New Developments in Vaccines, including Novel Vaccine Adjuvants

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Novartis Vaccines and Diagnostics

2010 Workshop on Protein Aggregation and Immunogenicity
Denver, CO, July 20-22
New Technologies in the Last 30 Years have Expanded the Range of Possible Vaccine Targets

- Empirical approach
  - Diphtheria
  - Tetanus
  - Pertussis
  - Rabies
  - Influenza
  - Smallpox
  - Polio

- Reverse vaccinology
  - MenB
  - GBS
  - GAS
  - Staph
  - H. pylori
  - S. aureus
  - C. difficile

- Next generation technology
  - HIV
  - HCV
  - RSV
  - Cancer

Glyco-conjugation
- MenACWY
- S. pneumoniae
- Hib
Licensed Vaccines Mostly Succeed due to Antibody Mediated Protection Against Pathogens with Low Variability.

- Influenza
- Diphtheria
- Tetanus
- Pertussis
- Haemophilus influenzae B
- Pneumococcus
- Meningococcus
- Pneumovirus
- Polio (IPV)
- Papillomavirus
- HAV
- HBV
- MMR
- Polio (OPV)
- Typhoid fever
- Staphylococcus
- Chlamydia
- Parasitic diseases

Reverse Vaccinology

Structural vaccinology, Novel adjuvants, Controlling the immune system

Many more targets for vaccines based on validated principles

A paradigm shift is necessary

Antigen variability

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We do not understand ‘Immunodominance’

- Many hypotheses
  - Immunodominant epitopes
    - Better fit to prevalent germ line antibodies
    - Share a common structural feature (flexibility, shape, charge)
  - Immunorecessive epitopes
    - Resemble self epitopes, so antibodies suppressed
    - Not accessible to antibody (canyon hypothesis)
    - Vulnerable to blocking by antibodies against surrounding epitopes
    - Transiently present in a conformationally flexible molecule

- Current attempts to manipulate are very basic
  - Delete immunodominant parts of the molecule
  - Cover immunodominant epitopes in an oligosaccharide “self-mask”
Neisseria Meningitidis - Pathogenesis

- Encapsulated, aerobic, Gram-negative bacteria
- Major cause of septicemia and meningitis
- Mainly in children, adolescents and young adults
  - Highest prevalence in young children
  - Second peak in adolescents
- Significant morbidity and mortality
  - 10 - 30% die
  - Of survivors, 10 - 20% have serious sequelae (amputations, deafness, mental retardation)
Protein/polysaccharide conjugate Vaccines for Meningococcus C Eliminated the Disease in the UK

Immunization with serogroup C conjugate vaccine in 15-17 yr olds began on 1November 1999
Meningococcus B (Men B)

Vaccinologists Have Tried for Many Years to Develop a Vaccine for Men B Without Success

- Polysaccharide vaccines
  - Failed: Self antigen - unable to trigger a response

- OMV\(^1\) vaccines
  - Failed: Insufficient strain coverage

- Purified proteins
  - Failed: Poorly immunogenic and insufficient strain coverage

\(^1\) Outer membrane vesicle

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Reverse Vaccinology Allowed the Identification of many Novel Men B Antigens

Based on the genome sequence of MC58, 600 ORFs that potentially encoded novel surface exposed or exported proteins were identified. Approximately 350 proteins successfully expressed in E.coli, purified, and used to immunize mice.

28 novel protein antigens with bactericidal activity were identified.

91 novel surface-exposed proteins identified.

Expression and purification of proteins.

Immunizations led to the identification of 91 novel surface-exposed proteins.
The Men B Vaccine Induces Bactericidal Antibodies Against a Panel of Strains Representative of the Men B Global Diversity
The Slow Pace of Adjuvant Development

- Alum and MPL (AS04) are the only adjuvants approved in the USA
- Many potent vaccine adjuvants have failed in clinical trials
MF59: a Potent and Safe o/w Emulsion Adjuvant

**Appearance:** milky white oil in water (o/w emulsion)

**Composition:**
- 0.5% Polysorbate 80
- 0.5% Sorbitan Triolate
- 4.3% Squalene
- Water for injection
- 10mM Na-citrate buffer

**Density:** 0.9963 g/ml

**Size:** 160nm

**Viscosity:** close to water, easy to inject
**Squalene is the Major Component of MF59**

- **Triterpenoid hydrocarbon oil** (C30H50) produced by plants, present in many foods
- **Produced by humans**, precursor to **cholesterol and steroid hormones**
- **Synthesized in liver and skin**, transported in the blood by VLDL and LDL
- **Secreted by sebaceous glands**
- **Used in cosmetics as a topical product and orally**
- **Commercially obtained from the liver of the most abundant species of shark**
MF59 Induces Enhanced H5N1 Antibody Responses

*P<0.01 vs. non-adjuvanted vaccine

MF59 Adjuvant Offers Significant Dose Sparing for H5N1 Vaccine

Keitel W. et al. Vaccine 2010;

*P<0.001 vs. non-adjuvanted vaccine
MF59 Acts Locally, Inducing Immune Activation

Cytokine Activity - GO:0005125
p = 2.03 x 10^{-18}

Cytokine Binding - GO:0019955
p = 2.14 x 10^{-10}

Mosca et al. PNAS 2008
MF59 Induces Local Cell Recruitment

CD11b$^+$ cells are recruited to the site of injection

Blue: PI  Red: αCD11b  Green: Utrophin
Antigen and MF59 are Transported to the Local Lymph Node

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>infl. monocytes</th>
<th>CD11b⁻ DC</th>
<th>CD11b⁺ DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3h-LN</td>
<td>0.0%</td>
<td>Q1-3 Q2-3</td>
<td>Q3-4 Q4-4</td>
</tr>
<tr>
<td>7h-LN</td>
<td>40.5%</td>
<td>Q1-3 Q2-3</td>
<td>Q3-4 Q4-4</td>
</tr>
<tr>
<td>24h-LN</td>
<td>30.4%</td>
<td>Q1-3 Q2-3</td>
<td>Q3-4 Q4-4</td>
</tr>
</tbody>
</table>

** MF59-DIO **

<table>
<thead>
<tr>
<th>Time</th>
<th>0h</th>
<th>7h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40.5% 80.6% 75.8%</td>
<td>5.6% 7.9% 69.5% 20.1%</td>
<td>5.6% 7.9% 69.5% 20.1%</td>
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</tbody>
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OVA-AlexaFluor 647

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Novartis
New Adjuvants will Exploit Synergy Between Immune Potentiators and Antigen Delivery Systems

- Delivery systems: Alum, MF59, PLG etc
- Immune potentiators: CpG, MPL, SMIPs etc
- Antigens: Recombinant proteins
- Long-lived B & T cell memory
Adjuvant Discovery: Screening for Identification of Small Molecule Immune Potentiators (SMIPs)

Focused or diverse libraries → TLR Screens → Hits → Confirmed hits → Secondary screens → Novel adjuvant candidates → Secondary screens → Structure activity assessment → Hit to lead optimization → Focused or diverse libraries

In vitro

In vivo

Adjuvant development → Lead candidates for advancement → Formulation and delivery → In vivo immunogenicity evaluation

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Advantages of SMIPs as New Generation Adjuvants

- Simple synthetic pathways
- Well defined chemical structure, easily manipulated
- 100 years of successful development as drugs
- Established safety profile
- Easily degraded and excreted
- Delivery systems are well established
New Generation Vaccines – co Delivery of Antigen and Immune Potentiators

Traditional Vaccine

Bacteria

~1 μm

Synthetic Vaccine

PLG Microparticle

SMIPs

~1 μm

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PLG Microparticles for co Delivery of Antigen (Men B) and TLR4 Agonist (MPL) in Mice

<table>
<thead>
<tr>
<th>Adjuvants</th>
<th>ELISA</th>
<th>Bactericidal activity (BCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLG/Men B</td>
<td>11,367</td>
<td>512</td>
</tr>
<tr>
<td>PLG/Men B + MPL (MPL added)</td>
<td>18,074</td>
<td>2048</td>
</tr>
<tr>
<td>PLG/MPL/Men B (MPL entrapped in PLG)</td>
<td>66,493</td>
<td>8192</td>
</tr>
</tbody>
</table>

PLG delivery of immune potentiatiors
1. Enhances potency
2. May improve safety – *remains to be proven*

- Vaccines for diseases of old age
  - Influenza
  - Nosocomial infections – Staph aureus etc (MRSA)
  - Alzheimers

- Therapeutic vaccines
  - Chronic infectious diseases (HIV, HCV etc)
  - Cancers (Viral – HBV, EBV, HPV, HCV)

- Life style vaccines
  - Smoking cessation
  - Hypertension
  - Diabetes

- Immune modulation vaccines
  - Allergies
  - Autoimmunity
Back ups
There are Significant Vaccine Knowledge Gaps

**What we know**

- Antibodies correlate with protection (function of antibodies very important)
  - Diphtheria
  - Tetanus
  - Meningococcus A, B, C, Y, W (BCA)
  - Pneumococcus (opsono)
  - Influenza (HI, neutralizing)
  - *Haemophilus influenzae*
  - Measles (neutralizing)
  - Hepatitis B

**What we do not know**

- The role of T cells
  - Th1/Th2 response
  - Cytotoxic T cells
  - Quality of T cells

- Mucosal immunity

- How adjuvants work

- Biomarkers other than antibodies
  - Translational medicine needed

- Link between antigen structure and immunogenicity
  - Immunodominant structures/epitopes
MF59 has More than 13 years of Clinical and Commercial Experience

Clinical experience
- Studied in 109 clinical trials
  - 64 influenza vaccine trials
  - 45 non-influenza vaccine trials
- Over 51,700 subjects enrolled
  - 37,600 received MF59-adjuvanted vaccine
  - 14,100 received non-adjuvanted comparator vaccine
- Subjects ranged in age from 6 months to 100 years

Commercial experience
- The MF59-adjuvanted vaccine Fluad was licensed in 1997
- More than 45 million doses of vaccine distributed commercially
- Post-marketing surveillance data published from more than 27 million doses of MF59-adjuvanted seasonal influenza vaccine
Antigen Entrapped in PLG has Failed – Antigen Adsorption Overcomes These Problems.*

- Exposure to organic solvents and solvent/water interfaces
- Exposure to high shear during preparation
- Inefficient encapsulation and low loading
- Low initial release
- Low pH within degrading microparticles
- Exposure to wet conditions at 37°C until released

* Antigen instability and degradation are common problems with entrapment in PLG microparticles, this is why we switched to antigen adsorption.
### Characteristics of Optimal Vaccine Adjuvants

<table>
<thead>
<tr>
<th>Potential problems</th>
<th>Ideal features - path to success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unacceptable safety issues</td>
<td>Safe, not associated with long term effects</td>
</tr>
<tr>
<td>Significant local reactions</td>
<td>Well tolerated</td>
</tr>
<tr>
<td>Complex, difficult to scale up, lack of reproducibility</td>
<td>Simple scale up and manufacturing, reproducible, easily characterized</td>
</tr>
<tr>
<td>Raw materials expensive or not available of suitable purity from reliable sources</td>
<td>Made from abundant inexpensive components</td>
</tr>
<tr>
<td>Non degradable, leaves long term residue at injection sites</td>
<td>Biodegradable and biocompatible</td>
</tr>
<tr>
<td>Difficult to formulate with diverse antigens, negative impact on antigen stability</td>
<td>Compatible with many different antigens</td>
</tr>
<tr>
<td>Inflexible, not easy to combine with additional components</td>
<td>Flexible, capable of co delivery of antigen and immune potentiator</td>
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