# Protease Activated Receptors 1 And 2 Mediate Swine Confinement Dust-Induced Lung Epithelial Inflammation

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Rationale: Workers in dusty agricultural environments such as concentrated animal feeding operations (CAFOs) are susceptible to the development of chronic inflammatory lung diseases. Extracts of dust collected from swine confinement facilities (HDE) contain protease activity and have potent pro-inflammatory properties. Protease-activated receptors (PARs) identified in airway epithelium are activated by serine proteases, and have been implicated in lung inflammatory and allergic responses. We hypothesized that lung epithelial PARs -1 and -2 are activated by the proteases found in CAFO dust, and that subsequent signaling events result in harmful inflammatory effects.
Methods: PAR1 and PAR2 were identified on bronchial epithelial cells (Beas-2B, and primary BEC) by immunocytological staining, flow cytometry and western blot. Beas-2B grown in serum-free culture were pretreated for 1 hour with various concentrations of the specific inhibitor of PAR1 (sch79797, 10 pM–10 nM) or PAR2 (FSLLRY-$NH_2$, 0.5–50 nM) prior to challenge with a 5% solution of HDE for 24 hours. In addition, cells were transfected with PAR1 or PAR2-specific siRNA constructs 18 hours before being treated with 5% HDE, protease-depleted HDE, or the above inhibitors +/- HDE for 24 hours. For both experiments, IL-6 and IL-8 were measured in culture supernates by ELISA. To examine HDE-induced effects on PARs surface expression, epithelial cells were challenged with 5% HDE for 30' to 18h, and were then immunostained for PAR1 and PAR2 for cytology and flow cytometry. PAR-specific inhibitors were also used to modulate HDE-induced ICAM-1 expression by flow cytometry.
Results: Both the chemical antagonists and siRNA knockdown of PAR1 and PAR2 inhibited dust-stimulated Beas-2B IL-6 and IL-8 release (PAR1 antagonist: 31 and 41%, and PAR2: 52 and 59%, siRNA: 28 and 41% inhibition of IL-6, IL-8, respectively, $p<0.05$ vs HDE alone). RNA interference did not affect the already blunted response caused by depletion of HDE proteases or inhibitors. Treatment of Beas-2B with HDE reduced the constitutive surface expression of both PAR1 and PAR2 by FACS within 30 minutes (MFI reduced 23% vs. control, $p<0.05$), This loss of receptor signal was gradually reversed from 2-18 hours later. The HDE-induced expression of the adhesion molecule ICAM-1 was also significantly inhibited by both PAR antagonists.
Conclusions: Protease constituents of agricultural dusts contribute to their potent pro-inflammatory properties. Disruption of PAR1 and PAR2 signaling decreased the damaging effects of dust-extract mediated inflammation, suggesting that modulation of PAR function may be a strategy for the treatment of environmental dust induced lung inflammation.

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