in the context of human malaria pathology and *Plasmodium* kinomics.

Hitherto undescribed in malaria parasites, a canonical pathway in eukaryotic cells relies on SNF1-related/AMPK kinases to respond to low energy levels by repressing anabolic pathways, and hence cell division, and activating backup energy-generating processes. The article by Mancio-Silva et al. [1] sheds new light on what may be a highly divergent nutrient-sensing pathway in *Plasmodium* spp, in which the kinase KIN may play the role of SNF1/AMPK. The authors reached their conclusion by summarising a large body of work in vivo and ex vivo with *Plasmodium berghei* (and corroborative experiments with *Plasmodium falciparum, Plasmodium yoelii,* and *Plasmodium chabaudi*). The initial observation is that infection of mice with *P. berghei* has different outcomes depending on whether the animals are fed ad libitum, or subjected to calorie restriction causing a 20% loss in body weight. Calorie-restricted mice seemed to be protected from a lethal infection with the *P. berghei* ANKA strain for quite some time, although they finally succumbed with the same parasitemia as the fully fed animals. The host inflammatory response is a major contributing factor of cerebral malaria pathogenesis, and, in line with the data from Mancio-Silva et al. [1], the levels of proinflammatory cytokines have been shown to be reduced in calorie-restricted mice, correlating with better infection outcomes [2]. How does this relate to human malaria? The effects of nutritional status on the outcome of the human disease are complex. A recent meta-analysis of 28 studies indicates that while malnutrition does not have a great impact on malaria morbidity, it could have a negative effect on malaria mortality and severity [3]. In fact, the effect of calorie restriction on rodent malaria pathology reported in [1] could be interpreted from a purely host-centric perspective, assuming that mice responded to fasting in well-established ways by burning fat and thus increasing levels of ketone bodies such as β-hydroxybutyrate (BHB), a molecule known to suppress inflammatory pathways [4], hence alleviating the cerebral manifestations of infection.

However, the authors also made the very interesting observation that calorie restriction led to a decrease in parasitemia (in contrast to the aforementioned earlier study [2], in which no effect of undervenourishment on parasitemia was detected). Furthermore, Mancio-Silva et al. [1] found that, under their experimental conditions, parasites (both *P. berghei* and *P. falciparum*), grown ex vivo for one cycle in the presence of serum from uninfected, calorie-restricted animals, produced fewer merozoites per schizont (median differences of three to five merozoites per schizont). The authors chose to investigate this intriguing response of the parasite to the host nutritional status in the rodent model. Untargeted transcriptomics revealed overexpression of a number of protein kinases in parasites from underweight animals. Their previous work in systematically deleting the nonessential kinome of *P. berghei* [5] provided mutant parasite strains to test the role of individual enzymes in the low-nutrient response. The reduction in merozoite yield per schizont as a response to ‘lean’ serum was abolished if the protein kinase KIN was genetically inactivated. The observation was confirmed to a limited extent in *P. falciparum* treatment of the human parasite with salicylate (an activator of AMPK activity) caused a reduction in merozoites per schizont similar to that caused by incubation in serum from caloric-restricted mice. The possible involvement of KIN could not be genetically tested in *P. falciparum,* however, as the authors’ attempts to generate a PKIN deletion mutant were unsuccessful (M. Mota, personal communication), in line with the results of a kinome-wide reverse genetics study in *P. falciparum* [6]. This is interesting, as it suggests that PKIN and...
PbKIN may have evolved divergently, with the former having acquired functions seemingly essential for completion of the intraerythrocytic cycle (in addition to the possible function in nutrient sensing inferred from the present study in *P. berghei*). The work did not address the nature of the serum component(s) that triggered the ‘low merozoite number per schizont’ response – it would be of great interest to measure SHB (see above) or cytokine levels in ‘lean’ serum, and to check whether these or other under-nourishment-associated circulating factors affect parasite replication in a PfKIN-dependent manner. Interestingly, the individual deletion of at least two different *P. falciparum* kinases, PIPK7 and Prck-5, confers a very similar phenotype, namely a decrease in the number of merozoites produced by each schizont [7,8]. Could these represent effectors of a KIN-dependent pathway? Also, KIN has been described to be predominantly expressed in gametocytes [9], raising the possibility of an effect of caloric restriction on malaria transmission efficiency, which would be well worth testing.

The implication of KIN is a most interesting observation, as it experimentally assigns a function to a kinase which until now could only be predicted to be related to yeast SNF1 and metazoan AMPKs from a weak phylogenetic association; it is important, to note that PfKIN and PbKIN may not be strict functional homologues, as mentioned above. Phylogenetic analyses point to three parasite kinases (including PfKIN) as distant relatives of SNF1/AMPKs [10]. AMPKs are typically composed of a catalytic α subunit and regulatory β and γ subunits. The latter are required to target the phosphorylating activity to the appropriate substrates and cellular locations. No gene encoding the regulatory polypeptides could be identified by the authors in a *Plasmodium* genome, but very intriguing experimental results described in the article provide evidence to link KIN to an SNF1-like function. The experiment consisted of complementing an SNF1-deficient yeast strain by expressing PbKIN. Although the wild-type sequence was unable to complement the lack of the yeast kinase, a constitutively active PbKIN mutant (carrying an acidic residue in lieu of an activating phosphothreonine) complemented the lack of SNF1 with almost 100% efficiency. This result would suggest that activated PbKIN is able to productively associate with yeast regulatory subunits and substrates, since it restores the ability to arrest growth in the absence of nutrients; in contrast, the parasite enzyme is presumably not able to be recognised by SNF1 activators, since the wild-type enzyme is unable to complement. To what signal KIN may be responding in parasites is still an open (and very interesting) question. The only nutrient directly compared between animals fed *ad libitum* and those on a diet was glucose, whose blood levels did not change much. Dietary supplementation with the sugar had a strong effect on body weight but a modest one on parasitemia or time of death, pointing to other serum components as regulators of KIN. Inflammation modulators, such as BHB [4] or cytokines, might conceivably be involved. Teasing out what is really happening may lead to delineate the evolutionary history of ancient nutrient-sensing pathways in these highly divergent eukaryotes, and it may perhaps even provide one of those still rare occurrences, a novel druggable and selective target for antimalarial intervention.

Cerebral malaria (CM) is the most severe form of malaria and causes high associated mortality. We propose a multistep process for CM pathology that is initiated by cytoadhesion of infected erythrocytes to the brain vasculature, followed by rupture and release of contents that complete the disruption of the blood–brain barrier.

According to the latest World Health Organization (WHO) estimates, in 2015 almost half of the world’s population was at risk of malaria, there were 212 million new cases, and more than 400 000 deaths occurred. Most of the

References


Forum

Rupture and Release: A Role for Soluble Erythrocyte Content in the Pathology of Cerebral Malaria

Julio Gallego-Delgado1 and Ana Rodriguez1,*

*Correspondence: christian.doerig@monash.edu (C. Doerig), http://dx.doi.org/10.1016/j.pt.2017.08.007

1Department of Microbiology, Infection & Immunity Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC3800, Australia

Cerebral malaria (CM) is the most severe form of malaria and causes high associated mortality. We propose a multistep process for CM pathology that is initiated by cytoadhesion of infected erythrocytes to the brain vasculature, followed by rupture and release of contents that complete the disruption of the blood–brain barrier.

According to the latest World Health Organization (WHO) estimates, in 2015 almost half of the world’s population was at risk of malaria, there were 212 million new cases, and more than 400 000 deaths occurred. Most of the