Financial sustainability is critical. Unfortunately, 'where malaria prospers most, human societies have prospered least' [18]. Although low-income countries are largely dependent on external financial support for vaccine implementation, they have increased their investment in immunization policy. However, this increase is insufficient to financially sustain malaria vaccination. In countries that receive support from Gavi, the Vaccine Alliance (a public–private global health partnership committed to increasing access to immunization in poor countries), the average price paid for vaccines by the government per child in 2015 ranged from $3.80 to $5.09 USD. A recent survey in a malaria-endemic area in Peru showed that 61% of the population interviewed would pay for their own malaria vaccine – but only if the price ranged from $0.36 to $2.00 [12]. Ultimately, since TBVs will most likely be used in extremely low-resource areas, where malaria is holoendemic or where there is intense seasonal transmission, it will almost certainly require full Gavi support.

## Acknowledgments

This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. We thank J. Patrick Gorres for assistance editing the manuscript.

## Disclaimer Statement

R.R. is a full-time employee of the GlaxoSmithKline group of companies.

[1]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD, USA
[2]GlaxoSmithKline, Via Fiorentina 1, 53100 Siena, Italy
[3]Texas Children's Hospital Center for Vaccine Development, Departments of Pediatrics and Molecular Virology and Microbiology, National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, USA

*Correspondence:
camila.coelho@nih.gov (C.H. Coelho) and
patrick.duffy@nih.gov (P.E. Duffy).

https://doi.org/10.1016/j.pt.2019.04.008

Published by Elsevier Ltd.

## References

1. Ojakaa, D.I. *et al.* (2011) Community perceptions of malaria and vaccines in the South Coast and Busia regions of Kenya. *Malar. J.* 10, 147
2. GBD 2017 Causes of Death Collaborators (2018) Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 392, 1736–1788
3. White, M.T. *et al.* (2015) Vaccine approaches to malaria control and elimination: Insights from mathematical models. *Vaccine* 33, 7544–7550
4. Talaat, K.R. *et al.* (2016) Safety and immunogenicity of Pfs25-EPA/Alhydrogel(R), a transmission blocking vaccine against *Plasmodium falciparum*: an open label study in malaria naive adults. *PLoS One* 11, e0163144
5. Sagara, I. *et al.* (2018) Safety and immunogenicity of Pfs25H-EPA/Alhydrogel, a transmission-blocking vaccine against *Plasmodium falciparum*: a randomised, double-blind, comparator-controlled, dose-escalation study in healthy Malian adults. *Lancet Infect. Dis.* 18, 969–982
6. WHO (2018) *World Malaria Report*, WHO
7. Ananian, S.A. (1945) Experimental data regarding malaria immunization with formol vaccine. *Mikrobiologiia* 14, 55–58
8. Richie, T.L. and Saul, A. (2002) Progress and challenges for malaria vaccines. *Nature* 415, 694–701
9. Coelho, C.H. *et al.* (2017) Advances in malaria vaccine development: report from the 2017 malaria vaccine symposium. *NPJ Vaccines* 2, 34
10. The RTS,S Clinical Trials Partnership (2014) Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. *PLoS Med.* 11, e1001685
11. Menaca, A. *et al.* (2014) Factors likely to affect community acceptance of a malaria vaccine in two districts of Ghana: a qualitative study. *PLoS One* 9, e109707
12. White, S.E. *et al.* (2018) Acceptability of a herd immunity-focused, transmission-blocking malaria vaccine in malaria-endemic communities in the Peruvian Amazon: an exploratory study. *Malar. J.* 17, 179
13. Chukwuocha, U.M. *et al.* (2018) Awareness, perceptions and intent to comply with the prospective malaria vaccine in parts of South Eastern Nigeria. *Malar. J.* 17, 187
14. Mtenga, S. *et al.* (2016) Stakeholders' opinions and questions regarding the anticipated malaria vaccine in Tanzania. *Malar. J.* 15, 189
15. Romore, I. *et al.* (2015) Assessment of parental perception of malaria vaccine in Tanzania. *Malar. J.* 14, 355
16. Standaert, B. and Rappuoli, R. (2017) 3. How comprehensive can we be in the economic assessment of vaccines? *J. Mark. Access. Health Policy* 5, 1336044
17. Brooks, A. *et al.* (2012) Implementing new health interventions in developing countries: why do we lose a decade or more? *BMC Pub. Health* 12, 683
18. Sachs, J. and Malaney, P. (2002) The economic and social burden of malaria. *Nature* 415, 680–685

## Spotlight

# Coalition Politics: Linking Malaria Transmission to Mosquito Reproduction

Sebastian Kirchner[1,*] and Andrew P. Waters[1,*]

**Female anopheline mosquito reproduction is intimately linked to the *Plasmodium* sporogonic cycle, whereby malaria parasites ostensibly compete for the same resources required for mosquito egg development. However, in a recent study, Werling and colleagues (*Cell* 2019;177:315–325) uncovered a parasitic strategy supporting coexistence, exploiting mosquito nutrients without affecting mosquito fitness and reproductivity.**

Malaria is a devastating parasitic disease, with 219 million cases and 435 000 deaths worldwide in 2017 [1]. It is caused by apicomplexan parasites of the genus *Plasmodium*, which, during their complex life cycle, transition between a mammalian host and a mosquito vector. During the blood feed of a female *Anopheles* mosquito on a *Plasmodium*-infected individual, gametocytes are taken up with the blood meal into the midgut. Gametocytes generate gametes that, once fertilized, quickly develop into motile ookinetes which cross the mosquito midgut epithelium and settle on its basal side (within 16–30 h after blood feeding). Ookinetes then transform into sessile oocysts, producing thousands of individual sporozoites within 10–14 days. Following oocyst rupture, sporozoites migrate to the salivary glands where they reside, ready to initiate a new infection when the mosquito feeds again.

For the female mosquito the sole purpose of the blood feed is to acquire essential nutrients required for production of its eggs (Figure 1A). Following a blood meal, mosquito egg development (vitellogenesis) within the ovaries is initiated through a cascade of hormones released from the mosquito brain [2]. This in turn triggers the synthesis of the steroid hormone ecdysone (E) in the ovaries. Hydroxylation of E into its active form, 20-hydroxyecdysone (20E), in the mosquito fat body, then promotes the synthesis of nutrient-transport

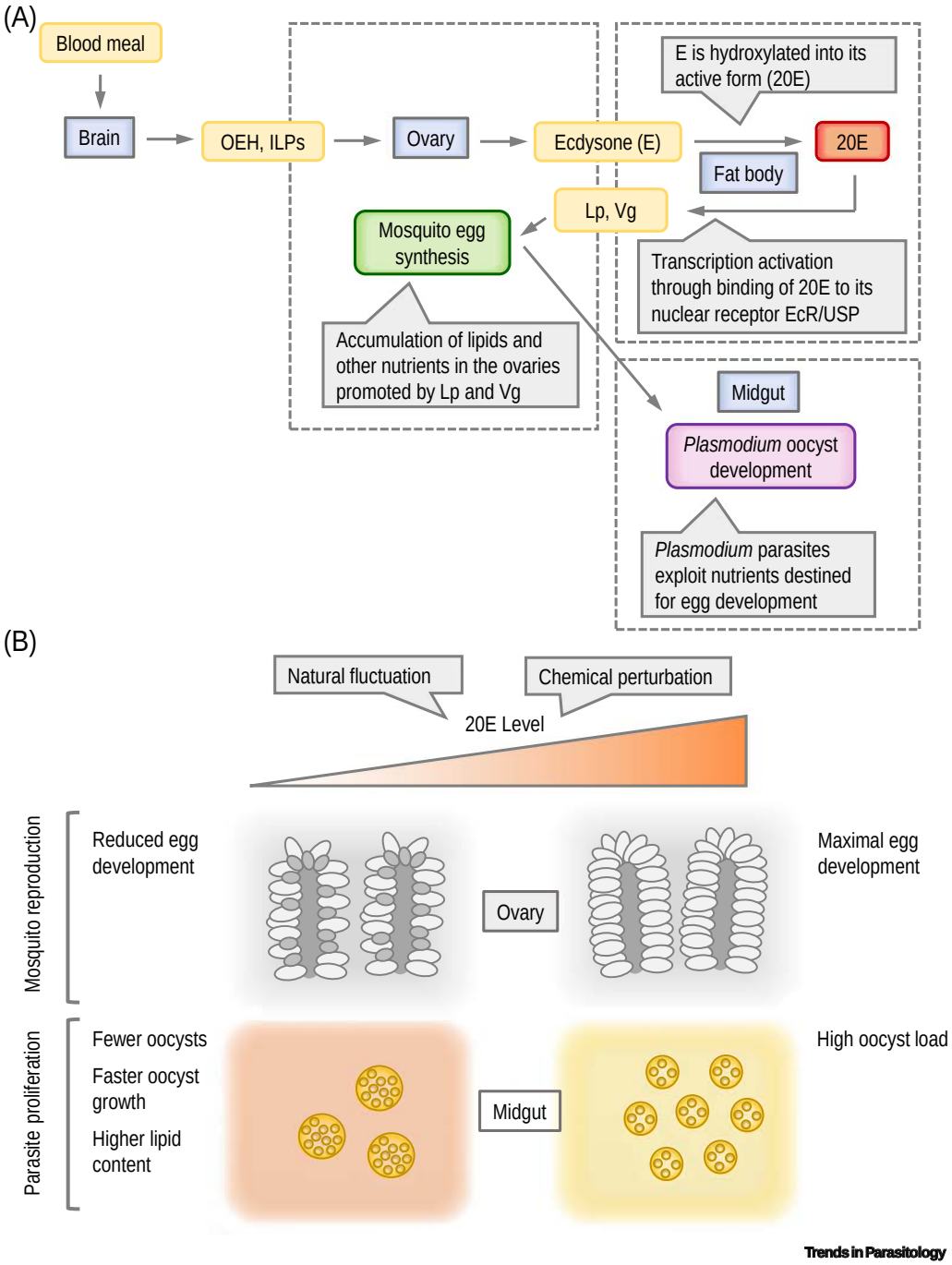


Figure 1. 20-Hydroxyecdysone (20E) Signalling Coordinates Mosquito Reproduction and *Plasmodium* Proliferation. (A) Following the blood meal of a female mosquito, a vast signalling cascade is activated, leading to the accumulation of 20E in the mosquito fat body. 20E triggers the synthesis of the nutrient transporters Lp and Vg, which accumulate in the ovaries and promote the uptake of lipids and other nutrients required for egg development. In *Plasmodium*-infected mosquitoes, developing parasites in the midgut utilize a mosquito lipid transporter (e.g., Lp) for proliferation and exploit nutrients destined for egg production. (B) Variation in 20E level (via natural fluctuation or experimental manipulation) affects egg numbers and parasite growth. When 20E level is low, mosquitoes produce fewer eggs, and *Plasmodium* parasites generate fewer oocysts compared to higher 20E levels. Additionally, a low 20E level leads to an accumulation of lipids in the midgut, promoting faster oocyst growth. Abbreviations: 20E, 20-hydroxyecdysone; E, ecdysone; EcR, ecdysone receptor; ILP, insulin-like peptides; Lp, lipophorin; OEH, ovarian ecdysiotropic hormone; USP, ultraspiracle; Vg, vitellogenin.

proteins and nutrient uptake within the ovaries, enabling the maturation of 100–150 eggs per mosquito. *Plasmodium* oocysts also exploit these nutrients, linking mosquito reproduction with parasite proliferation (Figure 1A). Maybe not unexpectedly, previous studies have pointed mostly towards a negative impact of *Plasmodium* infection on mosquito fitness and survival [3,4]. However, the exact relationship between resource exploitation by the parasite and potential costs for mosquito fitness and fecundity remained elusive.

A recent study by Werling and colleagues [5] sheds unexpected light on the link between reproductive fitness of the mosquito vector and *Plasmodium* infection. Rather surprisingly, the authors uncovered a positive relationship between the number of eggs produced by a female *Anopheles gambiae* mosquito and the number of *P. falciparum* oocysts in the same individual. Furthermore, transgenic mosquitoes, engineered to produce a greatly reduced number of eggs, also had significantly less *P. falciparum* oocysts, confirming the positive relationship noted above. The authors demonstrated that this positive correlation was dependent on 20E signalling and therefore retained when the 20E signalling pathway was disrupted through silencing of the 20E nuclear receptor EcR (dsEcR), resulting in reduced egg production and fewer oocysts (Figure 1B). The effect on egg number was also independent of infection, with uninfected and infected dsECR mosquitoes producing similar numbers of eggs. However, the fewer parasites in dsEcR mosquitoes grew faster and, at day 10 after an infected blood meal (pIBM), had produced larger oocysts than those in the control group. This unexpected, faster parasite growth also resulted in increased transmission potential, with dsEcR mosquitoes displaying a higher likelihood of having salivary glands colonized by sporozoites at day 10 pIBM and reducing what is called the extrinisic incubation period (EIP) of the sporozoite. These EIP-enhanced sporozoites were competent, performing comparably to their control counterparts in an *in vitro* infection assay.

In a search for the potential source of the observed faster parasite growth, the authors investigated the lipid level in dsEcR midguts. Several lipid species, as well as the level of the lipid transporter lipophorin (Lp), were significantly increased upon 20E disruption. Tellingly, when Lp and the 20E signalling pathways were silenced simultaneously, faster parasite growth was abolished. Additionally, in wild-type mosquitoes, the 20E level negatively correlated with oocyst size, which was again lost upon Lp silencing. When lipids were experimentally induced to accumulate in midguts through TAG lipase silencing, the oocysts grew more quickly. Overall, these results point towards an unexpected effect of 20E signalling on parasite developmental speed, likely resulting from lipid availability mediated by Lp. This is also in line with previous findings, where Lp has been found inside developing *Plasmodium* oocysts [6]. It would also explain why parasites grow faster if mosquito egg production is impaired, linking the activity of the 20E pathway to parasite growth benefitting from the excess of nutrients not destined for egg development. Currently, the authors have not established why oocyst numbers are reduced when egg development is impaired but speculate that immune (TEP1 independent) or somatic processes could be involved in a trade-off over resource allocation balancing immunity with egg production.

Overall, the results of Werling and coworkers elegantly expand the findings of another recently published study [7] which postulated that the model of noncompetitive parasitic resource exploitation minimizes fitness costs for the mosquito while maximizing parasite proliferation. This link between 20E signalling, egg production, and oocyst growth would allow the parasite to adapt its development to the host's status, optimizing its own fitness without harming the vector. *Plasmodium* parasites can grow faster if the female mosquito produces fewer eggs or generates more oocysts in a mosquito with more eggs (Figure 1B). Considering the potential use of gene-drive systems aiming to reduce the reproductive capacity of mosquitoes or especially heighten their immune status as a vector control strategy, this could have unexpected consequences favouring faster sporozoite production and transmission [8]. The nutritional status of the mosquito in the field may also become important: Werling's mosquitoes were all optimally fed. The outcome of a life-time of suboptimal blood feeding might well be to promote parasite growth, frequency of blood feeding, and thereby, transmission. Another open question which will require future work is to establish the mechanism behind 20E-dependent lipid accumulation and parasite growth in the midgut. How can the parasite adjust its growth pattern in a 20E-dependent manner? It will be fascinating to see whether *Plasmodium* uses mechanisms similar to those recently discovered for the mammalian host to sense the nutritional status of the vector and to adjust its proliferation rate accordingly [9]. Furthermore, it would be important to expand the findings of this study to other mosquito–*Plasmodium* combinations as well as to investigate their impact in other vector-borne pathogens.

[1]Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK

*Correspondence:
Sebastian.kirchner@glasgow.ac.uk (S. Kirchner) and andy.waters@glasgow.ac.uk (A.P. Waters).

https://doi.org/10.1016/j.pt.2019.05.003



**References**

1. WHO (2018) *World Malaria Report*. WHO
2. Roy, S. *et al.* (2018) Regulatory pathways controlling female insect reproduction. *Annu. Rev. Entomol.* 63, 489–511
3. Sangare, I. *et al.* (2014) Stress dependent infection cost of the human malaria agent *Plasmodium falciparum* on its natural vector *Anopheles coluzzii*. *Infect. Genet. Evol.* 25, 57–65

4. Ferguson, H.M. and Read, A.F. (2002) Why is the effect of malaria parasites on mosquito survival still unresolved? *Trends Parasitol.* 18, 256–261
5. Werling, K. *et al.* (2019) Steroid hormone function controls non-competitive *Plasmodium* development in *Anopheles*. *Cell* 177, 315–325
6. Atella, G.C. *et al.* (2009) The major insect lipoprotein is a lipid source to mosquito stages of malaria parasite. *Acta Trop.* 109, 159–162
7. Costa, G. *et al.* (2018) Non-competitive resource exploitation within mosquito shapes within-host malaria infectivity and virulence. *Nat. Commun.* 29, 3474
8. Hammond, A. *et al.* (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat. Biotechnol.* 34, 78–83
9. Mancio-Silva, L. *et al.* (2017) Nutrient sensing modulates malaria parasite virulence. *Nature* 547, 213–216

## Spotlight

# Initiating the T Cell Response to Liver-Stage Malaria

Komi Gbedande[1] and Robin Stephens[1,2,*]

**Kurup *et al.* (*Cell Host Microbe* 2019;25:565–577.e6) define the liver-based antigen-presenting cell driving CD8 T cell responses to mosquito transmission of *Plasmodium* spp., and show direct interaction of CD11c$^+$ cells with infected hepatocytes. We discuss this work in context, highlighting gaps and new approaches suggested by the work to target liver-stage vaccine antigens.**

Malaria remains an important parasitic infection in the world, with 217 million cases and 435 000 deaths in 2018. During the blood meal, infected *Anopheles* mosquitos introduce *Plasmodium* spp. sporozoites into the host dermis, which quickly transit to the liver of the host. The liver stage of *Plasmodium* is a potential target to induce immunity by vaccination. The mechanisms of immunity to the very effective, radiation-attenuated sporozoite (RAS) vaccination have been well characterized [1]. Here, we briefly review the host response to the liver stage of *Plasmodium* spp., and discuss the recent findings of Kurup *et al.* [2] on the role of hepatic inflammatory dendritic cells (DCs) in priming CD8 T cells against the liver stage of *Plasmodium yoelii* infection in this context.

## Liver-Stage Immunity to *Plasmodium* spp.

Liver-stage infection with *Plasmodium* spp. induces both innate and adaptive immune responses. The live parasite is now known to trigger interferon (IFN)-inducible genes in infected hepatocytes, recruiting CD8α$^+$ DCs and T cells to the liver more strongly than RAS [3,4]. Liver-localized DCs have been shown to take up both RAS and sporozoites after infection, and drive generation of T cell responses [1,4,5]. In humans, immune responses to liver-stage parasites have been measured. Antibodies to liver-stage antigens, such as LSA-3, are generated from the earliest exposures. The specificity for the antigens LSA-1 and STARP was present in most children of 8–10 years of age during a malaria-free rainy season, but fewer showed this specificity in the group with clinical disease [6]. These antibody responses likely require CD4 T cell help. However, most T cell epitopes tested to date have elicited responses in only a small fraction of infected people, though more are detected when epitopes are human leukocyte antigen (HLA)-matched [7]. Interactions between liver macrophages, or Kupffer cells, and the *Plasmodium* sporozoites are necessary for hepatocyte invasion. While this interaction induces cytokines [8], mechanisms of antigen presentation are unclear in the case of liver infection.

## CD11c$^+$ Cells Find Infected Hepatocytes and Prime CD8 T Cell Immunity

Although pathways of antigen presentation in the liver, including RAS vaccination, leading to *Plasmodium* immunity are beginning to be well understood, induction of immunity to infectious sporozoites has not been well explored [1,5]. The recent study by Kurup *et al.* identified a subset of hepatic CD11c$^+$ cells that present antigens upon mosquito transmission of *P. yoelii* and *P. berghei* infection [2]. While the frequencies of Kupffer cells (CD45$^+$ CD11c$^-$ MHC-II$^+$ F4/80$^+$ CD11b$^{int}$ CX3CR1$^{lo\text{-}int}$), macrophages (CD45$^+$ CD11c$^-$ F4/80$^+$ CD11b$^{hi}$ CX3CR1$^{hi}$), and monocytes (CD45$^+$ MHC-II$^{int}$ CD11c$^-$ F4/80$^-$ Ly6C$^+$) remain unchanged in the livers of infected mice, the numbers of CD11c$^+$ cells increased, suggesting a possible role for CD11c$^+$ DCs in antigen presentation [2,4]. The authors showed that CD11c$^+$ cells from the liver primed a CD8 T cell response to liver-stage-restricted antigens using CD11c-reporter and T cell receptor (TCR) retrogenic mice, and fluorescent parasites. While DCs in the liver have been observed with blood-stage parasites or RAS inside, tissue-localized antigen-presenting cells had not been previously observed in the less inflammatory liver stage. It had been hypothesized that hepatocyte apoptosis led to priming of liver-stage responses. However, it is now clear that Mda5 can initiate the intracellular response to *Plasmodium* infection, suggesting that the infection is not silent [3]. The authors carefully demonstrated that T cell priming requires hepatocyte invasion, ruling out an exclusive role for dead parasites as antigen for priming in the local lymph node. In addition, live video microscopy shows CD11c$^+$ cells taking up merosomes. Intriguingly, the cytokine, chemokine, and integrin signals used by DCs to find infected hepatocytes may also be used by T cells that could then find and kill them. These signals, which are not yet understood, could reduce the need for an extremely large number of CD8 T cells to be retained after vaccination to allow identification and elimination of the parasite. Though generally thought to inhibit liver-stage immunity, the inflammation caused in the liver from the blood stage of infection