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Research paper

Malaria infections: What and how can mice teach us

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ABSTRACT

Malaria imposes a horrific public health burden – hundreds of millions of infections and millions of deaths – on large parts of the world. While this unacceptable health burden and its economic and social impact have made it a focal point of the international development agenda, it became consensual that malaria control or elimination will be difficult to attain prior to gain a better understanding of the complex interactions occurring between its main players: *Plasmodium*, the causative agent of disease, and its hosts. Practical and ethical limitations exist regarding the ability to carry out research with human subjects or with human samples. In this review, we highlight how rodent models of infection have contributed significantly during the past decades to a better understanding of the basic biology of the parasite, host response and pathogenesis.

1. Introduction

Malaria still imposes a significant health and economic burden in large parts of the world, particularly in sub-Saharan Africa and Southeast Asia, where at least 200 million infections and over 600000 deaths are registered annually (WHO, 2013).

The disease is caused by protozoan parasites of the genus *Plasmodium*, which are transmitted by female Anopheline mosquitoes. During a blood meal the infected female mosquitoes deposit *Plasmodium* sporozoites in the mammalian skin. Within minutes to few hours after inoculation (Sinnis and Zavala, 2012) these highly motile forms enter the circulatory system and reach the liver where they infect hepatocytes establishing the so-called pre-erythrocytic phase of malaria infection. This phase of infection is completely asymptomatic and lasts in humans 5–17 days (the length varies according to *Plasmodium* species (Coatney et al., 1971)). Each sporozoite that infects the liver replicates into thousands of new parasites in a process called schizogony. Once parasite replication and cellularization are completed, the newly formed parasites, called merozoites, are released into the bloodstream and infect erythrocytes, initiating the erythrocytic stage of malaria infection (Prudencio et al., 2006). The cycles of parasite multiplication inside erythrocytes are shorter (24, 48 or 72 h, depending on parasite species), and causative of the classic symptoms of the disease (Coatney et al., 1971). When left untreated, the disease can eventually progress to severe syndromes and cause death (Haldar et al., 2007).

Human malaria can be caused by five *Plasmodium* species: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Of these, *P. falciparum* and *P. vivax* are the focus of intense research and targeting strategies due to the high mortality and/or morbidity they cause. *P. falciparum* is the most virulent species and is responsible for the vast majority of deaths in sub-Saharan Africa, primarily of young children and pregnant women.

P. vivax malaria is the most widespread and was previously considered a benign disease but is emerging as a potentially lethal condition outside of Africa (Baird, 2013), as current control measures are successfully reducing *P.*

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falciparum transmission (Cotter et al., 2013). Additionally, *P. vivax* is capable of forming cryptic forms called hyponozoites during the pre-erythrocytic stage that cause relapses months and even years after blood stage parasite clearance, contributing to the complexity of understanding and treating *P. vivax* malaria (Shanks and White, 2013; Kondrashin et al., 2014).

Despite major advances in the development and implementation of novel intervention strategies, the scientific community is still limited by substantial gaps in understanding the biology of *Plasmodium* and its complex interaction with the human host (The malERA Consultative Group on Basic Science and Enabling Technologies, 2011). Further studies addressing fundamental host–parasite interactions, as well as patho-physiological features of infection are necessary, but such studies are difficult to perform in humans.

2. The importance of addressing malaria infection experimentally

The study of human malaria involves a myriad of methods such as epidemiological analysis, population genetics, clinical studies of patients, both in field research studies and in hospital settings, as well as analyses of post-mortem biopsies. However, limitations exist regarding the ability to carry out research with human subjects or with human samples. For example, access to post-mortem tissues is hindered due to religious and cultural objection to autopsy and the lack of proper control subjects for most studies such as samples from infected patients that do not develop the pathology or die are some of the reasons that make research difficult. Moreover, the data obtained from post-mortem studies only represents the end stage of a long process, and the analysis of the sequence of events leading to pathology through monitoring the internal organs and environment is very limited; e.g. the study of the liver during the first phase of infection or the brain during cerebral malaria.

Despite their limitation and controversy on replicability of human disease, mouse models of malaria infection have been used for decades and have contributed significantly to a better understanding of the basic biology of the parasite, host response and pathogenesis. Several rodent-infectious *Plasmodium* parasites are available, *Plasmodium berghei*, *Plasmodium yoelii*, *Plasmodium chabaudi* and *Plasmodium vinckei*, each including several strains, which lead to distinct courses and outcomes of infection, depending on the host-mouse strain combination (see Box 1), raising questions about which, if any, of the mouse models can be extrapolated to understand human disease or diseases. Concerns exist about the translational utility of animal models in pathogenesis, immunity, vaccine development and drug discovery due to the heterogeneity observed with different parasite and mouse combinations (White et al., 2010; Craig et al., 2012). However, one can argue that the range of disease manifestations in the different mouse models should be considered as a reflection of the diversity of the human disease rather than a limitation (Langhorne et al., 2011). Undoubtedly, the availability of inbred/congenic/transgenic animals and the ability to manipulate and control different aspects of the host, including the immune system, make the mouse model a precious tool. Still, mice are not humans and the *Plasmodium* spp. that infect rodents are distinct from the ones that infect humans. As such, results arising from studies using rodent models should be interpreted with caution (White et al., 2010; Craig et al., 2012; Langhorne et al., 2011). Aware of the importance and simultaneously the limitation of the mouse models, there has been a constant search for mouse models that better reflect the different field situations (see Boxes 1 and 2).

2.1. How to address malaria infection experimentally

In addition to studying disease mechanisms, an advantage of using rodent models is the ease of maintaining the entire life cycle of the parasite in controlled and optimized laboratory conditions. The establishment and maintenance of laboratory *Anopheles stephensi* (as well as *Anopheles gambiae*) vector colonies and the development of transgenic parasite lines have allowed the dissection of processes occurring during transmission from the mosquito vector to the mammalian host, as well as studies of transmission from the mammalian host to the mosquito vector. Controlled infections can be initiated directly by mosquito bite or, alternatively, by intra-dermal or intra-venous injection of sporozoites. The infection can then be analyzed in the liver or be allowed to progress into the blood, and disease outcome can be monitored. It is also possible to bypass the skin and liver stages of infection by directly injecting parasitized red blood cells (pRBCs) intra-peritoneally or intravenously. The careful choice of transgenic parasite line determines the possibilities of analysis; e.g. the use of fluorescent parasites allows monitoring the infection and the parasite's interaction with host cells *in vivo* and in real-time (Gomes-Santos et al., 2012). When using chemiluminescent parasites, infection can be analyzed longitudinally over time (within the same infected animal) in a non-invasive (or minimally invasive) manner throughout liver stage into blood stage infection, where bioluminescence is correlated with the level of liver infection and with blood parasitemia (Ploemen et al., 2009; Zuzarte-Luis et al., 2014). Additionally, chemiluminescent parasites have also facilitated the study of infected erythrocyte sequestration (Franke-Fayard et al., 2006). Moreover, the combination of transgenic parasites (lacking or overexpressing parasite or exogenous molecules) with genetically engineered mice lacking key molecules (e.g. immune mediators or their receptors) has proved useful in deciphering the host response to parasite infection throughout the latter's life cycle.

Further technological progress such as the development of high-throughput *Omics* technologies employed to study *Plasmodium* infection at the DNA, RNA, protein, and metabolite levels has prompted further advances in our understanding of both host as well as parasite biology (Tarun et al., 2008; Albuquerque et al., 2009; Olszewski et al., 2009). Equally important was the recent development of clinical diagnostic techniques for small animals, including non-invasive imaging techniques such as computer tomography (CT) and magnetic resonance imaging (MRI), as well as monitoring systems for cardio-vascular and respiratory function (Penet et al., 2005; Martins et al., 2013). Such advances are crucial for the detailed characterization of malaria pathology in animal models and help determine the degree of similitude with the human pathology.