

# Immunization (II)

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# Objectives of this lecture

By the end of this lecture you will be able to:

- ① Recognize common adjuvants
- ② Describe how adjuvants work
- ③ Understand the roles of excipients

# Vaccine Formulation



# Antigen

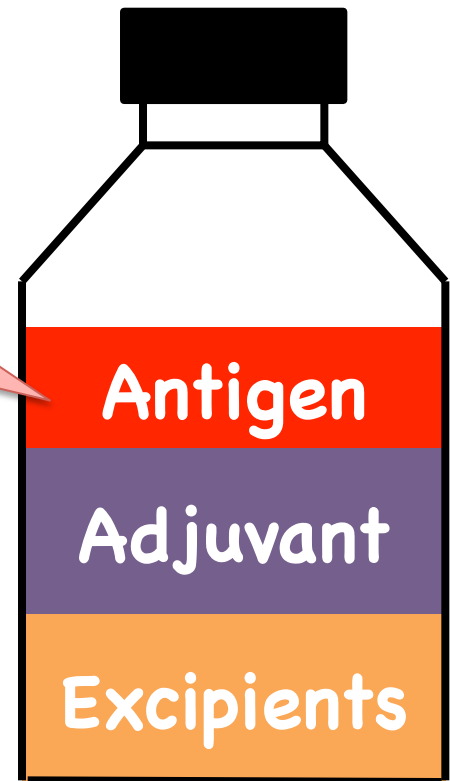
**Killed**

**Attenuated**

**Toxoid**

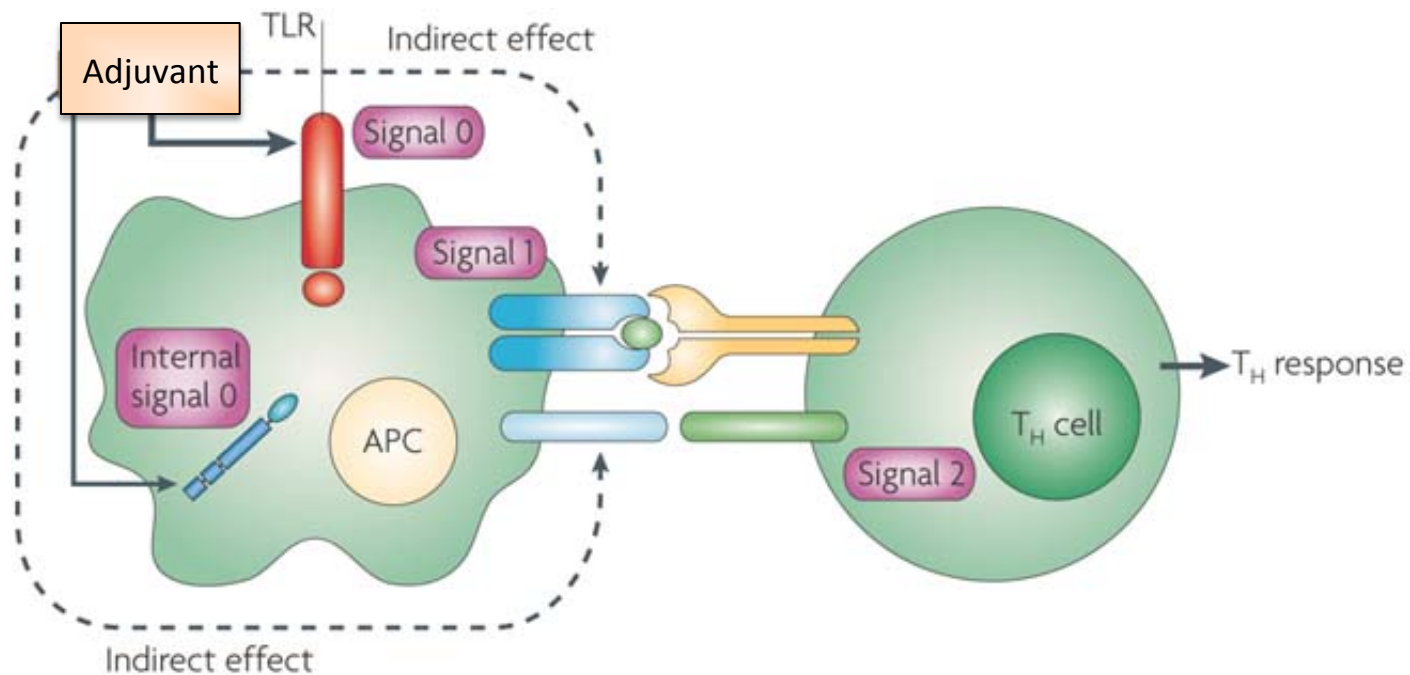
**Polysaccharide**

**Conjugate**



# Adjuvants

- Non-antigenic components that are designed to orient and augment the immune response



# Adjuvant

Aluminum salts

FCA

FIA

Squalene

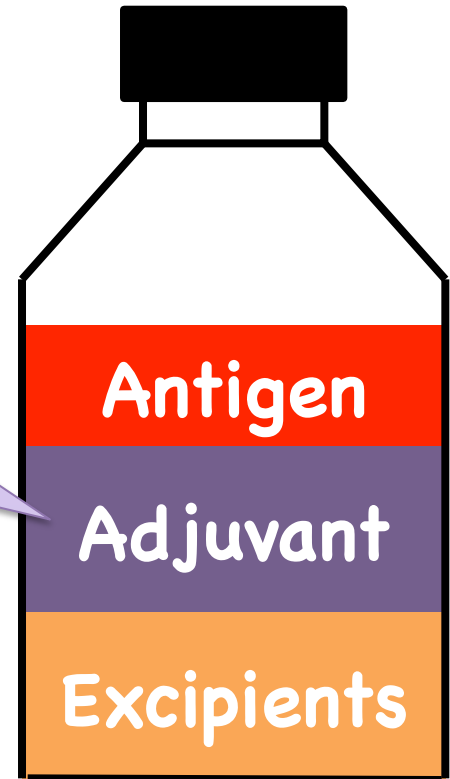
MDP

MPLA

CpG

Liposomes

ISCOM



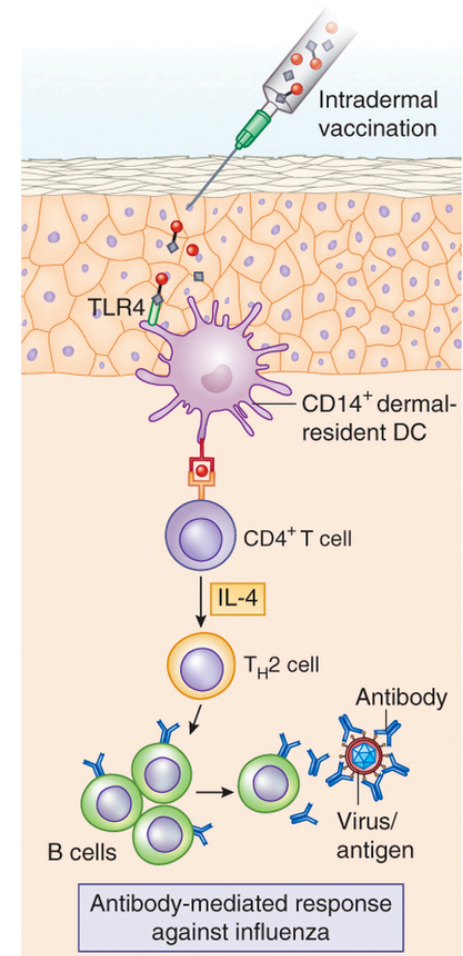
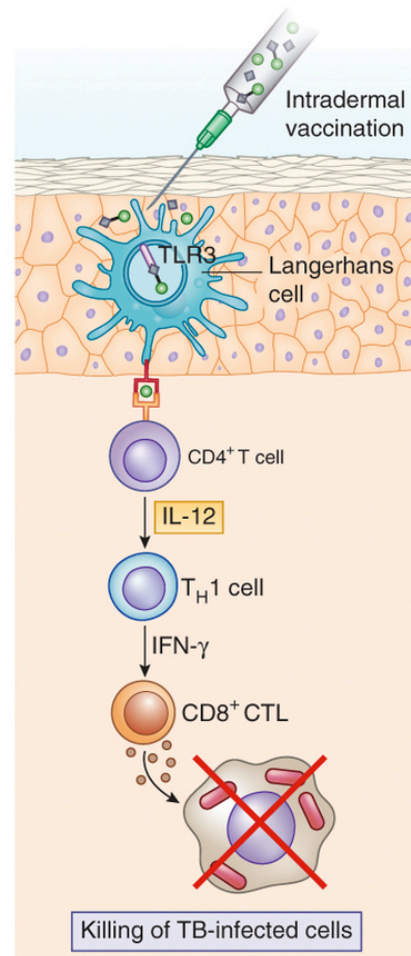
Antigen

Adjuvant

Excipients

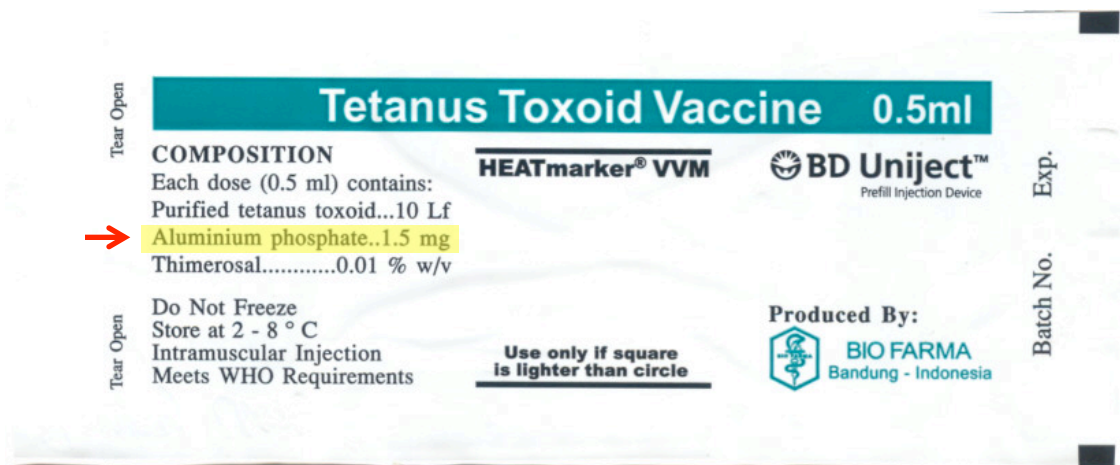
# Adjuvants

- Adjuvants can direct the immune system toward CMI or Humoral Immunity
- Adjuvants dictate the type of cytokines released by APCs and CD4<sup>+</sup> cells



# Aluminum Salts

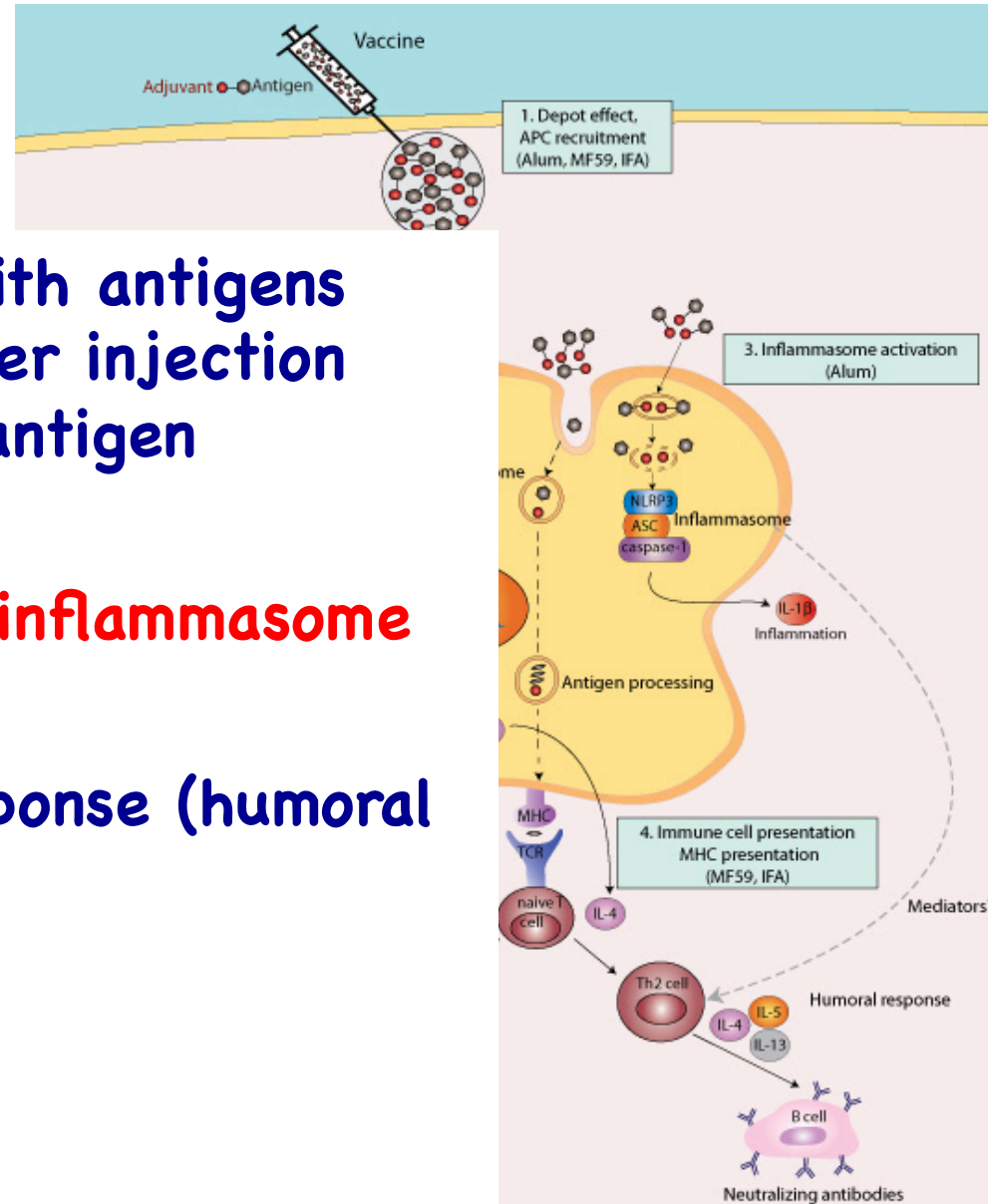
- Aluminum hydroxide
- Aluminum phosphate
- Alum (potassium aluminum sulfate)
- Commonly used adjuvants in many vaccines e.g. DPT, PCV, HAV, HPV





# Aluminum Salts

- Aluminum salts mixed with antigens form **insoluble depot** after injection and slowly release the antigen
- Aluminum salts activate **inflammasome**
- Stimulates antibody response (humoral immunity)



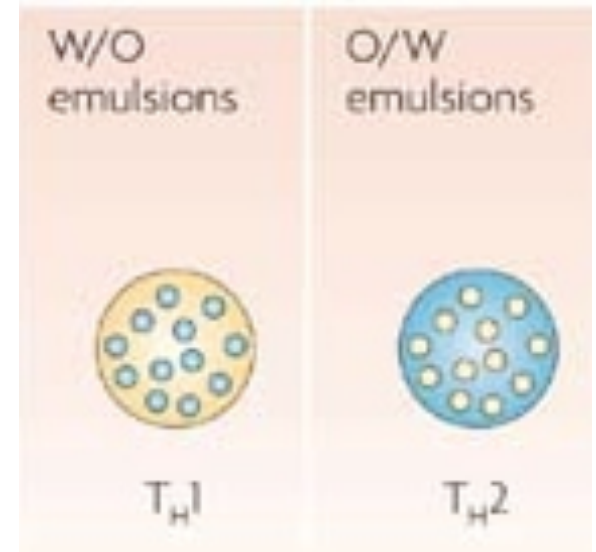
# Squalene

- Naturally occurring molecules. MF59 and ASO3 adjuvant contain 4.3% squalene in presence of Tween 80. Squalene is a safe but weak adjuvant
- It is commonly used in influenza vaccine in Europe but not in the US (**Gulf War syndrome**)



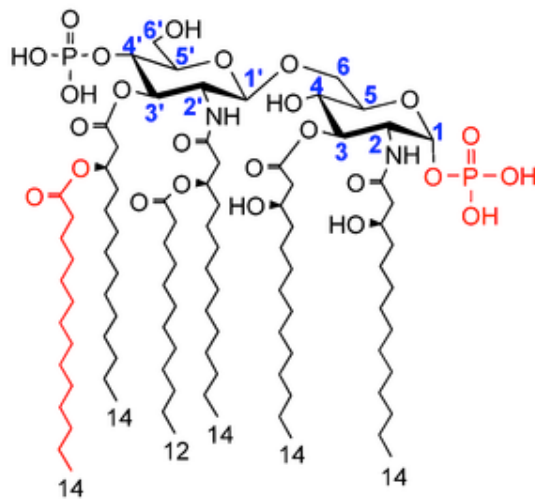
# Freund's Adjuvants

- **Freund's complete adjuvants (FCA):** it was the first adjuvant used. It is composed of **KILLED *Mycobacterium tuberculosis*** suspended in mineral oil w/o. Stimulates CMI but **cannot be used in human**
- **Freund's incomplete adjuvants (FIA or IFA):** it is o/w emulsion without mycobacteria. Stimulates antibody response

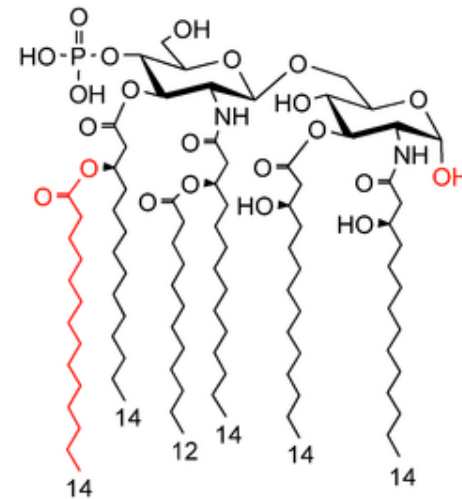


# Monophosphoryl Lipid A

- MPLA is a detoxified endotoxin lipid A fraction (lacking one phosphate group)
- It has no physiological toxicity but retains the adjuvant effect of parenteral endotoxin



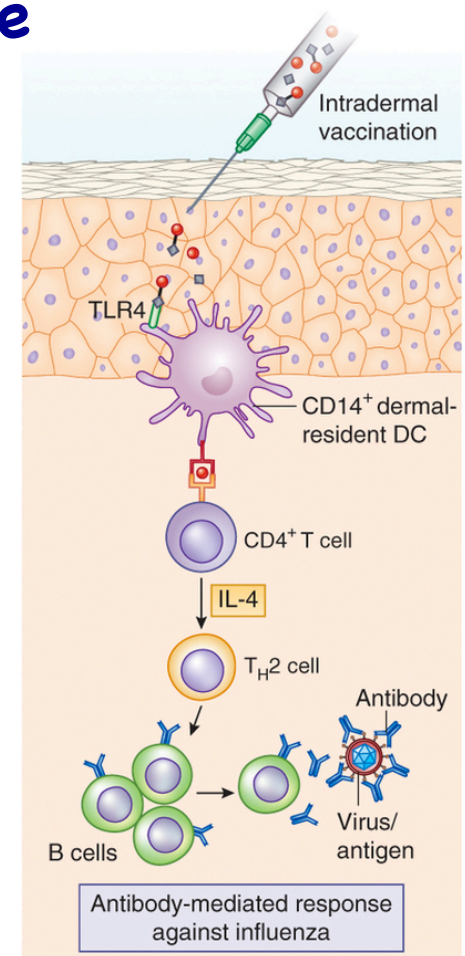
Lipid A



MPLA

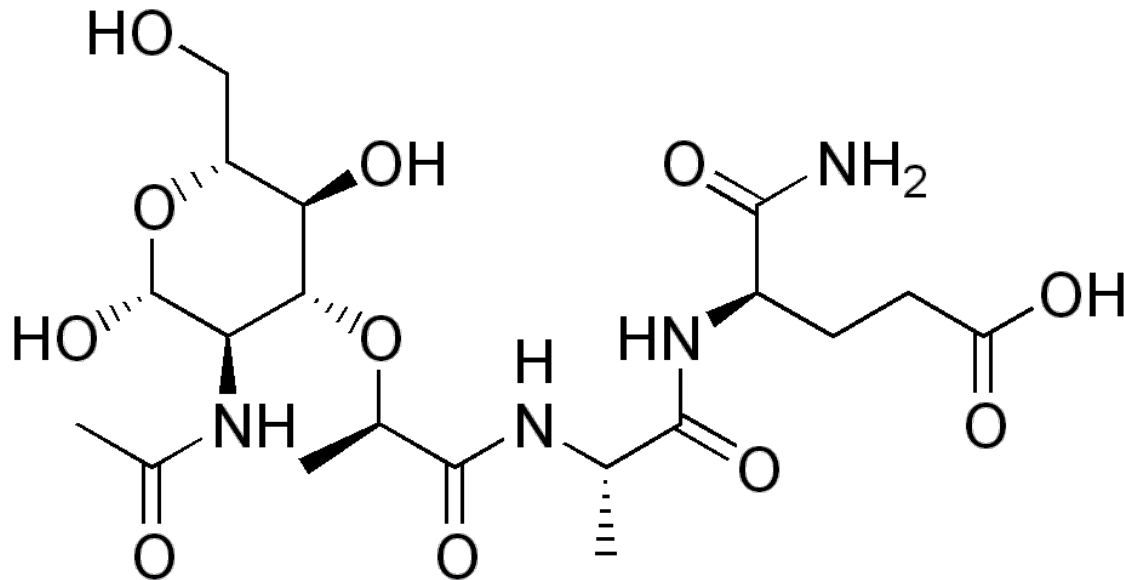
# Monophosphoryl Lipid A

- It upregulates costimulatory molecules (CD80 and CD86) on DC and macrophage through TLR4 stimulation
- Stimulate both  $T_H1$  and  $T_H2$
- Used in HBV in Europe and Cervarix<sup>®</sup>



# Muramyl Dipeptide

- MDP is a *Mycobacterium* cell wall fragment
- Non-toxic adjuvant
- If administered in saline → Humoral Immunity
- If mixed with glycerol → Strong CMI



# Oligodeoxynucleotides

- Unmethylated CpG sequence (part of bacterial DNA)
- CpG motifs are immunostimulatory sequence (ISS)

## CpG ODN Classes

NK cell activation



Humoral Immunity



CMI



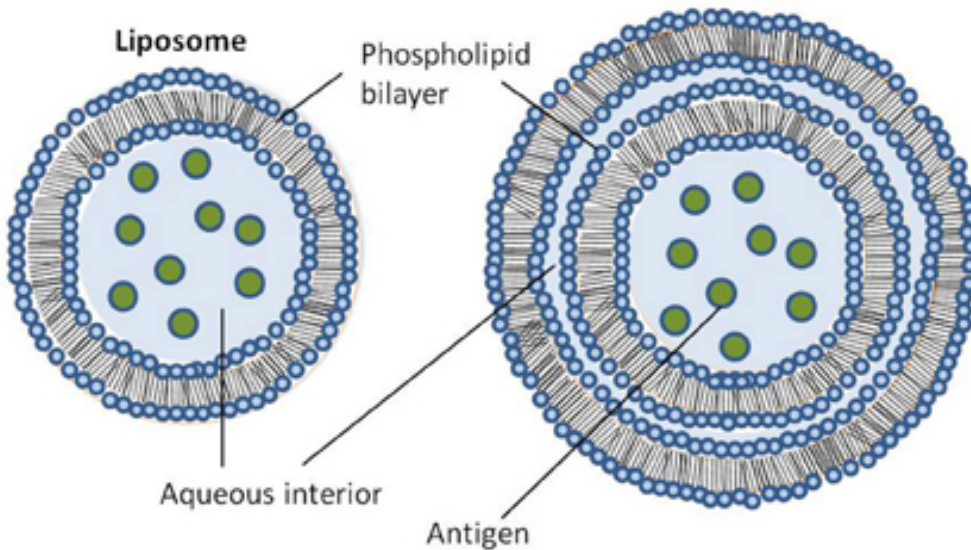
(n) Phosphorothioate link,

(N) Phosphodiester link,

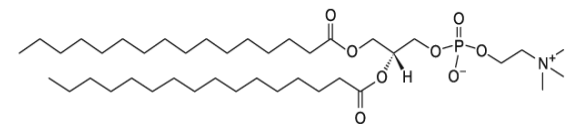
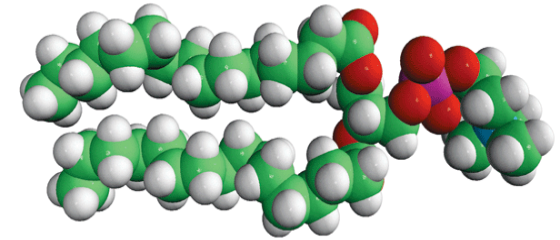
(c-g) CG dinucleotide

# Liposomes

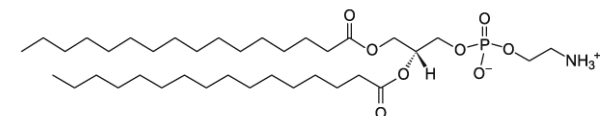
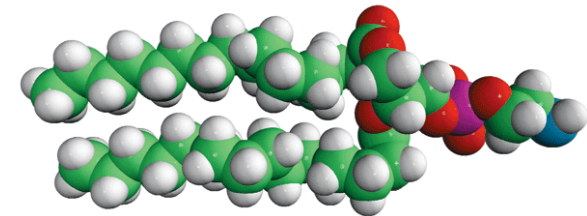
- Hollow vesicle consists of phosphatidylcholine (neutral) or phosphatidylethanolamine (cationic) bilayer



16:0 PC  
1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC)



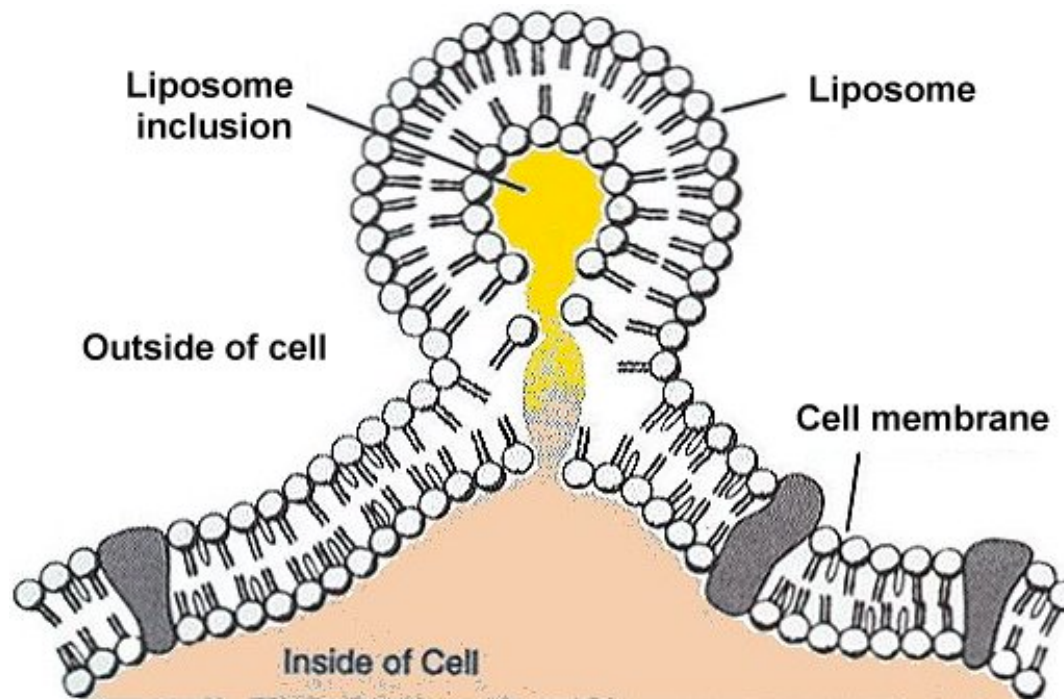
16:0 PE  
1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine (DPPE)





# Liposomes

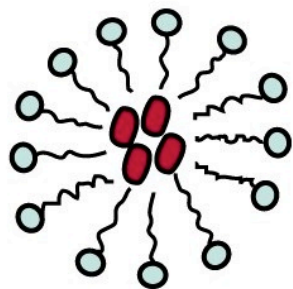
- Liposomes fuse with macrophage cell membrane and the antigen is internalized and processed for presentation



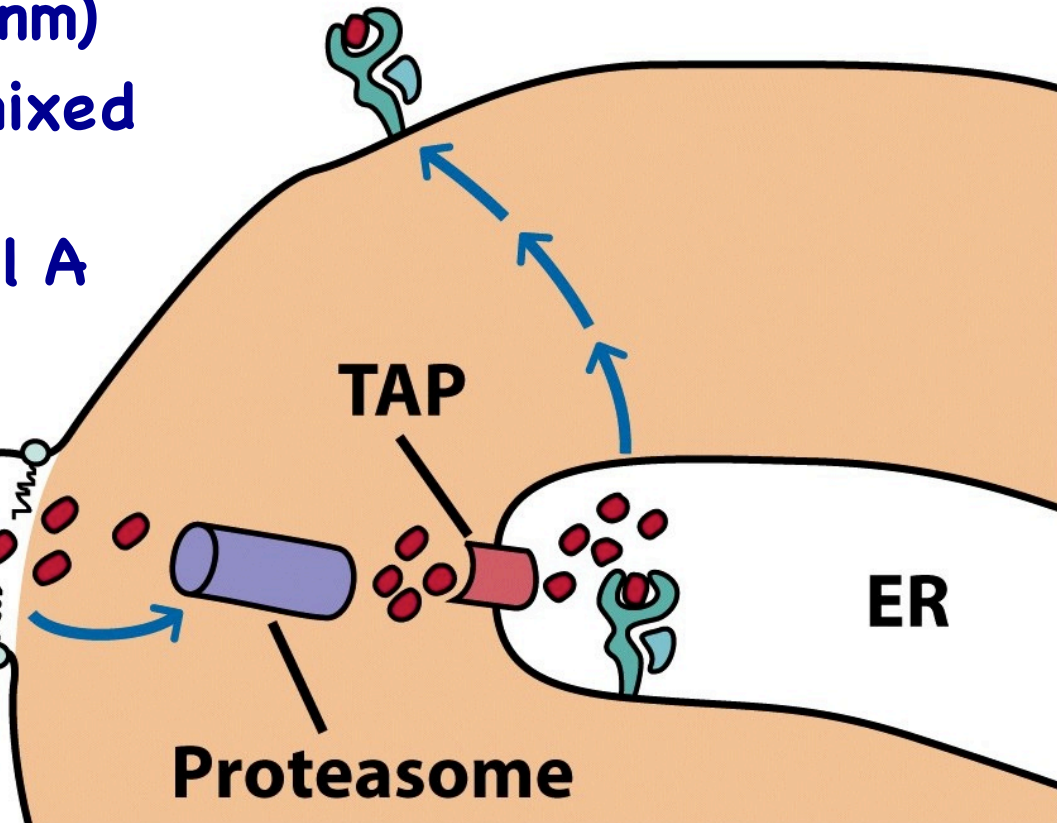
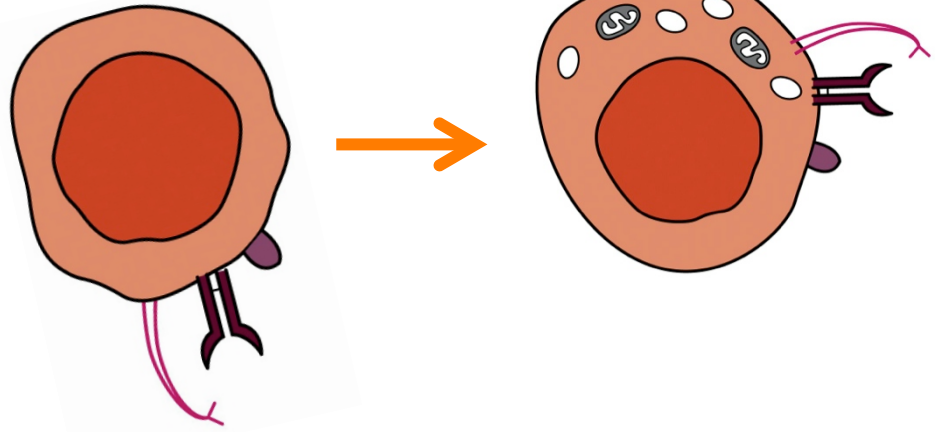
**Acceptance of liposome into cell**

# ISCOM™

- Immune Stimulating Complex (ISCOM)
- Spherical open-cage structure (around 40 nm)
- Self-assembly when mixed with cholesterol, phospholipids, and Quil A (saponin)



ISCOM



TAP

ER

Proteasome



# Excipients (additives)

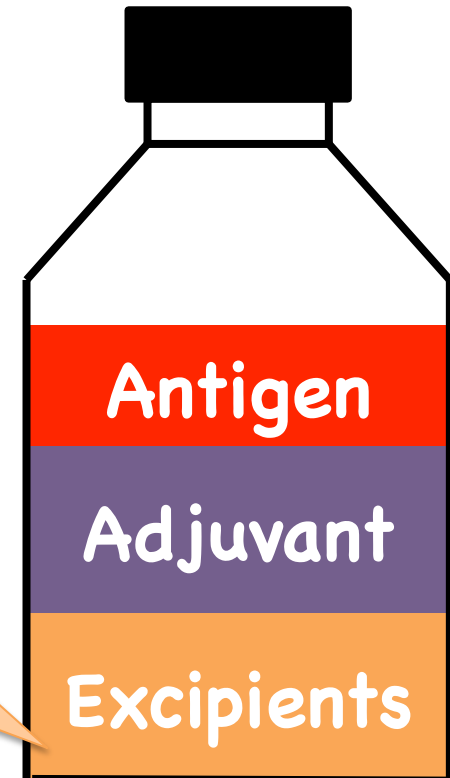
- Some are **intentionally** added to prevent microbial growth or to stabilize the vaccine
- Some are **remnants** of the manufacturing process

Antibiotics      Egg protein

Monosodium glutamate

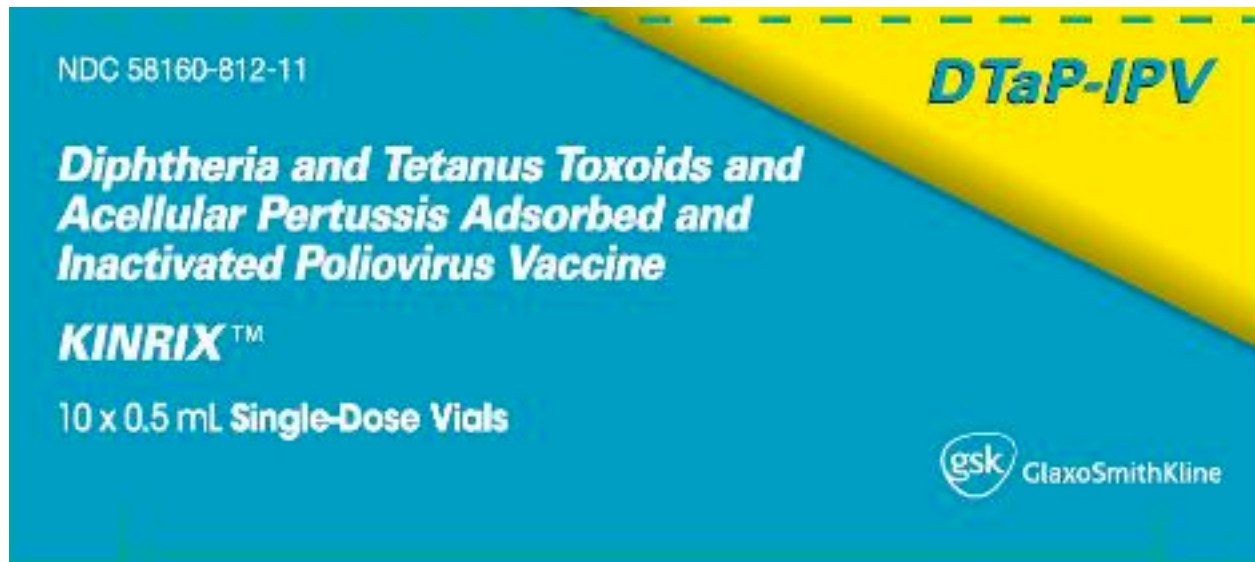
Gelatin

Latex



# Antibiotics

- Such as neomycin, polymyxin B, gentamicin, and streptomycin
- Added to **prevent contamination**
- Little risk of antibiotic-induced contact dermatitis at the site of injection



# Antibiotics

- Such as neomycin, polymyxin B, gentamicin, and streptomycin
- Added to **prevent contamination**
- Little risk of antibiotic-induced contact dermatitis at the site of injection

**Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze. Discard if frozen.**

Each 0.5 mL dose is formulated to contain 25 LF diphtheria toxoid, 10 LF tetanus toxoid, 25 mcg inactivated pertussis toxin, 25 mcg filamentous hemagglutinin, 8 mcg pertactin, 40 D-antigen Units (DU) Type 1 poliovirus, 8 DU Type 2 poliovirus, and 32 DU Type 3 poliovirus. Derived from *C. diphtheriae*, *C. tetani*, *B. pertussis*, and poliovirus strains Types 1, 2, and 3. Contains 4.5 mg of sodium chloride and aluminum adjuvant (not more than 0.6 mg aluminum by assay). Inactivated with glutaraldehyde and formaldehyde. Each dose contains  $\leq 0.05$  ng neomycin sulfate and  $\leq 0.01$  ng polymyxin B. **Contains no preservative.** See complete prescribing information for additional details.

Do not dilute; **shake well before using.** For intramuscular administration only.

**Dosage:** 0.5 mL equals one dose. See complete prescribing information for vaccination schedule.

*KINRIX* is a trademark of GlaxoSmithKline.

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Manufactured by **GlaxoSmithKline Biologicals**, Rixensart, Belgium

Distributed by **GlaxoSmithKline**, Research Triangle Park, NC 27709

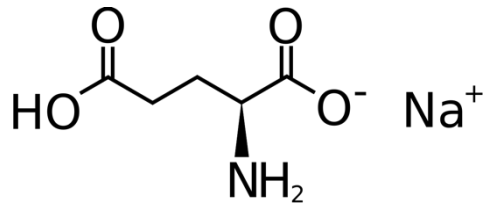
©2007, GlaxoSmithKline

# Egg Proteins

- Because the manufacturing process of some vaccines involve virus culture in eggs, remnants of **egg albumin** is usually found in the vaccine formulation
- Low risk of anaphylaxis in individuals with egg allergy
- Individuals with high sensitive to egg proteins must consult their physicians before taking the vaccine
- Example: seasonal flu shot

# Monosodium glutamate

- Used as a **stabilizer** in many vaccines




- **MSG causes Chinese Restaurant Syndrome (MSG symptom complex):**
  - Hives
  - Abdominal cramps
  - Nausea
  - Vomiting

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## Influenza Vaccine Live, Intranasal



### 2012-2013 Formula

**For Intranasal Administration Only**  
Contents: 10 pre-filled sprayers  
One 0.2 mL dose each (0.1 mL per nostril)

**STORE REFRIGERATED** at 2° - 8°C (35° - 46°F)  
Rx only



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Manufactured by MedImmune, LLC, One Medimmune Way, Gaithersburg, MD 20878 USA

Each 0.2 mL dose is formulated to contain 10<sup>6.5-7.5</sup> PFU (fluorescent focus units) of live attenuated influenza virus reassortants of each of the three strains recommended by the U.S. Public Health Service (USPHS) for the 2012-2013 season: A/California/7/2009 (H1N1), A/Mexico/361/2011 (H3N2), and B/Wisconsin/1/2010. Each 0.2 mL dose also contains monosodium glutamate, hydrolyzed porcine gelatin, egg protein (ovalbumin), and gentamicin sulfate (<0.015 mcg/mL). FLUMIST contains no preservatives. See the enclosed package insert for more information.



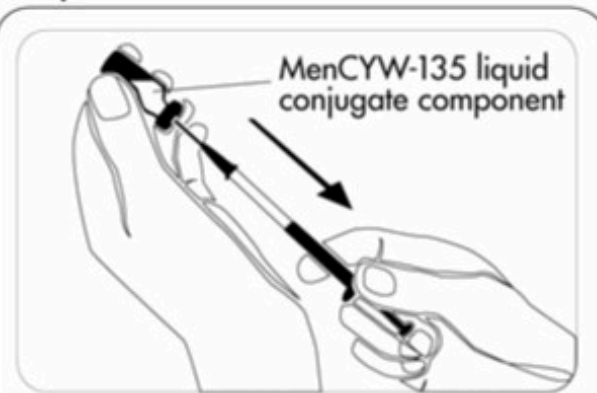
# Gelatin

- Produced by boiling (hydrolysis) of bones and connective tissues of cows and pigs
- It **protects** the vaccine from the effect of heat and cold
- Poorly-hydrolyzed gelatin in vaccines increase the risk of allergic reactions and anaphylaxis

# Latex

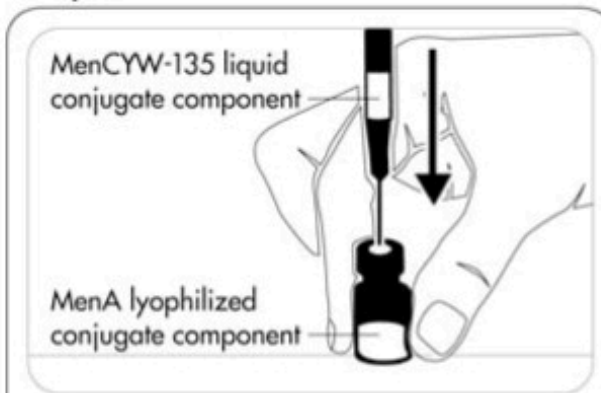
- Natural rubber is used in the manufacturing of syringe plungers and vial stoppers
- Although rare, a case of anaphylaxis due to latex allergy was reported after HepB vaccination

Step 1



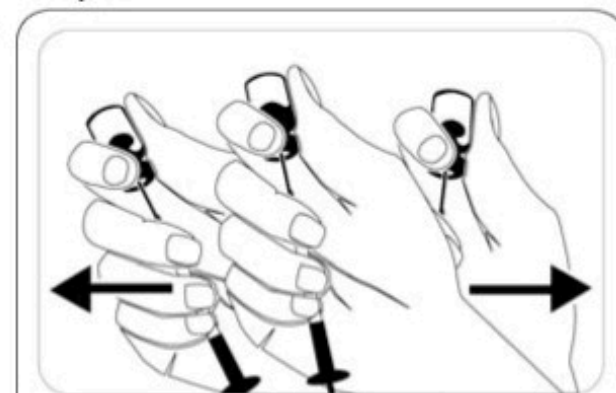
Withdraw all fluid from the vial containing **MenCYW-135 liquid conjugate component**

Step 2



Inject the MenCYW-135 liquid conjugate component into the vial containing **MenA lyophilized conjugate component**

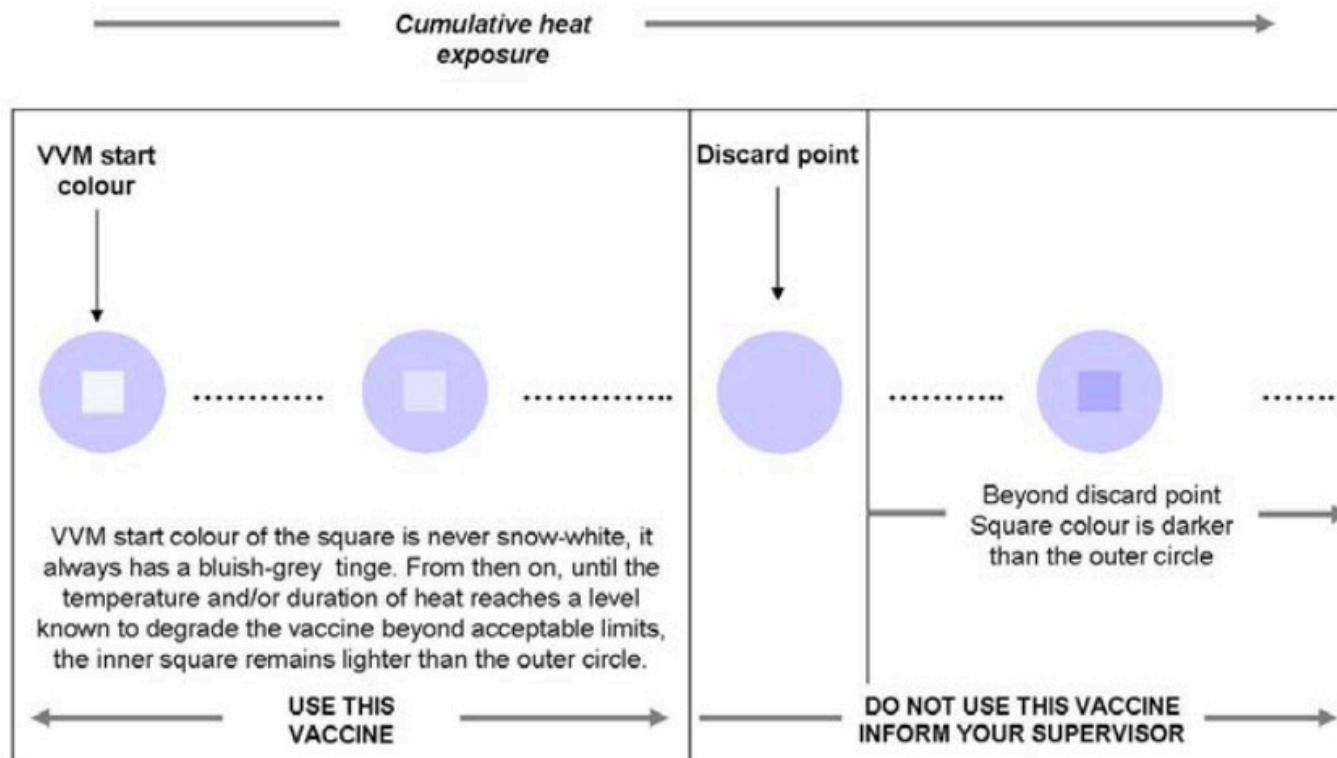
Step 3



Invert and shake the vial well until the vaccine is dissolved. Draw 0.5 mL into the syringe and administer as an intramuscular injection

# Vaccine Vial Monitor

- VVM is a thermochromic label put on vaccine vials
- It gets darker with heat

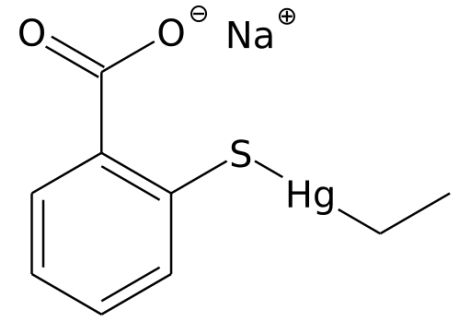


# Vaccine Vial Monitor

- VVM is a thermochromic label put on vaccine vials
- It gets darker with heat



# Thimerosal (ethylmercury)



- Also known as thiomersal
- A **preservative** that was included in vaccine formulations
- Controversially linked to autism in children
- Now phased out from routine children vaccines in the US and EU

**You are now able to:**

- ✓ Recognize common adjuvants
- ✓ Describe how adjuvants work
- ✓ Understand the roles of excipients