● ● ● **IMMUNOBIOLOGY**

Comment on Gramaglia et al, page 1669

Platelets: killers of parasites or patients?

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In this issue of *Blood*, Gramaglia et al[1] have provided valuable data on whether platelets are protective in malaria infection by directly killing *Plasmodium* parasites, or deleterious by driving adverse immune responses. Platelets are the cellular mediators of thrombosis. Although this is a very important biologic role, platelets are also the most numerous circulating cell with an inflammatory and immune modulatory function. The first, and still the best understood, disease context for platelet-driven inflammation is atherosclerosis. More recently, platelets have been shown to have important, largely adverse roles, in infectious disease processes such as sepsis, bacterial infections, viral infections, and parasitic infections, including infections with the malaria causative agent *Plasmodium*. With an increased appreciation for the immune and inflammatory functions of platelets in many diseases, there have been an increased number of studies, including study into platelets in malaria infection. There have been conflicting conclusions on whether platelets are "good or bad" players in malaria infection. Although there is a broad sense that platelets are proinflammatory and initiate or accelerate immune cell responses to infection in general, some reports have indicated a potential direct anti–*Plasmodium* parasite-killing role for platelets (see figure). This paper by Gramaglia et al provides a further understanding of whether platelets directly kill *Plasmodium* parasites, and if they do, does it actually limit the in vivo parasite burden.

The work of numerous laboratories has shown that platelets are key mediators of both immune responses and immune development. Platelets contain a large and diverse array of granule-stored chemokines and cytokines, and when stimulated, platelets also de novo produce inflammatory molecules that promote leukocyte activation and trafficking, as well as vascular inflammation. Although small in size ($\sim$2 μm in diameter), platelets have major immune effects due to their circulating
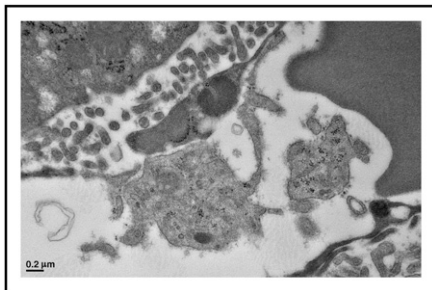
Platelets actively interact with liver endothelial cells in a *Plasmodium berghei* infection model. This platelet has formed a distinct contact interaction with the endothelial cell. These interactions occur in many tissue beds and with circulating immune cells contributing to the inflammatory pathogenesis of cerebral malaria. Electron microscopy image; scale bar, 0.2 μm.

numbers ($\sim$200 000/μL in humans) and the numerous inflammatory molecules they contain. Malaria-infected red blood cells (RBCs) can activate platelets that increase cerebral vasculature endothelial inflammation, leukocyte recruitment, and loss of the blood-brain barrier. Immune and endothelial cell responses to infection also induce vascular inflammation, more local platelet activation, and platelet interactions with an intact and inflamed endothelial cell layer, further driving platelet adhesion, activation, and an amplification of vascular inflammation. Direct antibacterial properties have been described for platelets, including bacteria engulfment and clearance and the release of microbicidal proteins from platelets,[2] leading to speculation that, similar to other immune cells, platelet immune responses may be protective and beneficial by directly clearing and killing pathogens.

So why the confusion on whether platelets are protective or deleterious in *Plasmodium* infection? Many studies have shown that in experimental cerebral malaria (eCM) platelets play a central role in the cerebral vascular disease pathogenesis,[3,4] and thus, platelets are

potentially "bad" in cerebral malaria. This concept is supported by human autopsy specimens.[5] However, a few recent studies concluded that platelets may have a direct role in killing *Plasmodium* parasites in infected RBCs.[6,7] This current study in *Blood* took a rigorous in vivo approach to show that although platelets drive an immune response to infection, they are unlikely to have direct protective in vivo parasite-killing functions.

There are many potential reasons for the different interpretations, including in vivo vs in vitro study, data analysis approaches, and interpretations, as well as the types of parasites used. Another very important factor is that we continue to better understand how platelets shape the immune response, and therefore, continue to gain a better understanding of how to best interpret in vivo platelet-related data. For example, the initial paper describing a potential direct parasite-killing role for platelets in malaria used an uncomplicated malaria (UM) model[6] in which the immune response to infection is very different than eCM immune responses. eCM models have significant platelet activation. UM models develop thrombocytopenia with less evidence of platelet activation. Immune and vascular responses to eCM infection are more important than the parasite itself in driving platelet activation. In addition, studies using mice that are genetically platelet-deficient must be carefully interpreted. We have recently described major immune alterations in platelet-deficient mice, including increased numbers of T-helper 17 type of T cells, and that the platelet chemokine PF4 regulates T-helper responses.[8] Therefore, interpretation of data from chronically platelet-deficient mice, particularly in more slowly developing infectious disease models like UM, must be carefully interpreted. The use of acute platelet depletion may be more appropriate in many situations, but again is complicated by the method used, such as introduction of a platelet-depletion antibody. This current study is very valuable in its thoughtful approach to measuring parasite burdens in acutely platelet-depleted and reconstituted mice. In addition, although models of eCM and UM are valuable in better understanding *Plasmodium*-related disease pathogenesis, separating the interpretation of each model and considering each independently are likely important distinctions due to their poorly understood differences in immune and vascular cell responses.

Any immune response is a double-edged sword: some are good for isolating, limiting, and clearing an infection, but exaggerated or prolonged immune responses can lead to tissue injury and long-term organ dysfunction. Understanding how platelets are active participants in well-validated model systems is therefore important to progress to a general understanding of vascular inflammatory disease pathogenesis.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

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● ● ● **LYMPHOID NEOPLASIA**

Is CMV in utero the first event in pediatric ALL?

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As reported in this issue of *Blood*, Francis et al hypothesized that a cytomegalovirus (CMV) infection in utero or a perinatal infection can initiate immune dysregulation during the critical development of fetal immune development and thereby play a role in childhood acute lymphoblastic leukemia (ALL).[1]

The DNA virome of 268 children who later developed ALL was characterized from neonatal blood spots using unbiased next-generation sequencing (NGS) and compared with the virome of 270 non–ALL controls. The authors found a higher prevalence of CMV infection in the group with ALL; it was also more prominent in the Hispanic group of ALL. Several human tumor DNA viruses are known to be involved in the development of a malignant clone. Viruses can persist in the lymphoid cells and suppress double-stranded DNA-break repair, also known as DNA damage response.[2,3] Because the DNA damage response protects the genome from accumulating deleterious mutations, downregulation is associated with an increased risk of clonal development. A viral infection may generate the aberrant clones of lymphocytes that precede ALL development.[4,5]

Even though epidemiological evidence proposes that ALL may be initiated by an in utero infection with a common pathogen, the identification of such a pathogen has not been made.[6-9] Unlike viral-specific methods, unbiased NGS can provide a holistic picture of the virome of ALL patients, thus facilitating identification of viral candidates that might lead to ALL development. The role of unbiased NGS in the study of the human virome has been notable during the past decade, aiding in the detection of known and unknown viruses from both isolated cases and from major disease outbreaks.

However, in a recent study published in the *British Journal of Cancer*, NGS was used to characterize the DNA virome present in neonatal blood spots, which were analyzed with the aim of searching for potential infectious agents in children who later developed ALL. No clear association between infections with DNA viruses in utero and development of ALL was found.[10]

In this study, ALL as a diagnostic group was analyzed. The authors state that they did not analyze ALL subgroups. It is generally agreed that subgroups of ALL have different biological characteristics. This, of course, has to be taken into consideration when interpreting the results.

Analysis of acute myeloid leukemia was complicated by the low incidence in the study sample and the later onset of disease, with few positive cases, despite patients being older at the time of diagnosis, meaning they would have acquired a CMV infection earlier.

Several other questions are raised by this study: if there was a higher prevalence of CMV infections, were there also more birth defects? Why would the Hispanic group be more vulnerable to CMV infections and development of ALL?

In summary, this paper reported interesting results that need to be explored further in a larger study, including information concerning birth defects, subgroup analysis of ALL, epidemiological data, and examination of the Hispanic group. Likewise, data from earlier studies where CMV infection was not found need to be reexamined.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

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