Effects of alpha 1 antitrypsin (A1AT) on pulmonary endothelial cell responses to pro-inflammatory cytokines

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Alpha-1 antitrypsin (A1AT) is a serine protease inhibitor that is primarily produced by the liver (Greene et al 2008) and released into circulation. It enters the lung and protects it from proteases such as neutrophil elastase which degrade the lung matrix leading to emphysema (Justin Ranes, MD). Chronic inflammation also contributes to disease progression. Although A1AT has been linked to negative regulation of the pro-inflammatory cytokine TNF-α, the role of A1AT in inflammation is not clearly defined. Therefore, we hypothesized that A1AT protects lung endothelial cells against pro-inflammatory cytokines. We serum starved lung endothelial cells 2h and then pretreated with purified human A1AT for 4h prior to treatment with rhTNF-a for up to 2h. Whole cell lysates were extracted and analyzed by Western blot. In a separate experiment, total RNA was extracted, reverse transcribed into cDNA and analyzed by real-time PCR. A1AT treatment alone decreased both baseline TNF-R1 and Ikβ-α protein levels. Upon stimulation with TNF-α, TNF R1 levels rapidly increased (5’) but the increase was delayed in the presence of A1AT (120’). While TNF-α alone lead to degradation of Ikβ-α, A1AT pretreatment lead to enhanced TNF-α induction of Ikβ-α. Using real-time PCR analysis we found that acute pre-stimulation with A1AT (4h) followed by acute TNF-a treatment (120’) lead to enhanced TNF-a induction of TNF-α gene expression. Interestingly, chronic stimulation with A1AT/TNF-α blocked TNF-α induction of TNF-α Gene expression. ICAM-1 expression was blocked during both acute and chronic stimulation. In conclusion A1AT may alter TNF-α signaling by regulating Ikβ-α degradation and preventing the TNF-α induced Increase in TNF R1. In the presence of TNF-α, A1AT enhances expression of the NF-kβ inhibitor and delays up-regulation of the TNF-α receptor which may explain why TNF-α induction of ICAM-1 gene expression is delayed with A1AT pre-stimulation. Understanding the anti-inflammatory mechanisms of A1AT is important for developing therapeutic strategies against emphysema.