytes and/or remove leucocytes	Differential centrifugation and leucocyte separation filter	15–20	Yes	Not expensive but patients suffering from such diseases are scarce in some latitudes of the world	Golenda et al. (1997)
		CF-11 column	>85	Not tested		Ocampo et al. (2002); Rodriguez et al. (2002); Urquiza et al. (2002)
Cord blood of newborns collected in heparin tubes	Cord blood was used to enrich reticulocytes and/or remove leucocytes	RCXL2 high-efficiency leucocyte reduction filters	ND	Not tested	Not expensive and recommended source	Goh et al. (2007)

(Continued)

Table 1 | Continued

Reticulocyte source and sample collection	Enrichment method	Yield (%)	Viability in *in vitro* culture	Expenses/suitability based on source	References
	CF-11 column	6.9–7.9	Yes		Udomsangpetch et al. (2007)
	70% isotonic Percoll gradient and a CF-11 column	57.8	Yes		Russell et al. (2011)
	Hypotonic sodium chloride solution and a CF-11 column	1–28	Yes		Grimberg et al. (2012)
Reticulocytes obtained by maturing erythroid cells isolated from cord blood by using 30–60% Percoll discontinuous gradients		ND	Yes		Panichakul et al. (2007)
Reticulocytes were obtained from cord blood by using 70% isotonic Percoll gradient. Leucocytes were removed using a CF-11 column. Sample was cryopreserved in liquid nitrogen with Glycerolyte 57		>40	Yes		Borlon et al. (2012)
Reticulocytes were obtained by maturing HSCs. Glycerolyte, Glycerol + liquid Sorbitol or IMDM/10% DMSO/40% FCS were used for cryopreservation		ND	Yes	Expensive but recommended source	Noulin et al. (2012)

ND: Not determined.

been used in other studies orientated towards evaluating reticulocyte maturation (Koury et al., 2005; Liu et al., 2010), as well as studying the partial proteome (Prenni et al., 2012).

Isolation from adult human blood

Even though the percentage of reticulocytes is low in adults, several enrichment techniques have been explored. Barnwell et al. (1989) obtained reticulocytes from human blood using a 62% Percoll gradient. The results showed that these cells supported *in vitro* parasite invasion and development (Barnwell et al., 1989). Norbert Lanners used the discontinuous Percoll/renografin-60 gradient technique in 1992 for separating reticulocytes from fresh human blood. Even though the amount of cells obtained was considerable (8–15%), they were fragile and did not support parasite development (Lanners, 1992). Once such disadvantage had been described, several research groups focussed their efforts on finding other sources and improving techniques for obtaining immature erythrocytes.

Reticulocytes, just like all cells, display a series of cell surface markers. It has been shown that transferrin receptors disappear during maturation, thereby suggesting that they are specific for immature red cells (Frazier et al., 1982). Bearing such difference in mind, a purification protocol using anti-transferrin antibodies coupled to magnetic beads was developed (Brun et al., 1990); the results revealed 15–42% reticulocyte enrichment. However, the difficulty of eluting cells coupled to beads has been the main factor limiting this method.

Blood from patients suffering haemochromatosis has a high percentage of reticulocytes (3–5%). Taking advantage of such proportion of cells, Golenda et al. (1997) used a differential centrifugation separation protocol. Leucocytes were removed by a leucocyte separation filter later on, achieving around 10–12 ml final volume having 15–20% reticulocytes per peripheral blood unit. These cells were able to support a short-term *P. vivax* culture.

Our group reported using blood from a patient suffering from β-thalassaemia in 2002, demonstrating

that such blood contained greater than 85% reticulocytes (Ocampo et al., 2002; Rodriguez et al., 2002; Urquiza et al., 2002). Samples obtained by venipuncture were washed and passed through a CF11 cellulose column to remove leucocytes and then used for identifying peptide sequences from *P. vivax* proteins binding with high affinity to target cells. Comparing reticulocyte and mature erythrocyte binding revealed that the former had specific receptors for the proteins being evaluated.

Isolation from cord blood

The animal and/or adult human blood reticulocyte isolation studies had limited application, given the intensive labour involved in the procedure and a lack of available patients in some parts of the world suffering from such diseases; this led to exploring other areas. Udomsangpetch et al. (2007) were the first to report that umbilical cord blood, containing a high percentage of reticulocytes (3–8%), supported an *in vitro P. vivax* culture which was maintained for a short period (not more than 30 days). Russel et al. (2011) then used a procedure for enriching cord blood reticulocytes using a 70% isotonic Percoll gradient. Leucocytes and platelets were removed from twenty-eight 20 ml volume samples using two rounds of filtering with a CF11 column. After being separated by a density gradient, an average of 57.8% reticulocytes contained in 191 μl blood was obtained; these were kept stable for 4 wk. This represented an advantage for culturing, as having a greater amount of reticulocytes in a small volume favours *P. vivax* invasion and growth.

Grimberg et al. (2012) developed a simple, rapid and easily used methodology for enriching reticulocytes from cord blood. The authors used selective enrichment on 10 cord samples using a hypotonic sodium chloride solution, taking advantage of the difference in reticulocytes' osmotic pressure compared with that of other types of cell. Reticulocytes increased by 1–28% after eliminating cells lysed by several washings and removing leucocytes by CF11. The cells maintained their integrity and were viable, demonstrated by analysing haemoglobin stability and *in vitro P. falciparum* culture assay. Even though this methodology is not suitable for use with normal adult blood, or for cryopreserving cells, it is clear that it represents an optimum procedure for obtaining cells for immediate use.

Maturation of haematopoietic cells

Extensive research focussed on studying the umbilical cord has led to determining that this is an important source of mesenchymal, somatic and haematopoietic stem cells (HSCs), this being of great importance in the field of biomedical and clinical research (Pelosi et al., 2012). HSCs can be differentiated in any type of cell present in the blood by the activity of specific differentiation factors; such cells can also be found in bone marrow or other tissues. Thinking about this line as a potential source of erythrocytes, Panichakul et al. (2007) introduced a method for obtaining a large amount of reticulocytes from HSC maturation into erythroid cells. Their results revealed a large amount of reticulocytes on day 14 determined by brilliant Cresyl blue staining; given their morphology, cell surface markers and susceptibility to *P. vivax* infection, the authors considered them to be completely viable. Although this method has led to fresh erythroid cells being obtained in different stages of maturation, it is very complex to use and is costly, meaning that it may have limited application in research groups working on malaria.

Reticulocyte cryopreservation

Although most reticulocytes are successfully recovered by using some of the methods mentioned in the 'Reticulocyte sources' section, their rapid maturation turns out to be a restrictive factor when using this type of cells in lengthy assays. Several studies focussed on reticulocyte preservation have been developed to overcome this problem. Borlon et al. (2012) analysed the possibility of cryopreserving reticulocytes obtained from cord blood; these cells were enriched according to the previously mentioned methodology (Russell et al., 2011), homogenised in 20% glycerol and then stored in liquid nitrogen. Once the cells had been unfrozen using the sodium chloride method, their ability to host infection was evaluated *in vitro.* The results showed that passing cells through liquid nitrogen did not affect their stability as they matured normally; cells were also viable and supported parasite invasion. A complementary study by Noulin et al. (2012) showed that erythrocytes obtained from HSC maturation could be cryopreserved for 1 year without damage to the receptors located on plasma membrane. Cryopreservation was successful using two media containing different preservative agents; the first consisted of 28%

glycerol, 3% sorbitol and 0.9% sodium chloride, whereas the other contained 10% DMSO/40% FCS (Noulin et al., 2012). The use of these methods represents an advantage concerning reticulocyte use in malaria research and in studies using analytical tools focussed on omic sciences, such as microarrays and/or proteomics.

Cell receptors characterised for *P. vivax*

Even though it is clear that some reticulocyte molecules are essential for the progress of *P. vivax* infection, their description, characterisation and the exact nature of molecular interactions with target cell proteins still remains unknown. Characterising cell surface receptors will provide valuable information for developing prevention strategies, mainly vaccines. In spite of the foregoing, advances in knowledge regarding reticulocyte receptors for *P. vivax* have notably lagged behind the body of knowledge concerning erythrocyte receptors for *P. falciparum* (Sim et al., 1994; Rayner et al., 2001; Goel et al., 2003; Maier et al., 2003; Li et al., 2004; Mayer et al., 2004; Kato et al., 2005; Mayer et al., 2009; Tham et al., 2010; Crosnier et al., 2011; Gunalan et al., 2011; Sahar et al., 2011; Triglia et al., 2011; Tham et al., 2012) (Figure 1) (Table 2).

The Duffy antigen receptor for chemokines (DARC) is the only reticulocyte receptor used by *P. vivax* during invasion which has been described and functionally evaluated to date (Figure 1). This molecule is a transmembrane glycoprotein which is expressed on the surface of various cells, including human erythrocytes and reticulocytes (Peiper et al., 1995; de Brevern et al., 2005). The DARC has 337 amino acids, having an estimated ∼35 kDa molecular weight and crosses the RBC membrane seven times. Its identity as a *P. vivax* receptor became known in a study involving the genotyping of individuals proving resistant to the disease which determined that their RBC lacked DARC expression (Miller et al., 1976). It has also been shown that this receptor is required for infection caused by *P. knowlesi* (Haynes et al., 1988; Adams et al., 1990) and *P. yoelii* (Swardson-Olver et al., 2002). The advent of 3D prediction and mutational studies has facilitated understanding the interaction between the DARC and its ligand, the *P. vivax* Duffy binding protein (VanBuskirk et al., 2004; Singh et al., 2006). Russel

Figure 1 | Red blood cell and reticulocyte receptors described to date with their respective *P. falciparum* and *P. vivax* binding ligands

Parasite ligands are shown in black and RBC receptors are shown in white letters, respectively. GPA, GPB and GPC, glycophorin A, B and C; CR1, complement receptor 1; DARC, Duffy antigen receptor for chemokines; EBA, erythrocyte binding antigen; EBL-1, erythrocyte binding like 1; RON-1, -2, -5, Rhoptry Neck Proteins -1, -2 and -5 which participate in forming the *tight junction* complex with AMA-1, apical merozoite antigen 1; MSP-1, merozoite surface protein 1; DBP, Duffy binding protein; RBP-1, -2, reticulocyte binding proteins -1 and -2. Y, Z, W and ? represent receptors not yet identified.
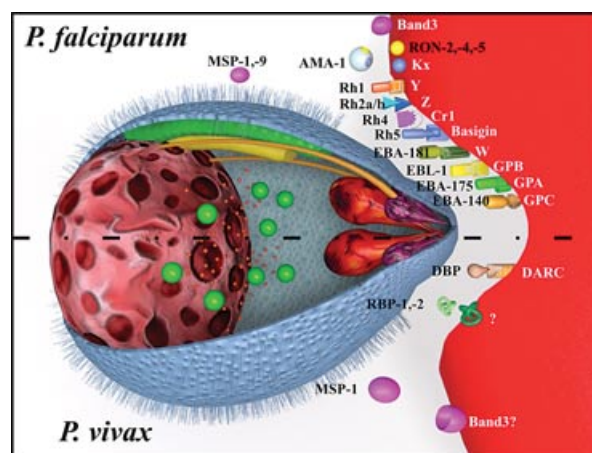
et al. (2011) have shown this receptor's functional importance *in vitro*. Consistent with assays by Barnwell et al. (1989) years ago, DARC interaction between the parasite ligand became inhibited on incubating reticulocytes with murine Fy6 antibodies directed against the receptor's N-terminal region, leading to the suggestion that DARC is an obligatory route for *P. vivax* invasion of reticulocytes.

The strong inclination of *P. vivax* for invading reticulocytes and its inability to infect mature RBC (which also express the Duffy antigen) suggest that this cell type's specificity could be attributed to an interaction with other membrane receptors. Several reports referring to identifying infection in Duffy-negative individuals (Ryan et al., 2006; Cavasini et al., 2007a; 2007b; Mendes et al., 2011) have supported the hypothesis that DARC is not the only component required for invasion by *P. vivax*, as initially proposed. Moreover, parasite proteins participating in target cell recognition and having a preference for

Table 2 | Red blood cell receptors required for *P. falciparum* and *P. vivax* invasion

Cell type	Receptor	Ligand	Ligand localisation	Reference
RBC	GPA	EBA-175	Micronemes	Sim et al. (1994)
	GPB	EBL-1	Micronemes	Mayer et al. (2009)
	GPC	EBA-140 (BAEBL)	Micronemes	Maier et al. (2003)
	W	EBA-181	Micronemes	Revised in Tham et al. (2012)
	Kx	AMA-1	Micronemes	Kato et al. (2005)
	Y	Rh1	Rhoptries	Rayner et al. (2001)
	Z	Rh2a/b	Rhoptries	Gunalan et al. (2011); Sahar et al. (2011); Triglia et al. (2011)
	CR1	Rh4	Rhoptries	Tham et al. (2010)
	Basigin (CD147)	Rh5/Ripr	Rhoptries	Crosnier et al. (2011)
	Band 3	MSP-1, -9	Surface	Goel et al. (2003); Li et al. (2004)
Reticulocyte	DARC	DBP	Micronemes	Peiper et al. (1995)
	Band3?	MSP-1	Surface	Rodriguez et al. (2002)
	?	RBP-1, -2	Rhoptries	Galinski et al. (1992); Cantor et al. (2001)

GPA, GPB and GPC, glycophorin A, B and C; CR1, complement receptor 1; EBA, erythrocyte binding antigen; EBL-1, erythrocyte binding like 1; AMA-1, apical merozoite antigen 1; MSP-1, merozoite surface protein 1. Y, Z, W and ? represent receptors not yet identified.

reticulocyte binding have been identified, including reticulocyte-binding proteins 1 and 2 (RBP-1, -2) (Galinski et al., 1992; Cantor et al., 2001) and merozoite surface protein 1 (MSP-1) (Rodriguez et al., 2002) (Figure 1). Previous studies have found that RBP-1 and MSP-1 contain peptides which bind to reticulocytes with high affinity; RBP-1 binds to 26 and 41 kDa receptors, whereas MSP-1 binds to 18 and 20 kDa molecular mass receptors (Rodriguez et al., 2002). These latter ones seem to be reticulocyte-specific because their molecular weight differs from that determined for Band 3, which has been considered as the MSP-1 receptor, according to experiments carried out with its *P. falciparum* homologue (Goel et al., 2003). Despite these studies, the receptors for MSP-1 in *P. vivax* as well as those for RBPs have not been experimentally characterised so far. The foregoing highlights the need for using different tools for characterising reticulocytes' surface molecules for understanding the molecular basis of their function as malaria parasite receptors. This will lead to proving the existence of an alternative *P. vivax* invasion route (Mons, 1990) and developing strategies for preventing the infection.

Perspectives

Improving methods to obtain large reticulocyte amounts is required to solve one of the main challenges in *P. vivax* malaria research: the characterisation of antigens with potential role in invasion. Various methods have been tested for cell separation in suspension, such as the use of immunomagnetic beads or selective separation by flow cytometry (Davies, 2012); these methods could be used for reticulocyte purification to prevent the damage caused to these cells when passing them through an isotonic Percoll gradient, a CF-11 column or when they are enriched using a hypotonic solution. This will facilitate obtaining a greater number of viable cells to use in *P. vivax in vitro* culture assays or studies focussed on understanding *P. vivax* infection dynamics.

Additional techniques orientated towards evaluating the identity of the molecules involved in protein–protein interactions between *P. vivax* and reticulocytes are thus needed. Among existing methodologies, using affinity etiquettes for purifying complexes, co-immunoprecipitation, chemical crosslinking, the two-hybrid system and confocal microscopy (Phizicky and Fields, 1995; Berggard et al., 2007; Wu et al., 2007) have proved of great use when molecularly analysing target cell–parasite protein interactions. Some of the approaches here mentioned, together with mass spectrometry and structural analysis, open the way forward for ascertaining the molecular basis for invasion and also for resolving the deficiencies which have arisen when studying antigens for inclusion in a potentially effective vaccine against malaria caused by *P. vivax*.

The 'omic' sciences are an interesting alternative when evaluating cell receptors facilitating parasite adhesion. Oligonucleotide microarrays and mass spectrometry assays have been used for studying

reticulocyte genes and proteins which are being expressed. Goh et al. (2007) used the microarray technique; they identify 107 genes, 103 of which were differentially expressed in umbilical cord reticulocytes. This profile provides an interesting source of information for carrying out probabilistic studies aimed at identifying surface receptors for the *P. vivax* proteins described to date (Patarroyo et al., 2012). Two mass spectrometry studies of immature murine erythrocyte membrane fractions have been carried out; the first reported 45 proteins having increased expression in a reticulocyte-enriched sample (Wooden et al., 2011), whereas the second identified 587 (Prenni et al., 2012), some of them being characterised as plasmatic membrane proteins. Such results provide invaluable information about the possible receptors which could be involved in their selectivity as host cells.

Furthermore, an open research line is the study of exosomes. These are internal vesicles located in multi-vesicular bodies which are involved in selective removal of several proteins during reticulocyte maturation and differentiation process to erythrocytes. A recent study has revealed that exosomes derived from reticulocytes infected with the non-lethal *P. yoelii* 17X strain contained antigens which were able to modulate the immune response, suggesting that these vesicles can play roles beyond those initially described, intercellular communication and antigen presentation among them (Martin-Jaular et al., 2011). Further studies to evaluate exosomes' importance in malaria research are in need, such as their detailed proteomic characterisation, seeking for reticulocyte receptors that make the *P. vivax* parasite infection so selective.

Conclusion

Although the study developed by Russell et al. (2011) has been the most promising for reticulocyte recovery since it has shown to be suitable for culturing *P. vivax in vitro*, further optimisation of target cell enrichment methods is needed to address several fundamental questions regarding both reticulocyte and parasite biology. Enrichment methods could be improved by avoiding the use of Percoll, CF-11 column or hypotonic solutions, and developing new approaches for recovering cells in suspension.

Advances in studying reticulocytes' biological aspects have provided valuable information for ascertaining these cells' genetic determinants regarding their susceptibility to different diseases, including infection caused by parasites. Further studies are required for understanding the precise role played by receptor–ligand interactions between cells and their main infectious agent, thereby also being useful for finding therapeutic targets and establishing other methods for preventing infection, including the development of chemically made vaccines.

Conflict of interest statement

The authors have declared no conflict of interest.

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