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EDITORIAL COMMENT

Interferon Interrupted

Are $P2Y_{12}$ Antagonists the Next Step to Target Platelets in Autoimmunity?

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$P2Y_{12}$ receptor antagonists (eg, clopidogrel, prasugrel, and ticagrelor) are commonly prescribed antiplatelet agents used to treat and avoid cardiovascular events. These drugs limit thrombosis by preventing adenosine diphosphate-mediated platelet activation via the $P2Y_{12}$ receptor, suppressing a well-established downstream signaling pathway that would usually support platelet aggregation and degranulation. Although the conventional mechanisms of $P2Y_{12}$ antagonists have been known for decades, growing evidence suggests that they also regulate inflammatory responses and that their roles extend beyond simply suppressing platelet (re)activity.

Systemic lupus erythematosus (SLE) is a chronic inflammatory multisystem autoimmune disease characterized by interferon (IFN)-driven responses that culminate in immune dysregulation and organ injury. SLE patients are particularly at risk for cardiovascular diseases, and often display increased levels of both baseline platelet activation and platelet sensitivity to agonists ex vivo.[1] Patients with SLE receiving $P2Y_{12}$ antagonists appear to have reduced circulating levels of platelet-derived CD40 ligand and fewer platelet-leukocyte aggregates,[2] both of which are hallmarks of SLE. In addition to increased (re)activity, platelets from patients with SLE display elevated gene expression of type I IFN, along with several IFN-related proteins, which are further enriched in patients with a history of vascular disease.[3] Such findings imply that platelets contribute to the inflammatory milieu of SLE beyond acute thrombotic events.

The ability of the systemic environment to modulate the platelet transcriptome is a growing topic with increasingly recognized importance, best highlighted in inflammatory diseases including COVID-19, sepsis, and SLE.[4] Changes in platelet RNA predominantly arise from transcriptional alterations within platelet-producing megakaryocytes (MKs). This link between the MK and platelet transcriptome allows researchers to study drug-induced changes to MK gene expression, which can be used as a surrogate for predicting the platelet RNA profile without the need for expensive animal models or fresh blood samples from patients. By differentiating $CD34^+$ hematopoietic progenitors into MKs, researchers can also bypass the difficulties of studying platelets in vitro, which are anucleate and cannot tolerate cell culture.

In this issue of *JACC: Basic to Translational Science*, Sowa et al[5] show that platelets derived from SLE patients have increased levels of several IFN-stimulated genes (ISGs), and that treating healthy volunteers with $P2Y_{12}$ antagonists downregulated baseline expression of ISGs in platelets. The investigators also demonstrated that a $P2Y_{12}$ antagonist could reduce ISG expression in MKs differentiated from $CD34^+$ hematopoietic progenitors both at baseline and in response to IFNα. These findings highlight a nonconventional role for $P2Y_{12}$ antagonists in regulating gene expression along the MK-platelet axis and suggest a novel mechanism through which $P2Y_{12}$ antagonists regulate IFN-driven inflammatory conditions. Interestingly, and in contrast to $P2Y_{12}$ antagonists, Sowa et al[5] found the transcriptome of both aspirin-treated MKs and platelets isolated from aspirin-treated individuals were largely unchanged, implying that transcriptional regulation by $P2Y_{12}$

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antagonists was not due to a common downstream mechanism of antiplatelet drugs.

The work of Sowa et al[5] and others highlight the exciting potential for adopting MKs as a surrogate for studying platelet biology. For example, the recent revolution in gene editing technologies has led to CRISPR-mediated screens of $CD34^+$ progenitor-derived MKs for the rapid assessment of genes associated with platelet function.[6] Although MKs are not simply "big platelets" and often lack conventional platelet responses to agonists (eg, aggregation), such approaches will likely become one of a growing number of tools to explore platelet biology.

Despite being able to take up RNA derived from other cells within the vascular niche, platelets predominantly inherit their RNA from MKs during thrombopoiesis. However, caution should be taken when comparing the transcriptome of 2 extremely different cells, and the assumption that MKs faithfully model the platelet transcriptome remains far from settled. This point is highlighted in the work of Sowa et al,[5] who demonstrate significant discrepancies between the MK and platelet transcriptome following $P2Y_{12}$ treatment (see their Figure 2C). These differences could derive from the nature of inhibition (in vivo for platelets, in vitro for MKs), the fact that platelets and $CD34^+$ progenitor-derived MKs did not originate from the same donor, or that there is some degree of selectivity when MKs package RNA transcripts into newly forming platelets. The extent and physiological relevance of RNA translation into protein by platelets during either their activation or quiescent transit through circulation also remains an important and largely unanswered question. It is therefore key that studies exploring the MK and platelet transcriptome include follow-up experiments that assess both the more consequential proteome and functional cellular responses.

In their work, Sowa et al[5] robustly demonstrate that a $P2Y_{12}$ inhibitor (AZD 1283) downregulates ISG expression in cultured MKs and provide evidence to suggest that such effects occur by suppressing the mammalian target of rapamycin signaling pathway. Whether this inhibition comes from direct action on $P2Y_{12}$ (inhibitory concentration at 50% = 11 nM) or from off-target effects at higher concentrations remains unknown. Interestingly, mammalian target of rapamycin signaling has already been shown to regulate both platelet aggregation and MK maturation.[7,8] Future detailed experiments would therefore deepen our understanding of the underlying mechanism by which $P2Y_{12}$ antagonists influence gene expression in MKs and their platelet progeny. The fact that, unlike platelets, very little is known about adenosine diphosphate/$P2Y_{12}$ signaling in MKs also offers an extra avenue for follow-up studies beyond SLE and inflammatory diseases.

In addition to the pharmacological blockade of $P2Y_{12}$ signaling described by Sowa et al,[5] platelets isolated from patients with SLE often present with lower levels of the $P2Y_{12}$ receptor at both the RNA and protein level.[9] This observation suggests an intrinsic protective response by MKs to limit the pathological effects of platelets in SLE. However, the molecule(s) responsible for triggering this response in the bone marrow of SLE patients remains unknown.

The current study by Sowa et al[5] makes important steps in understanding the complex inflammatory and thrombotic contributions of platelets to SLE, while providing further preclinical rationale for considering $P2Y_{12}$ antagonists as an adjuvant therapeutic approach. The hope for this and future work is that using MKs as a surrogate for platelet gene expression will mechanistically inform clinical trials (NCT02320357)[2] exploring the potential use of $P2Y_{12}$ inhibitors for the treatment and prevention of inflammatory diseases such as SLE and beyond.

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