Utilizing flow cytometry to determine inflammation-related receptors associated with nanotube-induced lung toxicity

Adam Fleddermann

Materials & Methods

To test this question an ex vivo experiment was designed using the flow cytometer on harvested type II pneumocytes. Rat lungs were harvested at 0.5 hr, 3 hrs (including control), 24 hrs, 1 week, and 30 days. The type II pneumocytes were collected from whole lung using elastase incubation.

Cells were fluorescently labeled (FITC) with anti-RAGE, TLR4 and TLR9 antibodies or appropriate isotype controls, and were fixed with 2% paraformaldehyde.

The samples were evaluated at Children’s Mercy Hospital at the Flow Cytometer Lab with the assistance of Ruth Morgan.

Background

Presence of damage associated molecular proteins (DAMPS) are associated with pulmonary exposure to single walled carbon nanotubes (SWCNTs).

Our studies of SWCNT delivered intratracheally (i.t.) to rats shows both acute (below) and chronic injury.

This tissue damage releases a nuclear “alarm protein,” high mobility group box protein 1 (HMGB1), which binds receptors. The receptor-HMGB1 complex induces cytokine release in the lung.

Which receptor(s) produce the carbon nanotube effects?

Several studies (1-3) suggest HMGB1 receptor effects.

Hypothesis: The receptor combining with it makes a ligand-receptor combination to stimulate cytokine upregulation.

Cytoxines can be either inflammatory or non-inflammatory.

Hypothesis: The type and amount of receptor available to bind HMGB1 influences the type of cytokine thus the nanoparticle effect on the lung.

Summary

This study utilized flow cytometry to determine the type and number of potential “alarm protein” receptors on pulmonary cells of rats exposed to SWCNT.

Our “alarm protein” studied was the HMGB1 response to sterile SWCNT, and the receptors RAGE, TLRs 4 and 9 were chosen based on referenced articles.

Both type II pneumocytes and macrophages were tagged with FITC labeled antibodies and corrected for non-specific reactions with isotype and no-nanoparticle controls.

We conclude that RAGE is the major HMGB1 receptor acutely, particularly at 3 hours in type II pneumocytes.

TLR4 shows a strong presence at 3hr, 1 week, and 30 days.

TLR9 showed low presence at all times.

Data on receptor levels will be compared to cytokines to determine whether hypothesis is accepted or rejected.

Limitations of this study include untested receptors such as TLR2, lack of repetition of assays, and lack of assays for soluble receptors.

This is a work in progress and these items are being added.

Hypothesis for Study

- HMGB1 produces no inflammation on its own
- The receptor combining with it makes a ligand-receptor combination to stimulate cytokine upregulation.
- Cytokines can be either inflammatory or non-inflammatory.
- Hypothesis: The choice and amount of receptor available to bind HMGB1 influences the type of cytokine thus the nanoparticle effect on the lung.

Results

Rage receptor, Type II pneumocytes

TLR-4 receptor, Type II pneumocytes

TLR-8 receptor, Type II pneumocytes

Shaded peaks are the lung cells stained with 3 receptor antibodies.

Open peaks are cells from the same lungs stained with control globulin (isotype control).

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