Nanoemulsions: New, Non-Inflammatory Mucosal Adjuvants

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What is a Nanoemulsion?

• High energy emulsification of:
  – Water
  – Oil
  – Organic Solvent
  – Surfactant
  – Cetylpyridinium Chloride

• Average Droplet Size
  – 350 nm
Mechanism of Action of Nanoemulsion-Based Vaccine

A. Vaccine Preparation
   - Nanoemulsion
   - Virus or bacteria
   - Antigen particle

B. Intranasal Delivery

C. Delivery of Vaccine to Nasal Mucosa and Maturation of Dendritic Cells

D. Nasal Mucosa After Triggering Innate and Adaptive Immunity
   - Receptor
   - Secondary lymphoid tissues
   - B-cell
   - T-cell
Incorporation of Anthrax Protective Antigen into NE Enhances Its Uptake into DC Line

Increased uptake of PA protein in the JawsII cells incubated in the presence of 0.001% NE (ELISA using anti-PA mouse serum)

Nanoemulsion-based Vaccines Have Been Evaluated in Animal Models Using...

- Recombinant proteins
  - rPA from *Bacillus anthracis*
  - HIV-1 gp120

- Purified proteins/VLPs
  - HepBsAg

- Whole cells
  - Vaccinia virus
Advantages to Nanoemulsion Adjuvants

• Needle-less administration

• Facilitated uptake by dendritic cells in nasal mucosa

• Stabilizes epitopes

• Induces mucosal immunity and a systemic Th1 cellular and humoral response that protects against subsequent disease challenge

• Non-inflammatory
Example of Inactivation: Nanoemulsion Disrupts Herpes Virus

- HSV-1 strain
- Nanodroplets surrounding and fusing with virus (1 min and 15 min)
- Disruption of viral envelope and tegument (30 min)
Mixing of HBsAg + Nanoemulsion: Incorporation of 22 nm VLPs into 350 nm Nanoemulsion Droplets

Antigens Incorporated in Nanoemulsion Droplets Have Enhanced Stability

**In vitro Analysis of HBsAg-NE Stability**

- **A.** Fresh 4°C, 25°C, 40°C
- **B.** Fresh 4°C, 25°C, 40°C
- **C.** Fresh 4°C, 25°C, 40°C

- **S** = Silver stain; **W** = Western blot

**Immunogenicity in Mice**

- **A.** 6 Weeks Storage
- **B.** 3 Months Storage
- **C.** 6 Months Storage

**HepBsAg-NE is stable for 3 months at 25°C**
rPA in Nanoemulsion Elicits Serum Anti-rPA IgG in Mice

All mice immunized IN with rPA-NE were seropositive with significantly higher antibody titers than immunizations with other adjuvants.

End point titers of serum IgG ranged from $10^4$ – $10^5$ after 2 IN immunizations

Pattern of IgG subtype antibodies is consistent with a Th1 polarized response

Anti-PA IgA and IgG Antibodies in Bronchoaveolar Lavage Fluid from Mice

Recombinant protective antigen from *B. anthracis* mixed with nanoemulsion induced mucosal IgA and IgG antibodies

HBsAg-NE produced an equivalent, durable immune response to HBsAg-Alum with high antibody titers maintained for 6 months and a higher avidity index upon maturation.
Antigen-Specific Induction of Cytokine Expression in PA-activated Splenocytes

Cytokine Expression in Splenocytes from BALB/c Mice Immunized with rPA-NE

Mean Conc (pg/ml) ± SEM

- IFN-γ
- TNF-α
- IL-2
- IL-4

Resting
rPA-stimulated

Immunization with rPA + nanoemulsion produces a Th1 cellular response

Nasal immunization with NE-killed vaccinia virus (VV) elicited a potent VV-specific CD8 T cell response.
Survival of Guinea Pigs Immunized with Nanoemulsion + rPA:
Intranasal Challenge with 10x LD₅₀ dose of *B. anthracis* Ames spores
7 weeks after First NE-rPA Immunization (1 day and 4 weeks)

70% protection using the NE-rPA vaccine– comparable to protection with rPA-alum administered IM

What about the Safety of Nanoemulsion-based Vaccines?
Nanoemulsion W$_{80}$5EC is Non-Toxic and Non-Inflammatory and Restricted to Immune Tissues

<table>
<thead>
<tr>
<th>Species/Strain$^a$</th>
<th>Treatment</th>
<th>Number of Doses</th>
<th>Dose Volume (µl)</th>
<th>Group Average Histopathological Score$^b$</th>
<th>Metabolic Analysis$^d$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NE (%)</td>
<td>HBsAg (µg)</td>
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<td>Nasal</td>
<td>Pulmon.</td>
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<tr>
<td>Mouse/CD-1</td>
<td>0</td>
<td>20</td>
<td>$2^e$</td>
<td>10</td>
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<tr>
<td></td>
<td>1</td>
<td>20</td>
<td>$2^e$</td>
<td>10</td>
<td>0</td>
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<tr>
<td></td>
<td>5</td>
<td>20</td>
<td>$2^e$</td>
<td>10</td>
<td>1.0 ± 0.9</td>
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<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>$2^e$</td>
<td>10</td>
<td>0.7 ± 1.1</td>
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<td>20</td>
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<td>$2^e$</td>
<td>10</td>
<td>1.4 ± 1.3</td>
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<td>0</td>
<td>$2^e$</td>
<td>10</td>
<td>1.1 ± 1.7</td>
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<tr>
<td>Mouse/BALB/c</td>
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<td>0</td>
<td>$4^f$</td>
<td>6</td>
<td>1.2 ± 0.4</td>
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<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>$7^g$</td>
<td>6</td>
<td>2.0 ± 1.0</td>
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<tr>
<td>Rat/Wistar</td>
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<td>32</td>
<td>$3^h$</td>
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<tr>
<td>Guinea Pig/Hartley</td>
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<td>32</td>
<td>$3^h$</td>
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<td>0</td>
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<td>Canine/Beagle</td>
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<td>$3^e$</td>
<td>200</td>
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<td>0</td>
<td>$3^e$</td>
<td>400</td>
<td>0</td>
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</table>

$^a$ Group size: CD-1 (n=10); BABL/c, Wistar rats and Hartley guinea pigs (n=5) and Beagles (n=1)

$^b$ Histological lesions were evaluated on a scale of 0-10 with +1 = single microscopic focus +2 = at least 2 microscopic foci, +3 = >3 foci or multiple locally extensive areas of pathology

$^c$ Other tissues evaluated include heart, liver, kidneys, spleen, esophagus, trachea, stomach, intestines, pancreas, and adrenals.

$^d$ Standard biochemical serum profile analysis; normal indicates all analytes fell within normal expected distributions per species.

$^e$ Administered every 2 weeks; $^f$ Administered every 15 minutes; $^g$ Administered every 4 hours; $^h$ Administered every 4 weeks.

Nanoemulsion W\textsubscript{80}5EC is Not Inflammatory to Mucosal Tissues of 4 Species

Murine Nasal Histology

A, B: Photomicrographs of H&E stained nasal epithelium collected from mice 14d following boost vaccination
C, D: Nasal epithelium collected 24h following boost with HepBsAg-NE

Nasal Histology of Rat (E, F), Guinea Pigs (G, H), and Dog (I, J)

E, F and G, H: 14d following boost vaccination
I, J: 24h following boost with HepBsAg-NE

Nanoemulsion-Based Vaccines

- Nanoemulsions facilitate delivery of antigens across the nasal mucosa and into dendritic cells leading to specific recognition by the immune system.
- Nanoemulsion adjuvants lead to persistence of antigen in the mucosa and the lymphoid organs.
- Nanoemulsion adjuvant induces mucosal immunity and a systemic Th1 cellular and antibody response that protects against subsequent disease challenge.
- Nanoemulsion-based vaccines are stable at room temperature.
- Nanoemulsions are non-toxic and non-inflammatory and are not found in non-immune organs (i.e. brain).
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