Vaccine Adjuvants

a Mini Review

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Adjuvant role in vaccine development

- The safest vaccine
- Provide maximal efficacy
- Require the least amount of antigen, and number of doses

Background

- Presently, *adjuvants are not licensed separately* from the formulated vaccine.
- The individual vaccine/adjuvant combination is licensed ➔ case by case evaluation.
- No general guidelines.
- Currently, the aluminum salt/gel-based *(alum) are the only adjuvants licensed for human use in the United States*.

Adjuvants: Some Challenges

Safety concerns

- Evaluating benefit vs. risks

Lack of universality

- Adjuvants are currently *not considered the active ingredient* in prophylactic vaccines
- Immune responses obtained with one antigen/adjuvant *cannot be extrapolated to another antigen* or even to the same combination given by different routes

Antigen delivery systems

- Insoluble aluminum compounds
- Calcium phosphate
- Liposomes
- Virosomes™
- ISCOMS®
- Microparticles (e.g., PLG)
- Emulsions (e.g., MF59™, Montanides)
- Virus-like particles & viral vectors

Immunopotentiators

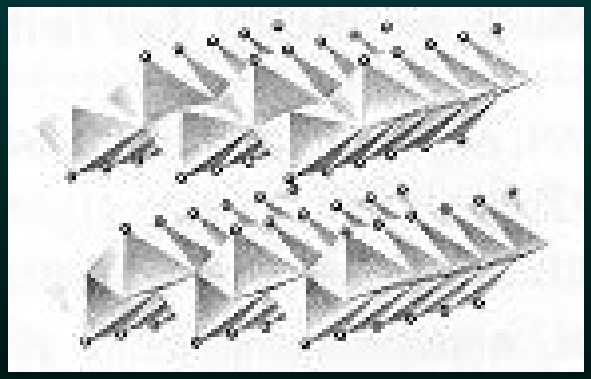
- MPL and synthetic derivates (Monophosphoryl Lipid A)
- MDP/MTP and derivatives (Muramyl di & tripeptides)
- Oligonucleotides (CpG, etc.)
- Double-stranded RNA (dsRNA) Hiltonol (PolyICLC)
- Saponins (Quils, QS-21)
- Cytokines & chemokines
- Alternative pathogen-associated molecular patterns (PAMPs) (*E. coli* heat labile enterotoxin (LT); flagellin)

Classification

- Mineral Salts – Alum
- Surface-Active Microparticles
- Bacterial Products
- Cytokines & Hormones
- Polyanions
- Carriers
- Living vectors
- Vehicles
- Antigen Constructs

Mineral Salts – Alum

- Aluminium hydroxide*
- Aluminium phosphate*
- Calcium phosphate*



* - used in humans

Surface-Active & Microparticles

- Nonionic polymeric surfactants (L101,L121)*
- Virosoms (PeviPRO™ & PeviTER™) *
- Saponin (QS-21) *
- Meningiciccal proteins (preteosomes) *
- Immune stimulating complexes (ISCOMs) *

* - used in humans

Bacterial Products

- Mycobacterium phlei (Detox)*
- Muramyl di & tripeptides (MDP/MTP) *
- Monophosphoryl lipid A (MPL)*
- Klebsiella pneumonia glycoprotein*
- Bacillus Calmette-Guerin*
- V. cholerae E. coli endotoxin*
- CpG oligodeoxynucleotides

* - used in humans

Cytokines & Hormones

- Interleukin -1, 2*, 6, 12
- Interferon – α *& γ*
- GM-CSF*
- HGH
- Lymphotactin

* - used in humans

Polyanions

- Dextran
- Double stranded polynucleotides

Carriers

- Tetanus toxoid*
- Diphtheria toxoid*
- Meningococcal B protein*
- Pseudomonas exotoxin A*
- Cholera toxin B subunit*
- Mutant heat labile enterotoxin of enterotoxigenic E. Coli*
- Hepatitis B virus core*
- Cholera toxin A fusion protein
- CpG dinucleotides
- Heat-shock proteins*
- Fatty acid
- KLH, BSA, Ovalbumin

* - used in humans

Living Vectors

- Vaccinia virus*
- Canarypox virus*
- Adenovirus*
- Attenuated Salmonella typhi*
- Bacillus Calmette-Guerin*
- Streptococcus gordonni*
- Herpes simplex virus
- Polio vaccine virus
- Rhinovirus
- Venezuelan equine encephalitis
- Yersinia enterocolitica
- Listeria monocytogenes
- Shigella
- Saccharomyces cerevisiae

* - used in humans

Vehicles

- Water in oil emulsion
  - mineral oil (Freud I)*
  - vegetable oil *
  - squalene & squalene*
- Oil in water emulsion
  - MF59 – squalene + Tween 80 + Span 85*
- Liposomes*
- Biodegradable polymer microspheres
  - Lactide, glycolide, PEG*
  - Polyphosphazenes*
  - Beta-glucan
  - Proteinoid microspheres

* - used in humans

Selected Mechanisms of Action

Adjuvant dependent & poorly understood

- Stimulation/prolongation of Ag APC uptake *(alum)*
- Attraction of MNC, DC, PMN - infiltration/enhancement

  Ag processing and presentation *(oil-in-water, MF 59)*
- Effects cellular membranes *(QS21)*
- TLR/Pattern Recognition Receptors PRR agonists
  - Release of pro-inflammatory cytokines
  - Enhance antibody & T cell responses
  - Engage negative feedback pathways

TLRs - Mechanism of action

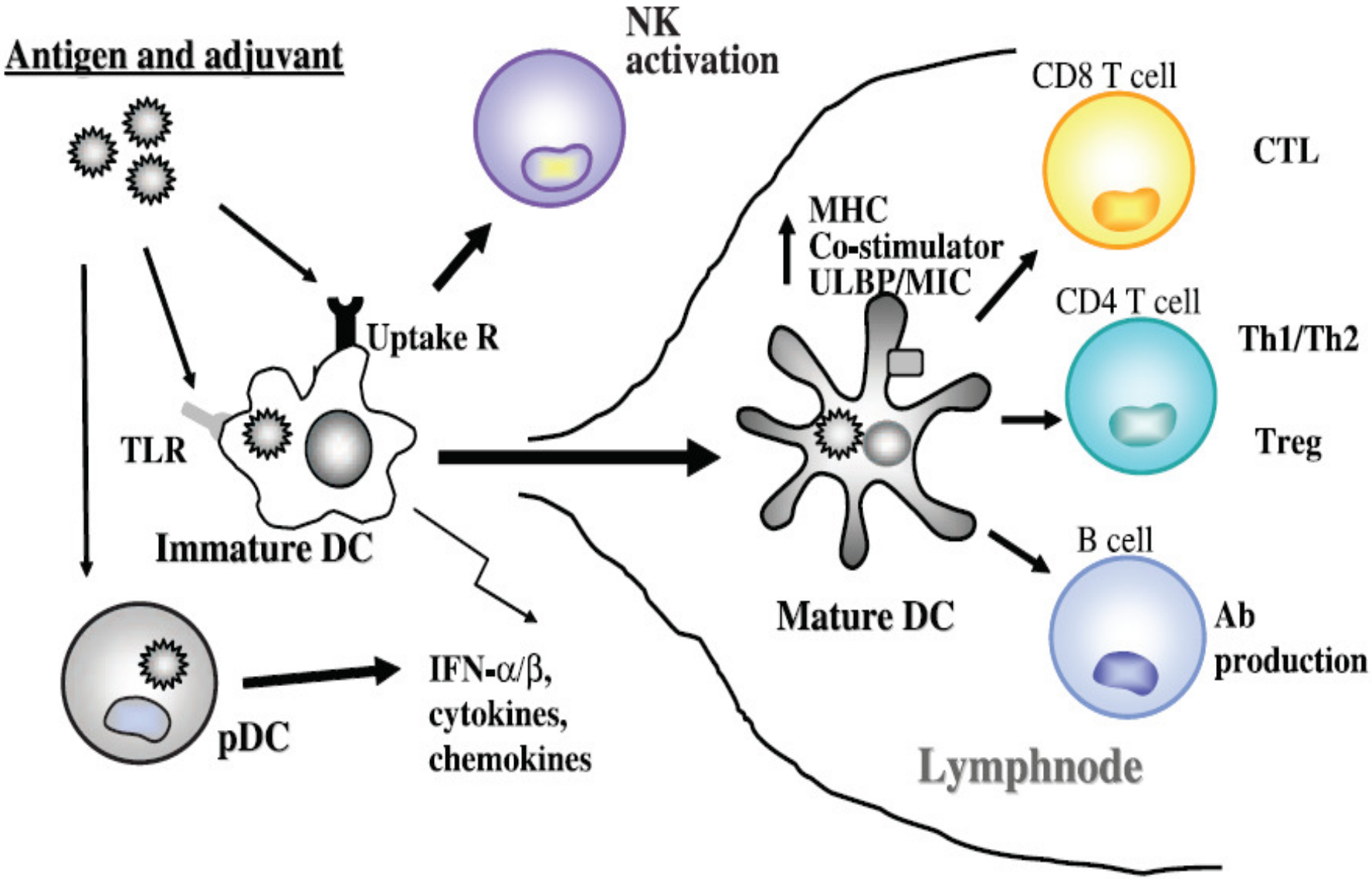


Figure 1. Role of human TLRs in mDC maturation followed by activation of various lymphocytes.

Immature dendritic cells (mDC) residing in local tissue phagocytose exogenous antigen (Ag) and pattern molecule (namely adjuant) and initiate the maturation process.

During maturation mDC induce IFNs, cytokines and chemokines, allow the upregulation of co-stimulators, NK-activating ligands (ULBP, MIC, etc) and MHC, and activate a variety of lymphocytes.

These maturation events are largely dependent on adjuvant properties. Also, adjuvant may participate in switching on of some unknown mechanisms which are essential in induction of CD8þ CTL by mDCs.

- Cytokines balance between **Th1** and **Th2,** (T-helper cells) activation.
- **Th1** primes a cell-mediated immune response that includes the activation of ***killer T cells***
- **Th2** primes an antibody response mediated by ***B cells***
- Different cytokines — and different adjuvants tilt the immune system toward characteristic points on the **Th1-Th2** continuum

Adjuvants - Th response

- **Alum - Th2** cytokines
- **MF59 -** squalene/water emulsion Chiron influenza vaccine **- Th2-**cytokines
- **CpG -** (bacterial cytosine- and guanine-rich unmethylated oligonucleotide motifs)**- Th1** cytokines
- **QS-21** - both **Th1** & **Th2-** cytokines

Human TLRs and their agonists

TLR$_{hu}$ -(PRR)	Ligands
TLR1	triacyl BLP
TLR2	PGN, BLP, Pam3Cys, peptidoglycan, lipoteichoic acid, zymosan, lipomannan, glycolipid, atypical LPS, Hsp60, porins
TLR3	dsRNA, poly I:C , poly ICLC (Hiltonol)
TLR4	LPS, Taxol, Lipid A mimetics (RC529), Hsp60, glycan, lipopeptidophosphoglycan, viral envelope proteins
TLR5	flagellin
TLR6	diacyl BLP, lipotechoic acid, zymosan, double stranded (viral) RNA
TLR7	ssRNA, Imidazoquinolines, imiquimod, 3M-019
TLR8	ssRNA, Imidazoquinolines, 3M-019
TLR9	unmethylated CpG DNA

Human TLR-specific signaling pathways

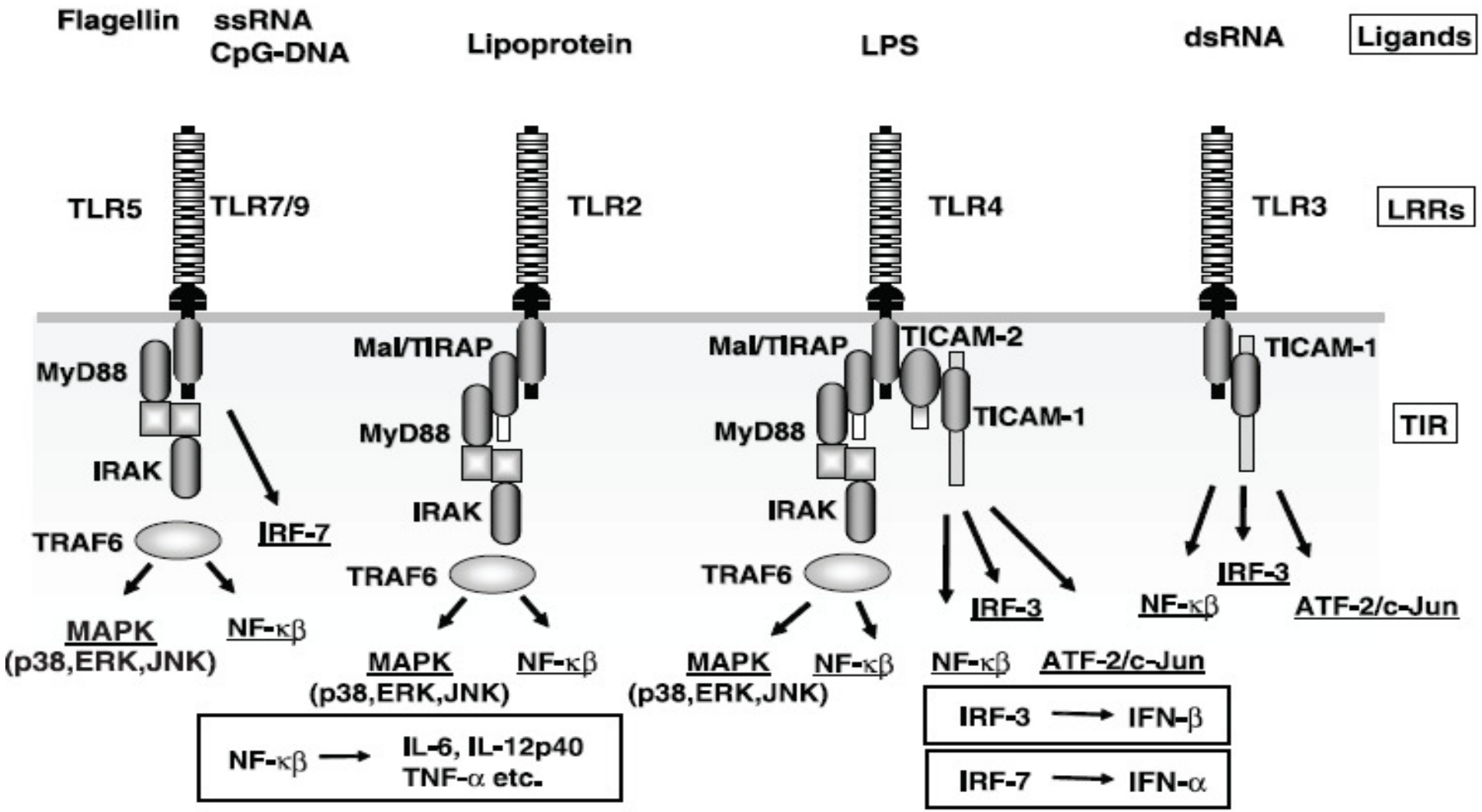


Figure 2. Association between human TLRs and adaptors determine each TLR-specific signaling pathway. Topology of the adaptor proteins in the TIR domains of TLR2, TLR3 and TLR4 is shown in the schema.

The complex consisting of each TIR and adaptors delivers TLR signaling to activate NF-kB and the IFN-b promoter (IRF-3).

In pDC, activation of TLR7 or 9 happens to activate IRF-7 in a MyD88-dependent way followed by induction of IFN-a. Representative ligands of TLRs are shown on the top.

Non TLR receptors

- **NLRs –** NOD like Receptors
- **RIG –**Retinoic acid inducible gene (RLH)
- **Scavenger receptors –** internalize polyanionic ligands
- **CLRs –** bind viruses through recognition sugar moieties (N-acetyl-glucosamine, mannose, N-acetyl-mannosamine, fucose, glucose
- **TREMs –** amplifiers of immune response – no known ligands

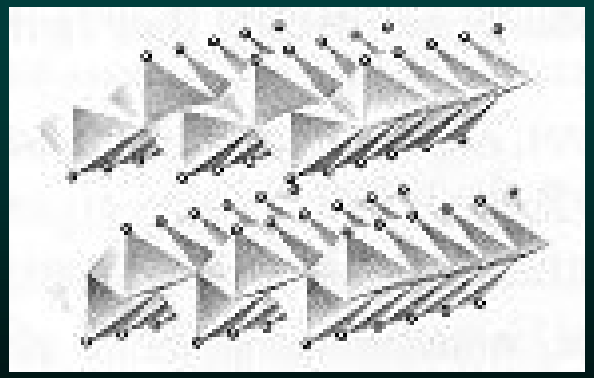
- A number of novel adjuvants have been under development and in preclinical evaluation for several decades and many of these are currently undergoing clinical investigation under U.S. Investigational New Drug (IND) applications

Alum - mechanism of action

- Uncertain, but *aluminum particles with the antigen are phagocytosed by macrophages activating them*.
- *Al is toxic & cause macrophage necrosis* in the lymph node.
- *Necrotic macrophages release their cytoplasmic contents*; alum-absorbed antigen and inflammatory mediators -- IL-1 and TNF into the lymph.
- This potent mix *stimulate* antigen-specific plasma cells and *antibody production* - **Th2**.

Limitations of alum.

- *Inability to induce cytotoxic T-lymphocyte (CTL)* - responses critical for viral protection and clearance



Oil-in-/Water-oil Emulsions General mechanism of action

- May include Montanide, Adjuvant 65, Lipovant 28, MF59, Drakeol/ISA-51, water-in-squalene emulsion (ISA-720), MPL and QS21-(SBAS-2), CFA35
- *Emulsion particles are irritants and cause local inflammation inducing macrophage invasion*.
- The oil particles with antigen are ingested by macrophages & move to drain in lymphnodes.

Limitations of oil-in-water emulsions

- Excessive reactogenicity and toxicity

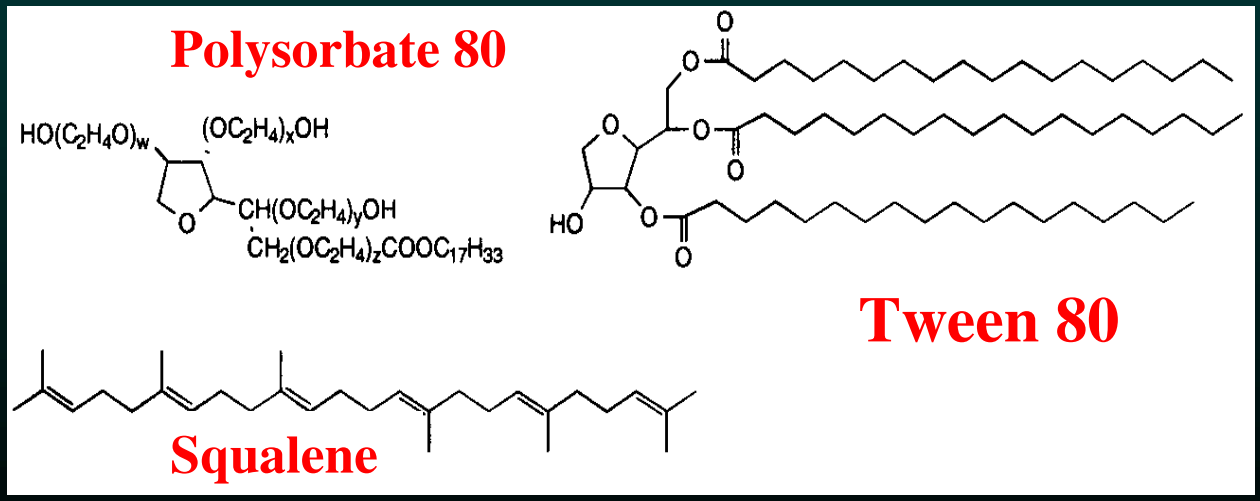
MF59™ * Mechanism of action

- MF59 -- oil-in-water emulsion => 4–5% w/v *squalene*, 0.5% w/v Tween 80, 0.5% Span 85, optionally, varying amounts of muramyl tripeptide phosphatidyl-ethanolamine (*MTP*-PE) => non-TLR receptors NOD-LRRs activator – **Th2**.
- Excessive reactogenicity and/or toxicity, MF59™ used in influenza vaccine (FLUAD) registered in Italy does not contain MTP.
- MF59™ superior to alum in inducing antibody responses to hepatitis B vaccine

* Chiron

Limitations of MF59 ™ *

- Injection *site pain and reactogenicity*, squalene may induce *chronic inflammatory arthritis* in genetically susceptible humans



* Chiron

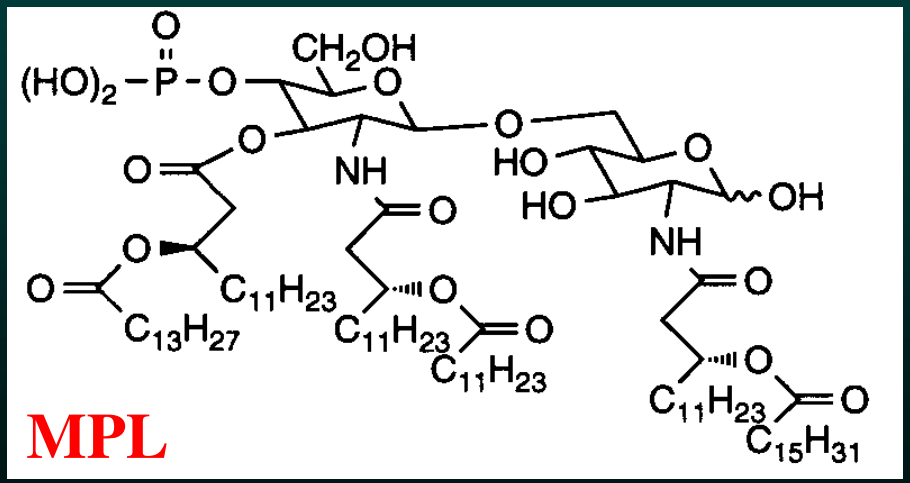
Monophosphoryl Lipid A (MPL)*
Mechanism of action

- **MPL** -- *detoxified **Lipid A** from Salmonella* minnesota R595, formulated with alum, QS21, liposomes, and emulsions - component of GSK's AS02 and AS04 adjuvants.
- Like LPS, MPL - ***TLR4 agonist*** on macrophages, releasing cytokines TNF, IL-2 and IFN-gamma ➔ ***Th1 responses***.
- Evaluated in cancer, genital herpes, HBV, malaria, HPV and allergies vaccines.
- *Approved vaccines* ➔ *melanoma vaccine in Canada*, a *hepatitis B vaccine for hemodialysis patients in Europe*, and an *HPV vaccine in Australia & US*.

***Corixa Corp**

Limitations of MPL*

- Significant reactogenicity, inconsistency of preparation, formulation, also cost.



*Corixa Corp

CpG
Mechanism of action

- Bacterial DNA CpG motifs, action due to ➔ *unmethylated **CpG** dinucleotides which are rare and methylated in vertebrate DNA.*
- *CpG's effect is mediated by **TLR9** receptors* expressed on B cells and dendritic cells ➔ release cytokines bias towards **Th1,** induction of CTL.
- CPG 7909, developed by Coley Pharmaceuticals, tested with an alum-adjuvanted Hepatitis B vaccine.

Limitations of CpG

- Flu-like symptoms, and headache
- Reactogenicity, toxicity, and safety ➔ barrier to acceptance of CpG adjuvants for human prophylactic vaccines.
- Could *exacerbate multiple sclerosis & systemic lupus erythematosus in susceptible individuals*

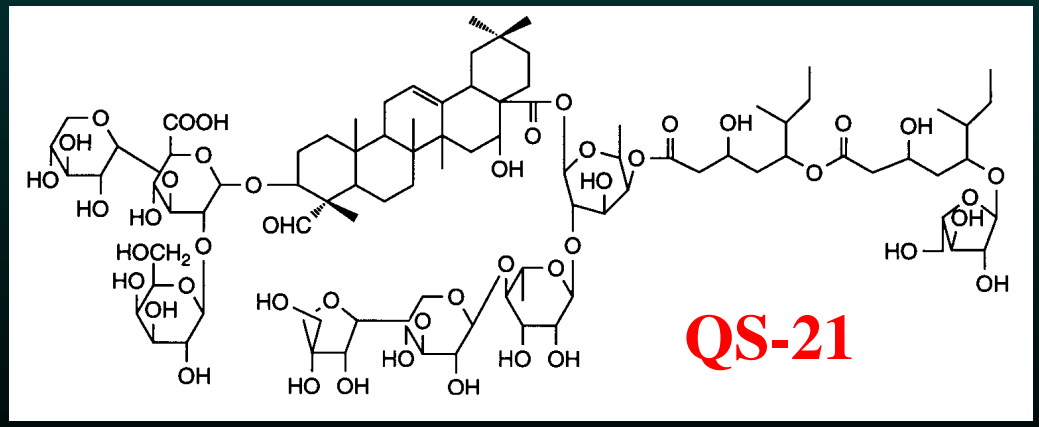
QS-21*-Mechanism of action

- **QS21** - *triterpenoid glycosides (saponins)* from the bark of the Quillaja Saponaria, induces **Th1** & **Th2** cytokines.
- *Saponins integrate into cell membranes through interaction with cholesterol*, creating pores for antigen entry.
- Clinical trials of cancer vaccines, HIV, influenza, herpes, malaria, & HBV

***Antigenics**

Limitations of QS-21*

- Pain on injection and granulomas, toxicity → *severe hemolysis*, unsuitable for human prophylactic.
- No advantage in antibody response compared with the unadjuvanted influenza vaccine.
- Malaria vaccine using QS21, two of 89 individuals developed severe vaccine allergy



*Antigenics

ISCOMs ®*
Mechanism of action

- *Complexes containing a saponin, a sterol, sterol cholesterol and phosphatidylethanolamine* (Quil A or QS21 optional)
- ISCOMs advantage → *reduced toxicity QS21* component (the saponin is bound to cholesterol and is less free to interact with cell membranes reducing QS21 hemolytic activity.)
- Generate CTL responses to HIV envelope glycoprotein and influenza hemagglutinin .
- Induce cytokines, IFN-g and IL-12 ability to skew immune responses in a **Th1** direction

*Iscotec

Limitations of ISCOMs ®*

- Cost, manufacturing difficulty, and stability, also reactogenicity, toxicity and safety concerns.
- Side effects in a Phase 1 human cancer trial included flu-like symptoms, fever and malaise.
- Reactogenicity and toxicity reflects the inclusion of Quil A or QS21 as an active ingredient.

*Iscotec

Liposomes - Mechanism of action

- **Liposomes** → phospholipid spheres encapsulating antigens, dual function; vaccine delivery vehicle & adjuvant, enhances *both humoral and cell-mediated immunity (**Th2** & **Th1**).*
- The mechanism → *fusion with the cell membranes of macrophages, enabling delivery* of antigens into the cytoplasm.
- Liposome-based vaccines based on virosomes are approved in Europe for hepatitis A and influenza - INFLUSOME-VAC.

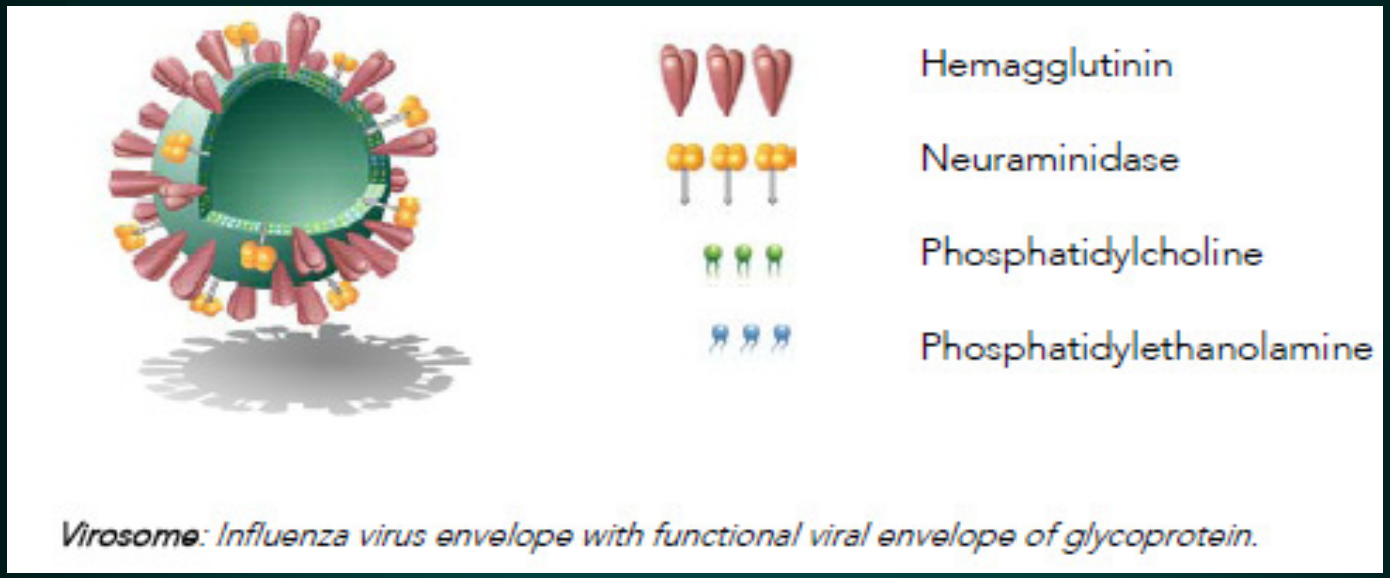
Limitations of liposomes

- Manufacturing difficulties, stability and high cost.
- *More antigen vehicles than true adjuvants* & require addition of immunostimulatory components such as MPL for potent adjuvant action.

Virosomes™

- Liposome-like spheres containing a membrane-bound *hemagglutinin* and *neuraminidase* (influenza virus).
- Facilitate fusion and uptake into antigen presenting cells (APC).
- The *delivery mimics a natural path and have an excellent safety profile*.
- *Hepatitis A* (Epaxal®) and *influenza* (Inflexal® vaccines registered in 45 countries

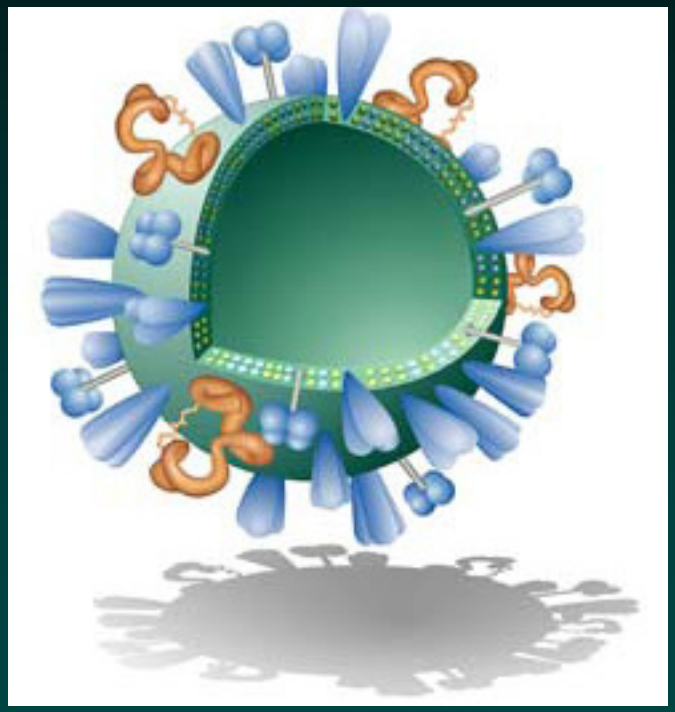
Virosomes™



***Virosome**: Influenza virus envelope with functional viral envelope of glycoprotein.*

Different antigens localization produces humoral **(PeviPRO™)** and/or a cellular **(PeviTER™)** immune response → prophylactic or a therapeutic vaccine

Virosomes™



PeviPro™

B-cells - antibodies

PeviTer™

cytotoxic T-cell response

Advax*
Mechanism of action

- *Nanocrystalline particles of inulin*, a natural plant-derived polysaccharide → a linear chain of fructose capped by a single glucose.
- Specific isoforms of inulin can enhance either humoral or cellular – **Th2** & **Th1** - ***immune responses without reactogenicity!!*.**
- High purity, heat stable, long shelf-life

***Vaxine Pty Ltd.**

Limitations of Advax*

- Presumption within the vaccine community →" adjuvant potency is proportionate to inflammation and reactogenicity".
- This dogma has arisen from *uncritical acceptance of the "danger hypothesis"*, which *suggests that immunogenicity is linked to activation of the innate immune system.*

***Vaxine Pty Ltd.**

VLPs - virus like particles

- The expression of viral structural proteins of envelope or capsid, can result in the spontaneous self-assembly of *virus like particles* (VLPs)

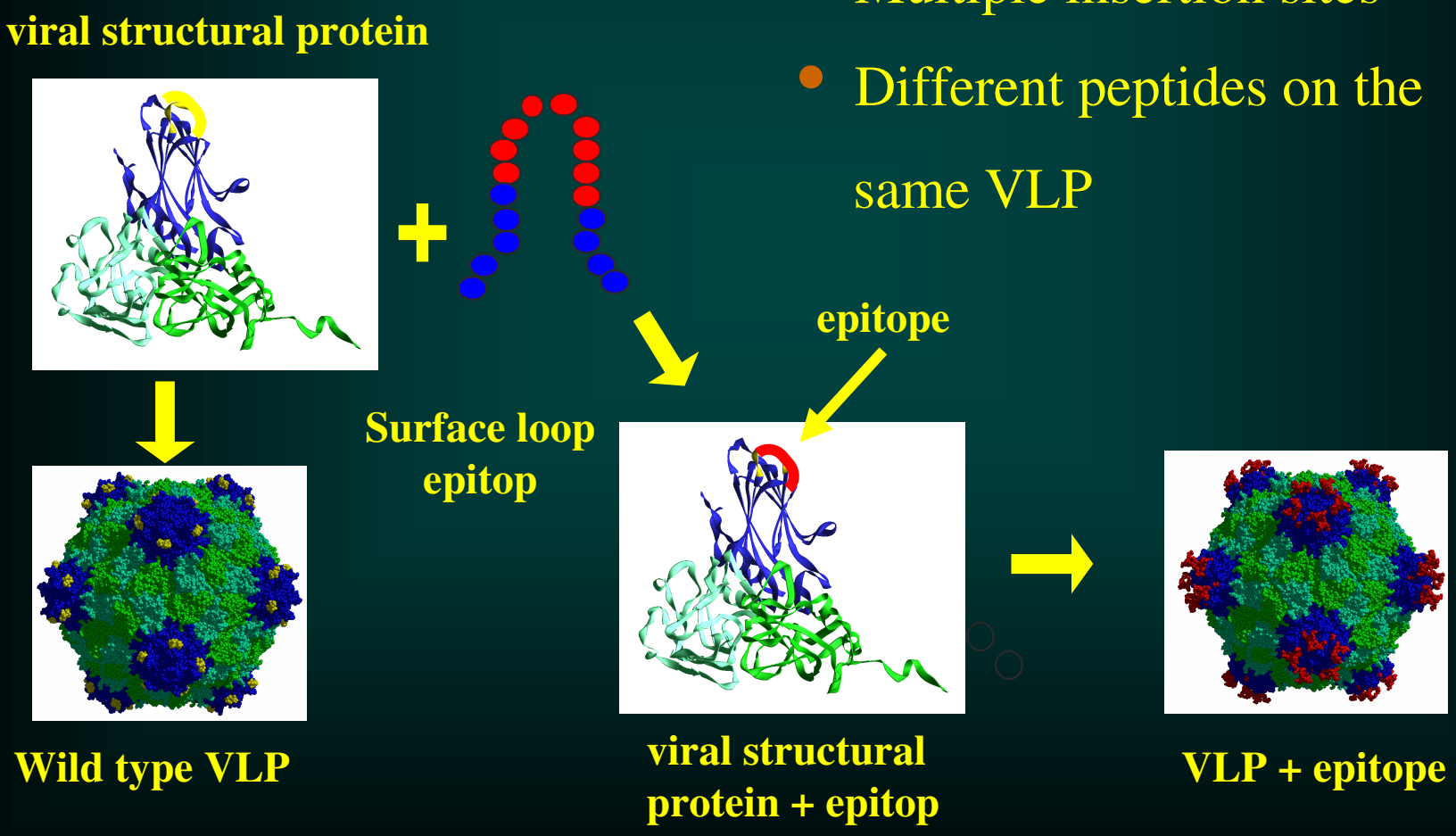
VLPs - virus like particles

- Powerful immune responses
  - *All arms of the immune system* (cellular, humoral, mucosal) activated
  - Th1 bias
- Multivalency & Flexibility
  - Accommodates peptide, protein small molecules antigens
- Broadly applicable
  - Infectious diseases – influenza, HPV, etc.
  - Chronic, non-infectious diseases - allergy

VLPs

Genetic fusion to display peptide antigens

- Multiple insertion sites
- Different peptides on the same VLP

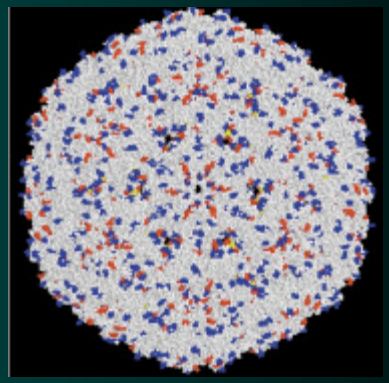


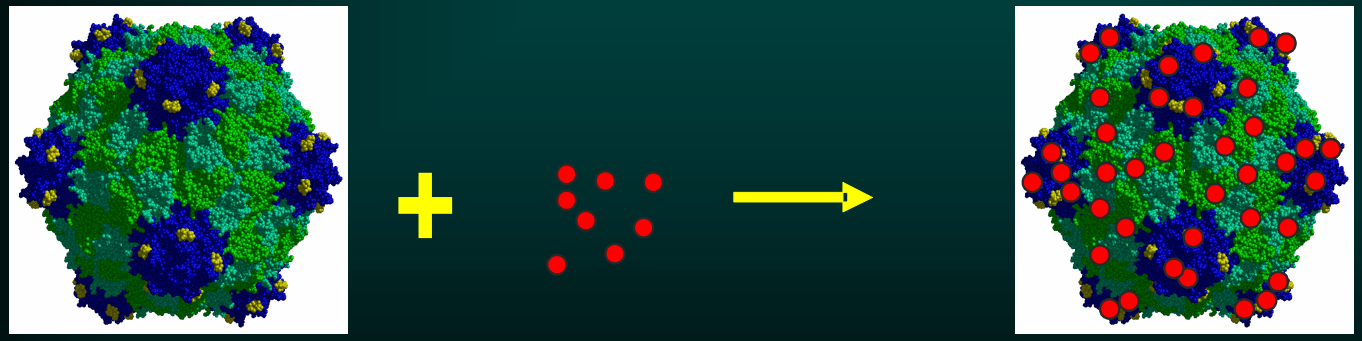
VLPs

Conjugation to display antigenic molecules

- Peptides, proteins, carbohydrates, nucleic acids, small molecules
- Covalent bonding - antigen to VLP
- Multiple conjugation sites & chemistries

Cysteines - SH
Lysines – ε NH_2
Carboxylates - COOH





VLP + Antigen → VLP antigen conjugate

Adjuvant Safety & tolerability

- The benefits of adjuvant in vaccines must be balanced against any increased reactogenicity or risk of adverse reactions.

- *Does increased adjuvant potency = need to increase reactogenicity and toxicity ?*

- Complete Freund's adjuvant (CFA), the gold standard of adjuvant potency => extreme reactogenicity and toxicity, no use in human vaccines & potential ban CFA in veterinary vaccines.

Vaccine vs adjuvant adverse effects

- **Local reactions -** injection site pain, inflammation, swelling, granulomas, sterile abscess formation, lymphadenopathy
- **Systemic reactions -** nausea, fever, *adjuvant arthritis*, *uveitis*, eosinophilia, allergic reactions, organ-specific toxicity, anaphylaxis, or immunotoxicity mediated by liberation of cytokines, immunosuppression, *induction of autoimmune diseases (Lupus, Arthritis)* .

Vaccine vs adjuvant systemic reactions

- Some systemic reactions such as *allergy and anaphylaxis* are clearly *due to the antigen*, others, such as *adjuvant arthritis,* may be *caused directly by or exacerbated by the adjuvant*.
- It can be *difficult to identify which* adverse reactions are *mediated by the antigen*, which by the *adjuvant*, and which by *both*.

Clinical Studies with Adjuvants

Recent or Ongoing

- Mineral salts/gels
- Oil-in-water emulsions (MF59™)
- Saponin-based (QS21)
- Microbial derivatives (MPL, CpG, LT)
- Endogenous human immunomodulators (cytokines)
- Virosomal/particle approaches (VPL)
- Combinations of these = “Adjuvant Systems”
- Viral like particles - VLP

Novel Adjuvants in Approved Vaccines

- **MPL** containing adjuvants:
  - ***AS04*** **–** hepatitis B vaccine (Fendrix) GSK

    HPV vaccine (Cervarix) GSK
  - ***AS01/AS02*** **–** *investigational* malaria vaccine(s)GSK
- **MF59™** - influenza vaccine (FLUAD *)N
- **VLP** - HPV vaccines – (Gardasil)M & (Cervarix) GSK

*** In EU**

Issues

- Currently available adjuvants ➔ “a random collection of immunostimulators”
- High reactogenicity and toxicity
- No diagnostic means to identify susceptible human sub-populations
- No systematic exploration of TLR agonists

The End