The Role of TLR signaling in the development of emphysema in ApoE-deficient mice

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Rationale: Emphysema and atherosclerosis are the leading causes of mortality in the United States. We have demonstrated that hypercholesterolemic Apoe-/- mice develop emphysematous changes in their lung. Recent studies have shown that Apoe-/- mice exhibit abnormal cholesterol efflux with activation of the TLR pathway in atherosclerotic lesions. The present study was conducted to examine the macrophages from the Apoe-/- mice and determine if TLR signaling was playing a role in the development of emphysema in these mice.

Methods: 8-week-old female Apoe-/- mice (n=6) were subjected to a Western diet for 10 weeks and compared to Apoe+/+controls (n=6). After sacrifice, their lungs were fixed for histological analysis. Total lung RNA was prepared for quantitative RT-PCR probed for MMP-9. Lung lysates were subjected to Western Blot analysis. Peritoneal macrophages were obtained from Apoe-/- and Apoe+/+mice and cultured in DMEM for 24 hours. Subsequently, they were treated with various TLR ligands: TLR2 [peptidoglycan (PGN), 2Î¼g/ml], TLR3 [polyinosine-polycytidylic acid (poly(I:C), 2.5Î¼g/ml], and TLR4 [lipid A, the active component of lipopolysaccharide, 100ng/ml].

Results: Elevated expression of Matrix Metalloproteinase-9 (MMP-9), which is a potent protease contributing to emphysema, was observed in the lungs of the Apoe-/- mice. In addition, the up regulation of IRAK1 and G-CSF, downstream targets of the TLR pathway was demonstrated in the lungs of Apoe-/- mice compared to Apoe+/+ mice, suggesting that TLR signaling is activated in the lungs of Apoe-/- mice. TLR4 ligand increased mRNA expression of MMP-9 by 8-fold in Apoe-/- macrophages and by 4-fold in wild-type macrophages. Interestingly, TLR2 and TLR3 ligands did not exhibit any effect on MMP-9 expression in Apoe-/- and wild-type macrophages. These data suggest that up regulation of MMP-9 observed in the lungs of Apoe-/- mice is TLR4 specific.

Conclusion: Augmented expression of downstream targets of TLR signaling in the lungs of Apoe-/- mice suggests that activation of the TLR pathway plays an important role in the pathogenesis of emphysema in these mice, possibly through up regulation of macrophage MMP-9 downstream of the TLR4 pathway.