**Characterization of Human Immortalized Cell-Based Blood-Brain Barrier Spheroid Models as a Tool for Brain Vascular Inflammation Studies**

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**Background and aims.** The blood-brain barrier (BBB), mainly formed by brain microvascular endothelial cells (BMECs), restricts entry of various substances from the blood into the brain. As BBB inflammation – characterized by barrier dysfunction and immune cell recruitment – contributes to various brain diseases, its modulation represents a promising therapeutic approach1). To facilitate such study, we have created human immortalized cell-based multicellular spheroidal BBB models (hiMCS-BBB) using three types of immortalized BBB cells: BMECs, astrocytes, and pericytes2). This study aimed to characterize their inflammatory response profiles and evaluate their applicability to drug development studies.

**Results and Discussion.** We first evaluated the inflammatory responses of the hiMCS-BBB models upon stimulation with the pro-inflammatory cytokines TNF-α and IFN-γ. Cytokine exposure markedly induced VCAM-1 and E-selectin expression at both mRNA and protein levels. Next, we performed cell adhesion assays using two representative immune cell lines: THP-1 (monocyte) and Jurkat (T lymphocyte) cells. The results showed that the number of cells adhering to cytokine-treated hiMCS-BBB models increased by 23.7- and 26.1-fold, respectively, compared with untreated controls. Furthermore, to evaluate the model’s applicability for drug evaluation, we employed natalizumab (20 ng/mL) and JPH203 (50 µM). The adhesion assay results showed that both drugs significantly suppressed inflammation-induced immune cell adhesion to 0.22- and 0.097-fold of the control levels (IgG4 or vehicle), respectively.

**Conclusion.** Our results demonstrate that the hiMCS-BBB models recapitulate inflammation-induced immune cell adhesion to the BBB, which can be suppressed by natalizumab and JPH203. These findings suggest their applicability for modeling BBB inflammation and evaluating therapeutics targeting it.

**References:**

(1) Patabendige et al., Biochem Soc Trans., 2023, 51(2):613-626.

(2) Kitamura et al., Biol Pharm Bull., 2021, 44(7):984-991