Vaccines: Antigens, Adjuvants Autogenous?

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What does it take to make an Efficacious Vaccine?
### Vaccine Formulation Components

- **Antigen(s)**
- **Adjuvant**
  - Immunostimulant
  - W/or w/o vehicle

### Antigen Selection and Right Inactivation Method

- **Right Antigens-MLV and Inactivated**
  - Viruses- field genotypes and biotypes
  - Bacteria- field subtypes
- **Inactivation Method-Inactivated**
  - Must be effective- inactivate
  - Must keep and even improve antigens

> "The best adjuvant will never correct the choice of the wrong epitope."
> Edelman & Tacket, 1990

### What is the Adjuvant Effect?

Adjuvants

> “Immunologist’s dirty little secrets” C. Janeway
Current Parenteral Adjuvants

Adjuvants being Tested in Clinical Trials

Adjuvant Effects

- Danger and immunostimulation-activation of dendritic cells
- Depot-controls release of antigen effects
- Protective- protects antigen from existing immune response
Danger Recognition, Antigen Presentation & Immunostimulation

- Direct Effect - Vehicle stimulates PRR Danger response
- Facilitate Phagocytosis - necessary for antigen presentation
- Induce cytokines and co-stimulatory molecules to drive Th1 vs Th2

Immunostimulation - Th1 vs Th2

Cellular Depot Effect

- Alums-
- Oil-Water
- Polymers - Polyesters
Polymer Vehicles

- Actually requires three phases
- Antigen in buffer
- Antigen-buffer trapped in oil droplets
- Oil droplets suspended in water

Protective Effect

- Oil in Water
  - Actually requires three phases
  - Antigen in buffer
  - Antigen-buffer trapped in oil droplets
  - Oil droplets suspended in water

Protect Antigen-Oil in Water
Oil-Water Adjuvant

1. Adjuvant/antigen complex injected
2. Dendritic cells ingest antigen/adjuvant complex
3. Antigens are then expressed on the surface of the dendritic cell
4. T-cells are activated to initiate an immune response
   - Cellular
   - Humoral
5. Plasma cells produce protective antibodies

Adjuvant

- Agent added to a vaccine to induce an enhanced immune responses to vaccine antigens
- Used for over 80 years
- Technology continually evolving
  - Advances in immunology
  - Advances in chemistry
  - Recombinant proteins and peptides can be poorly immunogenic

Role of Adjuvants in Vaccines

- Increase antibody responses
- Induce cell-mediated immunity
- Decrease the dose of antigen in the vaccine
- Decrease the number of doses of vaccine necessary
- Increase duration of immunity
- Overcome competition between antigens in combination vaccines
- Enhance immune responses in naive populations
Different Adjuvants – Different Strategies

Delivery
- Carriers which stabilize vaccine antigens and allow them to be present for extended periods of time.

Immune potentiator
- Exert direct stimulatory effects on immune cells and initiate the immune response through activation of innate immunity
- DANGER SIGNAL

The “Perfect” Adjuvant
- “Perfect” adjuvant achieves both strategies
  - Delivery - Enhance the amount of antigen reaching the cells that are responsible for the induction of the immune response
  - Immune potentiator - Directly activates these cells.
- Goal = Activate only the elements of the immune response required for protection, and avoid triggering a generalized activation of the immune response.
  In other words “Effective and Safe”

Categories of Adjuvants

Mineral Salts
Oil Emulsions
Particle Adjuvants
Immune Potentiators
Particle Adjuvants

- Membrane bound phospholipid vesicles (liposomes) with antigen trapped in lumen or incorporated in membrane
- Targets increased antigen uptake by APC
- ISCOMS (Immune Stimulating Complexes)
  - Liposomes containing saponin
- Virosomes
  - Liposomes prepared from viral membranes

Immune Potentiators

- Cytokines
  - IL-2, IL-12, IFN-γ – Drive Th1 response
  - IL-10 – Drive Th2 response

- Microbial Derivatives
  - PAMPS – Induce Th1 response
  - LPS – Interacts with APC, releases pro-inflammatory cytokines
  - CpG – Bacterial DNA

- Mucosal
  - Heat labile enterotoxin (LT)
  - Cholera toxin (CT)

- Saponins
  - Plant derivative – Primary function is induction of cytokines
  - Can be toxic in certain species (humans, mice)
  - Commonly used in veterinary vaccine formulations

Immunostimulatory Molecules

- Cytokines
  - IL-2, IL-4, IL-1 & IL-6, IL-12, IFN-gamma, IFN-alpha
- Chemical reagents
  - Levamisole (activates macrophages and induces migration of T cells to Peyer's patches)
  - Poly IC & Poly ICLC (mimics ds RNA), CpG- bind PRR -Danger Signals
- Bacterial Products
  - CT, LPS and LOS bind PRR-Danger signal
Adjuvants

Traditional – Main adjuvant platform were the mineral salts - primarily aluminum hydroxide

• Primary driver – Safety (injection site reactivity and anaphylaxis)
• Short withdrawal period
• Formulation - Large combinations, manufacturing

Today – New formulations – oil/water emulsions

• New formulation technologies
• Increased efficacy (Duration of immunity)
• Increased safety

Mineral Salts

• Primarily aluminum adjuvants
• Used extensively in veterinary vaccine products
• Most commonly used adjuvant in human vaccines
• Strong inducer of Th2 responses
• Examples
  – Aluminum hydroxide
  – Aluminum potassium sulfate (often called “Alum”) - Used in many toxoid formulations (Clostridial vaccines)

Aluminum Adjuvants

• Mode of action is not completely understood
  – Depot effect
  – Convert soluble antigens to particulate forms which are more readily phagocytized.
• Aluminum adjuvants cannot be frozen as it causes collapse of the gel resulting in gross aggregation and precipitation.
Oil Emulsions

- Detergent-stabilised emulsions of oil and water
- Precise mode of action is still not clearly understood
  - Depot
  - Induction of MHC responses
- Primarily used in veterinary vaccine formulations
- Gaining acceptance in Human vaccine formulations (MF59)
- 3 basic forms – multiple formulations
  - Oil in Water (O/W)
  - Water in Oil (W/O) (Freund’s)
  - Water in Oil in Water (W-O-W)

Oil Based Adjuvants Used in Veterinary Vaccines

- Water in Oil Emulsions (W/O) – antigen-containing water droplets within external continuous oil phase
- Oil in Water Emulsions (O/W) – emulsified oil droplets within continuous antigen-containing aqueous phase
- Water-in-Oil-in-Water Emulsions (W/O/W) – antigen-containing water droplets entrapped inside of larger oil droplets, which in turn dispersed in continuous antigen containing water phase
Oil Based Adjuvants Used in Veterinary Vaccines

- Microscopic view (100X) of emulsion structures: (A) W/O; (B) W/O/W; (C) O/W. (T. Jansen et al., Vaccine 2006, 5400-5405)

Water-in-Oil Emulsions (W/O)

**Pros**
- Retain antigen at the injection site
- Provide prolonged humoral immunity by constant provision of antigen to B-lymphocytes

**Cons**
- Often are not easy to inject due to the high viscosity
- May induce necro-ulcerative lesions at the injection site
- Onset of immunity is delayed

Oil-in-Water Emulsions (O/W)

**Pros**
- Low viscosity
- Easily tolerated and more safe compare to W/O vaccines
- Provide fast humoral immune response

**Cons**
- Induce predominantly short-term immune responses after single vaccination
Water-in-Oil-in-Water Double Emulsions (W/O/W)

**Pros**
- Double-emulsion adjuvant vaccines
- Intermediate type compared to W/O and O/W vaccines
- Provide faster humoral immune response (compared to W/O vaccines)
- Longer duration of immunity (compared to O/W vaccines)
- Less viscous, easily injected with reduced injection site reactions

**Cons**
- Physical stability of the emulsion is a limiting factor
- The optimization of emulsifiers is required to maintain acceptable stability throughout shelf life of the product

**Summary**
- Dendritic Cells are the key cell for the development of the immune response
- Adjuvant vehicle effects danger sequence
- Immunostimulatory molecules are probably only effective early in the primary immune response