Adjuvant

Adjuvant: An adjuvant is a substance which when used along with the vaccine improves the immune response against that vaccine. e.g., aluminium hydroxide, MF59, Saponin etc. The most appropriate adjuvant for a given vaccine antigen will depend to large extent on the type of immune response that is required for the protective immunity.

Adjuvants mediate their effects by any of the following mechanisms:

-increasing cellular infiltration
-inflammation
-trafficking to the injection site particularly for antigen presenting cells promoting the activation state of APCs by regulating MHC expressions, enhancing antigen presentation or
-inducing cytokine release.

Adjuvants enhance the immune response to vaccine antigens by

-decreases the antigen dose
-enhancing the speed and duration of immune response.
-stimulates mucosal immune response
-regulates antibody avidity and specificity by stimulating a strong CMI response.

Classification of Adjuvants:

a. Mineral salts
e.g., Aluminium hydroxide, Aluminium phosphate, Calcium phosphate, Alum.

b. Immuno-stimulatory Adjuvants
e.g., Cytokines -e.g., IL-12,IL-2,GM-CSF.
Saponins-e.g., AS-21
MDP derivatives
CPG Oligos, LPS, MPL, Polyphosphazenes.

c. Lipid Particles
e.g., Emostons-e.g.,Freund's,SAF,MF59.
Liposomes, Virosomes, Iscoms, Cochleates.

d. Mixed Adjuvant
e.g., Freund's complete adjuvant.

e. Microparticulate Adjuvants:
e.g., PLG microparticles,
Poloxamer particles, virus like particles.

f. Mucosal Adjuvants:
e.g. , Heat labile enterotoxin (LT)
Cholera toxin.
Mutant toxin-LTK63 &LTK72
Microparticles, Polymerized liposomes.

Common Adjuvants

Type	Adjuvant	Mode of Action
Mineral salts	Aluminium phosphate	Slow release antigen depot
	Aluminium hydroxide	Slow release antigen depot
	Alum	Slow release antigen depot
Water-in-oil emulsions	Freund's incomplete adjvant	Slow release antigen depot
Mixed Adjuvant	Freund's complete adjvant	Macrophage & T-cell activation
		Slow release antigen depot
Bacterial Fractions	Anaerobic Corynebacteria	Macrophage stimulator
	BCG	Macrophage stimulator
	Muramyl Dipeptide	Macrophage stimulator
	Bordetella pertussis	Lymphocyte stimulator
	LPS	Macrophage stimulator
Surface active agents	Saponin, Lysolecithin	Stimulates antigen processing
	Pluronic detergents	Stimulates antigen processing
Complex Carbohydrates	Acemannan	Macrophage stimulator
	Glucans, Dextran sulfate	Macrophage stimulator

A. Mineral salts: (Depot forming Adjuvants)

e.g., Aluminium hydroxide, Aluminium phosphate, Calcium phosphate, Aluminium potassium sulphate (Alum).

When antigen is mixed with one of these salts and injected into an animal. A macrophage rich granuloma forms in the tissues. The antigen within this granuloma slowly leaks into the body providing a prolonged antigenic stimulus. e.g., antigens are retained in the body for several weeks.

- These depot adjuvants influence only the primary immune response and have very little effect on secondary immune response. e.g., Alum-HS Vaccine.
- Most widely employed adjuvants in commercial veterinary vaccines.*These adjuvants are produced in the form of a colloidal suspension to which antigenic material is absorbed.*Stable on storage.
- They produce a small local granuloma on inoculation, do not track a large part of carcass, unsuitable for consumption.
- Most suitable type for animals at present.

B. Immunostimulatory Adjuvants:

They exert their effects at cytokine level through the activation of MHC molecules. e.g., Cytokines -e.g.,IL-12,IL-2,GM-CSF, Saponins-e.g.,AS-21, MDP derivatives, CPG Oligos, LPS, MPL, Polyphosphazenes.

Saponins: Saponins or Titerpenoid glycosides derived from Quillaja saponaria (Chilean soap bark tree). Saponins have been used as adjuvants for many years in veterinary vaccine.Qs-21 is a pure fraction of Quil A saponins with low toxicity isolated by Kensil and his colleagues in 1995. QS-21 is a potent adjuvant for cytotoxic T lymphocyte (CLT) induction,inducing Th1 cytokines(IL-2&IFN-g) and antibodies of the IgG2a isotype.

Saponins intercalate into cell membrane through interaction into cell membranes through interaction with cholesterol, forming pores, allows antigens to gain access to the cytoplasm for CLT induction.

QS-21 is an effective adjuvant for vaccine against pathogens that require a potent CTL response. e.g., used as an adjuvant for DNA vaccine, Recombinant Feline leukemia vaccine, FMD vaccine(stimulates T-cell activity),Anthrax vaccine (Saponin destroys the tissue at the site of injection so that anthrax spore germinates) .

ISCOMS-Immune Stimulating Complexes.

LPS/MPL: A second group of immunostimulatory adjuvants is derived from the LPS of Gram negative bacteria. The most extensively evaluated member of this family minophosphoryl lipid (MPL) is obtained from Salmonella Minnesota. MPL induces the synthesis and release of cytokines particularly IFNg, which promotes the generation of the response.

Bacterial DNA/CpGs Oligos: Bacterial DNA (not vertebrate DNA) has direct immune stimulatory effects on immune cells invitro. The immunostimulatory effect is due to the presence of unmethylated CpG dinucleotide, which are underrepresented and methylated in vertebrate DNA.

Unmethylated CpGs in the context of selective flanking sequences are thought to be recognized by cells of the immune system to allow discrimination of pathogen derived DNA from self DNA .

Bacterial DNA/CpGs trigger cells of the innate immune system including macrophages and dendritic cells.

CpGs is most potent for the induction of Th1 responses mainly through stimulating Tumour necrosis factor a(TNFa), IL1, IL6 &IL12 and through the expressioin of MHC molecules (costimulatory).

CpGs have significant potential as mucosally administered adjuvants.

Cytokines: As an alternative to the use of cytokine inducing adjuvants, cytokines may also be used directly. Cytokines that have been evaluated most extensively as adjuvants include-IL1,IL2,IFNg,IL12 & GM-CSF(Granulocyte Macrophage – Colony Stimulating Factor).

All of these molecules exhibit dose-related toxicity and because of their proteinaceous nature have stability problems, a short in vivo half life and relatively high cost of manufacture.

C. Particulate Adjuvant:

Particulate adjuvants have comparable dimensions to the pathogens that the immune system evolved to combat, therefore particulate adjuvants are naturally targeted for uptake by APCs to facilitate the induction of potent immune response.

e.g., Emulsions, MF59, Liposomes, Virosomes, virus like particles.

MF59: In 1980's oil-in-water (o/w) adjuvants formulation (SAF) was developed using bio-degradable oil (Squalane). This proved to be toxic, because of presence of a muramyl dipeptide (MPD) derivative. Subsequently a squalane o/w emulsion has been developed (MF59)

without the presence of additional immunostimulators, this formulation has proved to be a potent adjuvant. MF59 is an effective adjuvant in humans and can be recommended for the induction of potent antibody responses.

Liposomes/Virosomes: Liposomes have been evaluated both as adjuvants and as delivery systems for antigen and adjuvants.

Virosomes or fusogenic lyposomes are the liposomes with inserted virus fusion protein into liposomes by layer e.g.,immunopotentiating reconstituted influenza virosome (IRIV).

Modified liposomal structures termed **Cochleates** are also being evaluated as systemic and mucosal adjuvants in small animal models.

To reduce the dose of hemolytic Quil A adjuvant and to target the formulation directly to APCs, the immunostimulatory fractions from Saponaria (Quil A) have been corporated into lipid particles comprising cholesterol, phospholipids.

Freund's Incomplete Adjuvant:

Alternative method of forming depot is to incorporate the antigen in a water-in -oil emulsion called Freund's incomplete adjuvant. Oil stimulates a local, chronic inflammatory response and as a result a granuloma or abscess formed around the site of the inoculum. Antigen is slowly leached from the aqueous phase of the emulsion. Oil emulsion droplets may also be carried to other sites through the lymphatic system.

Freund's Incomplete Adjuvant contains-85% mineral oil + 15% Mannide monooleate.

Mixed Adjuvants:

Freund's Complete Adjuvant:

Killed tubercle bacilli (Mycobacterium tuberculosis/smegmatis) incorporated into the water in oil emulsion mix, is known as Freund's Complete Adjuvant. Extremly potent adjuvant.

Mode of action:-FCA not only forms slow release antigen depot but the Muramyl dipeptide MDP (present in tubercle bacilli) acts on macrophage to stimulate the production of IL1,which stimulates helper cell responses thereby enhancing the immunity. Responses only to thymus dependent antigens. Also stimulates Macrophages promoting cytotoxic phagocytic activity.

- FCA promote IgG production over IgM.
- FCA inhibits tolerance induction, favours delayed hypersensitivity reaction.
- FCA accelerates graft rejection and promotes resistance to tumours.
- Use of FCA is unacceptable in food animals not only because of mineral oil (oil spoils the meat) but also because the Mycobacteria in the adjuvant induce a positive skin reaction to tuberculin (drawback in areas where tuberculosis is controlled by skin testing).
- Endotoxins enhance antibody formation they have no effect on delayed hypersensitivity but can break tolerance and have general immunostimulatory activity.
- Endotoxins also stimulates macrophage production of IL1.
- Anaerobic Corynebacteria, Propionibacterium acnes promotes the release of IL1 leading to helper cell activation. Enhance antibacterial and anti-tumor activity.

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