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. ■

REFERENCES

1. Gramaglia I, Velez J, Combes V, Grau GER, Wree M, van der Heyde HC. Platelets activate a pathogenic response to blood-stage *Plasmodium* infection but not a protective immune response. *Blood*. 2017;129(12):1669-1679.

2. Kraemer BF, Campbell RA, Schwertz H, et al. Novel anti-bacterial activities of β-defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog*. 2011;7(11):e1002355.

3. van der Heyde HC, Gramaglia I, Sun G, Woods C. Platelet depletion by anti-CD41 (αIIb) mAb injection early but not late in the course of disease protects against *Plasmodium berghei* pathogenesis by altering the levels of pathogenic cytokines. *Blood*. 2005;105(5):1956-1963.

4. Srivastava K, Cockburn IA, Swaim A, et al. Platelet factor 4 mediates inflammation in experimental cerebral malaria. *Cell Host Microbe*. 2008;4(2):179-187.

5. Grau GE, Mackenzie CD, Carr RA, et al. Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. *J Infect Dis*. 2003;187(3):461-466.

6. McMorran BJ, Marshall VM, de Graaf C, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science*. 2009;323(5915):797-800.

7. McMorran BJ, Wieczorski L, Drysdale KE, et al. Platelet factor 4 and Duffy antigen required for platelet killing of Plasmodium falciparum. *Science*. 2012;338(6112):1348-1351.

8. Shi G, Field DJ, Ko KA, et al. Platelet factor 4 limits Th17 differentiation and cardiac allograft rejection. *J Clin Invest*. 2014;124(2):543-552.

DOI 10.1182/blood-2017-01-764621

● ● ● LYMPHOID NEOPLASIA

Comment on Francis et al, page 1680

Is CMV in utero the first event in pediatric ALL?

Britt Gustafsson KAROLINSKA INSTITUTET

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. ■

REFERENCES

1. Francis SS, Wallace AD, Wendt GA, et al. In utero cytomegalovirus infection and development of childhood acute lymphoblastic leukemia. *Blood*. 2017;129(12):1680-1684.

2. Chabay PA, Preciado MV. EBV primary infection in childhood and its relation to B-cell lymphoma development: a mini-review from a developing region. *Int J Cancer*. 2013;133(6):1286-1292.

3. Weitzman MD, Ornelles DA. Inactivating intracellular antiviral responses during adenovirus infection. *Oncogene*. 2005;24(52):7686-7696.

4. Boggs SS. The hematopoietic microenvironment: phylogeny and ontogeny of the hematopoietic microenvironment. *Hematology*. 1999;4(1):31-44.

5. Ornelles DA, Gooding LR, Garnett-Benson C. Neonatal infection with species C adenoviruses confirmed in viable cord blood lymphocytes. *PLoS One*. 2015;10(3):e0119256.

6. Bogdanovic G, Jernberg AG, Priftakis P, Grillner L, Gustafsson B. Human herpes virus 6 or Epstein-Barr virus were not detected in Guthrie cards from children who later developed leukaemia. *Br J Cancer*. 2004;91(5):913-915.

7. Gustafsson B, Bogdanovic G. Specific viruses were not detected in Guthrie cards from children who later developed leukemia. *Pediatr Hematol Oncol*. 2007;24(8):607-613.

8. Gustafsson B, Carstensen J. Evidence of space-time clustering of childhood acute lymphoblastic leukaemia in Sweden. *Br J Cancer*. 1999;79(3-4):655-657.

9. Gustafsson B, Jernberg AG, Priftakis P, Bogdanovic G. No CMV DNA in Guthrie cards from children who later developed ALL. *Pediatr Hematol Oncol*. 2006;23(3):199-205.

10. Bogdanovic G, Pou C, Barrientos-Somarribas M, et al. Virome characterisation from Guthrie cards in children who later developed acute lymphoblastic leukaemia. *Br J Cancer*. 2016;115(8):1008-1014.

DOI 10.1182/blood-2017-01-758730

Comment on Deppermann et al, page 1702

Platelet granules in vascular integrity

Simon F. De Meyer KU LEUVEN CAMPUS KULAK KORTRIJK

In this issue of *Blood*, Deppermann et al dissect the role of platelet granule secretion in maintaining vascular integrity during inflammation.[1] The authors show that mice lacking platelet granule secretion do not bleed in skin or lung inflammation models. Lack of platelet release however resulted in increased brain hemorrhage after experimental stroke. The latter finding is of clinical importance when designing novel therapies to improve stroke outcome.

It is well known that platelets are crucial for stopping bleeding. Platelets prevent excessive posttraumatic blood loss at sites of vascular injury by forming a platelet plug. Upon exposure of the subendothelial extracellular matrix, platelets are recruited to the site of injury and become activated, resulting in firm adhesion and subsequent platelet aggregation. The molecular mechanisms underlying the formation of a hemostatic platelet plug are relatively well understood: upon exposure of the subendothelial matrix, platelets either interact directly with matrix proteins (eg, via glycoprotein VI [GPVI] and α2β1 to collagen) or bind to von Willebrand factor (VWF) that is deposited at the site of injury. Transient interactions between platelet GPIb and VWF support platelet tethering at sites of high shear stress. Firm adhesion and subsequent aggregation is mediated by activated integrin receptors such as αIIbβ3. G-protein–coupled receptors mediate activation signals after being triggered by soluble agonists such as thrombin, thromboxane A2, and adenosine 5′-diphosphate, which reinforce thrombus propagation.

Interestingly, newer insights reveal that platelets also safeguard a different form of hemostasis by maintaining vascular integrity during acute inflammation.[2] It was recently shown that single platelets seal vascular breaches caused by neutrophils.[3] In contrast to our understanding of vascular injury-induced thrombus formation, much less is known about the mechanisms used by platelets to prevent inflammation-induced hemorrhage. Intriguingly, the process by which platelets maintain vascular integrity at the site of inflammation is independent from the ability of platelets to form a hemostatic platelet plug. Indeed, neither the adhesion receptors GPIb and αIIbβ3, nor signaling via G-protein–coupled receptors are necessary to maintain vascular integrity in inflamed organs.[4,5] The immunoreceptor tyrosine-based activation motif receptors GPVI and CLEC2 on platelets have been identified as crucial mediators supporting vascular integrity.[3,5] However, the exact triggers that induce platelet signaling and the downstream effector mechanisms involved in the prevention of inflammatory bleeding remain unclear. Platelet components released from intracellular storage granules have been suggested to be implicated in this process.[6]

To address the role of platelet granule content in maintaining vascular integrity in inflammation, Deppermann et al generated *Unc13d*$^{-/-}$/*Nbeal2*$^{-/-}$ mice.[1] Platelets from these mice are unable to secrete their α- or dense-granule content. The authors used these mice in models of lung inflammation, skin inflammation, and brain infarction. Similar to previous studies, intradermal hemorrhage was observed in platelet-depleted wild-type (WT) mice at the site of inflammation. Strikingly, no bleeding was observed in the inflamed skin of *Unc13d*$^{-/-}$/*Nbeal2*$^{-/-}$ mice. Analogous results were observed in lung inflammation. These experiments show that release of α or dense granules is not necessary to maintain vascular integrity at sites of acute inflammation in skin and lung. Much different results were however obtained in the stroke model used by the authors. Indeed, when subjected to transient middle cerebral artery occlusion, *Unc13d*$^{-/-}$/*Nbeal2*$^{-/-}$ mice were prone to intracranial bleeding in the infarcted areas. Cerebral hemorrhage in these mice resulted in a significantly increased mortality compared with WT animals. In an elegant approach using platelet transfusion experiments, the authors showed that the observed effects of combined Munc13-4 and Nbeal deficiency were related to the platelet-specific secretion effects and not to potential defects in other cells.

The results from Deppermann et al are important in 2 ways. First, this study shows that platelets use different pathways to ensure hemostasis in different inflammatory settings and vascular beds. Second, the results demonstrate that platelet granule release is important to safeguard hemostasis during stroke injury. The latter insight might become particularly relevant for the development of novel treatment of ischemic stroke. Maintaining cerebral hemostasis during a stroke is of high clinical relevance because intracranial bleeding often leads to aggravation of the disease state and increase of mortality. Strategies to prevent or treat acute ischemic stroke should not increase the risk cerebral bleeding. In this context, anti-thromboinflammatory therapeutics have shown promising preclinical results.[7] Thromboinflammation causes progressive ischemic brain damage via complex pathways that include early platelet adhesion and activation but not platelet aggregation.[7] Importantly, the release of α or dense granules also contributes to thromboinflammatory brain injury.[8,9] Correspondingly, in the current study, Deppermann et al observed reduced infarct volumes and fewer neurological deficits in those *Unc13d*$^{-/-}$/*Nbeal2*$^{-/-}$ mice that did not die of intracranial hemorrhage. Hence, although preventing platelet granule release might seem an attractive strategy to reduce