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REFERENCES

1. Olson TL, Cheon H, Xing JC, et al. Frequent somatic *TET2* mutations in chronic NK-LGL leukemia with distinct patterns of cytopenias. *Blood*. 2021;138(8):662-673.
2. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.
3. Pastoret C, Desmots-Loyer F, Drillet G, et al. Linking the KIR phenotype with *STAT3* and *TET2* mutations to identify chronic lymphoproliferative disorders of NK cells. *Blood*. 2021;137(23):3237-3250.
4. Epling-Burnette PK, Liu JH, Catlett-Falcone R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest*. 2001;107(3):351-362.
5. Jerez A, Clemente MJ, Makishima H, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. *Blood*. 2012;120(15):3048-3057.
6. Galm O, Yoshikawa H, Esteller M, Osieka R, Herman JG. SOCS-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma. *Blood*. 2003;101(7):2784-2788.
7. Ley TJ, Miller C, Ding L, et al: Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-2074.
8. Smith AE, Mohamedali AM, Kulasekararaj A, et al. Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. *Blood*. 2010;116(19):3923-3932.
9. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012;44(11):1179-1181.
10. Sun X, Hasanali ZS, Chen A, et al. Suberoylanilide hydroxamic acid (SAHA) and cladribine synergistically induce apoptosis in NK-LGL leukaemia. *Br J Haematol*. 2015;168(3):371-383.

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PLATELETS AND THROMBOPOIESIS

Comment on Han et al, page 674

Decitabine revives Treg function in ITP

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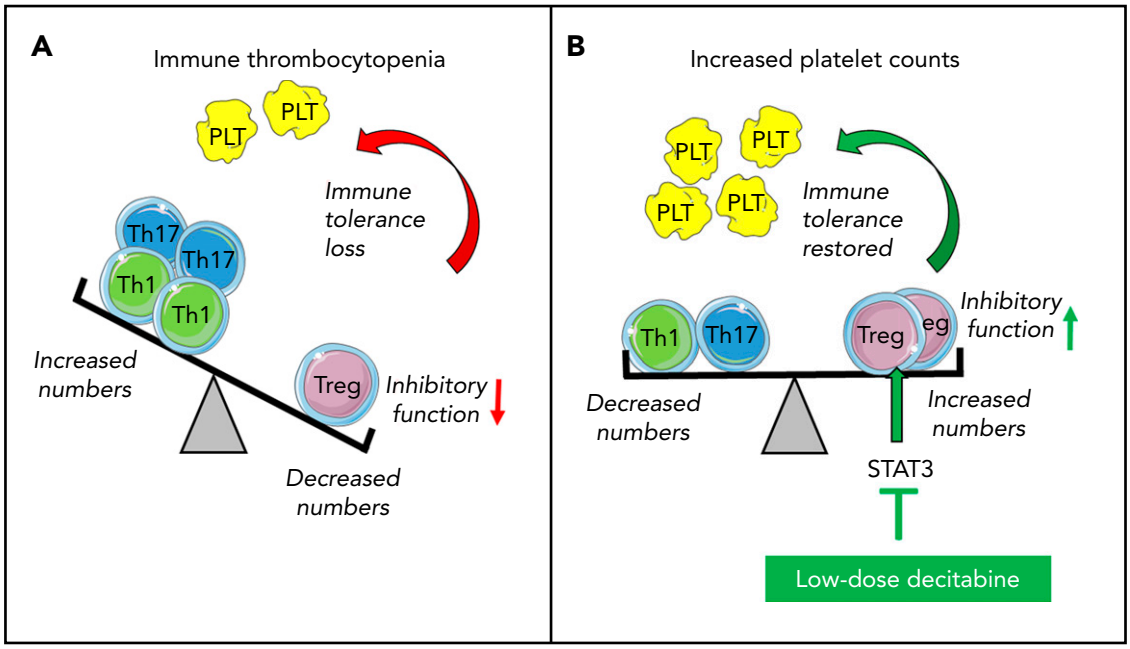
In this issue of *Blood*, Han et al describe that the hypomethylating agent decitabine increases platelet counts in immune thrombocytopenia (ITP) by restoring T-cell homeostasis through modulation of regulatory T cells (Tregs), thereby increasing their numbers and enhancing their immunosuppressive function.[1]

ITP is an acquired autoimmune bleeding disorder that is characterized by an isolated thrombocytopenia (peripheral blood platelet count $<100 \times 10^9$/L).[2] Clinical symptoms include petechiae, mucosal bleeding, purpura, and, rarely, intracranial hemorrhages, resulting in a reduced health-related quality of life.[3] Therapeutic management of ITP has proven to be challenging because of its heterogeneous nature and incompletely understood pathophysiology.[3,4] Although the exact and likely multiple mechanisms that are involved in the pathogenesis of ITP have not been fully elucidated, they culminate in peripheral platelet destruction resulting from antiplatelet antibodies and/or $CD8^+$ cytotoxic T cells or in bone marrow–megakaryocyte impairment resulting in decreased platelet production.[3] Therapeutic strategies in ITP are primarily aimed at decreasing platelet antibody production, preventing platelet clearance, or stimulating thrombopoiesis. However, central in the development of ITP is the loss of immune tolerance due to a quantitative and qualitative impairment in $CD4^+CD25^+FoxP3^+$ Tregs, which is related to the excessive expansion of $CD4^+$ T helper 1 (Th1) and Th17 cells.[4,5] Therefore, it has been suggested that restoring immune tolerance in ITP, by reversing Treg impairment and suppressing Th1 and Th17 cells, may be the key to a sustained therapeutic response.[3,4]

Decitabine (5-aza-2′-deoxycytidine) is a hypomethylating agent that promotes cell differentiation and maturation at low doses; it is used for the treatment of myelodysplastic syndrome.[6] It was previously found that low-dose decitabine could promote megakaryocyte maturation and platelet production in in vitro cultures with plasma from healthy controls and with plasma of more than half of patients with ITP.[7] Very recently, it was demonstrated that low-dose decitabine may inhibit cytotoxic T lymphocyte–mediated platelet destruction through the programmed cell death protein 1 signaling pathway.[8] The use of low-dose decitabine for treatment of ITP has also been explored. For example, a prospective multicenter study evaluating the efficacy of low-dose decitabine in adult patients with refractory ITP was conducted; the primary response was defined as a platelet count $\geq 30 \times 10^9$/L and at least a twofold increase in the baseline platelet count and absence of bleeding (partial response) or a platelet count $\geq 100 \times 10^9$/L (complete response).[9] The outcomes of that study showed a complete response in 8 of 45 patients (18%) and a partial response in 15 of 45 patients (33%). For the responding patients, relapse-free survival rates at 6, 12 and 18 months were observed 87% (20/23 patients), 61% (14/23 patients), and 39% (9/23 patients), respectively.[9]

In the current study, Han et al further explored the immunomodulatory potential of decitabine in ITP with a focus on Tregs. They used in vitro cellular assays in combination with a murine model of active ITP, which allowed them to probe antibody- and T-cell–mediated ITP in vivo.[10] In vitro, peripheral blood mononuclear cells from patients with ITP and healthy controls were isolated and cultured with different concentrations of decitabine. Low-dose decitabine (100 nM) significantly elevated the percentage of Tregs in the cultures from ITP patients. Furthermore, the division index of effector T cells from patients with ITP was significantly decreased after coculture with Tregs and low-dose decitabine compared with Tregs alone. Because decitabine itself did not affect the proliferation of effector T cells, these data suggested that the



Immunomodulatory therapeutic mechanism of low-dose decitabine in ITP. (A) In ITP there is a quantitative and qualitative impairment in Tregs, with an expansion of Th1 and Th17 cells, resulting in loss of immune tolerance leading to thrombocytopenia. (B) Low-dose decitabine treatment in ITP, through inhibition of STAT3, modulates Tregs, increasing their numbers and suppressive function. Additionally, Th1 and Th17 cells are suppressed. Collectively, this leads to a rebalanced T-cell homeostasis, resulting in a restored immune tolerance that allows platelet counts to increase. PLT, platelet.

immunosuppressive function of Tregs was enhanced by low-dose decitabine. Further analysis in a murine model of active ITP revealed that low-dose decitabine could increase the platelet counts. Strikingly, this therapeutic response was associated with increased numbers of Tregs in the spleen and with a decrease in Th1 and Th17 cells (as percentages of $CD4^+$ T cells). To confirm that the mode of action of decitabine was from the effects on Tregs, the investigators performed elegant Treg-deletion and -depletion experiments in the active ITP mouse model (Treg deletion by magnetic beads in transferred splenocytes and Treg depletion in vivo using anti-CD25 antibody treatment). This revealed that Treg deletion and depletion could negate the effect of decitabine on increasing platelet counts and restoring T-cell subpopulations, indicating that low-dose decitabine modulates T-cell homeostasis via Tregs. Importantly, Han et al also observed an increase in percentages of Tregs, decreases in Th1 and Th17 cells, and an enhanced suppressive function of Tregs, in peripheral blood samples of patients with ITP treated with decitabine. Finally, the authors investigated the contribution of the Jak-STAT and PI3K-AKT signaling pathways, which play important roles in the maintenance of $CD4^+$ T-cell homeostasis. They used STAT3 and AKT inhibitors in vitro and in the mouse model of active ITP. These data demonstrated that in vitro, no differences in the percentages of Tregs, and in vivo, no differences in platelet counts were found in the STAT3 inhibitor plus decitabine group vs the STAT3 inhibitor group (in contrast to the AKT inhibitor). This suggests that, in ITP, low-dose decitabine may restore Tregs through inhibition of STAT3.

In summary, Han et al provide important insights into the mechanism of low-dose decitabine in ITP. They demonstrate that low-dose decitabine restores immune tolerance in ITP by reversing Treg impairment through inhibition of STAT3 and by suppressing Th1 and Th17 cells (see figure). Further studies are needed to validate these findings, and clinical trials are now warranted to establish the potential role of low-dose decitabine in the treatment of ITP.

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REFERENCES

1. Han P, Hou Y, Zhao Y, et al. Low-dose decitabine modulates T-cell homeostasis and restores immune tolerance in immune thrombocytopenia. *Blood.* 2021;138(8):674-688.
2. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood.* 2009;113(11):2386-2393.
3. Zufferey A, Kapur R, Semple JW. Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). *J Clin Med.* 2017;6(2):16.
4. Semple JW, Rebetz J, Maouia A, Kapur R. An update on the pathophysiology of immune thrombocytopenia. *Curr Opin Hematol.* 2020;27(6):423-429.
5. Semple JW, Kapur R. Platelet immunology from the inside out. *ISBT Sci Ser.* 2020;15(3):315-319.
6. van den Bosch J, Lübbert M, Verhoef G, Wijermans PW. The effects of 5-aza-2'-deoxycytidine (decitabine) on the platelet count in patients with intermediate and high-risk myelodysplastic syndromes. *Leuk Res.* 2004;28(8):785-790.
7. Zhou H, Hou Y, Liu X, et al. Low-dose decitabine promotes megakaryocyte maturation and platelet production in healthy controls and immune thrombocytopenia. *Thromb Haemost.* 2015;113(5):1021-1034.
8. Han P, Yu T, Hou Y, et al. Low-dose decitabine inhibits cytotoxic T lymphocytes-mediated platelet destruction via modulating PD-1 methylation in immune thrombocytopenia. *Front Immunol.* 2021;12:630693.
9. Zhou H, Qin P, Liu Q, et al. A prospective, multicenter study of low dose decitabine in adult patients with refractory immune thrombocytopenia. *Am J Hematol.* 2019;94(12):1374-1381.
10. Chow L, Aslam R, Speck ER, et al. A murine model of severe immune thrombocytopenia is induced by antibody- and CD8+ T cell-mediated responses that are differentially sensitive to therapy. *Blood.* 2010;115(6):1247-1253.

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