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Review Article

Validity of Modelling Cerebral Malaria in Mice: Argument and Counter Argument

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Abstract The clinical manifestations of *Plasmodium falciparum* infection of humans that are collectively recognised as cerebral malaria produce profound changes in mental status and induce coma. The histopathological hallmark of this encephalopathy is the sequestration of cerebral capillaries and venules with both infected and uninfected erythrocytes. The underlying cause of cerebral malaria is the subject of vigorous debate. A major reason for this is that human brain tissue is only available *post mortem*. In order to dissect the pathology of this acute disease, therefore, a number of models using murine malarias have been developed. While these have undoubtedly proved useful in helping to identify immunological mechanisms involved, recognition of the differences between the pathological processes during cerebral malaria in mice and man has led to some researchers questioning the validity of extrapolating findings from mouse models to the human condition with a view to informing therapeutic interventions. In turn, this has provoked lively debate within the malaria research community. This commentary sets out our current understanding of cerebral disease in humans and evaluates what meaningful contribution the study of mouse models has made to this knowledge.

Keywords *Plasmodium*; cerebral malaria; mouse model

Science is seldom more exciting than when it is sparking controversy. Recently, White *et al.* [39] have caused quite a commotion amongst the malaria research community by calling into question the relevance of the murine model of cerebral malaria to the pathology of the corresponding condition in humans. While the host-parasite relationship in humans has been difficult to determine, the pliability of murine malaria models has enabled valuable contributions to the understanding of the pathogenesis of disease. Although no single model reflects precisely malaria infection in humans, different models collectively provide important information on the mechanisms of protective immunity and immunopathogenesis [36]. It is in light of this that models of cerebral malaria have been developed [14].

Cerebral malaria is the most profound manifestation of severe malaria in humans, which arises from infection with the protozoan parasite *Plasmodium falciparum*. It presents as an acute encephalopathy with three primary symptoms: impaired consciousness with non-specific fever; generalised convulsions and neurological sequelae; and an initially rousable and then unrousable coma. If a person is not treated, cerebral malaria is fatal in 24–72 hours [23]. The pathophysiology of this syndrome is not fully understood and has been the subject of ongoing discussion [2,5,13,17,35]. As the underlying cause of the symptoms of cerebral malaria, the two proposed mechanisms of erythrocyte sequestration and mechanical blockage [2] and of pro-inflammatory cytokine induction [8] both receive support. A hypothesis to unify these potential causes of microcirculatory dysfunction was recently advanced [38]. This proposes that infected erythrocyte antigens activate platelets that, in turn, contribute to the activation of the inflammatory response and increased levels of endothelial cell adhesion molecules. Raised expression of the latter results in further infected erythrocyte sequestration and marked local inflammation that may disrupt the brain microvasculature. Importantly, this cannot be repaired readily because of haemostasis dysfunction caused by the pro-coagulant environment that has been created.

Over the last three decades fundamental studies to examine the pathogenesis of human cerebral malaria have increasingly utilised the accessibility of, and ease of manipulation and intervention afforded by, experimental murine malaria infections. The relevance of these models, however, has been subject periodically to critical appraisal [13,35,39]. Although a number of murine models of severe malaria are available, few bear valid comparison with *P. falciparum* cerebral malaria on the basis of all recognised parasitological, morphological and molecular criteria. Most notably, there is no host-parasite combination in which

the intensive intra-cerebral sequestration of erythrocytes and modest inflammation that is observed in the human condition, at least *post mortem*, is accurately reproduced [27]. That said, they have highlighted the importance of immune mediators, including tumour necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ) and nitric oxide (NO), in modulation of cerebral pathology. Infection of CBA/Ca mice with *P. berghei* ANKA strain, for instance, provides a model in which striking cerebral complications develop, causing so-called "murine cerebral malaria". Manifestations shared with its human counterpart include the onset of a reversible coma, severe neurological dysfunction and diffuse cerebral microvascular abnormalities (Table 1). As *P. berghei* ANKA infected erythrocytes intrinsically lack the ability to cytoadhere, this form of cerebral disease is notable for the fact that there is little or no parasite sequestration. However, there is an amplification of expression of cytoadherence receptors in cerebral capillaries which leads to a substantial sequestration of leukocytes [35].

With this model, it has been shown that excessive production of TNF-α is a key factor in the pathogenesis of murine cerebral malaria. Treatment of *P. berghei*-infected mice with anti-TNF antibody was found to prevent the onset of cerebral symptoms and pathology [18]. The involvement of TNF-α was further implied by the subsequent report that the plugging of cerebral blood vessels with monocytes seen in murine cerebral malaria could be reproduced by administering high doses of recombinant TNF-α to normal CBA/Ca mice [19]. However, at least some of the attempts to corroborate this finding have failed to do so; injection of TNF-α instead mimics the pathology seen in terminal *P. vinckei* infections, in which cerebral involvement is absent (and which may act as a more suitable model of other manifestations of falciparum malaria, such as hypoglycaemia and liver damage) [6,7]. It is apparent, therefore, that it is not the systemic level of TNF-α which is important in cerebral malaria, but rather the local level of this cytokine produced by sequestered monocytes within the microcirculation of the brain. These differences between pathological manifestations associated with infection of mice with different parasites, *P. berghei* ANKA and *P. vinckei*, and between the findings of different groups performing similar experiments, serve to highlight the complexities of cerebral malaria and the need for further fundamental studies aimed at establishing the locality within the brain of cytokine production (including cytokines other than TNF-α) and of induction of adhesion molecules. That these are intimately linked has been shown by the observation that increased production of TNF-α upregulates the endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and of CD36, with subsequent sequestration of cells expressing CD11a (lymphocyte function-associated antigen 1, LFA-1) [11,33]. Circulating cells which bear CD11a include neutrophils, monocytes, and lymphocytes, as well as platelets. An indication of the relevance of these murine studies to our understanding of the critical factors involved in the pathogenesis of cerebral malaria comes from a clinical study in which anti-TNF-α therapy inhibited the biological activity of TNF-α in children with cerebral malaria, causing most notably a reduction in fever [25]. As such, this report represents a bridge between experimental and clinical investigations, and provides evidence from *P. falciparum* infection in humans to support ideas originating from studies of murine cerebral malaria.

As TNF-α is known to induce NO, this molecule has been proposed as a contributor to malaria-associated pathology, particularly the coma accompanying cerebral malaria [9]. It is hypothesised that NO released by vascular endothelial cells stimulated by TNF-α diffuses into the brain, where it disrupts the regulation of glutamate-induced neural NO, resulting in alteration of neurotransmission and, consequently, coma. This theoretical framework, which has come to be known as the "cytokine theory" of cerebral malaria, has met with opposition from those working with *P. berghei* ANKA [32]. It was reported that there is no influence of NO inhibitors on the development of cerebral malaria in this model, even upon direct intracranial administration [1,24,32], implying that NO blockade, *in vivo*, is not able to protect against pathology. While these differences may appear to be irreconcilable, Grau and de Kossodo [17] proposed that NO may mediate early changes in cerebral malaria, such as neurotransmission disturbances, when the neurological syndrome is still reversible, but that NO is unlikely to be involved in the actual processes causing neurovascular damage at the advanced stages of the condition. These investigators performed an elegant study to examine the relationship between susceptibility and resistance to cerebral malaria and Th1-cell versus Th2-cell regulated cytokine mRNA expression, *in vivo* [12]. Strains of mouse susceptible (CBA/J) and resistant (BALB/c) to cerebral malaria had an identical ability to produce TNF-α. In contrast, susceptibility to *P. berghei* ANKA infection was accompanied by the upregulation of IFN-γ gene expression, and conversely, the expression of two cytokines potentially able to antagonise the effects of TNF-α, interleukin 4 (IL-4) and transforming growth factor beta (TGF-β), was significantly downregulated.

Overproduction of IFN-γ may be of direct relevance to the development of cerebral malaria as it is considered that, in synergy with TNF-α, this leads to the activation of endothelial cells, macrophages [16] and microglial cells [29]. Endothelial cells would then upregulate a range of adhesion molecules, notably ICAM-1, which has been implicated in the pathogenesis of cerebral malaria [20]. This begs the question of whether susceptibility to cerebral

Observation	Human	Murine
Loss of vascular cell integrity/tissue oedema	+	+
Congestion of microvessels with infected erythrocytes	+	−/+
Haemorrhages	+	+
Mononuclear cell adherence to, or extravasation through, the vascular endothelium	−/+	+
Astrocyte response (redistribution, astrogliosis, activation, apoptosis)	?	+
Microglia and perivascular macrophage response (redistribution, morphological changes, activation)	+	+
Pro-inflammatory cytokine expression	+	+
Neurological complications, including convulsions, paralysis, coma	+	+

Modified from Medana *et al.* (reference [28])

Table 1: Summary of pathology of cerebral malaria in the brain of humans and mice infected, respectively, with *Plasmodium falciparum* and *P. berghei* ANKA.

malaria correlates with a Th1 cell pattern of cytokine production. Although data available are consistent with a predominant Th1 cell response in mice developing cerebral complications following infection with *P. berghei* ANKA the cytokine profile from resistant mice does not match conclusively with a Th2 cell response. It is tempting to make a link between IFN-γ overproduction, and by implication Th1 cell involvement, and susceptibility to human cerebral malaria, but it may be equally argued that murine models are not of the greatest relevance to the field situation [39]. Observations from epidemiological studies, of a higher capacity of malaria-specific IFN-γ production by peripheral blood $CD4^+$ T cells among non-immune compared to immune individuals [4], do, however, lend support for this proposal. It remains to be seen whether Th1 cell activation is a prerequisite for *P. falciparum* malaria cerebral pathogenesis. The relationship needs to be established between the ability of cells of the immune system to produce certain cytokine patterns and the premunition status or the susceptibility to disease of the host. In this regard, continuing the use of the *P. berghei* ANKA model should prove invaluable, by means of intervention experiments, in determining why, where, and when each of the factors instrumental to cerebral malaria is involved in pathogenesis.

Although sequestration of parasites in brain capillaries is a constant feature of human cerebral malaria, its absence from current murine models of cerebral malaria is their main drawback, making them inappropriate for studying the molecular basis of cytoadherence. However, one report has highlighted the possibility of providing a more relevant model by mixing infections of *P. chabaudi* and *P. berghei* ANKA [21]. These parasites each contribute one of the two features considered necessary to produce cerebral sequestration: cytoadherence to, and upregulation of, cerebral endothelial receptors for sequestration, respectively. While *P. chabaudi* is cytoadherent in the liver and spleen, cerebral cytoadherence is absent [10], suggesting that although the parasite is intrinsically capable of cytoadherence, it is probably unable to upregulate cytoadherence receptors in the brain. The concurrent infection of *P. berghei* ANKA and *P. chabaudi*, however, produced cerebral cytoadherence of the latter, believed to be caused by this parasite "hijacking" receptors otherwise adhered to by leukocytes in infections with *P. berghei* ANKA alone [21]. The concept that the necessary features for sequestration may be dissociated is important as it may explain the many discrepancies observed between murine models and human malaria, as well as between clinical severity and *in vitro* assays. As all the murine malarias were derived from a limited number of original isolates, then maintained for many generations by blood passage in artificial hosts, conditions were sustained in which a selection pressure could favour certain characters ahead of others within a single parasite population. In contrast, natural infections of humans generally comprise a mixture of several different populations [37] and each isolate may therefore contain a pool of different intrinsic characters. Given this rationale, it is possible to reason why the full manifestations of cerebral malaria are often observed in *P. falciparum* infection but only partially so in currently available models of murine cerebral malaria.

While they do not come close to matching the condition of human malaria infection in its entirety, experimental models can mirror specific facets which may be examined *ante mortem*. For instance, the recent work of Desruisseaux *et al.* [15] and Lacerda-Queiroz *et al.* [26] has revealed the utility of *P. berghei* ANKA in studying long-term neurocognitive defects, demonstrating that memory dysfunction and behavioural impairment correlate with brain inflammation and haemorrhage, as well as with microglial activation and leukocyte migration. These findings thereby support the argument that murine models do indeed recapitulate effectively human disease. Certainly, through their judicious use, it can be reasonably claimed that our understanding of cerebral malaria has been significantly advanced. Further, there is sufficient scope for investigations underpinning anti-disease vaccine design and other therapeutic interventions for this to continue.

This is a view shared by a significant proportion of malaria experts, some of whom have mounted a robust defence to recent criticism of the use of murine cerebral malaria as a translational research tool [3, 22, 30, 31, 34]. Importantly, by choosing to ignore invasive studies in experimental models, as White *et al.* [39] would direct us to do, the opportunity for malaria research to benefit from a "bench to bedside" approach in this key area of global public health may be missed.

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