Review

Plasmodium vivax and the importance of the subtelomeric multigene *vir* superfamily

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***Plasmodium vivax* is responsible for more than 100 million clinical cases yearly. Unlike *P. falciparum*, in which infected red blood cells cytoadhere via variant proteins, avoiding passage through the spleen, *P.-vivax*-infected reticulocytes seem not to cytoadhere. However, a variant subtelomeric multigene *vir* family has been identified in *P. vivax*. Thus, questions remain about how *P. vivax* circulates through the spleen and the role of Vir proteins. In this review, the importance of the *vir* multigene superfamily is reviewed in the light of the completion of the entire genome sequence of *P. vivax* and from data gathered from experimental infections in reticulocyte-prone non-lethal malaria parasites and natural *P. vivax* infections.**

Biological and epidemiological aspects of malaria

Plasmodium vivax is the most widely distributed human malaria parasite, with an at-risk population of 2.5 billion people. Although the exact burden of disease caused by *P. vivax* is still a matter of debate [1,2], it is likely that this burden has been underestimated and that 100–300 million clinical cases each year are due to this parasite [3]. Moreover, the appearance of chloroquine-resistant *P. vivax* parasites [4], clinical severity including deaths associated exclusively with *P. vivax* [5] and global warming indicate that the burden of *P. vivax* will increase in the next few years. Notably, outside sub-Saharan Africa, *P. vivax* accounts for more than half of all malaria cases in South and Southeast Asia, Central and South America, the Middle East, and eastern and southern Africa, where it is responsible for a large socioeconomic burden. In most of these regions, *P. vivax* is sympatric with *P. falciparum*, the most virulent human malaria parasite, and mixed infections commonly are diagnosed. This concurrence reinforces the widely held, although not completely accepted [6], view that immunity in malaria is species-specific. This view is further sustained by solid evidence in experimental malaria infections of neurosyphilitic patients [7]. In this scenario, it is possible to speculate that an effective vaccine against *P. falciparum* will not cross-protect against *P. vivax*. Rather, it will probably create new opportunities for *P. vivax* infections, and this reinforces the need to develop *P.-vivax*-specific vaccines or to include formulations that offer protection against both species.

The search for vaccines against *P. vivax* remains a formidable challenge; presently, there are only two *P. vivax* subunit vaccine candidates and a modest number of other candidates being tested in preclinical trials [8]. This is in striking contrast to *P. falciparum,* for which more than 70 different vaccine formulations have been produced, of which 23 are undergoing clinical trials [9]. Furthermore, there are fundamental biological differences between *P. vivax* and *P. falciparum* that can guide the rational search of vaccine candidates and drug targets that are unique to the biology of *P. vivax* (Table 1). A prime example of this is the *P. vivax* Duffy-binding protein (PvDBP). It is well established that most human black populations from sub-Saharan Africa are refractory to infections by *P. vivax* because they do not present the Duffy-blood-group receptor for reticulocyte invasion [10]. Although this paradigm is now being challenged by recent studies indicating that *P. vivax* parasites are able to invade Duffy-negative individuals [11,12], molecular approaches to determine the ligand molecule of the Duffy group led to the discovery of the PvDBP [13] and its receptor-binding domain, the Duffy-antigen receptor for chemokines [14]. Several complementary studies led to the production of a correctly folded version of PvDBP in *Escherichia coli* [15], and preclinical trials in nonhuman primates have been conducted [16]. In addition, the crystal structure from the Duffy-binding-like domain of *Plasmodium knowlesi* revealed that crucial amino acid residues involved in ligand–receptor interactions are mostly protected from neutralizing antibodies [17]. Studies that further investigate the unique aspects of

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Table 1. Main biological differences between *Plasmodium vivax* and *Plasmodium falciparum*

Plasmodium vivax	*Plasmodium falciparum*
100–300 million yearly clinical cases [3].	300–500 million yearly clinical cases.
Usually non-lethal and non-severe; recently, however, this situation seems to be changing [5].	Lethal and severe, responsible for 1.7–2.4 million deaths each year, mostly in children under five years old.
Continuous *in vitro* culture not available, although promising attempts are now reported [62].	Continuous *in vitro* culture available since 1976 [63].
Invades preferentially, if not exclusively, reticulocytes [64].	Invades all red blood cells.
Requires the Duffy blood group as the receptor for entrance into reticulocytes [10].	Has multiple entrance pathways and receptors [65].
Gametocytes appear in peripheral blood before clinical symptoms.	Gametocytes appear in peripheral blood after clinical symptoms.
Chloroquine resistance first reported in 1989 [4].	Chloroquine resistance first reported in late 1950s [4].
Chloroquine resistance is not associated with mutations of the *pvcrt-o* (*P. vivax* orthologue of *pfcrt*) gene [66].	Chloroquine resistance is associated with mutations of the *pfcrt* (*P. falciparum* chloroquine-resistance transporter) gene [67].
Surface membrane of infected reticulocytes suffers invaginations, called caveola vesicles [68].	Surface membrane of infected red blood cells suffers protrusions, called knobs [69].
Sporozoites can remain within hepatocytes in dormant stages known as hypnozoites [70].	Sporozoites within hepatocytes undergo immediate schizogony with no dormant stages.
Hypnozoites cause clinical relapses.	There are no clinical relapses.
Widely accepted that there is no sequestration in the capillaries of internal organs.	There is sequestration in the capillaries of internal organs.
Passage through the spleen of all asexual blood stages.	Avoids passage through the spleen of mature asexual blood stages.
Genome contains two DNA components with two major isochores with GC-content of 18% and 30% [71].	Genome contains one single DNA component with GC-content of 18% [71].
Subtelomeric regions of complete assembled chromosomes of *P. vivax* lack complex repeats [22].	Subtelomeric regions of *P. falciparum* have complex repeats, such as rep20 [72].

P. vivax biology, therefore, might lead to other vaccine candidates and alternative control strategies.

The subtelomeric *vir* multigene superfamily

Analysis of a chromosome end from a *P. vivax* wild isolate contained within a yeast artificial chromosome (YAC) led to the discovery of a subtelomeric multigene superfamily termed *vir* (*P. vivax* variant genes) with an estimated 600–1000 copies per haploid genome [18]. Moreover, sequence-similarity analyses of *vir* genes contained within this YAC revealed that 16 of the 32 *vir* genes could be clustered into six different subfamilies termed A–F, whereas the others remained as singletons. In addition, structural analysis revealed that most of these 32 *vir* genes displayed a common three-exon structure with a first small exon lacking canonical signal peptide sequences, followed by a highly polymorphic exon and a third exon encoding a putative cytosolic domain. Formation of a conserved transmembrane (TM) domain was predicted after *in silico* splicing of the second intron. Confocal laser scanning microscopy has demonstrated that Vir proteins localize to the surface of infected reticulocytes. Their variant nature and subtelomeric location have led to the suggestion that Vir proteins are mainly involved in antigenic variation.

Analysis of the expressed *vir* repertoire in natural infections from individual parasites demonstrated that there is no allelic exclusion of *vir* genes and no clonal expression of Vir proteins at the surface of individually infected reticulocytes. Moreover, first-time infected *P. vivax* patients had naturally acquired humoral immunoglobulin G (IgG) responses capable of cross-reacting against different Vir proteins [19,20]. Thus, whereas these data are consistent with a major role of *vir* genes in immune evasion, they are inconsistent with a predominant role in the strict sense of antigenic variation. These data are in agreement with the view that *vir* genes might mainly be involved in another undiscovered immune-evasion mechanism [21].

The recent annotation of the complete *vir* gene repertoire from the *P. vivax* Salvador I (Sal I) strain genome revealed that the number of *vir* genes was much lower than originally predicted [22]. Instead of 600–1000 genes, a total of 346 *vir* genes, including 80 fragments and/or pseudogenes, were annotated. Presently, it is not clear whether this discrepancy reveals differences between parasites obtained from infected patients and parasites maintained in monkeys or an overestimation of the *vir* repertoire in the original calculations. Regardless, *vir* genes remain the largest subtelomeric multigene family of *P. vivax* [22]. Moreover, in addition to subfamilies A–F, six new subfamilies termed G–L have been identified, 82 genes remain unclustered and analysis of all of their structures revealed genes with different numbers of exons (1–5) and different sizes (156–3434 bp) (Figure 1a). Furthermore, probabilistic modelling and predictions of protein and gene structures revealed the existence of protein motifs conserved among Vir proteins (Figure 1b). Noticeably, unlike *var* genes, *vir* genes were not found in internal regions of the genome.

The *Plasmodium* interspersed repeats gene superfamily

The *Plasmodium* interspersed repeats (PIR) multigene superfamily is the largest family to date with homologous genes in human, monkey and rodent malaria parasites (Table 2). Homologous genes in *P. vivax*, *Plasmodium*

Table 2. The *Plasmodium* PIR multigene superfamily

Species	Family	Total genes	Location	Ref.
P. vivax	*vir*	346	Subtelomeric	[22]
P. falciparum	*rifin/stevor*	177	Subtelomeric and internal	[72]
P. knowlesi	*kir*	68	Subtelomeric and internal	[28]
P. yoelii	*yir*	838	Subtelomeric	[73]
P. berghei	*bir*	180	Subtelomeric	[29]
P. chabaudi	*cir*	138	Subtelomeric	[29]

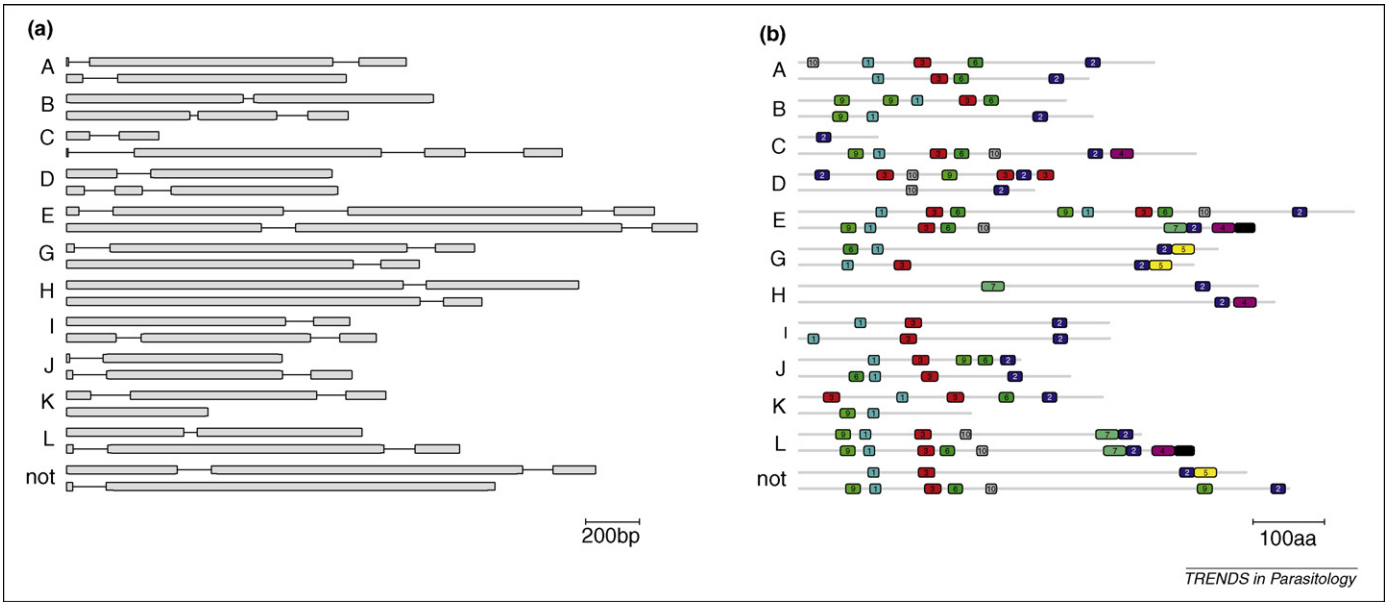

Figure 1. The *Plasmodium vivax* subtelomeric *vir* multigene superfamily. **(a)** Schematic representation of gene models from the different subfamilies (two of each) A–G, H–L and unclustered genes (not). Exons are depicted as boxes and introns as lines. Scale in nucleotides (base pairs, bp). **(b)** Schematic representation of the deduced proteins from the genes depicted in (a) displaying conserved amino acid motifs. 1, CIYLNYWLYDQI; 2, VVGVMMTFFFLYKFTP (TM membrane); 3, KERKDLHDYFKNYDTIKC (PEXEL-like); 4, KRKGKIFEHNYYEEYEKELAMYGSE; 5, VGAFFRGGRGRVHRIPRSFHGQFPG; 6, CEKYCTYVTYIKSLYE; 7, IADSPGTLGTVHEELDSNFFRNIIM; 8, TFLDSQMDRYYLNYQPDQDSYY; 9, VKELCKKLVRNLKKIS; 10, YDPKDLLSKLDC [22]. Scale in amino acids (aa).

berghei and *Plasmodium yoelii* are mostly transcribed by late trophozoites [23]. Amino acid similarities range from 30% to 50% between *Plasmodium chabaudi*, *P. yoelii* and *P. berghei* and from 20% to 30% between *P. chabaudi* and *P. vivax*, and all members contain a conserved three-exon structure. Gene members were named according to their species: *vir* (*P. vivax*), *cir* (*P. chabaudi*), *bir* (*P. berghei*) and *yir* (*P. yoelii*). Gene structural analysis and protein structural predictions in conjunction with probabilistic modelling on a whole-genome scale subsequently identified other members of this multigene superfamily: the *rif/stevor* genes in *P. falciparum* and the novel *kir* genes in *Plasmodium knowlesi* [24]. Notably, these analyses demonstrated that *vir* genes are closely related to *kir* genes and not to the *P. knowlesi sica-var* genes or the *P. falciparum var* genes, which are both involved in antigenic variation.

A similar multi-character computational analysis was later used to further analyze *vir* [25] and *yir* [26] genes. Both analyses revealed many common structural aspects of these gene families, such as different subfamilies and groups, and a more complex structure than the originally described three-exon common structure, including genes with varying numbers of exons, sizes and architecture. Moreover, *yir* genes display complex transcription patterns, including alternative splicing [26]; whether *vir* genes use this mechanism for control of gene expression remains to be determined. In addition, analysis of *yir* gene-expression profiles in experimental rodent infections has revealed that this expression is influenced by the immune status of the host [27]. These data support the use of the *yir* multigene family from *P. yoelii* as a model system to try to unveil the function of *vir* genes. Studies of *kir*, *cir* and *bir* genes are more limited [28,29]; however, they further reinforce the importance of monkey and rodent models to advance our knowledge of the evolution and function of the PIR multigene superfamily.

Subcellular localization of Vir proteins

Malaria parasites have developed sophisticated mechanisms for protein export within red blood cells to target different subcellular compartments. These include the use of an amino acid motif (Arg-Xaa-Leu-Xaa-Glu/Gln/Asp) by *P. falciparum*, which has been termed PEXEL/VSP (*Plasmodium* export element/vacuolar transport signal) and is used as an intracellular signal that is necessary to transport malarial proteins to the surface of infected red blood cells and the cytosol [30,31]. Strikingly, sequence analysis of the entire *vir* gene repertoire from Sal I showed that only 160 deduced Vir proteins possess PEXEL-like motifs [22]. Similar results were observed in genome-wide analyses of *yir* [26] and *rif* [32] genes, which showed that many genes lack PEXEL motifs, indicating an alternative pathway for exportation to the infected red blood cell membrane or different subcellular localization, as shown for Rifins [33].

Based on confocal microscopy images obtained from smears of a single wild isolate and using immune serum raised against a conserved peptide sequence from subfamily D, Vir proteins were originally thought to be exclusively located at the surface of infected reticulocytes [18]. However, *in silico* analysis of protein domains and secondary structures from parasite sequences obtained directly from patients revealed that subfamily A is related to the SURFIN subtelomeric *P. falciparum* multigene family and that Vir subfamily D contains 2TM domains similar to the Pfmc-2TM multigene family [25]. Of note, SURFIN proteins are located at the surface of merozoites and infected red blood cells [34], whereas Pfmc-2TM proteins are located mainly at Maurer's clefts [35]. These data, together with the lack of PEXEL motifs in a notable number of Vir proteins, indicate that these proteins might have subcellular localizations other than the surface membrane of infected reticulocytes. Determining the subcellular localization of Vir proteins remains an essential aspect

of *P. vivax* biology for unravelling the biological function(s) of this subtelomeric multigene superfamily.

Role of the spleen and Vir proteins

Pathology in malaria is associated with the capacity of infected red blood cells to escape immune responses and establish chronic infections. In the case of *P. falciparum*, cytoadherence of infected red blood cells containing mature stages of the parasite in the deep capillaries of internal organs avoids passage through the spleen, where these cells would be targeted for destruction. Notably, cytoadherence is mediated by the *var* multigene family [36]. In the case of *P. vivax*, it is widely accepted that there is no cytoadherence of infected reticulocytes, even though this human malaria parasite contains the *vir* multigene superfamily. Although this lack of sequestration in *P. vivax* is now being challenged [21,37], the question remains as to how *P. vivax* is able to escape spleen clearance to establish chronic infections and whether Vir proteins have a role in such an escape.

The role of the spleen in modulating expression of variant proteins in malaria was first shown in *P. knowlesi*, whereby splenectomized monkeys no longer expressed variant antigens on the surface of the infected red blood cells [38]. Similar observations were made afterwards in *P. falciparum*, *Plasmodium fragile* and *P. chabaudi* [39–42]. In the case of natural infections of *P. falciparum*, tissue sequestration in splenectomized patients (as detected by the presence of mature stages in peripheral blood circulation) is largely impaired [43]. This concurs with the presently accepted view that the spleen plays a major part in modulating the expression of variant proteins in malaria.

The spleen is a lymphoid organ that is adapted to selectively clear abnormal red blood cells, particles from the blood and infectious agents, including malaria. Histopathological studies have documented the structure and function of the spleen under normal and pathological conditions, including experimental infections with different rodent malaria parasites in mice [44]. Notably, in reticulocyte-prone non-lethal murine models of malaria such as *P. yoelii* strain 17X in BALB/c mice, a situation that resembles *P. vivax* infections, the 'open' circulation of the spleen is suddenly and temporarily changed to a 'closed' circulation because of the formation of syncitial layers of contractile fibroblasts that form physical barriers, termed 'barrier cells' [44]. In striking contrast, formation of 'closed' circulation owing to the formation of barrier cells is not detected in the lethal *P. yoelii* strain 17XL.

A hypothesis has been proposed that suggests that *P. vivax*-infected reticulocytes induce the formation of spleen barrier cells to actively and selectively cytoadhere to them through conserved Vir subfamily proteins or unidentified ligand(s), in addition to inducing spleen erythropoiesis to compensate for anaemia [21]. This way, infected reticulocytes that are physically protected from direct contact with spleen macrophages escape spleen macrophage clearance (Figure 2). Moreover, because dismantling of barrier cells can take weeks or even months, infected reticulocytes protected by these barriers prolong parasitism and establish chronic infections. An alternative model has been proposed, in which infected reticulocytes increase their deformability, facilitating spleen-clearance escape [45].

Several lines of indirect experimental evidence from infections of Balb/c with *P. yoelii* 17X (reticulocyte-prone non-lethal) and 17XL (normocyte-prone lethal) strains support the barrier cell formation hypothesis. First, a comprehensive study to determine the transcriptome of spleen cells from Balb/c mice infected with the *P. yoelii* 17X and 17XL strains demonstrated that only the 17X strain dramatically induced the expression of genes involved in erithropoiesis [46]. Thus, only the non-lethal strain elicits the formation of spleen reticulocytes, the host cells for invasion. The study also found several genes annotated under molecular terms of Gene Ontology corresponding to cell adhesion molecule activities that are only expressed in the spleen of mice infected with the *P. yoelii* 17X strain. Whether some of these genes are molecular markers and/or participate in the formation of barrier cells remains to be determined.

Second, Balb/c mice immunized with a recombinant merozoite surface protein 8 of *P. yoelii* were protected against a lethal challenge with the 17XL strain [47]. Interestingly, protection was associated with a change in cell tropism from normocytes to reticulocytes and the capacity of parasitized cells to 'be passed into/or to be sequestered' within the red pulp of the spleen [47]. Moreover, these changes were associated with upregulation of a subset of *yir* genes. Thus, it is clear that the *P. yoelii* lethal strain was capable of establishing chronic infections by changing its cell tropism to reticulocytes, by being channelled to the red pulp of the spleen and by upregulating particular *yir* sequences.

Third, large differences in expression-level profiles of spleen macrophage receptors in experimental infection of Balb/c mice with *P. yoelii* 17X and 17XL strains have been observed by relative quantification of real-time PCR, indicating that a different structural remodelling of the spleen in the 17X strain controls parasitaemia and impedes hyperactivation of the mouse immune system, avoiding death of the host and enabling the establishment of chronic infections [48].

Together, these data support the hypothesis that in reticulocyte-prone non-lethal malaria, such as *P. vivax*, modifications of the function and architecture of the spleen play a key part in spleen-clearance escape and establishment of chronic infections. Direct evidence of the role of Vir proteins in these mechanisms in *P. vivax*, however, will have to await results from experimental infections of monkeys with *P. vivax*, prospective histopathological analyses of human spleens obtained from cases of *P. vivax* patients with spleen rupture or sudden death, and attempts to use the recently described isolated-perfused human spleen system [49].

Correlates of Vir sequences and particular clinical outcomes

The availability of the complete genome of *P. vivax* has created the possibility of using two complementary whole-genome approaches to look for correlates of *vir* sequences and clinical syndromes.

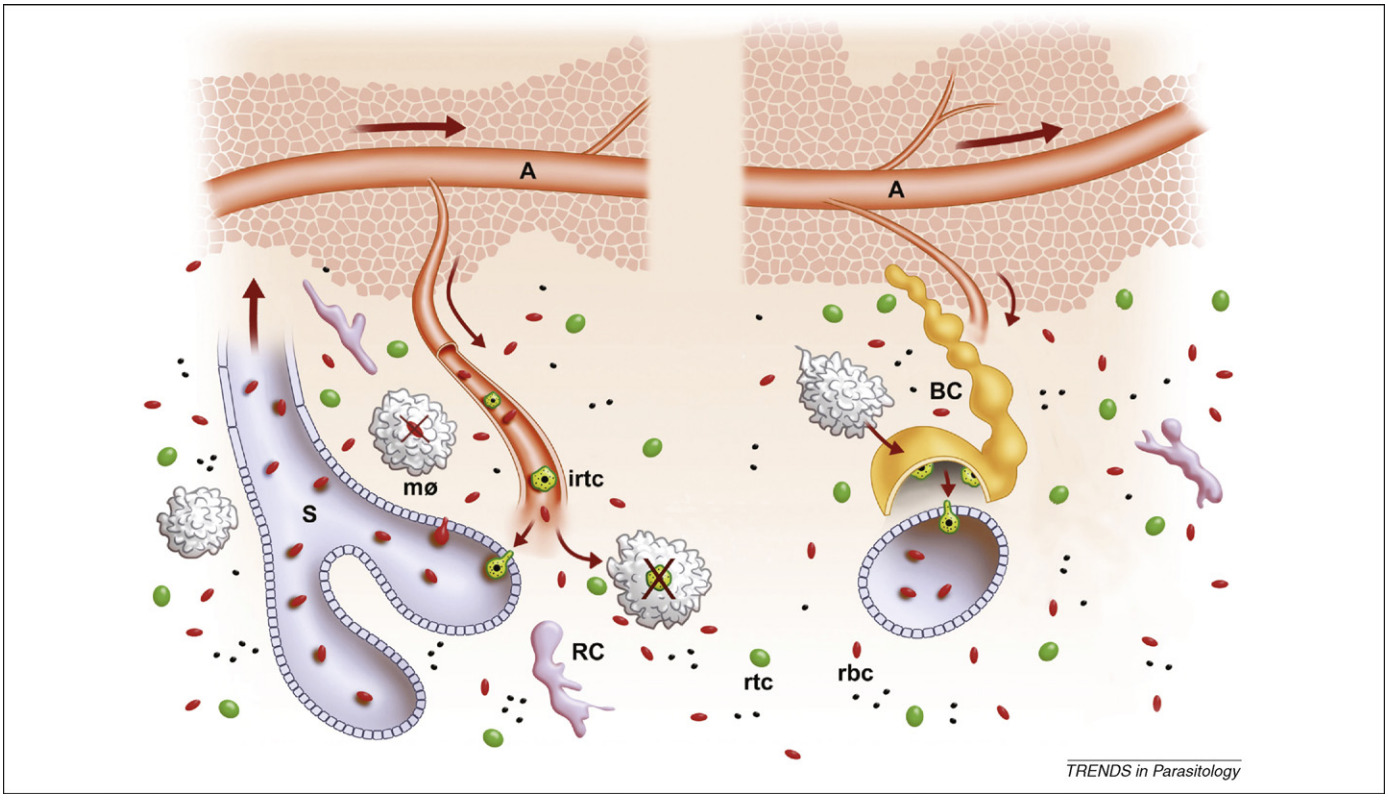

Figure 2. Proposed model of Vir protein function in spleen-specific cytoadherence and clearance evasion. Normal open circulation in the spleen releases blood into the reticular mesh, where damaged erythrocytes or *P. vivax*-infected reticulocytes are destroyed by spleen macrophages (left-hand side). A model has been proposed in which infected erythrocytes escape spleen clearance by increasing their deformability, enabling passage through adjacent endothelial cells into the venous sinus lumen [45]. This alternative proposed hypothesis in which *P. vivax* induces spleen structural remodelling, including the formation of barrier cells and 'closed' circulation, is shown on the right-hand side [21]. In doing so, *P. vivax*-infected reticulocytes specifically cytoadhere to barrier cells via conserved Vir protein subfamilies or an unidentified ligand, protecting themselves from macrophage spleen clearance. Because dismantling of barrier cells can take weeks or even months, this mechanism will facilitate prolonging parasitism and establishment of chronic infections. Abbreviations: A, artery; S, venous sinus lumen; RC, reticular cells; rtc, reticulocytes; rbc, red blood cells; irtc, infected reticulocyte; mØ, macrophages; BC, barrier cells.

Prospective longitudinal studies of immune sera

Prospective longitudinal studies of immune sera from actively identified asymptomatic *P. vivax* patients can determine whether there is a correlation between protection and/or reduced risk of infection with particular Vir protein sequences. Such an approach has been reported extensively in studies of naturally acquired immunity against different antigens in *P. falciparum* [50] and recently was reported in *P. vivax* for the PvMSP1 protein and the Duffy-binding protein [51,52]. These studies, however, will require the establishment of high-throughput methodologies for the production of large numbers of soluble Vir proteins. Such approaches now seem feasible in light of the recently described wheat germ expression system, which readily solubilizes malarial proteins [53], and the production of protein arrays displaying large numbers of recombinant proteins for immunoscreenings [54].

Gene expression analysis of the vir gene repertoire of parasites from patients with different clinical symptoms

Such analyses have been recently reported in *P. falciparum* to study parasite clones with different phenotypes [55,56]. In addition, particular expression patterns associated with cerebral and severe malaria have been identified in rodent models [57,58].

The use of microarrays in *P. vivax* is yet to be reported, but the complete genome sequence enabled several groups to join efforts to design a first-generation chip containing one oligonucleotide per 2 kb of exon sequences from Sal I (Figure 3). Pilot experiments with commercially subcontracted slides with this design tested whether such technology is feasible by using total RNA from *P. vivax* isolates, in which parasitaemia is rather low and human leukocytes represent a major source of contamination (data deposited at the National Center for Biotechnology Information Gene Expression Omnibus, or GEO [see http://www.ncbi.nlm.nih.gov/geo/], as GEO Series accession number GSE11075). A total of 719 oligonucleotides of relevance to this review and representing *vir* sequences were spotted on this array. Of those, 353 corresponded to 147 annotated genes from the genome of Sal I, and 122 corresponded to unique *vir* sequences from wild isolates spotted in triplicate. Remarkably, close to 94% (138 of 147 *vir* genes) of the analyzed *vir* gene repertoire from Sal I was expressed. A gene was defined as expressed if at least one of its probes presented an intensity signal greater than the intensity of the 95th percentile obtained from negative controls. There was overlap of expressed *vir* genes in parasites obtained from monkeys and those obtained from patients from the Brazilian Amazon (Figure 3). Thus, 59% (86 of 147) and 38% (56 of 147) of the labelled RNAs from parasites of two different patients cross-hybridized to *vir* gene sequences Sal I. Although much work needs to be done to validate these findings, these results demonstrate that microarray technology using long-synthetic oligonucleotides can now

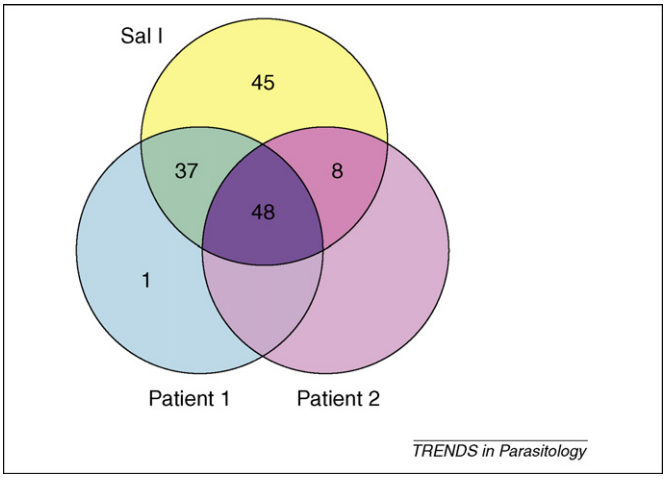

Figure 3. P. *vivax* microarray and *vir* genes. Venn diagram showing the number of *vir* sequences significantly expressed above the detection limit in each experiment. Each experiment compares *P. falciparum* to either *P. vivax* Sal I, a *P. vivax* isolate from one patient (patient 1) or a second *P. vivax* isolate from another patient (patient 2). Experimental data were deposited in GEO (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession number GSE11075. The design of this long synthetic oligonucleotide microarray representing all exon sequences from the genome of *P. vivax* Sal I strain at a density of 1 oligonucleotide per 2 kB of exon sequences received scientific input from the following people: Zbynek Bozdech, Nanyang Technological University Singapore; Jane M. Carlton, NYU Medical Center; Manuel Llinás, Princeton University; Hernando A. del Portillo, Barcelona Centre for International Health Research; and Akhil Vaydia, Drexel University College of Medicine. The complete oligo list, generated by Zbynek Bozdech and Guangan Hu using the OligoRankPick algorithm [74], is available at http://zblab.sbs.ntu.edu.sg/vivax/index.html. Printed microarrays are available through the National Institute for Allergy and Infectious Diseases/National Institutes of Health-funded Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute (http://pfgrc.jcvi.org/index.php/microarray/array_description/plasmodium_vivax/version1.html).

be fully exploited in *P. vivax* by using parasite RNA obtained from wild isolates.

Vir vaccine development

Vaccines against variant surface proteins in *P. falciparum* first met scepticism because of their variant nature. However, it has now been established that these vaccines can induce protection against severe disease [59], disrupt rosetting, protect against sequestration [60] and have a key role in preventing malaria in pregnancy [61]. Analysis of naturally acquired humoral immune responses of *P. vivax* patients against Vir proteins revealed that patients infected for the first time already had IgG antibodies capable of reacting against different proteins [19,20]. This indicates that several Vir proteins are expressed in a primary infection and/or that there are Vir sequences capable of eliciting cross-reacting antibodies. Analysis of the complete *vir* gene repertoire from Sal I revealed the existence of conserved amino acid motifs among the *vir* repertoire [22] (Figure 1b). Whether these motifs are immunogenic in natural infections remains to be determined.

Concluding remarks

Data gathered since the discovery of the subtelomeric *vir* multigene superfamily in *P. vivax* strongly indicate that its main function is not antigenic variation in the strict sense. Rather, it is proposed that Vir proteins might be involved in a novel and undiscovered immune-evasion mechanism that enable this parasite to escape spleen clearance and establish chronic infections. The complete genome sequence from the *P. vivax* Sal I strain and the availability of monkey and rodent malaria species containing *vir* orthologous multigene families will facilitate molecular studies to address this essential aspect of *P. vivax* biology. Ultimately, unveiling how *P. vivax* establishes prolonged parasitism, in addition to determining whether Vir proteins play a part, will guide rational approaches for alternative control strategies against this neglected and not benign human malaria parasite.

Update

Recently, Bozdech and co-workers have reported the use of microarrays in *P. vivax* [75].

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