

# The Effects of Inflammatory Neutrophils on Endothelial Cell Integrity

Hou, Jacqueline (School: Blacksburg High School)

Endothelial vascular leakage is responsible for the pathogenesis of diverse acute and chronic inflammatory diseases, such as COVID-19 and influenza, heart disease and arteriosclerosis. In particular, COVID-19 infection exacerbates fluid extravasation to the surrounding tissues, eventually leading to systemic inflammatory diseases that induce sepsis and multiorgan failure. These processes are mediated by inflammatory cytokines and first-responding leukocytes, such as neutrophils; however, the effects of neutrophils on endothelial cell integrity mechanisms have not been well examined to date. In this in vitro study, it is shown that naive neutrophils cause significant leakage in brain endothelial tissue, the most restrictive vascular barrier, through the integrated analysis of three perspectives: dye-leakage assay, cell morphology, and molecular biology. An optimized Evans Blue Dye Assay was developed to determine the interaction of murine neutrophils and endothelial cells in vitro; when tested, it was revealed that the endothelial cell barrier was disrupted significantly by neutrophils. Under light microscopy, endothelial cells cultured with naive neutrophils shrink and demonstrate finger-like morphology with gaps. Neutrophils negatively affect the intactness of endothelial cells by reducing expression of adhesion and tight junction endothelial markers E-Cadherin, Catenin beta-1, ESAM, and JAM-1, examined via flow cytometry. Collectively, this data challenges the current understanding that naive neutrophils are non-inflammatory, significantly altering targeted therapeutic intervention for inflammatory diseases such as COVID-19. The methodology of this study provides an optimized in vitro assay to advance the study of drug development affecting endothelial vasculature.