

# VACCINE ADJUVANTS



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# INTRODUCTION

A **vaccine** is a biological preparation that improves immunity to a particular disease.

A vaccine may contain one or more of the following

- ❖ Organisms inactivated by chemical or physical means whilst retaining adequate immunogenic properties;
- ❖ living organisms that are naturally avirulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties;
- ❖ antigens extracted from or secreted by the infectious agent ;
- ❖ plasmid DNA;
- ❖ antigens produced by chemical synthesis in vitro.

## *Vaccine adjuvants:*

A vaccine adjuvant is a component that potentiates the immune response to an antigen and/or modulates it towards the desired immune response.

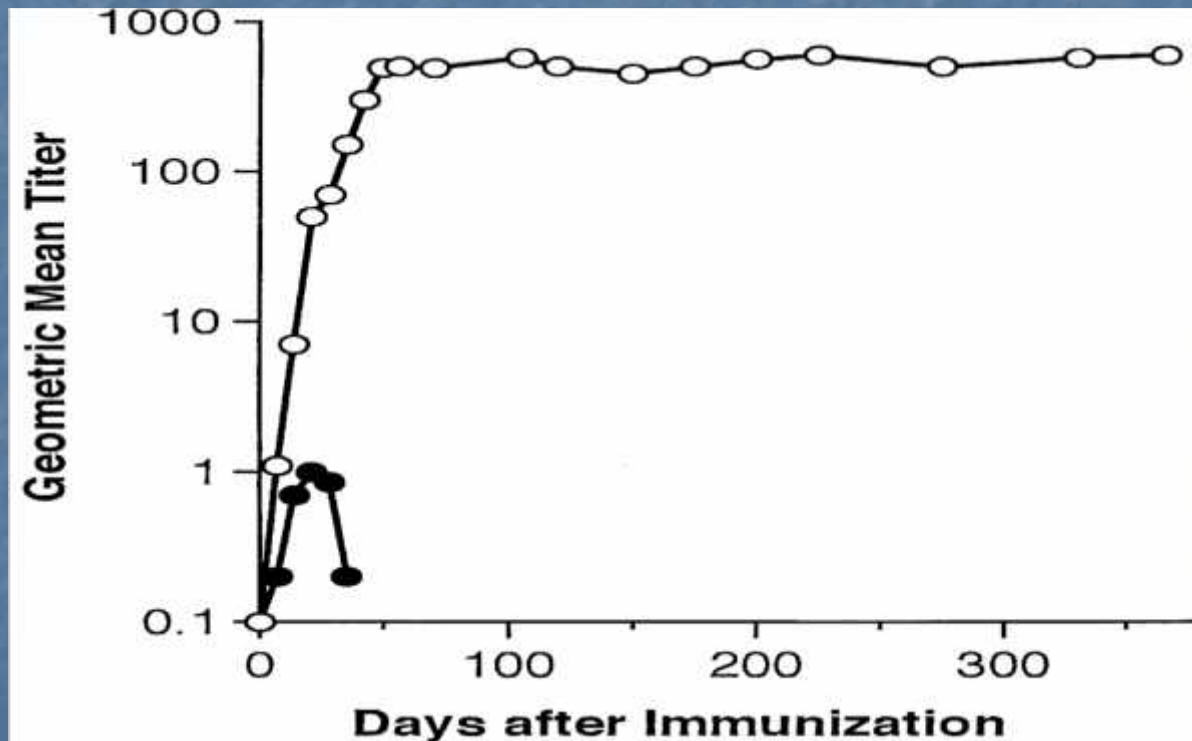
In the traditional vaccines impurities or other components of organisms act as adjuvants,

For example diphtheria-tetanus- pertussis (DTP) vaccine contains two potent adjuvants from whole cell pertussis vaccine (LPS and PT), whole cell typhoid and cholera vaccines have potent adjuvants (LPS and cholera toxin).

Therefore, purified, synthetic vaccines require potent adjuvants.

## Effect of adjuvants on immune response

Many potentially protective antigens are weak immunogens. Protein antigens injected in saline typically produced weak and transitory antibody responses while those injected in effective adjuvants produced strong and sustained responses



## ***MECHANISM OF IMMUNE RESPONSE***

- Particulate and soluble antigens are efficiently internalized by phagocytosis and macropinocytosis, respectively.
- The internalized antigens are recognized by Toll -like receptors (TLRs) on dendritic cells, which leads to potentiation of T-cell priming.
- CD8+ and CD4+ T cells express receptors that recognize fragments of antigens (peptides) associated with MHC class I and II, respectively. Antigen degradation and peptide loading onto MHC molecules occurs intracellularly in APCs.

- The TH cell requires 2 signals for activation:
  1. T cell receptor with the MHC class II – complexed antigen
  2. interleukin-1, which is produced by the APC.
- The activated TH cells forms interleukin-2 and other cytokines required for B cell activation.
- The TC cells are activated when they contact antigens presented along with MHC class I molecules.

## *Properties of an ideal adjuvant*

- Safe
- The preparation would elicit a protective immune response with weak antigens including polysaccharide-protein conjugates with lower doses of antigens and with fewer injections.
- promote an appropriate immune response, namely cellular or antibody immunity depending on requirements for protection
- The adjuvant would be stable with regard to adjuvanticity and toxicity without any interaction with the antigen.
- It would be biodegradable and immunologically inert.
- cheap to produce



# *Uses of adjuvants*

*Adjuvants can be used for various purposes:*

- **Adjuvants have been used with conventional vaccines to elicit early, high and long-lasting immune response.**
- **Adjuvants are very important for purified, synthetic vaccines which are poorly immunogenic.**
- **To reduce the amount of antigen or the number of immunizations needed for protective immunity**
- **Synthetic and subunit vaccines will be expensive to produce. With the use of adjuvants, less antigen may be required to stimulate the immune response, thus saving cost of vaccines**
- **As antigen delivery systems for the uptake of antigens by the mucosa**
- **Adjuvants received much attention due to their ability to selectively modulate the immune response to elicit humoral and/or cellular immune responses**

# *Classification*

On the basis of adjuvanticity, vaccine adjuvants can be grouped into substances

1. Causing depot formation at the site of injection - For example, mineral compounds, oil-based adjuvants, liposomes;
2. Acting as delivery vehicles for the antigens which may help in targeting antigens to immune competent cells - For example, liposomes, oil adjuvants;
3. Acting as immunostimulators - For example, Freund's complete adjuvant (FCA), muramyl dipeptide(MDP), lipopolysaccharide (LPS), lipid A, monophosphoryl lipid A (MPL), pertussis toxin (PT), cytokines.

## *Mechanisms of adjuvant action*

Adjuvants may exert their immune-enhancing effects according to the following immune-functional activities:

1. Adjuvants help in the translocation of antigens to the lymph nodes where they can be recognized by T cells.
2. Adjuvants provide physical protection to antigens which grants the antigen a prolonged delivery.
3. Adjuvants help to increase the capacity to cause local reactions at the site of injection, inducing greater release of danger signals by chemokine releasing cells such as helper T cells and mast cells.
4. Adjuvants are believed to increase the innate immune response to antigen by interacting with Toll-like receptors (TLRs) on accessory cells.

## ***Aluminum-containing adjuvants:***

E.g.; aluminum phosphate, aluminum hydroxide and alum-precipitated vaccines

### ***Mechanism of action***

1. Formation of a repository or depot of antigen in tissues.
  2. Direct effect on APCs – increased immune response to DNA vaccines when mixed with aluminum phosphate adjuvants.
  3. Direct activation of dendritic cells.
- ✓ Aluminum hydroxide has been found to be a more potent adjuvant than aluminum phosphate.
  - ✓ Aluminum hydroxide showed higher adsorption of tetanus toxoid and diphtheria toxoid than aluminum phosphate at room temperature at a pH of 6.0

***Adsorption mechanisms:*** The main mechanisms by which aluminum-containing adjuvants adsorb antigens are:

- ***electrostatic attraction*** - based on isoelectric point
- ***hydrophobic forces*** - tested by ethylene glycol
- ***ligand exchange*** – occurs with phosphorylated antigens

## *MF 59: a oil-in-water emulsion*

MF59 is a low-oil-content o/w emulsion.

### **Composition of MF59 :**

0.5% Tween 80 - water-soluble surfactant

0.5% Span 85 - oil -soluble surfactant

4.3% squalene oil

water for injection

10 nM Na-citrate buffer

- ❖ The oil used for MF59 is squalene, a naturally occurring biodegradable and biocompatible oil.
- ❖ MF59 contains 2 nonionic surfactants, Tween 80 and Span 85.
- ❖ Citrate buffer is also used in MF59 to stabilize pH.

## Mechanism of action of MF59:

- ❖ The emulsion acts as a direct delivery system and was responsible for promoting the uptake of antigen into antigen-presenting cells (APCs).
- ❖ A direct effect on cytokine levels in vivo has been observed.
- ❖ Recent studies have confirmed the ability of MF59 to have a direct effect on immune cells, triggering the release of chemokines and other factors responsible for recruitment and maturation of immune cells.

# Freund's adjuvant

- Freund, in 1937, demonstrated the adjuvant effect of mineral (paraffin) oil mixed with killed *Mycobacteria*, referred to as Freund's complete adjuvant (FCA). The water-in-oil emulsion without *Mycobacteria*, known as Freund's incomplete adjuvant (FIA), has been used in a number of veterinary vaccines.
- The mode of action of FIA was attributed to depot formation at the site of injection and slow release of the antigen with stimulation of antibody-producing cells. Injection of FIA and antigen at separate sites did not increase the immune response. The antigen must be trapped within water droplets (aqueous phase) in the lipid emulsion for augmentation of the immune response.
- FIA was used in humans, particularly with influenza and killed poliomyelitis vaccines enhancing their immunogenicity. FIA is not currently used in humans because of the side effects such as local reactions at the site of injection (granuloma and cyst formation), oil-induced neoplasmas in mice.

## Microorganism - derived adjuvants

- Bacterial or fungal substances constitute a productive source of potential adjuvants.
- Bacterial cell wall peptidoglycan or LPS enhances the immune response.
- This adjuvant activity is mediated through activation of Toll-like receptors (TLRs).
- Different species of bacteria used as a source of adjuvants include *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulorum*, *Bordetella pertussis* and *Neisseria meningitidis*.
- The adjuvants obtained from microorganisms are:
  - a) muramyl dipeptide (MDP)
  - b) lipid A
  - c) trehalose dimycolate (TDM).



- ❖ The major adjuvant activity of these bacteria is mediated by *N*-acetyl muramyl-1-alanyl-d-isoglutamine, also called muramyl dipeptide (MDP).
- ❖ In saline, MDP mainly enhances humoral immunity, whilst when incorporated into liposomes or mixed with glycerol it induces strong cellular immunity. Compounds with adjuvant activity derived from MDP include treonin-MDP.
- ❖ Another important group of compounds derived from the cell wall of Gram-negative bacteria is LPS. The major structural element of LPS responsible for their adjuvant effect is lipid A.
- ❖ In low acid conditions, lipid A can be hydrolyzed to obtain monophosphoryl lipid A (MPL), a compound which retains the adjuvant activity of lipid A with reduced toxicity.
- ❖ Another extract from bacterial walls is trehalose dimycolate (TDM), an adjuvant which simulates both humoral and cellular responses.

# *ISCOMS*

- ISCOMs are 40 nm large particles made up of saponins (Quil A), lipids, cholesterol and antigen, held together by hydrophobic interactions between the first three components.
- Cholesterol is the ligand that binds to saponin forming 12 nm rings.
- These rings are fixed together by lipids to form the spherical nanoparticles. Hydrophobic or amphipathic antigens can be incorporated into this complex.
- They are versatile and flexible delivery systems with increased efficiency of antigen presentation to B cells and uptake by the APC. vaccines are potent inducer of both humoral and cellular (CD4+ and CD8+ T-cell) immune responses.

- The use of saponins in ISCOMs-based vaccines retains the adjuvant activity of the saponin component but with a reduced toxicity.
- The ISCOMATRIX adjuvant is identical to ISCOMs except that it does not contain antigen. This adjuvant can be mixed with antigens and has some of the advantages of ISCOMs such as the preferential targeting of antigen to APC.
- However, the response obtained differed from that of ISCOMs vaccination in that the ISCOMATRIX induced a Th2-like response, whereas the ISCOMs-based vaccine induced a mixed Th1/Th2 response

# *Liposomes*

- Liposomes are synthetic spheres comprised by lipid bilayers that can encapsulate antigens and act as both a vaccine delivery vehicle and adjuvant.
- The potency of liposomes depends on the number of lipid layers, electric charge, composition and method of preparation
- Depot formation at the site of injection and efficient presentation of antigens to macrophages.
- Both humoral and cell-mediated immune responses have been elicited by liposomes.
- Immunostimulators such as LPS and MDP when encapsulated within liposomes show enhanced adjuvanticity with reduced side effects.
- However, phospholipid liposomes have certain limitations such as sensitivity to host phospholipases, instability on storage, high cost of manufacture and difficulty in scale up of production.

- To overcome these problems non-phospholipid liposomes are developed. These non-phospholipid liposome vesicles, composed of dioxyethylenecetyl ether, cholesterol and oleic acid, were evaluated with human vaccine antigens (tetanus and diphtheria toxoids) in rabbits and mice.
- Tetanus and diphtheria toxoids encapsulated in or mixed with these liposomes elicited antitoxin levels similar to those elicited by antigens given with FCA or adsorbed onto aluminum adjuvants.

## virosomes

Another type of liposomes, referred to as virosomes, contain a membrane bound hemagglutinin and neuraminidase derived from influenza virus, and serve to amplify fusogenic activity and therefore facilitate the uptake into APCs and induce a natural antigen-processing pathway.

## *POLY(LACTIDE-CO-GLYCOLIDE) MICROPARTICLES*

- Surface charged poly(lactide-*co*-glycolide) (PLG) microparticles with surface adsorbed antigen(s) can also be used to deliver antigen into APC.
- PLG microparticles are effective for the induction of cell-mediated immunity.
- The preparation of cationic and anionic PLG microparticles which have been used to adsorb a variety of agents, including plasmid DNA, recombinant proteins and immunostimulatory oligonucleotides resulted in the induction of significantly enhanced immune responses in comparison to alum.

## Nucleic acid based adjuvants

- Bacterial DNA shows direct immunostimulatory effects on immune cells *in vitro*. This immunostimulatory effect is due to the presence of unmethylated CpG dinucleotides.
- CpG motifs are recognized by the Toll-like receptor (TLR) in mammalian cells, inducing the secretion of type I interferons and IL-12 by cells of the innate immune system, promoting a Th1 cellular response and preventing allergic responses. Therefore, CpG-containing DNA-based molecules would be useful for therapeutic applications and also for adjuvanting other types of vaccines.

# *Mucosal adjuvants*

The main function of *mucosal adjuvants* is breaking tolerance and inducing an immune response.

- ✓ *soluble small immunopotentiating mucosal adjuvants* - CpG; Imiquimod and Resiquimod
- ✓ *soluble protein immunopotentiating mucosal adjuvants* – mutants of heat-labile enterotoxin from *E.coli*
- ✓ *Miscellaneous* – bioadhesives e.g. chitosan, carbopol



# Cytokines

- Cytokines are small proteins that are released in response to immunological stimuli and function in regulating immune activity and homeostasis.

E.g. GM-CSF, IL-12, IL-2, IL-4, IL-15, IL-7, interferons, TNF- $\alpha$ .

- Granulocyte-macrophage colony stimulating factor (GM-CSF) enhances the immune response by activating and recruiting APC.
- However, the practical application of GM-CSF as an adjuvant has been limited by the requirement for multiple doses, toxicity and the immunogenicity of heterologous cytokines.
- On the other hand, the direct application of IL-12 and other cytokines as soluble proteins has proven effective as mucosal adjuvants.

## Problems in development of adjuvants

- **Limited adjuvanticity:** For example, aluminum compounds did not exhibit an adjuvant effect when used with typhoid vaccine, influenza haemagglutinin antigen.

For preliminary evaluation of adjuvants, the use of vaccine antigens such as tetanus toxoid, diphtheria toxoid, pertussis toxoid at doses which are not maximal for that animal model or minimal threshold doses is recommended. Diphtheria toxoid has been found particularly useful, as it is a poor immunogen.

- **Animal models:** There are no reliable animal models for many diseases against which vaccines are being developed.

-inulin, which has been shown to be a good adjuvant, did not show much adjuvanticity with diphtheria toxoid in CD-1 outbred mice, whereas it was a good adjuvant in inbred Balb/c and C57 mice.

- **Problems with assays**

## ***SIDE EFFECTS OF VACCINE ADJUVANTS:***

- Toxicity and adjuvant activity must be balanced to obtain maximum immune stimulation with minimal adverse effects.
- Majority of adjuvants produce some effects
  - local reactions
    - the inflammatory response
    - local pain and tissue lysis
    - granulomas and hypersensitivity reactions
  - systemic effects

# *Conclusion*

- ✓ **Adjuvants are essential for the development of new and improved vaccines.**
- ✓ **The development of successful vaccine adjuvants has been a constant balancing act between safety and immunogenicity, delivery and immunostimulation.**
- ✓ **The design and selection of new adjuvants will have to face some major hurdles:**
  - **understanding of the mechanisms of adjuvanticity,**
  - **development of appropriate delivery systems.**

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