NIOSH Pilot Research Project | Symposium 2013

Endotoxin Exposures in Size-specific Airborne Particles In & Out of The Workplace

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Background

Microbial contaminants in size-selective airborne particles

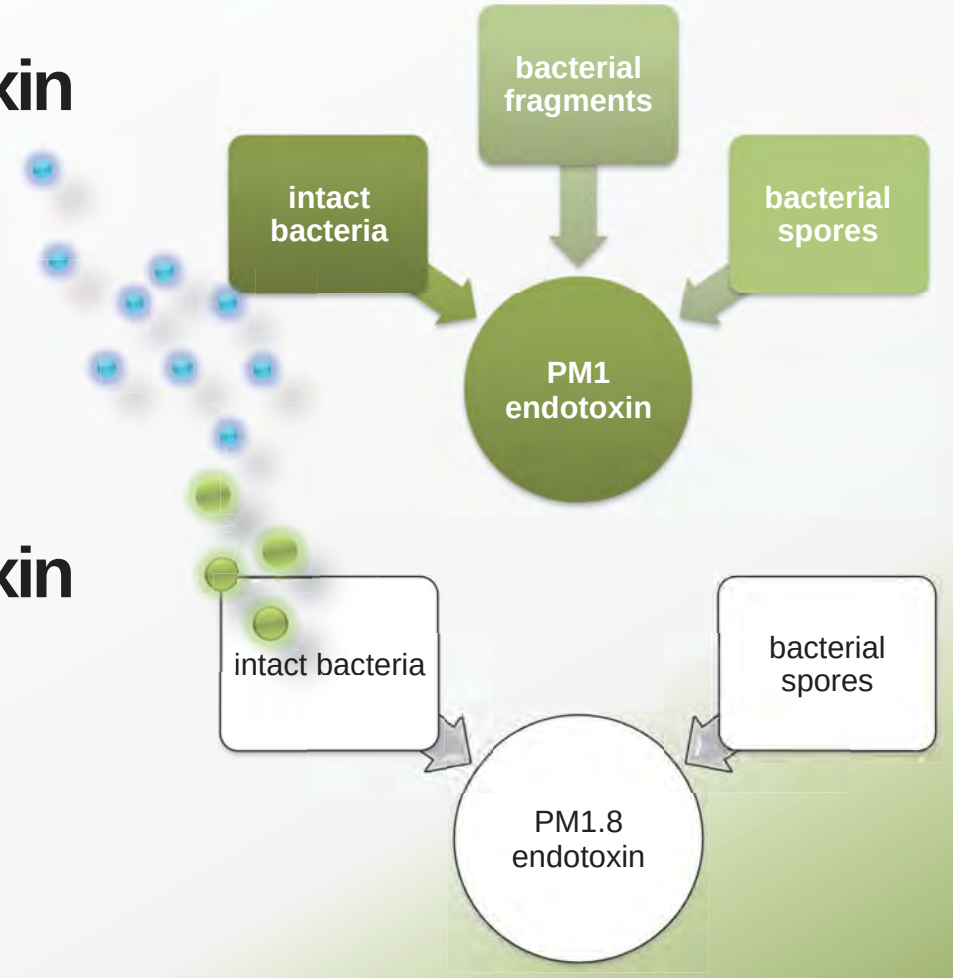
endotoxin exposures in size-specific airborne particles in & out of the workplace

PM1.0 endotoxin

d_a<1.0 µm

PM1.8 endotoxin

d_a=1.8 µm



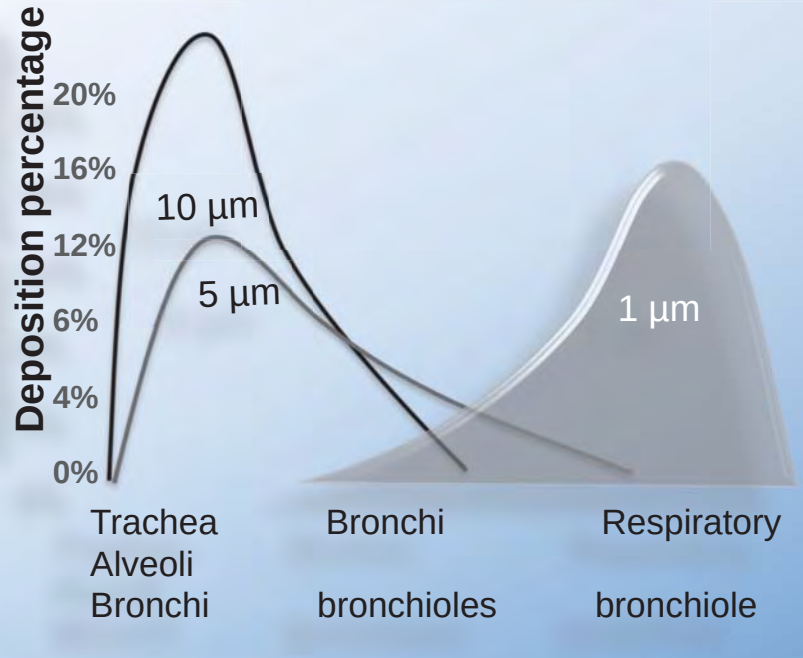
Background

Health relevance

Exposures to Airborne PM1 Microbial Particles Are More Relevant for Assessing Health Outcomes

PM1 microbial particles

- can penetrate up to and deposit on alveolar ducts
- may have effects on
  - inflammatory mediators
  - airway inflammation



Background

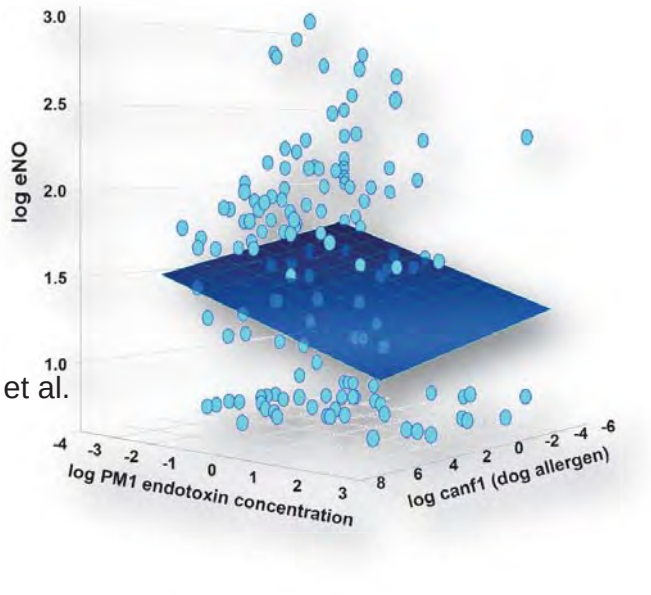
Previous study

Predictors of eNO level in non-asthmatics

Airway response to PM1 endotoxin exposures

NON-ASTHMATIC CHILDREN (n=146)				
Predictors of eNO	parameter estimate	95% CI		p-value
PM1 endotoxin	**-0.14**	**-0.24**	**-0.04**	**<0.01**
Dog allergen (+)	-0.39	-0.74	-0.04	0.03

HUD-CCAAPS, LeMasters, Reponen, et al.



PM1 endotoxin exposure is associated with significantly less airway inflammation in non-asthmatic school age children….

.....biologically unexplained

Hypothesis

Induction of nitrative stress and the inflammatory response in human bronchoepithelial cells with endotoxin exposures (at equivalent concentrations) would vary significantly depending on the size of endotoxin particles (i.e., d_a<1.0 and >1.8µm).

Specific Aims

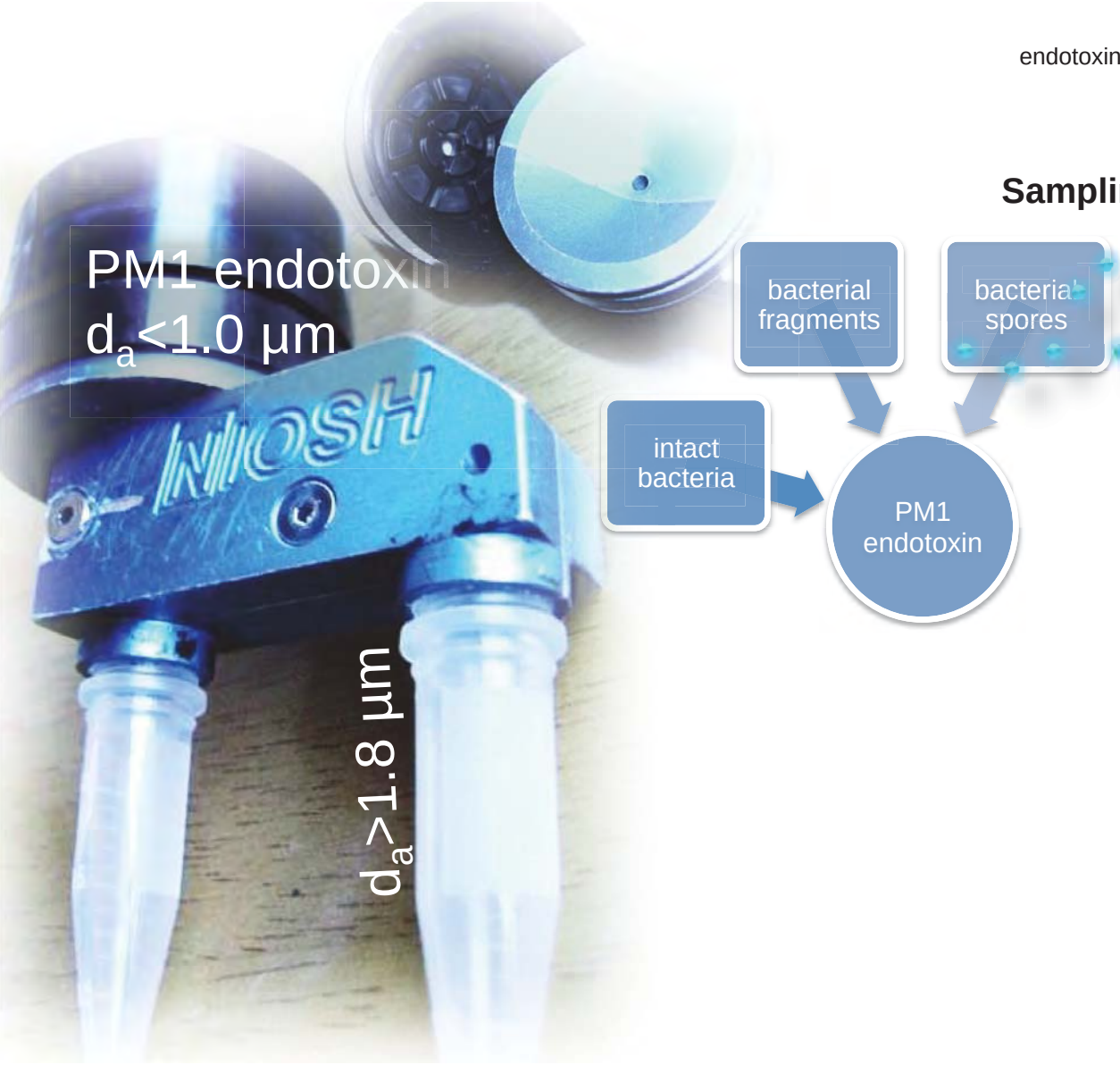
Aim 1: To determine nitrative stress induction and

Aim 2: To determine gene expression regulating inflammatory responses

in human bronchoepithelial cells after exposures to (similar concentrations of) endotoxin derived from size-specific airborne particles (i.e., in particles < 1 um and >1.8 um).

Methods

Sampling of Size-specific Airborne Endotoxins



Bio-aerosol cyclone sampler cyclone designed to deposit aerosols into standard micro centrifuge tubes and a back-up filter

Endotoxin extracted from particles using non-ionic detergent (Tween 80)

Methods

Analysis of Size-specific Airborne Endotoxins

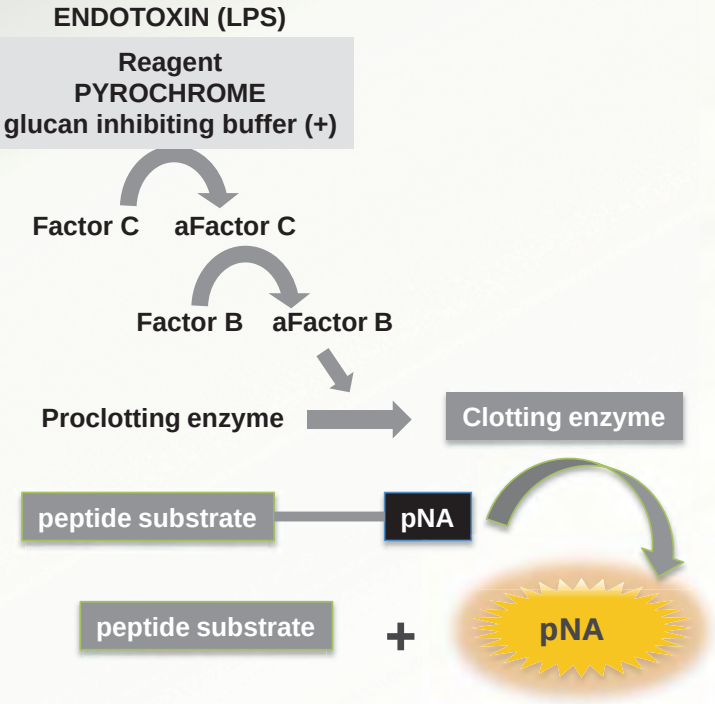
Limulus Amebocyte Lysate (LAL) Assay for Quantification of Endotoxins

activated clotting enzyme

synthetic chromogenic substrate that releases a chromophore when substrate is broken

activity measured by fluorescent intensity at 405 nm

rate of optical density increase proportional to concentration of endotoxin / glucan



Limit of detection: 0.0526 EU/ml

Results

Endotoxin concentrations in size-specific particles:

Particle size	Endotoxin concentration (EU/mL)
<1.0	34.47
>1.8	399

Methods

In Vitro Nitrative Stress Assays

– Human bronchoepithelial cells grown cover-slips, 5% CO2, 37°C

– Loaded (stained) with nitrative stress indicator dye, DAF-FM*, ×30min and washed ×30min

– Cells were treated with endotoxin extracts from particles <1.0 µm and >1.8 µm

– Images under fluorescent microscope (ex/em 488/514 nm) were taken before and after treatment

– Nitric oxide (NO) produced by cells was quantified by emitted fluorescence.

- DAF-FM essentially nonfluorescent until it reacts with NO to form a fluorescent compound and thus fluoresces more in presence of NO produced endogenously. (NO detection limit for DAF-FM ~3 nM)

– Fluorescent intensities after treatment (F) were compared to baseline intensities before treatment (F0)

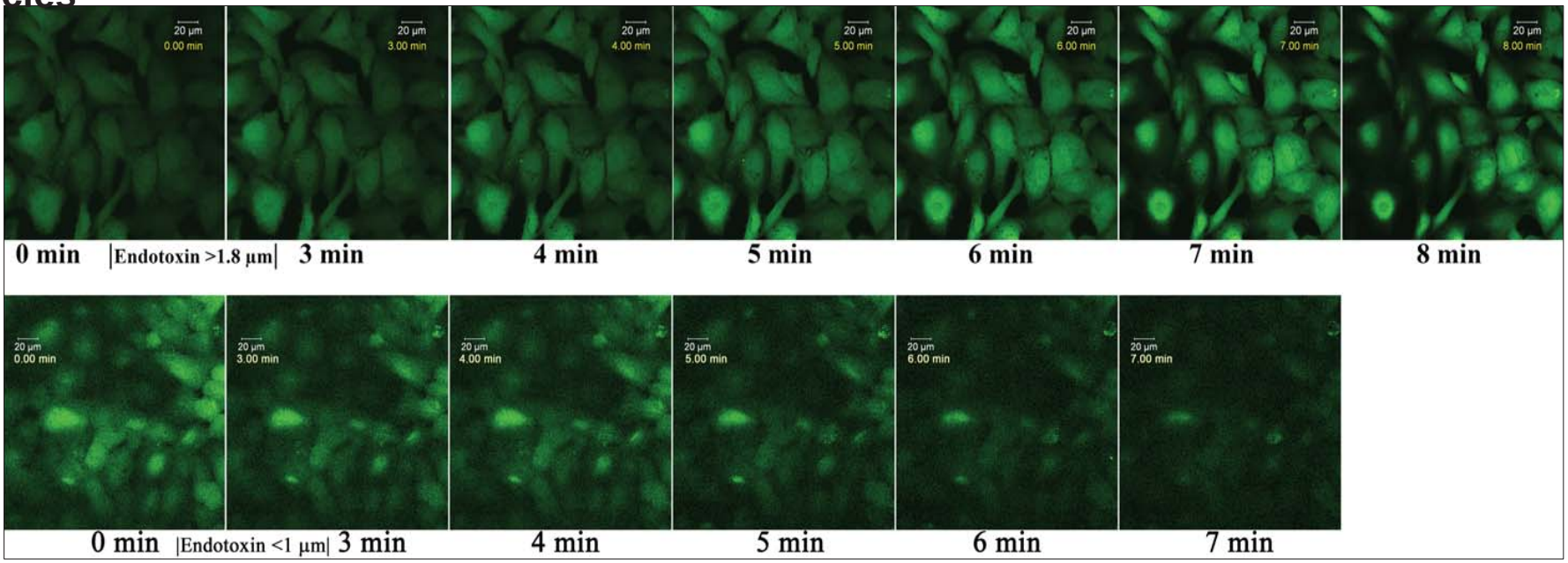
*4,5-diaminofluorescein diacetate

Results

Induction of nitrative stress associated with endotoxin exposures in size-specific particles

Endotoxin da>1.8 µm

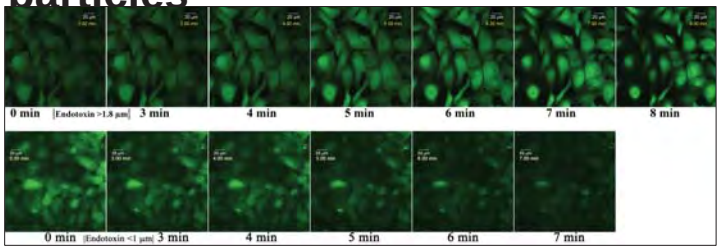
Endotoxin da<1.0 µm



Nitrative stresses were measured in BEAS-2B by observing fluorescence under fluorescence microscope (excitation/emission spectra 488/514 nm) every 1 minute following exposures to endotoxin extracts derived from size-specific particles.

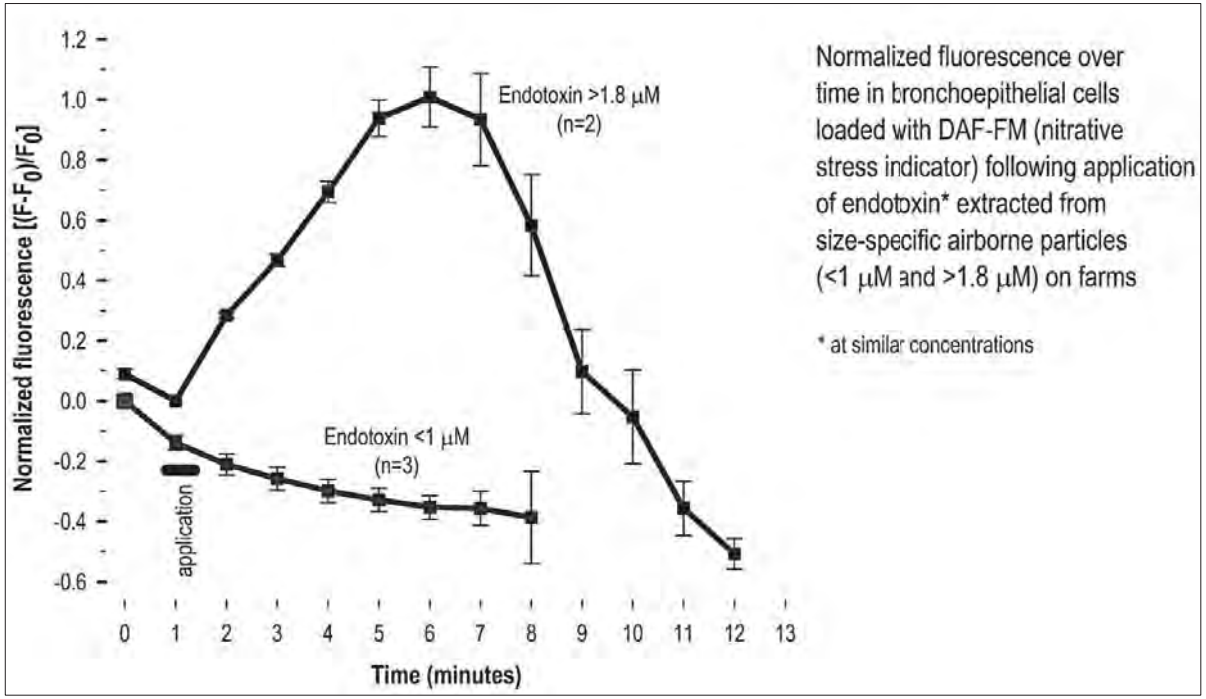
Results

Induction of nitrative stress associated with endotoxin exposures in size-specific particles



- Exposures to endotoxin in particle < 1µm did not result in a significant increase in fluorescence compared to pre-treatment (baseline) fluorescence.
- In contrast, exposures to endotoxins extracts from particle size > 1.8 µm resulted in an increase in fluorescence over 4-5 minutes.

Conclusion: Findings are suggestive of nitrative stress induction in BEAS-2B cells following exposures to endotoxin in particles >1.8 µm but not in particles <1 µm.



Methods

Determination of relative changes in inflammatory gene expression after treatment with endotoxin in size-specific particles

– Cells were treated with similar concentrations of endotoxin extracts from size specific particles x20 min

– Cells were lysed

– mRNA extracted

– mRNA to cDNA

– cDNA amplification TaqMan® gene expression array for inflammatory pathway

Results

Relative gene expression in cells exposed to endotoxin in particles with d_a<1µm vs. cells exposed to endotoxin in particles with d_a>1.8 µm

Induction of cytokines and over-expression of receptors mediating inflammation were compared following exposures to endotoxin in airborne particles with da<1 µm and >1.8 µm about 20 minutes after such exposures in vitro on BEAS-2B cell lines.

Exposures to endotoxin in smaller particles resulted in:

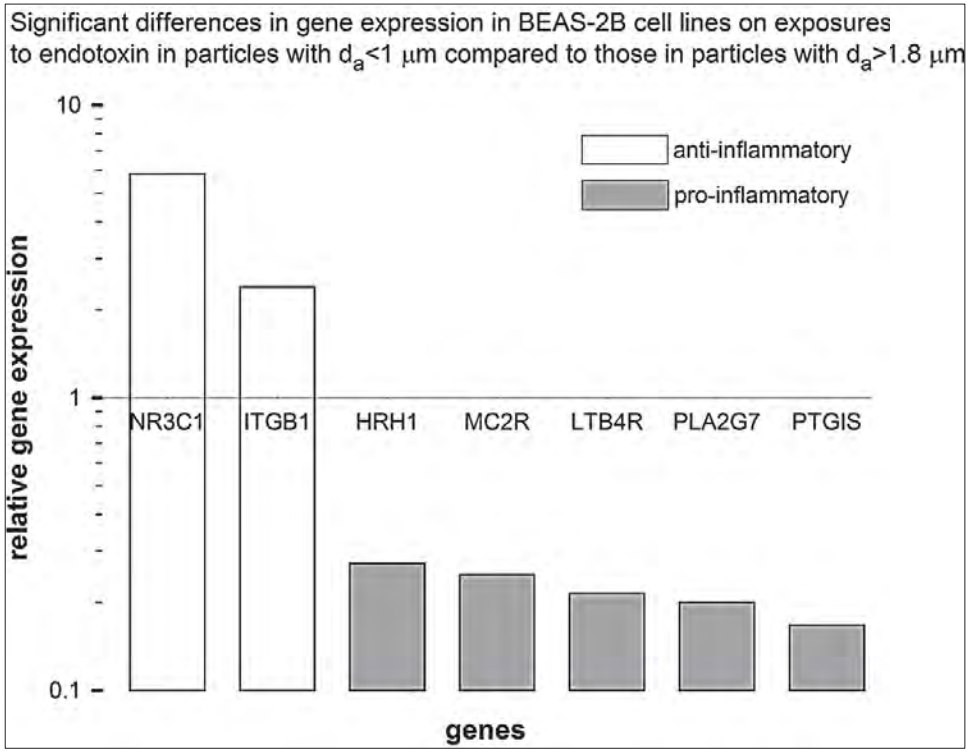
relative overexpression of genes for

- glucocorticoid receptors (NR3C1) and
- integrin beta 1 (ITB1) **….anti-inflammatory genes**

but down-regulation of genes for

- Leukotriene B4 receptors (LTB4R)
- Histamine 1 receptors (HRH1)
- Melanocortin receptors (MC2R)
- Prostacyclin synthase (PTGIS)
- Platelet activating factor acetylhydrolase (PLA2G7)

…….pro-inflammatory genes



Conclusion

Airway epithelial cells exposed to endotoxin in airborne particles with d_a<1.0 µm

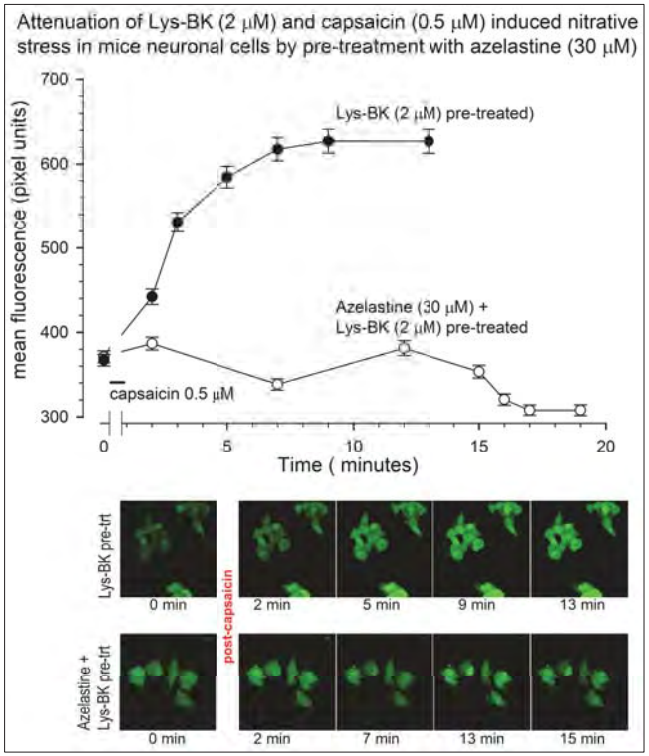
- suffer less nitrative stress
- over-expressing anti-inflammatory genes and down-regulating pro-inflammatory genes

than those exposed to iso-concentrate endotoxin in particles with d_a>1.8µm

Discussion

- With this in vitro study we thus **verified our previous findings obtained in epidemiologic studies** (i.e., children living in homes with higher concentrations of PM1 endotoxin have less airway inflammation.
- This study, however, did not determine the **complex interactions of different inflammatory mediators (e.g., TNFα, histamine, endotoxins) and channels mediating inflammation (e.g., TRP, HT, TLR)** that ultimately determine the overall inflammatory response of bronchial epithelium.
- Interpretations are limited by the relatively small number of experiments performed contingent with the resources available.

Research to Practice (r2p)



Transient Receptor Potential Vanilloid Type 1 are non specific ion channels; present in airway epithelial cells

Endotoxins up-regulate and activate TRPV1 (Sadofsky et al., 2012). In our supplementary study we have determined that TRPV1 activation in Bradykinin pre-treated cells induces nitrative stresses.

- **Exposures to endotoxin in particles with da<1.0 m are less hazardous**
- **However, endotoxin exposures in particles with d_a>1.8 µm should be monitored among workers with pre-existing allergic diseases with endogenous bradykinin-induced hypersensitivity of airway epithelium**
- **exposures to endotoxin (da>1.8 µm) in these susceptible workers may induce severe nitrative stresses.**

Sadofsky, L.R., et al., Inflammatory stimuli up-regulate transient receptor potential vanilloid-1 expression in human bronchial fibroblasts. Exp Lung Res, 2012. 38(2): p. 75-81.

Impact Statement

Comprehensive approaches towards environmental exposure assessment

This study demonstrates newer approaches towards environmental monitoring for workers in health-care settings, zoos or farms where **exposures through airborne endotoxins in size-specific particles** would be a significant concern.

The National Occupational Research Agenda (NORA) Priority Area

Endotoxin exposure is often an ignored but significant occupational exposure among

- **agricultural workers**
- **healthcare workers and**
- **animal handling occupations**

that may impact acute or chronic health of workers.

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