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## Caspase-Dependent Septic Pulmonary Microvascular Endothelial Cell Barrier Dysfunction is Associated with Vascular Endothelial-Cadherin Disruption

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**Background:** Sepsis often causes dysfunction of pulmonary microvascular endothelial cells (PMVEC) leading to severe pulmonary edema. We previously reported in a murine model of sepsis that lung injury, including PMVEC barrier dysfunction, was strongly caspase-dependent, and associated with PMVEC apoptosis. The mechanism through which caspases mediate PMVEC barrier function, however, was not determined. The classic role of caspases is the initiation and execution of apoptosis; however, caspases have important biological functions other than apoptosis. Indeed, caspases can cleave multiple targets, including proteins associated with cell-cell junctions.

**Objective:** To identify the mechanisms through which caspases regulate septic PMVEC barrier dysfunction.

**Hypothesis:** PMVEC barrier dysfunction in sepsis is mediated by caspase-dependent disruption of inter-PMVEC junctions.

**Methods:** Human PMVEC cultured alone or in co-culture with neutrophils (PMN) were stimulated with cytomix (equimolar tumour necrosis factor  $\alpha$ , interleukin 1 $\beta$  and interferon  $\gamma$  to mimic septic conditions). PMVEC barrier function was assessed by flux of Evans blue labelled-albumin in a transwell assay. Specific microscopic localization of leak was determined by imaging paracellular fluorescein isothiocyanate labelled-avidin leak across PMVEC monolayers in biotin-coated wells. PMVEC apoptosis was examined using 3 markers: caspase activation (FLICA staining), loss of cell membrane polarity (Annexin V staining), and DNA fragmentation (terminal deoxynucleotidyl transferase dUTP nick end labelling [TUNEL] staining). PMVEC cell-cell junctions were examined using immunofluorescence with antibodies against vascular endothelial (VE)-cadherin.

**Results:** We found that co-culture of PMVEC with PMN significantly disrupts PMVEC barrier function under both basal and septic conditions vs. PMVEC cultured alone. Compared to cytomix-treated PMVEC alone, the presence of PMN also significantly enhanced septic PMVEC caspase activation (FLICA staining), but this increase did not appear to be associated with any change in PMVEC apoptosis (Annexin V or TUNEL staining) vs. PMVEC cultured alone. We also found that microscopic paracellular leak co-localized around cells positive for caspase activity, and was highly associated with disruption of circumferential PMVEC surface VE-cadherin staining (97.1%). Moreover, PMVEC treatment with Q-VD, a pan-caspase inhibitor, rescued the septic barrier dysfunction, microscopic leak, and VE-cadherin disruption.

**Conclusion:** Our data demonstrates that neutrophil presence enhances caspase-dependent septic PMVEC barrier dysfunction independently of PMVEC apoptosis partly through disruption of VE-cadherin.

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