MiSAC matters
MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE
50th Anniversary Articles

Malaria Parasite Transmission: Starting the Journey from A to B and Back Again

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Malaria is a disease that everyone has heard of. Most people can think of an historical figure who is recorded as being killed by malaria, such as Genghis Khan or Alexander the Great. Napoleonic battles have been shaped by malaria and German antimalarial drug research was a subject of Allied espionage during World War 2. Today, you occasionally hear of a Premiership footballer (usually African) succumbing to a bout of the disease which prevents him playing for a few weeks.

However, the truth is that malaria is still one of the greatest killers amongst today's infectious diseases. It kills >400,000 young Africans (typically aged under 5), infecting 200 million people each year worldwide (source: 2019 WHO report), and causing significant global economic damage. Despite reports of vaccine trials, there is no properly effective vaccine due to a combination of parasite diversity, parasite use of antigenic variation and impaired immune memory. Furthermore, new drugs are urgently needed as parasite resistance to all past and current antimalarial formulations is increasing.

Malaria is caused by single-cell protozoan parasites from the genus *Plasmodium* that reside in a variety of host cells as they progress through their complicated life cycle. The oftentimes fatal symptomology occurs during the blood phase where the parasite cyclically invades erythrocytes, multiplying asexually therein before bursting out to reinitiate the process. Consequently, *Plasmodium* might induce anaemia, compromise multiple organs and its voracity for glucose can invoke host metabolic disorders.

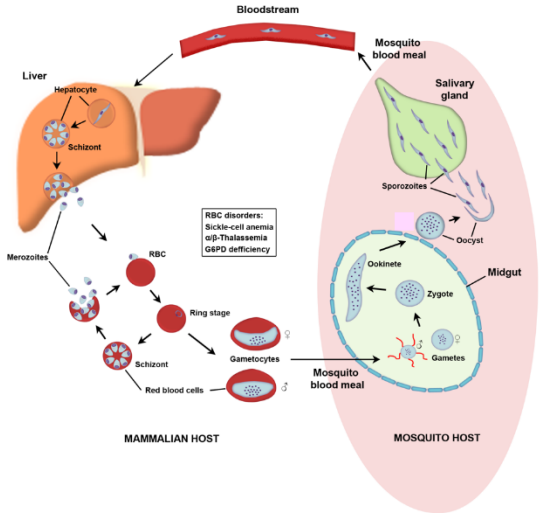

Figure 1. The life cycle of *Plasmodium*

We will consider that the initiation of the life cycle occurs with the injection of infectious parasite forms (sporozoites) by the mosquito into the mammalian blood stream. Successful sporozoites invade hepatocytes where they differentiate and multiply before emerging back into the blood stream as merozoites which are capable of invading erythrocytes. Erythrocyte invasion initiates the pathogenic intraerythrocytic cycle (IEC) phase of the disease which consists of invasion, growth, multiplication and erythrocyte rupture, the latter initiating the paroxysms characteristic of the disease. Human infectious *Plasmodium* species typically take 48 hours to complete the IEC. Human erythrocyte cell disorders that offer partial protection against malaria are indicated. Human disease results from hypoglycaemia, tissue damage stemming from hypoxia and immune dysregulation due to parasite sequestration, encephalopathy and respiratory distress. Sexual development (gametocytogenesis) is initiated in the IEC as an alternative form of development resulting in the formation of relatively stable gametocytes that circulate in the bloodstream. Transmission is initiated when the female mosquito takes a blood meal and the initial events are described in the main text. The zygote morphs into an ookinete that is both motile & invasive and harnesses both of these properties to pass through the basement membrane and basal lamina of the mosquito midgut wall where it encysts forming an oocyst. Within the cyst, parasite multiplication and differentiation occurs once more resulting in the production of over 10,000 sporozoites. Sporozoites are also motile and migrate from the ruptured oocyst to the salivary glands in preparation for the completion of transmission when the next blood meal takes place.

This article is one of a number invited as part of the MiSAC 50th anniversary celebrations. The articles are written by experts and are both up to date and relevant to microbiology in schools. MiSAC is grateful to all contributing authors.

There are many species of *Plasmodium* infecting primates, birds and even lizards. Five species are known to infect humans, of which *P. falciparum* is easily the most lethal, and all are transmitted by the bite of a female anopheline mosquito (see typical life cycle, **Figure 1**).

Transmission, *i.e*. to humans from the mosquito vector, is dependent upon parasite sex. In order to transmit, the parasite must generate separate sexual precursor forms, a male and female gametocyte in the human blood stream which in turn must be taken up by the mosquito as part of her blood meal. Once in the mosquito midgut, reduced temperature and an increase in pH stimulate the previously quiescent gametocytes to undertake their transformation to gametes (**Figures 1 & 2**). Successful fertilization and zygote formation are essential for the further development of the parasite in the mosquito and ultimately transmission to a new host.

What triggers gametocyte production, known as commitment, in human blood? Commitment entails a developmental switch, a complete reprogramming of parasite development to produce sexually reproductive cells that are physically very distinct and have a very different biological purpose - transmission! Commitment is a highly regulated process representing an investment (sacrificing growth and multiplication) by the parasite since the chances of both a male and female gametocyte becoming part of a blood meal are infinitesimally small.

Commitment, gametocyte production, can be varied, depending on circumstances (*e.g*. increasing in a sick or malnourished host) and the parasite senses host serum components such as lysophosphatidylcholine (LPC) that regulate commitment. The parasite cellular signalling events that result in gametocytogenesis are unclear but ultimately stimulate the production of a master regulator protein, a transcription factor called AP2G that rewires the gene expression programme of the blood stream asexual parasite so that gametocytes of both genders develop. When the gene encoding AP2G (denoted *ap2g* by convention) is transcribed and critical levels of AP2G are achieved within the parasite, it will form a (male or female) gametocyte.

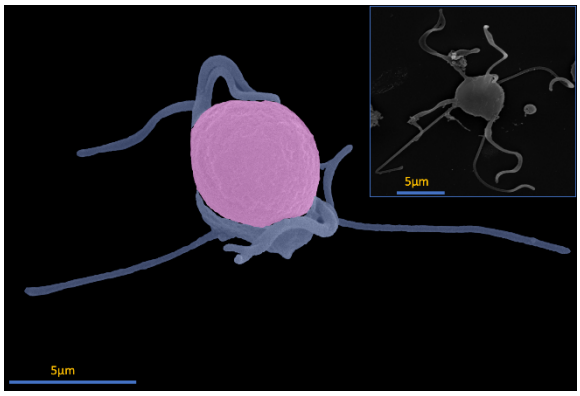

Figure 2. Plasmodium gamete formation ("gametogenesis")
Main image: false-coloured electron micrograph of female (pink, foreground) and male (blue, background and insert) gametes. Upon activation in the mosquito midgut, the initial event is the rupture of the erythrocyte and emergence of the gametes. Whilst the female macrogamete is then ready to be fertilized, the male undergoes a remarkable transformation ("exflagellation" *ie*, the departure of the 8 motile male gametes from the residual body of the ruptured male gametocyte). The entire 23 Mb genome is replicated through three cycles in 10 minutes, packaging the resulting 8 freshly replicated nuclei into motile, filamentous microgametes that swim within the insect in search of the female. Microgametes leave behind the residual body as the process of exflagellation completes. Although receptors and ligands that aid gamete mutual recognition have been identified, it is unknown how gametes find each other in the context of a blood meal. In the main picture, engagement of the female with the male gametes, prior to fertilisation, has been initiated so rapidly that the process of exflagellation has still not been completed.

Images produced by Dr Leandro Lemgruber from parasite preparations supplied by Dr Katie Hughes, both of whom are affiliated with WCIP.

The ap2g gene is itself regulated epigenetically which means *ap2g*, when silent, is effectively hidden from the transcriptional apparatus through DNA/chromatin folding. The DNA is wrapped around nucleosomes which are themselves composed of two copies of four proteins called histones. The effect is that the DNA is then organized like beads on a string and the degree of compaction is controlled by specific chemical modifications to the histones to which more proteins can bind. This control can be very

regionalized to specific genes and is effected by a whole suite of enzymes that catalyse the addition and removal of these chemical modifications and affect expression of the local genes. These enzymes offer very promising targets for drug development that could not only block activation of *ap2g* but also other proteins critical for parasite growth and survival.

The signalling pathways that stimulate gametocytogenesis and liberate *ap2g* ultimately control these histone-modifying enzymes and might also be targeted but currently remain completely undefined. In our research group, we have made genetically-modified parasites that allow us to switch on *ap2g* at will and in all cells generating populations of pure gametocytes that allow us to look at the very early stages of sexual development. This we expect will allow us to uncover what drives the gametocytes to become either male or female and with that understanding may come interventions that will block transmission.

We've come a long way since 1896 when Alphonse Laveran first observed exflagellation with his microscope, thereby realizing that parasite forms within red blood cells were the agents of malaria. However, there are still many secrets to uncover and exploit as we seek new ways and targets to combat this highly successful and still very lethal parasite.

AUTHOR PROFILE

Professor Andy Waters has been a Wellcome Trust Principal Research Fellow since 2008 on his return to the UK from The Netherlands and Director of the Wellcome Centre for Integrative Parasitology (WCIP) at the University of Glasgow since 2012. He is a fellow of the Royal Society of Edinburgh and a member of the European Molecular Biology Organisation. He and his colleagues are interested in malaria parasite transmission from host to vector, attempting to uncover the molecular detail behind the processes that prepare and initiate development of transmissible forms of the malaria parasite. WCIP considers fundamental parasite biology as well as polymorbid aspects of disease involving tropical parasites in combination with other infectious and non-infectious diseases. WCIP partners with research organisations in the Tropics where parasites have their greatest impact.

Acknowledgements

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