Abstract of Dissertation submitted by MHD YOUSUF YASSOUF

Title: Biphasic effect of mechanical stress on lymphocyte activation

Japanese title: リンパ球活性化に対する機械的ストレスの二相性効果

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Introduction:

Various mechanical cues, such as shear stress and hydrostatic pressure, regulate the biological properties of cells, especially in the circulatory and immunological systems. Previous studies suggest that mechanical cues can promote the activation of different immune cells, including myeloid cells and lymphocytes, which contribute to countering infection. In contrast, clinical studies indicate worse outcomes and severity of serious infections in hypertensive patients. Therefore, we aimed to investigate the role and underlying mechanisms of elevated mechanical stress, specifically elevated pathological levels of hydrostatic pressure, in the activation of lymphocytes.

Materials and Methods:

Lymphocytes were collected from the mouse spleen and incubated without (Resting) or with stimulation by LPS or (phorbol 12-myristate 13-acetate/ionomycin) PMA/I (Stimulated) to test their activation response. We used a hydrostatic pressure system capable of subjecting cells to 50 mmHg pressure for 3 hours, and as a control, cells were placed at atmospheric pressure. In some experiments, Yoda1 was used to study the effect of Piezo1 mechanosensitive ion channel activation. The activation of lymphocytes was evaluated based on the expression of CD69 in CD4+, CD8+, and CD19+ subsets by flow cytometry; the levels of IL2, IFN-γ, and TNF-α in supernatants by ELISA; and the levels of *Tnf*, *Ifng*, *Il1b*, *Il2*, *Il4*, *Il10* by RT-qPCR.

To further understand the mechanism, the nuclear and cytoplasmic levels of the transcription factors NFAT1 and HIF-1α in CD4+ and CD8+ lymphocytes were detected using fluorescent antibodies and DAPI staining, followed by quantitative analysis of the fluorescence intensity using Image J software. The transcription factors *Jun* and *Fos* levels were assessed by RT-qPCR. As hypertension imposes elevated hydrostatic pressure on circulating leukocytes, RNA sequencing data from peripheral blood mononuclear cells of COVID-19 patients, with and without hypertension, was retrieved from the GEO database and analyzed to test differential expression.

Results:

Resting CD8+ lymphocytes subjected to 50 mmHg hydrostatic pressure for 3 hours significantly increased the expression of CD69, *Ifng* mRNA, and the secretion of IFN-γ. However, hydrostatic pressure reduced CD69 expression, mRNA levels of *Ifng, Il2, Il4, Il10,* and secretion of IL-2, IFN-γ, and TNF-α in lymphocytes stimulated with PMA/I or LPS. PIEZO1 activation using Yoda1 increased CD69 expression in resting CD8+ cells while inhibited it in PMA/I-stimulated lymphocytes. Yoda1, on the other hand, intriguingly increased IFN-γ and TNF-α secretion in both resting and PMA/I-stimulated lymphocytes.

Hydrostatic pressure induced HIF-1α nuclear localization in resting lymphocytes but inhibited *Hif1a* mRNA enhancement in PMA/I-stimulated lymphocytes. Additionally, Hydrostatic pressure promoted NFAT1 nuclear localization in both resting and PMA/I-stimulated lymphocytes, while increased *Jun* mRNA level in resting lymphocytes and decreased it in PMA/I-stimulated lymphocytes.

According to our RNA sequencing analysis, compared to normotensive COVID-19 patients, hypertensive COVID-19 patients had lower *JUN* and *CD69* expression at the treatment stage but conversely had higher *JUN* and *CD69* expression at the rehabilitation stage.

Discussion:

Our experimental results showed a biphasic effect of mechanical stress on lymphocyte activation, as hydrostatic pressure can slightly induce the activation of resting CD8+ lymphocytes but impair the activation of lymphocytes in response to exogenous stimulation. In searching for underlying mechanisms, we found that while PIEZO1 activation and hydrostatic pressure have similar effects on resting lymphocytes, they have opposite effects on IFN-γ and TNF-α secretion from PMA/I-stimulated lymphocytes, implying that hydrostatic pressure affects lymphocyte activation via other mechanisms. Given HIF1-α importance for the effector functions of lymphocytes, the alteration of HIF1-α is consistent with the observed biphasic impact of hydrostatic pressure.

The hydrostatic pressure-induced mild activation of resting CD8+ lymphocytes was accompanied by increased NFAT1 and Jun, whereas hydrostatic pressure suppression of stimulated lymphocytes' activation was accompanied by an imbalance between increased NFAT1 and decreased Jun, implying that hydrostatic pressure regulates Jun. Since Jun is essential for the proper activation of lymphocytes, the hydrostatic pressure-induced alteration of Jun expression may explain the impaired lymphocytes' activation response in this study. As hydrostatic pressure is commonly elevated in various pathological conditions, the findings from our study provide mechanistic insight into the increased risk of serious infection in individuals with comorbidities involving elevated hydrostatic pressure levels.