In-Vitro Mechanistic Approach to Understand LPS Toxicity in and out of Workplace

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Rationale

Pattern recognition receptors such as membrane bound Toll-Like Receptors (TLRs) and cytoplasmic Nod-like receptors (NLRs) of surface epithelial cells and antigen presenting cells detect airborne pathogens and activate innate immune response providing the first line of defense against these inflammatory agents. Pathogen-associated molecular patterns, such as lipopolysaccharide (LPS) in bacterial cell walls and bacterial flagella, acts as ligands to trigger for downstream pathways that release inflammatory mediators such as TNF-α and interleukins (IL1β, IL2, IL6 and IL18). TLR dimerization by PAMPs is critical for subsequent downstream signaling mediated by inducible nitric oxide synthase (iNOS) to cause oxidative or nitrative stresses that lead to tissue inflammation. Effect of size-specific airborne LPS on these inflammatory pathways, however, has been under-researched.

Methods

Human bronchoepithelial cells (BEAS2B) will be maintained in BEBM at 37°C, 5% CO2 and ambient O2 (21%). Cells plated in 6-well plates will be treated 24 hours later with LPS extracts from PM1 and T1 (>1.8 μM) fraction of Bio-aerosol sampler (NIOSH). Untreated cells will used as controls. In separate experiment either CM-H2DCFDA (oxidative stress indicator) or DAF-FM (nitrative stress indicator) will be loaded into cells (×30 minutes) and subsequently will be observed for fluorescence intensity under confocal microscopy. Intensity being proportional to stress mediators will be indicator of the extent of cellular stresses upon exposure to particular size-specific LPS. IL-1β, IL-6, TNF-α and iNOS mRNA and protein expressions will be measured in the in cultured media of naïve and size-specific-LPS treated BEAS2B.

Expected results

TLRs on APCs dimerize in order to convey intracellular signals that produce inflammatory mediators. Larger LPS particles from T1 fraction would be more effective at causing such dimerization than smaller PM1 LPS. Thus cells exposed to T1 LPS is expected to produce more fluorescence than that exposed to PM1 indicating that later cause less airway inflammation. Compared to cells exposed to T1-LPS, the IL-1β, IL-6, TNF-α, iNOS mRNA and protein expression will be significantly higher in than cells exposed to PM1-LPS.

Conclusion

These in-vitro experiments would help in understanding LPS-toxicity from airborne size-specific LPS particles that are of specific relevance to health care, farm and animal care workers.

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