Editorial

Plasmodium falciparum gametocytes: playing hide and seek

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Malaria is a major cause of morbidity and mortality worldwide, annually it affects more than 240 million people and is responsible for 600-800 thousand deaths (1). *Plasmodium falciparum (P. falciparum)* causes the most severe form of malaria and is responsible for most of these deaths, particularly in young children and pregnant women. The malaria parasite has a complex life cycle requiring both human and mosquito hosts, but the clinical manifestations only occur while it is undergoing asexual reproduction within the hosts' red blood cells (RBC). A small proportion of the asexual parasite population enters the sexual pathway and develops into male and female sexual forms called gametocytes which are essential for transmission to the mosquito vector.

Most malaria eradication programs have focused on eliminating the asexual stage of the parasite, and it is only recently that the focus has shifted to transmission blocking strategies. While this has had the effect of increasing research efforts into the sexual parasite stages our knowledge of gametocyte biology still lags behind that of asexual stage parasites.

P. falciparum gametocytes undergo a long maturation period of 7 to 10 days during which they develop from stage I to stage V gametocytes. Mature stage V gametocytes are the only stage which circulates within the host's blood stream, making them available for uptake by the mosquito vector, with immature stages (I-IV) sequestering away from the peripheral circulation in a manner similar to mature asexual stage parasites.

There has been significant speculation over the last few years in regard to two fundamental questions about the biology of *P. falciparum* gametocytes. Firstly, how do gametocytes escape detection and elimination by the host immune system when they do not appear to express either *P. falciparum* knob-associated histidine-rich protein (KAHRP) or *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on their surface (2,3), both of which are essential for cytoadherence of asexual stage parasites under the flow conditions found within the microcirculation (4). Secondly, as they are not present in peripheral blood except as mature stage V gametocytes, where do gametocytes sequester during their long development phase given their lack of a cytoadherence complex?

The study undertaken by Joice and colleagues (5) and published in the July 9th 2014 issue of *Science Translational Medicine* addresses the second of these questions in some detail. The authors present a detailed analysis of malaria patient specimens to determine the relative abundance of gametocytes in different body tissues. They used qRT-PCR and immunohistochemistry to examine samples from an existing tissue collection of malaria autopsy cases and in accordance with previous studies (6,7) found high parasite densities in the spleen, brain, gut and heart. Gametocyte levels were also high in these organs, and additionally in the bone marrow. When differences in vascularity across organs was taken into account the authors found a significantly higher proportion of gametocytes vs total parasites in the bone marrow (44.9%), in comparison to the gut (12.4%), the brain (4.8%) and other organs.

These observations are not surprising given that the bone marrow has long been considered a potential site for gametocyte sequestration. Smalley *et al.* (8) showed that the density of immature gametocytes in bone marrow smears prepared from children infected with *P. falciparum*

was 10× greater than in peripheral blood. The presence of gametocytes in the bone marrow was confirmed by Farfour *et al.* (9) who went on to demonstrate that the majority of immature gametocytes (stage II-IV) were found in the extra vascular component of the bone marrow.

In 2013 a study by Aguilar *et al.* (10) reached a similar conclusion that immature gametocytes sequester in the bone marrow. However Joice and colleagues went a step further than Aguilar in revealing that the incidence of gametocyte carriage is significantly higher in the bone marrow when compared to other organs. Joice and colleagues confirm and extend the findings of these previous studies, they present data demonstrating that early stage gametocytes are enriched in the bone marrow and report finding very few parasites positive for the early gametocyte stage marker Pfs 16 in the blood vessels of the bone marrow with these cells being found almost exclusively in the extravascular spaces. This strongly supports the observation by Farfour *et al.* that immature gametocytes are found in extravascular spaces of the bone marrow, in close proximity to erythroblasts (9). Joice and colleagues also went on to perform *in vitro* studies which confirm that gametocytes can form and develop in erythroid precursor cells, something that was eluded to by the work of Peatey *et al.* in 2013 (11).

The authors also examined the co localization of parasites with bone marrow macrophages and found that in the cases examined the number of gametocytes inside macrophages was significantly lower than the number outside, confirming the findings of previous *in vitro* studies (12) and adding further weight to the idea that developing sexual stage parasites are less susceptible to phagocytosis than asexual stage parasites.

There is now considerable evidence to support the theory that immature gametocytes are sequestered in areas such as the extracellular spaces of the bone marrow which experience reduced circulatory flow and may provide protection from the host immune system despite the lack of the cytoadherence complex.

This well executed study brings together a number of pieces of the jigsaw and adds considerable weight to the theory that the development of *P. falciparum* gametocytes takes place outside of the blood stream, in the bone marrow.

Nonetheless, questions remains as to why, given the successful use of sequestration by asexual parasites, in avoiding immune surveillance, gametocytes do not express either PfEMP1 or KAHRP on the surface of the infected red cell and how they do in fact sequester without these molecules. Joice and colleagues suggest that developing gametocytes may be retained in extravascular space through binding interaction with erythroblastic islands and/or through their increased rigidity.

Early studies did assume that early stage gametocytes sequestered via parasite ligands expressed on the surface of the infected RBC (2) in a manner similar to asexual cytoadherence (13), but this theory of cytoadherence in the bone marrow was subsequently disproven (14). Other ligands such as rifins were also postulated once it became apparent that gametocytes did not express either KAHRP or PfEMP1 (15,16). But a link between this protein and gametocyte sequestration has not been demonstrated.

One plausible explanation for a lack of parasite ligands on the surface of the gametocyte infected RBC is that unlike mature *P. falciparum* schizonts which rupture to release merozoites, which then invade new erythrocytes, and thus shed the host RBC membrane in the process, gametocytes do not shed the host RBC as they develop to maturity. The presence of PfEMP1 on the surface of a mature gametocyte could conceivably hinder free circulation in the microvasculature, thus making mechanical retention in a privileged niche the likely mechanism by which immature gametocytes are prevented from circulating freely within the host blood stream. This hypothesis is supported by the observations that developing gametocytes undergo significant changes in their deformability as they age (11,16) becoming highly rigid during their early development followed by a relatively rapid transition at maturity when they have a similar deformability as uninfected red cells.

Other evidence, such as the apparent increased commitment to gametocytogenisis in the presence of immature erythrocytes (11,17) also supports the hypothesis that the extravascular spaces of the bone marrow are where the majority of gametocytes form. Thus it is possible that the signal for gametocytogenisis is not anaemia, and the increased presence of reticulocytes per se, as was once postulated, but rather that the presence of immature erythrocytes acts as a signal that informs the invading merozoite that it is within this privileged niche.

In conclusion, Joice *et al.* have presented evidence of gametocyte development in the haematopoietic system of the human bone marrow and have shown that gametocytes are enriched in the extravascular components. In addition they show that gametocytes can form and develop in erythroid precursor cells and that that immature gametocytes are less susceptible to phagocytosis than asexual stage parasites. They suggest that developing gametocytes may be retained in the extra vascular space through binding

interaction with erythroblastic islands and/or through their increased rigidity. In doing so they take us one step further towards understanding the complex and mysterious development of *P. falciparum* gametocytes. As we gain further understanding of the apparently alternative pathway used by *P. falciparum* to develop its sexual stages we may discover novel ways in which to hamper this development.

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