

are major mediators of death in sepsis. *Nat Med.* 2009; 15(11):1318-1321.

8. Fuchs TA, Bhandari AA, Wagner DD. Histones induce rapid and profound thrombocytopenia in mice. *Blood.* 2011;118(13):3708-3714.

9. Motto DG, Chauhan AK, Zhu G, et al. Shigatoxin

triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *J Clin Invest.* 2005;115(10):2752-2761.

10. Clark SR, Ma AC, Tavener SA, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med.* 2007;13(4):463-469.

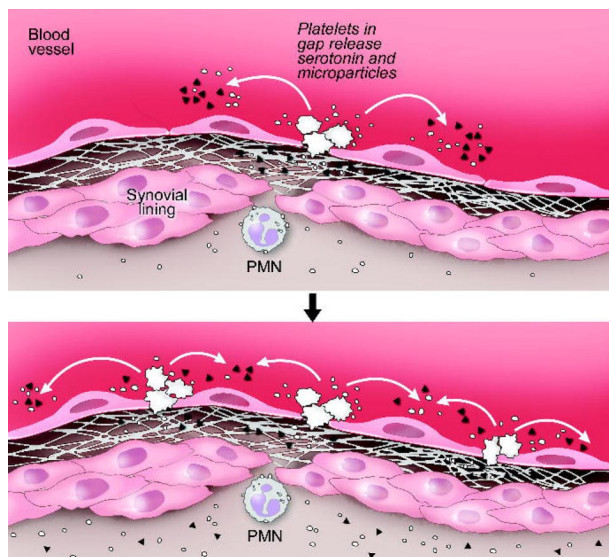
## ● ● ● VASCULAR BIOLOGY

Comment on Cloutier et al, page 1334

# The functional dissonance of platelets

Enming Joseph Su and Daniel A. Lawrence UNIVERSITY OF MICHIGAN MEDICAL SCHOOL

In this issue of *Blood*, Cloutier et al answer a long-standing but unappreciated question about the influence of platelets on vascular permeability.<sup>1</sup>



**Proposed pathway for the formation of gaps and amplification of the vasculature permeability by platelets during arthritis.** (Top panel) Gaps between endothelial cells in arthritic joints are formed. The GPVI-expressing platelets are activated by the leakage during disease. Note that the precise anatomic location of platelet activation, and the route by which microparticles enter the joint remains speculative.  $\Delta$  indicates serotonin and  $\circ$ , platelet microparticle. Professional illustration by Steve Moskowitz, Advanced Medical Graphics. See Figure 7 in the article by Cloutier et al that begins on page 1334.

**T**he role of platelets in responding to vascular injury and the prevention blood loss is very well understood. Cloutier and colleagues have now used fluorescent microspheres, 2-photon microscopy, and genetically modified mice to convincingly demonstrate that in addition to their role in hemostasis, platelets can also promote vascular leakage. More importantly, using a model of rheumatoid arthritis, they present data that uncoupled vascular permeability from inflammation, and their data suggest that platelets can increase vascular permeability directly, through the release of serotonin (see figure).

The concept of platelet-induced vascular permeability is not new; more than 40 years

ago Nachman et al reported that platelet granule extracts were capable of inducing vascular permeability.<sup>2</sup> However, despite efforts to identify the molecular components responsible for this activity,<sup>3</sup> the molecular mechanism whereby platelets promote vascular permeability have remained elusive. Nevertheless, over the years circumstantial evidence has supported the notion that platelets can induce vascular leakage.<sup>4,5</sup> Here, Cloutier et al present convincing evidence that endothelial gap formation in arthritic vessels depends on the presence of platelets, and that this activity is independent of the inflammation normally seen in rheumatoid arthritis. Specifically, animals treated with platelet-

depleting antibodies showed significantly less vascular leakage than controls after the onset of arthritis, as demonstrated by the direct injection of fluorescent microspheres. In addition, inducing inflammation with injections of IL-1 $\beta$  did not change the outcome of platelet-depleting antibody treatment on vascular permeability, suggesting that platelets and not inflammation were the cause of increased vascular permeability in rheumatoid arthritis.

Interestingly, the size of microspheres that gained access to arthritic joints in this study appeared to be limited to 0.45  $\mu\text{m}$  to 0.84  $\mu\text{m}$ , a range that is very similar to serotonin-induced endothelial gaps seen in vessels in the cremaster muscle identified by electron microscopy (0.1–0.8  $\mu\text{m}$ ).<sup>6</sup> Because platelet-dense granules are known to contain high concentrations of serotonin,<sup>7</sup> Cloutier et al investigated whether serotonin in platelets was associated with vascular permeability in rheumatoid arthritis. These studies demonstrate that unlike patients with osteoarthritis, patients with rheumatoid arthritis have both more platelet microparticles and serotonin in their synovial fluid. They also found that direct injection of serotonin into healthy mice induced vascular leakage reminiscent of arthritic animals. These data suggest that platelet-derived serotonin was important in endothelial gap formation. To test this hypothesis, Cloutier et al took advantage of mice deficient in the serotonin transporter (SERT), which enables platelets to take up and store serotonin.<sup>8</sup> Using the SERT-deficient mice, Cloutier et al convincingly demonstrated that mice with low levels of serotonin in their platelets had significantly reduced fluorescent microspheres accumulation in their joints during arthritis development. Finally, Fluoxetine, a psychiatric drug that inhibits the uptake of serotonin, significantly reduced vascular leak in their rheumatoid arthritis mouse model. This observation is consistent with a previous report by Sacre et al on the efficacious effect of Fluoxetine in rheumatoid arthritis but may offer a different mechanism.<sup>9</sup>

Overall, this novel finding that platelet-induced vascular permeability is mediated via serotonin in rheumatoid arthritis signifies a change in the view about how platelets can affect vascular integrity. More importantly, understanding this new pathway may generate new treatment options for diseases such as rheumatoid arthritis.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

## REFERENCES

1. Cloutier N, Paré A, Farndale RW, et al. Platelets can enhance vascular permeability. *Blood*. 2012;120(6):1334-1343.
2. Nachman RL, Weksler B, Ferris B. Increased vascular permeability produced by human platelet granule cationic extract. *J Clin Invest*. 1970;49(2):274-281.
3. Nachman RL, Weksler B, Ferris B. Characterization of human platelet vascular permeability-enhancing activity. *J Clin Invest*. 1972;51(3):549-556.
4. Kirsch CM, Brokaw JJ, Prow DM, White GW. Mechanism of platelet activating factor-induced vascular leakage in the rat trachea. *Exp Lung Res*. 1992;18(4):447-459.
5. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest*. 2006;116(12):3211-3219.
6. Majno G, Palade GE. Studies in inflammation. 1. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J Biophys Biochem Cytol*. 1961;11:571-605.
7. Chatterjee D, Anderson GM. The human platelet dense granule: serotonin uptake, tetrabenazine binding, phospholipid and ganglioside profiles. *Arch Biochem Biophys*. 1993;302(2):439-446.
8. Mercado CP, Kilic F. Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. *Mol Interv*. 2010;10(4):231-241.
9. Sacre S, Medghalchi M, Gregory B, Brennan F, Williams R. Fluoxetine and citalopram exhibit potent anti-inflammatory activity in human and murine models of rheumatoid arthritis and inhibit toll-like receptors. *Arthritis Rheum*. 2010;62(3):683-693.