Cerebral malaria remains a major cause of death for African children, and mechanistic insights regarding the establishment of brain pathology are greatly needed. Expression of specific domains of parasite’s var genes promoting brain adhesion of infected erythrocytes had been previously identified, but binding specificities and the receptor preference in the brain endothelial cells had not been fully described. The study by Storm et al (2019) in this issue of EMBO Molecular Medicine demonstrates that binding to brain endothelial cells via EPCR and ICAM-1 is increased in parasites causing cerebral malaria compared to parasites causing uncomplicated malaria. Furthermore, expression levels of var genes encoding the CIDRz1 domain with EPCR affinity correlate with the receptor-dependent binding to brain, but not dural endothelial cells, highlighting the important role of EPCR in cerebral malaria pathology.

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See also: J Storm et al (February 2019)

Despite a substantial decrease of the malaria burden since 2010, the latest WHO analysis indicates a stalling of the progress in recent years, in reducing both the number of malaria cases and the number of deaths caused by Plasmodium falciparum. Cerebral malaria is a major cause of P. falciparum-caused deaths, and several mechanisms have been proposed to be involved, including obstruction of microvessels by sequestered infected red blood cells (iRBCs), endothelial dysfunction, immune activation and release of pro-inflammatory cytokines, dysregulation of coagulation pathways, blood–brain barrier permeability or brain swelling. Although the mechanisms leading to cerebral malaria are not fully described, it appears that both parasite and host factors contribute and that iRBCs binding to the brain endothelial play an important role. The study by Storm et al links cerebral malaria-causing parasites with a higher binding of iRBCs in the brain endothelium through endothelial protein C receptor (EPCR; Storm et al., 2019).

Remodelling of the host cell by P. falciparum and expression of parasite proteins on the surface of iRBCs allows its sequestration to the endothelium preventing recognition and clearance by the spleen (Smith et al., 1995). Adhesion of iRBCs is primarily mediated by P. falciparum erythrocyte membrane protein 1 (PFEMP1), encoded by the var genes, of which each parasite possesses ~60 mutually exclusive expressing genes. Binding of iRBCs can be quantified in static or flow conditions and measured by testing parasite adhesion to purified or recombinant versions of the receptors, or to primary endothelial cells from different tissues, allowing dissecting binding specificities in the former, and a better representation of the in vivo receptor distribution in the latter. Binding properties of different PFEMP1s have been linked to single functional cysteine-rich adhesion domains called Duffy binding-like (DBL) and cysteine-rich interdomain region (CIDR). All parasites carry a similar repertoire of var genes, conferring each parasite a similar array of possible receptor binding phenotypes. PFEMP1s are known to bind mainly host endothelial cell receptors EPCR or CD36, via their CIDRz1 or CIDRz2-6 domains, respectively. This phenotype dichotomy is maintained by a genetic organization of var genes with the subtelomeric group A var genes encoding EPCR-binding PIEMP1 and telomeric group B and centromeric group C encoding CD36-binding PIEMP1. Most parasites also carry chimeric group B/A var genes encoding a conserved tandem domain arrangement (domain cassette, DC) known as DC8, which also binds EPCR. Some PIEMP1 of both groups A and B bind ICAM-1 via DBLβ domains (Lavstsen et al., 2003). In addition, all parasites carry one gene encoding the unusual VAR2CSA PIEMP1 binding to placental chondroitin sulphate A and expressed by parasites causing pregnancy malaria.

In years preceding the identification of EPCR as a PIEMP1 receptor, transcription of var genes encoding DC8/DC13 (groups B/A and A, respectively) were linked with parasites causing cerebral malaria, and parasites selected in vitro to bind brain endothelial cells. Lavstsen et al introduced for the first time a set of 42 primer pairs targeting specific CIDR and DBL subtypes and compared var gene transcript levels in children with uncomplicated malaria and different forms of severe malaria. The degenerate primers were designed to target conserved var sequences specific for different domain subclasses. With this tool and parasites from 88 children, the authors showed that vars containing DC8 and to a lesser extent DC13 domains were consistently increased in parasites from patients with severe malaria compared with uncomplicated malaria patients (Lavstsen et al., 2012). Avril et al (2012) at the same time reported that P. falciparum iRBCs panned on human brain microvascular endothelial cells (HBMEC)
altered their binding phenotype and selected three var genes, including two that encoded DC8 PiEMP1. Also at the same time, Claessens et al analysed transcription of isogenic pairs of parasites selected on brain endothelial cells or unselected parasites with a chip containing oligonucleotide probes for multiple var genes from different P. falciparum strains. The multiple comparisons converged in outcome and indicated that on all selections, one or two vars showed markedly increased transcription in the brain endothelial adherent parasites and these were consistently group A var genes (Claessens et al., 2012). However, in none of the above-mentioned studies the receptor affinity of the different PiEMP1 was determined.

It was only later that EPCR was identified by Turner et al as the molecular target of parasites isolated from severe malaria cases in a screening of recombinantly expressed human endothelial receptors and binding inhibition by receptor blockade. Subdomains CIDRz1.1 and CIDRz1.4 were identified to mediate EPCR binding (in DC8 and DC13 respectively), and surface plasmon resonance experiments showed that the binding occurred at significantly higher strengths as EPCRs binding to its natural ligand activated protein C (Turner et al., 2013). In a later study, Lau et al (2015) found, using structural analyses, that all but two CIDRz1 subtypes (CIDRz1.2 and CIDRz1.3 found in the var1 pseudogenes) bind to EPCR.

More recently parasites causing cerebral malaria were shown to express var genes encoding for PiEMP1s that bind EPCR and ICAM-1 in static binding assays (Tuikue Ndam et al., 2017). Parasites from children with cerebral or uncomplicated malaria were characterized in their transcribed var gene and the binding properties of iRBC collected on admission. High expression levels of PiEMP1 predicted to bind EPCR were significantly more frequent among parasites of patients with cerebral malaria than among uncomplicated malaria cases. And, when the binding capacity to CD36, ICAM-1 or EPCR was investigated, adhesion to EPCR and ICAM-1 was more common in cerebral malaria-causing parasites than in uncomplicated malaria-causing parasites. Furthermore, parasite isolates from cerebral malaria patients more frequently exhibited binding to both ICAM-1 and EPCR, whereas binding to CD36 was more frequent in uncomplicated malaria than cerebral malaria patients (Tuikue Ndam et al., 2017).

However, in this study a direct link between var genes encoding for PiEMP1 binding to EPCR and adhesion to brain cells of cerebral malaria-causing parasites continued to be elusive.

In this issue of EMBO Molecular Medicine, Storm et al (2019) now used a microchannel flow adhesion assay to enquire how inhibition of iRBC cytoadhesion through blocking ICAM-1, CD36 or EPCR on brain or dermal endothelial cells affects binding of parasites causing cerebral malaria or uncomplicated malaria. Parasites collected from children with cerebral malaria or uncomplicated malaria were allowed to bind to HBMEC or human dermal microvascular endothelial cells (HDMEC) in flow conditions. The data show that cerebral malaria-causing parasites bind better to HBMEC than uncomplicated malaria-causing parasites; no such difference is observed when either type of parasite is overlaid on HDMECs. Interestingly, cerebral malaria-causing parasites bind equally well to HBMEC and HDMEC, but uncomplicated malaria-causing parasites adhere better to HDMEC than to HBMEC at least through ICAM-1 binding (Fig 1). These observations are in line with earlier autopsy studies showing that CM patients have widespread parasite sequestration in most other organs apart from the brain.

Storm et al also investigated how the blocking of different endothelial receptors would affect binding of cerebral malaria- or uncomplicated malaria-causing parasites. ICAM-1 was blocked with antibodies in both HBMEC and HDMEC, while CD36 was blocked in HDMEC, and in all conditions, antibodies could block ~50% of adhesion independently of the parasites originating from cerebral malaria or uncomplicated malaria cases. EPCR binding of iRBCs from cerebral malaria or uncomplicated malaria cases was reduced in HBMEC after blocking the receptor with a recombinant protein version (rEPCR), but in the dermal endothelial cells this effect was only visible for parasites originating from cerebral malaria cases. When assessing the inhibition ability of rEPCR on the different cell types and parasites origins, the authors found a trend towards a higher inhibition of binding of cerebral malaria-causing parasites than uncomplicated malaria-causing parasites to HBMEC, and this difference did not reach statistical significance.

In parallel, the authors characterized the var gene transcripts in each of the field isolates used in the above-mentioned adhesion assay. Performing qPCR with a recently improved set of var typing primer pairs targeting specific CIDR and DBL subtypes normalized to a housekeeping gene, the authors calculated the relative level of var transcripts to the level of the housekeeping gene transcript in each sample. Interestingly, when var transcript levels were correlated with iRBCs binding capacities to HBMEC or HDMEC, it showed a strong positive correlation between % binding attributed to EPCR binding and expression of EPCR-binding var domains by parasites from cerebral malaria patients, supporting that cerebral malaria is indeed promoted by EPCR binding of parasites to the brain endothelium.

Adding on to previous reports, Storm et al attempted to recapitulate in the small in vitro flow chambers how parasites expressing different var genes lead to different clinical outcomes by comparing their binding abilities to brain or dermal endothelial cells through CD36, ICAM-1 and EPCR. Importantly, Storm et al use in their assays iRBCs in the 1st-3rd cycle after blood collection expressing the native full-length PiEMP1s, and endothelial cell lines naturally expressing their receptors, avoiding possible affinity artefacts introduced by analyses of individual recombinant PiEMP1 domains on plates coated with recombinant protein (Azasi et al., 2018). The data obtained are indeed supportive of EPCR being an important endothelial receptor responsible for iRBCs binding in cerebral malaria cases, alongside ICAM-1 (Storm et al., 2019).

None of the main endothelial receptors to which PiEMP1s bind is selectively expressed on the brain endothelium. Thus, it is most likely that cerebral malaria-causing parasites do not adhere specifically to the brain, but rather bind to all EPCR and ICAM-1 expressing tissues, or even additional other receptors, such as gC1qR, which has been associated with severe malaria and rosetting, but remains to be investigated in the context of cerebral malaria (Mayor et al., 2011). EPCR binding in the brain endothelium by PiEMP1 subdomains may also increase brain pathology by contributing to inflammation and brain swelling. The competitive effect of PiEMP1 subdomains decreases protein C activation, possibly resulting in increased blood coagulation, inflammation, recruitment of immune cells to the endothelium and obstruction of the endothelial barrier (Turner et al., 2013).
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