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Category: Predoc
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Title: Diesel Exhaust Particles Reduce Airway Epithelial Barrier Integrity through a Reduction of the Tight Junction Protein Tricellulin
Abstract: Early life exposure to airborne particulate matter (PM) has been increasingly linked to the development of Asthma. While changes to the expression of junctional proteins in lung epithelium has been seen in asthmatics, the impact of PM on their expression has received little attention. We investigated whether exposure to diesel exhaust particles (DEP), a major component of PM, would affect epithelial barrier function by reducing the expression of the tight junction protein Tricellulin. Standard reference material 2975 Diesel Particulate Matter (DEP) was purchased from NIST and suspended in culture media. Monolayers of the human bronchial epithelial cell line 16HBE14o- were grown on collagen coated Transwell inserts and exposed to 5 to 50 µg/cm2 DEP for 6 to 24 hours. Changes in barrier function were assessed by measuring transepithelial electrical resistance (TEER) and permeability of 4 kDa FITC-Dextran. Changes in tight junction protein levels were assessed by Western blotting of whole cell lysates. Neonatal BALB/c mice (postnatal day 3-5) were exposed to 251 ± 27 ug/m3 aerosolized DEP or filtered air for 2 hours per day for 5 consecutive days and sacrificed 2 weeks later. Lungs were collected, homogenized and analyzed by Western blot or RT-qPCR. Exposure to 25 and 50 µg/cm2 DEP significantly reduced epithelial barrier function as measured by reduced TEER and increased permeability to 4 kDa FITC-Dextran at both 6 and 24 hours post exposure without inducing any detectable cytotoxicity. Co-treatment with the antioxidant N-Acetyl Cysteine (NAC) had no effect on this DEP induced barrier dysfunction. These barrier changes coincided with a significant reduction of Tricellulin protein as measured by Western blot by six hours post exposure in 25 and 50 µg/cm2 DEP exposed cells. Neonatal BALB/c mice (pnd 3-5) exposed to aerosolized DEP presented with a significant reduction in Tricellulin in the lung two weeks post exposure as measured by Western blot and RT-qPCR. Taken together, exposure to DEP caused a significant reduction in the expression of the tight junction protein Tricellulin. This reduction corresponds to a significant reduction in barrier function in vitro as measured by reduced TEER and increased permeability to 4 kDa FITC-Dextran without inducing cytotoxicity. Neonatal exposure to DEP caused a lasting reduction of Tricellulin at both the mRNA and protein level, suggesting early life exposure to DEP may cause a stable change in lung barrier structure and function.