Anti-Oxidant Gene Therapy in a Rat Sepsis Model Delivered by Adeno-Associated Virus
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Introduction
Lung sepsis, caused by a gram-negative bacterial infection, has a high mortality rate and currently has few effective therapies. It is typified by recruitment of neutrophils to the lungs in response to IL-8 secretion, which produce inflammatory cytokines, leukotrienes and reactive oxygen species (ROS)[1]. These ROS can kill bacteria, but are also associated with injury to the host tissue via cell membrane oxidation and induction of apoptosis or necrosis[2]. Here we have constructed an adeno-associated viral vector, infection by which elicits expression of human superoxygen dismutase (SOD). SOD has three isoforms, of which SOD 3 is most commonly found on the lung epithelial cell surface or free in the bronchio-alveolar fluid (BAL), hence we expected the best results from this gene[3].

Materials and Methods
AAV serotype 6 particles expressing SOD3 were generated by transfection of 293T cells and subsequent purification by fractionation. Animals were anaesthetised, administered 1 x 1011 viral particles intratracheally and allowed to recover for 72 hours. Animals were re-anaesthetised and a lung sepsis injury was induced through intrathecal administration of 0.25mg/kg LPS. With continuous anaesthesia, lung physiology and blood parameters were assessed over a 4 hour time period. At sacrifice, lung fluid and tissue were harvested for subsequent analysis for expression of transgene, secretion of inflammatory markers, and histological examination.

Results
Animals that received intra-tracheal AAV6 containing the EC-SOD transgene demonstrated substantially greater lung EC-SOD expression. Overexpression of pulmonary EC-SOD decreased endotoxin induced bronchoalveolar lavage (BAL) total protein levels and Interleukin-6 concentrations. EC-SOD attenuated the decrease in dynamic compliance, as assessed by peak airway pressures following endotoxin induced injury. Peak airway pressure was significantly lower in animals that received EC-SOD compared to both other groups. Quantitative stereological analysis demonstrated that EC-SOD decreased the degree of histologic injury compared to both other groups. Animals that received EC-SOD demonstrated a decreased acinar tissue volume fraction and an increased acinar air-space volume fraction compared to animals that received no vector, or received a null transgene. (Figure)

Discussion and Conclusions
We have demonstrated that AAV serotype 6 is an effective agent for the transduction of rat lung epithelial cells when delivered intratracheally. We further show that SOD 3 mRNA and protein can be detected at high levels in the lung tissue after 72 hours. Rats that are challenged with lipopolysaccharide (LPS) show significantly improved blood oxygenation and reduced BAL inflammatory cytokine load when treated with AAV6-SOD3 with respect to either vehicle or AAV6-null virus.

References
3. Adapted from www.signumbiosciences.com/

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Disclosures
The authors have nothing to disclose.