International Journal for Parasitology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Invited Review

Plasmodium vivax vaccine research – we've only just begun

Wai-Hong Tham [a,b], James G. Beeson [c,d], Julian C. Rayner [e,*]

[a] The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
[b] Department of Medical Biology, University of Melbourne, Parkville, Victoria 3052, Australia
[c] Macfarlane Burnet Institute of Medical Research, 85 Commercial Road, Melbourne, Victoria 3004, Australia
[d] Central Clinical School and Department of Microbiology, Monash University, Victoria, Australia
[e] Wellcome Trust Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom

ARTICLE INFO

Article history:
Received 29 May 2016
Received in revised form 24 September 2016
Accepted 29 September 2016
Available online xxxx

Keywords:
Malaria
Plasmodium vivax
Vaccine
Erythrocyte invasion

ABSTRACT

Plasmodium vivax parasites cause the majority of malaria cases outside Africa, and are increasingly being acknowledged as a cause of severe disease. The unique attributes of *P. vivax* biology, particularly the capacity of the dormant liver stage, the hypnozoite, to maintain blood-stage infections even in the absence of active transmission, make blood-stage vaccines particularly attractive for this species. However, *P. vivax* vaccine development remains resolutely in first gear, with only a single blood-stage candidate having been evaluated in any depth. Experience with *Plasmodium falciparum* suggests that a much broader search for new candidates and a deeper understanding of high priority targets will be required to make significant advances. This review discusses some of the particular challenges of *P. vivax* blood-stage vaccine development, highlighting both recent advances and key remaining barriers to overcome in order to move development forward.

1. Still looking for the on-ramp in the *Plasmodium vivax* vaccine roadmap

Plasmodium falciparum and *Plasmodium vivax* both emerged from African apes early during human evolution (Liu et al., 2010, 2014), but their subsequent history has been very different. While *P. falciparum* cases are still concentrated in Africa, with a relatively light, although highly impactful, footprint in the rest of the world, *P. vivax* parasites dominate Asia and the Americas, causing the majority of malaria outside Africa (Gething et al., 2012). Detailed clinical studies have made it clear that while the majority of *P. vivax* cases are asymptomatic, *P. vivax* infections can be severe and result in mortality (Baird, 2013). This revelation, coupled with concerns about emerging *P. vivax* drug resistance, have led to a renewed interest in this neglected tropical disease, and concerted discussions about a *P. vivax*-specific elimination strategy. The biologically distinct life cycle of *P. vivax*, with high transmission potential and a dormant liver stage, the hypnozoite, make it clear that control strategies will not simply be able to be transferred from *P. falciparum* (Mueller et al., 2009). While effective treatments for blood-stage *P. vivax* exist, primarily artemisin-combination therapies (ACTs), treatment for hypnozoites is limited to one drug (primaquine) that is contra-indicated by glucose-6-phosphate dehydrogenase (G6PD) deficiency, a common phenotype in many malaria endemic regions (Baird et al., 2012).

Given the unknown burden of hypnozoites, which can cause relapse and trigger blood-stage infections even in the absence of continued transmission, an effective blood-stage vaccine would be a particularly useful tool for *P. vivax* elimination, as well as reducing the burden of disease. However, there is currently no effective vaccine for *P. vivax*. This statement, which has been made in one guise or another in the introduction to almost every review on malaria vaccine development for the last 10 years, in fact understates the current situation for *P. vivax*. Whereas *P. falciparum* vaccine research has progressed inexorably forward, moving to large-scale testing of panels of antigens and with one candidate reaching Phase III trials (Rts, 2015), the majority of *P. vivax* vaccine development is resolutely still in early preclinical development (Mueller et al., 2009). Human clinical trials have to date only been carried out for two *P. vivax* antigens. A vaccine based on the pre-erythrocytic candidate Circumsporozoite Protein (PvCSP) was well-tolerated in a recent Phase I/IIa trial, inducing antibody and cell-mediated immune responses and resulting in a delay in the median prepatency period for all vaccinated individuals (Bennett et al., 2016). While the prepatency period was delayed by up to 2 days, no sterile protection was achieved, and just as with the development of CSP-based vaccines for *P. falciparum* has taken an extended period of time, it is clear that much more work awaits

* Corresponding author.
E-mail address: jr9@sanger.ac.uk (J.C. Rayner).

http://dx.doi.org/10.1016/j.ijpara.2016.09.006
0020-7519/

for *P. vivax*. The only other candidate to reach Phase I, the transmission-blocking candidate Pvs25, induced antibody responses that could block *P. vivax* transmission (Malkin et al., 2005; Wu et al., 2008), but again much work is required to move beyond this initial study. All other potential antigens remain resolutely at the preclinical stage, although in the case of one blood-stage candidate, *P. vivax* Duffy Binding Protein (PvDBP), this preclinical development has been extensive (reviewed in (Mueller et al., 2015), and a Phase Ia trial of a virally vectored PvDBP vaccine (de Cassan et al., 2015) has recently been completed (https://clinicaltrials.gov; trial NCT01816113). The detailed advancement of only a single blood-stage candidate is in marked contrast to the more extensive testing of candidates for *P. falciparum*, where the number of candidates under active investigation reflects the complexity of the erythrocyte invasion process, which involves proteins located in multiple intracellular organelles (Fig. 1). If we are to advance blood-stage *P. vivax* vaccine development in any significant manner, new tools and detailed studies of emerging candidates will be needed. This review discusses the emergence of some such tools and identifies the key gaps that remain on the path to *P. vivax* vaccine development.

2. Broader – expanding the range of candidates using new tools

Malaria vaccine research has an extensive history and is based on seminal findings that productive immunity can be generated following repeated experimental exposure to parasite infection (Collins and Jeffery, 1999) or by passive transfer of antibodies (Cohen et al., 1961). Identifying the best vaccine antigens has fixated the field ever since, especially since the true scale of potential targets was revealed with the publication of the first *P. falciparum* genome in 2002 (Gardner et al., 2002), followed in 2008 by the *P. vivax* genome (Carlton et al., 2008). It has taken some years for the scale of *Plasmodium* vaccine research to begin to match the scale of the *Plasmodium* genome, with the vast majority of research focussing on only a handful of antigens, which not coincidentally were also among the first *Plasmodium* genes cloned (Conway, 2015). A significant portion of this lag can be attributed to the fact that almost any vaccine candidate research requires expression of recombinant antigens and *Plasmodium* proteins have been consistently some of the most difficult proteins to express. The percentage of Adenine (A) and Thymine (T) nucleotides in some *Plasmodium* genomes can exceed 80%, which makes manipulating *Plasmodium* DNA into expression constructs difficult and results in amino acid biases that are not always well tolerated by the standard eukaryotic or prokaryotic protein expression systems. Added to this is the apparent lack of N-linked glycosylation in *Plasmodium* parasites but presence of canonical N-linked glycan sites within their antigens, which means that their expression in some eukaryotic systems can result in aberrant glycosylation and production of distinctly non-native proteins. For too many years, those that aspired to express *Plasmodium* proteins were routinely frustrated by insoluble pellets and vanishingly small yields.

Two developments have radically improved our ability to express *Plasmodium* proteins. Perhaps most significantly, if also most trivially, the cost of DNA synthesis has dropped dramatically in recent years. This has facilitated molecular cloning and allowed researchers to easily optimise both AT content and codon usage to suit the specific expression system that they are using. In parallel, much more scalable expression systems have been developed, allowing expression of up to hundreds of genes simultaneously and the first systematic screens of potential *Plasmodium* vaccine antigens and immune targets. A widely applied approach has been the use of an *Escherichia coli* cell-free in vitro transcription-translation system, which enabled printing of slides with hundreds of antigens (Doolan et al., 2008) and large-scale screening of epidemiological cohorts for *P. falciparum* antigens (Crompton et al., 2010; Dent et al., 2015). While bacterial in vitro transcription–translation systems have the advantage of scale, they have the disadvantage of producing proteins in an extracellular reducing environment and expressed proteins can thus lack disulphide bonds, post-translational modifications or other elements of native tertiary structure. Linear epitopes are preserved but some conformational and functional epitopes may be lost, which is a particular concern for extracellular proteins which include most *Plasmodium* vaccine targets. Proteins produced in this platform for use on arrays are not purified, which presents other limitations. An alternative in vitro transcription–translation approach is the wheat-germ cell free expression system, which has been shown to successfully produce active proteins with tertiary structure, and vaccines based on this platform induce functional immune responses (Miura et al., 2013; Tsuboi et al., 2010). Large-scale wheat-germ protein

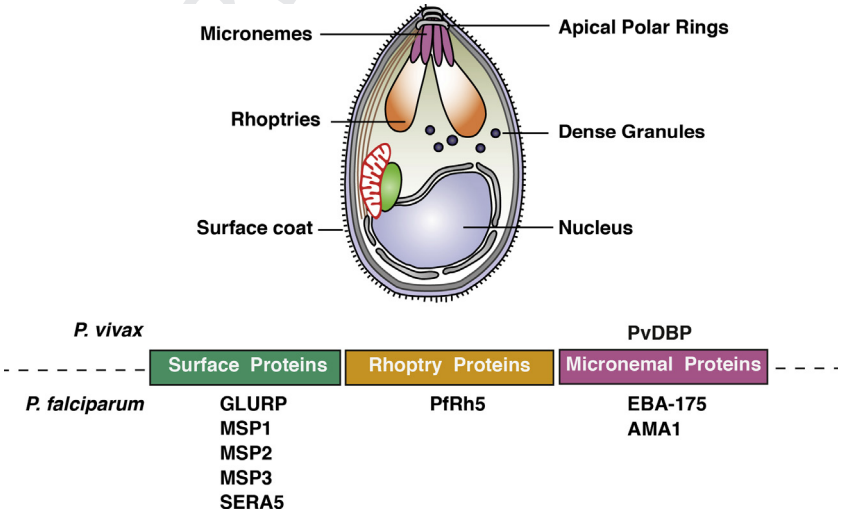

Fig. 1. A schematic representation of a *Plasmodium* merozoite showing several of the major structures and organelles. *Plasmodium vivax* and *Plasmodium falciparum* antigens currently in clinical development are shown according to their respective localisation to either the surface coat, rhoptries or micronemes. PvDBP, *P. vivax* Duffy Binding Protein; EBA, erythrocyte-binding antigen; AMA, apical membrane antigen; PfPH5, *P. falciparum* reticulocyte binding-like homologue 5, GLURP, glutamate-rich protein of *P. falciparum*; MSP, merozoite surface protein; SERA, serine repeat antigen.

Please cite this article in press as: Tham, W.-H., et al. *Plasmodium vivax* vaccine research – we've only just begun. Int. J. Parasitol. (2016), http://dx.doi.org/10.1016/j.ijpara.2016.09.006