“Immunization has been a great public health success story. The lives of millions of children have been saved, millions have the chance of a longer healthier life, a greater chance to learn, to play, to read and write, to move around freely without suffering”.

Nelson Mandela,

HALMUTHUR SAMPATH KUMAR
Vaccine Immunology Laboratory, CSIR-IICT, Hyderabad, India
Why do we need Adjuvants??

Definition: An adjuvant is a chemical substance that can be added to a vaccine in order to enhance immune response.

- Traditional self adjuvanted vaccines based on attenuated live organisms being invasive in nature provides efficient delivery to antigen-presenting cells and Various naturally occurring components of the pathogens stimulate the innate immune system.

- The majority of recent vaccines represent highly purified subunit components of pathogens lack most of the features of the original pathogens, such as immunostimulatory components, and the ability to replicate and produce high level of antigens. Therefore, they are usually poorly immunogenic and need adjuvants to improve immunogenicity.

SLOW PATH TO THE DISCOVERY OF ADJUVANTS
Antigen

- Formulation process
- Formulation excipients
- Device & container
- Delivery route
- Storage
- Delivery system
- Immune potentiator

Adjuvant

About 40
Approx tuberculo develop

Source: WHO annual report
Small Molecules as Vaccine Adjuvants  

Natural, Semisynthetic, Synthetic

**Adjuvant Discovery Cycle**

- **Compound Collections**
- **Primary Assays**  
  - In vitro efficacy and Toxicity
- **Chemical Synthesis**  
  - Indirect
  - Direct
- **Design**
- **Lead Compound and SAR**
- **Structural Characterization, Protein-Ligand complex**
- **In vivo Efficacy, Toxicity**  
  - Secondary Assay like metabolism, bioavailability etc.
- **Clinical Candidate**
Adjuvant Design-Multiple Roles of Adjuvants

Multiple Roles of Adjuvants
• Depot Formation
• Rapid antigen delivery into APC
• Effective antigen processing/presentation
• Humoral and cell mediated immunity
• Immune Memory
• Complimentary immune response to specific antigen

Benefits of Adjuvants
• Replacement of Live Vaccine with Subunit Vaccine- New Vaccines
• Permits use of a much smaller quantity of Antigen- Affordable
Plants with Immunomodulatory Potential as possible source of Adjuvants??

- *Tinospora cardifolia*
- *Vitex negundo Linn*
- *Withania somnifera*
- *Picrorhiza kurroa*
1. Picroside II (PK-II) = $R_1 - O - \text{phenyl} - O - R_2 - H$

2. Picroside I (PK-I) = $R_1 - H - \text{phenyl} - R_2 - H$

3. Catalpol = $R_3 - H - R_2 - H$

**Picrorhiza kurroa**

**IL-4 (pg/ml)**

**Primary titre**

- **HBsAg**
- **Alum + HBsAg**
- **RLJ-NE-299A + HBsAg**

**Secondary titre**

- **IgG1**
- **IgG2a**
- **IgG1**
- **IgG2a**

**Adjuvant doses µg/ml**

- **Normal control**
- **Alum alone 1.45mg**
- **HBsAg alone 20ug**
- **Standard vaccine**
- **Alum 1.45mg + 20ug**
- **0.312ug 299A**
- **0.625ug 299A**
- **1.25ug 299A**
- **2.5ug 299A**
- **5ug 299A**
- **10ug 299A**
- **20ug 299A**
- **40ug 299A**

**IgG antibody titre MIL/U**

**IL-10**, **IFN-gamma**, **IL-5**, **TNF-alpha**, **IL-2**

**% change**

**Adjuvant doses µg/ml**

- **Normal control**
- **1.45 mg Alum + 20ug HBsAg**
- **80 µg FCA + 20ug HBsAg**
- **2.5 µg 299A + 20ug HBsAg**

**1.45 Alum + 20m**
Lipase Catalysed Regioselective Acylation of Picroside-II

Halmuthur Kumar et al., *Vaccine*, 28 (2010) 8327–8383
Regio-selective acylation of Agnuside

Candida antarctica, THF, molecular sieves (4Å)

Vitex negundo Linn

**Figure**

A) IgG titre

<table>
<thead>
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<th>Doses (μg/ml)</th>
<th>15th day</th>
<th>28th day</th>
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<tr>
<td>Alum</td>
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<tr>
<td>OVA (100 μg)</td>
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b) IgG1

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<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA</td>
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<tr>
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c) IgG2a

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<th>Doses (μg/ml)</th>
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<tbody>
<tr>
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<tr>
<td>100</td>
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Plant Based Adjuvants: Drawbacks

- Availability of plant in abundance
- Isolation process to be economical

- Alternative to expensive adjuvants from plant sources
- In expensive Synthetic route to plant based adjuvants
  QS21 is highly expensive
- Scaffold modification a promising strategy
- Mode of action to be established
Pattern Recognition Receptors (PRRs)

- Toll-like receptors
- NOD /RIG receptors
- Mannose binding lectin (MBL)
- C-reactive protein

Functions of PRRs:
- Opsonization, activation of complement and coagulation cascades,
- Phagocytosis,
- Activation of pro-inflammatory signaling pathways,
- Apoptosis
Strategy for Harnessing Innate Immunity

- Combination of delivery system and TH1 effectors
- Safe adjuvants with reduced toxicity - Modify known 1
PAMPs

- **Conserved** among microbes
- **Known as** Pathogen-Associated Molecular Patterns (PAMPs)
- PAMPs are recognized by **plants** as well as **animals**, meaning this innate response **arose before the split**
- **Recognized by the complimentary** PRR
- **Only** vertebrates have evolved an **adaptive immune response**
Dendritic cell TLRs and their Agonists

- Lipoprotein, unconventional LPS (bacteria)
- Peptidoglycan, zymosan, LAM (bacteria, fungi)
- Mycoplasmal lipoprotein (bacteria)
- Enterobacterial LPS (bacteria)
- Flagellin (bacteria)
- Profilin (protozoa)

- TLR1
- TLR2
- TLR3
- TLR4
- TLR5
- TLR6
- TLR7
- TLR8
- TLR9

- MDP
- NOD2
- DAP
- NOD1

- ssRNA (virus)
- dsRNA (virus)
- CpG DNA (bacteria)
**Microbial Pattern Recognition Ligands**

**Virtues**
- Powerful PRR specificity
- Humoral and cell mediated immunity
- High activity at low doses
- Overall Th1/Th2 balance

**Drawbacks**
- High pyrogenicity
- High Toxicity
- Often induce auto immunity

- Lipoteichoic acid (LTA)
- MDP
- PAMP
- MALP
- MP Lipid A
- PIMS
Immune response derived from select PAMPs and TCR Ligands

- **Th1**
  - QS-21 (Saponins)
  - Muramyl dipeptide
  - Th1
    - IL2, IFN-γ
  - TNF-α

- **Th2**
  - Pam3cys (Lipopeptide)
  - TLR2
  - Th2
    - IL-4, 6
    - IgG, CD8+

- **α-Galactoceramide**
  - (Spingolipid)
  - NK Cell α,β-TCR
  - Th2
    - IL-4, 5, 10
    - IgG, CD8+

- **Imiquimod**
  - (Imidazoquinoline)
  - TLR7/8

- **Monophosphoryl lipid**
  - (Liposaccharide)
  - TLR 4, 2
  - Th1
    - IL2, IFN-G
  - Th2
    - IL-4, 6
    - IgG, CD8+

- **Muramyl dipeptide**
  - (Glycopeptide)
  - TLR 4, 2
  - NOD-2
Synthetic lipopeptides like MALP have demonstrated great potential as a vaccine strategy for eliciting cellular and humoral immunity.

**PAM3CYS** binds and activates dendritic cells by engagement of Toll like receptor 2 (TLR 2). as well as its less hydrophobic nature, indicate that PAM3CS could also be used as a self-adjuvanting moiety (Derived from *Micoplasma Fermentan*)
Ligand field expansion through diversity oriented synthesis approach leads to adjuvants with varying degree of immunogenicity.
Click-Chemistry Approach to Novel triazole tethered carbohydrate Pam3Cys conjugates

Click Chemistry Propargyl bromide

Carbohydrate

>94%

Lactose azide

82%

Propargyl bromide

K2CO3

Zn, CH3COO

H2O 2h

80%

Yield=80%

Et3N DCM DMAP 70%

NaBH4 MeOH

Tosyl chloride,

BF3 Et2O Triethyl orthoformate, 12h 80%

THF: Water (9:1) 75%

NaIO4

Na, Liquid ammonia

L-cystine 60%

NaHCO3, 1,4 dioxane 75%

Boc anhydride, water

KI DMF 80%

Phenacyl bromide

KF, DMF, 1h 70%

Cyclohexanone

20%
Novel TLR2 Agonistic Triazole tethered Pam2Cys carbohydrate conjugates ADJUVANTS.

Immunological Evaluation of Pam3Cys conjugates as TLR2 agonists

Carbamate analogues of Lysine lipopeptides elicit Adjuvanticity

Human TLR-2 reporter gene assay

Antigen specific antibody response

Ismail Tabasum and Halmuthur Kumar et al., International Immunopharmacology 11 (2011) 1855–1863
MDP (MurNAc-L-Ala-D-isoGln, also known as muramyl dipeptide), is the minimal bioactive peptidoglycan motif common to all bacteria and the essential structure required for adjuvant activity in vaccines. MDP is recognized by NOD2 (CARD15) a member of the family of Nod-like receptors (NLRs, also known as CATERPILLER), characterized by a nucleotide-oligomerization domain (NOD) and ligand-recognizing leucine-rich repeats.
SYNTHESIS OF NOVEL MURAMYL DIPEPTIDE ANALOGUES:
Immunological Evaluation of Muramyl dipeptide analogues as vaccine adjuvants

Reagents and conditions: a) BnOH, BF3.Et2O, 8 h, 80 °C, 90%; b) PhCH(Ome)2, DMF, 7 h, 80 °C, 87%; c) KOH, EtOH, 7 h, 130 °C, 85%; d) RCOOH, DCC, DMAP, CH2Cl2, 0 °C to rt, 4 h, 96%; e) (S)-(−)-2-Chloropropionic acid, NaH, THF, 65 °C, overnight, 93%; f) 6, EDCI, HOBr, THF, DIPEA, 0 °C to rt, 6 h, 88%; g) H2, Pd/C, AcOH, H2O, rt, 12 h

IgG response against OVALBUMIN

HBsAg specific antibody response
α-GalCer is a glycolipid antigen derived from extracts of Japanese marine sponge *agelas mauritianus*. It activates *i*-NKT Cells when presented by CD1d and produces large amount of cytokines.

Sugar hydroxyls interact with NKT cell TCR with strong H-bonding network.

C26 chain extend into A’ pocket of CD1d Which can accommodate up to 80 carbon length.

C14 chain extend into F’ pocket of CD1d.

Provide anchor to CD1d/GalCer complex.

Structure Activity Relationship (SAR) of α-Galactosylceramide.
Immunological Evaluation of α-GalCer Analogues

Galactosyl azidoPhosphoglycerine

In vitro Splenocyte stimulation assay

Cytokines secretion by mouse splenocytes when stimulated with α-GalCer and its analogues with concentration of 1000, 100 and 10 ng/ml. IL-2, IFN-γ and IL-4 production was measured after 48 hrs treatment.
**In vitro CD1d antigen presentation assay**

IL-2 expression by iNKT hybridoma cells incubated with CD1d protein on treatment with compounds at a concentration of 100 ng/ml for 16 h.

**In vivo kinetic release of cytokines**

IFN-γ and IL-4 production by BALB/c mice on *in vivo* treatment of compounds at a concentration of 1 μg after 2, 12, 24, and 48 h.

Hydrogen Bond Interaction with CD1d

Ligand

GalCer
Asp-151, Thr-154

Ceramide-1
Gln-150, Asp-151, Asp-80, Arg-79

Ceramide-2
Asp-151, Thr-154

**In vitro** CD1d antigen presentation assay

**Fig:** IL-2 secretion by murine hybridomas were stimulated by 1000 ng/ml glycolipids loaded on CD1d coated plates. CD1d molecules (10 µg/ml in PBS) were coated in a 96-well plate by incubation of 1 h at 37°C. IL-2 release was measured after 16 h of culture in a sandwich ELISA.
Kinetics of cytokine release into serum following the injection of glycolipids. BALB/c male mice were injected intravenously with 1 µg of Glycolipids and then the serum was collected at the indicated times for cytokine profiling.
Novel saponins as Adjuvants

Uniqueness of QS21
1. Alternating HLB
2. Presence of branched trisaccharide
3. Aldehyde on triterpinoid

Possibilities
• Simplify the structure
• Introduce formyl group
• Change the triterpinoid
Design, Synthesis, and Evaluation of 1,2,3-Triazole-tethered carbohydrate triterpenoid Glycolipids as Vaccine Adjuvants

B. Debabrata and Halmuthur Kumar et al., Arch. Pharm. Chem. Life Sci. 2015, 348, 1–15
QS 21 inspired Saponins Mimics as Vaccine Adjuvants

Two fold increase in antibody titer compared to QS-21.

Low hemolysis and Low membrane toxicity.
Docking approach to Adjuvant Design

<table>
<thead>
<tr>
<th>TLRs</th>
<th>Active site residues</th>
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<tr>
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<td>Trp258</td>
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<tr>
<td>TLR2</td>
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<tr>
<td>TLR10</td>
<td>Trp256</td>
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Oleic acid nanoemulsion for nasal vaccination: Impact on adjuvanticity based immune response

Vaccine Immunology Laboratory at CSIR-IICT
SUMMARY

- DC receptor agonists are the best choice for developing adjuvant libraries
- Diversity oriented approach to library generation gives leads to efficacious and inexpensive adjuvants
- Rational approach to design leads to less toxic adjuvants
- Vaccine delivery systems for non invasive administration
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