Nanotechnology in Immunology and Allergy

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

Medical Nanotechnology Timeline
Relative sizes of cells and their components

- Small Molecule
- Virus
- Bacterium
- Animal Cell
- Plant Cell

cm = 10^{-2} m
mm = 10^{-3} m
μm = 10^{-6} m
nm = 10^{-9} m
Å = 10^{-10} m

1 Å 1 nm 10 nm 100 nm 1 μm 10 μm 100 μm 1 mm 1 cm

Electron microscope
Nanotechnology
Light microscope

Michigan Nano Institute for Medicine and Biological Sciences
Size of Various Materials and Synthetic Particles (in Nanometers)

- Gold Nanoparticle: 20 nm
- Antibody: 11 nm
- Albumin: 7 nm
- Iron Oxide Nanoparticle: ~1 nm
- Peptide: 0.5 nm
- Salicylic Acid: 0.5 nm
Types of Nanoparticles

- Fullerines
- Gold Nanoshells
- Fe$_3$O$_4$ Nanocrystals
- Starch
- Quantum Dots
Uniformity of Dendrimers
Dendrimer Size Comparison
Barriers to Supermolecular Therapeutics

Contact: charles.lumsden@utoronto.ca
Nanoscale Barriers to Targeted Therapeutics

<20 nM

<150 nM
Membrane Structure and Immune Sampling
a Delivery of antigens

Draining lymph node
Why do nanoparticles act uniquely in biological systems?

– Nanomaterials migrate in tissue because of size

– Nanostructured materials are unusual in recognition and characteristics.

– Combinations of materials (drugs, immune regulators) have mixed effects

– Therefore, the size, structure and composition of nanomaterials are unique and important.
Three Areas Where Nanomaterials Have Unique Potentials for Modifying the Immune System

• Activating innate immunity
• Inducing specific immune responses
• Suppression of immune responses
Three Areas Where Nanomaterials Have Unique Potentials for Modifying the Immune System

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Viruses are Nanoparticles

Satellite Tobacco Mosaic Virus
5 μs
5 μs

Satellite Panicum Mosaic Virus
25 μs

Satellite Tobacco Necrosis Virus
7 μs

Brome Mosaic Virus
5 μs
5 μs

Poliovirus
11 μs

Bacteriophage φX174 Procapsid

Reovirus Core
1.5 μs

50 nm

Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign
<table>
<thead>
<tr>
<th>Size</th>
<th>&lt;5 nm</th>
<th>10–20 nm</th>
<th>50–100 nm</th>
<th>&gt;150 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticle</td>
<td>Dendrimer</td>
<td>Polymer</td>
<td>DNA polypeptide</td>
<td>Liposome</td>
</tr>
<tr>
<td>Bioactivity</td>
<td>Partition like small molecules and filter through the kidney</td>
<td>Escape the vasculature, infiltrate the tissues and lymphatics like proteins</td>
<td>Penetrate the mucosal membranes and the skin and are taken up into cells.</td>
<td>Taken up mainly into phagocytic cells</td>
</tr>
</tbody>
</table>

Nature Reviews | Immunology
Lipid Nanoemulsion Enhances Antigen Internalization into Dendritic Cells

Recombinant Protective Antigen of Anthrax (rPA)

rPA-FITC / NE

rPA-FITC

Control

HBsAg Uptake – Western Blot

Legend
1. Control cell extract
2. HBsAg only
3. HBsAg-W805EC 0.001%
4. HBsAg-W805EC 0.005%
5. HBsAg-P4075EC 0.001%
6. HBsAg-P4075EC 0.005%
a Delivery of antigens

- 20–1000 nm
- 20–1000 nm
- <20–30 nm
- <20–30 nm

- Apoptotic body

- DC

- Draining lymph node
Ultrastructure of the Nasal Epithelium

- Vesicles are seen in the nasal epithelial cells.
- The tight junctions remain intact.
A new Route of Immune Activation?

Calreticulin expression (immunogenic apoptosis)

Makidon, Euro J. Immunol 2012
Nanoemulsion Enhances Delivery to Immune System In Vivo @ 24 hrs

GFP Alone

Nasal Epithelium

Submandibular Lymph Nodes

Thoracic Lymph Node

GFP With NE
Nanoparticles Also Enhance the Retention of Antigen
Imaging of Mice Administered Intranasal RFP

RFP with Saline

RFP with Nanoemulsion
Three Areas Where Nanomaterials Have Unique Potentials for Modifying the Immune System

• Activating innate immunity
• Inducing specific immune responses
• Suppression of immune responses
<table>
<thead>
<tr>
<th>Compound</th>
<th>Size</th>
<th>Medical application</th>
<th>Mechanism</th>
<th>Current use</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus-like particles</td>
<td>15–30 nm</td>
<td>Vaccine carrier and adjuvant</td>
<td>Repetitive antigen display, structural or molecular mimicry of virus, particle size-dependent tissue penetration and trafficking to lymphatics, and TLR activation</td>
<td>In humans and animals</td>
<td>4,5,14,15,18,32</td>
</tr>
<tr>
<td>MF59 (squalene oil-in-water emulsion)</td>
<td>165 nm</td>
<td>Vaccine adjuvant</td>
<td>Neutrophil, monocyte and DC recruitment, antigen uptake, and the induction of humoral and T\textsubscript{H}1-type immune responses</td>
<td>In humans</td>
<td>48</td>
</tr>
<tr>
<td>Nanoemulsion W\textsubscript{50}5EC (soybean oil-in-water emulsion)</td>
<td>400 nm</td>
<td>Vaccine adjuvant</td>
<td>Antigen uptake by and activation of epithelial cells and DCs, TLR2 and TLR4 activation, local cytokine production, mucosal antibody responses and T\textsubscript{H}1, T\textsubscript{H}2 and T\textsubscript{H}17 cell responses</td>
<td>In humans and animals</td>
<td>57,59,67,68</td>
</tr>
<tr>
<td>Poly(lactide-co-glycolide) nanoparticles</td>
<td>100–200 nm</td>
<td>Vaccine carrier and adjuvant when combined with bioactive immunomodulators</td>
<td>Encapsulation for sustained local antigens and co-mediator release</td>
<td>In mice</td>
<td>139,140</td>
</tr>
<tr>
<td>Nanogel (cholesterol-bearing hydrophobized pullulan nanoparticles)</td>
<td>30–40 nm</td>
<td>Vaccine carrier or delivery vehicle</td>
<td>Antigen entrapment in a hydrated nanogel matrix for slow release, delivery to APCs and induction of tumour-specific T cells and antibody responses</td>
<td>In humans and mice</td>
<td>141,142,143–145</td>
</tr>
<tr>
<td>Cationic liposomes</td>
<td>200–1,000 nm</td>
<td>Vaccine carrier</td>
<td>Encapsulation and targeted antigen delivery or uptake by APCs, and recruitment of monocytes to the injection site</td>
<td>In humans and mice</td>
<td>74,75,76,146</td>
</tr>
<tr>
<td>Immune-stimulating complexes</td>
<td>40 nm</td>
<td>Vaccine carrier and adjuvant</td>
<td>Targeting, antigen uptake and activation of DCs</td>
<td>In humans and mice</td>
<td>147,148</td>
</tr>
</tbody>
</table>

APC, antigen-presenting cell; DC, dendritic cell; T\textsubscript{H}, T helper; TLR, Toll-like receptor.
Virus Binding and Entry into Cells

Nature Reviews Microbiology 9, 369-381 (May 2011)
### Table

<table>
<thead>
<tr>
<th>Size Range</th>
<th>Material Type</th>
<th>Penetration Path</th>
</tr>
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<tbody>
<tr>
<td>50–100 nm</td>
<td>DNA polplex</td>
<td>Penetrate the mucosal membranes and the skin and are taken up into cells.</td>
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<td>&gt;150 nm</td>
<td>Liposome</td>
<td>Taken up mainly into phagocytic cells</td>
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</table>

**Nature Reviews | Immunology**
c Repetitive antigen display

- Microbial antigen
- CpG
- Microbial antigen
- CpG
- Synthetic nanoparticle
- Endosome
- CpG
- TLR9
- APC
- Activation of innate receptors

- BCR
- BCR activation
- Microbial antigen
- Production of long-lived high-affinity neutralizing antibodies
Toll-like Receptors (TLRs)

- diacyl lipopeptides
- triacyl lipopeptides
- flagellin
- LPS

TLR-6  TLR-2  TLR-1  TLR-2  TLR-5  TLR-4

plasma membrane

endosome membrane

- dsRNA
- ssRNA
- CpG DNA

Endosome
Toll–Like Receptors Aggregate into Lipid Rafts with Signaling Molecules
TREM-1 and TLR4 associate together in the lipid rafts upon PMN stimulation and activate IRAK1 in human PMN. Purified PMNs were stimulated with anti-TREM-1 (1 µg ml$^{-1}$) for 2 min or with LPS (1 µg ml$^{-1}$) for 5 min before the immunofluorescence staining as described in Methods.
Influenza Antigen Localized in Oil Phase

20% Nanoemulsion

20% Nanoemulsion + 30 µg Fluzone

Cross-section TEM of Nanoemulsion
Nanoemulsion Incorporates Antigen

Nanoemulsion Adjuvant Mixed With Hepatitis B Antigen

19,000x Magnification
Increase in $\text{CD86}^{\text{high}}$ in CD11c/CD86 MDDC

Control

W805EC 0.0001%

W805E 0.001%

P4075EC 0.0001%

PMA/Iono

MNDIMBS
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Modifying the Specific T Cell Response with Adjuvant

- Immunostimulation
  - a) Antigens
  - b) TLR ligands
  - c) Cytokines

- Delivery
  - a) Enhanced antigen uptake
  - b) Signal transduction

- Repetitive antigen display
  - BCR ligation

- Upregulation of chemokine and co-stimulatory receptors

- TCR ligation

- Repetitive display

- CTL
  - T cell help
  - IFNγ

- Tn1 cell
  - IL-1
  - IL-12
  - IL-6
  - TGFβ

- Tn17 cell
  - IL-17
  - IL-5
  - IL-6
  - IL-10

- Tn2 cell
  - IgG
  - IgA

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RSV Vaccine History

- Formalin-inactivated (FI) RSV vaccine in infants in the ‘60s led to severe Th2 cytokine mediated eosinophilic disease (IL-4, 5, 13).

- Alternative vaccine strategies have used cold-adapted live vaccines and recombinant vaccinia expressing F or G RSV proteins.

- Not clear that a killed or subunit vaccine be used for effective immunization.
Vaccination of Mice with NE-RSV Enhanced Viral Clearance in RSV Challenged Animals

Day 4 after RSV Challenge

A

# Copies per 10⁵ GAPDH

Control RSV Vaccine

RSV G

Control RSV Vaccine

RSV F

Control RSV Vaccine

RSV N

RSV-NE Vaccination Reduced Mucus and Expression in the Lung Upon Virus Challenge

Day 4 after RSV Challenge

B
Control RSV
Vaccine

PAS

C

Muc5ac

Fold + over Uninfected

Uninfected Control RSV Vaccine

Gob5

Fold + over Uninfected

Uninfected Control RSV Vaccine
Nanoparticle-RSV and Formalin Inactivated-RSV Vaccination Cytokine Responses Differ

Day 4 after RSV Challenge

Fold + (over uninfected)

**II13**

Fold + (over uninfected)

**II4**

Fold + (over uninfected)

**Ifng**

Fold + (over uninfected)

**II17**

* *
Severe Histopathology Was Associated with Formalin but Not Nanoparticle RSV Vaccination

A

No Vacc | NE-RSV | FI-RSV

Day 4 after RSV Challenge

B

Muc5ac

Fold + (over uninfected)

No Vacc | NE-RSV | FI-RSV

Gob5

Fold + (over uninfected)

No Vacc | NE-RSV | FI-RSV
Migration of Eosophils is Associated with Formalin but not Nanoparticle RSV Vaccination

Day 4 after RSV Challenge
Three Areas Where Nanomaterials Have Unique Potentials for Modifying the Immune System

• Activating innate immunity
• Inducing specific immune responses
• Suppression of immune responses
Nanomaterials Have Capabilities for Suppressing Immunity

- Specific uptake by immune cells
  - Poisons cells
- Suppressive receptor activation
- Ability to deliver small molecules
  - Drugs and immunosuppressants
a Direct immunosuppressive effect

- Carbon nanotube 4 nm x 1 μm
- Nanoemulsion 400 nm
- TLR 5 nm
- pH-sensitive silica nanotube with immunosuppressant 35 nm x 100 nm
- PLGA with immunosuppressant 400 nm

b Delivery

- Increased number and function
- Decreased function or apoptosis of APCs

Decreased number and function

Increased number and function

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Direct Immunosuppressive Activities of Nanomaterials

• Carbon nanotubes and fullerines
  – Direct dendritic and T cell suppression in mouse models

•Activation of TLR 9i and certain NOD-like receptors by functionalized nanoparticles
  – Data in cells and plants
NOD Like Receptors Can Alter Immune Response

Table 1. Proposed functional classification of NLRs

<table>
<thead>
<tr>
<th>Activity</th>
<th>Triggers</th>
<th>NLRs</th>
<th>Outcomes</th>
<th>Proposed DC effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcriptional regulation</td>
<td>Cytokines</td>
<td>CIITA, NLRC5</td>
<td>MHC Class I and II transcription</td>
<td>Antigen presentation</td>
<td>[73,109]</td>
</tr>
<tr>
<td>TLR-like activity</td>
<td>PAMP recognition</td>
<td>NOD1, NOD2</td>
<td>Synergistic induction of inflammatory signaling cascade (NF-κB, MAPK)</td>
<td>Activation (Figure 1b)</td>
<td>[46,49,110]</td>
</tr>
<tr>
<td>Inflammasome nucleation</td>
<td>DAMP or PAMP sensing</td>
<td>NLRP1, NLRP2, NLRP3, NLRP6, NLRP7, NLRP12, NLRC4/NAIP2,5</td>
<td>IL-1β secretion, IL-18 secretion, Pyroposis</td>
<td>Enhanced T cell priming?</td>
<td>[14,22,62,111–113]</td>
</tr>
<tr>
<td>Signaling modulator</td>
<td>?</td>
<td>NLRP2, NLRP4, NLRP6, NLRP12, NLRX1, NLRC3</td>
<td>Inhibit NF-κB pathways</td>
<td>Modification of TLR-induced maturation</td>
<td>[55,57,60,63,66,114,115]</td>
</tr>
<tr>
<td>Signaling molecule?</td>
<td>?</td>
<td>NLRP10, NLRP12</td>
<td>Migration</td>
<td>Licensing (Figure 1b)</td>
<td>[90,94]</td>
</tr>
</tbody>
</table>

Activation of CD24 and Siglec-10 PAMPs Repress Tissue Damage Induced Immunity

Immunosuppressive Drug Delivery by Nanomaterials

• PGLA delivery of IL4 and TGF beta
  – Dendritic and T cell suppression in mouse models

• Specific delivery of immunosuppressive drugs to immune cells by functionalized nanoparticles
Macrophages and clinical disease

Heart Disease

Cancer

Metabolic disease

Arthritis
Rheumatoid Arthritis

• Autoimmune Disease with joint inflammation
  – Symptoms: swelling, pain, stiffness, redness
  – Joint deformity, destruction and functional disability

• 1.3M people diagnosed in U.S.
Computer Model of a Tri-functional Dendrimer

**Methotrexate**
(ester-linked therapeutic agent)

**Folic acid**
(amide-linked targeting agent)

**Fluorescein**
(detecting agent)

**G5-polyamidoamine**
(dendrimer platform)

5 nM
Effect of Dendrimer-FA-MTX on RA– Rat model

A

Ankle Diameter (inches)

Study Day

Normal

Vehicle

MTX

G5-FA-MTX-1

G5-FA-MTX-2

B

Ankle Diameter AUC

Arthritis

Vehicle

MTX

Normal

G5-FA-MTX-1

G5-FA-MTX-2

C

Paw Weight (gram)

Arthritis

Vehicle

MTX

Normal

G5-FA-MTX-1

G5-FA-MTX-2

D

Body Weight Change (gram)

Arthritis

Vehicle

MTX

Normal

G5-FA-MTX-1

G5-FA-MTX-2
Effect of G5-FA-MTX on RA Histopathology

Inflammation
Pannus
Cartilage Damage
Bone Resorption
Average

Vehicle  G5-FA-MTX-1  G5-FA-MTX-2  Free MTX
Summary: Nanomaterials Have Unique Capabilities for Altering Immunity

….due to their

• Size
• Structuring of materials
• Specific uptake by immune cells
• Ability to deliver small molecules
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