Progress in Neurobiology 91 (2010) 140–151

Contents lists available at ScienceDirect

Progress in Neurobiology

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

Microvesiculation and cell interactions at the brain–endothelial interface in cerebral malaria pathogenesis

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ARTICLE INFO

Article history:
Received 16 February 2009
Received in revised form 2 April 2009
Accepted 5 January 2010

Keywords:
Cerebral malaria
Cell–cell interactions
Microparticles
Endothelium

ABSTRACT

Cerebral malaria (CM) is still a major world health problem whose pathogenic mechanisms remain incompletely understood. After reviewing some particularities of anti-malarial immunity, we focus here on the neurovascular aspects of CM. We specifically address the central role of endothelial activation and alteration in disease pathogenesis. We discuss the respective roles of "mediator-induced" versus "host cell-induced" mechanisms of endothelial alteration. The former include cytokines, chemokines and their receptors, while the latter encompass cells located inside and outside the vessel, notably glial cells. We also present evidence for a pathogenic role for membrane microparticles (MP) in CM, based on studies in African patients and in a recognised mouse model. Intervention studies on MP production, via either gene knockout or pharmacological inhibition, can prevent the neurological syndrome and its associated mortality, suggesting potential new therapeutic avenues.

Contents

Abbreviations: ABCA1, ATP-binding cassette transporter A1; CM, cerebral malaria; CM-R, CM-resistant; CM-S, CM-susceptible; EC, endothelial cells; HBEC, human brain endothelial cells; MP, microparticles; MRI, multimodal resonance imaging; PfEMP-1, *P. falciparum* erythrocyte membrane protein-1; PRBC, parasitised red blood cells; PS, phosphatidylserine; PbA, *Plasmodium berghei* ANKA; *P. falciparum*, *Plasmodium falciparum*.

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doi:10.1016/j.pneurobio.2010.01.007

1. Cerebral malaria, still a major world health problem

Malaria remains a major problem of public health, since WHO estimates indicate that each year in excess of 300 million people are infected by *Plasmodium falciparum* worldwide (Snow et al., 2005). More than two million African children die each year of severe malaria, mainly cerebral malaria (CM) and severe malarial anaemia. Currently there are 109 malaria-endemic countries with more than three billion individuals at risk

of infection. (See http://www.malariaeliminationgroup.org/for additional information.)

Even if they are constantly the focus of numerous studies, available preventive methods are still not sufficient to reduce mortality and morbidity. In addition, the numbers of cases have increased dramatically over the past few decades, in part due to the development and geographical spread of resistance to the main anti-malarial drugs, notably chloroquine (Beales et al., 2000).

There are four known species of *Plasmodium* that are pathogenic in humans: *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale* and *P. falciparum*. Recently there also have been sporadic cases of infection with *P. knowlesi*, primarily a pathogen of macaques, in South-East Asia. The blood-stage cycle of *P. falciparum* causes the most severe manifestations of the disease and accounts for the majority of deaths. Approximately one to two percent of *P. falciparum* infections result in a life-threatening neurological complication known as cerebral malaria. Infections with *P. falciparum* that cause neurological involvement and respiratory distress lead to a poor prognosis, with the majority of patients moribund (Marsh et al., 1996). General consensus exists for the implementation of adjunct therapy with anti-parasitic treatment to minimise cognitive impairment and mortality. However, children developing CM despite the rapid administration of adjunct therapy undermine the importance of this approach (Newton and Krishna, 1998).

The incidence of CM is highly dependent on the immune status of the population and the frequency of transmission. Children aged 2–4 years are at very high risk in malaria-endemic regions characterised by a high transmission, such as sub-Saharan Africa (Reyburn et al., 2005), but in very high transmission areas there is a tendency towards severe malaria anaemia rather than cerebral malaria in younger children. In this instance cerebral malaria appears to be a disease of the semi-immune. Areas that are characterised by a low transmission have a higher incidence of CM in older children and adults (Reyburn et al., 2005).

CM is a multisystem multi-organ dysfunction that involves other complications besides those pertaining to the brain. The blood-borne *P. falciparum* has access to multiple organs via circulation. Typical presentation includes fever, malaise, headache, joint and body ache, and delirium. Major complications seen in CM include severe anaemia, spontaneous bleeding, severe metabolic acidosis, jaundice, renal failure and pulmonary oedema (Beales et al., 2000). The incidence of these complications depends on the endemic region and age group to which an individual belongs (Idro et al., 2005).

CM is a clinical syndrome defined by the presence of unarousable coma, with the exclusion of all encephalopathies of other causes, and the presence of asexual forms of *P. falciparum* in the blood (Beales et al., 2000). There are notable clinical differences between CM in African children, the population in which over 90% of life-threatening cases develop (Beales et al., 2000), and in non-immune adults. The main neurological features are coma, seizures and respiratory distress. In children, coma usually develops rapidly after a seizure, whereas in adults the development is slower and takes 2–3 days. Seizures and convulsions are commonly present in the reported history of over 80% of children compared to 20% of adults. Evidence for mortality associated with multiple and prolonged seizures has accumulated, as well as for associated neurocognitive deficits. High intracranial pressure and brain swelling are common, accounting for brainstem signs. Neurological signs that are characteristic of CM include changes in ocular movements and reaction, retinal abnormalities (retinopathy), altered pupillary size, abnormal respiration, particularly hyperventilation, and changes in posture that can be due to decerebration or decortication (Idro et al., 2005, 2007). Neurocognitive deficits amongst African children are found with an increased frequency following a CM episode (John et al., 2008), and possible mechanisms are discussed later in this review.

2. Pathogenic mechanisms: neurovascular aspects of CM

Three main theories have been proposed to explain the pathogenesis of CM: a "mechanical" theory, an "immunological" theory, and a combination of both, as reviewed elsewhere (Combes et al., 2006; Hunt and Grau, 2003; Schofield and Grau, 2005). However, the fine mechanisms of this complex syndrome remain incompletely understood.

In terms of histopathology, the central feature of CM is the preferential sequestration of parasitised red blood cells (PRBC) in the cerebral microvasculature (Newton and Krishna, 1998). This sequestration is a normal step in the life cycle of the parasite and occurs in all deep vessels. However specific localisation of the parasites in the brain seems to be a complex feature involving both expression of human adhesion molecule isoforms and parasitic PfEMP1 proteins polymorphism. In contrast to the peripheral blood, all mature stages of the parasite are seen within these vessels, both in adults (Aikawa, 1988; MacPherson et al., 1985; Spitz, 1946), and in African children (Lemercier et al., 1966). The distended venules are more prominent in the grey matter, where they appear evenly distributed. Other neuropathological features of CM include petechial haemorrhages in the brain parenchyma, ring haemorrhages and, rarely, Dürck's granulomas. Immunohistochemical and electron microscopy studies have shown that widespread cerebral endothelial cell activation and morphological changes occur in CM, as well as, sometimes, focal endothelial cell damage and necrosis (Beales et al., 2000; Coltel et al., 2004).

2.1. Some particularities of anti-malarial immunity

The immune response during malaria is unique. It can be described as a non-sterilising immunity or transient anti-disease immunity, named "premunition" (Sergent and Parrot, 1935). Patients in endemic areas submitted to multiple infectious mosquito bites will acquire, over time, an immunity that will persist as long as there is an exposure to the parasite. This could explain why children and non-immune travellers are the populations at higher risk of developing severe malaria (Schofield and Grau, 2005). This type of partial immunity will limit the inflammatory response of the host to the parasite and allow the development of an anti-disease immunity: a patient can still be infected but is able to control the parasite burden and show little or no sign of malarial infection. However, if the exposure to the infection becomes less stringent, for example after moving to a non-endemic country, this immunity is lost and the patient is at risk of developing severe malaria again if he becomes re-infected (Artavanis-Tsakonas et al., 2003). Balance between anti-parasite immunity and anti-disease immunity (mainly IgM-dependent) triggers this reappearance of symptoms and signs. It is not clear whether control of parasites is lost during the time of non-exposure or if only anti-disease immunity is weakened. CM is associated with a TH1 immune response, in which IFN-γ and TNF, CD4, CD8 and NK cells play major roles, as reviewed extensively elsewhere (Artavanis-Tsakonas et al., 2003; Hunt and Grau, 2003; Schofield and Grau, 2005).

Understanding the pathogenesis of CM is important, and this is best achieved through a combined approach involving *ex vivo* studies using patient samples (blood, plasma, post-mortem specimens), *in vitro* modelling of the interactions between the various cells and their released mediators, and *in vivo* testing of hypotheses using an animal model of CM (de Souza and Riley, 2002; Hunt and Grau, 2003) (Combes et al., 2005b). The respective advantages of each model have been detailed elsewhere (Combes