Convulsions Due to Increased Permeability of the Blood-Brain Barrier in Experimental Cerebral Malaria Can Be Prevented by Splenectomy or Anti–T Cell Treatment

C. C. Hermesen, E. Mommers, T. van de Wiel, R. W. Sauerwein, and W. M. C. Eling

Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands

Experimental cerebral malaria (ECM) can be induced in C57Bl mice by infection with *Plasmodium berghei* K173 parasites. Behavioral changes shortly before they die of ECM may reflect disturbance of the integrity of the blood-brain barrier (BBB). Folic acid elicits strong convulsive activity if the permeability of the BBB is increased. Administration of folic acid to mice during development of ECM induced convulsions. Interventions known to prevent fatal outcome from ECM, such as splenectomy or treatment with anti-CD4 or anti-CD8 monoclonal antibodies, also prevented sensitivity to folic acid–induced convulsions. In addition, infected mice with ECM and sensitive to folic acid–induced convulsions, recovered from this sensitivity after treatment with anti–T cell antibodies within 4 h. These data suggest that disturbance of the permeability of the BBB can be reversed and depends on the involvement of T cells.

C57Bl mice infected with *Plasmodium berghei* K173 parasites die of experimental cerebral malaria (ECM) [1]. Mice that develop ECM exhibit behavioral changes during the last 24 h of life that reflect convulsive activity [2]. In this model, ECM is associated with leukocyte adherence to the microvascular endothelium, edema, and petechiae in the brain [3, 4]. Using Evans blue or Monastral blue as indicators of the integrity of the blood-brain barrier (BBB), Thumwood et al. [5] and Neill and colleagues [6, 7] reported increased vascular permeability in mice that developed ECM (*P. berghei ANKA*–infected CBA mice) but not in mice without cerebral malaria (CBA mice infected with *P. berghei* K173 parasites) except at a very late stage of the disease [7].

Convulsions are common in childhood malaria and are associated with severity of disease and mortality [8]. Recent observations show that childhood convulsions are particularly associated with malaria, but not necessarily in combination with fever, suggesting that *Plasmodium falciparum* parasites have epileptogenic properties [9]. Sequestration and adherence of *P. falciparum*–infected erythrocytes in the cerebral vasculature [10] may disturb the permeability of the BBB [11, 12], permitting the entry of epileptogenic factors into brain parenchyma.

Hommes et al. [13] demonstrated that strong, rapidly lethal convulsions could be evoked in rats by injection of folic acid if the BBB was disturbed. This technique of provoking strong epileptic activity by folic acid treatment if the permeability of the BBB was increased was applied in our model of ECM.

In this study, we assessed the effect of folic acid treatment on mice with ECM and of interventions by splenectomy with anti-CD4 and anti-CD8 monoclonal antibodies (MAbs).

**Materials and Methods**

**Mice.** Female C57Bl/6J mice, ages 6–10 weeks, were obtained from specific pathogen-free colonies maintained at the Central Animal Facility, University of Nijmegen. All mice were housed in plastic cages and received water and standard RMH food (Hope Farms, Woerden, Netherlands) ad libitum.

**Parasite.** *P. berghei* K173 (originally obtained from W. Kretschmar, Tübingen, Germany) has been maintained by weekly transfer of parasitized erythrocytes (PE) from infected into naive mice for >30 years. Experimental mice were infected intraperitoneally (ip) with $10^7$ PE from blood of infected donors.

**Parasitemia.** Thin blood films were made from tail blood stained with May-Grünwald and Giemsa solutions, and the proportion of parasitized red blood cells was determined.

**Detection of ECM.** ECM was detected as described by Curfs et al. [3]. In brief, 95% of C57Bl mice infected with $10^5$ *P. berghei* K173 parasites die early in the second week after infection. One day before death a progressive hypothermia develops that is strongly correlated with development of hemorrhages in the brains as observed by histology [1]. The parasitemia in mice that die of ECM is between 5%–10%, and such mice have a 20% reduction in hematocrit. Mice that show a transient hypothermia (in most cases >32°C) survive this critical period but die in the third week or later after infection with signs of severe anemia and a parasitemia of 20%–40% but without detectable cerebral pathology as determined by light microscopy.

**End-stage disease.** Mice infected with *P. berghei* K173 parasites exhibit a rapidly decreasing body temperature and usually die within 24 h [1] about 8 or 9 days after infection. Mice are considered to be in end-stage disease when their body temperature drops to $\approx$35.5°C.
Results

Induction of convulsions by folic acid in P.berghei–infected mice. Convulsions after folic acid administration were not observed in normal C57Bl mice. In the absence of folic acid treatment, P. berghei–infected mice did not show obvious convulsions. C57Bl mice infected with P. berghei K173 PE developed convulsions after injection of 5 mg of folic acid at day 8 or 9 after infection, depending on body temperature. There was a significant difference (P < .05) in mean body temperatures of infected mice that developed (32.1 ± 2.8°C) or did not develop convulsions (36.3 ± 2.1°C) after folic acid treatment. Of 28 infected mice, 26 (93%) with body temperature ≤35.5°C and 2 (16.7%) of 12 with a temperature >35.5°C developed generalized convulsions (P < .05). The proportion of mice that developed convulsions decreased when ≤5 mg of folic acid was injected in infected mice with body temperatures ≤35.5°C (data not shown). Thus, in the standard test to determine sensitivity to induction of folic acid–dependent convulsions, mice with body temperatures ≤35.5°C were given 5 mg of folic acid iv.

Pretreatment of infected mice with body temperatures ≤35.5°C with the anticonvulsant phenobarbitone neither affected parasitemia nor prevented ECM (n = 7), but it protected all mice (n = 8) against development of convulsions induced by a subsequent injection of folic acid.

Discussion

C57Bl mice infected with P. berghei K173 develop ECM characterized by leukocyte adherence to the vascular endothelial lining, edema, and petechiae in the brain [1], suggesting a progressive loss of BBB integrity. These results show that mice during development of ECM become sensitive to the induction of convulsions by folic acid treatment and that protection against ECM by splenectomy or anti–T cell treatment also protects against development of convulsions after folic acid treatment. Moreover, when mice in end-stage disease are treated

### Table 1. Effect of splenectomy (day 1) or anti-CD4 or anti-CD8 T cell treatment (days −2 and 2) on prevention of experimental cerebral malaria (ECM) and of convulsions induced by folic acid treatment in Plasmodium berghei–infected mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice protected against ECM/total (%)</th>
<th>No. of mice* without convulsions/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/5</td>
<td>0/8</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>3/3 (100)*</td>
<td>17/28 (61)</td>
</tr>
<tr>
<td>Anti-CD4 (day −2/2)</td>
<td>5/6 (83)*</td>
<td>9/9 (100)</td>
</tr>
<tr>
<td>Anti-CD8 (day −2/2)</td>
<td>3/4 (75)*</td>
<td>10/15 (67)</td>
</tr>
</tbody>
</table>

* Only small nos. of mice were used as controls because protection against ECM by these treatments was previously shown [1, 14].

### Table 2. Effect of treatment with anti-CD4 or anti-CD8 monoclonal antibodies (MAbs) of Plasmodium berghei K173–infected mice with body temperature of 30–35.5°C on development of folic acid–induced convulsions.

<table>
<thead>
<tr>
<th>Hours after MAb administration</th>
<th>Treatment with folic acid was administered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-CD4*</td>
</tr>
<tr>
<td>4</td>
<td>5/9 (56)</td>
</tr>
<tr>
<td>24</td>
<td>5/9 (56)</td>
</tr>
</tbody>
</table>

NOTE. Data are nos. of mice with no convulsions/no. treated with folic acid (%).

* 0.3 mg/0.2 mL anti-CD4 or anti-CD8 MAb injected intraperitoneally once.
with anti-CD4 or anti-CD8 MAbs, the evolution of ECM is disrupted [14] and they recover within 4 h from sensitivity to folic acid–induced convulsions.

In another experimental murine model of ECM [2], convulsions occurred in end-stage disease. In our model, behavioral changes (progressively inert with ruffled fur, humped back, and locomotor disturbances) were not explicit enough for further analysis. Therefore, we injected folic acid, which induces convulsions if the BBB is damaged [13], to analyze the hypothesis that during development of ECM the permeability of the BBB is increased and causes all or part of the accompanying behavioral changes observed. Only infected mice developing ECM were sensitive to folic acid treatment. This could be prevented by treatment with the anticonvulsant phenobarbitone, suggesting that the permeability of the BBB is increased during development of ECM.

Thumwood et al. [5] and Neil and Hunt [6] also found an increased permeability for Evans blue or Monostral blue of the BBB in late stage ECM development in P. berghei ANKA–infected mice. Neil et al. [7] observed an increased permeability only in terminally ill CBA or DBA mice infected with P. berghei K173 parasites that did not develop cerebral symptoms. The absence of ECM in their experiments may relate to their use of DBA or CBA mice (we used C57Bl/6J mice) or to the number of parasites used for infection (10^6/mouse in their model vs. 10^7 in ours). Our previous experiments showed that the proportion of mice that develop ECM decreases as more parasites are used to infect mice [3], which may explain why Neil and colleagues [6, 7] observed neither ECM nor increased permeability until the mice were terminally ill.

Although ECM cannot readily be compared with P. falciparum–induced childhood malaria, it is of interest that Waruiru et al. [9] describe malaria-specific convulsions in P. falciparum–infected children.

Previous work showed that splenectomy [1] or treatment with either anti-CD4 or anti-CD8 T cell MAbs [14] prevented development of ECM in P. berghei K173–infected mice. A close correlation between sensitivity to folic acid–induced convulsions and development of ECM is supported by the observation that prevention of ECM by splenectomy or anti–T cell treatment also prevented sensitivity to induction of convulsions by folic acid (table 1). These data support and complement the observation of Neil and Hunt [15], who prevented an increase in the permeability of the BBB by treatment with dexamethasone, which in our model successfully prevents ECM when given prior to day 5 of infection [1]. Treatment with anti-CD4 or anti-CD8 MAbs in our model prevented ECM [14] and also prevented development of convulsions after folic acid treatment 4 or 24 h later (table 2).

Why treatment with anti–T cell MAbs is not 100% successful remains to be determined. In some mice with body temperature ≤35.5°C, anti–T cell treatment may be ineffective because of preexisting fatal petechiae as observed by light and electron microscopy [1, 4]. This may also explain why recovery from sensitivity to folic acid–induced convulsions was not always successful and did not increase when folic acid treatment was delayed to 24 h after anti–T cell treatment.

In summary, our results suggest that mice developing ECM suffer from an increasing permeability of the BBB that may lead to petechiae. CD4 and CD8 T cells are involved in the increase of permeability, probably by triggering reactions that are more directly involved in damage.

Acknowledgments

We thank G. Poelen, T. van den Ing, and Y. Brom for skilled biotechnical assistance.

References