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Host-pathogen interactions in malaria: cross-kingdom signaling and mitochondrial regulation

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Plasmodium parasites, unicellular alveolates in the Phylum Apicomplexa, are the causative agents of malaria. Their development is a complex interplay among multiple, distinct parasite life stages and host cells in humans and mosquitoes, organisms that are separated by more than 200 million years of evolution. Despite this vast biological divide, malaria parasites have adapted to a life that is dictated by networks of host signaling pathways and mitochondrial physiology that are remarkably conserved in humans and mosquitoes. Among the most important and most well-studied of the malaria parasites affecting humans is *Plasmodium falciparum*, which causes significant pathology in humans and more modest, although biologically important, pathology in the mosquito host. Rather than a coincidence of convergent host responses, we would suggest that these fundamental malaria parasite-host interactions reflect Apicomplexan radiation and adaptation to parasitism of invertebrate hosts, which preceded the appearance of bloodfeeding and parasitism of vertebrate hosts [1]. In these divergent hosts, the parasite has adapted to patterns of insulin/insulin-like growth factor signaling (IIS), regulation by conserved host protein kinases, and changes in host mitochondrial function that can alter parasite development. Accordingly, we suggest that parasite survival in the invertebrate host depended on the adaptation of parasites to pathways that were similar enough in vertebrate hosts to facilitate survival in these additional hosts over the course of evolutionary time. Further, we argue that a closer examination of malaria parasites within their primeval insect hosts can reveal the most fundamental aspects of host-pathogen interactions in malaria and, hence, provide the key to the development of novel therapeutics that can both cure human disease and block transmission to the mosquito host. To this end, we discuss host-malaria parasite interactions in the context of networked processes of IIS, activation of mitogen-activated protein kinases (MAPKs) and protein kinase C (PKC) isoforms, and bioenergetics.

Insulin/insulin-like growth factor signaling (IIS) in malaria

The highly conserved IIS pathway is comprised of MAPK- and a phosphatidylinositol 3-kinase/Akt-dependent branches that play critical roles in the regulation of growth, longevity, and immunity in vertebrates and invertebrates [2 and 3]. Indeed, the majority of IIS proteins and their interactions are conserved between humans and mosquitoes [4–11, 12•, 13, 14•• and 15••]. In humans, IIS can be induced by members of the insulin superfamily of peptide hormones, which include insulin and insulin-like growth factors (IGF) I and II, and seven relaxin family insulin-like peptides (ILPs) [16]. ILPs have also been identified in *Anopheles gambiae* 17] and in *Anopheles stephensi* 7], key mosquito vectors of malaria in sub-Saharan African and in India and parts of Asia, respectively.

IGF-1 is abundant in human blood (0.11–0.13 μM) and its bioavailability is tightly regulated by IGF binding proteins [18] due to its pleiotropic effects on apoptosis, autophagy, and stem cell renewal [19 and 20]. During malaria parasite infection, serum IGF-1 levels decrease significantly in humans and correlate with disease severity and anemia [21]. Ingestion of low serum concentrations of IGF-1 by *A. stephensi* extends lifespan by inhibiting apoptosis and decreasing damage to the midgut, while simultaneously enhancing mitochondrial function [15••]. This is similar to observations made in mice, where repression of IGF-1 signaling induces resistance to apoptosis by oxidative stress and extends lifespan [19]. Low levels of human IGF-1 in ingested serum also repress the phosphorylation of the MAPK extracellular signal-regulated kinase (ERK) in the mosquito midgut, thereby enhancing midgut synthesis of reactive nitrogen and oxygen species (RNOS) and resistance of *A. stephensi* to *P. falciparum* 15••]. In contrast, physiological concentrations of IGF-1 lead to sustained RNOS production and enhanced resistance of *A. stephensi* to *P. falciparum*, but also cause damage leading to midgut epithelial dysplasia [15••].

In contrast to IGF-1, insulin levels in healthy humans are significantly lower (17–590 pM), but can rise by as much as 10–35-fold during malaria parasite infection [22 and 23]. This may be due, in part, to the presence of insulin-mimetic *P. falciparum*-derived glycosylphosphatidylinositols (*Pf*GPIs). *Pf*GPIs tether parasite cell surface proteins, but are produced in vast excess of this need [24], presumably to act as signaling mediators to manipulate host biology. *Pf*GPIs induce hypoglycemia [25] and can reverse much of the pathology associated with type 2 diabetes [26, 27 and 28]. However, *Pf*GPIs synergize with insulin signaling [29], which can also inhibit nuclear factor (NF)-κB-dependent innate immune responses [30, 31 and 32]. The inhibition of innate immunity is responsible, in part, for the increased susceptibility of diabetics to opportunistic infections and malaria [33, 34• and 35]. As in humans, activation of IIS in *A. stephensi* by insulin results in the inhibition of NF-κB-dependent immunity and increased susceptibility to malaria parasite infection [4, 6 and 12•]. Human insulin and parasite-derived products also induce endogenous *A. stephensi* ILP production [7], which can further dampen NF-κB-mediated immunity [36]. In sum, these studies highlight the conserved nature of IIS between humans and mosquitoes and suggest that *Plasmodium* parasites may have evolved to manipulate, and benefit from, this conservation.

Protein kinase-dependent regulation of host-parasite interactions

In addition to IIS activation by *Pf*GPIs, these parasite molecules along with parasite hemozoin act as pathogen-associated molecular patterns (PAMPs) to activate MAPK signaling in both mammalian and mosquito hosts. While activation of IIS by *Pf*GPIs may benefit the parasite through subversion of host cell signaling [25, 26, 27, 28, 37, 38, 39, 40 and 41], activation of Toll-like receptor signaling in mammalian immune cells by *Pf*GPIs also precipitates a protective host response [37 and 38]. In particular, triacylated *Pf*GPIs are recognized by Toll-like receptor 1 (TLR1) and TLR2, while diacylated *Pf*GPIs are recognized by TLR2/TLR6 heterodimers [38]. TLR ligation recruits adapter proteins including myeloid differentiation factor 88 (MyD88), TIR-domain-containing adaptor protein-inducing IFN-β (TRIF), and TRIF-related adaptor molecule (TRAM [42]), which collectively activate NF-κB-dependent activation via ERK, *c*-Jun *N*-terminal kinase (JNK), and p38 MAPK [43]. In this context, *Pf*GPIs-mediated TLR-dependent signaling induces proinflammatory cytokine production by macrophages [44] and dendritic cells [45].

In an analogous fashion, *Pf*GPIs function as an early signal of parasite infection in *A. gambiae* and in *A. stephensi*. In *A. stephensi*, *Pf*GPIs induce ERK phosphorylation in the midgut within minutes of ingestion [8]. From studies with *A. gambiae*, this signaling may be Toll-initiated to activate NF-κB-dependent anti-parasite responses, including synthesis RNOS and antimicrobial peptides [8 and 46]. Hence, in both mammals and mosquitoes innate immunity to parasite infection appears to depend on PAMP-mediated ERK activation of NF-κB-dependent signaling. Hemozoin is a by-product of parasite degradation of hemoglobin that accumulates in the parasite digestive vacuole and induces activation of p38 MAPK-, ERK-, and NF-κB-dependent signaling, but not JNK signaling in murine macrophages and monocytes [47, 48, 49, 50 and 51]. In human monocytes, hemozoin activates p38 MAPK- and NF-κB-dependent signaling [52 and 53]. In contrast to ERK signaling, which is more typically associated with cell survival, both JNK and p38 MAPK signaling induce stress responses that can contribute to host pathology. Consequences of increased p38 MAPK activation in response to *P. falciparum* include endothelial dysfunction, heightened TLR2 responsiveness, elevated plasma lysozyme levels, and overproduction of inflammatory cytokines [52, 53, 54• and 55]. In *A. stephensi*, *P. falciparum* infection rapidly activates p38 MAPK signaling in the mosquito midgut, which precipitates decreased transcription of a variety of NF-κB-dependent innate immune genes [56]. Conversely, delivery of small molecule inhibitors (SMIs) of p38 MAPK via the bloodmeal significantly enhances immune gene expression and reduces *P. falciparum* development in *A. stephensi* 56]. While p38 MAPK-dependent signaling increases parasite burden in the mosquito host, resulting pathology from this burden appears to be offset by p38 MAPK-enhanced host survival during infection [56], a situation that may attest to the relatively longer evolutionary relationship of malaria parasites with their invertebrate hosts. Collectively, these observations suggest that therapeutic use of p38 MAPK inhibitors could reduce disease pathology in human hosts and reduce parasite development and transmission by mosquitoes that feed on treated patients[1].

In *A. stephensi* cells, hemozoin activates not only ERK but also atypical PKCζ, which likely regulates the synthesis of RNOS in the mosquito midgut [9]. The genomes of *A. stephensi*

and *A. gambiae* encode six PKC genes – PKCδ, PKCε, PKCζ, PKD, PKN, and an indeterminate conventional PKC [57]. Pan-inhibition of PKCs in *A. stephensi* via provision of SMIs in the bloodmeal had no effect on expression of immune genes, but significantly increased midgut barrier integrity and decreased development of *P. falciparum* 57]. These data suggest that PKC-dependent signaling during infection negatively regulates epithelial barrier function in the mosquito to promote parasite development. Intriguingly, PKC signaling also regulates barrier function in human malaria. In particular, PKCθ- and JNK-dependent signaling are required for the development of microvascular and neuronal pathology, respectively, through disruption of the blood-brain barrier in an experimental murine model of cerebral malaria [58 and 59]. This pathology can be reduced, increasing mouse survivorship, through treatment of parasite-infected mice with SMIs that block p38 MAPK, PKC or JNK signaling [60• and 61]. Together with our data from the mosquito host [56], these observations suggest that protein kinase SMIs could be leveraged for drug treatment to reduce disease pathology in humans and to block parasite transmission in mosquitoes that feed on treated patients.

Mitochondrial physiology during malaria parasite growth and development

Mitochondria reside at the center of cell signaling, immunity and basic intermediary metabolism and control stress responses [62••] as well as the degree of the proinflammatory immune responses fueled by the balance between glycolysis and mitochondria-derived ATP (oxidative phosphorylation or OXPHOS) [63, 64, 65•• and 66]. Most studies of PAMP signaling during infection have focused on the phosphorylation of mitochondria-associated apoptotic proteins [67, 68, 69 and 70]. However, mitochondria are involved in the host response to infection or tissue damage not only via apoptosis, but also through bioenergetics [63, 64, 65••, 71, 72•• and 73] and these latter effects have been ascribed to the translocation and/or activation of critical protein kinases [74, 75, 76 and 77]. For instance, activation and translocation of PKCε to mitochondria (in the presence of redox active cofactors) inhibits the pyruvate dehydrogenase complex (PDHC) and decreases OXPHOS [78]. In addition to PKCε, the MAPKs ERK, JNK, and p38 MAPK can modulate mitochondria function in response to diverse stimuli [79] including infection [80] in a variety of biological models [69, 81, 82 and 83]. Collectively, these data suggest that conserved host protein kinases can regulate parasite development and disease severity in malaria by altering mitochondria-dependent host immunity.

Analogous networking between immunity and mitochondrial biology is evident in the mosquito host from our studies. Following infection with *P. falciparum*, *A. stephensi* midgut PKCε and PKCδ exhibited reciprocal expression [57] a pattern similar to that reported for the reciprocal mitochondrial regulation of PHDC by PKCε and PKCδ [78]. Hence, an infection-driven mosquito “signalosome” of PKCε, PKCδ, JNK and p38 MAPK may

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transduce information between mitochondria and other cellular compartments to modulate not only mitochondrial homeostasis but also host immunity. In *A. stephensi*, inhibition of p38 MAPK signaling with SMIs significantly enhanced RNOS and an array of anti-parasite immune genes and reduced protein synthesis machinery and OXPHOS [56]. Hence, *P. falciparum*-induced activation of p38 MAPK signaling in the mosquito midgut appears to facilitate parasite infection through reduced anti-parasite immune defenses and enhanced host protein synthesis and bioenergetics to improve both host and parasite survival, and ultimately, transmission. In contrast, sustained midgut activation of IIS-associated Akt in transgenic *A. stephensi* resulted in decreased OXPHOS with decreased mitophagy and accumulation of dysfunctional mitochondria – analogous to over-activation of Akt in mammals [84 and 85] – with increased resistance to *P. falciparum* infection and reduced lifespan [14••]. Given that sustained activation of Akt inhibits autophagy and mitochondrial biogenesis [84 and 85], we predicted that overexpression of phosphatase and tensin homolog (PTEN), which opposes Akt signaling, would upregulate mitochondrial biogenesis to improve both resistance and fitness. Indeed, midgut overexpression of PTEN in transgenic *A. stephensi* resulted in enhanced resistance to *P. falciparum* infection with increased midgut barrier integrity and lifespan relative to non-transgenic controls [11]. Similarly, inhibition of PKC-dependent signaling in *A. stephensi* increased midgut barrier integrity and decreased *P. falciparum* infection in the absence of any change in NF-κB-dependent anti-parasite defense genes [57], consistent with a role of NF-κB in energy homeostasis [86•]. Notably, PKCs regulate mitochondrial biogenesis via IIS, suggesting that PKC inhibition through IIS leads to increased mitochondrial biogenesis and/or function to enhance the midgut barrier for resistance to *P. falciparum* infection in *A. stephensi*.

Conclusions

Collectively, these observations suggest that the relationship between mitochondria and the immune response to *Plasmodium* infection is conserved in human and mosquito hosts (Figure 1). Hence, targeting conserved protein kinase signaling pathways that regulate the balance between immunity and mitochondrial genes [63, 64, 65•• and 73] may influence host-pathogen interactions with potential to (i) minimize disease severity and/or parasitemia, (ii) decrease gametocytogenesis, and (iii) block malaria parasite transmission to mosquitoes. Furthermore, this same balance can impact genotype by environment interactions. In particular, insecticide resistance in a wide variety of insects, including mosquitoes, has been associated with higher expression of mitochondrial gene products related to mitochondrial respiratory chain and ATP production [87, 88 and 89], mitochondrial NADPH-dependent xenobiotic catabolism [90, 91 and 92], and glutathione *S*-transferases (GSTs) [93]. GST isoforms can function as activators or inhibitors of JNK and ERK/p38 MAPK/IKK pathways in *D. melanogaster* 94], suggesting that protein kinase targeting could be leveraged to generate therapeutics for treatment of malaria in the human host that can directly modulate insecticide resistance, immune response, and bioenergetics in mosquitoes that feed on treated patients.

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Highlights

- Mosquitoes and humans share many responses to malaria parasite infection.
- Conserved signaling regulates barrier and mitochondrial function during infection.
- Parasite success in both insect and human hosts likely depends on this conservation.
- This biology can be translated to novel drugs with transmission blocking activity.

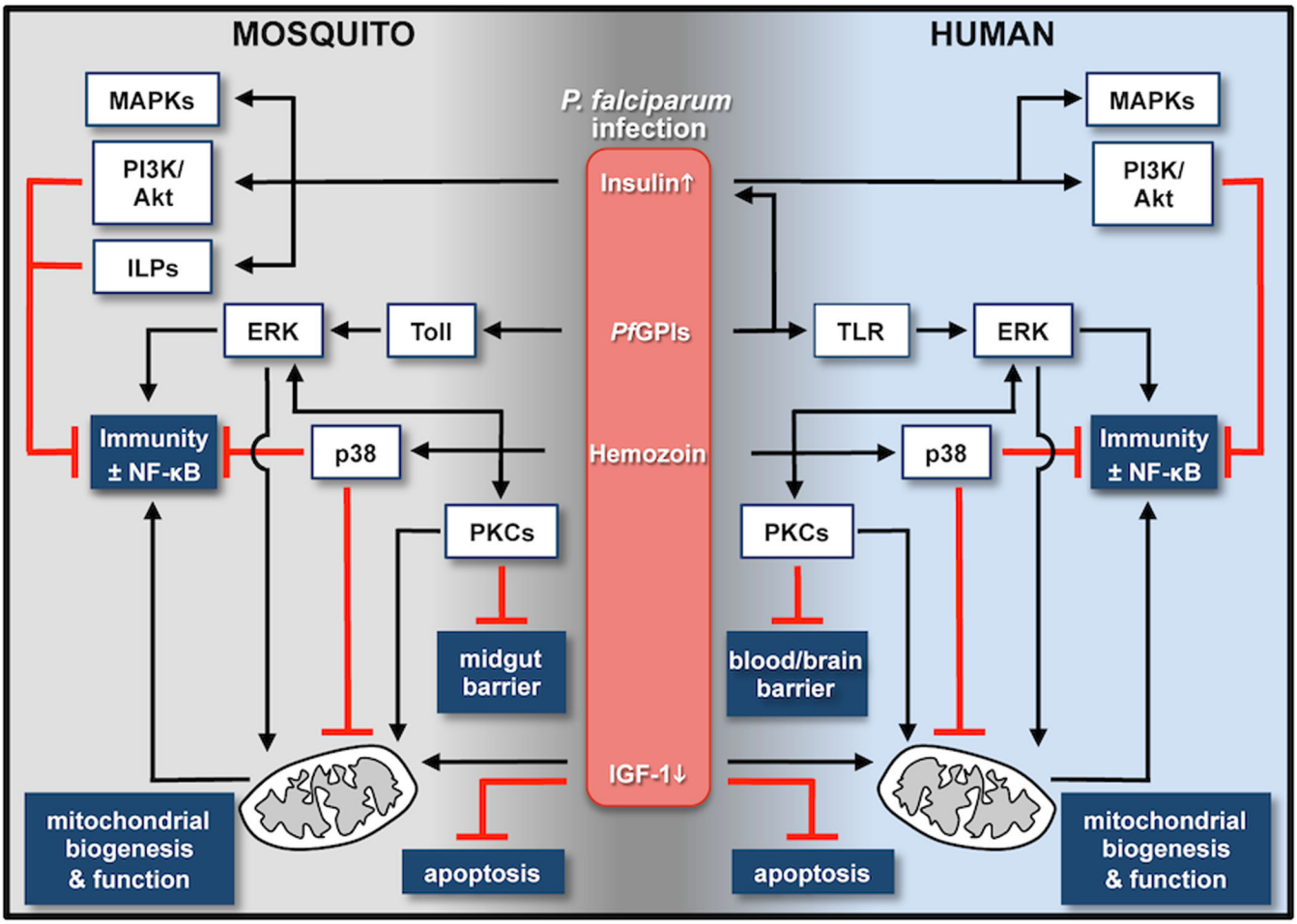

Figure 1.

Host-pathogen interactions in malaria. During infection with *P. falciparum*, both human and mosquito hosts exhibit responses that reflect physiological changes to infection (insulin/IGF-1) and to parasite PAMPs (PfGPIs, hemozoin). In particular, remarkably conserved protein kinase signaling pathways are networked to regulate epithelial and endothelial barrier function, which can dictate infection success and pathology, as well as mitochondrial biogenesis and function to control immunity through NF-κB-dependent and -independent responses.