and during follow-up in chronic lymphocytic leukemia. *Haematologica.* 2011;96(8):1161-1169.

4. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. *Leukemia.* 2007;21(12):2442-2451.
5. Baliakas P, Jeromin S, Iskas M, et al; ERIC, the European Research Initiative on CLL. Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. *Blood.* 2019;133(11):1205-1216.
6. Thompson PA, O'Brien SM, Wierda WG, et al. Complex karyotype is a stronger predictor than del(17p) for an inferior outcome in relapsed or refractory chronic lymphocytic leukemia patients treated with ibrutinib-based regimens. *Cancer.* 2015;121(20):3612-3621.
7. Leeksma AC, Baliakas P, Moysiadis T, et al. Genomic arrays identify high-risk chronic lymphocytic leukemia with genomic complexity: a multi-center study. *Haematologica.* 2021;106(1):87-97.
8. Campo E, Cymbalista F, Ghia P, et al. *TP53* aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. *Haematologica.* 2018;103(12):1956-1968.
9. Rossi D, Khiabanian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. *Blood.* 2014;123(14):2139-2147.
10. Navrkalova V, Plevova K, Hynst J, et al. LYmphoid NeXt-Generation Sequencing (LYNX) Panel: A Comprehensive Capture-Based Sequencing Tool for the Analysis of Prognostic and Predictive Markers in Lymphoid Malignant Tumors. *J Mol Diagn.* 2021;23(8):959-974.

DOI 10.1182/blood.2021013285

PLATELETS AND THROMBOPOIESIS

Comment on Lee-Sundlov et al, page 2408

pDC as a modulator of platelet production

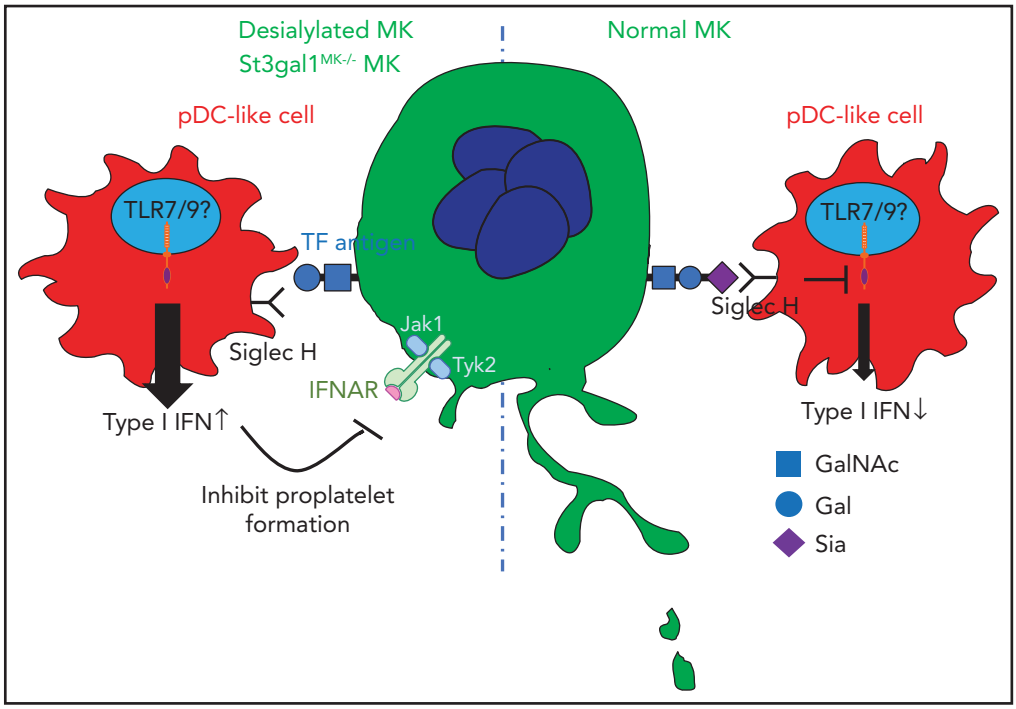
Taisuke Kanaji | Scripps Research

In this issue of *Blood*, Lee-Sundlov et al[1] demonstrate a novel surveillance mechanism of megakaryocyte (MK) sialylation by plasmacytoid dendritic cell (pDC)-like cells and its regulatory effects on platelet production via type I interferon (IFN-I) signaling.

Desialylation has been recognized as a mechanism for platelet clearance in various conditions, including infection and immune thrombocytopenia (ITP), and plays a role in the removal of aged platelets.[2] However, it remains unclear whether, and if so how, MK desialylation affects thrombopoiesis. The study by Lee-Sundlov et al identified a novel surveillance system of MK sialylation status by pDC-like immune cells, leading to inhibition of platelet production from MKs. Targeted deletion of *O*-glycan sialyltransferase (St3gal1), specifically in MK lineage (St3gal1$^{MK-/-}$), generated a mouse model with increased Thomsen-Friedenreich (TF) antigen expression on MKs. TF antigen, which is normally masked by terminal sialylation, becomes exposed when St3gal1 is deleted. The St3gal1$^{MK-/-}$ mice had thrombocytopenia with platelet counts at ~50% of the control mice. Interestingly, thrombocytopenia in St3gal1$^{MK-/-}$ mice was reversed by treatment with dexamethasone or targeted deletion of Jak3, suggesting an immune-mediated component. Antibody-mediated cell depletion studies and RNASeq identified unique pDC subtypes with increased transcripts of immunoglobulin rearrangement genes specifically in St3gal1$^{MK-/-}$ mice. pDC clusters identified in St3gal1$^{MK-/-}$ bone marrow (BM) also showed enrichment in IFN-I gene sets. The authors further showed that thrombocytopenia in St3gal1$^{MK-/-}$ mice could be restored or ameliorated by treatment with antibodies against Siglec (sialic acid–binding immunoglobulin-like lectin) H.[3] By coculturing pDCs with St3gal1$^{MK-/-}$ MKs, it was shown that pDCs inhibit thrombopoiesis through secretion of IFN-I and potentially through involvement of Siglec H (see figure). Based on these results, the authors concluded that the sialic acid moiety of MKs regulates platelet production via immune cells, mainly CD4$^+$ pDC-like immune cells in the BM.

The most important finding of this article is the recognition of desialylated MK by immune cells, specifically those with a pDC-like signature. The regulatory effect of these cells on platelet production was elegantly demonstrated by an ex vivo coculture study. Further studies are needed to prove this hypothesis by depleting pDCs in vivo. pDCs express endosomal Toll-like receptor (TLR) 7/9, which senses microbial or self-DNA/RNA, promoting secretion of large quantities of IFN-I. In this study, enhanced IFN-I secretion was noted when pDCs were cocultured with St3gal1$^{MK-/-}$. Blockade of IFN-I ameliorated thrombocytopenia in St3gal1$^{MK-/-}$ mice. Of note, enhanced colocalization of CD4$^+$ cells with MKs was observed in the St3gal1$^{MK-/-}$ BM. Thus, it is intriguing to speculate that pDCs recognize and ingest a part of the desialylated MKs. Internalization of ingested MK fragments and trafficking to endosomes may contribute to TLR activation and IFN-I secretion. In this scenario, ingested desialylated MKs may also help potentiate antigen-specific T-cell responses, bridging innate and adoptive immunity in cases of ITP. In the normal state of sialylated *O*-glycan on MKs, Siglec H may inhibit IFN-I secretion. The contribution of Siglec H needs to be further investigated using Siglec H knockout mice. It is not yet known which component of MKs activates TLRs (TLR7 and/or TLR9) in pDCs to activate IFN-I signaling. Although the major agonists for TLR7 are pathogen-derived single-stranded RNAs, host-derived RNAs (eg, microRNAs [miRNAs] and transfer RNAs [tRNAs]) have also been known to serve as endogenous agonists to activate endosomal TLRs. Thus, it is feasible to speculate that MK RNAs, including miRNA and tRNA, activate TLR7 in pDCs.[4] Alternatively, mitochondrial DNA of MKs might stimulate TLR9.[5] Further investigations are necessary to test these hypotheses .

This study also identified increased anti-TF antigen antibodies in pediatric patients with ITP, suggesting pDC-mediated inhibition of thrombopoiesis as a part of the mechanism inducing thrombocytopenia. Although the platelet antigen is not determined in the patients studied in this article, it is well established that binding of the anti-GPIbα antibody causes desialylation of platelet GPIbα.[6] Thus, it is likely



TF antigen on MK is normally masked by terminal sialylation but becomes exposed when sialic acids are depleted (desialylated) or St3gal1 is genetically deleted. pDC-like immune cells sense TF antigen exposure on MKs, which induces IFN-I secretion and inhibits platelet release (left). In the steady state of sialylated *O*-glycan on MKs, IFN-I secretion is inhibited, possibly via Siglec H, and normal thrombopoiesis occurs (right).

that binding of the autoantibodies induces desialylation of platelet membrane proteins, leading to TF antigen exposure and enhanced production of anti-TF antigen antibodies. If the anti-GPIbα antibody binds not only to platelets but also to MKs in the BM,[7] pDCs in the BM and possibly in the spleen might recognize desialylated MKs and activate the IFN-I pathway. In addition to the roles in the progression of disease, pDCs may also play a role in the initiation of ITP.[8] ITP in pediatric patients is often preceded by viral infections. Viral or bacterial infections cause desialylation and TF antigen exposure on MKs, which can be recognized and ingested by pDCs. Ingested MK components translocated to endosomes may activate TLR-IFN-I signaling and also trigger T-cell responses, potentiating the production of antiplatelet antibodies. Further investigation is warranted to determine the role of pDCs in the pathophysiology of ITP. The study by Lee-Sundlovet paves the way to understand the molecular mechanisms of immune-mediated and other causes of thrombocytopenia.

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

REFERENCES

1. Lee-Sundlov MM, Burns RT, Kim TO, et al. Immune cells surveil aberrantly sialylated *O*-glycans on megakaryocytes to regulate platelet count. *Blood*. 2021;138(23):2408-2424.
2. Grozovsky R, Giannini S, Falet H, Hoffmeister KM. Regulating billions of blood platelets: glycans and beyond. *Blood*. 2015;126(16):1877-1884.
3. Blasius AL, Colonna M. Sampling and signaling in plasmacytoid dendritic cells: the potential roles of Siglec-H. *Trends Immunol*. 2006;27(6):255-260.
4. Pawar K, Shigematsu M, Sharbati S, Kirino Y. Infection-induced 5'-half molecules of tRNA-HisGUG activate Toll-like receptor 7. *PLoS Biol*. 2020;18(12):e3000982.
5. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464(7285):104-107.
6. Li J, van der Wal DE, Zhu G, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nat Commun*. 2015;6(1):7737.
7. Morodomi Y, Kanaji S, Won E, Ruggeri ZM, Kanaji T. Mechanisms of anti-GPIbα antibody-induced thrombocytopenia in mice. *Blood*. 2020;135(25):2292-2301.
8. Davison LM, Jørgensen TN. Sialic acid-binding immunoglobulin-type lectin H-positive plasmacytoid dendritic cells drive spontaneous lupus-like disease development in B6.Nba2 mice. *Arthritis Rheumatol*. 2015;67(4):1012-1022.

DOI 10.1182/blood.2021012975