Nanotoxicity in Cells of the Immune System

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Nanotoxicity in Cells of the Immune System

Abstract
Nanoparticles (NPs) provide a new medical approach to drug therapy. As with every new approach, safety precautions need to be taken, and the immediate and long-term effects for many NPs are still unclear. When administering a medical treatment into the human body, the first issue that needs to be addressed is host detection of the medicine and inflammation as a possible result of the treatment. If a new NP treatment causes inflammation before it releases its medicine, that treatment may be ineffective, even damaging to the patient. Small metallic and organic particles have been shown to elicit an inflammatory responses in humans. In order to gauge the immune cell reaction to commonly-used nanoparticles, we exposed leukemic *Mus musculus* macrophages to copper, silver, iron and lipid NPs. We measured cell death and cytokine production to assess the affects of these nanoparticles.

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First Supervisor
Fernando Ontiveros Phd.

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Subject Categories
Hemic and Immune Systems | Immune System Diseases | Nanomedicine | Nanotechnology | Pharmaceutics and Drug Design | Tissues
Nanotoxicity in cells of the immune system
by Jonathan Pelc
What is Nanotoxicity?

The study of the toxicity of nanomaterials.

Let’s visualize the nano-scale.
Visualizing the Nano-scale

~ 19 mm
or
0.019 m

100,000x difference

~ 19 nm

1.8 m (5'11")
If our nanoparticles were as big as a penny...

180 km (~110 miles)
Here is an example of how scale matters
Macro-scale Gold
Gold at the \textit{nano-scale}

Increasing size of nanoparticle
Why nanoparticles?

• They are everywhere.
We want to ask whether nanoparticles may cause damage to our cells

\[\text{nanoparticles} + \text{cell} = \text{cell death? inflammation?}\]
What cells will we use?
RAW 264.7

- Established from *Mus Musculus (mouse)* leukemia blast cells

*Rapidly-dividing macrophage cell line*
Qdot-nanoparticles phagocytosed by RAW cells
Quantifying Toxicity

- Cell Death
Quantifying Toxicity – Cell Death

• Some is normal, but too much can be damaging.

• Two kinds that are relevant.
Quantifying Toxicity – Cell Death

• Apoptosis

A clean, controlled, building demolition.
Quantifying Toxicity – Cell Death

• Necrosis

Release of proteases and other digestive enzymes
Quantifying Toxicity

• Cell Death
• Activation of immune cells
Quantifying Toxicity – Activation of immune cells

- Causes Inflammation
How did we measure cell death and inflammation?

- Annexin V and PI (propidium iodide) for Cell Death
- IL-6 and IL-1β for Immune Activation
Cell Death

- Apoptosis

Membrane is not permeable, but it changes its arrangement.

Annexin V binds re-arranged membrane and fluoresces.

PI bounces off.
Cell Death

- Necrosis

Membrane integrity is compromised

PI binds to DNA and fluoresces
How do we measure PI and Annexin V?

Flow Cytometry
• PI measurement example:

Low levels of necrosis

High levels of necrosis
Cu and Ag nanoparticle exposure led to a modest increase in cell necrosis
Interestingly, we did not detect any apoptosis
Interestingly, we did not detect any apoptosis.

Cell necrosis leads to inflammation.
Activation of immune cells

Toll-like Receptors

Pathogens

Nanoparticles

Sterile Particles

Inflammasome

IL-6 production

IL-1β production
We found no evidence for the production of IL-1β.
We found no evidence for the production of IL-1β
What about IL-6?

Pathogens → Toll-like Receptors → IL-6 production

Nanoparticles?
E. coli LPS elicits secretion of IL-6 in a dose-dependent manner.
*S. minnesota* LPS does not provide reliable measurements.
Now we can compare LPS induction of IL-6 to commonly-used nanoparticles

- Copper
- Silver
- Iron
- Lipid
Ag early results pointed to the absence of inflammation

![Graph showing IL-6 (O.D.) results for different treatments.](Image)

Results by B. Battaglia
We are still working on Ag
Similarly, Cu and Fe IL-6 results are so far inconclusive.

<table>
<thead>
<tr>
<th>JP RAW</th>
<th>Test Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>Control (-)</td>
</tr>
<tr>
<td>0.057</td>
<td>Control (+)</td>
</tr>
<tr>
<td>0.045</td>
<td>Cu 25 uL</td>
</tr>
<tr>
<td>0.086</td>
<td>50 uL</td>
</tr>
<tr>
<td>0.039</td>
<td>Fe 25 uL</td>
</tr>
<tr>
<td>0.035</td>
<td>50 uL</td>
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</table>
Results were not reproducible

<table>
<thead>
<tr>
<th>IL-6 OD</th>
<th>Test Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.367</td>
<td>100 uL LPS</td>
</tr>
<tr>
<td>0.043</td>
<td>10 ul LPS (priming)</td>
</tr>
<tr>
<td>0.007</td>
<td>Cu</td>
</tr>
<tr>
<td>0.015</td>
<td>10 uL (200ng)</td>
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<tr>
<td>0.010</td>
<td>Cu</td>
</tr>
<tr>
<td>0.013</td>
<td>Fe</td>
</tr>
<tr>
<td>0.010</td>
<td>50 uL (1000ng)</td>
</tr>
<tr>
<td>0.010</td>
<td>Cu + priming</td>
</tr>
<tr>
<td>0.010</td>
<td>10 uL</td>
</tr>
<tr>
<td>0.028</td>
<td>Cu</td>
</tr>
<tr>
<td>0.047</td>
<td>Fe + priming</td>
</tr>
<tr>
<td></td>
<td>50 uL</td>
</tr>
</tbody>
</table>
Lipid Nanoparticles on the other hand, may elicit increased IL-6 production.
But it cannot be confirmed yet…
Nanotoxicity

• Exposure to Copper and Silver nanoparticles led to increased immune cell necrosis.
• None of the particles led to apoptosis nor to IL-1β production.
• In terms of IL-6 production, so far results for any of the nanoparticles are inconclusive.
Future Directions

• Further explore the effects of lipid nanoparticles.
• Explore potential toxicity induced by other nanoparticles (Gold, carbon nanotubes, etc.)
• Transition from cancerous immune cell lines towards primary macrophages.
Special Thanks

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• Science Scholars for convincing me to come to this school.
• And Dr. Ontiveros for guiding me with the research.
Most scientists regarded the new streamlined peer-review process as “quite an improvement.”