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Title: Regulation of vascular endothelial growth factor expression in a mouse model of acute respiratory distress syndrome (ARDS)

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Body: ARDS is a common condition in critically ill. It's characterized by barrier dysfunction, edema, and leukocyte influx. Vascular endothelial growth factor (VEGF) A is responsible for alveolar integrity but also important for permeability regulation. The role of VEGF in ARDS is a matter of on-going discussion We examined the regulation of VEGFA expression after induction of lung injury in a murine model of intratracheal endotoxin instillation. Mice were sacrificed at 0, 24, 48 and 72h. Lungs were lavaged (BAL) and embedded. By laser microdissection we isolated precapillary small arterioles and alveolar septa. Total mRNA was isolated and realtime RT-PCR performed. Expression of VEGFA was evaluated in whole lung homogenates and the different cell types by mRNA levels and VEGF protein. Expression of VEGF receptors flt-1 and flk-1 was also investigated. Leukocytes were counted and VEGF protein was measured by ELISA in BAL. After lung injury we found a massive leukocyte influx in the alveolar space within 24h, which was not cleared within 72h. Concentrations of VEGFA protein were highly elevated in BAL after 24h and returned to baseline within 72h. At all times we could not detect any regulation on mRNA level of either VEGFA or its receptors in whole lung homogenates or in endothelial cells. In the alveolar epithelium mRNA of VEGFA and flk-1 was decreased within 4h and remained low. In this model of lung injury the regulation of VEGFA and its receptor flk-1 occur predominantly in the alveolar epithelium, whereas we detected no regulation in the endothelium. This knowledge might be crucial for therapeutic approaches targeting VEGF-pathways in ARDS.