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Citation

Published Version
doi:10.1371/journal.ppat.1002401

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The Role of Animal Models for Research on Severe Malaria

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In light of the recent controversies over the role of animal models for research into the development of new treatments for severe malaria, particularly cerebral disease, a group of scientists came together to discuss the relative merits of a range of animal models and their overlap with the complex clinical syndromes of human disease. While it was not possible to fully resolve differences over the utility of the Plasmodium berghei ANKA model of experimental cerebral malaria, the meeting did bring the two research communities closer together to identify further work to provide information needed to validate the model and revitalise the development of other animal models displaying features of human pathology. The driving force behind this was the desire to ensure better translation of experimental findings into effective treatments for severe malaria.

Introduction

In 2010 there were a number of publications discussing the relevance of rodent models for the study of human cerebral malaria (HCM). These were prompted by an initial discussion paper from Professor Nick White and colleagues (“The murine cerebral malaria phenomenon” [1]) in which the suggestion was made that the Plasmodium berghei ANKA (PbA) mouse model of experimental CM (ECM) did not replicate the pathophysiology of cerebral disease in humans, and which was followed closely by a set of papers supporting the ECM approach [2–5]. This has resulted in some polarisation of the research community on this topic and an occasionally uncritical application of some of the perceived conclusions of this initial paper to other areas of malaria research using rodent animal models. This Wellcome Trust–supported workshop brought together experts (Table 1) from both sides of the debate and those with expertise related to non-human primate (NHP) malaria to discuss the clinical features of severe malaria in humans and how various animal models might best be used to advance our knowledge of this important disease and the development of therapeutic measures.

What Are the Clinical and Pathological Features of Severe Malaria?

An important aspect of falciparum malaria that has not been always fully appreciated is the heterogeneity of disease patterns in humans. Frequently the terms “severe” and “cerebral” when used in conjunction with the term “malaria” have been thought to be interchangeable, but severe malaria is made up of a range of clinical syndromes, several of which do not involve coma. Severe malaria shows both clinical heterogeneity between patients and the patterns of pathophysiology observed between adults and children. Therefore it is important when dealing with studies on severe malaria that a reliable and consistent definition of disease is used. Detailed clinical criteria for the diagnosis of severe malaria have been defined by the World Health Organization [6].

Most studies on severe malaria have focused on infection by the malaria parasite Plasmodium falciparum. Adults with severe malaria show signs of profound metabolic derangement and cerebral malaria, and may have placental malaria and (unlike children) multi-organ failure, including kidney and liver dysfunction. For children, severe disease includes metabolic derangement, cerebral malaria, and severe malarial anaemia. Frank pulmonary oedema is less common in children. Severe malaria (particularly lung involvement, thrombocytopenia, or anaemia, but without coma), although less frequent, can be caused by Plasmodium vivax [7,8]. Plasmodium knowlesi cases also frequently develop severe malaria, with limited clinical data and autopsy findings from a single case report appearing to show similarities to P. falciparum pathology but without coma [9].

A principal feature of HCM from nearly all post-mortem studies is the packing of sequestered infected erythrocytes (infected red blood cells [IRBC]) in the brain microvasculature [10,11], associated with microvascular obstruction [12]. This may also be associated with microhaemorrhages, although these are also seen in non-cerebral malaria cases and other...
Table 1. Participants at the Meeting.

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<tr>
<th>Name</th>
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<tr>
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<td>Liverpool School of Tropical Medicine, UK</td>
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<td>National Institutes of Health, USA</td>
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<td>Chris Engwerda</td>
<td>Queensland Institute of Medical Research, Australia</td>
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doi:10.1371/journal.ppat.1002401.t001

Mortality in paediatric HCM occurs very rapidly, with 50% of deaths being recorded within 24 hours of admission and nearly all fatalities by 48 hours. In adults a similar level of early mortality is observed, with about 50% of deaths occurring in the first 48 hours post admission, but subsequent deaths can take place later due to other complications such as renal failure or incidental pneumonia. Patients with HCM tend to have higher parasitaemias, and some studies have also suggested a larger parasite biomass (as estimated by plasma PfHRP2 levels [21]). However, infections with hyper-parasitaemia without manifestations of HCM also occur. A key point in interpreting these findings is the location of the tissue burden of sequestered parasites in severe disease, and the immune mechanisms that attenuate disease.

Information on the histological pattern of severe malaria is still very restricted, particularly from childhood deaths, in part due to the difficulty in accessing relevant human material (see below). Given the multiple aetiologies of severe disease, this type of information will be critical in helping us to understand the relative contribution of different pathologies to the clinical signs observed in severe malaria. A consistent pathological finding in HCM at autopsy, unique to this disease, is the presence of sequestered iRBC that pack brain blood vessels. Indeed, patients who die soon after admission to a health care facility may have brain microvascular sequestration as the only significant pathological feature of CM. However, observation and correlation of neuropathological features with pre-mortem clinical diagnosis can be affected by multiple factors such as time to death, treatment, and other systemic complications of severe malarial infection, or may be compromised by misdiagnosis. In this regard there are some differences between adults and children, for example, in the degree of brain swelling and oedema observed using imaging techniques, and the accumulation of leukocytes and platelets in cerebral microvessels, with significant levels seen in some cases of paediatric HCM but very few observed in adults, despite clear evidence (for instance, from circulating cytokine levels) of a vigorous pro-inflammatory host response. Astroglial activation and blood–brain barrier changes are also commonly observed in HCM, so inflammatory responses elicited within the brain parenchyma may be more subtle than merely accumulation of monocytes or other leukocytes [22,23]. Data are also available for iRBC sequestration and host responses in non-brain tissues that represent other sites for maturation of *P. falciparum* within erythrocytes [10].

Adhesion-related pathology in *P. falciparum* malaria is evident in placental infections in pregnant women. Here, placental sequestration of iRBC and the onset of fetal impairment and disease in women protected by immunity is linked to the appearance of a set of parasite variants expressing a class of PIEMP1 proteins known as VAR2CSA [24] with atypical structure and domain composition [25,26]. A key feature of these parasite variants is their ability to bind to chondroitin sulphate A (CSA) in the placenta [27], where they are thought to induce fetal disease through as yet unknown inflammatory pathways [28].

**Important Issues in Research on Severe Malarial Disease and the Use of Animal Models**

Two key questions, “what is the pathophysiology of severe disease?” and “how do people living in malaria endemic regions become protected from severe disease?”, cannot be answered fully by observations of infected humans. While studies on the clinical definition and histopathology of HCM and placental malaria have provided information regarding correlates of pathogenesis, the exact mechanisms whereby malaria parasites cause severe disease and how immunity protects against disease despite infection remain unknown. Parasites with complex life cycles such as *Plasmodium* species have evolved multiple and redundant strategies to deflect and evade the host immune response, so it is unlikely that protection will be correlated with the ability to recognise and neutralise a single...
antigen [29]. In the case of *P. falciparum*,
anti-disease immunity develops relatively
quickly, perhaps after only a few infec-
tions, but this immunity is incomplete, still
allowing lower density blood stage infec-
tion that is accompanied by either mild or
no symptoms [30]. Immunity is also linked
to levels of exposure so that children
appear to acquire anti-disease immunity
at a very early age in areas of high
transmission (and have a distinct pattern
of severe disease, dominated more by
severe malarial anaemia rather than
HCM) [31]. By contrast, malaria naive
adults and older children encountering
the parasite for the first time produce a
different defence response. Linked to
immunity is the question of the role of
inflammation in the pathogenesis of severe
disease. Investigations of this issue require
not only longitudinal clinical studies, but
also the analysis of multiple host responses.
Thus, it is no surprise we do not fully
understand the mechanism of protection
either from severe disease or infection
itself.

Mechanistic studies in animal models
may provide information on the processes
of, and protection from, severe malarial
disease, and there are good examples
where they have provided insights into
features and characteristics of human
malaria infection. Although it is unlikely
that any single model will reproduce the
complexity and spectrum of disease and
immunity observed in human malaria
infections, there are parallels between
some human and animal presentations of
malaria infection (and disease). However,
the choice of the host/parasite combina-
tion is important (see Table S1).

**Validity of the Rodent *P. berghei*
ANKA Model (PbA Mouse Model) for
Human Cerebral Malaria?**

In some ways, this is one of the most
challenging questions for the malaria
research community and accordingly
has resulted in a degree of polarisation
of viewpoints. The correct choice of a
combination of experimental animal host
and a malaria parasite able to appropri-
ately mimic the disease pattern seen in
humans is essential. However, it is also
very clear that animal models allow for
more detailed examination of multiple and
specific pathophysiological processes caused
by malaria infection that is not possible for
clinical studies of human malaria through
the level and scope of experimental
observation and intervention that can be
applied to animals. In discussing the role
of the PbA mouse model of HCM
specifically, the issue that recurred
throughout the meeting concerned the
degree of sequestration of iRBC in the
brains and other organs of *P. berghei*
ANKA-infected mice. Several groups
provided data that supported the accumu-
lation of iRBC in a number of organs,
sometimes including the brain, and that
higher parasite accumulation was seen in
mice susceptible to ECM. However, the
key piece of information missing was direct
histological evidence of substantial para-
sitised erythrocyte sequestration
packaging brain microvessels in a similar pattern
to that observed with HCM.

The consensus of most participants was
that *P. berghei* ECM, in contrast to HCM,
is associated with marked accumulation of
leukocytes, but not with prominent se-
questration of cytoadherent mature tro-
phozoite/schizont iRBC in brain vessels
[32]. However, a number of participants
emphasised that recent data have provided
evidence of increased iRBC accumulation
during ECM in multiple organs, including
the brain [4]. It was felt that more
research is needed to define this phenotype
of iRBC accumulation during ECM.

ECM is associated with increased accu-
mulation of immune cells in the brain
(particularly monocyte/macrophages and
T cells). In particular, recruitment of
CD8+ T cells to the brain late in disease
pathogenesis, and production of the cyto-
lytic molecules granzyme B and perforin,
is critical for ECM development [33]. In
general, it was agreed that in the absence
of prominent parasitised erythrocyte se-
questration in the brain it is not appropri-
ate to use the PbA mouse model for testing
interventions that may reverse iRBC
adhesion to relieve microvascular obstruc-
tion (a hallmark of HCM). However, since
in both humans and rodents vascular
obstruction, either by iRBC, leukocytes,
or by both, appears to be associated with
features of CM, the PbA mouse model
may be useful to analyse the relationship
between reversal of vessel blockage and
inflammation in CM. The types of endo-
thelial cell microenvironment changes
induced by cytoadherence and inflamma-
tion are not the same [34], although they
share several processes, and as HCM
encompasses more than one pathologic
entity it is possible that disease may be
caused by different vascular conditions.
Thus, there is room for investigating these
different mechanisms in relation to human
disease. For HCM, both post-mortem and
in vitro evidence show that cytoadherence
of iRBC to brain endothelium has specific
functional effects [35–37].

ECM is clearly an inflammatory syn-
drome with local vascular endothelial
activation with inflammatory cytokines
playing an essential role, which has
obvious differences and some similarities
to the clinical and pathological features of
HCM, for example, the signs of vascular
inflammation/damage seen in some cases
of paediatric HCM [13]. Some studies
have suggested associations between high
levels of cytokines and severe malaria
[38,39], but these have been challenged
recently by work showing that high levels
of pro-inflammatory cytokines such as
tumor necrosis factor (TNF) are poor
indicators of HCM in African children
and that markers of localised endothelial
cell inflammation (e.g., Angiopoietin 1/
Angiopoietin 2 ratio) are more strongly
associated [40,41]. It is possible that
inflammatory processes play an essential
role in a subset of clinical patients.
Therefore, (mechanistic) studies of ECM
in PbA mouse models may provide
insights into inflammatory processes and/
or immune responses and their role in
HCM pathology. An alternate view,
strongly articulated at the meeting, was
that ECM in the PbA mouse model was
so very different to HCM that the
pathological and therapeutic interpreta-
tions derived from it were largely irrele-
vant for the understanding and treatment
of HCM. It was suggested that future
experimental studies in the mouse and
other animal models (i.e., NHPs) would
be better directed towards pathological
processes that could be shown to be
similar in human infection and the animal
models. Other features of ECM, such as
PbA-iRBC adhesion to CD36, may have
relevance to HCM. Although CD36 is
expressed at very low levels on cerebral
endothelial cells in the human and mouse,
it is abundantly expressed on platelets,
macrophages (cells that accumulate in the
brain in ECM), on endothelium in other
vascular compartments, and is also
present on microparticles. The role of plate-
lets and microparticles in human malaria
has become a major topic of research
[42,43] and may become more relevant
to identifying potential interventions for
HCM.

One of the benefits of the discussion
about ECM studies at the meeting was a
clarification of how inhibitor or interven-
tion studies of adjuvant treatments in
mice should be interpreted. In most
studies, the inhibitors/drugs are given
before the development of neurological
symptoms, thereby providing information
on disease processes in ECM but not
necessarily on the identification of viable
therapies. The validity of carrying out
human clinical trials, often in underpow-
cytoadherence. While this “double transgenic” model is not available yet, human graft systems have been investigated using SCID mice, as transient models allowing perfusion of *P. falciparum* iRBC in human dermal tissue in a format where detailed microscopy is facilitated [52], and in investigating the role of Fc receptors in malaria [53]. The graft model allows detailed examination of the interaction between parasites and the host in a three-dimensional tissue, rather than the two-dimensional ex-vivo models commonly used. Complex humanised models for longer-term experiments on *P. falciparum* infections in NOD/SCID mice have also been described [54]. Results suggest that these do not show cytoadherence [55] or ECM, and so they cannot be used for HCM research. Uptake of graft models has been extremely limited and further work is needed to validate their potential use and to develop the protocols to extend the range of tissues available.

Non-Human Primate Models for HCM or Severe Malarial Disease

Given the greater genetic relatedness of primate hosts and primate parasites, the importance of NHP and malaria parasite combinations as appropriate systems for researchers to study ECM was also discussed at the workshop. Further, it was noted that a degree of reluctance to use NHP models in malaria research has led to a reduction in the resources needed to support such research. If NHP systems are capable of providing models of HCM and other types of severe disease in human malaria, then significant investment will be required to grow and sustain this research community, train young researchers, and provide the resources for wider participation of clinical and laboratory oriented investigators.

Several host/parasite combinations were discussed (Table 1): *Plasmodium coatneyi* is a simian parasite that in Japanese macaques exhibits phenotypic characters that mimic *P. falciparum* iRBC sequestration, rosetting, and clinically severe disease in humans; however, it does not show cytoadherence [55]. In addition, *P. coatneyi* produces a “severe disease” that is almost always lethal to this host. Mature *P. knowlesi* infections in rhesus monkeys partially sequester in a variety of tissue sites via the variant-antigen products of the large var-like SICAvar multi-gene family [64,65], although it is clear that severe disease in the *P. knowlesi* rhesus macaque model can mimic some clinical syndromes of *P. falciparum* severe disease in humans, it is questionable whether it is a useful model for HCM, as neither adhesion of iRBC to cerebral vessels or clinical signs of HCM have been noted. Although it is unclear if *P. knowlesi* in rhesus monkeys is a satisfactory model for HCM, from initial reports it is certain to be a model for human severe disease caused by this parasite [9,70,71]. *P. knowlesi* in the olive baboon (*Papio anubis*) [72] has been suggested as an HCM model because of apparent cerebral iRBC sequestration and neurological signs in terminal infections, but this needs further investigation.*P. falciparum* will also infect and cause severe disease (anaemia, acute nephritis, cardiopathy, metabolic collapse) and death in neotropical monkeys such as various species of *Aotus* (owl monkeys).

There are, however, limitations to this primate model since symptoms of CM and cerebral vascular sequestration have not been noted in New World monkeys infected with *P. falciparum.*

What Models Are Available for Other Aspects of Malaria Research?

At the meeting, brief mention was made of models for placental malaria, acute respiratory distress (ARDS), anaemia, and immunity. The lack of the var multi-gene family in rodent and some primate malaria species (i.e., *Plasmodium cynomolga*, which is a model for *P. vivax*) means that the roles of the encoded variant antigens in placental sequestration cannot be investigated. However, NHP models such as *P. coatneyi* in rhesus monkeys or *P. falciparum* in *Aotus* monkeys are also available; and *P. coatneyi* expresses the comparable variant antigen multi-gene family [J. Barnwell and

What Other Rodents Models of Human Cerebral Malaria Are Available or Possible?

With questions raised about the relevance of the PbA mouse model for HCM, a consideration of alternative rodent models was undertaken. Improvements based on genetic manipulations to the mouse model were discussed, such as a humanised rodent system. Schizonts of *P. berghei* sequester and express parasite ligands on the surface membrane of iRBC [51]. The characterisation and the genetic modification of *P. berghei* ligands involved in binding to host receptors might offer novel possibilities in the development of small-animal models for analysing the sequestration properties of *P. falciparum* ligands that have hitherto only been examined in vitro. This could be performed by, for example, substituting *P. berghei* ligands with PiEMP-1 proteins or domains. Using in vivo imaging in conjunction with such “falciparamised” *P. berghei* parasites in mice expressing human receptors (e.g., human ICAM-1) may create an in vivo screening system for testing inhibitors that block *P. falciparum*
M. Galinski, unpublished data). Furthermore, analysis of the effects of inflammatory responses and parasite accumulation/sequestration in the placenta and foetus may provide insights into the role of pro-inflammatory cytokines in malarial placental pathology [74–77]. Major limitations of many mouse models are the lack of a natural chronic infection, with the exception of Plasmodium chabaudi, and the ability of some host/parasite systems to develop almost complete immunity after a single infection. Investigation of the effects of malaria on multiple pregnancies is therefore not possible in most rodent models except in drug-induced chronic P. berghei infection in mice, which is one of the best described models for rodent placental malaria. A “humanised” PbHa mouse model may also be developed for analysing sequestration properties during pregnancy. Transgenic P. berghei parasites may be generated that express Pf VAR2CSA or CSA binding domains on the surface of iRBC. Such parasites in combination with a “humanised” placental malaria mouse model may offer a screening system for in vivo testing inhibitors (small molecule inhibitors, antibodies) that block P. falciparum sequestration.

Several recent studies indicate that P. berghei in mice can be models to investigate malaria-associated lung pathology [78–81]. For these models it was felt that, as with CM, detailed comparisons between lung pathology in the rodent models and lung pathology in humans are required to validate their use for developing therapies/interventions.

Models for anaemia were also considered to be important to support human clinical studies. P. chabaudi [82] and P. yoelii 17xNL in mice and P. berghei in Brown Norway rats (C. Janse and B. Franke-Fayard, unpublished data) were briefly mentioned as possible models for malarial anaemia. However, the lack of a real chronic infection and the rapid acquisition of sterilising immunity preclude the use of many rodent models to study anaemia in chronic infection, which would be more reflective of severe malaria in children. However, mechanisms of disruption of bone marrow haematopoiesis [83] and dyserythropoiesis in acute infection can be readily investigated.

Based on mainly unpublished reports, several NHP models offer opportunities for studying severe anaemia in both acute and chronic infection in regards to clarifying the mechanisms and interventions for clearance dynamics of uninfected RBC, bone marrow differentiation, and dyserythropoiesis in severe malarial anaemia. Simian malarias such as P. coatneyi in acute infections produce profound anaemia that is life-threatening due to clearance of uninfected erythrocytes and a dyserythropoiesis that does not allow a compensatory reticulocytosis [A. Moreno and M. Galinski, unpublished data], as well as severe anaemia later on during chronic severely recrudescent infections that can last for months or over a year (W. Collins, unpublished data). P. cynomolgi, like P. vivax, also produces a profound anaemia during acute infections (A. Moreno and M. Galinski, unpublished data). Severe lethal anaemia also develops in semi-immune neotropical primates with P. falciparum infections going into chronic infection and upon reinfection or challenge of animals induced into a semi-immune state [J. Barnwell and W. Collins, unpublished data]. P. vivax infections in neotropical monkeys also develop severe anaemia during acute and chronic infections [84,85].

It was generally agreed that rodent models can provide relevant information on mechanisms of host defence and immunity to all stages of the infection. A recent example of this is the demonstration of suppression of liver stage malaria parasite development in the presence of a blood stage infection [86]. P. chabaudi and P. yoelii in mice and P. berghei in Brown Norway rats show self-resolving infections with low peak parasitaemias and anaemia. It is generally assumed that immunity in humans and NHPs develops slowly over many months and incompletely, in contrast to rodents where immunity develops rapidly and is frequently sterilising. Only P. chabaudi in mice [87] and P. berghei in rats exhibit low density chronic infections for prolonged periods (2–3 months), whereas in humans and NHPs single infections may last years and decades. One feature in common to human, NHP, and rodent infections is that protective immune responses are generally species-, genotype-, and variant-specific, although in the case of variant-specific immunity some of the antigens that are products of multi-gene families (e.g., PfEMP1 and PIR) exhibit evolutionarily distinct sequences and characteristics in the species and strains where they exist. Humans are generally exposed to different strains, genotypes, and variants of the parasite, whereas in most experimental model studies the same cloned parasite line or a single strain/isolate is used for repeated infections. It was emphasised that when humans are similarly re-exposed to the same strain/genotype, acquisition of immunity is generally similar to that of mice. A recommendation therefore was that studies on immunity to re-infection and the effectiveness of immunological memory should be carried out in mice or monkeys using different strains of the same parasite species. More immunological studies in mouse/rat peripheral blood, for comparison with humans, would also be valuable. Another important point of experimental model studies was that direct injection of infected erythrocytes to initiate infections was normal practice in the study of immune mechanisms and immunopathology. However, this does not reproduce the sequence of the natural infection where infection starts with a mosquito bite and inoculation of sporozoites. It was discussed here that blood stage infections of some Plasmodium species may look very different and be considerably less virulent when initiated via the natural route compared with injection of iRBC. This should be taken into account when translating findings from animal models to humans.

At the meeting the use of animal models for development and testing of vaccines and drugs was only briefly mentioned. Discussion of this area was outside the scope of the meeting, but it was acknowledged that animal models have played a major role in the development of anti-malarial therapies, including those targeting different stages in the parasite life cycle [2].

Conclusions and Recommendations

There was universal support for stronger interactions between groups working on human and animal studies, both to improve current models and to promote more realistic translation of laboratory findings into clinical studies.

The importance of animal models in malaria research was recognised by all participants. However, it was not possible to reach a consensus on the role of the P. berghei mouse model for studies on HCM. The basis of the disagreement centred on the relative importance of iRBC adhesion to brain endothelial cells in causing HCM pathology. There is evidence to support the presence of P. berghei iRBC in cerebral vessels in ECM, but this accumulation represents a minor (if any) concentration of iRBC compared to the peripheral parasitaemia, unlike the levels of adherent iRBC seen with P. falciparum. In the absence of this hallmark of human CM, and based on observations of pathologic changes to
endothelial cells on interaction with iRBC, it was the view of some of the participants that the mouse/PbA system was not a useful model of HCM. However, it was pointed out that we do not know that P. falciparum cytoadherence causes HCM and its role in pathology may have been exaggerated. At the same time, comparisons of ECM and HCM have revealed many similarities (although the extent of these was also contentious), and therefore the PbA mouse model can provide a platform for detailed mechanistic experiments in this field. Thus, the overriding conclusion of this meeting was that there are presently genuine differences of opinion on this topic, but this was coupled with the genuine willingness indicated by all parties at the meeting to try overcoming these differences by collaborating. Specifically, these centered around two major areas: 1) A more careful use of the term “drug” when applied to interventions used in ECM, particularly when these were applied prior to the physiological signs of cerebral disease in mice, although the validity of the use of inhibitors to understand pathological processes was appreciated. 2) A bi-directional exchange of resources and knowledge to provide better information about the complex pattern of human disease to scientists using animal models and better access for this latter community to human tissues in order to validate findings obtained from model systems. The major issues raised at the meeting are summarised in Box 1.

The main recommendations from the meeting were:

(i) Coordinated pathological studies across species

As with many disciplines, one of the major confounders of research is the lack of standardisation. This is particularly clear with the mouse/PbA model, where differences between parasite lines and environmental factors, such as host diet, can influence features of pathology. Thus, there was a general consensus that tissue specimens from animal models of CM produced in different laboratories need to be analysed using uniform methods of histology and quantification. While it was questioned whether the mouse/PbA model is appropriate to derive cytoadherence-based therapies for CM, it does not preclude this model as a vehicle to investigate possible inflammatory processes in severe disease, including CM. Further research is needed to link animal and human studies to allow a comparison of the pathology of disease across a spectrum of clinical syndromes and therefore to develop the much needed animal models that can drive research into the development of interventions for severe malaria. The mouse model may be useful in studying tissues where inflammation is known to play a significant role in human pathology, such as the lung. But in some clinically important syndromes of human malaria such as malaria-associated acute renal failure in adults, very little data are available on the clinical or pathological correlates in animal models. However, NHP infections in rhesus monkeys involving several simian malaria species can cause acute renal failure.

(ii) More use of primates and better access to primates

Strengths of studies on NHPs are that their physiology and acquisition of immunity to infection are more similar to humans than mice, and their size may also permit a wider range of studies. Also, within limits, neotropical (New World) primates can be infected with human P. falciparum and P. vivax (as well as P. knowlesi). Infections with P. fragile, P. coatneyi, and P. knowlesi in certain macaque species are potential models of CM, anaemia, placental malaria, and ARDS and other severe malaria syndromes in humans [80]. NHP models can also facilitate studies of malaria parasite multi-species co-infections and co-morbidities, e.g., affect of worm infections on the severity of malaria, and overlapping syndromes, e.g., metabolic acidosis, anaemia, etc. One drawback is that the currently available NHP models, such as P. coatneyi in Japanese or rhesus macaques, exhibit cerebral sequestration but it is not clear how similar they are to HCM.

NHP models of vaccine efficacy (using GMP material) have been reasonably predictive of human clinical trials (albeit mainly negative, as are most clinical trials). Unlike rodent models, they do provide access to some of the life cycle stages that are not available in vitro, including hypnozoites.

NHP studies for malaria research require specialised scientific centres with the regulatory framework, access to NHP animals, and expertise to conduct such research. The group recognised that investment in existing NHP research centres would be a valuable and cost-effective way forward, coupled with a scheme to provide “placements” for scientists from collaborating laboratories. This would ensure an enhanced and vibrant scientific environment for malaria research within the NHP centres and disseminate the potential for NHP research to the community.

(iii) Clear discrimination between intervention and mechanistic studies

One of the more fundamental problems of animal models highlighted by recent publications has been the confusion between studies aimed at collecting mechanistic information on disease processes and studies aimed at screening for potential therapies against severe disease, perhaps most graphically demonstrated by the figures of 38 of 42 interventions for ECM working but none of 16 being successful in HCM. The limitations associated with the application of therapies before clinical signs, often used in PbA mouse model studies, need to be recognised. Relevant animal models are highly desirable, as studies of humans with severe malaria are by definition limited to situations where clinical interventions are the priority. Thus, interpretations of the result of therapies preventing CM must be done in the context of anti-malarial drug treatment and other supportive measures.

(iv) Facilities to support research on severe malaria

One of the major outcomes of the workshop was the requirement for further development of facilities for research.

Box 1. Major Issues from the Meeting

- The mouse and human malaria research communities need to work together more closely to support better translation of laboratory studies into clinical leads.
- It was not possible to resolve the disagreements over the use of the P. berghei ANKA mouse model for human cerebral malaria, particularly in the area of cytoadherence-associated pathology. It was agreed that researchers using the P. berghei model should be cautious in their interpretation of inhibitor studies, particularly where the intervention is given prior to symptoms.
- The spectrum of malarial disease in humans is broad and we do not fully understand the pathological mechanisms.
- Development and standardisation of animal models, including renewed investment in non-human primate systems, is a priority.
We need to understand more about human disease and immunity and communicate more effectively with researchers working on animal studies so that we can identify appropriate models for detailed mechanistic studies. Apart from a general need for more work in this area, two specific areas were identified for further development:

(v) Tissue biobanking

Our lack of knowledge of the detailed pathology of human disease is part of the problem in identifying relevant animal models. To address this, the creation of a tissue biobank was proposed. This resource would make material from humans with malaria available to the broader research community so that validation of initial observations from well-characterised clinical specimens could be examined from a variety of perspectives using novel technologies. There are clearly ethical and logistic issues associated with this, but the benefit accrued in terms of validating animal studies by having good access to human tissues was deemed to outweigh the effort that would be required to set this up properly. The use of tissue microarrays, where multiple cases can be examined on a single slide, may facilitate this approach.

(vi) Standardisation of animal models

It was proposed that a repository of standard parasite lines and protocols relating to the maintenance/ passage of parasite lines and their hosts should be created. There should also be an opportunity for members of the research community to share information (particularly unpublished data that is not widely available), including data such as a comparison of parasite sequences.

Supporting Information

Table S1 Animal host/malaria parasite combinations for the study of human malaria pathogenesis.

(DOC)

Acknowledgments

We wish to thank all the participants for their time and energy in a robust and constructive discussion. We would particularly like to thank Paul Hagen for agreeing to chair the meeting and for doing this in an energetic, effective, and balanced way, and Eleanor Riley for her support and advice in convening the meeting.

References

4. Riley EM, Cooper KN, Helmby H, Hafalla JC, de Souza JB, et al. (2010) Neuroprotection of humans with malaria available to the tissue biobank was proposed. This resource would make material from human tissues was deemed to outweigh the effort that would be required to set this up properly. The use of tissue microarrays, where multiple cases can be examined on a single slide, may facilitate this approach.

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