CARBON NANOPARTICLE LUNG EFFECTS: HMGB1 AND PATTERN RECOGNITION RECEPTORS AND CYTOKINES

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BACKGROUND

• Nanotube technology is present in our everyday life: stronger dental implants, sunscreens, smaller electronics
• Exposure to nano-technology is known to induce cytotoxicity in lung cells; the nature of these effects is poorly understood
• **Hypothesis:** the initial cytotoxic effect of single wall carbon nanotubes (CN) is produced by cellular release of damage-associated pattern recognition receptors (PRRs), and the earliest measure being the high mobility group box 1 (HMGB1) protein
• HMGB1, extracellularly, exerts its actions as a ligand for pattern recognition receptor activation in target cells.
• Our in vitro, ex vivo and in vivo data suggests this action triggers subsequent cytokine upregulation.

METHODS

Purified Single Wall Nanotubes (CN) SES Research, Houston, TX, USA. Short CN with small amounts iron (Fe) and cobalt (Co) as impurities determined by Energy Dispersive Spectrometry (EDS) performed in-house

**IN VIVO:**
- Rat model dosage 25 µg endotoxin free CN in 50 µL volume i.t. (n=45)
- Samples harvested: BAL, pleural fluid, plasma, lung tissue, viable lung cells
- Assays: Tissue and BAL

**RESULTS**

- RAW blue cells express a “secreted embryonic alkaline phosphatase” (AP) gene inducible by NFkB
- RAW blue cells in culture were exposed to BALs from CN-exposed rats
- AP is detected at 620 nm on cell supernatants
- Significant amounts of NFkB were stimulated by the BALs harvested at 3 and 24 hr following nanoparticle exposure of the animals (graphic in next column)

**CONCLUSIONS**

- These experiments show that HMGB1 is a vital, early marker of sterile nanoparticle damage
- These experiments show that HMGB1 is present in BAL of nanoparticle exposed rats at levels activating downstream pattern recognition receptors (PRRs)
- Use of RAW blue cells show these pathways, not previously shown for DAMP markers
- This demonstration was performed largely in cell culture, although in vivo toxicity of CN is inferred

**SOURCES/REFERENCES**

1. Raw BLUE CELLS: INVITROGEN Corp. (San Diego CA) Derived from RAW 264.7 macrophages. Expresses a secreted alkaline phosphatase gene inducible by NFkB transcription factors. A Reagent allows a blue color to be developed when the NFkB pathway is stimulated. Assays Performed at UMKC with in house reagents.

2. Interleukin 10 assayed in house by commercial R&D ELISA kit using bronchoalveolar lavages from rats exposed to carbon nanoparticles on UMKC IACUC protocol 1104.

3. HMGB1 ELISA measured in house with a commercial Japanese ELISA kit