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Prolonged Activation of STAT3 Mediates the IL6-Induced Loss of Stress Fibers and Increase in Endothelial Permeability

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Interleukin 6 (IL6) promotes endothelial barrier breakdown in vitro, as well as, in vivo. However, the signaling mechanisms that mediate this response are not fully understood. IL6 trans-signaling promotes a prolonged activation of JAK/STAT3 in human umbilical vein endothelial cells (HUVECs) that lasts for at least 24 hours, leading to a sustained increase in endothelial permeability. IL6-induced STAT3 phosphorylation and loss of barrier function is prevented when cells are pre-treated with the JAK inhibitor, ruxolitinib, suggesting that JAK activity is required for these responses. Furthermore, siRNA-mediated STAT3 knockdown attenuates the loss of IL6-induced loss of barrier function, demonstrating a critical role for STAT3 in this response. Immunofluorescence analysis showed that this change in barrier function correlates with a marked loss of junctional ZO-1 (but not changes in ZO-1 protein expression). Phalloidin staining demonstrates that IL-6 also promotes a JAK, and STAT3, dependent loss of actin stress fibers and a marked reduction of the cortical actin signal. A prolonged activation of STAT3 is required for this response, because treatment of HUVECs with ruxolitinib up to 4 hours after activation of IL6 trans-signaling, completely reverses the IL6-induced loss of barrier function. STAT3 activation kinetics are regulated by a negative feedback loop that is mediated by the suppressor of cytokine signaling 3 (SOCS3). Consistently, siRNA-mediated SOCS3 knockdown increases IL6-induced STAT3 phosphorylation in HUVECs and exacerbates the IL6-induced increase in permeability. Collectively, our data shows that prolonged activation of STAT3 is required for the IL6-induced barrier breakdown, and loss of junctional ZO-1, cortical actin and actin stress fibers in endothelial cells.

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