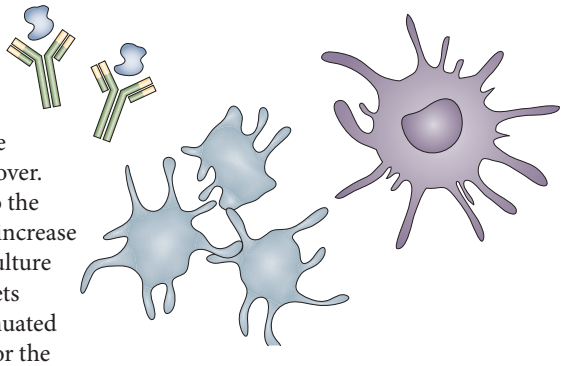
CONNECTIVE TISSUE DISEASES

Activated platelets as a target for SLE therapy?

New research linking platelet activation, immune complexes (ICs) and interferon (IFN)-α in the pathogenesis of systemic lupus erythematosus (SLE) could provide support for the use of antiplatelet strategies as adjuvant therapy for the disease. Previous research has shown that levels of soluble CD154 (sCD154) are elevated in the sera of patients with SLE, and that these levels correlate with disease activity. "However," points out Pierre Duffau, lead author of the paper reporting the findings in *Science Translational Medicine*, "the origin of the sCD154 remained somewhat of a mystery." The investigators hypothesized that the source could be CD154 released from the surface of activated platelets, and that platelet-derived CD154 could drive SLE disease activity through the IFN-α system.

In *ex vivo* experiments, they confirmed that platelets isolated from patients with active SLE are activated, as characterized by overexpression of CD154, and that CD154 levels correlated with disease severity. In addition, platelets isolated from healthy controls exposed to sera from patients with SLE were activated in a dose-dependent fashion. Notably, this activation could be inhibited by prior incubation with an anti-CD32 antibody or by depleting the SLE sera of IgG; by contrast, blocking IFN-α, CD154 or interleukin-6 had no such inhibitory effect. Western blot analysis showed that IgG was found in platelet lysates from patients with SLE but was "barely detectable" in those with rheumatoid arthritis or in healthy individuals. Together, the findings pointed to the platelets being activated by ICs through a CD32-dependent mechanism.

But what about the consequences of platelet activation in SLE? Duffau *et al.* used flow cytometry and confocal microscopy to demonstrate that activated platelets aggregate with circulating antigen-presenting cells—including monocytes and plasmacytoid dendritic cells (pDCs; a major source of type I IFN in SLE). In addition, incubating purified pDCs with ICs alone induced some IFN production, but the simultaneous presence of activated platelets increased the level of production several times over. The addition of $CD154^+$ L-cells to the pDC–IC culture also induced an increase in IFN-α secretion, whereas co-culture of pDCs, ICs and activated platelets with an anti-CD40 antibody attenuated the increase, highlighting a role for the CD154–CD40 pathway in potentiating IFN-α production.



To target the mechanisms of platelet activation *in vivo*, the authors turned to the NZBxNZW(F1) and MRL/*lpr* mouse models of lupus. Similar to the mechanisms of platelet activation in human disease, NZBxNZW(F1) mice show elevated serum levels of sCD154 and occurrence of platelet–DC aggregates, and sera from these mice activate normal platelets, leading to increased production of IFN-α by stimulated pDCs. Platelet depletion reduced inflammatory infiltrates and signs of glomerulonephritis in these mice. By contrast, the kidneys of animals transfused with activated platelets had increased IC deposits and inflammatory infiltrates. Similarly, daily administration of clopidogrel, an inhibitor of platelet activation, improved disease measures and overall survival in NZBxNZW(F1) and MRL/*lpr* mice.

"The findings provide a link between CD154 and IFN-α, two important cytokines in the pathogenesis of SLE," says Duffau, "and strongly highlight antiplatelet treatment as a valuable therapy for lupus." Lars Ronnblom of Uppsala University agrees that platelets undoubtedly have a role in SLE pathogenesis, but maintains that their role in the IFN response remains far from certain. As an example, he points out, type I IFN exacerbates disease in NZBxNZW(F1) mice and ameliorates disease in the MLR/*lpr* model, whereas inhibition of platelets improved disease severity in both these models in the study by Duffau *et al.* "A more direct experiment would be to take an SLE patient with an IFN-α signature, administer clopidogrel to block platelets *in vivo*, and see if the IFN signature decreases or disappears," suggests Ronnblom.

George Tsokos of Harvard Medical School commends the report for bringing together several findings that were known to researchers in the field, although he too suggests that the full story of platelet activation in SLE could yet prove more complex than outlined in this paper. Nonetheless, Tsokos agrees that the results confirm the need to include antiplatelet strategies in the treatment of patients with SLE: "I think there is sufficient evidence to try platelet inhibitors or platelet depletion as an adjuvant treatment in patients with SLE, particularly those with lupus nephritis."

Duffau *et al.* plan to undertake a clinical trial of clopidogrel in patients with SLE. "Lupus patients are prone to premature cardiovascular disease, which is a leading cause of mortality," says Duffau. "Thus, a long-term antiplatelet strategy may affect not only the immune aspects of lupus pathogenesis but also cardiovascular problems related to the disease."

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Original article Duffau, P. *et al.* Platelet CD154 potentiates interferon-α secretion by plasmacytoid dendritic cells in systemic lupus erythematosus. *Sci. Trans. Med.* **2**, 47ra63 (2010)