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The role of EPCR in the pathogenesis of severe malaria

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Abstract

Of the five *Plasmodium* species that infect humans, infection with *P. falciparum* is the most lethal, causing severe malaria syndromes, that result in over half a million annual deaths. With parasites becoming increasingly resistant to artemisinin there is an urgent need for new preventative and therapeutic options, for which understanding the mechanisms that cause death and disability in malaria is essential. The recent discoveries that certain variants of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on infected erythrocytes are intimately linked to the precipitation of severe malaria syndromes and that these PfEMP1 variants contain EPCR binding domains provides new opportunities to improve our understanding of the molecular mechanisms responsible for the pathogenesis of severe malaria. EPCR is known for its essential role in the protein C (PC) system and for its ability to support the cytoprotective effects of activated protein C (APC) that result in vascular and tissue protective effects in many organ systems of the body, including the brain, lung, kidney, and liver. Observations that binding of PfEMP1 to EPCR results in an acquired functional PC system deficiency support the new paradigm that EPCR plays a central role in the pathogenesis of severe malaria. Thus, targeting of the PfEMP1-EPCR interaction and restoring the functionality of the PC system may provide new strategies for the development of novel adjuvant therapies for severe malaria.

Keywords

Protein C; Endothelial protein C receptor; Malaria; Plasmodium falciparum; vascular endothelium

The anticoagulant and cytoprotective functions of the PC system are essential for the regulation of coagulation, vascular inflammation, and endothelial permeability [1, 2]. The endothelial protein C receptor (EPCR), named after its ability to recruit PC to the endothelial surface, is central to a functional PC system and binds both protein C and activated protein C with similar affinity [2, 3]. EPCR facilitates the activation of PC by the thrombin-

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thrombomodulin complex [2-4], but also facilitates APC-mediated cytoprotective effects on cells that involve activation of protease activated receptors (PAR) 1 and 3 [1, 5, 6]. These cytoprotective effects of APC that include anti-apoptotic and anti-inflammatory activities, beneficial alterations of gene expression profiles and protection of endothelial and vascular barrier functions provide important contributions to APC's beneficial effects in multiple *in vivo* disease and injury models [2, 7]. Notably, APC cytoprotective activities provide neuroprotective effects that include protection of the blood brain barrier as well as anti-inflammatory and anti-apoptotic activities within the neurovascular unit, nephroprotective effects, and anti-inflammatory effects in the lung, and thus, these activities of APC might be directly relevant to the complications associated with severe malaria [7-9].

Malaria

Approximately half the world population is at risk for contracting malaria due to infection with *Plasmodium* parasites, with an estimated 1.2 billion people living mostly in the African Region and the South-East Asia Region being at high risk. Of the ~200 million annual cases of malaria, infection with *P. falciparum* is the most dangerous and lethal, responsible for over 0.5 million annual deaths [10, 11]. The clinical outcome of malaria due to *P. falciparum* infection ranges from no or minor symptoms to severe malaria with complications such as severe anemia, respiratory distress, and cerebral malaria [10, 12, 13]. Even with the latest antimalarial and supportive treatments available, cerebral malaria is fatal in around 15% of the cases, and survivors often suffer long-term neurological impairments [14, 15].

Upon inoculation of the host by an infected feeding mosquito, the initial parasites (sporozoites) target the liver to begin an elaborate lifecycle [10]. When the infected hepatocytes (liver schizonts) rupture, thousands of parasites (merozoites) enter the bloodstream and rapidly invade circulating erythrocytes. The infected erythrocytes (IE) would normally be cleared by the spleen if it were not for an elaborate scheme designed to evade splenic clearance that involves expression of parasite proteins on the surface of IEs that mediate cytoadhesion of the IEs to the endothelium [10]. This cytoadhesion of IEs is the hallmark of *P. falciparum* infection and permits the reproduction and maturation of parasites inside IEs, relatively protected against host defense systems. Under the pressure of the growing and replicating parasites, the IEs burst in cycles of ~48 hours, thereby releasing new merozoites into the bloodstream that rapidly invade additional erythrocytes fueling an exponential growing infection until generally after 3-4 cycles severe complications become apparent [10].

EPCR as vascular adhesion receptor for IE

Upon IE invasion, the parasites express parasite-encoded genes on the surface of the IE, among them the notorious *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). These PfEMP1 molecules are responsible for binding several host receptors on vascular (endothelial) cells, including ICAM-1, CD36, and EPCR [16, 17]. PfEMP1 are large (280 kDa) multi-domain proteins consisting of 2-10 *Duffy-binding-like* (DBL) and Cysteine-rich Inter Domain Region (CIDR) domains, each consisting of different subgroups based on sequence similarity [18]. Strong evidence indicates that the particular subset of CIDR

domains expressed by the parasite determines the difference between uncomplicated vs. severe malaria syndromes and that CIDRα1 domains that bind EPCR are intimately linked to the occurrence of severe malaria syndromes [19-24]. PfEMP1 variants are also defined by the domain cassette (DC) categorization in which DC8 or group B/A PfEMP1 variants and group A PfEMP1 that include DC13, both contain EPCR-binding CIDRα1 subtypes [25]. However, all CIDRα1 subtypes appear to conform to the same structure and molecular interaction with EPCR, indicating that the subdivision of CIDRα1 variants reflects advantageous extremes of diverse serotypes rather than divergent EPCR binding affinities [26-28].

Specific receptor interactions remain to be elucidated for many of the PfEMP1 domains, but the CIDRα1-EPCR interaction studies and other data suggest that domain type classification by sequence reflects structural and functional specialization of domain types. Thus, CIDRα1 domains bind EPCR and are associated with severe malaria, CIDRα2-6 domains bind CD36 and are associated with uncomplicated malaria, and ICAM-1 binding appears to be exclusive but not absolute for DBLβ domains and has an as of yet unclear association to disease severity [16, 17, 24, 29-32].

Additional contributions of EPCR to the pathogenesis of severe malaria

Sequestering IEs provoke a strong and focused inflammatory response caused by alterations in the microenvironment and disruption of local blood flow that result in vascular dysfunction, ischemia, vascular leakage including of the blood-brain barrier (BBB), edema, and may ultimately manifest in organ failure, respiratory distress, renal failure, coma, and death [33-35]. EPCR has important function in the PC system and thereby potentially provides a direct link between severe malaria and the PC system [1-3, 36]. The anticoagulant and cytoprotective activities of APC provide a natural threshold for prothrombotic and proinflammatory alterations in normal physiology. However, functional EPCR greatly enhances the activation of PC by the thrombin-thrombomodulin complex and is required for the initiation of cytoprotective signaling by APC-mediated activation of PAR1 and PAR3 [1-3].

This prompts the question whether the targeting of EPCR by PfEMP1 directly contributes to the pathogenesis of severe *P. falciparum* malaria in addition to the complications associated with parasite sequestration [16, 35]. Indeed, in vitro studies with purified CIDRα1 domains and IE with selected *P. falciparum* laboratory strains confirm the loss of EPCR functionality upon CIDRα1 or IE binding that includes loss of PC and APC binding to EPCR, inhibition of EPCR-mediated PC activation and obstruction of APC-mediated endothelial barrier protective effects (Figure 1A) [27, 28, 37]. Thus, binding of PfEMP1 to EPCR results in an acquired functional PC system deficiency supporting the new paradigm that EPCR plays a central role in the pathogenesis of severe malaria. However, with the exception of a few case reports for beneficial effects of recombinant wt-APC (Xigris) treatment in severe malaria [38-40], no studies in patients or in animals in vivo have been performed and thus a direct contribution of EPCR to the pathogenesis of severe *P. falciparum* malaria in vivo remains to be established.

Unfortunately, the lack of an animal model mimicking parasite host interactions that occur with *P. falciparum* in humans represents a major hurdle for addressing the role of EPCR in the pathogenesis of severe malaria. PfEMP1 molecules are unique to malaria parasites infecting humans and the great African apes [41] and *P. berghei* used in the murine *P. berghei* ANKA model of experimental cerebral malaria does not express PfEMP1 molecules and thus does not epitomize the sequestration of IEs in the microvasculature comparable to human *P. falciparum* malaria [42]. New animal models that encompass PfEMP1-mediated cytoadhesion of IEs as a critical part of *P. falciparum*-induced severe malaria are urgently needed in order to validate EPCR as a primary IE receptor, to address the contributions of EPCR inactivation to the pathogenesis of severe malaria, and to test the efficacy of potential novel EPCR-focused adjunctive therapies for severe malaria (see below).

Development of new adjunctive therapies focusing on EPCR

With each new *P. falciparum* infection, the human immune system is presented with a set of PfEMP1 adhesion proteins that differ in their primary amino acid sequence to that of any previous infection. However, due to the opposing selective pressures for diversification to avoid immune recognition vs. conservation to maintain receptor binding affinity, all parasites appear to carry similar repertoires of PfEMP1 proteins which may be different by sequence but conform to conserved protein structures allowing EPCR binding [26]. Since the receptor is not under selective pressure for diversification, EPCR-based targeting approaches may provide viable adjunctive therapeutic strategies to combat severe malaria.

An E86A-soluble (s)EPCR decoy strategy was recently proposed to attenuate the binding and adhesion of CIDRα1 and IEs to cellular EPCR [37]. Since Glu86 is not required for CIDRα1 binding to EPCR, the E86A-sEPCR reduces the adhesion of CIDRα1 and IE binding to cellular EPCR similar to wt-sEPCR and enables barrier-protective effects of APC in presence of CIDRα1 (Figure 1B) [16, 37]. Glu86 is however required for (A)PC binding to EPCR, and the E86A mutation prevents competition of the decoy for (A)PC-binding to vascular EPCR [43, 44]. However, validation of E86A-sEPCR as potential decoy strategy for severe malaria requires new animal models that encompass PfEMP1-mediated cytoadhesion of IEs as a critical part of *P. falciparum*-induced severe malaria.

Concluding remarks

In summary, PfEMP1 binding to EPCR has been associated with severe malaria and induced an acquired insufficiency of the PC system to generate APC and to mediate cytoprotective effects of APC. These in vitro data suggest that the obstruction of EPCR by IE sequestration contributes directly to the pathogenesis of severe malaria. Thus, targeting the PfEMP1-EPCR interaction and restoring the functionality of the PC system may provide new strategies for the development of novel adjuvant therapies for severe malaria. However, new animal models that encompass PfEMP1-mediated cytoadhesion of IEs are urgently needed to support and validate the ongoing efforts in this area.

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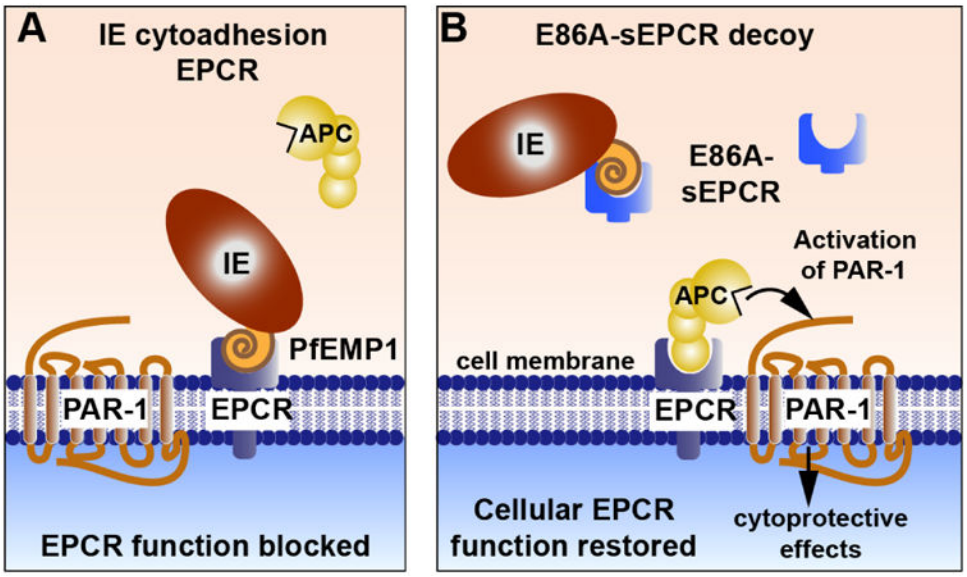

Figure 1. Schematic model of IE cytoadhesion to EPCR and the E86A-sEPCR decoy strategy

A) IEs expressing PfEMP1 that contain a CIDRα1 domain bind to EPCR on endothelial cells and block access of PC and APC to EPCR. The ensuing acquired deficiency of PC system functions likely contributes to the pathogenesis of severe malaria by leaving the affected endothelium vulnerable to the vicious cycle of inflammation, thrombosis, and loss of vascular integrity caused by IE sequestration. B) E86A-sEPCR acts as a decoy for cellular EPCR to saturate available CIDRα1 domains thereby preventing binding of IEs to cellular EPCR. Since E86A-sEPCR is devoid of PC and APC binding, it permits normal interaction of PC and APC with cellular EPCR to restore the anticoagulant and cytoprotective functions of the PC system. This figure was adapted from Petersen et al. [37] with permission.