Crucial role for Protein kinase C alpha and arginase activation in the induction of pulmonary endothelial hyperpermeability by pneumolysin

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Rationale: Infections with *Streptococcus pneumoniae* are accompanied by a pulmonary endothelial hyperpermeability. Death in pneumonia patients can occur when tissues are sterile and correlates with the presence of bacterial virulence factors, the most important one of which is the pore-forming toxin pneumolysin (PLY). Unfortunately, to date mechanisms of PLY-induced pulmonary endothelial hyperpermeability, as well as potential therapeutic options to tackle its activity, remain elusive. To investigate the mechanism of PLY-induced endothelial dysfunction and to identify novel therapeutic targets and treatment options.

Methods: We have evaluated PLY-induced endothelial hyperpermeability in an *in vitro* culture system, using monolayers of human lung microvascular endothelial cells, as well as in a 6h *in vivo* mouse model, using intratracheal PLY instillation.

Results: PLY induces an endothelial hyperpermeability in human lung microvascular endothelial cells *in vitro*, an event which is preceded by a RhoA/Rac1 imbalance and an increased myosin light chain (MLC) phosphorylation. The PKC-α/β inhibitor GÖ6976 inhibits the permeability increasing effect of PLY and the toxin moreover induces a time-dependent activation of PKC-α, indicating an implication of this PKC isoform. PLY moreover leads to an increased arginase activity in the endothelial cells, which, upon inducing eNOS uncoupling leads to a reduced NO and an increased ROS generation. Intratracheal PLY instillation causes a significantly increased endothelial permeability in mice *in vivo*, as assessed by Evans Blue incorporation. The lectin-like domain of TNF, which can be mimicked by the 17 amino acid TIP peptide, is able to inhibit the PLY-induced PKC-a activation, arginase activity and endothelial hyperpermeability.

Conclusion: These results identify PKC-a as a potential upstream and arginase as a potential downstream therapeutic target during G^+-associated pulmonary hyperpermeability and moreover indicate that the TNF-derived TIP peptide is able to blunt its activation.