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

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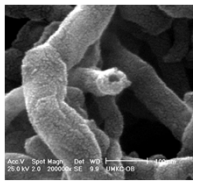
BACKGROUND

- 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



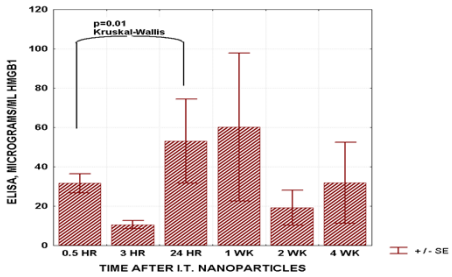
IN VIVO:
- Rat model dosage 25 µg endotoxin free CN in 50 µL volume i.t. (n=45)
- Samples harvested: BAL, pleural fluid, plasma, lung tissue, viable lung cells
- Assays: Tissue and BAL

Histology post CN in animals,
- ELISA or HMGB1 cytokines.
- Flow cytometry for HMGB1 receptors in lung cells
- Western blots for receptors.

RAW BLUE CELLS:
- In vitro: Purified HMGB1 activates PRRS in cells
- Ex vivo: HMGB1 containing BAL activates PRRS in RAW Blue cell nuclear fraction (Western blot)
- In vitro: to demonstrate HMGB1 role as PRR

IN VIVO

HMGB1 , NUCLEAR CHAPERONE, APPEARS IN BAL AFTER INTRATRACHEAL CN EXPOSURE

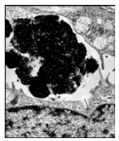


Identify HMGB1 receptors : flow cytometry and Western Blot

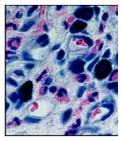
Type II pneumocytes and BAL macrophages from rats exposed to CN at 0.5, 3, 24 hr and 4 weeks
lung (Western blots)

Evaluate lung cell pathology after CN

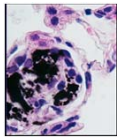
TEM – nanofiber indents Nuclear membrane



Eosinophils in lung 30 min after CN



Lung granuloma 30 days after CN



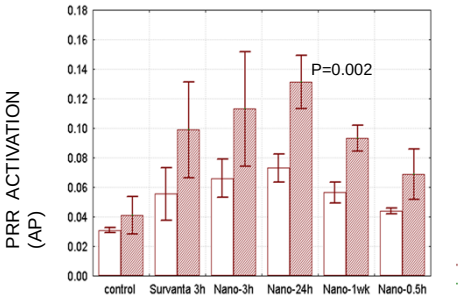
IN VITRO, EX VIVO

- 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

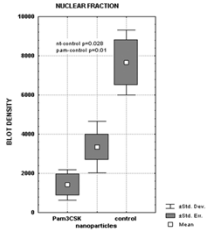
(graphic in next column)

RESULTS

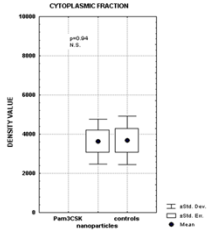
RAW BLUE CELLS CULTURED WITH ALVEOLAR LAVAGES FROM NANOPARTICLE EXPOSED RATS



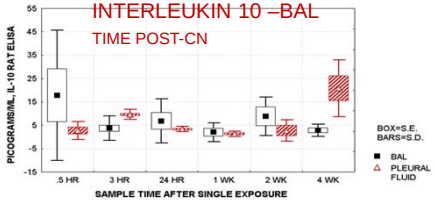
WESTERN BLOT FOR HMGB1 RELEASE FROM CN-EXPOSED RAW BLUE CELLS



Nuclear fraction , macrophages stimulated with CN (or Pam3CSK a TLR 2 agonist) Detected with anti-HMGB1 p=0.02



Cytoplasmic fraction from NE-PER-separated cell fractions shows HMGB1 was released from cell



SUMMARY

In vivo
- HMGB1 the nuclear chaperone is released into BAL by CN containing no LPS (0.03 EU)
- CN-stimulated lungs produce measurable HMGB1 in < 30 minutes
- 3 hr after CN, HMGB1 cell-bound receptors upregulated; cytokines elevated in BAL and pleural fluid
  -IL-10 highest at 30 days
- Lung histopathology highest at 3 and 24 hours

In vitro / ex vivo
- Purified HMGB1 protein activates PRRs in RAW blue cells (Stably transfected macrophages)
- CN-triggered HMGB1 (from BALs) induced t expression of AP in cultured RAW blue cells.
- CN lung effects implies a role for HMGB1 via activation of PRRs through TLRs, RAGE and cytokine stimulation
- HMGB1-mediated PRR activation offers new mechanisms to explore regarding CN –induced pulmonary inflammation

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 . Express a secreted alkaline phosphatase gene inducible by NF-kB transcription factors. A
Reagent allows a blue color to be developed when the NF-kB 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

*Data previously presented at Experimental Biology 2011 Washington DC and Leukocyte Biology 2011, Kansas City