

Bone marrow reticulocytes: a Plasmodium vivax affair?

Alfredo Mayor^a and Pietro Alano^b

^aISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain; ^bIstituto Superiore di Sanità, Rome, Italy

Malleret and colleagues show the importance of the bone marrow in *Plasmodium vivax* biology by proving the preferential infection of young reticulocytes, generally restricted to the bone marrow, which experience accelerated maturation post-invasion¹.

Plasmodium vivax mainly invades reticulocytes, a heterogeneous population of red blood cell precursors, characterized by a reticular network of residual RNA, whose maturation is indicated by the decreasing expression of the transferrin receptor CD71. This host cell specificity shapes *P. vivax* pathobiology and the strict requirement for reticulocytes has hampered the establishment of an *in vitro* culture system for this parasite. By sorting different developmental stages of cord blood reticulocytes for use in an *ex vivo* invasion assay, Malleret and colleagues show that *P. vivax* merozoites prefer the youngest of the young erythrocytes. The immature CD71+ reticulocytes, generally restricted to bone marrow, were more efficiently invaded than older CD71- reticulocytes, principally found in peripheral blood. This was puzzling, as *P. vivax* ring stage parasites from patients were instead predominantly found in CD71- reticulocytes. Analysing the first steps of *P. vivax* invasion of CD71+ reticulocytes, Malleret and colleagues revealed a remarkably rapid remodeling of *P. vivax* invaded reticulocytes, leading to increased deformability, accelerated loss of CD71 and replacement of clathrin pits by distinctive caveolae nanostructures.

The narrow tropism towards CD71+ immature reticulocytes, produced and largely residing in the bone marrow², raises the intriguing possibility that *P. vivax* invasion may mainly take place in this organ rather than in the circulating blood. The authors accordingly suggest that increased deformability associated with the accelerated reticulocyte aging might contribute to the trafficking of mature parasites into this organ and the egress of infected reticulocytes into the peripheral blood system. As pointed out in the paper, the accumulation of *P. vivax* infections in the bone marrow extravascular spaces may explain early observations of patients scored negative for *P. vivax* in peripheral blood but positive in bone marrow biopsies³. Several case reports described recurrences of *P. vivax*, as well as *Plasmodium falciparum*, following bone marrow transplantations⁴. Together with the evidences provided by Malleret and

colleagues, these observations suggest that bone marrow can be a site where *P. vivax* parasites may hide and develop.

Recent reports have placed the host-parasite interplay in the bone marrow at the center of pathophysiology and transmissibility of the other main human parasite *P. falciparum* by demonstrating the enrichment of late parasite stages and immature gametocytes in this organ, with the latter readily found in the bone marrow extravascular compartment⁵⁻⁷. The current study shows the potential of the bone marrow as a niche where *P. vivax* invasion can occur before reentering the circulation. Intriguing similarities may deserve further attention. Most of the erythroid precursors containing *P. falciparum* immature gametocytes in the marrow stroma in autopsy specimens were CD71⁻⁷, suggesting a similar parasite-mediated acceleration of host cell differentiation. Moreover, the rapid increase in deformability of the *P. vivax* infected immature reticulocytes, proposed to facilitate migration through the bone marrow sinusoidal lining, parallels the analogous reduction in the rigidity of erythrocytes infected with *P. falciparum* mature sexual stages, which may help their release from the bone marrow sequestration sites to peripheral circulation⁸.

The study by Malleret and colleagues raises several questions with far-reaching implications for *P. vivax* biology which may have clinical impact. First, what is the role of bone marrow as a reservoir of *P. vivax* parasites and what are the implications for strategies to eliminate malaria? The bone marrow is emerging as a site where both *P. vivax* and *P. falciparum* can hide and sustain infection and transmission, posing new challenges for malaria elimination initiatives directed at identifying and treating all *Plasmodium* infections. Second, through which mechanisms do infected reticulocytes transverse the bone marrow sinusoidal capillaries to the primary hematopoietic sinus? The authors argue that only young reticulocytes that have migrated out of the red bone marrow would be available for *P. vivax* invasion, unless invasion principally occurs in the extravascular space of the red bone marrow. The latter scenario would imply a two-way journey of *P. vivax* merozoites and freshly invaded reticulocytes through the bone marrow sinusoidal capillaries, similarly to what has been proposed for *P. falciparum*⁷. Third, to what extent anaemia or inflammatory reactions, common during *P. vivax* infection, are triggered by the bone marrow tropism and might disrupt the sinusoidal lining permitting cellular transit? A relationship has been suggested between hematological disturbances and *P. falciparum* development in bone marrow⁵. This raises further questions on how this interplay in bone marrow is modified in the two parasite species once clinical malaria ceases in asymptomatic infected individuals, as

these will stand as the last, most challenging parasite reservoir to be attacked to achieve malaria eradication. Fourth, how does *P. vivax* accelerate reticulocyte aging? Do loss of the CD71 clathrin pits and the formation of microvesicles contribute to the rapid host cell remodeling, and are there common mechanisms to affect deformability of reticulocytes by *P. vivax* and of erythrocytes by *P. falciparum* gametocytes⁸? Finally, do parasites in the bone marrow reach some degree of refractoriness to treatment, as shown for *Salmonella typhi*⁹, malignant hematopoietic cells and epithelial tumor cells that metastasize to bone¹⁰? Investigating these topics will allow us to begin to dissect the role of the bone marrow in the *P. vivax* biology and the manifestation of disease.

Bone marrow, which accounts for approximately 5% of the body weight in humans, generates all hematopoietic cells circulating in peripheral blood. Although functional alterations of the bone marrow under pathogen attacks are largely unknown, it is tempting to speculate that even small changes in this delicate environment may lead to significant modification in the cellular constituents in peripheral blood and tissues. An important role for bone marrow is emerging in the pathobiology of *P. falciparum* malaria. Malleret and colleagues expand a possibly similar role in *P. vivax*, requiring the assessment of how this organ contributes to the parasite biomass and the pathogenesis in this parasite. As for *P. falciparum*⁷, investigations of post mortem samples from *P. vivax* malaria cases would be of great value.

Figure legend: Proposed models for *P. vivax* (A) and *P. falciparum* (B) development in the bone marrow. A) Adapted from Malleret et al¹. *P. vivax* infects stage III reticulocytes that have egressed by diapedesis to the sinusoidal capillary lumen. Alternatively, *P. vivax* merozoites or *P. vivax*-infected erythrocytes might enter the red bone marrow compartment leading to invasion of CD71+ reticulocytes. Accelerated reticulocyte aging increases host cell deformability for subsequent endothelial crossing towards blood circulation. B) Adapted from Joice et al⁷. Presence of immature gametocytes in the bone marrow extravascular space may be explained by erythrocytes infected by stage I gametocytes or sexually committed ring stages entering the bone marrow stroma through the endothelial lining. Alternatively, asexual schizonts which develop in the extravascular compartment may produce sexually committed merozoites invading erythroid precursors, whose host cell remodeling may share possible similarities with that of *P. vivax*. Mature gametocyte-infected host cells cross the endothelial barrier to reenter the circulation. Professional illustration by Patrick Lane, ScEYEnce Studios.

Conflict of interest declaration: Authors do not have any conflict of interest.

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