

CYTOKINES IN LUNG FOLLOWING STERILE DAMAGE BY NANOPARTICLES

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BACKGROUND

- Carbon nanoparticles (CN) are among the strongest substances known and carry remarkable electrical potential.
- These characteristics, among many other unique properties, have made CN a huge academic and industrial interest.
- The potential uses for CN range from making stronger and lighter aircrafts to creating paper batteries.
- However, there has been evidence of potential toxicity secondary to respiratory exposure to CN.

INTRODUCTION

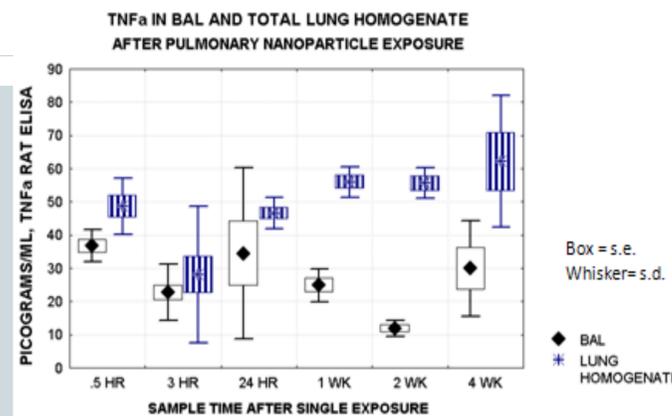
- My research rotation included gathering ELISA data on cytokines as part of a CN study.
- Intratracheal (i.t.) administration of CN was accompanied by acute inflammation in the airspaces and lung parenchyma.
- The alarm marker of cell damage, HMGB1, binds its receptors, Toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE).
- Together the HMGB1-receptor complex up-regulates pro-inflammatory or anti-inflammatory cytokines.
- Following i.t. CN exposure, we measured the subsequent release of inflammatory Interleukin-6 (IL-6) and Tumor Necrosis Factor- α (TNF α) and anti-inflammatory Interleukin-10 (IL-10).

METHODS AND MATERIALS

- Sources of body fluids measured in this report included bronchoalveolar lavage (BAL), pleural fluid and plasma from rats administered 25 micrograms of carbon nanoparticles suspended in 50 microliters fluid (either rat serum or pediatric surfactant).
- 0.5 hours to 30 days after receiving nanoparticles, rats were sacrificed by lab staff using approved protocol.
- Fluids were immediately harvested and frozen for batch testing.
- Templates for 96-well Elisa plates were made, and thawed aliquots of samples were arranged in the same format.
- Standard curves were included in all runs for quantitative measurement.
- The rat specific ELISA kits were obtained from R&D systems.
- Buffers, dilutions, amounts and incubation times were performed exactly as recommended by the manufacturer.
- Plates were read on a Powerwave BioTek reader and analyzed by BioTek KC4 software.
- Graphs were made in Statistica where ELISA data were keyed and analyzed.

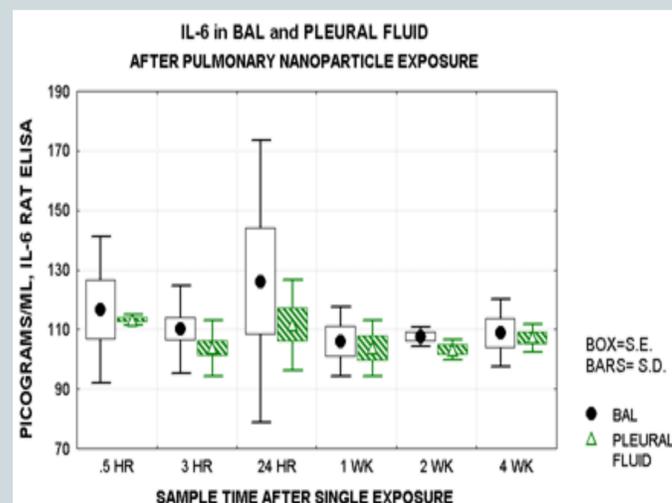
TNF α

- This cytokine is associated with early systemic inflammatory response syndrome following severe trauma.
- Clinical studies show direct relation between BAL levels of TNF α and severity of pulmonary illness (i.e. lung compliance and hypoxemia).
- We hope to help clarify TNF α 's role in pathogenesis of pulmonary nanoparticle exposure.



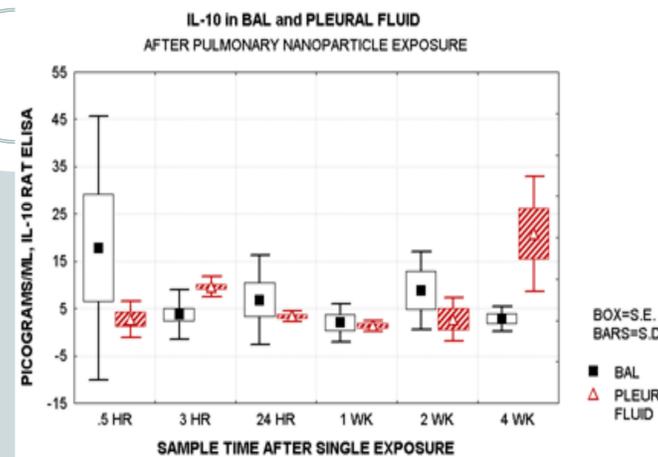
IL-6

- This cytokine is pro-inflammatory and can promote the evolution into a chronic inflammatory state.
- Overall, IL-6 is pivotal in inflammation with systemic repercussions.
- It is slower to respond to nanoparticles than TNF α but provides a greater chance for chronicity.



IL-10

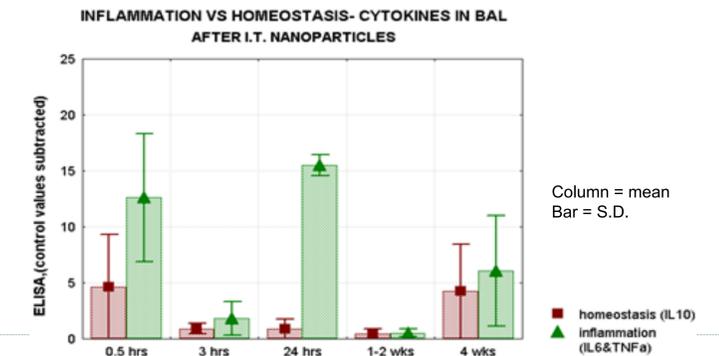
- This highly potent anti-inflammatory cytokine inhibits production of other cytokines by macrophages.
- Some suggest that the inhibition of inflammatory responses may encourage infection.
- Correlating the titers with lung histopathology, our studies of this cytokine tend to emphasize anti-inflammatory activity.



RESULTS

- Cytokines TNF α , IL-10, and IL-6, known to respond to HMGB1-receptor complexes, were elevated at all time periods (p=0.04).
- Pro-inflammatory TNF α** increased in BAL at 0.5 hours, decreased over two weeks, then increased again at four weeks. Different than BAL, TNF α remained elevated over four weeks in lung homogenate.
- Pro-inflammatory IL-6** predominated at 24 hours, slightly declined over two weeks, and then started to rise again at four weeks.
- Anti-inflammatory IL-10** initially increased in BAL at 0.5 hours, then decreased and remained low over four weeks. Different than BAL, IL-10 increased at four weeks in pleural fluid.
- TNF α was increased in lung homogenate that is different from BAL. We have begun evaluating different cytokines and lung proteins by Western blot using the lung homogenates, which will further explore this difference.

SUMMARY CHART



CONCLUSIONS

- We found that sterile intratracheal (i.t.) introduction of CN to rats produced both acute and long-term pulmonary inflammation.
- Immediate cell damage released "alarm" protein HMGB1, which bound to receptors and stimulated IL-6, IL-10, and TNF α .
- The late (4 week) rise in both pro- and anti-inflammatory cytokines remains to be clarified.
- We hypothesize that the nanoparticles may be released from dying phagocytes (macrophages) at 4 weeks, and acute effects may be repeating. This will be evaluated in longer-term studies.
- No clear correlation was found between HMGB1 binding to TLR4 and RAGE receptors and the subsequent release of pro- and anti-inflammatory cytokines. This suggests that another receptor is involved.

WHAT'S NEXT?

- I have received a Sarah Morrison Research Grant to further investigate CD24 as a potential receptor involved in the cytokine response following CN administration.
- CD24 has been found to be involved in several cancers; could it be involved with initiating lung cancer following CN exposure?

REFERENCES

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