Hog Confinement Dust Enhances Neutrophil Adherence to Airway Epithelial Cells.

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Rationale: Persons exposed to swine confinement facilities have evidence of neutrophilic airway inflammation. We previously reported that dust extract from swine facilities is a potent stimulant of interleukin–8 (IL–8) from airway epithelial cells in vitro that is mediated by protein kinase C (PKC) isoforms alpha and epsilon. We hypothesized that dust extract also modulates the ability of neutrophils to adhere to airway epithelium. Methods: Confluent human bronchial epithelial cell monolayers (BEAS–2B) were pre–treated with one of the following agents: anti–ICAM–1 neutralizing antibody or PKC–α or –ε specific inhibitors (Gö6976, RO–318220, respectively) before being exposed to 5% hog confinement dust extract (HDE) for up to 24h. Two epithelial cell lines with inactive PKCα or PKCε (PKCαDN, PKCεDN respectively) were also used. Fresh blood neutrophils (PMN, 300x105/well) labeled with the fluorescent dye calcein–AM were allowed to adhere to the BEAS–2B for 30 minutes. Adherent cells were quantified photometrically. Six wells were averaged for each condition; experiments were repeated 3 times. Multiple comparisons were analyzed using ANOVA with Bonferroni posttest. Results: Adhesion of PMN to BEAS–2B cells treated with HDE was increased in both a time– and dose–dependent fashion. Inhibition of BEAS–2B PKCα using Gö6976 or the PKCαDN cell line resulted in a 55.4%, and 51.4% reduction in PMN adherence, respectively ($p<.01$). Likewise, PKCε inhibition by RO–318220 or PKCεDN cells resulted in a 55.8% and 58.1% decrease, respectively ($p<.05$). Neutralizing antibody blockade of ICAM–1 (1:50 dilution) reduced PMN adherence dose–responsively (68.63%, $p<.01$). Conclusions: Dust extract mediates PMN adherence predominantly by influencing the epithelium and involves PKC isoforms α and ε and ICAM–1.

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