Nanomaterials Unique Potential for Activating Innate Immunity

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and Director
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Disclosures:
Former employee, MERCK
Former CEO and current stockholder, NanoBio Co.

Reference:
Applications of nanotechnology for immunology.
Nature Reviews Immunology. 13: 529-605, 2013
Nanomaterials Activate Innate Immunity

• Good
  – Provides vaccine adjuvants
  – Better understanding of innate function

• Bad
  – Most common cause of toxicity
  – Cannot predict how any specific nanoparticle will react….

Viruses are Nanoparticles

![Diagram of viruses as nanoparticles]

Delivery of antigens

- [Diagram showing the delivery of antigens through different size ranges]
RSV-NE Vaccination Reduced Mucus and Expression in the Lung Upon Virus Challenge

Day 4 after RSV Challenge

Nanoparticle-RSV and Formalin Inactivated-RSV Vaccination Cytokine Responses Differ

Migration of Eosophils is Associated with Formalin but not Nanoparticle RSV Vaccination

Day 4 after RSV Challenge
Severe Histopathology Was Associated with Formalin but Not Nanoparticle RSV Vaccination

Day 4 after RSV Challenge

Nanoemulsion Enhances Delivery to Immune System In Vivo @ 24 hrs
A new Route of Immune Activation?

Calreticulin expression (immunogenic apoptosis)

Uptake of Apoptotic Epithelial Cells by Macrophages

Cytokine Expression Following Intranasal Treatment with NE
Hierarchical Clustering of Changes in Gene Expression in JAWSII Cells (6 hrs)

- **Red**: up-regulated
- **Green**: down-regulated

Over 2 fold increase
>1400 probe sets

### Changes in Gene Expression
JAWS II Cell Line

<table>
<thead>
<tr>
<th>6 hrs</th>
<th>Over 2 fold change</th>
<th>%</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>W805EC</td>
<td>1412</td>
<td>3.13</td>
<td>898</td>
</tr>
<tr>
<td>W805EC+PA</td>
<td>1234</td>
<td>2.74</td>
<td>730</td>
</tr>
<tr>
<td>P4075EC</td>
<td>154</td>
<td>2.30</td>
<td>19</td>
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<tr>
<td>P4075EC+PA</td>
<td>389</td>
<td>1.09</td>
<td>34</td>
</tr>
<tr>
<td>PA</td>
<td>27</td>
<td>0.99</td>
<td>17</td>
</tr>
<tr>
<td>PMA/Iono</td>
<td>1318</td>
<td>4.22</td>
<td>1346</td>
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</table>

### 24 hrs

<table>
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<tr>
<th></th>
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<th>Up</th>
<th>Down</th>
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<tbody>
<tr>
<td>W805EC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>W805EC+PA</td>
<td>5207</td>
<td>11.54</td>
<td>1034</td>
</tr>
<tr>
<td>P4075EC</td>
<td>938</td>
<td>2.38</td>
<td>548</td>
</tr>
<tr>
<td>P4075EC+PA</td>
<td>508</td>
<td>1.25</td>
<td>548</td>
</tr>
<tr>
<td>PA</td>
<td>125</td>
<td>1.25</td>
<td>545</td>
</tr>
<tr>
<td>PMA/Iono</td>
<td>1294</td>
<td>2.42</td>
<td>707</td>
</tr>
</tbody>
</table>

JAWS GeneChip Microarray 430.2
45102 probesets, 34800 gene transcripts

Over 2 fold change
>1000 probe sets

**Red**: up-regulated
**Green**: down-regulated
Increase in CD86<sup>high</sup> in CD11c/CD86 MDDC

Control

W805EC 0.0001%

W805EC 0.001%

W805EC 0.001%

P4075EC 0.0001%

PMA/Iono

NF-κB activation via TLR2 and TLR4 receptors in HEK293 TLR+ clones

OD<sub>650nm</sub>

HTR2  HTR3  HTR4  HTR5  HTR7  HTR9

A/6 hours

LPS (1725)

NE (241)

A/24 hours

NE (1154)

LPS (924)

B/24 hours

NE 24 hours (1154)

189

965

1536

C/6 hours

LPS (1725)

W805EC

D/ W805EC

LPS
Antibody Responses are Maintained in TLR 2/4 and MD88 Knock out Mice

Pathways Required for Induction of Immune Responses after Intranasal Delivery of W05SEC Nanoemulsion

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Antibody</th>
<th>Th-1</th>
<th>Th-2</th>
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<tbody>
<tr>
<td>WT</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MyD88−/−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>TLR2−/−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>TLR4−/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>IL-12−/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>IL-12Rβ1−/−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CD86−/−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>CD80−/−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CD40−/−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</table>
Cytokine Differences are Seen in Knock Out Mice

Activation of Mucosal Innate Immunity
- Involves epithelial and dendritic cells
- Induces unique patterns of immunity
- May be process that predates specific PAMPS

- Nanoparticle Toxicity
  - the dark side of the macrophage activation
The establishment of such a model provides an important platform to investigate chronic lung disease, and osteopontin null mice exhibit reduced granuloma formation. Furthermore, osteopontin is up-regulated in granulomatous disease, and is implicated in extracellular matrix remodeling.

Entry of Small Nanoparticles into Immune System

Novel Marine Model of Chronic Granulomatous Lung Inflammation Elicited by Carbon Nanotubes

<table>
<thead>
<tr>
<th>Type</th>
<th>Nanoparticle</th>
<th>Diameter</th>
<th>Entry Site</th>
<th>Phagocytosis</th>
<th>Expression</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>Vesicle</td>
<td>20-100 nm</td>
<td>Quinones</td>
<td>100%</td>
<td>Rest</td>
</tr>
<tr>
<td>B</td>
<td>Spherical</td>
<td>20-100 nm</td>
<td>Receptors</td>
<td>95%</td>
<td>Rest</td>
</tr>
<tr>
<td>C</td>
<td>Nucleus</td>
<td>&lt;20-30 nm</td>
<td>Chemokines</td>
<td>90%</td>
<td>Rest</td>
</tr>
</tbody>
</table>

TABLE 1. mRNA FOLD CHANGE OF SELECTED CYTOKINES FROM CHRONIC LUNG INFLAMMATION TISSUE COMPARED WITH SHAM EXPOSED MICE

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>3</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.5</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2</td>
</tr>
<tr>
<td>CCL2</td>
<td>6</td>
</tr>
<tr>
<td>CCL22</td>
<td>5</td>
</tr>
<tr>
<td>CCL20</td>
<td>7</td>
</tr>
<tr>
<td>CCL5</td>
<td>2</td>
</tr>
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</table>

Am J Respir Cell Mol Biol Vol 45. pp 858–866, 2011
Macrophages and Nanoparticle Induced Granulomas and Fibrosis

- Nanoparticles induce granulomas and fibrosis when they activate macrophages and induce monokines (as compared to IL2 and other T cell cytokines)

- Macrophage activation can be suppressed by steroids and PPARγ in animal models (Huizar et al. Respiratory Research 2013, 14:7)

Case 2: In the beginning.

Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma

Y. Song*, X. Li* and X. Gu*

[Image of histological sections]
How to Treat Nanoparticle Exposed Individuals with Granulomas?

- Patients with granulomatous disease from multiple etiologies (including CVID/TNFR defects)
- Infliximab has been highly effective in these situations

Acknowledgements

MNIMBS Group
- James Baker, Jr., MD
- Nick Lucey, Ph.D.
- Jessica O’Keefe, Ph.D.
- Anna Berens, PhD
- Jola Kubacka-Latshu, PhD
- Pascale Lonsardi, PhD
- Paul Maltoni, PhD
- Andrey Myc, PhD
- Douglas Smith, PhD
- Su He Wang, PhD
- Pan Wung, PhD
- Peter Cox, MD
- Crystal Peacocke
- Yangyi Fan, PhD
- Jeffrey Green
- Katarzyna Janczak
- Jeffrey Landers
- Shelly Leung
- Catherine Mullen
- Trent Road

NanoBio Corporation
- Frank Mazzucchi, MD, PhD, MBA
- Susan Ouellet, PhD
- Lyzofu Wu, PharmD, PhD
- Rob Hagen, PhD
- Laetitia Cadene, MS
- Ann McCarthy
- Ross Evans
- Jessica Hanus, MS
- Jennifer Sun, MS
- Shelly Asiah

HTS Lab, University of Michigan
Center for Chemical Genomics, LSI
- Martha Ancell, BS
- Steve VanderRoest, MS
- Steven Searcy

NIAID
Basic Immunology Branch
- Wolfgang Leitner, MSc, PhD
- Lynda Ovodovit, PhD

Doan, Haas and Wheeler Foundations