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Mosquitoes Reset Malaria Parasites

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Serial blood passage of Plasmodium universally increases parasite virulence, which can be reversed by mosquito transmission. How mosquitoes reset Plasmodium virulence has been unknown. We have shown that mosquito transmission modifies expression of Plasmodium subtelomeric multigene families, including those that code for variant surface antigens (VSA), and transforms the systemic immune response to blood-stage infection. In this way, the mosquito regulates malaria disease severity. Here, we present a model in which expression of multigene families is reset by epigenetic reprogramming of Plasmodium within the mosquito. This prepares the malaria parasite for entry into a new unknown host and transforms the early parasite–host interactions that shape disease severity. Studying the molecular mechanisms that operate outside the human host to regulate Plasmodium virulence is therefore a priority.

Historical Perspective

It has been recognised for decades that serial blood passage of Plasmodium through rodents, primates, or humans universally increases parasite virulence. In 1917, induced malaria was first used as pyretic therapy for neurosyphilis, with Plasmodium vivax routinely inoculated to elicit a mild form of disease. Yet passage through the human host elevated parasitaemia and exacerbated disease, increasing the requirement for chemotherapeutic intervention [1]. Blood passage of Plasmodium knowlesi or Plasmodium cynomolgi, whether through human volunteers or nonhuman primates, similarly elevated parasite densities and disease severity [2–4]. And serial blood passage of every rodent malaria parasite species increased parasitaemia and pathogenicity [5–8]. On the other hand, it has been assumed for decades that mosquito transmission resets Plasmodium virulence [8]. At the Horton Mental Hospital, a pioneering centre for malaria therapy, Plasmodium strains were maintained by mosquito transmission to preserve their clinical and parasitological features [9]. Nevertheless, direct evidence that mosquito transmission resets Plasmodium virulence, and a mechanism to explain this phenomenon, have been missing [6,10,11].

Mosquito Transmission Resets Plasmodium Virulence

We have recently shown that mosquito transmission modifies gene expression in blood-stage malaria parasites and in this way resets Plasmodium virulence [12]. Whereas serial blood passage of Plasmodium chabaudi leads to hyperparasitaemia and severe disease in laboratory mice, mosquito transmission of serially blood-passaged parasites leads to a low-grade, chronic, recrudescent infection with minimal pathology. Attenuation of virulence is not parasite clone- or dose-dependent and therefore cannot be explained by bottlenecks during mosquito transmission [13]. Instead, attenuation of the blood-stage parasite is dependent upon host genotype
and an intact host immune response and associates with increased expression of Plasmodium subtelomeric multigene families, including those that code for VSA (Box 1). Mosquito transmission therefore modifies expression of parasite virulence genes and transforms host immunity in the pathogenic blood-stage of infection. As such, the mosquito vector both transmits malaria and regulates disease severity.

Epigenetic Reprogramming of Plasmodium

By recognising this key function of the mosquito, new research avenues open that can accelerate our understanding of the pathogenesis of human malaria. It is first important to delineate where, when, and how mosquito transmission modifies expression of Plasmodium virulence genes. This is likely to be a consequence, at least in part, of necessary changes in gene expression for progression through each step of the life cycle in both vector and host. However, epigenetic reprogramming of Plasmodium provides a mechanism by which expression of virulence genes could be reset within the vector. Heritable chromatin modifications control transcription of subtelomeric multigene families in the blood-stage parasite and can thus promote adaptation of malaria parasites to their host. Nevertheless, global erasure of epigenetic marks following gamete fusion in the mosquito could reset expression of multigene families and thus prepare Plasmodium for entry into a new unknown host (Fig 1).

Resetting Plasmodium gene expression could be particularly important when transmission is seasonal, and parasites undergo an extended period of host adaptation in a chronically infected individual before their return to the mosquito. In this context, it is important to know whether parasite virulence increases in the chronic phase of infection, as has been observed in human volunteers. Thus, serial blood passage per se may not increase Plasmodium virulence; an alternative explanation is that virulence increases with time elapsed from the mosquito. This will be observed only in a new host and when mosquito transmission is bypassed.

Immune Control of Plasmodium Virulence

By resetting Plasmodium gene expression, the mosquito can also control how blood-stage parasites elicit the systemic immune response in a new host. Mosquito transmission attenuates P. chabaudi virulence because merozoites that emerge from the liver induce an immune response that can rapidly control parasite growth without collateral damage. This contrasts with the host response to serially blood-passaged parasites that causes severe immunopathology. Does

Box 1. Mosquito Transmission Modifies Expression of Plasmodium Virulence Genes in Human Malaria

Transcriptional profiling and proteomic analysis of cultured Plasmodium falciparum demonstrates that the diversity and magnitude of rifin and var gene expression is increased in sporozoites (isolated from mosquito salivary glands) as compared to merozoites, trophozoites, or gametocytes. Furthermore, 53 of 59 var genes were transcribed in a single human volunteer infected with P. falciparum by mosquito bite just five days after merozoite egress from the liver, and diversity of var gene expression has been shown to decrease in human volunteers after blood passage. Collectively, these data support a model in which expression of Plasmodium subtelomeric multigene families is increased as parasites transit through the mosquito and subsequently decreases with time elapsed from the vector.
increasing expression of *Plasmodium* VSA explain how the mosquito can transform the elicited host immune response? Or does mosquito transmission change the context in which blood-stage parasites initiate host immunity (e.g., by modifying invasion, cytoadherence, or sequestration)? Furthermore, it remains possible that immune priming and/or regulation during the pre-erythrocytic stages of infection can subsequently modify the systemic immune response to the blood-stage parasite. In all scenarios, the early immune response, elicited in the context of a mosquito bite, can shape malaria disease severity. In turn, the developing immune response is likely to influence expression of *Plasmodium* virulence genes and could therefore also directly regulate parasite pathogenicity.

**Improving Models of Malaria**

Mosquitoes reset malaria parasites and can be used to strengthen the relevance of mouse models to human malaria. We should therefore aim to initiate experimental infections by the natural route of transmission wherever possible. We should also strive to study combinations of vector, parasite, and host that exist in nature to validate or improve our current experimental systems. Mouse models are important for interrogating the pathogenesis of malaria because they can answer research questions that cannot be addressed directly in humans. Moreover, relevant mouse models can act as a bridge between human studies. For example, vector regulation of *Plasmodium* virulence was first observed in human volunteers and subsequently reproduced and delineated in mice; the molecular mechanisms that operate within the mosquito to regulate *Plasmodium* virulence can now be dissected with human malaria parasites.

To this end, inoculation of human volunteers with *Plasmodium* is a powerful experimental model [18,19]. In this setting, it is possible to look for evidence of epigenetic reprogramming of *P. vivax* in laboratory-reared anopheline mosquitoes fed on infected volunteers. Interrogating
expression and regulation of subtelomeric multigene families in gametocytes as they circulate, transmit, and then pass through each developmental checkpoint of sporogony is a priority. So, too, is examining how route of transmission influences the systemic host response to blood-stage infection. For this, the immune response to *P. falciparum* can be compared in peripheral blood obtained from human volunteers infected via mosquito bite versus direct inoculation of blood-stage parasites (isolated just 6–8 days after liver egress [20]). Nevertheless, mice are absolutely required to observe the interactions between parasites and the immune system that shape disease severity because these interactions occur in tissues, such as spleen. We should therefore aim to identify mouse models that share a common immune signature of infection in whole blood with human malaria and use these models to delineate the immune response to *Plasmodium* in relevant tissues.

**Concluding Remarks**

A mosquito is not simply a flying syringe. Mosquitoes reset malaria parasites in preparation for entry into a new unknown host and thereby regulate *Plasmodium* virulence. Furthermore, they are a mixing pot for the generation of new recombinant parasites and can thus transmit previously unseen virulent strains. By studying events within the mosquito, we will accelerate our understanding of malaria disease severity.

**References**

1. James SP, Nicol WD, Shute PG. Clinical and Parasitological Observations on Induced Malaria: (Section of Tropical Diseases and Parasitology). Proc R Soc Med. 1936; 29(8): 879–94. PMID:19990731
2. Chin W, Contacos PG, Collins WE, Jeter MH, Alpert E. Experimental mosquito-transmission of *Plasmodium knowlesi* to man and monkey. Am J Trop Med Hyg. 1968; 17(3): 355–6. PMID:4385130
3. Coatney GR, Elder HA, Contacos PG, Getz ME, Greenland R, Rossan RN, et al. Transmission of the M strain of *Plasmodium cynomolgi* to man. Am J Trop Med Hyg. 1961; 10: 673–8. PMID:13694174
4. Hartley EG. Increased virulence of *Plasmodium cynomolgi* bastianellii in the rhesus monkey. Trans R Soc Trop Med Hyg. 1969; 63(3): 411–2.
5. Dearsly AL, Sinden RE, Self IA. Sexual development in malarial parasites: gametocyte production, fertility and infectivity to the mosquito vector. Parasitology. 1990; 100 Pt 3: 359–68. PMID:2194152
6. Knowles G, Walliker D. Variable expression of virulence in the rodent malaria parasite *Plasmodium yoelii yoelii*. Parasitology. 1980; 81(1): 211–9. PMID:7422362
7. Mackinnon MJ, Read AF. Selection for high and low virulence in the malaria parasite *Plasmodium cha- baudi*. Proc Biol Sci. 1999; 266(1420): 741–8. PMID:10331293
8. Yoeli M, Hargreaves B, Carter R, Walliker D. Sudden increase in virulence in a strain of *Plasmodium berghei yoelii*. Ann Trop Med Parasitol. 1975; 69(2): 173–8. PMID:1098585
9. Covell G, Nicol WD. Clinical, chemotherapeutic and immunological studies on induced malaria. Br Med Bull. 1951; 8(1): 51–5. PMID:14944815
10. Alger NE, Branton M, Harant J, Silverman PH. *Plasmodium berghei* NK65 in the inbred A-J mouse: variations in virulence of *P. berghei* demes. J Protozool. 1971; 18(4): 598–601. PMID:5133123
11. Mackinnon MJ, Bell A, Read AF. The effects of mosquito transmission and population bottlenecks on virulence, multiplication rate and rosetting in rodent malaria. Int J Parasitol. 2005; 35(2): 145–53. PMID:15710435
12. Spence PJ, Jarra W, Lévy P, Reid AJ, Chappell L, Brugal T, et al. Vector transmission regulates immune control of *Plasmodium vivax*. Nature. 2015; 521: 486–93. doi:10.1038/nature14403 PMID:25271963
13. Cortes A, Crowley VM, Vaquero A, Voss TS. A view on the role of epigenetics in the biology of malaria parasites. PLoS Pathog. 2012; 8(12): e1002943. doi:10.1371/journal.ppat.1002943 PMID:23271963
16. Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. Nat Struct Mol Biol. 2013; 20(3): 282–9. doi: 10.1038/nsmb.2489 PMID: 23463313
17. Rovira-Graells N, Gupta AP, Planet E, Crowley VM, Mok S, Ribas de Pouplana L, et al. Transcriptional variation in the malaria parasite *Plasmodium falciparum*. Genome Res. 2012; 22(5): 925–38. doi: 10.1101/gr.129692.111 PMID: 22415456
18. McCarthy JS, Griffin PM, Sekuloski S, Bright AT, Rockett R, Looke D, et al. Experimentally induced blood-stage *Plasmodium vivax* infection in healthy volunteers. J Infect Dis. 2013; 208(10): 1688–94. doi: 10.1093/infdis/jit394 PMID: 23908484
19. Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can accelerate clinical malaria vaccine development. Nat Rev Immunol. 2011; 11(1): 57–64. doi: 10.1038/nri2902 PMID: 21179119
20. Cheng Q, Lawrence G, Reed C, Stowers A, Ranford-Cartwright L, Creasey A, et al. Measurement of *Plasmodium falciparum* growth rates in vivo: a test of malaria vaccines. Am J Trop Med Hyg. 1997; 57(4): 495–500. PMID: 9347970
21. Le Roch KG, Zhou Y, Blair PL, Grainger M, Moch JK, Haynes JD, et al. Discovery of gene function by expression profiling of the malaria parasite life cycle. Science. 2003; 301(5639): 1503–8. PMID: 12893887
22. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the *Plasmodium falciparum* life cycle. Nature. 2002; 419(6906): 520–6. PMID: 12368866
23. Wang CW, Hermsen CC, Sauerwein RW, Arnot DE, Theander TG, Lavstsen T. The *Plasmodium falciparum* var gene transcription strategy at the onset of blood stage infection in a human volunteer. Parasitol Int. 2009; 58(4): 478–80. doi: 10.1016/j.parint.2009.07.004 PMID: 19616120
24. Peters J, Fowler E, Gatton M, Chen N, Saul A, Cheng Q. High diversity and rapid changeover of expressed var genes during the acute phase of *Plasmodium falciparum* infections in human volunteers. Proc Natl Acad Sci U S A. 2002; 99(16): 10689–94. PMID: 12142467