Immune Responses and Vaccine Development

"VaxForm consulting envisions a world where formulation takes a leading role in providing solutions to development of vaccines against life threatening diseases."

29Oct2012
History of Vaccination

- Smallpox – *Variola*
- Only disease to be eradicated
History of Vaccination

- **China late 1600s**
- *The Golden Mirror of Medicine*
  - Listed 4 methods of smallpox inoculation
    - Plug the nose with powdered scabs on cotton wool
    - Powdered scabs blown into the nose
    - Undergarments of an infected child put on a healthy child for several days
    - Cotton smeared with the contents of a vesicle and stuffed into the nose.
History of Vaccination

- **Variolation**
  - Introduction of dried pus from smallpox pustules into the skin of the patient.

- **India, Turkey, India, America in the 1700’s**
  - George Washington ordered recruits to the continental army to undergo variolation to prevent smallpox.

- **2-3% of people variolated died of smallpox**
History of Vaccination

- **Vaccination: Live attenuated organisms**
  - late 1700s – early 1800s
- **Benjamin Jesty, Edward Jenner**
  - Inoculation of humans with cowpox to prevent smallpox infection.
- **Louis Pasteur**
  - Inoculation of animals/humans with attenuated anthrax, cholera, and rabies.
History of Vaccination

- Vaccination – Killed whole organisms
  - Late 1800s
- Protein vaccines
  - Early 1900’s
    - Diphtheria and Tetanus toxoids
- Recombinant proteins/conjugates
  - Modern Era
    - Hepatitis B, HPV – virus like particles
    - Hib, pneumo, meninge – conjugates
    - Flumist, Varivax – live attenuated virus
Success of Vaccination

- Eradication of smallpox
- Near eradication of polio
- Reduced rates of vaccine preventable diseases
What happens after vaccination?

- Activation of Innate and Adaptive immunity
The Course of an Infection

- **Innate Response**
- **Initiation of adaptive response**
- **Level of Infection**
- **Duration of Infection**
- **Infection Cleared**

- **adaptive response**
Innate Immunity

- First line of defense.
- Response is not microorganism specific.
- Granulocytes are the primary innate immune cells:
  - Macrophages
  - Neutrophils
  - Eosinophils
  - Basophils
  - Mast Cells
Pathogen Recognition

- Cells of innate immunity have receptors that recognize pathogens.
  - Toll-like receptors
  - Mannose receptor
  - Glycan receptor

- Pathogen recognition results in engulfment and cytokine/chemokine release.
  - Cause inflammation
  - Recruit additional leukocytes to the site of infection
  - Direct the form of the adaptive immune response.
Toll-like Receptors (TLRs) Recognize Conserved Molecular Patterns on Pathogens

- TLR2
- TLR1
- TLR6
- TLR4
- TLR5
- MyD88
- TRIF
- TLR3
- TLR7/8
- TLR9
- MyD88
- NF-κB, ISREs, AP1

- 1/2/6 – peptidoglycan
- 3 – double stranded RNA
- 4 – LPS
- 5 – Flagellin
- 7/8 – single stranded RNA
- 9 – CpG DNA

Cytokines, Chemokines, IFNs
Antigen Presenting Cells

- Are a link between innate and adaptive immunity.
- Professional APCs
  - Dendritic cells
  - Macrophages
  - B-cells
- Antigens are degraded and presented to T-cells on MHC I & II molecules.
What Happens Next?

- Migration to lymph nodes for presentation to T-cells.
Immunological Synapse formed in the lymph node
CD4 Co-Receptor

- Stabilizes interactions with MHC II.

T-cell

Activation of transcription factors → Antigen Presenting Cell
CD8 Co-Receptor

- Stabilizes interactions with MHC I.
T-cell activation is not Complete Without Co-Stimulation

- B7 on the APC binds with CD28 on the T-cell to provide the co-stimulation signal.
- IL-2 induces T-cell proliferation.
Antigen Recognition Without Co-Stimulation Results in Anergy

- Cells are unable to produce IL-2 for proliferation
- Ensures tolerance to self tissues
Activated CD4+ T-cells Differentiate

- $T_h1$ – Cell mediated immunity
- $T_h2$ – Humoral mediated immunity
- $T_h17$ – Implicated in auto-immunity, protection from bacterial & fungal infection
- Differentiation dependent on cytokines and peptide binding strength

![Diagram showing differentiation of CD4+ T-cells into $T_h1$, $T_h2$, and $T_h17$.]
Intracellular microorganism destroyed

T-Helper Type 1

Th1

Activation

Macrophage

Macrophage
T-Helper Type 2

Activation

Neutralizing antibody secretion
Activation of CD8+ T-cells

- IL-2 induces T-cell proliferation.
- Differentiates into a cytotoxic T-cell
If CTL matches its target antigen it induces apoptosis in the target cell.

Apoptosis is induced by release of lytic granules and expression of Fas ligand.
T-cell Mediated Cytotoxicity

- **Lytic granules contain:**
  - Perforin: Forms membrane pores in target cell
  - Serine proteases: degrades cellular proteins
T-cell Mediated Cytotoxicity

- CTL migrates to new target.
- Target cell dies.
Components of Vaccine Formulations

- Antigen – entity in which it is desired to direct the immune response against.
- Adjuvant – Enhances the desired immune response.
- Buffer – Maintains appropriate pH.
- Excipients – Isotonicity and stabilization
## A Rational, Systematic Approach for the Development of Vaccine Formulations

<table>
<thead>
<tr>
<th>Preclinical Phase</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III and beyond</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biophysical Characterization</strong></td>
<td><strong>Stabilizer Screening</strong></td>
<td><strong>Adjuvant Interactions</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Sterile Filtration, Container Interactions</strong></td>
<td><strong>Real Time / Accelerated Stability</strong></td>
<td><strong>Troubleshooting</strong></td>
</tr>
</tbody>
</table>

**VaxForm LLC**
Biophysical Characterization

- Utilizing diverse analytical techniques to characterize the physical stability of an antigen.
- Empirical phase diagram approach
- Define suitable pH, Buffer species, ionic strength
Stabilizer Screening

- High Throughput Screening of GRAS excipients.

- Excipient 2 would be selected as a stabilizer for further development.

<table>
<thead>
<tr>
<th>Excipient Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acids</td>
<td>Arginine, Aspatate, Glycine, Glutamate, Lysine, Proline,</td>
</tr>
<tr>
<td></td>
<td>Ascorbic Acid, EDTA, Malic Acid</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>α-cyclodextrin, β or γ 2-hydroxypropyl-cyclodextrin</td>
</tr>
<tr>
<td>Cyclodextrins</td>
<td>Albumin, Gelatin</td>
</tr>
<tr>
<td></td>
<td>Sucrose, Trehalose, Lactose, Dextrose, Glycerol, Sorbitol, Mannitol</td>
</tr>
<tr>
<td>Proteins</td>
<td>Brij, Pluronic, Tween</td>
</tr>
<tr>
<td>Sugars/Sugar Alcohols</td>
<td></td>
</tr>
<tr>
<td>Surfactants</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing optical density vs. temperature for control, Excipient 1, Excipient 2, and 1+2]
Adjuvants

- Enhance the potency of vaccines.
- Aluminum salts are the most common.
- Emulsions have been approved in the EU.
Aluminum Adjuvant Interactions

- Adsorption profile with aluminum adjuvants
- Adsorption stability
- Importance of adsorption for immunogenicity
- Differentiation of the immune response with alternative adjuvants
Product Contact Material Interactions

- Interaction with product contact materials can impact vaccine formulations
  - Filters, glass, stainless steel, plastic, etc..
- Leachables and Extractables
Real Time/Accelerated Stability

- Correlation of real time and accelerated conditions.
- Early decisions on whether formulation is suitable to obtain shelf life goals.
- Support for temperature excursions during shipment and storage of clinical material.
Product Support

- Vaccines are not always stored properly.
- Issues with cold chain
  - Human error
  - Power outage
  - Lack of space
Thank You

- Questions?