Role of Endoplasmic Reticulum Stress in the Regulation of Induction of Lung Inflammatory Mediators by Organic Dust

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Rationale: Exposure to organic dust is a risk factor for the onset and progression of respiratory diseases with chronic inflammation playing a major role in the disease pathogenesis. Our published studies have shown that organic dust from industrial poultry farms induces lung inflammatory mediators via a cellular pathway involving increased reactive oxygen species (ROS) generation, activation of protein kinases and NF-κB and STAT-3. Elevated ROS generation is known to increase endoplasmic reticulum (ER) stress leading to unfolded protein response (UPR). As ER stress and UPR play important roles in immune regulation and disease development, we hypothesized that ER stress is involved in organic dust regulation of lung inflammation.Methods: The effects of poultry organic dust extract on ER stress sensors IRE-1α, ATF6, and PERK and inflammatory mediators (IL-6, IL-8, IL-1β and ICAM-1) in Beas2B and normal human bronchial epithelial (NHBE) cells and in mouse lungs were analyzed by realtime qRT-PCR, ELISA and western blotting. Effects on cell toxicity were determined by trypan blue staining. Chemical inhibitor and siRNA knockdown studies were performed to investigate the induction of IRE-1α and inflammatory mediators. Results: In Beas2B cells, organic dust extract increased IRE-1α protein levels, whereas ATF6 levels were decreased and PERK levels remained unaffected. Similar results for IRE-1α were observed in NHBE cells and in mouse lungs. Increase in IRE-1α levels was due to increase in mRNA levels. Treatment with dust extract rapidly increased phospho-IRE-1α levels indicating IRE-1α activation. Chemical inhibition and siRNA knockdown studies indicated that induction of IRE-1α was dependent on NF-κB and STAT-3. Studies also showed that TLR2 and Myd88 control induction of IRE-1α by dust extract. Induction of IRE-1α levels was suppressed by NOX inhibitor VAS 2870 indicating the involvement of ROS. IRE-1α knockdown in Beas2B cells reduced induction of IL-6 and IL-8 but had no effect on proIL-1β and ICAM-1protein levels. Additionally, IRE-1α knockdown had no effect on NF-κB and STAT-3 activation.Conclusion:. Our studies have indicated that ER stress sensor IRE-1α is induced by organic dust and is an important regulator of organic dust induced lung inflammation. Induction of IL-6 and IL-8 levels associated with IRE-1α knockdown occurred independently of NF-κB and STAT-3 indicating perhaps the involvement of posttranscriptional mechanisms.Acknowledgement: Supported by Centers for Disease Control and the National Institute of Occupational Safety and Health Grant U54 OH007541.

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