EXPERIMENTAL MODELS OF CEREBRAL MALARIA

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INTRODUCTION

• Human Cerebral Malaria (CM) is defined as unrousable coma in *P. falciparum* malaria in the absence of an alternative or additional cause of altered consciousness.

• Ethically, it is difficult to follow the course of CM within the human brain; hence the theories of the pathogenesis of this disease are often based on data from animal models of which mice and monkeys provide the major models.
INTRODUCTION II

• Human malaria parasites, *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* do not easily develop in experimental hosts.

• Many natural malaria parasite/host systems develop chronic infections without cerebral involvement.

• These two facts have significantly led to the current situation where most experimental data on CM is generated from experimental malaria parasites in experimental hosts.
Characteristics of Human Cerebral Malaria

- Sequestration of parasitised erythrocytes (pRBCs)
- Accumulation of intravascular cell infiltrates such as leukocytes and platelets
- Upregulation of adhesion molecules such as ICAM-1
- Production of cytokines
- Presence of cytotoxic CD8+ T-lymphocytes
- Activation of brain astrocytes, pericytes and microglia leading to chemokine recruitment
Knowledge gaps

- Sequence of pathological events leading to CM are poorly characterised
- It is unclear how the blood brain barrier is crossed
- Do we have reliable medication against CM?
- Does a patient completely recover from CM without any future consequences?
- How do we standardize approaches to studying CM?
Knowledge gaps II

- Is vascular obstruction an immunopathological process and are cytokines responsible for the changes observed?
- What is the involvement of the kynurenine pathway of tryptophan metabolism in CM pathogenesis?
- Why are the tissue protection mechanisms normally mounted by the host overwhelmed during CM?
- Experimental models probably hold the key
Why experimental models

• What is an experimental model?
Why experimental models

- A model is just that, a model. A system for (experimental) demonstration
Why experimental models

• Investigations are easily done under controlled conditions
• Easier to justify ethical considerations
• It is unlikely that one model will mirror all the human aspects of the condition

It is not easy to select the best experimental model:
• They have been used for many years
• Their features have been documented
• Work on them has defined the current paradigm
• They are easy to work with
• They are easily available from suppliers
Criteria for a good laboratory parasite model for malaria

- It should be relevant to human malaria
- It should offer the ability to study the biology of the parasite at the cellular and molecular levels in different hosts
- *In vitro* culture asexual forms possible
- Posibility for highly synchronised infections and cultures
- Cyclical passages in the laboratory possible
- Immunological interaction with host (natural/experimental) studied
- Variety of well defined clones and lines available that exhibit natural phenotypes
- Variety of research tools, mAbs, probes etc available
- Purification of major relevant life-cycle stages in large numbers possible
- Combined *in vivo* and *in vitro* research possible in normal laboratory
- Complete *in vitro* development of all vertebrate stages
- Culture of early/late sporogonic stages practicable
Experimental Murine Malaria Marasites

• Of the common malaria species, *Plasmodium berghei, P. yoelii, P. vinckei* and *P. chabaudi* have been isolated from murine rodents and infect laboratory rats and mice.

• The range of differences between rodent parasites is probably less than that which exists between the malaria parasites of man; such differences are relatively minor and most are of little importance to those using rodent parasites as models of the human disease.
## Major Experimental Murine Malaria Parasites

<table>
<thead>
<tr>
<th>PARASITE</th>
<th>NATURAL HOST</th>
<th>EXPERIMENTAL HOSTS</th>
<th>MAJOR LAB STRAINS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. berghei</em></td>
<td>Thicket rats</td>
<td>Mice, rats, hamsters</td>
<td>K173, ANKA, NK65</td>
</tr>
<tr>
<td><em>P. yoelii</em></td>
<td>Thicket rats</td>
<td>Mice, rats, hamsters</td>
<td>17X</td>
</tr>
<tr>
<td><em>P. vinckei</em></td>
<td>Thicket rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. chabaudi</em></td>
<td>Thicket rats</td>
<td>mice</td>
<td>54X</td>
</tr>
</tbody>
</table>
## Characteristics of Murine CM Models

<table>
<thead>
<tr>
<th>PLASMODIUM SPECIES</th>
<th>MOUSE STRAIN</th>
<th>NEUROPATHOLOGY</th>
<th>SEQUESTRATION</th>
<th>CYTOKINES INVOLVED</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. berghei</em> (ANKA)</td>
<td>CBA/ca</td>
<td>CM+</td>
<td>pRBC</td>
<td>TNF, IFN-γ</td>
</tr>
<tr>
<td><em>P. berghei</em> (ANKA)</td>
<td>BALB/c</td>
<td>CM-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. berghei</em> (ANKA)</td>
<td>C57BL/6</td>
<td>CM+</td>
<td>Leucocyte</td>
<td>TNF, IFN-g</td>
</tr>
<tr>
<td><em>P. berghei</em> (ANKA)</td>
<td>DBA/2</td>
<td>CM+ (mild + recovery)</td>
<td>Leucocyte</td>
<td></td>
</tr>
<tr>
<td><em>P. berghei</em> (ANKA)</td>
<td>BALB/c X C57BL/6</td>
<td>CM+ (CM-) Age dependent</td>
<td>pRBC + Leucocyte</td>
<td>IFN-g</td>
</tr>
<tr>
<td><em>P. berghei</em> (K173)</td>
<td>C57BL/6</td>
<td>CM+</td>
<td>Leucocyte</td>
<td>IFN-g</td>
</tr>
<tr>
<td><em>P. yoelii</em> (17XL)</td>
<td>Swiss</td>
<td>CM+</td>
<td>pRBC</td>
<td></td>
</tr>
<tr>
<td><em>P. yoelii</em> (17XL)</td>
<td>BALB/c</td>
<td>CM+</td>
<td>pRBC</td>
<td></td>
</tr>
<tr>
<td><em>P. yoelii</em> (17XL)</td>
<td>DBA/2</td>
<td>CM-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Brain Histopathology Human and Mouse CM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human CM</th>
<th><em>P. berghei</em> ANKA model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain haemorrhage</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Plugging of microvessels</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sequestration of pRBC</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Knobs on pRBC</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Sequestration of leukocytes</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Necrosis of microvessels</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CAM upregulated</td>
<td>ICAM-1, VCAM-1, CD36, TSP, CD36, E-selectin</td>
<td>ICAM-1, VCAM-1</td>
</tr>
<tr>
<td>Overexpression of MHC</td>
<td>Class I and Class II</td>
<td>Class I and Class II</td>
</tr>
<tr>
<td>Upregulation of TNF receptors</td>
<td>p75</td>
<td>p75</td>
</tr>
</tbody>
</table>
Non-human Primate Model

*Cerebral malaria in the rhesus monkey (Macaca mulatta): observations on hostpathology* Ibiwoye et al., 1993

- *Macaca mulatta* inoculated with a virulent strain of *P. knowlesi*
- 1 week later 4/5 animals developed acute malaria and died
- PM showed marked CV congestion and widespread plugging of capillaries and venules by heavily parasitized erythrocytes mixed with uninfected erythrocytes
- EM major changes in adherence of large numbers of parasitized erythrocytes / macrophages to swollen microvascular ECs
- ↑ fibroblasts and deposition of collagen bundles in ECM around damaged and parasite-packed CMV
# Cerebral Malaria in Monkeys

<table>
<thead>
<tr>
<th>Plasmodium species</th>
<th>Monkey host</th>
<th>pRBC sequestration</th>
<th>Adhesion Molecule expressed</th>
<th>Cytokine involvement</th>
<th>Neurology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. coatneyi</em></td>
<td><em>Macaca mulatta</em></td>
<td><em>Brain</em></td>
<td>ICAM-1, CD36, TSP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. fragile</em></td>
<td><em>M. mulatta</em></td>
<td><em>Brain (rosette formation)</em></td>
<td>ICAM-1, CD36, TSP</td>
<td>ND</td>
<td>Fitting, coma</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td><em>Saimiri sciureus</em></td>
<td><em>Multi-organ (rosette formation)</em></td>
<td>ICAM-1, CD36, TSP</td>
<td>ND</td>
<td>Fitting, coma</td>
</tr>
<tr>
<td><em>P. knowlesi</em></td>
<td><em>M. mulatta</em></td>
<td><em>Brain</em></td>
<td>?</td>
<td>?</td>
<td>Mild coma</td>
</tr>
<tr>
<td><em>P. coatneyi</em></td>
<td><em>M. fuscata</em></td>
<td><em>Brain</em></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><em>P. knowlesi</em></td>
<td><em>Papio anubis</em></td>
<td><em>Brain</em></td>
<td>ND</td>
<td>ND</td>
<td>Coma</td>
</tr>
</tbody>
</table>
A new Baboon model

*Experimental infection of the olive baboon (Paplio anubis) with *P. knowlesi*: severe disease accompanied by cerebral involvement (Ozwara et al, 2003)*

- *P. anubis* were infected with *P. knowlesi* H strain erythrocytic parasites
- Acute infection: development of multiple system organ dysfunction and cerebral involvement
- Chronically infected animals: spleen moderately enlarged
- *P. knowlesi* parasitemia profile in baboons and rhesus monkeys was comparable
- Some clinical symptoms of baboons and *P. falciparum*-infected humans were similar
P. knowlesi in Baboon
P. knowlesi in Baboon
**In vitro** models for cytoadherence during CM

<table>
<thead>
<tr>
<th>System</th>
<th>ICAM-1</th>
<th>CD36</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUVEC</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C32</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Monocytes</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Platelets</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Stably transfected cells (CD36)</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Stably transfected cells (ICAM-1)</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>HD-MVEC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HB-MVEC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HBEC-51</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BB19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HL-MVEC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey brain MVEC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mouse brain MVEC</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Retinal wholemount</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Knowledge gained from *in vivo* models

- Pathogenesis of CM is influenced by host genetics, parasite genetics, nutritional status and intercurrent infections
- The concepts defined in experimental CM might lead to further investigation in the human disease
- Cytokine overproduction contributes to brain vascular pathology
- TNF and IFN-g are important mediators in the pathogenesis of CM
- T lymphocytes play a significant role in development of murine CM
- Cytokine induced phenotypic changes of brain microvasculature endothelial cells represent a key event in the sequestration of pRBCs and leukocytes
Further knowledge gained from experimental models

- A role of platelets in TNF-induced microvascular pathology

- Protection from CM in TNF receptor p75-deficient mice but not p55-deficient mice

- Brain microvasculature endothelial cells derived from CM susceptible and CM-resistant mice exhibit differential responsiveness to TNF and IFN-g
Strengths of Murine Models

• Expected incidence of CM is high and reproducible

• Highlighted importance of immune system in the modulation of CM

• Useful by means of intervention experiments, in determining why, where and when each of the factors instrumental to CM is involved in pathogenesis
Limitations of Murine Models

- In Murine CM, the condition is fatal while it is potentially reversible in human
- Cells sequestering in the brain are monocytes and not parasitised erythrocytes
- Murine malaria parasites have uncertain phylogenetic relationship with human Plasmodia which calls into question their relevance as biochemical or molecular models
Similarities between murine and human CM

- Clinical signs of nervous system dysfunction and cerebral pathology
- Cytokine overproduction contributes to brain vascular pathology
- Endothelial cells are involved in both systems
- Similar immune response pathways
Strengths of Primate Models

• Close phylogenetic relationship with human

• Invaluable tool for the study of parasitised erythrocyte sequestration

• Parasitised erythrocytes and brain vascular complications such as haemorrhages are closely similar to human CM
Limitations of Primate Models

- Onset of CM difficult to determine
- Expected incidence of CM is low and variable
- High cost of acquisition and maintenance
- Lack of genetically modified animals
- Complex ethical considerations
Way Forward for Models of CM

Models should enable an understanding of the mechanisms involved in CM. This will in turn lead to:

• Crucial leads to development of new preventive and curative therapies as wells as defining prognostics markers
• The possibility of vaccinating against toxins that induce inflammatory cascades leading to CM related complications
• Identifying the role of T cells in the pathogenesis of CM
Major References

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• Clin Microbio Rev 14(2001)810-20
• Microbes Infection 4(2002)291-300
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