Epidermal Growth Factor Receptor Phosphorylation and Downstream Signaling by an Aqueous Extract of Hog Barn Dust in Airway Epithelial Cells.

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Rationale: Workers in swine confinement facilities are prone to chronic inflammatory lung disease. We previously reported that the epidermal growth factor receptor (EGFR) is required for stimulation of IL–6 and IL–8 secretion by cultured airway epithelial cells in response to an aqueous extract of settled dust from these facilities (HDE, hogbarn dust extract) [AmJRespCritCareMed,177,A158,2008]. Thus we hypothesized that HDE would lead to EGFR phosphorylation and downstream activation of ERK/MAPK signaling. Methods: Beas–2B human bronchial epithelial cells were treated with HDE in the absence or presence of signaling pathway inhibitors, and EGFR and ERK1/2 phosphorylation were then assessed by immunoblotting. Results: Exposure of cells to 5% HDE stimulated EGFR phosphorylation at 5 and 15 min, and this increased phosphorylation was sustained up to 18 hr. EGFR phosphorylation induced by 10 ng/ml EGF was greater in magnitude at 5 and 15 min but the response was of shorter duration. The EGFR tyrosine kinase inhibitor AG1478 (10 μM) prevented EGFR phosphorylation by both EGF and HDE, implicating autophosphorylation. The matrix metalloproteinase (MMP) inhibitor GM6001 (25 μM) did not block HDE–induced EGFR phosphorylation, consistent with its failure to block HDE stimulation of IL–6 and IL–8 release. HDE stimulation of downstream signaling was evidenced by phosphorylation of ERK1/2 upon 5 min exposure; this ERK phosphorylation was inhibited by AG1478, indicating EGFR involvement, and by the MEK inhibitor U0126 (10 μM). Conclusions: HDE induces both rapid and sustained EGFR phosphorylation, and ERK1/2 is in turn activated downstream of EGFR activation. Further studies to identify the MMP–independent pathway by which HDE activates EGFRs and the roles of ERK1/2 in HDE effects may lead to new insights into disease mechanisms and possible new therapeutic approaches.

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