Ligand-receptor involvement in the pulmonary inflammation by sterile nanoparticles

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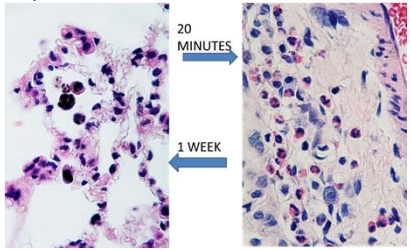
BACKGROUND

- Carbon nanoparticles (CN) are persistent in vivo and distribute widely following pulmonary exposure.
- Massive eosinophilia was observed 20 min after a sterile intratracheal (i.t.) dose of 25 µg CN in 50 µL serum to rats.



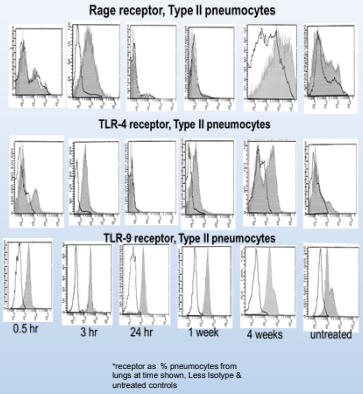
- To evaluate the extent of this cellular effect, we chose the nuclear chaperone, high mobility box protein (HMGB1), which is released into the airways when cells are damaged and become necrotic.
- Our damage marker, high mobility group box protein 1 (HMGB1), showed both acute (3-24 hours) and chronic (1-4 weeks) peaks with corresponding increases in cytokine concentration, notably Interleukin-6 (IL-6).

The question: Is in vivo pulmonary response to sterile CN a function of which cytokines are triggered by the ligand (HMGB1)-receptor combination, and, does the lung response (cytokine type; quantity) relate to the receptor identity?

Literature Search

A MEDLINE search was performed using the following terms: HMGB1 Protein, Pattern Recognition Receptors, Interleukin-6, Toll-Like Receptor 4, Toll-Like Receptor 9, and RAGE.

FLOW CYTOMETRY



*receptor as % pneumocytes from lungs at time shown, Less Isotype & untreated controls

% total cells*	Time after NT
0.5	0.5 hr
64.6	3 hr
0	24 hr
2.1	1 wk
0.4	4 wk

% total cells*	Time after NT
9.8	0.5 HR
50.1	3 HR
0	24 HR
45.8	1 WK
30.2	4 WK

% total cells*	Time after NT
9.9	0.5 hr
12.3	3 hr
6.5	24 hr
13.3	1 week
10.2	4 weeks

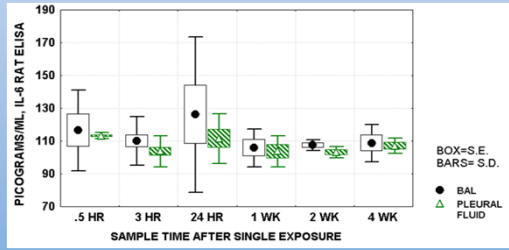
METHODS

- Purified CN was given as a sterile intratracheal (i.t.) dose of 25 µg CN in 50 µL serum to rats (n= 6-7 rats/time period).
- Lungs, plasma, and bronchoalveolar lavage (BAL) were analyzed at 0.5 hr, 3 hr, 24 hr, 1 - 2 weeks, or 4 weeks after one dose of CN.
- BAL, pleural fluid, plasma and lung homogenates were measured by ELISA for ligand HMGB1 and inflammatory cytokine IL-6 at times following CN dosage.
- Type II pneumocytes were isolated and 3 HMGB1 cell-bound receptors were measured by flow cytometry: Toll-like receptors 4 and 9 (TLR4, 9) and receptor for advanced glycation (RAGE).
- HMGB1 and cytokines were measured by ELISA on BAL, pleural fluid, and plasma.
- Since the 3 receptors may also bind ligand HMGB1 when all are soluble in the lung milieu (thereby reducing HMGB1 combination to cell bound PRRs which stimulate cytokines) soluble receptor presence was confirmed by Western blot.

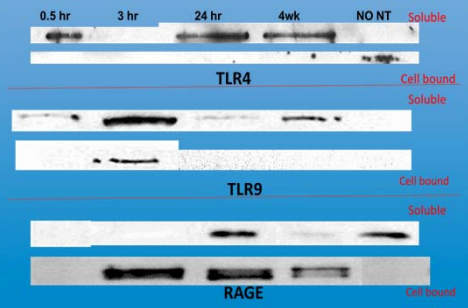
RESULTS

- Western blot data showed increases in soluble TLR-4 at 0.5 hr, 24 hr, and 4 wks.
- TLR-9 was the major soluble PRR present at 3 hr
- Cell bound RAGE was highest at 3 hr and 24 hr, with residual elevation persisting at 4 wks.
- BAL HMGB1 (pg/mg protein) was elevated at 0.5 and 24 hours, but low at 3 hours ($p<0.04$).
- Type II Pneumocyte TLR-4 and RAGE were both low at 0.5 and 24 hours and high at 3 hours ($p=0.05$).
- IL-6 and TNF-α in BAL followed HMGB1 values until 4 weeks when both showed an increase.
  - This indicates a possible internal re-exposure.

HMGB1 IN BAL AND PLEURAL FLUID



Representative Western blots, soluble and cell-related



Hmgb1 RECEPTORS,

0.5 hr	3 hr	24 hr	4 weeks
Rage			
Flow 0.5%	64.6%	0%	0.4%
Cell bound 0	31,719 pixels in density peak.	32,000	1,000
Western blot			
TLR-4			
Flow 9.8%	50.1%	0	30.2%
Cell bound 0	0	0	0
Western blot			
TLR-9			
Flow 9.9%	12.3%	6.5%	10.2%
Cell bound 0	813	0	0
Western blot			

CONCLUSIONS

- **Based on the Western blot data:**
- We predicted cytokine levels to be elevated at 3 and 24 hrs post-CN exposure and absent at 0.5 and 4 weeks.
- Actual results showed levels of IL-6 were elevated at 0.5, 24, and 4 weeks following exposure, indicating a disconnect between cytokine response and cell bound PRR identity.
- Data suggest that subsequent inflammation by a common inflammatory cytokine is not predicted by level of HMGB1 receptors on the pneumocytes or in lung homogenates of CN-treated rat lung.
- The rise in inflammatory cytokines at 4 weeks without additional CN exposure hints at a chronic effect.

REFERENCES

1. Herndon B et al, E. Exp Biology 2009, Poster B289, New Orleans April 20, 2009
2. Tian J et al. Nature Immunol. 8: 487-96, 2007.
3. Hori O et al.. J. Biol Chem 270: 25752-61, 1995.
4. Park JS, et al, J Biol Chem 2004: 279:7370-77

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