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POSTER ABSTRACTS

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

Terbutaline Modulates Macrophage Homeostasis and Macrophage-T-Cell Interactions in Immune Thrombocytopenia

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Background: Increased platelet destruction by macrophage phagocytosis contributes to immune thrombocytopenia (ITP). Neural immune modulation in autoimmune diseases has been given considerable interest. Our preliminary studies showed a decreased sympathetic innervation in the bone marrow and spleen of ITP mice and that abnormal T cell immunity caused by sympathetic modulation promoted the progression of ITP (*Blood Advances 2019; J Thromb Haemost, 2023*). Moreover, previous studies indicated a potential interaction between T cells and macrophages (*Immunity, 2017*). However, the regulation of spleen sympathetic denervation on macrophage function and the interaction between splenic macrophages and T cells under the sympathetic modulation in ITP remain unknown.
Methods: Whole-tissue immunolabeling and 3D imaging of the human spleen were performed to compare the sympathetic distribution between ITP with splenectomy and non-ITP with traumatic splenectomy. RNA sequencing was used to detect the transcriptional changes and metabolomics and seahorse extracellular flux analyses were carried out to determine the metabolic reprogramming in splenic macrophages of ITP mice. The expression of cell surface markers of macrophages and T cells and cytokines was assessed by flow cytometry, western blot, and enzyme-linked immunosorbent assay. Chemical sympathectomy with 6-hydroxydopamine (6-OHDA), a β2-AR agonist terbutaline, and a β2-AR antagonist ICI 118,551 were used to detect the sympathetic modulation. Genetic lineage tracing was used to compare serum norepinephrine (NE) and its origin in the spleen of WT mice with or without chemical sympathectomy.
Results: A panicle-shaped sympathetic neural architecture was revealed in the spleen of both ITP and non-ITP patients. The distribution of sympathetic nerves in the spleen and the level of serum NE were both significantly decreased in ITP patients. Immunofluorescence staining revealed an anatomical colocalization between sympathetic nerves, macrophages, and T cells in the spleen, suggesting potential sympathetic modulation of macrophages and a potential interaction between splenic macrophages and T cells.
RNA-sequencing data of splenic macrophages in ITP mice compared with wild type (WT) mice revealed higher expression of M1-specific genes, including *IL1b*, *Tnf* and *IL6*, and lower expression of M2-specific genes, including *IL10* and *Arg1*, indicating an M1 polarization tendency. In splenic macrophages isolated from ITP mice, we observed an enrichment of glycolysis pathway genes compared to WT mice, including *Hk2*, *Pkm2*and *Ldha*. An increase in the extracellular acidification rate and a decrease in the oxygen consumption rate were also found in splenic macrophages of ITP mice, indicating a switch to glycolysis.
To investigate the interaction between splenic macrophages and T cells under sympathetic modulation in ITP, we measured the expression of cytokines that might have an influence on macrophage polarization and T cell differentiation and observed upregulation of interleukin (IL)-12, IL-18 and interferon (IFN)-γ. We also used macrophages from ITP mice and WT mice as

antigen-presenting cells for naïve CD4$^+$ T cells, which were interrogated for IFN-γ production following stimulation with OVA$_{323-339}$. We observed increased IFN-γ production and upregulation of IL-12 and IL-18 in response to macrophages from ITP mice, and we also observed diminished production of IL-12, IL-18 and IFN-γ when pretreated with aIL-12 mAb and aIL-18 mAb.

Moreover, chemical sympathectomy with 6-OHDA was carried out in WT mice and significantly decreased serum NE and platelet counts at D7 and D14 were observed. Increased expression of M1-specific markers and upregulation of IL-12, IL-18 and IFN-γ were also determined. The β2-AR agonist terbutaline was shown to decrease the expression of proinflammatory factors and inhibit M1 polarization, and its effects were reversed by the β2-AR antagonist ICI 118,551.

Conclusions: Splenic sympathetic denervation-mediated proinflammatory M1 polarization, metabolic alterations, and proinflammatory interactions between macrophages and T cells were involved in ITP pathogenesis. The β2-AR agonist terbutaline was demonstrated to restore the homeostasis of splenic macrophages, indicating a novel potential ITP treatment strategy.

Disclosures No relevant conflicts of interest to declare.

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