Comment on Aguilar et al, page 959

Uncovering the hideout of malaria sexual parasites

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In this issue of *Blood*, Aguilar and colleagues[1] present the result of a timely and much-needed investigation aimed to unveil the hidden sites of maturation of *Plasmodium falciparum* transmission stages in human malaria infections.

More than 30 years after a similar study solely relying on parasite morphology on a small group of Gambian children,[2] Aguilar and colleagues[1] conduct a rigorous analysis with state-of-the-art tools on samples of peripheral blood and bone marrow aspirates from 174 anemic children from a region of moderate to perennial malaria transmission and provide molecular evidence confirming the preferential distribution of *P falciparum* immature sexual stages in the human bone marrow.

Recent renewed emphasis on the eradication of malaria has highlighted the need for novel interventions to target the human malaria parasite during transmission from the human host to the mosquito. Transmission of malaria parasites relies on the sexual stages, the gametocytes, which circulate in peripheral blood and are taken up by the mosquito during a blood meal. *P falciparum* gametocytes do not freely circulate in the peripheral blood for their entire intraerythrocytic development, which instead requires sequestration of the immature gametocyte-infected erythrocytes in internal organs of infected individuals for as long as 10 days. How and where *P falciparum* gametocytes sequester in humans are fundamental unanswered questions in malaria biology, and a better understanding of the mechanisms of gametocyte sequestration and circulation is essential to enable the development of new tools to disrupt malaria transmission.

The current work of Aguilar and colleagues uses a newly developed stage-specific quantitative reverse-transcriptase polymerase chain reaction method to quantitatively detect early, intermediate, and mature gametocyte-stage transcripts in bone marrow vs peripheral blood. Results of this work provide evidence for the higher prevalence and abundance of immature gametocytes in bone marrow samples, whereas mature gametocyte are more abundant and prevalent in samples from peripheral blood. In addition, Aguilar and colleagues[1] analyze correlations of demographic and clinical parameters with gametocyte prevalence and density in both tissues. Their results indicate that severe anemia and dyserythropoiesis are independently associated with a higher prevalence of mature gametocytes in bone marrow, suggesting a relationship between hematologic disturbances and gametocyte development in this tissue. The study, conducted on a cohort of mainly asymptomatic children, also contains relevant information for the epidemiology of malaria transmissibility. Although it is now recognized that microscopy generally underestimates gametocyte presence in peripheral blood, this work interestingly shows that the same method detects a 6-fold-higher prevalence of gametocytes in bone marrow. On one hand, this further confirms the need for molecular methods to identify the reservoir of malaria transmission in asymptomatic populations, and on the other hand it suggests the importance to reveal and possibly quantify the presence of the sequestered gametocyte nurseries to predict evolution and dynamics of malaria transmissibility in the populations under survey.

Although the virtual absence of immature gametocytes in peripheral blood is described from the early days of malariology, almost nothing is known about sites and mechanisms of immature gametocyte sequestration. Only a few early analyses of postmortem specimens and a recent clinical report provide morphologic evidence indicating bone marrow and spleen as the organs where immature gametocytes are most readily detectable.[2-5] These studies were of limited scale and used standard light microscopy as the only method for gametocyte detection and stage differentiation. In this respect, Aguilar and colleagues have the merit of readdressing this fundamental issue with state-of-the art tools. However, because their study does not inspect the presence of gametocytes in tissues other than bone marrow and peripheral blood, we hope that this work will fuel novel efforts in investigating if gametocytes can develop and mature in other organs of the infected host. For instance some of the early postmortem observations and a recent clinical case report on a splenectomized patient suggest that immature gametocytes may also mature in the spleen.[3,4,6] Despite the different physiological functions of such organs, sinusoids in the bone marrow and in the spleen, where blood circulation occurs at a reduced flow rate, may provide suitable sites for host-cell interactions and the establishment of the early sequestering gametocytes that do not have the same adhesive properties of asexual stages.[7]

The described localization of immature gametocytes in the bone marrow and the association between gametocyte presence and some functional parameters of this organ raise the question of *P falciparum*–host interactions in this microenvironment. A recent clinical case report from a patient with subacute *P falciparum* malaria importantly revealed the presence of immature gametocytes in extravascular spaces of bone marrow, in close proximity to erythroblasts and adipocytes.[5] Although this result needs to be confirmed in a larger scale and possible confounding effects of clinical symptoms or drug treatment on the histologic results need to be ruled out, this observation surely falls on a fertile ground for new hypotheses on the physiological relevance of such previously unreported localization of *P falciparum* immature sexual stages. Rather than representing just one of the possible parasite sequestration sites, it is tempting to speculate that bone marrow represents instead a privileged site for a complex crosstalk of gametocyte-infected erythrocytes with endothelial and nonendothelial cells. Such an interplay could master several processes from the extravascular development of immature gametocytes to their release in circulation at maturity, taking advantage of recently described changes in

their cellular mechanical properties during gametocytogenesis.[8-10]

The renewed attention dedicated to the biology of gametocytes is significantly improving our basic understanding of how malaria parasites are maintained and propagated. One example of such progress are the observations by Aguilar and colleagues, which have important practical implications in designing how parasite transmission stages can be effectively targeted by current and future antimalarial drugs or vaccines.

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Kolyada et al, page 1090

In APS, two A1's are better than one!

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In this issue of *Blood*, Kolyada and colleagues elegantly demonstrate the therapeutic utility of a novel, synthetically constructed molecule, the A1 dimer (A1-A1), in preventing anti-β2 glycoprotein I (anti-β2GPI) autoantibody-mediated thrombosis in 2 distinct murine antiphospholipid syndrome (APS) thrombosis models. Current therapies for thrombotic APS entail long-term anticoagulation, with the associated risk of bleeding complications. The findings presented by Kolyada et al raise the possibility of perhaps using this agent to treat thrombotic APS patients in the future, allowing for a reduction in bleeding risk.[1]

APS is an important cause of acquired thrombophilia in the community, characterized by predisposition to both venous and arterial thrombosis, as well as obstetric complications. The laboratory diagnostic criteria for APS require the demonstration of persistently elevated levels of so-called antiphospholipid antibodies (aPL Abs) detected either by the anticardiolipin (aCL) or anti-β2GPI enzyme-linked immunosorbent assays (ELISAs) or lupus anticoagulant assays (LACs).[2] The term aPL Abs is a misnomer, as the major autoantigen to which APS patient autoantibodies (Abs) bind is β2GPI, a plasma protein that binds to negatively charged phospholipids such as cardiolipin.[3] Anti-β2GPI Abs bind domain I of the β2GPI molecule[4] (see figure).

Patient-derived anti-β2GPI Abs mediate direct thrombogenic effects via multiple mechanisms, which have been delineated using both in vitro and in vivo experiments (extensively reviewed elsewhere).[5] An important mechanism is the ability of anti-β2GPI Abs to dimerize β2GPI molecules on a number of cell-surface receptors leading to activation of prothrombotic downstream pathways in platelets, endothelial cells, and monocytes. One such receptor on platelets is ApoER2 (see figure). The in vivo importance of this receptor has been delineated using ApoER2 knockout mice, whereby the ability of passively transferred Abs from APS patients to potentiate thrombosis was abrogated in the knockout mice compared with the wild type.[6]

Kolyada and colleagues have used, in an innovative way, insights gleaned from previous work which has shown that β2GPI via its fifth domain binds the A1 portion of ApoER2.[7] They have synthesized a dimer, composed of 2 A1 molecules joined by a flexible linker, and have demonstrated its ability to inhibit anti-β2GPI Ab/dimerized β2GPI complexes from binding negatively charged phospholipids and ApoER2 in vitro, in a manner that is more potent than using A1 in the monomeric form.[8] A previous study by the research group of the late Silvia Pierangeli demonstrated that the A1 monomer is able to effectively inhibit thrombus potentiation induced by the passive infusion of Abs from APS patients into a nonautoimmune murine thrombosis model.[6] One of the key questions that remained for Kolyada and colleagues, in order for them to extend their in vitro work,[8] was whether the A1 dimer they have developed is effective in ameliorating anti-β2GPI Ab-mediated thrombus potentiation in vivo.

In this study, the research group of Kolyada, Porter, and Beglova have addressed this question using 2 distinct murine APS in vivo thrombosis models.[1] They studied the effects of A1-A1 and control peptide on laser-induced thrombus size in the autoimmune strain, NZW × BXSB *yaa* F_1 male mice. This murine strain is significant because it is the only known strain that spontaneously develops a syndrome that is analogous to human APS on a background of systemic lupus erythematosus.[9] The male mice spontaneously develop aPL Abs, including anti-β2GPI Abs, with epitope specificity identical to anti-β2GPI Abs affinity purified from APS patients.[4,10] Kolyada et al determined that significant levels of anti-β2GPI Abs spontaneously develop at ~8 weeks of age in this strain of male mice, rising further at 10 weeks of age, then staying at a plateau between 10 and 16 weeks.[1] Thrombus-inducing laser injuries to the cremasteric arteriolar vessels were administered in a systematic manner to mice