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POSTER ABSTRACTS

311.DISORDERS OF PLATELET NUMBER OR FUNCTION: CLINICAL AND EPIDEMIOLOGICAL

High Dimensional Single-Cell Profiling Identifies Immune and Metabolic Heterogeneity in Immune Thrombocytopenia

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Introduction
Primary immune thrombocytopenia (ITP) is an autoimmune disease characterized by excessive platelet destruction and decreased platelet production. Ongoing efforts to characterize the pathophysiology of ITP have yielded certain insights, but the details of the immune and metabolic characteristics of the disease remain elusive. Tacrolimus has been demonstrated to be a promising therapeutic option for ITP patients in our previous report (*63rd ASH, 2021*), but the mechanisms are incompletely understood.
Methods
A total of 15 patients diagnosed with ITP and 8 healthy control (HC) individuals were enrolled between March and July 2023 in this study. Peripheral blood from ITP patients was collected at two time points: when admitted to the cohort without previous tacrolimus treatment and when receiving tacrolimus treatment for at least 28 days. We employed mass cytometry (cytometry by time-of-flight, CyTOF) with a panel of 27 phenotypic, 7 metabolic, and 8 functional markers to explore the immune landscape, metabolic characteristics, and phosphorylated signaling states at single-cell resolution. Additionally, a 10x Genomics platform was applied to perform single-cell RNA sequencing (scRNA-seq). Primary findings from our transcriptomic and proteomic analyses were further confirmed and extended by flow cytometry and RT-PCR.
Results
Using t-distributed stochastic neighbor embedding, a CyTOF panel of 42 markers partitioned the 32 clusters into 7 subsets (CD4+ T cells, CD8+ T cells, B cells, NK cells, monocytes/macrophages, dendritic cells, and $\gamma\delta$ T cells). Quantification of immune cell distribution across cell clusters showed a relatively higher level of CD8+ T cells and lower CD4+:CD8+ T-cell ratios in the patients with ITP than in the HC individuals. Additionally, the proportion of NK cells was significantly lower in patients than in control individuals. With further investigation of CD4+ and CD8+ T cells, we observed a decrease of naïve T cells and Treg cells and skewed accumulation of Th1 cells and Tc1 cells in patients with ITP relative to HC individuals.
We further investigated the metabolic and functional status of T cells with CyTOF. One-carbon metabolism (MTHFD2 and SHMT2) was found to be elevated in Th1 cells from ITP patients compared with those from HC individuals. Based on CyTOF analysis as well as flow cytometry validation, a subgroup of Th1 cells with elevated ERK1/2 phosphorylation and MTHFD2 expression was speculated to be associated with the pathogenesis of ITP. A longitudinal study demonstrated that the dysregulated immune subset exhibited HC-like expression after treatment with tacrolimus.
To gain further insights into the molecular characteristics of immune cell subsets and metabolic status that are differentially expanded in ITP patients, we performed scRNA-seq analysis on peripheral blood mononuclear cell (PBMC) isolated from 5 ITP patients pre- or posttreatment with tacrolimus and 3 HC individuals. Similar to the CyTOF data, a relative increase in Th1 cells and memory T cells and a reduction in naïve T cells and Treg cells were found in the ITP patient group compared with

the HC group. Among the 9 subclustered T-lineage cell types, the CD4+ CXCR3+ IFNγ+ Th1 cells with functional pathways such as TCR pathway activation, IL-17 signaling pathway, and MAPK signaling pathway draw our attention. Next, we examined the metabolic status of immune cells of ITP patients to elucidate the underlying mechanisms of dysfunction of immune cells. Our analysis suggested that aberrant metabolism including glucose metabolism, nucleotide metabolism, and one-carbon metabolism caused by Th1 cells were involved in the dysregulated immune response in ITP. Moreover, ITP patients had elevated levels of metabolic checkpoints (mTOR, HIF-1α, and c-Myc) and low expression of immune checkpoint regulators (CTLA4, TIM3, and LAG3) in Th1 cells. Patients who recovered from tacrolimus treatment had a lower proportion of Th1 cells and lower expression levels of metabolic enzymes than nonresponsive patients.

Conclusion

Using integrated single-cell proteomic and transcriptomic profiling, this study depicts the broad immune and metabolic landscape of PBMC from ITP patients. In particular, our data unveil that inflammatory Th1 cells play a critical role in the pathophysiology of ITP and suggest a novel therapeutic opportunity of tacrolimus towards reprogramming immune metabolism.

Disclosures No relevant conflicts of interest to declare.

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