overt ALL, and likely mediated by activation-induced cytidine deaminase and recombination-activating genes.[7] Models of these dynamics have been based on epidemiological and animal research that links ALL risk with reduced microbial exposure in early life.[8] Thus, one of the most consistent epidemiological findings is the association of daycare center attendance with a 20% to 25% reduced risk of childhood ALL.[9] Importantly, immune system maturation in early postnatal life may not just reflect infectious exposures because abnormal profiles of inflammatory markers can already be detected in neonatal blood spot samples.[10]

Although traditional genome-wide association study analyses have granted us some insight into the biological pathways involved in leukemogenesis, a deeper understanding of childhood leukemia development will require integration of large-scale screening of cord blood samples,[6,10] mapping the infectious burden in early life through population-based registers that provide data on known risk factors for ALL, such as birth weight, birth order, sibship size, and daycare center attendance, as well as hospital admissions and antibiotic use, and linkage of these data with genomic profiling of both host and tumor DNA.

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Comment on Zhao et al, page 730

More than one pathway: novel treatment for ITP

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In this issue of *Blood*, Zhao et al explored the role of low doses of the histone deacetylase inhibitor (HDACi) chidamide in restoring immune tolerance in patients with immune thrombocytopenia (ITP).[1]

For their investigation, the authors used both an animal model and a translational model with patient samples. Their work adds to the knowledge about the pathophysiology of ITP and explores a novel therapeutic avenue for patients with refractory disease who need alternative therapies. The authors recognized that $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells are reduced in ITP. That, combined with the knowledge that use of low-dose HDACi's restores Treg cell populations in patients with graft-versus-host disease and other autoimmune conditions, led them to hypothesize that these agents may be useful as therapy for ITP.[2,3] The Zhao et al study was a proof-of-concept study that investigated this novel approach to treating ITP.

By starting treatment at the time of antiplatelet antibody exposure in a passive ITP murine model, the authors were able to ameliorate thrombocytopenia at 72 and 120 hours. In this model, ITP is induced in mice by giving animals antiplatelet antibodies, which simulates some, but not all, of the characteristics of patients with ITP. Therefore, the authors used a second animal model of ITP, in which mice that lacked certain platelet antigens were used as donor mice to cause an immune response, which could then be transferred by harvesting spleen cells and infusing these splenocytes. This active ITP model more accurately simulated a severe chronic ITP scenario and showed development of durable thrombocytopenia (lasting ~28-35 days) with associated bleeding mortality. By using this model, the authors were again able to ameliorate thrombocytopenia by administering chidamide beginning with the infusion of splenocytes. This therapy increased the number of Treg cells in the splenocytes and effectively improved the thrombocytopenia. Even more relevant to clinical use, mortality from bleeding rates was reduced in mice treated with chidamide. In a translational experiment, the authors used peripheral blood mononuclear cells from 8 patients and 8 healthy controls and cultured these cells with low-dose chidamide, thus demonstrating that cells from patients with ITP responded by increasing the number of Treg cells in culture.

The authors then explored additional mechanisms by which HDACi's might ameliorate thrombocytopenia in ITP. They demonstrated ex vivo that chidamide treatment of macrophages decreased macrophage phagocytosis of antibody-coated platelets, which supports a role of HDACi's in modulating macrophage activity and

providing a potential additional mechanism by which these drugs might be effective in ITP. Recently, similar therapy aimed at modulating monocyte and macrophage activity against platelets through the splenic tyrosine kinase (SYK) inhibitor fostamatinib has been approved by the US Food and Drug Administration for treating ITP.[4] Although they do not alter the production of antiplatelet antibodies, strategies intended to reduce phagocytosis hold promise for patients with refractory disease, many of whom have been treated with myriad other therapies that modulate platelet count without success.

Zhao et al highlight the role of CTLA4 in the pathogenesis of ITP. CTLA4 is an immune checkpoint responsible for downregulating the immune response by blocking the interaction of B7 proteins with CD28, which ultimately limits T-cell activation. The link between CTLA4 and ITP has recently been investigated by observing the increased expression that occurs after treatment with corticosteroids.[5,6] Although CTLA4 gene polymorphisms have been implicated in autoimmune conditions, no connection was established between CTLA4 gene polymorphisms and the development of ITP in children.[7,8] Here the authors provide some translational evidence for a role of CTLA4 in a small group of patients with ITP (n = 10); patients with ITP demonstrated lower expression of CTLA4 than controls, which was related to reduced histone acetylation of H3K27. After treatment with low-dose chidamide, enhanced expression of CTLA4 on T cells was associated with increased histone acetylation. The Zhao et al study is the first to propose the mechanism of CTLA4 impairment in ITP as it relates to histone acetylation. Further study will be required to determine whether CTLA4 plays a role in all patients with ITP or is a marker in particular subsets of patients with risk of additional autoimmune disease, but the Zhao et al study provides some intriguing preliminary data for exploring mechanisms of loss of tolerance.

The earliest understanding of the pathogenesis of ITP included splenic macrophage phagocytosis of antibody-coated platelets. But until recently, therapies targeting this component of ITP were limited. HDACi's have an impact on macrophage phagocytosis and they also show promise of restoring immune balance by increasing Treg cells and CTLA4 expression. All of these changes further reduce macrophage platelet destruction through immune downregulation. By intervening with HDACi's at ITP induction in these animal models, the authors were able to modulate the course of ITP and provide interesting data on the disease mechanism and a potential novel therapy. Because specific triggers of ITP in human disease are still not known, the direct application of HDACi's to modulate platelet counts in human ITP will require further study, especially regarding the role of HDACi's in established ITP.

First-line therapy often fails for ~60% to 80% of adult patients with ITP.[9] Options for managing these patients include splenectomy, thrombopoietin-receptor agonists, rituximab and, more recently, fostamatinib.[4,10] Response rates to these treatments are not universal, and each therapy has its own limitations. In their study, Zhao et al describe mechanisms by which some patients with ITP may have decreased numbers of Treg cells, and they explore a potential novel therapeutic avenue. Future research in ITP should continue to address the mechanisms responsible for platelet destruction and the development of novel therapies. Perhaps as we gain awareness of the various mechanisms responsible for ITP, we may be able to target treatment to patient-specific defects. Carrying the data presented here forward from bench to bedside will be essential for determining the response rate in patients, the impact on health-related quality of life, and the durability of response.

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