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COMMENTARY

The emerging role of the human bone marrow as a privileged developmental niche for the transmission stages of the malaria parasite *Plasmodium falciparum*

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Abstract

The spread of malaria relies on the ability of the Plasmodium parasites to be transmitted from infected individuals to the Anopheles mosquito vectors. Recent work on the most lethal of the malaria parasites, *Plasmodium falciparum*, identified the infected human bone marrow as a preferential site for the localization and maturation of the parasite transmission stages, the gametocytes. These findings unveil a complex host parasite interplay and an unsuspected role of the bone marrow microenvironment in the successful transmission of the malaria parasite and have major implications in developing and targeting future interventions to block the transmission of *P. falciparum*.

Key words

- malaria
- Plasmodium transmission
- gametocytes
- bone marrow
- stromal microenvironment

The present years represent a turning point in the fight against malaria. The year 2015 was landmarked by World Health Assembly and the Roll Back Malaria Program as the target year to achieve a substantial decrease in malaria incidence and mortality and the startpoint of the new Sustainable Development Goals [1], which include a renewed effort to fight malaria and the transmission of the Plasmodium parasite by the Anopheles mosquito. Today, despite the success of having achieved an estimated reduction of malaria incidence and mortality respectively of 36 and 60% in the past 15 years, the global burden of this disease is still unbearable. WHO reports that last year 1.2 billion people in 97 countries have been at a high risk of being infected by Plasmodium, that 214 million contracted the disease and that the parasite, mostly *Plasmodium falciparum*, killed one person, in most cases a child under 5 years, every minute [2].

The unicellular protozoan parasite Plasmodium has a complex life cycle alternating between Anopheles mosquitoes and humans. The infection starts with the bite of an infected mosquito which injects sporozoites in the skin, from which they migrate to the bloodstream and reach the liver. Asexual reproduction in the hepatocytes yields thousands of parasites which infect erythrocytes in the bloodstream, starting indefinite rounds of intraerythrocytic asexual replication. Asexual parasites cause the pathology of malaria, whose most severe outcomes are severe anemia and cerebral malaria, the latter caused by the accumulation of parasites in the brain microvasculature. Besides proliferation, at every asexual cycle a small fraction of parasites differentiates into the non-dividing gametocytes, the Plasmodium sexual stages responsible for parasite transmission to the Anopheles mosquito vector. Male and female gametocytes transform into gametes within minutes after being engorged by the mosquito, egress from the red blood cells, fertilize and produce a motile ookinete. This cell traverses the mosquito gut wall and transforms into an oocyst where thousands of sporozoites are produced, which migrate to the insect salivary glands, ready to start a new cycle at the next mosquito bite.

Amongst the five malaria parasite species infecting humans, *P. falciparum* has the unique feature that large parts of both the asexual and the sexual development occur sequestered in internal body organs, away from peripheral circulation and from the critical checkpoint represented by the passage through the spleen [3]. In the 44 hour long asexual cycle, the young asexual parasites modify the erythrocyte surface to mediate the adhesion of the infected red blood cell to the endothelial lining of the microvasculature in several organs. In the 10 day long sexual development, another distinguished feature of *P. falciparum*, the maturation of the

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intraerythrocytic gametocytes also occurs sequestered in internal organs and only the mature gametocytes are seen in the peripheral blood circulation. Whereas asexual parasites have been described to sequester in virtually all organs examined [4], the sites of gametocyte sequestration have been poorly explored and only a few observations from the early years of malariology [5, 6] and the 1980s [7] provided some evidence that the immature sexual stages are commonly found in the human bone marrow.

After the decades in which this fundamental aspect of the parasite life cycle has been virtually ignored, the present years are likely to represent a turning point also in our basic understanding of the hiding strategies used by *P. falciparum* to ensure gametocyte maturation and parasite transmission from the human to the mosquito host. Recent major breakthrough observations shed light on this still obscure part of the *P. falciparum* life cycle: three reports based on the examination of *ex-vivo* and autopsy specimens from infected individuals altogether confirmed with specific molecular markers that the human bone marrow is a primary site of the sequestration and the maturation of *P. falciparum* gametocytes [8-10]. The diverse source of the biological samples examined, ranging from the case of one asymptomatic individual [8], to a cohort of infected children enrolled in a study on anemia [9], to organ specimens from lethal cases of malaria [10] supports the general conclusion of these observation.

The fact that the bone marrow appears as a major site for the sequestration and maturation of *P. falciparum* gametocytes raises several questions on how the sexual stage parasites are able to establish and to maintain for several days the sequestration in this tissue. Previous work aiming to address these questions reported that *P. falciparum* gametocytes from the earliest detectable stage of differentiation, in contrast to asexual stages, do not expose parasite ligands on the infected red blood cell surface and fail to significantly adhere on human endothelial cells from several organs, including the bone marrow [11-13]. This rules out that the key mechanism of immature gametocyte sequestration is the adhesion to endothelial cells in the bone marrow microvasculature and suggests that the divergent strategies of remodeling the host red blood cells in asexual and sexual development most likely influence the mechanisms used by the two parasite types to home to and/or to be maintained in specific organs and tissues of the infected body.

To further complicate this scenario, two of the above reports revealed an unexpected localization of the immature gametocytes in the bone marrow, which were readily observed in the extravascular spaces of this tissue, embedded amongst several stromal host cell types such as adipocytes, erythroblasts and macrophages [8, 10]. In addition, comparing stage and number of parasites found inside and outside macrophages in the bone marrow autopsy specimens, one report observed that immature gametocytes appear less susceptible than asexual parasites to be phagocytized [10]. This observation is consistent with the aforementioned low antigenic profile specific of the young sexual stages and also strongly suggests that immature gametocytes may adopt specific mechanisms to escape host immune cell recognition to guarantee their long survival and maturation in the stromal microenvironment.

The localization of immature gametocytes in the bone marrow extravascular compartment also raises the fundamental questions of how, and at what stage precisely, do these parasites reach the extravascular spaces, and how do gametocytes reenter blood circulation at maturity. The ability of the endothelium of the bone marrow microvasculature and sinusoids to sustain bidirectional cell trafficking or to release in circulation the reticulocytes produced in the extravascular hematopoietic islands is well documented. In contrast, to our knowledge, the extravasation of mature erythrocytes in the bone marrow stromal spaces is not documented and their presence in this compartment is not physiological. This does not provide obvious cues on the possible mechanism(s) used by erythrocytes infected with early sexual stages to contact and traverse the bone marrow endothelial barrier and reach the stromal microenvironment. Nevertheless, in addition to the mechanism used by the asexual stage parasite to adhere to the vascular endothelium in virtually all body organs [14], several types of physical and molecular cross talk between parasitized red blood cells and the endothelium have been described. In the bone marrow, an ultrastructural study reported an intriguing interdigitation of cellular processes emanating from endothelial cells with *P. falciparum* asexual parasites [15]; one study on brain endothelial cells described the transfer of parasite proteins and a progressive elaboration of endothelial cell membranous structures induced by the parasite [16]; finally, one study described that extracellular vesicles derived from *P. falciparum* infected red blood cells are able to modulate gene expression and the barrier properties of endothelial cells [17]. It is therefore conceivable that early gametocytes engage signaling and physical interactions with the bone marrow sinusoid endothelium which elicit the passage of these infected erythrocytes either between cells in a permeabilized endothelial layer and/or through the endothelial cell bodies via diapedesis [18].

At the end of the process of sexual differentiation, the ability of the mature gametocytes to traffic in the opposite direction to reenter in the blood circulation may be explained by a similar cross talk, but cues also derive from recent studies revealing that cell mechanical properties of the infected erythrocyte change during the long gametocyte maturation [19-21]. It is noticeable that a distinct switch in deformability accompanies the transition from the rigid, immature to the highly deformable, mature gametocytes [19], which the parasite could exploit to traverse the endothelium during intravasation. The described physical proximity of the extravascular immature gametocytes to the hematopoietic islands [10] may lead to speculate that the maturation and intravasation of the gametocytes may be somehow coordinated with the maturation of erythroblasts and with the process of endothelial crossing performed by the newly produced reticulocytes.

The recent evidence of a preferential accumulation

of immature gametocytes of *P. falciparum* in the human bone marrow and of their presence and likely ability to mature in the extravascular stromal compartment of this tissue will require intense investigations to elucidate the cellular and molecular mechanisms involved. This is a challenging task, partly for the difficulty to recapitulate in experimental systems the complex architecture and the interplay between several cell types characterizing the bone marrow microenvironment [22]. Experimental systems have been however developed and used to investigate physiology of this compartment *in vitro* [23] and in a bone marrow *in vivo* model in humanized mice [24, 25]. Using *P. falciparum* parasites, and in particular transgenic lines in which different life cycle stages are specifically marked by fluorescent [26] or bioluminescent [27, 28] reporters, in these or similar systems is a promising route to provide answers to these questions.

This information will be critical to update, after over one century of malariology, our understanding of the *P. falciparum* life cycle, by choosing between several models recently put forward to describe the development of the parasite in this organ [3, 29]. An intriguing one is based on the observations that *P. falciparum* asexual parasites can proliferate and that gametocytes can develop within erythrocyte precursors [10, 30] and that efficiency of gametocyte production is increased in reticulocyte rich blood [31] and in erythrocyte precursors [32]. These data would altogether be consistent with the hypothesis that the bone marrow microenvironment could host a population of replicating asexual parasites characterized by a pronounced commitment to produce gametocytes. This parasite population would be formally equivalent to a lineage of stem cells in balance between self renewal (asexual replication) and differentiation in terminally differentiated cells (the gametocytes). In this model, the bone marrow stroma, which physiologically host hematopoietic stem cells and the ensuing lineages, but can also act as a niche for the proliferation of neoplastic cells, has been evolutionary hijacked by *P. falciparum* as an ideal microenvironment to host this ancillary part of the parasite life cycle, and yet absolutely critical for its successful transmission to the mosquito host.

Compelling evidence is accumulating to propose the intriguing scenario that the human bone marrow is a privileged site for *P. falciparum* gametocyte maturation and for previously unsuspected regulatory interactions of immature gametocytes and sexually committed asexual stage parasites with endothelial cells and with non-endothelial cell types such as erythrocyte precursors, mesenchymal cells and macrophages. In this important time in the fight against malaria, the research on the fundamental biology of the parasite is warning on the existence of yet unexplored forms and sites critical for parasite development and transmission, but it is also geared to identify and target the molecular players of this *P. falciparum* deadly "hide and seek" game.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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